

ANTIBODIES AGAINST ILT2 AND USE THEREOF

FIELD OF INVENTION

[0001] The present invention is in the field of monoclonal antibodies and modulating the immune response to cancer.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims priority from PCT Patent Application PCT/IL2020/050889, filed August 12, 2020; United States Provisional Patent Application 63/145,604, filed February 4, 2021; and United States Provisional Patent Application 63/149,371, filed February 15, 2021. The disclosures of those priority applications are incorporated by reference herein in their entirety.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. The electronic copy of the Sequence Listing, created on August 10, 2021, is named 022548_WO091_SL.txt and is 105,080 bytes in size.

BACKGROUND OF THE INVENTION

[0004] Immunoglobulin-like transcript 2 (ILT2), also known as leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1), LIR1 and CD85j, is a cell surface protein expressed on immune cells and is known to inhibit the immune response. The protein contains 4 IgC domains in the extracellular region and 4 intracellular ITIM domains. It is a member of the ILT family, which is made up of ILT1, ILT2, ILT3 and ILT4. ILT2 is most similar to ILT4, having ~80% homology. Known ligands of ILT2 include MHC-I as well as non-classical MHC molecules such as HLA-F, HLA-G, HLA-B27 and UL18 (human CMV). The strongest known interactor of ILT2 in humans is HLA-G1.

[0005] HLA-G1 is widely expressed on the surface of various cancer cells, including breast, cervical, CRC (colorectal cancer), lung, gastric, pancreatic, thyroid and ovarian cancer cells as well as glioblastoma multiform cells and melanoma cells. Its expression is associated with poor clinical outcomes. Further, ILT2 expression on immune cells in the tumor microenvironment has been associated with poor clinical response to oncolytic immune therapy, even when HLA-G1 is

not present. Harnessing the immune response as a weapon against cancer and for cancer surveillance is a promising avenue for cancer prevention and treatment. However, ILT2 presents a roadblock to effective immune therapy. Treatment modalities that can circumvent the ILT2-HLA-G1 axis, as well as HLA-G1-independent functions of ILT2, are greatly needed.

SUMMARY OF THE INVENTION

[0006] The present disclosure provides monoclonal antibodies that bind to ILT2 and inhibit ILT2-mediated immune suppression, as well as pharmaceutical compositions comprising the same. Also provided are methods of treating cancer comprising administering the compositions described herein, methods of producing the antibodies, antigen-binding fragments, and compositions described herein, and methods of increasing the efficacy of PD-1/PD-L1 based therapy.

[0007] According to one aspect, there is provided a monoclonal antibody or antigen-binding fragment that binds to a sequence of human immunoglobulin-like receptor subfamily B member 1 (ILT2) selected from SEQ ID NOs: 41-44 and 68-70.

[0008] According to some embodiments, the sequence is selected from SEQ ID NOs: 68-70.

[0009] According to some embodiments, the sequence is SEQ ID NO: 71 or 72.

[0010] According to some embodiments, the antibody or antigen-binding fragment binds to SEQ ID NOs: 71 and 72.

[0011] According to some embodiments, the antibody or antigen-binding fragment of the present disclosure binds ILT2 and inhibits direct interaction between the ILT2 and beta-2-microglobulin (B2M).

[0012] According to some embodiments, the antibody or antigen-binding fragment inhibits interaction of ILT2 and an HLA protein or MHC-I protein via the inhibition of ILT2 direct interaction with B2M.

[0013] According to some embodiments, the HLA is HLA-G.

[0014] According to some embodiments, the antibody is an IgG4 antibody and comprises a heavy chain constant region of a human IgG4 antibody comprising S228P and L235E mutations (Eu numbering).

[0015] According to one aspect, there is provided a monoclonal anti-ILT2 IgG4 antibody comprising three heavy chain CDRs (CDR-H1-3) and three light chain CDRs (CDR-L1-3) comprising:

- a. SEQ ID NOs: 13-18, respectively,
- b. SEQ ID NOs: 1-6, respectively, or

c. SEQ ID NOs: 7-12, respectively, and

wherein the antibody comprises a heavy chain constant region of a human IgG4 antibody and comprises one or both of the S228P and L235E mutations in the heavy chain constant region (Eu numbering).

[0016] According to some embodiments, the antibody of the present disclosure comprises a heavy chain variable domain comprising an amino acid sequence selected from SEQ ID NOs: 19, 21, and 23 or an amino acid sequence at least 95% identical thereto.

[0017] According to some embodiments, the antibody of the present disclosure comprises a light chain variable domain comprising an amino acid sequence selected from SEQ ID NOs: 20, 22, 24, and 45 or an amino acid sequence at least 95% identical thereto.

[0018] According to some embodiments, the X in SEQ ID NO: 15 is A and the heavy chain comprises a variable domain sequence selected from SEQ ID NOs: 28 and 56-59, or an amino acid sequence at least 95% identical thereto.

[0019] According to some embodiments, the light chain comprises a variable domain sequence selected from SEQ ID NOs: 24 and 60-62, or an amino acid sequence at least 95% identical thereto.

[0020] According to some embodiments, the heavy chain constant region comprises SEQ ID NO: 55, or an amino acid sequence at least 95% identical thereto.

[0021] According to some embodiments, the heavy chain comprises SEQ ID NO: 48 or an amino acid sequence at least 95% identical thereto and the light chain comprises SEQ ID NO: 49 or an amino acid sequence at least 95% identical thereto.

[0022] According to some embodiments, the heavy chain comprises SEQ ID NO: 51 or an amino acid sequence at least 95% identical thereto and the light chain comprises SEQ ID NO: 49 or an amino acid sequence at least 95% identical thereto.

[0023] According to some embodiments, the heavy chain comprises SEQ ID NO: 52 or an amino acid sequence at least 95% identical thereto and the light chain comprises SEQ ID NO: 49 or an amino acid sequence at least 95% identical thereto.

[0024] According to some embodiments, the heavy chain comprises SEQ ID NO: 64 or an amino acid sequence at least 95% identical thereto and the light chain comprises SEQ ID NO: 49 or an amino acid sequence at least 95% identical thereto.

[0025] According to some embodiments, the heavy chain comprises the amino acid sequence SEQ ID NO: 65 or an amino acid sequence at least 95% identical thereto and the light chain comprises SEQ ID NO: 66 or an amino acid sequence at least 95% identical thereto.

[0026] According to some embodiments, the heavy chain comprises SEQ ID NO: 67 or an amino acid sequence at least 95% identical thereto and the light chain comprises SEQ ID NO: 49 or an amino acid sequence at least 95% identical thereto.

[0027] According to some embodiments, the heavy chain comprises SEQ ID NO: 53 or an amino acid sequence at least 95% identical thereto and the light chain comprises SEQ ID NO: 54 or an amino acid sequence at least 95% identical thereto.

[0028] According to one aspect, there is provided a monoclonal antibody comprising a heavy chain and a light chain comprising SEQ ID NOs: 48 and 49, respectively.

[0029]

[0030] According to some embodiments, the antibody or antigen-binding fragment of the present disclosure is for use in at least one of binding ILT2, inducing/enhancing an anti-tumor T cell response, increasing T cell proliferation, reducing cancer-induced suppressor myeloid activity, increasing natural killer cell cytotoxicity, increasing macrophage phagocytosis, increasing generation of M1 inflammatory macrophages, decreasing generation of M2 suppressor macrophages, increasing dendritic cell number in a tumor microenvironment, increasing dendritic cell activation, treating an HLA-G expressing cancer, and treating a MHC-I expressing cancer.

[0031] According to some embodiments, the antibody or antigen-binding fragment of the present disclosure is for use in combination with an opsonizing agent for treating an HLA-G or MHC-I expressing cancer.

[0032] According to some embodiments, the antibody or antigen-binding fragment of the present disclosure is for use in combination with an anti-PD-L1/PD-1 based therapy (e.g., immunotherapy) for treating an HLA-G or MHC-I expressing cancer. In certain embodiments, the anti-PD-L1/PD-1 based therapy is pembrolizumab therapy.

[0033] According to one aspect, there is provided a pharmaceutical composition comprising an antibody or antigen-binding fragment of the present disclosure.

[0034] According to one aspect, there is provided a method of treating an HLA-G or MHC-I expressing cancer in a subject in need thereof, the method comprising administering to the subject a pharmaceutical composition of the present disclosure or an antibody or antigen-binding fragment of the present disclosure.

[0035] According to one aspect, there is provided a method of increasing efficacy of an anti-PD-L1/PD-1 based therapy against a cancer cell expressing HLA-G, MHC-I or both in a subject in need thereof, the method comprising administering to the subject receiving anti-PD-L1/PD-1 based therapy a pharmaceutical composition of the present disclosure or an antibody or antigen-binding fragment of the present disclosure.

[0036] According to some embodiments, the method further comprises administering to the subject an opsonizing agent.

[0037] According to some embodiments, the opsonizing agent is an EGFR inhibitor, optionally wherein the EGFR inhibitor is cetuximab.

[0038] According to some embodiments, the method further comprises administering to the subject an anti-PD-L1/PD-1 based therapy (e.g., immunotherapy). In certain embodiments, the anti-PD-L1/PD-1 based immunotherapy is pembrolizumab therapy.

[0039] According to one aspect, there is provided a method of identifying an antibody that competes for binding to ILT2 with a reference antibody whose heavy and light chains comprise SEQ ID NOs: 48 and 49, respectively, the method comprising: contacting a library of antibodies with a polypeptide sequence comprising an ILT2 sequence selected from SEQ ID NOs: 41-44 and 68-70, and selecting from the library an antibody that binds the ILT2 sequence, thereby obtaining an antibody that competes for binding to ILT2 with the reference antibody.

[0040] According to one aspect, there is provided a method for producing an agent (e.g., an ILT2-binding protein or molecule), the method comprising: obtaining an agent that binds to a sequence of human ILT2 selected from SEQ ID NOs: 41-44 and 68-70; or obtaining a host cell comprising one or more nucleotide sequences encoding an agent that binds to a sequence of human ILT2 selected from SEQ ID NOs: 41-44 and 68-70, and culturing the host cell under conditions that allow expression of the agent, thereby producing the agent. According to some embodiments, the agent binds to a sequence selected from SEQ ID NOs: 68-70. According to some embodiments, the agent binds to one or both of SEQ ID NOs: 71 and 72. According to some embodiments, the agent binds to SEQ ID NOs: 71 and 72.

[0041] According to one aspect, there is provided an agent produced by a method of the present disclosure.

[0042] According to one aspect, there is provided an isolated nucleic acid molecule encoding an antibody or antigen-binding fragment of the present disclosure. In some embodiments, the nucleic acid molecule is an expression vector.

[0043] According to one aspect, there is provided a host cell comprising an isolated nucleic acid molecule of the present disclosure.

[0044] Further embodiments and the full scope of applicability of the present disclosure will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the present disclosure, are given by way of illustration only, since various

changes and modifications within the spirit and scope of the present disclosure will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

- [0045] **Fig. 1.** Histograms depicting the expression of ILT2 on lymphocytes. Commercial antibody #1 at a 5 µg/mL final concentration was used. Binding is depicted as black histogram while isotype control staining is shown with a light grey histogram.
- [0046] **Fig. 2.** Histograms depicting expression of ILT2 on various immune cells. Commercial antibody #1 at a 5 µg/mL final concentration was used. Binding is depicted as black histogram while isotype control staining is shown with light grey histogram.
- [0047] **Figs. 3A-3C.** (3A) Table of cancer indications from the TCGA database in which ILT2 RNA is over-expressed. (3B) Dot plot of correlation between MDSC (myeloid-derived suppressor cell) enrichment in tumors and ILT2 expression. A bar graph depicting the correlation between M2 enrichment and ILT2 expression is also presented. (3C). Scatter plot of the percent of various immune cells which express ILT2 in different tumors.
- [0048] **Figs. 4A-4B.** (4A) Bar graph of the percent of cases for various cancers that are HLA-G positive as determined by immunohistochemistry (IHC). (4B) Scatter plots of HLA-G IHC score for various cancers.
- [0049] **Fig. 5.** Scatter plot of soluble HLA-G levels in various cancers.
- [0050] **Figs. 6A-6H.** (6A) Sequences of the heavy and light chains of three anti-ILT2 antibodies. CDRs as determined by the KABAT system are underlined. (6B) Line graph of the binding of humanized 15G8 antibodies to ILT2 expressed on the surface of BW cells transfected with human ILT2. The graph indicates the fold above background (FAB) levels of the different tested antibodies in comparison to secondary antibody only. (6C-6F) Bar graphs measuring phagocytosis as mean fluorescent intensity (MFI) of phagocytic events for (6C) A375-HLA-G, (6D) COLO-320-WT, (6E) COLO-320-WT and (6F) COLO-320-HLA-G cancer cells cocultured with macrophages in the presence of 15G8 antibodies. (6G-6H) Bar graphs of percent cytotoxicity from NK cell line cells cocultured with various cancer cell lines expressing (6G) A375-HLA-G and (6H) A253 WT in the presence of 15G8 antibodies.
- [0051] **Figs. 7A-7E.** (7A) Table of antibody binding values to ILT2 and ILT2 family members. (7B) Histograms of antibody binding to ILT2 on the cell surface of BW cells transfected with human ILT2. (7C) Line graph of the binding of chimeric and humanized 19E3 (left panel) and of chimeric and humanized 15G8 (right panel) to ILT2 expressed on the surface of BW cells transfected with human ILT2. (7D) Immunostaining on gastric cancer samples with the 19E3

antibody. (7E) Scatter plot of percent of various immune cells which express ILT2 in PBMC samples from healthy controls and cancer patients using the 15G8 humanized antibody.

[0052] Figs. 8A-8P. (8A) Bar graph of percent blocking for each ILT2 antibody and a positive control (PC, GHI/75 antibody). (8B) Histogram of ILT2-biotin binding to cells expressing HLA-G in the presence of an ILT2 blocking antibody. The binding of ILT2-biotin to the cells was determined using Streptavidin-PE by flow cytometry analysis. No antibody (grey line), 15G8 (light grey line), isotype control (black line). (8C) Line graph of blocking activity of the 15G8 humanized antibody as determined by ILT2-biotin binding to cells expressing HLA-G. (8D) Line graph of the blocking activity of chimeric and humanized 19E3 (left panel) and of chimeric and humanized 15G8 (right panel) as determined by the binding of ILT2-biotin to cells expressing HLA-G in the presence of the antibodies. (8E) Bar graph of mouse IL-2 secretion from cells expressing an ILT2 signaling reporter construct in the presence of HLA-G-expressing cells and the presence or absence of ILT2 blocking antibodies. PC=positive control (GHI/75 antibody). (8F) Line graph of blocking activity of the 15G8 humanized antibody as determined by reporter assay. (8G) Bar graph of mouse IL-2 secretion from cells expressing an ILT2 signaling reporter construct in the presence or absence of ILT2 blocking antibody and a positive control antibody. (8H-8K) Bar graph of human IL-2 secretion from Jurkat cells (8H) lacking ILT2, or (8I-8K) expressing ILT2 cocultured with A375 cancer cells (8I) with only MHC-I expression or (8J-8K) expressing both MHC-I and exogenous HLA-G in the presence or absence of ILT2 blocking antibody and a positive control (8I-8J) pan-HLA antibody or (8K) HLA-G specific antibody. (8L-8N) Bar graphs of human IL-2 secretion from Jurkat cells expressing ILT2 cultured with A375 cancer cells expressing HLA-G in the presence or absence of (8L) the 15G8 antibody, (8M) the GHI/75 antibody and (8N) the HP-F1 antibody. (8O-8P) Dot plots of expression of activation markers (8O) phosphorylated ZAP70 and (8P) phosphorylated Syk in TIL cells and NK cells, respectively, incubated with HLA-G-positive cancer cells with and without the presence of 15G8 antibody.

[0053] Figs. 9A-9D. (9A) Bar graph measuring phagocytosis as percent from control of HLA-G expressing cancer cells cocultured with macrophages in the presence of ILT2 antibodies as determined by a FACS-based method. (9B) Line graph of real-time phagocytosis of cancer cells by macrophages in the presence of the ILT2 antibodies as determined by an Incucyte® system. (9C) Bar graphs measuring phagocytosis as percent from control of various HLA-G and MHC-I expressing cancer cells cocultured with macrophages in the presence of the ILT2 antibody 15G8. (9D) Bar graph of phagocytosis by macrophages cocultured with A253-HLA-G cells in the presence of ILT2 antibodies, cetuximab (Erbix®), hIgG control or their combinations.

[0054] **Figs. 10A-10B.** Bar graphs of IFN γ secretion and granzyme B secretion from activated CD8 T cells co-cultured with (10A) wild-type 721.221 cells or HLA-G expressing 721.221 cells or (10B) HLA-G expression A375 cells in the presence of ILT2 antibodies.

[0055] **Figs. 11A-11H. (11A-11B)** Bar graphs of percent cytotoxicity from NK cell line cells cocultured with various cancer cell lines expressing (11A) HLA-G and (11B) MHC-I in the presence of ILT2 antibodies. (11C-11D) Bar graphs of (11C) Granzyme B and (11D) IFN γ secretion from NK cell line cells co-cultured with H&N cancer and melanoma cells respectively in the presence of the 15G8 ILT2 antibodies. (11E-11F) Bar graphs of (11E) IFN γ expression and (11F) CD107A expression in ILT2 positive primary NK cells incubated with target cancer cells in the presence of ILT2 antibodies. (11G-11H) Scatter plots of individual expression showing correlation of ILT2 positive cells and (11G) IFN γ expression and (11H) CD107A expression in response to ILT2 antibodies.

[0056] **Fig. 12.** Line graphs of HLA-DR and CD80 expression (MFI) as determined by flow cytometry in macrophages which were differentiated from monocytes isolated from healthy donors into M0, M1 or M2 macrophages in the presence of IgG or anti-ILT2 antibody. The number of patients which displayed increased expression of the specified marker in comparison to control IgG is indicated for each condition tested.

[0057] **Figs. 13A-13C. (13A)** Bar graph of phagocytosis by macrophages co-cultured with various primary tumor cells. (13B-13C) Bar graphs of dose dependent phagocytosis of primary tumor cells isolated from a (13B) RCC patient and a (13C) H&N patient by autologous macrophages in the presence of a humanized antibody of the present disclosure.

[0058] **Figs. 14A-14L. (14A)** Dot plots of ILT2 and PD-1 expression in tumor cells (left panels) and PBMCs (right panels) from an RCC and esophageal cancer patient. (14B-14C) Box and whisker plots of (14B) PD-1 and (14C) ILT2 RNA expression in CD8 T cell populations in the TME of CRC patients. (14D-14E) Dot plots of (14D) ILT2 expression in CD8 T cells from peripheral blood of healthy donors and of (14E) ILT2 and PD-1 expression in TILs from esophageal cancer. (14F) Scatter plot of the increase in membranal CD107a on PBMCs from 10 healthy donors activated with Staphylococcal Enterotoxin B (SEB) in the presence of 15G8, anti-PD-1 antibody or a combination of the two. (14G) Bar charts of CD107a increase in expression in exemplary PBMCs from 3 donors. (14H-14J) Bar charts of levels of inflammatory cytokine (14H) IFN γ , (14I) TNF α , (14J) GM-CSF secretion from activated PBMCs cocultured with various primary cancer cells in the presence of anti-PD-1 antibody, humanized anti-ILT2 antibody or both. (14K-14L). Bar charts of levels of IFN γ secretion from T cells cocultured with (14K) dendritic cells or (14L) macrophages in a mixed lymphocyte reaction.

[0059] **Figs. 15A-15F.** (15A) Line graphs of tumor volume of HLA-G and MHC-I expressing tumors grown in immunocompromised mice supplemented with human macrophages and anti-ILT2 antibodies. (15B) Illustration of mice treatment schedule for preventing lung tumors. (15C) Photographs of lungs from immunocompromised mice inoculated with HLA-G positive cancer cells with or without human PBMC and an ILT2 antibody. (15D) Scatter plot summarizing the data from 15C. (15E) Illustration of mice treatment schedule for treating already established lung tumors. (15F) Box and whisker plot of tumor weights.

[0060] **Figs. 16A-16F.** (16A-16F) Box and whisker plots of (16A) CD107A expression in total CD8 T cells, (16B) CD107A expression in T_{EMRA} cells, (16C) CD69 expression in NK cells, (16D) CD69 expression in total CD8 T cells, (16E) CD107 expression in T_{EMRA} cells and (16F) CD69 expression in combination treated NK cells in mice that received PBMC from donors with low or high levels of ILT2 in their T_{EMRA} cells or NK cells, respectively. * is a $P < 0.005$. ** is a $P < 0.0005$. *** is a $P < 0.0001$.

[0061] **Figs. 17A-17F.** (17A) Illustration of treatment schedule for humanized NSG mice inoculated with head and neck cancer and treated with anti-ILT2 or control antibodies. (17B) Line graph of tumor weight from IgG and anti-ILT2 treated mice. (17C-17E) Bar graphs of (17C) baseline ILT2 levels in peripheral CD8 T cells in mice that responded (R) or did not respond (NR) to BND-22 treatment. Intra-tumoral post-treatment (17D) CD107A expression, (17E) M1/M2 ratio and (17F) total CD80 positive dendritic cell number in the four mice treated with anti-ILT2 antibody.

[0062] **Figs. 18A-18F.** (18A) Partial sequence of ILT2 showing residues with significant predicted binding. These residues are divided in four categories as a function of their raw probability to belong to the epitope. Stars indicate locations of selected mutations. (18B-18C) 3D renderings of ILT2 surface structure showing (18B) the location of the residues from 18A and (18C) the four main interaction regions on ILT2. (18D-18F) 3D ribbon or surface diagrams of ILT2 showing (18D) the epitope of the 15G8 antibody and the epitope of the 3H5, 12D12 and 27H5 antibodies from WO2020/136145 (left upper circle), as well as a secondary epitope of the 3H5 antibody (right upper circle) (18E-18F) and interaction of the 15G8 epitope on ILT2 with B2M in complex with (18E) HLA-A or (18F) HLA-G.

[0063] **Figs. 19A-19G.** Distribution of protection ratios for the free ILT2 against ILT2-15G8 complex. (19A) Bar chart of data derived from tryptic digestion of ILT2 protein. The median of the distribution is 1.5, the mean is 1.37. (19B) Bar chart of data derived from trypsin and Asp-N digestion of ILT2 protein. The median of the distribution is 1.58, the mean is 1.48. Selected protected regions are marked. (19C-19G) Dose-response plot comparison of the free ILT2

peptides against the complex. Lines with squares and circles denote the control and complex respectively. **(19C)** Peptide 56-71: free ILT2 $K = 9.09 \text{ s}^{-1}$, complex $K = 2.31 \text{ s}^{-1}$ indicating a 3.94-fold decrease as a result of complex formation. **(19D)** Peptide 57-71: free ILT2 $K = 6.93 \text{ s}^{-1}$, complex $K = 1.97 \text{ s}^{-1}$ indicating a 3.52-fold decrease as a result of complex formation. **(19E)** Peptide 84-100: free ILT2 $K = 1.26 \text{ s}^{-1}$, complex $K = 0.37 \text{ s}^{-1}$ indicating a 3.41-fold decrease as a result of complex formation. **(19F)** Peptide 57-66: free ILT2 $K = 4.32 \text{ s}^{-1}$, complex $K = 0.72 \text{ s}^{-1}$ indicating a 6-fold decrease as a result of complex formation. **(19G)** Peptide 91-100: free ILT2 $K = 0.7 \text{ s}^{-1}$, complex $K = 0.14 \text{ s}^{-1}$ indicating a 5-fold decrease as a result of complex formation.

[0064] **Fig. 20.** Graph of ILT2-Fc binding to human B2M in the presence of 15G8 or a non-relevant antibody in an ELISA assay.

[0065] **Figs. 21A-21D.** **(21A-21B)** Bar graphs of % increased phagocytosis as compared to IgG control of **(21A)** A375-HLA-G and **(21B)** SKMEL28-HLA-G cancer cells cocultured with macrophages in the presence of various anti-ILT2 antibodies. **(21C-21D)** Line graphs of competition ILT2 binding ELISAs using biotinylated 15G8 antibody in the presence of competing unbiotinylated **(21C)** GHI/75, HP-F1 and **(21D)** MAB20172 and 15G8 antibodies.

DETAILED DESCRIPTION OF THE INVENTION

[0066] The present disclosure is directed to monoclonal antibodies or antigen-binding fragments and pharmaceutical compositions that bind ILT2 and inhibit ILT2-mediated immune suppression. Methods of treating cancer and enhancing PD-1/PD-L1 immunotherapy are also provided.

[0067] The present disclosure is at least partially based on the surprising finding that ILT2 antagonism acts synergistically with PD-1 and PD-L1-based immunotherapies to combat cancer cells. Specifically, it was found that ILT2-blocking antibodies in combination with anti-PD-1 antibodies increased pro-inflammatory cytokine secretion by immune cells. This increase was not merely additive, but rather greater than the sum of the effects of each agent individually. Indeed, for at least one cytokine a de novo increase was observed, where neither agent alone had any effect. This combined treatment allows for conversion of cancers from PD-1/PD-L1 refractory to PD-1/PD-L1 responsive.

[0068] It was further surprisingly found that the level of ILT2 expression in the immune cells of patients was correlated to the effectiveness of the ILT2 blocking therapy. Responders to the therapy had high ILT2 levels, while non-responders had low ILT2 levels. In particular, ILT2 levels on circulating CD8-positive T cells were predictive of treatment outcome.

[0069] Lastly, the antibodies of the present disclosure were found to bind a unique epitope within the ILT2 interdomain between the D1 and D2 domains. This region is known to be the interaction domain between ILT2 and beta-2-microglobulin (B2M). The antibodies of the present disclosure are the first known antibodies to directly block this interaction. Further, the antibodies of the present disclosure were found to have immuno-stimulating effects not reported for other anti-ILT2 antibodies. The present antibodies were able to modulate the immunosurveillance of T cells, NK cells, dendritic cells and macrophages against MHC-I (e.g., HLA-G) expressing cancer cells. In particular, for the first time, an anti-ILT2 antibody used as a monotherapy was shown to enhance phagocytosis of cancer cells.

Antibodies

[0070] In a first aspect, the present disclosure provides an antibody comprising three heavy chain CDRs (CDR-H) and three light chain CDRs (CDR-L), wherein: CDR-H1 comprises the amino acid sequence set forth in SEQ ID NO: 1 (DHTIH), CDR-H2 comprises the amino acid sequence as set forth in SEQ ID NO: 2 (YIYPRDGSTKYNEKFKG), CDR-H3 comprises the amino acid sequence as set forth in SEQ ID NO: 3 (TWDYFDY), CDR-L1 comprises the amino acid sequence as set forth in SEQ ID NO: 4 (RASESVDSYGNSFMH), CDR-L2 comprises the amino acid sequence as set forth in SEQ ID NO: 5 (RASNLES), and CDR-L3 comprises the amino acid sequence as set forth in SEQ ID NO: 6 (QQSNEDPYT). In some embodiments, the antibody further comprises a heavy chain constant region of an IgG4 antibody (e.g., a human IgG4).

[0071] In another aspect, the present disclosure provides an antibody or antigen-binding fragment comprising three heavy chain CDRs (CDR-H) and three light chain CDRs (CDR-L), wherein: CDR-H1 comprises the amino acid sequence set forth in SEQ ID NO: 7 (GYTFTSYGIS), CDR-H2 comprises the amino acid sequence as set forth in SEQ ID NO: 8 (EIYPGSGNSYYNEKFKG), CDR-H3 comprises the amino acid sequence as set forth in SEQ ID NO: 9 (SNDGYPDY), CDR-L1 comprises the amino acid sequence as set forth in SEQ ID NO: 10 (KASDHINWLA), CDR-L2 comprises the amino acid sequence as set forth in SEQ ID NO: 11 (GATSLET), and CDR-L3 comprises the amino acid sequence as set forth in SEQ ID NO: 12 (QQYWSTPWT). In some embodiments, the antibody further comprises a heavy chain constant region of an IgG4 antibody (e.g., a human IgG4).

[0072] In another aspect, the present disclosure provides an antibody or antigen-binding fragment comprising three heavy chain CDRs (CDR-H) and three light chain CDRs (CDR-L), wherein: CDR-H1 comprises the amino acid sequence set forth in SEQ ID NO: 13 (SGYYWN),

CDR-H2 comprises the amino acid sequence as set forth in SEQ ID NO: 14 (YISYDGSNNYNPSLKN), CDR-H3 comprises the amino acid sequence as set forth in SEQ ID NO: 15 (GYSYYYAMDX) wherein X is selected from A, C and S, CDR-L1 comprises the amino acid sequence as set forth in SEQ ID NO: 16 (RTSQDISNYLN), CDR-L2 comprises the amino acid sequence as set forth in SEQ ID NO: 17 (YTSRLHS), and CDR-L3 comprises the amino acid sequence as set forth in SEQ ID NO: 18 (QQGNTLPT). In some embodiments, the antibody further comprises a heavy chain constant region of an IgG4 antibody (e.g., a human IgG4).

[0073] In some embodiments, SEQ ID NO: 15 is GYSYYYAMDA (SEQ ID NO: 25). In some embodiments, SEQ ID NO: 15 is SEQ ID NO: 25 and the antibody or antigen-binding fragment is a humanized antibody. In some embodiments, SEQ ID NO: 15 is GYSYYYAMDS (SEQ ID NO: 26). In some embodiments, SEQ ID NO: 15 is SEQ ID NO: 26 and the antibody or antigen-binding fragment is a humanized antibody. In some embodiments, SEQ ID NO: 15 is GYSYYYAMDC (SEQ ID NO: 27). In some embodiments, SEQ ID NO: 16 is SEQ ID NO: 27 and the antibody or antigen-binding fragment is a murine antibody.

[0074] In another aspect, there is provided an antibody or antigen-binding fragment that binds a human leukocyte immunoglobulin-like receptor subfamily B member 1 (ILT2) interdomain between domains D1 and D2.

[0075] In another aspect, there is provided an antibody or antigen-binding fragment that binds to a sequence of ILT2 selected from VKKGQFPISITWEH (SEQ ID NO: 41), LELVVTGAYIKPTLS (SEQ ID NO: 42), VILQCDSQVAFDGFS (SEQ ID NO: 43), WYRCYAYDSNSPYEW (SEQ ID NO: 44), KGQFPISITWEHAGR (SEQ ID NO: 68), GQFPISITWEHAGR (SEQ ID NO: 69), and SESSDPLELVVTGAYIK (SEQ ID NO: 70). In some embodiments, the antibody or antigen-binding fragment may bind to 1, 2, 3, 4, 5, 6, or all 7 of said sequences.

[0076] In another aspect, there is provided an antibody or antigen-binding fragment that binds ILT2 and inhibits interaction between ILT2 and B2M.

[0077] In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a polyclonal antibody. In some embodiments, the antibody is a murine antibody. In some embodiments, the antibody is a humanized antibody. As used herein, a "humanized" antibody refers to an antibody that has a human backbone, but with CDRs that are derived or taken from a non-human antibody. In some embodiments, during humanization the CDRs may become altered but are generally still derived from the CDRs of the non-human parental antibody. In some embodiments, the antigen-binding fragment is a single chain antibody. In some embodiments, the antigen-binding fragment is a single domain antibody.

[0078] In some embodiments, the antibody comprises an IgG4 constant region. In some embodiments, the antibody is a humanized antibody comprising a human IgG4 constant region. In some embodiments, the IgG4 constant region is an engineered IgG4 constant region and contains mutations relative to wildtype IgG4 constant region. In some embodiments, a human IgG4 constant region comprises the amino acid sequence:

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ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES
KYGPPCPSCP APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED
PEVQFNWYVD GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK
CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPQSQEEMTK NQVSLTCLVK
GFYPSDIAVE WESNGQPENN YKTTTPVLDS DGSFFLYSRL TVDKSRWQEG
NVFSCSVMEH ALHNHYTQKS LSLSLGK (SEQ ID NO: 50).
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In some embodiments, a human IgG4 constant region consists of SEQ ID NO: 50. In some embodiments, a human IgG4 constant region comprises at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to SEQ ID NO: 50. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, a human IgG4 constant region comprises at least 95% identity to SEQ ID NO: 50. In some embodiments, a human IgG4 constant region comprises at least 97% identity to SEQ ID NO: 50. In some embodiments, a human IgG4 constant region comprises at least 99% identity to SEQ ID NO: 50.

[0079] In some embodiments, the IgG4 constant region comprises at least one mutation. In some embodiments, the mutation decreases binding to an Fc receptor, such as Fc gamma receptor (FcγR), or decreases Fab-arm exchange of the IgG4 antibody. In some embodiments, the mutation is of serine 108. In some embodiments, serine is mutated to proline. In some embodiments, serine 108 is serine 108 of SEQ ID NO: 50 (or S228 according to Eu numbering). Mutation of serine 108 to proline is also known as the S228P mutation. In some embodiments, the mutation is of leucine 115 (or L235 according to Eu numbering). In some embodiments, leucine is mutated to glutamic acid. In some embodiments, leucine 115 is leucine 115 of SEQ ID NO: 50. Mutation of leucine 115 to glutamic acid is also known as the L235E mutation. It will be understood by a skilled artisan that the exact numerical position of any amino acid in the constant region of the heavy chain will depend on the length of the variable region of the heavy chain. As such, the position of the serine is given as 108 within the constant region and the position of leucine is given as 115 (see, e.g., SEQ ID NO: 50). It should further be understood that modifications to the constant region (e.g., addition or deletion of a base) can also alter the numerical position, but that any analog or derivative of IgG4 is also envisioned so long as it includes the recited mutation. In some embodiments, the IgG4 constant region comprises a plurality of mutations. In some embodiments,

the IgG4 constant region comprises mutations of serine 108 and leucine 115. In some embodiments, the IgG4 constant region comprises S108P and L115E mutations (or S228P and L235E mutations according to Eu numbering).

[0080] In some embodiments, the IgG4 constant region comprises the following amino acid sequence, where mutations to wildtype human IgG4 constant region (S228P and L235E; Eu numbering) are shown in boxes:

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ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES
KYGPPCPECP APEFEGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVDSQED
PEVQFNWYVD GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK
CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK
GFYPSDIAVE WESNGQPENN YKTTTPVLDS DGSFFLYSRL TVDKSRWQEG
NVFSCSVMEH ALHNHYTQKS LSLSLGK (SEQ ID NO: 55).
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In some embodiments, the human IgG4 constant region comprises or consists of SEQ ID NO: 55. In some embodiments, the antibodies of the present disclosure comprise SEQ ID NO: 55. In some embodiments, the heavy chain constant region of the antibodies of the present disclosure comprise SEQ ID NO: 55. In some embodiments, the heavy chain constant region of the antibodies of the present disclosure consists of SEQ ID NO: 55. In some embodiments, the IgG4 constant region is an analog or derivative of SEQ ID NO: 55, e.g., containing further mutations that improve the manufacturability or reduce immunogenicity of the antibody. In some embodiments, an IgG4 constant region comprises at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to SEQ ID NO: 55. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, an IgG4 constant region comprises at least 95% identity to SEQ ID NO: 55. In some embodiments, an IgG4 constant region comprises at least 97% identity to SEQ ID NO: 55. In some embodiments, an IgG4 constant region comprises at least 99% identity to SEQ ID NO: 55.

[0081] In some embodiments, an analog of the IgG4 constant region further comprises at least one other mutation. In some embodiments, the at least one other mutation is in SEQ ID NO: 55. In some embodiments, the at least one other mutation is not at position 108. In some embodiments, the at least one other mutation is not at position 115. In some embodiments, the at least one other mutation is not at position 108 or 115. Other mutations of IgG4 are well known in the art and any of these may be employed. Examples of other mutations include, but are not limited to, E233P, F234A, L235A, G237A, P239G, F243L, T250Q, T250E, M252Y, S254T, T256E, E258F, D259I, V264A, D265A, F296Y, T307A, T307Q, V308W, V308Y, V308F, Q311V, K317Q, A330R, E356K, K370Q, K370E, E380A, R409K, V427T, M428L, M428F, H434K, N434S, N434A,

N434H, N434F, H435R, Y436H, K439E, L445P, deletion of G236, deletion of G446, and deletion of K447 (numbers are given according to the Eu numbering). Once again, the numerical position given for these mutations is the common notation (Eu numbering) but will depend on the length of the variable region of the heavy chain. Within SEQ ID NO: 50, these positions correspond to E113, F114, L115, G117, P119, F123, T130, M132, S134, T136, E138, D139, V144, D145, F176, T187, V188, Q191, K197, A210, E236, K250, E260, R289, V307, M308, H313, N314, H315, Y316, K319, L325, G326, and K327. It will also be understood that specific combinations of mutations may be employed, such as E233P/F234A/L235A/G236del/G237A or S228P/F234A/L235A/G237A/P238S.

[0082] In some embodiments, the antibody or antigen-binding fragment binds ILT2. In some embodiments, ILT2 is human ILT2. In some embodiments, ILT2 is mammalian ILT2. In some embodiments, ILT2 is primate ILT2 (e.g., cynomolgus monkey ILT2). In some embodiments, ILT2 is murine ILT2. In some embodiments, the antibody or antigen-binding fragment binds an extracellular domain of ILT2. In some embodiments, the antibody or antigen-binding fragment binds the ligand pocket of ILT2. In some embodiments, the ligand is B2M. In some embodiments, the ligand is not an HLA. In some embodiments, the ligand is HLA. In some embodiments, the HLA is HLA-G. In some embodiments, the ligand is not an MHC. In some embodiments, the ligand is MHC. In some embodiments, the MHC is MHC class I (MHC-I). In some embodiments, the antibody or antigen-binding fragment binds an ILT2 interdomain. In some embodiments, the interdomain is the interface between the D1 and D2 domains. In some embodiments, the interdomain is the hinge domain between the D1 and D2 domains. In some embodiments, the interdomain does not comprise the N-terminal domain of D1. In some embodiments, the interdomain is from amino acids 54-184 of SEQ ID NO: 31. In some embodiments, amino acids 54-184 of SEQ ID NO: 31 comprise the interdomain. In some embodiments, the interdomain is from amino acids 90-184 of SEQ ID NO: 31. In some embodiments, amino acids 90-184 comprise the interdomain. In some embodiments, the antibody or antigen-binding fragment binds an epitope within the interdomain. In some embodiments, the epitope comprises at least 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 97, 99 or 100% of the interdomain. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the epitope is within D2. In some embodiments, the antibody or antigen-binding domain binds an epitope in D2. In some embodiments, the epitope is at least partially in D2. In some embodiments, the antibody or antigen-binding domain binds an epitope at least partially in D2. In some embodiments, the epitope spans D1 and D2. In some embodiments, the antibody or antigen-binding fragment does not bind an ILT2 domain that interacts with the $\alpha 3$ domain of HLA-G.

[0083] In some embodiments, ILT2 is mammalian ILT2. In some embodiments, ILT2 is human ILT2. In some embodiments, ILT2 has the amino acid sequence provided in NCBI Reference Sequence: NP_006660.4. In some embodiments, ILT2 has the following amino acid sequence:

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MTPILTVLIC LGLSLGPRTH VQAGHLPKPT LWAEPGSVIT QGSPVTLRCQ
GGQETQEYRL YREKKTALWI TRIPQELVKK GQFPIPSITW EHAGRYRCYY
GSDTAGRSES SDPLELVVTG AYIKPTLSAQ PSPVNSGGN VILQCDQVA
FDGFSLCKEG EDEHPQCLNS QPHARGSSRA IFSVGPVSPS RRWWYRCYAY
DSNSPYEWSL PSDLLELLVL GVSCKPSLSV QPGPIVAPEE TLTLCQGS DA
GYNRFVLYKD GERDFLQLAG AQPQAGLSQA NFTLGPVSR S YGGQYRCYGA
HNLSSSEWSAP SDPLDILIAQ QFYDRVLSLV QPGPTVASGE NVTLLCQS QG
WMQTFLLTKE GAADDPWRLR STYQSQKYQA EFPMPGVTSA HAGTYRCYGS
QSSKPYLLTH PSDPLELVVS GPSGGPSSPT TGPTSTSGPE DQPLTPTGSD
PQSGLGRLHG VVIGILVAVI LLLLLLLLLL LILRHRQ GK HWTSTQRKAD
FQHPAGAVGP EPTDRGLQWR SSPAADAQEE NLYAAVKHTQ PEDGVEMDTR
SPHDEDPQAV TYAEVKHSRP RREMASPPSP LSGEFLDTKD RQAEEDRQMD
TEAAASEAPQ DVTYAQLHSL TLRREATEPP PSQEGPSPAV PSIIATLAIH
(SEQ ID NO: 31).

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[0084] In some embodiments, ILT2 has the amino acid sequence provided in NCBI Reference Sequence: NP_001075106.2. In some embodiments, ILT2 has the amino acid sequence provided in NCBI Reference Sequence: NP_001075107.2. In some embodiments, ILT2 has the amino acid sequence provided in NCBI Reference Sequence: NP_001075108.2. In some embodiments, ILT2 has the amino acid sequence provided in NCBI Reference Sequence: NP_001265328.2.

[0085] In some embodiments, the D1 domain of ILT2 comprises or consists of the amino acid sequence

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GHLPKPTLWA EPGSVITQGS PVTLCQGGQ ETQEYRLYRE KKTALWITRI
PQELVKKGF PIPSITWEHA GRYRCYSGD TAGRSESSDP LELVVTGA
(SEQ ID NO: 46).

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In some embodiments, the D1 domain of ILT2 comprises or consists of amino acids 24-121 of SEQ ID NO: 31. In some embodiments, the D2 domain of ILT2 comprises or consists of the amino acid sequence

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YIKPTLSAQP SPVNSGGNV ILQCDQVAF DGFSLCKEGE DEHPQCLNSQ
PHARGSSRAI FSVGPVSPSR RWWYRCYAYD SNSPYEWSLP SDLLELLVLG
V (SEQ ID NO: 47).

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In some embodiments, the D2 domain of ILT2 comprises or consists of amino acids 122-222 of SEQ ID NO: 31. In some embodiments, the interdomain of ILT2 comprises amino acids Gln41,

Lys65, Trp90, Gly120, Ala121, Val122, Ile123, Gln148, Val149, Ala150, Phe151, Asp201, Asn203, and Glu207 of SEQ ID NO: 31. In some embodiments, the epitope comprises amino acids Gln41, Lys65, Trp90, Gly120, Ala121, Val122, Ile123, Gln148, Val149, Ala150, Phe151, Asp201, Asn203 and Glu207 of SEQ ID NO: 31. In some embodiments, the epitope comprises at least 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 amino acids selected from amino acids Gln41, Lys65, Trp90, Gly120, Ala121, Val122, Ile123, Gln148, Val149, Ala150, Phe151, Asp201, Asn203, and Glu207 of SEQ ID NO: 31.

[0086] In some embodiments, the epitope comprises at least 10 amino acids selected from amino acids Gln41, Lys65, Trp90, Gly120, Ala121, Val122, Ile123, Gln148, Val149, Ala150, Phe151, Asp201, Asn203, and Glu207 of SEQ ID NO: 31. In some embodiments, the antibody or antigen-binding fragment binds to the ILT2 sequence provided in SEQ ID NO: 41. In some embodiments, the antibody or antigen-binding fragment binds to the ILT2 sequence provided in SEQ ID NO: 42. In some embodiments, the antibody or antigen-binding fragment binds to the ILT2 sequence provided in SEQ ID NO: 43. In some embodiments, the antibody or antigen-binding fragment binds to the ILT2 sequence provided in SEQ ID NO: 44. In some embodiments, the antibody or antigen-binding fragment binds a 3-dimensional epitope comprising residues from at least two of SEQ ID NOs: 41, 42, 43 and 44 (e.g., a 3-dimensional epitope comprising at least two of SEQ ID NOs: 41, 42, 43 and 44). In some embodiments, the 3-dimensional epitope comprises residues from at least 3 of SEQ ID NOs: 41, 42, 43 and 44 (e.g., a 3-dimensional epitope comprising at least 3 of SEQ ID NOs: 41, 42, 43 and 44). In some embodiments, the 3-dimensional epitope comprises residues from SEQ ID NOs: 41, 42, 43 and 44 (e.g., a 3-dimensional epitope comprising SEQ ID NOs: 41, 42, 43 and 44).

[0087] In some embodiments, the antibody or antigen-binding fragment binds to SEQ ID NO: 41 (e.g., to an ILT2 epitope comprising or within SEQ ID NO: 41). In some embodiments, the antibody or antigen-binding fragment binds to SEQ ID NO: 42 (e.g., to an ILT2 epitope comprising or within SEQ ID NO: 42). In some embodiments, the antibody or antigen-binding fragment binds to SEQ ID NO: 43 (e.g., to an ILT2 epitope comprising or within SEQ ID NO: 43). In some embodiments, the antibody or antigen-binding fragment binds to SEQ ID NO: 44 (e.g., to an ILT2 epitope comprising or within SEQ ID NO: 44). In some embodiments, the antibody or antigen-binding fragment binds to SEQ ID NO: 68 (e.g., to an ILT2 epitope comprising or within SEQ ID NO: 68). In some embodiments, the antibody or antigen-binding fragment binds to SEQ ID NO: 69 (e.g., to an ILT2 epitope comprising or within SEQ ID NO: 69). In some embodiments, the antibody or antigen-binding fragment binds to SEQ ID NO: 70 (e.g., to an ILT2 epitope comprising or within SEQ ID NO: 70). In some embodiments, the

antibody or antigen-binding fragment binds to a 3-dimensional epitope comprising residues from at least two of SEQ ID NOs: 41-44 and 68-70 (e.g., to a 3-dimensional epitope comprising at least two of SEQ ID NOs: 41-44 and 68-70). In some embodiments, the 3-dimensional epitope comprises residues from at least 3 of SEQ ID NOs: 41-44 and 68-70 (e.g., the 3-dimensional epitope comprises at least 3 of SEQ ID NOs: 41-44 and 68-70). In some embodiments, the 3-dimensional epitope comprises residues from at least 4 of SEQ ID NOs: 41-44 and 68-70 (e.g., the 3-dimensional epitope comprises at least 4 of SEQ ID NOs: 41-44 and 68-70). In some embodiments, the 3-dimensional epitope comprises residues from SEQ ID NOs: 41, 42, and 68-70 (e.g., the 3-dimensional epitope comprises SEQ ID NOs: 41, 42, and 68-70). In some embodiments, the antibody or antigen-binding fragment binds to a sequence selected from SEQ ID NOs: 68-70 (e.g., to an epitope comprising or within any one of SEQ ID NOs: 68-70).

[0088] In some embodiments, the antibody or antigen-binding fragment binds to the sequence GQFPIPSITW (SEQ ID NO: 71) (e.g., to an ILT2 epitope comprising or within said sequence). In some embodiments, the antibody or antigen-binding fragment binds to the sequence ELVVTGAYIK (SEQ ID NO: 72) (e.g., to an ILT2 epitope comprising or within said sequence). In particular embodiments, the antibody or antigen-binding fragment binds to SEQ ID NOs: 71 and 72 (e.g., to an epitope comprising or within SEQ ID NOs: 71 and 72). In some embodiments, the antibody or antigen-binding fragment binds to a sequence selected from SEQ ID NOs: 68-72 (e.g., to an epitope comprising or within said sequence). In some embodiments, the antibody or antigen-binding fragment binds to a sequence selected from SEQ ID NOs: 41-44 and 68-72 (e.g., to an epitope comprising or within said sequence). In some embodiments, SEQ ID NO: 71 is an epitope within SEQ ID NO: 41. In some embodiments, SEQ ID NO: 71 is an epitope within SEQ ID NO: 68. In some embodiments, SEQ ID NO: 71 is an epitope within SEQ ID NO: 69. In some embodiments, SEQ ID NO: 72 is an epitope within SEQ ID NO: 42. In some embodiments, SEQ ID NO: 72 is an epitope within SEQ ID NO: 70.

[0089] In some embodiments, the antibody or antigen-binding fragment binds an ILT2 epitope comprising a residue of ILT2 selected from Q18, G19, K42, L45, S64, I65, T66, W67, E68, G97, A98, Y99, I100, Q125, V126, A127, F128, D178, N180, S181, and E184. In some embodiments, the antibody or antigen-binding fragment binds an ILT2 epitope comprising a residue of ILT2 selected from G97, A98, Y99, I100, Q125, and V126. In some embodiments, the antibody or antigen-binding fragment binds an ILT2 epitope comprising a plurality of residues of ILT2 selected from Q18, G19, K42, L45, S64, I65, T66, W67, E68, G97, A98, Y99, I100, Q125, V126, A127, F128, D178, N180, S181, and E184. In some embodiments, the antibody or antigen-binding fragment binds an ILT2 epitope comprising a plurality of residues of ILT2 selected from

G97, A98, Y99, I100, Q125 and V126. In some embodiments, the antibody or antigen-binding fragment binds at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 residues selected from Q18, G19, K42, L45, S64, I65, T66, W67, E68, G97, A98, Y99, I100, Q125, V126, A127, F128, D178, N180, S181, and E184. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the antibody or antigen-binding fragment binds 1, 2, 3, 4, 5, or 6 residues selected from G97, A98, Y99, I100, Q125 and V126. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the antibody or antigen-binding fragment binds G97, A98, Y99, I100, Q125, and V126. It will be understood that the ILT2 residue numbers used herein are with respect to SEQ ID NO: 31.

[0090] In some embodiments, the antibody or antigen-binding fragment is an ILT2 antagonist. In some embodiments, the antibody or antigen-binding fragment is not an ILT2 agonist. In some embodiments, antagonism is of ILT2-mediated immune suppression. In some embodiments, the antibody or antigen-binding fragment inhibits ILT2-mediated immune suppression. In some embodiments, the antibody or antigen-binding fragment inhibits ILT2 signaling.

[0091] In some embodiments, the antibody or antigen-binding fragment inhibits interaction between ILT2 and B2M. In some embodiments, the interaction is direct interaction. In some embodiments, the antibody or antigen-binding fragment inhibits ILT2 contact with B2M. In some embodiments, the contact is direct contact. In some embodiments, the antibody or antigen-binding fragment inhibits interaction between ILT2 and HLA. In some embodiments, the antibody or antigen-binding fragment inhibits interaction between ILT2 and MHC. In some embodiments, the MHC is HLA. In some embodiments, the HLA is HLA-G. In some embodiments, the antibody or antigen-binding fragment inhibits interaction between ILT2 and HLA, MHC or both. In some embodiments, the antibody or antigen-binding fragment inhibits interaction between ILT2 and HLA, MHC or both via inhibition of ILT2 interaction with B2M. In some embodiment, the interaction is mediated by B2M. In some embodiments, the antibody indirectly inhibits interaction with HLA, MHC or both via inhibition of interaction with B2M. In some embodiments, the interaction is B2M mediated interaction. In some embodiments, the antibody or antigen-binding fragment inhibits interaction between ILT2 and a B2M/HLA complex. In some embodiments, the antibody or antigen-binding fragment inhibits interaction between ILT2 and a B2M/MHC complex. In some embodiments, the complex comprises a B2M monomer. In some embodiments, the complex comprises an HLA or MHC monomer. In some embodiments, the complex comprises a B2M dimer. In some embodiments, the complex comprises an HLA or MHC dimer.

[0092] In some embodiments, ILT2-mediated immune suppression is suppression of an immune cell. In some embodiments, the immune cell is selected from a T cell, a macrophage, a dendritic cell and a natural killer (NK) cell. In some embodiments, ILT2-mediated immune suppression is suppression of a T cell, a macrophage, a dendritic cell and an NK cell. In some embodiments, ILT2-mediated immune suppression is suppression of a T cell, a macrophage and an NK cell. In some embodiments, the T cell is a CD8-positive T cell. In some embodiments, the T cell is a T_{EMRA} cell (terminally differentiated effector memory cell re-expressing CD45RA). In some embodiments, the immune cell is selected from a CD8-positive T cell, a T_{EMRA} cell, a dendritic cell, a macrophage, and a natural killer (NK) cell. In some embodiments, the immune cell is a T cell. In some embodiments, the immune cell is an NK cell. In some embodiments, the immune cell is a macrophage. In some embodiments, the macrophage is a tumor-associated macrophage (TAM). In some embodiments, the immune cell is a dendritic cell. In some embodiments, the dendritic cell is a tolerogenic dendritic cell. In some embodiments, the immune cell is a peripheral blood immune cell. In some embodiments, the immune cell is a peripheral blood mononuclear cell (PBMC). In some embodiments, the immune cell is an intratumor immune cell. In some embodiments, the immune cell is an immune cell in the tumor microenvironment (TME). In some embodiments, ILT2-mediated immune suppression is suppression of macrophage phagocytosis. In some embodiments, ILT2-mediated immune suppression is suppression of NK cell cytotoxicity. In some embodiments, ILT2-mediated immune suppression is suppression of T cell cytotoxicity. In some embodiments, ILT2-mediated immune suppression is suppression of T cell proliferation. In some embodiments, ILT2-mediated immune suppression is suppression of immune cell proliferation.

[0093] In some embodiments, the antibody or antigen-binding fragment does not bind a member of the leukocyte immunoglobulin-like receptor subfamily B other than ILT2. In some embodiments, the antibody or antigen-binding fragment is specific to ILT2. An antibody is said to specifically bind to an antigen when the K_D is $\leq 1 \mu\text{M}$, preferably $\leq 100 \text{ nM}$ or $\leq 10 \text{ nM}$. In some embodiments, the antibody or antigen-binding fragment preferentially binds to ILT2. In some embodiments, the antibody or antigen-binding fragment does not inhibit a member of the leukocyte immunoglobulin-like receptor subfamily B other than ILT2.

[0094] As used herein, “increased binding efficacy” refers to specific binding to a target or antigen that is greater than the binding of an isotype control. In some embodiments, increased binding is an increase of at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000% in binding efficacy. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, increased binding is the presence of binding as

compared to an isotype control that has no binding. Binding of an antibody to a specific domain will be well known to a person of skill in the art. Antibody binding can be assayed in any way known to one skilled in the art, including but not limited to: X-ray crystallography, immunoprecipitation, immunoblotting, competition assays, surface plasmon resonance, and kinetic exclusion assays. In some embodiments, increased binding efficacy is specific binding.

[0095] An antibody or antigen-binding fragment, variant, or derivative disclosed herein can be said to bind a target antigen, e.g., ILT2, with an on rate (k_{on}) of greater than or equal to $10^3 \text{ M}^{-1} \text{ sec}^{-1}$, $5 \times 10^3 \text{ M}^{-1} \text{ sec}^{-1}$, $10^4 \text{ M}^{-1} \text{ sec}^{-1}$ or $5 \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}$. Each possibility represents a separate embodiment of the present disclosure. An antibody or antigen-binding fragment, variant, or derivative disclosed herein can be said to bind a target antigen with a K_D of 10^{-6} M or stronger, whereas most antibodies have typical K_D of at least 10^{-9} M . In some embodiments, K_D is a measure of affinity. It will be understood by a skilled artisan that that stronger binding, which is higher affinity, is binding with a lower K_D . In some embodiments, the antibody or antigen-binding fragment, variant, or derivative disclosed herein binds a target antigen with a K_D of between 10^{-6} M and 10^{-12} M . In some embodiments, the antibody or antigen-binding fragment, variant, or derivative disclosed herein binds a target antigen with a K_D of between 10^{-6} M and 10^{-11} M . In some embodiments, the antibody or antigen-binding fragment, variant, or derivative disclosed herein binds a target antigen with a K_D of between 10^{-6} M and 10^{-10} M . In some embodiments, the antibody or antigen-binding fragment, variant, or derivative disclosed herein binds a target antigen with a K_D of between 10^{-6} M and 10^{-9} M . In some embodiments, the antibody or antigen-binding fragment, variant, or derivative disclosed herein binds a target antigen with a K_D of between 10^{-7} M and 10^{-12} M . In some embodiments, the antibody or antigen-binding fragment, variant, or derivative disclosed herein binds a target antigen with a K_D of between 10^{-8} M and 10^{-12} M . In some embodiments, the antibody or antigen-binding fragment, variant, or derivative disclosed herein binds a target antigen with a K_D of between 10^{-9} M and 10^{-12} M . In some embodiments, the antibody or antigen-binding fragment, variant, or derivative disclosed herein binds a target antigen with a K_D of between 10^{-7} M and 10^{-11} M . In some embodiments, the antibody or antigen-binding fragment, variant, or derivative disclosed herein binds a target antigen with a K_D of between 10^{-8} M and 10^{-11} M . In some embodiments, the antibody or antigen-binding fragment, variant, or derivative disclosed herein binds a target antigen with a K_D of between 10^{-9} M and 10^{-11} M . In some embodiments, the antibody or antigen-binding fragment, variant, or derivative disclosed herein binds a target antigen with a K_D of between 10^{-7} M and 10^{-10} M . In some embodiments, the antibody or antigen-binding fragment, variant, or derivative disclosed herein binds a target antigen with a K_D of between 10^{-8} M and 10^{-10} M . In some embodiments, the antibody or antigen-binding

fragment, variant, or derivative disclosed herein binds a target antigen with a K_D of between 10^{-9} M and 10^{-10} M. In some embodiments, the antibody or antigen-binding fragment, variant, or derivative disclosed herein binds a target antigen with a K_D of between 10^{-7} M and 10^{-9} M. In some embodiments, the antibody or antigen-binding fragment, variant, or derivative disclosed herein binds a target antigen with a K_D of between 10^{-8} M and 10^{-9} M.

[0096] In some embodiments, the antibody or antigen-binding fragment comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 19

(QVQLQQSDAELVKPGASVKISCKVSGYTFTDHTIHWMKQRPEQGLEWIGYIYPRDGSTKYNEKFKGKATLTADKSSSTAYMQLNSLTSEDSAVYFCARTWDYFDYWGGTTLTVSS).

In some embodiments, the heavy chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 19. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the heavy chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 19. In some embodiments, the antibody or antigen-binding fragment comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 21

(QVQLQQSGAELARPGASVKLSCKASGYTFTSYGISWVKQRTGQGLEWVGEIYPGSGNSY YNEKFKGKATLTADKSSSTAYMELRSLTSEDSAVYFCARSNDGYPDYWGQGTTLTVSS).

In some embodiments, the heavy chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 21. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the heavy chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 21. In some embodiments, the antibody or antigen-binding fragment comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 23

(DVQLQGSGPGLVKPSETLSLTCSVTGYTSITSGYYWNWIRQFPGKKLEWMGYISYDGSNN YNPSLKNRITISRDTSKNQFSLKLNSVTAADTATYYCAHGYSYYYAMDXXWGQGTSTVTS

S), wherein X is selected from A, C and S. In some embodiments, the heavy chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 23. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the heavy chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 23.

[0097] In some embodiments, the antibody or antigen-binding fragment comprises a light chain comprising the amino acid sequence of SEQ ID NO: 20

(DIVLTQSPASLAVSLGQRATISCRASESVDSYGNFSFMHWYQQKPGQPPLLIYRASNLESGI PARFSGSGSRDFTLTINPVEADDVATYYCQQSNEDPYTFGGGTKLEIK). In some

embodiments, the light chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 20. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the light chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 20. In some embodiments, the antibody or antigen-binding fragment comprises a light chain comprising the amino acid sequence of SEQ ID NO: 22

(DIQMTQSSSYLSVSLGGRVTITCKASDHINNWLAWYQQKPGNAPRLISGATSLETGVPSRFSGSGSGKDYTLSTSLQTEDVATYYCQQYWSTPWTFGGGTKLEIK). In some embodiments, the light chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 22. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the light chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 22. In some embodiments, the antibody or antigen-binding fragment comprises a light chain comprising the amino acid sequence of SEQ ID NO: 24

(DIQMTQSPSSLSASVGDRVTITCRTSQDISNYLNWYQQKPGKAVKLLISYTSRLHSGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPTFGQGTKLEIK). In some embodiments, the light chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 24. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the light chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 24. In some embodiments, the antibody or antigen-binding fragment comprises a light chain comprising the amino acid sequence of SEQ ID NO: 45

(DIQMTQTSSLSASLGDRVTISCRTSQDISNYLNWYQQKPDGTVKLLISYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPTFGSGTKLEIK). In some embodiments, the light chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 45. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the light chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 45.

[0098] In some embodiments, SEQ ID NO: 23 is

DVQLQGSGPGLVKPSETLSLTCSVTGYTSITSGYYWNWIRQFPGKKLEWMGYISYDGSNNYNPSLKNRITISRDTSKNQFSLKLNSVTAADTATYYCAHGYSYYYAMDAWGQGTSVTVSS (SEQ ID NO: 28). In some embodiments, SEQ ID NO: 23 is SEQ ID NO: 28 and the antibody or antigen-binding fragment is humanized. In some embodiments, the heavy chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 28. Each possibility represents a separate embodiment of the present disclosure. In

some embodiments, the heavy chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 28. In some embodiments, SEQ ID NO: 23 is

DVQLQGSGPGLVKPSETLSLTCSVTGYSITSGYYWNWIRQFPGKKLEWMGYISYDGSNN
YNPSLKNRITISRDTSKNQFSLKLNSVTAADTATYYCAHGYSYYYAMDSWGQGTSTVTS
S (SEQ ID NO: 29). In some embodiments, SEQ ID NO: 23 is SEQ ID NO: 29 and the antibody

or antigen-binding fragment is humanized. In some embodiments, the heavy chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 29. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the heavy chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 29. In some embodiments, SEQ ID NO: 23 is

DVQLQGSGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYISYDGSNN
YNPSLKNRISITRDTSKNQFFLKLNSVTSEDTATYYCAHGYSYYYAMDCWGQGTSTVTVSS
(SEQ ID NO: 30). In some embodiments, SEQ ID NO: 23 is SEQ ID NO: 30 and the antibody or

antigen-binding fragment is murine. In some embodiments, the heavy chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 30. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the heavy chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 30.

[0099] In some embodiments, SEQ ID NO: 15 is SEQ ID NO: 25 and the heavy chain comprises or consists of SEQ ID NO: 28. In some embodiments, SEQ ID NO: 15 is SEQ ID NO: 25 and the heavy chain comprises

DVQLQGSGPGLVKPSETLSLTCSVTGYSITSGYYWNWIRQFPGKKLEWMGYISYDGSNN
YNPSLKNRITISRDTSKNQFSLKLSSVTAADTATYYCAHGYSYYYAMDAWGQGTTVTVS
S (SEQ ID NO: 56). In some embodiments, the heavy chain comprises an amino acid sequence at

least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 56. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the heavy chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 56. In some embodiments, the heavy chain variable region consists of SEQ ID NO: 56. In some embodiments, the heavy chain variable region consists of a sequence with at least 95% identity to SEQ ID NO: 56. In some embodiments, SEQ ID NO: 15 is SEQ ID NO: 25 and the heavy chain comprises

DVQLQGSGPGLVKPSETLSLTCSVTGYSITSGYYWNWIRQPPGKGLEWMGYISYDGSNN
YNPSLKNRITISRDTSKNQFSLKLSSVTAADTATYYCAHGYSYYYAMDAWGQGTTVTVS
S. (SEQ ID NO: 57). In some embodiments, the heavy chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 57. Each

possibility represents a separate embodiment of the present disclosure. In some embodiments, the heavy chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 57. In some embodiments, the heavy chain variable region consists of SEQ ID NO: 57. In some embodiments, the heavy chain variable region consists of a sequence at least 95% identical to SEQ ID NO: 57. In some embodiments, SEQ ID NO: 15 is SEQ ID NO: 25 and the heavy chain comprises QVQLQGSGPGLVKPSETLSLTCSVTGYSITSGYYWNWIRQPPGKGLEWMGYISYDGSNNYNPSLKNRVTISRDTSKNQFSLKLSSVTAADTATYYCAHGYSYYYAMDAWGQGTTVTVSS (SEQ ID NO: 58). In some embodiments, the heavy chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 58. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the heavy chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 58. In some embodiments, the heavy chain variable region consists of SEQ ID NO: 58. In some embodiments, the heavy chain variable region consists of a sequence at least 95% identical to SEQ ID NO: 58. In some embodiments, SEQ ID NO: 15 is SEQ ID NO: 25 and the heavy chain comprises QVQLQGSGPGLVKPSETLSLTCTVTGYSITSGYYWNWIRQPPGKGLEWIGYISYDGSNNYNPSLKNRVTISRDTSKNQFSLKLSSVTAADTATYYCAHGYSYYYAMDAWGQGTTVTVSS (SEQ ID NO: 59). In some embodiments, the heavy chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 59. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the heavy chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 59. In some embodiments, the heavy chain variable region consists of SEQ ID NO: 59. In some embodiments, the heavy chain variable region consists of a sequence at least 95% identical to SEQ ID NO: 59. In some embodiments, the heavy chain comprises a sequence selected from SEQ ID NOs: 28 and 56-59. In some embodiments, the heavy chain comprises a sequence selected from SEQ ID NOs: 56-59. In some embodiments, the heavy chain variable region consists of a sequence selected from SEQ ID NOs: 28 and 56-59. In some embodiments, the heavy chain variable region consists of a sequence selected from SEQ ID NOs: 56-59.

[0100] In some embodiments, the heavy chain comprises

DVQLQGSGPGLVKPSETLSLTCSVTGYSITSGYYWNWIRQFPGKKLEWMGYISYDGSNNYNPSLKNRITISRDTSKNQFSLKLNSVTAADTATYYCAHGYSYYYAMDAWGQGTSTVTS SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGKTYTCNVDPKPSNTKVDKRVESKYGPPCPPCPAPEFEGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK

NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEG NVFSCSVMHEALHNHYTQKSLSLGLGK (SEQ ID NO: 48). In some embodiments, the heavy chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 48. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the heavy chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 48. In some embodiments, the heavy chain consists of SEQ ID NO: 48. In some embodiments, the heavy chain consists of a sequence at least 95% identical to SEQ ID NO: 48. In some embodiments, the heavy chain comprises

DVQLQGSGPGLVKPSETLSLTCSVTGYSITSGYYWNWIRQFPGKKLEWMGYISYDGSNN YNPSLKNRITISRDTSKNQFSLKLNSVTAADTATYYCAHGYSYYYAMDSWGQGTSTVTS SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVTVTPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFEGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEG NVFSCSVMHEALHNHYTQKSLSLGLGK (SEQ ID NO: 51). In some embodiments, the heavy chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 51. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the heavy chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 51. In some embodiments, the heavy chain consists of SEQ ID NO: 51. In some embodiments, the heavy chain consists of a sequence at least 95% identical to SEQ ID NO: 51. In some embodiments, the heavy chain comprises

DVQLQGSGPGLVKPSETLSLTCSVTGYSITSGYYWNWIRQFPGKKLEWMGYISYDGSNN YNPSLKNRITISRDTSKNQFSLKLNSVTAADTATYYCAHGYSYYYAMDCWGQGTSTVTS SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVTVTPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFEGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEG NVFSCSVMHEALHNHYTQKSLSLGLGK (SEQ ID NO: 52). In some embodiments, the heavy chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 52. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the heavy chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 52. In some embodiments, the heavy chain consists of SEQ ID

NO: 52. In some embodiments, the heavy chain consists of a sequence at least 95% identical to

SEQ ID NO: 52. In some embodiments, the heavy chain comprises

DVQLQGSGPGLVKPSETLSLTCSVTGYSITSGYYWNWIRQFPGKKLEWMGYISYDGSNN
YNPSLKNRITISRDTSKNQFSLKLSSVTAADTATYYCAHGYSYYYAMDAWGQGTTVTVS
SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVTVTPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCAPEFEGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEG
NVFSCSVMEALHNHYTQKSLSLGLK (SEQ ID NO: 64). In some embodiments, the heavy

chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or

100% identical to SEQ ID NO: 64. Each possibility represents a separate embodiment of the

present disclosure. In some embodiments, the heavy chain comprises an amino acid sequence at

least 95% identical to SEQ ID NO: 64. In some embodiments, the heavy chain consists of SEQ ID

NO: 64. In some embodiments, the heavy chain consists of a sequence at least 95% identical to

SEQ ID NO: 64. In some embodiments, the heavy chain comprises

DVQLQGSGPGLVKPSETLSLTCSVTGYSITSGYYWNWIRQPPGKGLEWMGYISYDGSNN
YNPSLKNRITISRDTSKNQFSLKLSSVTAADTATYYCAHGYSYYYAMDAWGQGTTVTVS
SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVTVTPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCAPEFEGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEG
NVFSCSVMEALHNHYTQKSLSLGLK (SEQ ID NO: 65). In some embodiments, the heavy

chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or

100% identical to SEQ ID NO: 65. Each possibility represents a separate embodiment of the

present disclosure. In some embodiments, the heavy chain comprises an amino acid sequence at

least 95% identical to SEQ ID NO: 65. In some embodiments, the heavy chain consists of SEQ ID

NO: 65. In some embodiments, the heavy chain consists of a sequence at least 95% identical to

SEQ ID NO: 65. In some embodiments, the heavy chain comprises

QVQLQGSGPGLVKPSETLSLTCTVTGYSITSGYYWNWIRQPPGKLEWIGYISYDGSNNY
NPSLKNRVTISRDTSKNQFSLKLSSVTAADTATYYCAHGYSYYYAMDAWGQGTTVTVSS
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVTVTPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCAPEFEGGPSVFL

FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGN
VFSCSVMHEALHNHYTQKSLSLGLGK (SEQ ID NO: 67). In some embodiments, the heavy
chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or
100% identical to SEQ ID NO: 67. Each possibility represents a separate embodiment of the
present disclosure. In some embodiments, the heavy chain comprises an amino acid sequence at
least 95% identical to SEQ ID NO: 67. In some embodiments, the heavy chain consists of SEQ ID
NO: 67. In some embodiments, the heavy chain consists of a sequence at least 95% identical to
SEQ ID NO: 67. In some embodiments, the heavy chain comprises

QVQLQGSGPGLVKPSETLSLTCSVTGYSITSGYYWNWIRQPPGKGLEWMGYISYDGSNN
YNPSLKNRVTISRDTSKNQFSLKLSSVTAADTATYYCAHGYSYYYAMDAGWGQGTITV
SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVTVPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFEGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQE
GNVFSCSVMHEALHNHYTQKSLSLGLGK (SEQ ID NO: 106). In some embodiments, the
heavy chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98,
99 or 100% identical to SEQ ID NO: 106. Each possibility represents a separate embodiment of
the present disclosure. In some embodiments, the heavy chain comprises an amino acid sequence
at least 95% identical to SEQ ID NO: 106. In some embodiments, the heavy chain consists of SEQ
ID NO: 106. In some embodiments, the heavy chain consists of a sequence at least 95% identical
to SEQ ID NO: 106. In some embodiments, the heavy chain comprises

QVQLQQSGAELARPGASVKLSCKASGYTFTSYGISWVKQRTGQGLEWVGEIYPGSGNSY
YNEKFKGKATLTADKSSSTAYMELRSLTSEDSAVYFCARSNDGYPDYWGQGTTLTVSSA
STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVTVPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFEGGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNV
FSCSVMHEALHNHYTQKSLSLGLGK (SEQ ID NO: 53). In some embodiments, the heavy
chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or
100% identical to SEQ ID NO: 53. Each possibility represents a separate embodiment of the

present disclosure. In some embodiments, the heavy chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 53. In some embodiments, the heavy chain consists of SEQ ID NO: 53. In some embodiments, the heavy chain consists of a sequence at least 95% identical to SEQ ID NO: 53.

[0101] In some embodiments, the antibody comprises a kappa light chain. In some embodiments, the constant region of the light chain is a kappa constant region. In some embodiments, the kappa constant region comprises

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 63). In some embodiments, the kappa constant region consists of SEQ ID NO: 63. In some embodiments, the kappa constant region is an analog or derivative of SEQ ID NO: 63. In some embodiments, a kappa constant region comprises at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to SEQ ID NO: 63. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, a kappa constant region comprises at least 95% identity to SEQ ID NO: 63. In some embodiments, a kappa constant region comprises at least 97% identity to SEQ ID NO: 63. In some embodiments, a kappa constant region comprises at least 99% identity to SEQ ID NO: 63.

[0102] In some embodiments, the light chain comprises

DIQMTQSTSSLSASVGDRVTITCRTSQDISNYLNWYQQKPGKAVKLLISYTSRLHSGVPSR FSGSGSGTDYTLTISSLQPEDFATYFCQQGNTLPTFGQGTKLEIK (SEQ ID NO: 60). In some embodiments, the light chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 60. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the light chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 60. In some embodiments, the light chain variable region consists of SEQ ID NO: 60. In some embodiments, the light chain variable region consists of a sequence at least 95% identical to SEQ ID NO: 60. In some embodiments, the light chain comprises

DIQMTQSPSSLSASVGDRVTITCRTSQDISNYLNWYQQKPGKAVKLLISYTSRLHSGVPSR FSGSGSGTDYTLTISSLQPEDFATYFCQQGNTLPTFGQGTKLEIK (SEQ ID NO: 61). In some embodiments, the light chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 61. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the light chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 61. In some embodiments, the light chain variable region consists of SEQ ID NO: 61. In some embodiments, the light chain variable region

consists of a sequence at least 95% identical to SEQ ID NO: 61. In some embodiments, the light chain comprises

DIQMTQSPSSLSASVGDRVTITCRTSQDISNYLNWYQQKPGKAVKLLISYTSRLHSGVPSR
FSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPTFGQGTKLEIK (SEQ ID NO: 24). In some embodiments, the light chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 24. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the light chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 24. In some embodiments, the light chain variable region consists of SEQ ID NO: 24. In some embodiments, the light chain variable region consists of a sequence at least 95% identical to SEQ ID NO: 24. In some embodiments, the light chain comprises

DIQMTQSPSSLSASVGDRVTITCRTSQDISNYLNWYQQKPGKAPKLLISYTSRLHSGVPSR
FSGSGSGTDYTLTISSLQPEDFATYFCQQGNTLPTFGQGTKLEIK (SEQ ID NO: 62). In some embodiments, the light chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 62. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the light chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 62. In some embodiments, the light chain variable region consists of SEQ ID NO: 62. In some embodiments, the light chain variable region consists of a sequence at least 95% identical to SEQ ID NO: 62. In some embodiments, the light chain comprises a sequence selected from SEQ ID NOs: 24 and 60-62. In some embodiments, the light chain comprises a sequence selected from SEQ ID NOs: 60-62. In some embodiments, the light chain variable region consists of a sequence selected from SEQ ID NOs: 24 and 60-62. In some embodiments, the light chain comprises a sequence selected from SEQ ID NOs: 60-62. In some embodiments, the light chain variable region consists of a sequence selected from SEQ ID NOs: 60-62.

[0103] In some embodiments, the light chain comprises

DIQMTQSPSSLSASVGDRVTITCRTSQDISNYLNWYQQKPGKAVKLLISYTSRLHSGVPSR
FSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPTFGQGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNFPYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSK
ADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 49). In some embodiments, the light chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 49. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the light chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 49. In some embodiments, the light chain consists of SEQ ID

NO: 49. In some embodiments, the light chain consists of a sequence at least 95% identical to SEQ ID NO: 49. In some embodiments, the light chain comprises

DIQMTQSPSSLSASVGDRVITTCRTSQDISNYLNWYQQKPGKAVKLLISYTSRLHSGVPSR
 FSGSGSGTDYTLTISSLQPEDFATYFCQQGNTLPTFGQGTKLEIKRTVAAPSVFIFPPSDEQL
 KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKA
 DYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 66). In some embodiments, the
 light chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99
 or 100% identical to SEQ ID NO: 66. Each possibility represents a separate embodiment of the
 present disclosure. In some embodiments, the light chain comprises an amino acid sequence at
 least 95% identical to SEQ ID NO: 66. In some embodiments, the light chain consists of SEQ ID
 NO: 66. In some embodiments, the light chain consists of a sequence at least 95% identical to
 SEQ ID NO: 66. In some embodiments, the light chain comprises

DIQMTQSSSYLSVSLGGRVTITCKASDHINNWLAWYQQKPGNAPRLLISGATSLETGVPS
 RFSGSGSGKDYTLISLQTEDVATYYCQQYWSTPWTFGGGTKLEIKRTVAAPSVFIFPPS
 DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLT
 LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 54). In some embodiments,
 the light chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98,
 99 or 100% identical to SEQ ID NO: 54. Each possibility represents a separate embodiment of the
 present disclosure. In some embodiments, the light chain comprises an amino acid sequence at
 least 95% identical to SEQ ID NO: 54. In some embodiments, the light chain consists of SEQ ID
 NO: 54. In some embodiments, the light chain consists of a sequence at least 95% identical to
 SEQ ID NO: 54. In some embodiments, the light chain comprises

DIQMTQSTSSLSASVGDRVITTCRTSQDISNYLNWYQQKPGKAVKLLISYTSRLHSGVPSR
 FSGSGSGTDYTLTISSLQQEDFATYFCQQGNTLPTFGQGTKLEIKRTVAAPSVFIFPPSDEQ
 LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSK
 ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 107). In some embodiments,
 the light chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98,
 99 or 100% identical to SEQ ID NO: 107. Each possibility represents a separate embodiment of
 the present disclosure. In some embodiments, the light chain comprises an amino acid sequence at
 least 95% identical to SEQ ID NO: 107. In some embodiments, the light chain consists of SEQ ID
 NO: 107. In some embodiments, the light chain consists of a sequence at least 95% identical to
 SEQ ID NO: 107. In some embodiments, the light chain comprises

DIQMTQSPSSLSASVGDRVITTCRTSQDISNYLNWYQQKPGKAPKLLISYTSRLHSGVPSR
 FSGSGSGTDYTLTISSLQPEDFATYFCQQGNTLPTFGQGTKLEIKRTVAAPSVFIFPPSDEQL

KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKA
DYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 108). In some embodiments, the light chain comprises an amino acid sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to SEQ ID NO: 108. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the light chain comprises an amino acid sequence at least 95% identical to SEQ ID NO: 108. In some embodiments, the light chain consists of SEQ ID NO: 108. In some embodiments, the light chain consists of a sequence at least 95% identical to SEQ ID NO: 108.

[0104] In some embodiments, the antibody comprises a heavy chain comprising SEQ ID NO: 48 and a light chain comprising SEQ ID NO: 49. In some embodiments, the antibody comprises a heavy chain consisting of SEQ ID NO: 48 and a light chain consisting SEQ ID NO: 49. In some embodiments, the antibody consists of a heavy chain comprising SEQ ID NO: 48 and a light chain comprising SEQ ID NO: 49. In some embodiments, the antibody consists of a heavy chain consisting of SEQ ID NO: 48 and a light chain consisting of SEQ ID NO: 49. In some embodiments, the antibody is 15G8-13.

[0105] In some embodiments, the antibody comprises a heavy chain comprising SEQ ID NO: 51 and a light chain comprising SEQ ID NO: 49. In some embodiments, the antibody comprises a heavy chain consisting of SEQ ID NO: 51 and a light chain consisting SEQ ID NO: 49. In some embodiments, the antibody consists of a heavy chain comprising SEQ ID NO: 51 and a light chain comprising SEQ ID NO: 49. In some embodiments, the antibody consists of a heavy chain consisting of SEQ ID NO: 51 and a light chain consisting of SEQ ID NO: 49. In some embodiments, the antibody is 15G8-13 with a serine at position 108.

[0106] Antibodies with any combination of the above-described heavy and light chains are contemplated by the present disclosure.

[0107] In some embodiments, the antibody comprises a heavy chain comprising SEQ ID NO: 52 and a light chain comprising SEQ ID NO: 49. In some embodiments, the antibody comprises a heavy chain consisting of SEQ ID NO: 52 and a light chain consisting SEQ ID NO: 49. In some embodiments, the antibody consists of a heavy chain comprising SEQ ID NO: 52 and a light chain comprising SEQ ID NO: 49. In some embodiments, the antibody consists of a heavy chain consisting of SEQ ID NO: 52 and a light chain consisting of SEQ ID NO: 49. In some embodiments, the antibody is 15G8-13 with a cysteine at position 108.

[0108] In some embodiments, the antibody comprises a heavy chain comprising SEQ ID NO: 64 and a light chain comprising SEQ ID NO: 49. In some embodiments, the antibody comprises a heavy chain consisting of SEQ ID NO: 64 and a light chain consisting SEQ ID NO: 49. In some

embodiments, the antibody consists of a heavy chain comprising SEQ ID NO: 64 and a light chain comprising SEQ ID NO: 49. In some embodiments, the antibody consists of a heavy chain consisting of SEQ ID NO: 64 and a light chain consisting of SEQ ID NO: 49. In some embodiments, the antibody is 15G8-23.

[0109] In some embodiments, the antibody comprises a heavy chain comprising SEQ ID NO: 65 and a light chain comprising SEQ ID NO: 66. In some embodiments, the antibody comprises a heavy chain consisting of SEQ ID NO: 65 and a light chain consisting SEQ ID NO: 66. In some embodiments, the antibody consists of a heavy chain comprising SEQ ID NO: 65 and a light chain comprising SEQ ID NO: 66. In some embodiments, the antibody consists of a heavy chain consisting of SEQ ID NO: 65 and a light chain consisting of SEQ ID NO: 66. In some embodiments, the antibody is 15G8-32.

[0110] In some embodiments, the antibody comprises a heavy chain comprising SEQ ID NO: 67 and a light chain comprising SEQ ID NO: 49. In some embodiments, the antibody comprises a heavy chain consisting of SEQ ID NO: 67 and a light chain consisting SEQ ID NO: 49. In some embodiments, the antibody consists of a heavy chain comprising SEQ ID NO: 67 and a light chain comprising SEQ ID NO: 49. In some embodiments, the antibody consists of a heavy chain consisting of SEQ ID NO: 67 and a light chain consisting of SEQ ID NO: 49. In some embodiments, the antibody is 15G8-53.

[0111] In some embodiments, the antibody comprises a heavy chain comprising SEQ ID NO: 53 and a light chain comprising SEQ ID NO: 54. In some embodiments, the antibody comprises a heavy chain consisting of SEQ ID NO: 53 and a light chain consisting SEQ ID NO: 54. In some embodiments, the antibody consists of a heavy chain comprising SEQ ID NO: 53 and a light chain comprising SEQ ID NO: 54. In some embodiments, the antibody consists of a heavy chain consisting of SEQ ID NO: 53 and a light chain consisting of SEQ ID NO: 54. In some embodiments, the antibody is 19E3.

[0112] In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for use in treating or ameliorating cancer in a subject in need thereof. In some embodiments, the cancer is an HLA-G positive cancer. In some embodiments, the cancer is an MHC-I positive cancer. In some embodiments, the cancer is an HLA-G expressing cancer. In some embodiments, the cancer is an MHC-I expressing cancer. In some embodiments, the cancer is a solid cancer. In some embodiments, the cancer is a tumor. In some embodiments, the cancer is selected from hepato-biliary cancer, cervical cancer, urogenital cancer (e.g., urothelial cancer), testicular cancer, prostate cancer, thyroid cancer, ovarian cancer, nervous system cancer, ocular cancer, lung cancer, soft tissue cancer, bone cancer, pancreatic cancer, bladder cancer, skin cancer,

intestinal cancer, hepatic cancer, rectal cancer, colorectal cancer, esophageal cancer, gastric cancer, gastroesophageal cancer, breast cancer (e.g., triple negative breast cancer), renal cancer (e.g., renal carcinoma), head and neck cancer, leukemia and lymphoma. In some embodiments, the cancer is selected from breast cancer, hepato-biliary cancer, cervical cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, hepatic cancer, lung cancer (e.g., non-small cell lung cancer), renal cancer, skin cancer (e.g., melanoma or squamous cell carcinoma), urogenital cancer, and pancreatic cancer. In some embodiments, the cancer is selected from breast cancer, hepato-biliary cancer (e.g., hepatocellular carcinoma, gallbladder cancer, cholangiocarcinoma, etc.), cervical cancer, colorectal cancer (e.g., KRAS wild-type colorectal cancer), esophageal cancer, gastric cancer, head and neck cancer, hepatic cancer, lung cancer, renal cancer, skin cancer, urogenital cancer, pancreatic cancer and leukemia.

[0113] In some embodiments, an antibody or antigen-binding fragment of the present disclosure is for use in shifting a tumor microenvironment from immunosuppressive to immunostimulatory. In some embodiments, said shifting the tumor microenvironment comprises one or more of: inducing/enhancing an anti-tumor T cell response, increasing T cell proliferation, reducing cancer-induced suppressor myeloid activity, increasing dendritic cell (DC) activation, increasing dendritic cell homing to the tumor, increasing macrophage phagocytosis, increasing generation of M1 macrophages, decreasing generation of M2 macrophages and increasing NK cell activity. In some embodiments, an antibody or antigen-binding fragment of the present disclosure is for use in increasing a T cell response against a cancer cell. In some embodiments, the T cell response comprises increased pro-inflammatory cytokine secretion. In some embodiments, the T cell response comprises increased cytotoxicity. In some embodiments, the T cell response comprises increased T cell proliferation. In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for use in increasing macrophage phagocytosis of a cancer cell. In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for use in increasing dendritic cell homing to a tumor or cancer. In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for use in increasing macrophage phagocytosis. In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for use increasing macrophage phagocytosis of the cancer. In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for use in increasing generation of M1 macrophages. In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for use in decreasing generation of M2 macrophages. In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for use in increasing NK cell cytotoxicity against a cancer cell. In some embodiments, the antibody or antigen-binding fragment

of the present disclosure is for use in reducing cancer-induced suppressor myeloid activity. In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for use in reducing tolerogenic dendritic cell (DC) activity. In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for increasing M1 monocyte activity or number. In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for decreasing M2 monocyte activity or number. In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for increasing generation of M1 macrophages. In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for decreasing generation of M2 macrophages. In some embodiments, M1 monocytes/macrophages are inflammatory macrophages/monocytes. In some embodiments, M2 monocytes/macrophages are suppressor macrophages/monocytes. In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for increasing DC number in a tumor. In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for increasing recruitment of DCs to a tumor. In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for increasing DC recruitment to a tumor. In some embodiments, to a tumor is to a tumor microenvironment (TME). In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for increasing DC activation. In some embodiments, increasing DC activation comprises decreasing tolerogenic dendritic cell activity. In some embodiments, the antibody or antigen-binding fragment of the present disclosure is for increasing antigen presentation.

[0114] In some embodiments, the antibody or antigen-binding fragment induces in a subject at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 anti-cancer effects. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the antibody or antigen-binding fragment induces in a subject at least 2 effects. In some embodiments, the antibody or antigen-binding fragment induces in a subject at least 3 effects. In some embodiments, the antibody or antigen-binding fragment induces in a subject at least 4 effects. In some embodiments, the effects are selected from: increased NK cell cytotoxicity, increased T cell cytotoxicity, increased T cell proliferation, increased macrophage phagocytosis, increased generation of M1 macrophages, decreased generation of M2 macrophages, increased dendritic cell homing to a tumor of the cancer, and increased dendritic cell activation. In some embodiments, the effects are selected from: a) increased NK cell cytotoxicity; b) increased T cell cytotoxicity, proliferation or both; c) increased macrophage phagocytosis, increased generation of M1 macrophages, decreased generation of M2 macrophages or a combination thereof; and d) increased dendritic cell homing to a tumor of the cancer, increased dendritic cell activation and a combination thereof. In some

embodiments, cytotoxicity is cytotoxicity against a cancer. In some embodiments, phagocytosis is phagocytosis of a cancer or cancer cells. In some embodiments, the antibody or antigen-binding fragment induces in a subject an anti-cancer effect on T cells, NK cells, dendritic cells, and macrophages. In some embodiments, the antibody or antigen-binding fragment induces in a subject an anti-cancer effect on at least 3 of T cells, NK cells, dendritic cells, and macrophages. In some embodiments, the antibody or antigen-binding fragment induces the effect as a monotherapy. In some embodiments, the antibody or antigen-binding fragment induces the effect without combination.

[0115] In some embodiments, increased cytotoxicity comprises increased pro-inflammatory cytokine secretion. Pro-inflammatory cytokines are well known in the art and include, but are not limited to: IL-1, IL-1B, IL-6, TNF α , IFN γ , MCP-1, IL-12, IL-18, IL-2, IL-15, IL-17, IL-21 and granulocyte-macrophage colony stimulating factor (GM-CSF). In some embodiments, the pro-inflammatory cytokine is selected from IL-6, interferon gamma (IFN γ) and GM-CSF. In some embodiments, the pro-inflammatory cytokine is GM-CSF.

[0116] An “anti-ILT2 antibody,” an “antibody which recognizes ILT2,” or an “antibody against ILT2” is an antibody that binds to ILT2, with sufficient affinity and specificity. In some embodiments, an anti-ILT2 antibody has ILT2 as the antigen to which it binds.

[0117] An “antigen” is a molecule or a portion of a molecule capable of eliciting antibody formation and being bound by an antibody. An antigen may have one or more than one epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

[0118] The term “antigenic determinant” or “epitope” according to the present disclosure refers to the region of an antigen molecule that specifically reacts with a particular antibody. Peptide sequences derived from an epitope can be used, alone or in conjunction with a carrier moiety, applying methods known in the art, to immunize animals and to produce additional polyclonal or monoclonal antibodies. Immunoglobulin variable domains can also be analyzed using the IMGT information system ([www://imgt.cines.fr/](http://imgt.cines.fr/)) (IMGT®/V-Quest) to identify variable region segments, including CDRs. See, e.g., Brochet et al., *Nucl Acids Res.* (2008) J6:W503-508.

[0119] Kabat et al. also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of “Kabat numbering” to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, “Kabat numbering” refers to the numbering

system set forth by Kabat et al., U.S. Dept. of Health and Human Services, “Sequence of Proteins of Immunological Interest” (1983).

[0120] In some embodiments, the antibody or antigen-binding fragment is for use in combination with another agent. In some embodiments, the use in combination with another agent is for treating an HLA-G and/or MHC-I expressing cancer. In some embodiments, the agent is an opsonizing agent. In some embodiments, the agent is an anti-PD-1 and/or anti-PD-L1 agent. In some embodiments, the antibody or antigen-binding fragment is for use in combination with anti-PD-1/PD-L1 based therapy.

[0121] As used herein, an “opsonizing agent” is any agent that can bind to a target cell (e.g., a cancer cell, a cell harboring an intracellular pathogen, etc.) and opsonize the target cell. For example, any antibody that can bind to a target cell, where the antibody has an Fc region, is considered to be an agent that opsonizes a target cell. In some embodiments, an opsonizing agent is an antibody that induces antibody dependent cellular phagocytosis (ADCP). Examples of opsonizing agents include, but are not limited to anti-CD47 antibodies, anti-CD20 antibodies, anti-HER2 antibodies, anti-EGFR antibodies, anti-CD52 antibodies and anti-CD30 antibodies. In some embodiments, the opsonizing agent is selected from rituximab (Rituxan®), trastuzumab (Herceptin®), pertuzumab (Perjeta®), cetuximab (Erbix®), and panitumumab (Vectibix®). In some embodiments, the opsonizing agent is an anti-EGFR antibody. In some embodiments, the opsonizing agent is cetuximab.

[0122] As used herein, an “anti-PD-1/PD-L1 therapy,” and a “PD-1/PD-L1 therapy” are synonymous and used interchangeably and refer to a therapeutic regime that comprises blockade of the PD-1 and PD-L1 signaling axis. In some embodiments, the cancer is a PD-L1 positive cancer. In some embodiments, PD-1/PD-L1 therapy is PD-1/PD-L1 immunotherapy. In some embodiments, the PD-1/PD-L1 therapy is PD-1/PD-L1 blockade. In some embodiments, the PD-1/PD-L1 therapy is an agent that blocks PD-1 based immune inhibition. In some embodiments, the PD-1/PD-L1 therapy comprises an anti-PD-1 blocking antibody (e.g., selected from nivolumab (Opdivo®), pembrolizumab (Keytruda®) and cemiplimab (Libtayo®)). In some embodiments, the PD-1/PD-L1 therapy comprises an anti-PD-L1 blocking antibody (e.g., selected from atezolizumab (Tecentriq®), avelumab (Bavencio®), and durvalumab (Imfinzi®)). In some embodiments, the PD-1/PD-L1 therapy increases immune surveillance. In some embodiments, the PD-1/PD-L1 therapy is an anti-cancer therapy. In some embodiments, the PD-1/PD-L1 therapy increases tumor immune surveillance. Unless otherwise indicated, the term “antibody” (also referred to as an “immunoglobulin”) encompasses monoclonal antibodies and antibody fragments (also referred to herein as antibody portions) so long as they exhibit the desired biological activity.

In certain embodiments, the use of a chimeric antibody or a humanized antibody is also encompassed by the present disclosure. In the context of “an antibody or an antigen-binding fragment thereof,” the term “antibody” refers to a full antibody having two heavy chains and two light chains.

[0123] The basic unit of the naturally occurring antibody structure is a heterotetrameric glycoprotein complex of about 150,000 Daltons, composed of two identical light (L) chains and two identical heavy (H) chains, linked together by both noncovalent associations and by disulfide bonds. Each heavy and light chain also has regularly spaced intra-chain disulfide bridges. Five human antibody classes (IgG, IgA, IgM, IgD and IgE) exist, and within these classes, various subclasses (e.g., IgG1, IgG2, IgG3, and IgG4), are recognized based on structural differences, such as the number of immunoglobulin units in a single antibody molecule, the disulfide bridge structure of the individual units, and differences in chain length and sequence. The class and subclass of an antibody is its isotype.

[0124] The amino terminal regions of the heavy and light chains are more diverse in sequence than the carboxy terminal regions, and hence are termed the variable domains. This part of the antibody structure confers the antigen-binding specificity of the antibody. A heavy variable (VH) domain and a light variable (VL) domain together form a single antigen-binding site, thus, the basic immunoglobulin unit has two antigen-binding sites. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains (Chothia et al., *J Mol Biol.* (1985) 186, 651-63; Novotny and Haber, *Proc Natl Acad Sci USA* (1985) 82:4592-6).

[0125] The carboxy terminal portions of the heavy and light chains form the constant domains i.e. CH1, CH2, CH3, CL. While there is much less diversity in these domains, there are differences from one animal species to another, and further, within the same individual there are several different isotypes of antibody, each having a different function.

[0126] The term “framework region” or “FR” refers to the amino acid residues in the variable domain of an antibody, which are other than the hypervariable region amino acid residues as herein defined. The term “hypervariable region” as used herein refers to the amino acid residues in the variable domain of an antibody, which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a “complementarity determining region” or “CDR.” The CDRs are primarily responsible for binding to an epitope of an antigen. The extent of FRs and CDRs has been precisely defined (see, Kabat et al.). In some embodiments, CDRs are determined using the KABAT system. In some embodiments, CDRs are determined using the Chothia system. In some embodiments, the Chothia system is the enhanced Chothia system (Martin system).

[0127] The monoclonal antibodies herein specifically include “chimeric” antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. 4,816,567; and Morrison et al., *Proc Natl Acad Sci USA* (1984) 57:6851-5). In addition, complementarity determining region (CDR) grafting may be performed to alter certain properties of the antibody molecule including affinity or specificity. A non-limiting example of CDR grafting is disclosed in U.S. Pat. 5,225,539.

[0128] Chimeric antibodies are molecules, the different portions of which are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region. Antibodies which have variable region framework residues substantially from human antibody (termed an acceptor antibody) and complementarity determining regions substantially from a mouse antibody (termed a donor antibody) are also referred to as humanized antibodies. Chimeric antibodies are primarily used to reduce immunogenicity in application and to increase yields in production, for example, where murine mAbs have higher yields from hybridomas but higher immunogenicity in humans, such that human/murine chimeric mAbs are used. Chimeric antibodies and methods for their production are known in the art (for example, PCT patent applications WO 86/01533, WO 97/02671, WO 90/07861, WO 92/22653, and U.S. Pats. 5,693,762, 5,693,761, 5,585,089, 5,530,101 and 5,225,539). As used herein, the term “humanized antibody” refers to an antibody comprising a framework region from a human antibody and one or more CDRs from a non-human (usually a mouse or rat) immunoglobulin. Parts of a humanized immunoglobulin, except possibly the CDRs, are substantially identical to corresponding parts of natural human immunoglobulin sequences. In some cases, however, specific amino acid residues, for example in the framework regions, may be modified, so as to optimize performance of the humanized antibody. Importantly, the humanized antibody is expected to bind to the same antigen as the donor antibody that provides the CDRs. For further details, see e.g., U.S. Pat. 5,225,539 assigned to Medical Research Council, UK. The terms “a framework region from an acceptor human immunoglobulin” and “a framework region derived from an acceptor human immunoglobulin,” and similar grammatical expressions, are used interchangeably herein to refer to a framework region or portion thereof that has the same amino acid sequence of the acceptor human immunoglobulin.

[0129] The term “monoclonal antibody” or “mAb” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variants that may arise during production of the monoclonal antibody, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are uncontaminated by other immunoglobulins. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies. The monoclonal antibodies to be used in accordance with the methods provided herein may be made by the hybridoma method first described by Kohler et al., *Nature* (1975) 256:495, or may be made by recombinant DNA methods (see, e.g., U.S. Pat. 4,816,567). The monoclonal antibodies may also be isolated from phage antibody libraries using the techniques described in Clackson et al., *Nature* (1991) 352:624-8 and Marks et al., *J Mol Biol.* (1991) 222:581-97, for example.

[0130] The mAb of the present disclosure may be of any immunoglobulin class including IgG, IgM, IgE or IgA. A hybridoma producing a mAb may be cultivated *in vitro* or *in vivo*. High titers of mAbs can be obtained from *in vivo* production where cells from the individual hybridomas are injected intraperitoneally into pristine-primed Balb/c mice to produce ascites fluid containing high concentrations of the desired mAbs. mAbs of isotype IgM or IgG may be purified from such ascites fluids, or from culture supernatants, using column chromatography methods well known to those of skill in the art.

[0131] “Antibody fragment,” “antigen-binding fragment,” and “antigen-binding portion” are used synonymously and comprise a portion of an intact antibody, preferably comprising the antigen-binding region thereof. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments, scFvs, diabodies, tandem diabodies (taDb), linear antibodies (e.g., U.S. Pat. 5,641,870, Example 2; Zapata et al., *Protein Eng.* (1995) 8(10):1057-62); one-armed antibodies, single variable domain antibodies, minibodies, single-chain antibody molecules, multispecific antibodies formed from antibody fragments (e.g., including but not limited to, Db- Fc, taDb-Fc, taDb-CH₃, (scFV)₄-Fc, di-scFv, bi-scFv, or tandem (di,tri)-scFv), and bi-specific T cell engagers (BiTEs).

[0132] Papain digestion of antibodies produces two identical antigen-binding fragments, called “Fab” fragments, each with a single antigen-binding site, and a residual “Fc” fragment,

whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

[0133] “Fv” is the minimum antibody fragment that contains a complete antigen-recognition and antigen-binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0134] The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear at least one free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments that have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0135] The “light chains” of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains.

[0136] Depending on the amino acid sequence of the constant domain of their heavy chains, antibodies can be assigned to different classes. There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy chain constant domains that correspond to the different classes of antibodies are called α , δ , ϵ , γ , and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0137] “Single-chain Fv” or “scFv” antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. In some embodiments, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains that enables the scFv to form the desired structure for antigen-binding. For a review of scFvs, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0138] The term “diabodies” refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain (VH - VL). By using a linker that is too short

to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are Natl. Acad. Sci. USA, 90:6444-6448 (1993).

[0139] The term “multispecific antibody” is used in the broadest sense and specifically covers an antibody that has polyepitopic specificity. Such multispecific antibodies include, but are not limited to, an antibody comprising a heavy chain variable domain (VH) and a light chain variable domain (VL), where the VH/VL unit has polyepitopic specificity, antibodies having two or more VL and VH domains with each VH/VL unit binding to a different epitope, antibodies having two or more single variable domains with each single variable domain binding to a different epitope, full length antibodies, antibody fragments such as Fab, Fv, dsFv, scFv, diabodies, bispecific diabodies, triabodies, tri-functional antibodies, antibody fragments that have been linked covalently or non-covalently. “Polyepitopic specificity” refers to the ability to specifically bind to two or more different epitopes on the same or different target(s).

[0140] The present disclosure also provides multispecific antibodies (e.g., bispecific antibodies) having the binding specificity (e.g., comprising the antigen-binding portions, such as the six CDRs or the VH and VL) of an anti-ILT2 antibody described herein. In some embodiments, the multispecific antibody additionally has the binding specificity of another, distinct antibody, which may target ILT2 or a different protein such as a cancer antigen or another cell surface molecule whose activity mediates a disease condition such as cancer. Multispecific antibodies and their preparation are known in the art. In some embodiments, a multispecific antibody described herein is used in place of an anti-ILT2 antibody described herein in a therapeutic method, kit, or article of manufacture described herein.

[0141] The monoclonal antibodies of the present disclosure may be prepared using methods well known in the art. Examples include various techniques, such as those in Kohler, G. and Milstein, C, Nature 256: 495-497 (1975); Kozbor et al., *Immunology Today* (1983) 4:72; Cole et al., pg. 77-96 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985).

[0142] Besides the conventional method of raising antibodies *in vivo*, antibodies can be generated *in vitro* using phage display technology. Such a production of recombinant antibodies is much faster compared to conventional antibody production and they can be generated against an enormous number of antigens. Furthermore, when using the conventional method, many antigens prove to be non-immunogenic or extremely toxic, and therefore cannot be used to generate antibodies in animals. Moreover, affinity maturation (i.e., increasing the affinity and specificity) of recombinant antibodies is very simple and relatively fast. Finally, large numbers of different antibodies against a specific antigen can be generated in one selection procedure. To generate

recombinant monoclonal antibodies one can use various methods all based on display libraries to generate a large pool of antibodies with different antigen recognition sites. Such a library can be made in several ways: One can generate a synthetic repertoire by cloning synthetic CDR3 regions in a pool of heavy chain germline genes and thus generating a large antibody repertoire, from which recombinant antibody fragments with various specificities can be selected. One can use the lymphocyte pool of humans as starting material for the construction of an antibody library. It is possible to construct naive repertoires of human IgM antibodies and thus create a human library of large diversity. This method has been widely used successfully to select a large number of antibodies against different antigens. Protocols for bacteriophage library construction and selection of recombinant antibodies are provided in the well-known reference text *Current Protocols in Immunology*, Colligan et al. (Eds.), John Wiley & Sons, Inc. (1992-2000), Chapter 17, Section 17.1.

[0143] Non-human antibodies may be humanized by any methods known in the art. In one method, the non-human complementarity determining regions (CDRs) are inserted into a human antibody or consensus antibody framework sequence. Further changes can then be introduced into the antibody framework to modulate affinity or immunogenicity.

[0144] In some embodiments, antibodies as described herein are neutralizing antibodies. "Neutralization," as discussed here, is defined as the reduction in protein function by antibodies of the present disclosure. In one embodiment, "neutralization," as discussed here, is binding of antibodies to the surface of immune cells, preferably to immature and mature myeloid lineage derived cells, T cells and NK cells, thereby blocking the propagation of inhibitory signals inside these cells and conferring a less suppressive phenotype and function.

[0145] In some embodiments, the antibody or antigen-binding fragment of the present disclosure is the agent of the present disclosure.

[0146] In some embodiments, the present disclosure provides nucleic acid sequences encoding the antibody of the present disclosure. In one embodiment, an antibody as described herein is encoded by a nucleic acid molecule comprising a nucleotide sequence having at least 75% identity to a nucleotide sequence as described herein. In one embodiment, an antibody as described herein is encoded by a nucleic acid molecule comprising a nucleic acid sequence having at least 80% identity to a nucleic acid sequence as described herein. In one embodiment, an antibody as described herein is encoded by a nucleic acid molecule comprising a nucleic acid sequence having at least 85% identity to a nucleic acid sequence as described herein. In one embodiment, an antibody as described herein is encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to a nucleic acid sequence as described herein. In one embodiment, an

antibody as described herein is encoded by a nucleic acid comprising a nucleic acid sequence having at least 95% identity to a nucleic acid sequence as described herein.

[0147] By another aspect, there is provided a nucleic acid sequence encoding an antibody or antigen-binding fragment of the present disclosure.

[0148] By another aspect, there is provided a nucleic acid molecule encoding an antibody or antigen-binding fragment of the present disclosure.

[0149] In some embodiments, a nucleic acid sequence encoding a variable region of a heavy chain of an antibody or antigen-binding fragment of the present disclosure comprises a nucleic acid sequence selected from:

cagggtcagctgcagcagctggagctgagctggcgaggcctggggcttcagtgaaagctgtcctgcaaggcttggctacaccttcacaagctatggtataagctgggtgaagcagagaactggacagggccttgagtggttgagagattatcctggaagtggtaattctactacaatgagaagtcaagggcaaggccactgactgcagacaaatcctccagcacagcgtacatggagctccgagcctgacatctgaggactctgcggtctatttctgtgcaagatcgaatgatggttaccctgactactggggccaaggcaccactctcacagtctctca (SEQ ID NO: 32),
gatgtacagcttcaggggtcaggacctggcctcgtgaaaccttctcagtcctctgtctctcacctgctctgtcactggctactccatcaccagtgttattactggaactggatccggcagttccaggaaacaaactggaaatggatgggctacataagctacgatgtagcaataactacaacctctctcaaaaatcgaatctccatcactcgtgacacatctaagaaccagttttcctgaagttgaattctgtgacttctgaggacacagccacataattactgtgccatggttactcatattactatgctatggactgctgggtcaaggaaacctcagtcaccgtctctca (SEQ ID NO: 33),
gatgtccagctgcaggctctggccctggactggttaagccttcgagacactgtccctgacctgctctgtgaccggctactctatcacctccggctactactggaactggatcagacagttccccggcaagaaactggaaatggatgggctacatctctacgacggctccaacaactacaacccagcctgaagaaccggatcacatctctcgggacacctccaagaaccagttctccctgaagctgaactccgtgaccgctgccgataccgctactactactgtgtcacggctactcctactactacgccatggatgctggggccagggcacatctgtgacagtgctctct (SEQ ID NO: 34) and

cagggtcagctgcaacagctgcagcgtgagttggtgaaacctggagcttcagtgaaagatacctgcaaggtttctggctacaccttcactgacctactattcactggatgaagcagaggcctgaacagggcctggaatggattggatattatcctagagatggtagtactaagtacaatgagaagttcaagggaaggccacattgactgcagacaaatcctccagcacagcctacatgcagctcaacagcctgacatctgaggactctgcagctctattctgtgcaagaacctgggactactttgactactggggccaaggcaccactctcacagtctctca (SEQ ID NO: 35).

[0150] In some embodiments, a nucleic acid sequence encoding a variable region of a light chain of an antibody or antigen-binding fragment of the present disclosure comprises a nucleic acid sequence selected from

gacattgtctgaccaatctccagcttcttggctgtgtctctagggcagagggccaccatacctgcagagccagtgaaagtgttagattatggcaatagttttatgactggtaccagcagaaaccaggacagccacccaaactcctcatctatcgtgcatccaacctagaatctgggatccctgccagggtcagtggcagtggtctaggacagacttcacctcaccattaatcctgtggaggctgatgatgttgaacctattactgtcagcaagttaatgaggatccgtacacgttcggagggggaccaagctggaataaaa (SEQ ID NO: 36),
gatatccagatgacacagactacatcctcctgtctgcctctctggagacagagtcacatcagttgcaggacaagtccaggacattagcaatt

atftaaactggtatcagcagaaaccagatggaactgttaaactcctgatctcctacacatcaagattgcactcaggagtgcccatcaagggtcagt
ggcagtgggcttggaacagattattctctcaccattagcaacctggagcaagaagatttgccacttactttgccaacagggtatacgttcc
cacgttcggctcggggacaaagtggaaataaaa (SEQ ID NO: 37),
gacatccagatgacctagctctcctctgtctgtggtggcgacagagtgaccatcacctgtcggacctctcaggacatctccaact
acctgaactggtatcagcagaaacccggcaaggccgtgaagctgtgatctcctacacctccagactgcactctggcgtgcccctccagattt
ctggctctggatctggcaccgactacacctgacctcagttctgtcagcctgaggacttcgccactactactgtcagcagggaacacct
gcctactcttggccagggcaccaagctggaaatcaag (SEQ ID NO: 38) and
gacatccagatgacacaattctcctactgtctgtatctctaggaggcagagtcaccattacttgaaggcaagtgcaccattaataattgg
ttagcctggtatcagcagaaaccaggaaatgctcctaggtcttaatatctgtgtgcaaccagtttgaaactggggtcctcaagattcagtg
cagtggtatctggaaggattacactctcagcattaccagttctcagactgaagatgttgctacttattactgtcaacagtattggagtactccgtgg
acgttcggtggaggcaccaagctggaaataaaa (SEQ ID NO: 39).

[0151] In some embodiments, a nucleic acid sequence encoding a heavy chain of an antibody or antigen-binding fragment of the present disclosure comprises or consists of
gatgtccagctgcaaggctctggccctggactggttaagccttcgagacactgtccctgacctgtctgtgaccggctactctatcacctccg
gtactactggaactggatcagacagttccccggcaagaaactggaatggatgggctacatctctacgacggctccaacaactacaacccc
agcctgaagaacccgatcacatctctcgggacacctccaagaaccagttctccctgaagctgaactccgtgaccgctgccataccgtctac
ctactactgtgtcacggctactctactactacgccatggatgcttggggccagggcacatctgtgacagtgtcctctgtcttccaccaaggga
ccctctgtgtccctctggctcctgtctccagatccacctgtagctctaccgtgtctgtggctgctggtcaaggattacttctgagcctgtga
ccgtgtcttgaactctggtgtctgtacctccggcgtgcacacattccagctgtgtgctgcagtcctccggcctgtactctgtctctgtctgtga
ccgtgcttctagctctctgggaccaagacctacacctgtaacgtggaccacaagcctccaacaccaagggtggacaagcgctggaatct
aagtacggccctcctgtctcctcatgtctgtctccagaattcgaaggcggcccttcctgttctctccaaagcctaaggacacctgat
gatctctcggacccctgaagtgcctgctgtggtggtgatgtgtctcaagaggaccccgaggtgcagttcaattgtgtgacgtgacggcgtgg
aagtgcacaacgccaagaccaagcctagagagggaacagttcaactccacctacagagtgtgtccgtgctgacctgtctgcaccaggattg
gctgaacggcaagagtacaagtgaaggtgtccaacaaggcctgcctagctccatcgaaaagacctatccaaggctaagggccagcc
tcgggaacctcaggtttacacctgcctccaagccaagaggaaatgaccaagaatcaggtgtcactgacatgcctcgtgaagggtcttctacc
ctccgatatcgccgtggaatgggagttaattggccagccagagaacaattacaagacaacccctcctgtgctggactccgacggctcttcttc
ctgtattcccgtgacctggacaagtccagatggcaagagggaacgtgttctcctgtcctgtgatgcagaggccctgcacaatcactac
accagaagtcctgtctctgtcctgggcaaa (SEQ ID NO: 109). In some embodiments, the nucleic acid
sequence encoding a heavy chain of an antibody or antigen-binding fragment of the present
disclosure further comprises a sequence encoding a signal peptide. In some embodiments, the
sequence encoding a signal peptide is an N-terminal sequence. In some embodiments, the
sequence encoding a signal peptide comprises or consists of
atggatctgctgcacaagaacatgaagcacctgtggttcttctgtctgtggtggcgtcctagatgggtgtgtct (SEQ ID NO:
110). In some embodiments, the sequence encoding a heavy chain of an antibody or antigen-

binding fragment of the present disclosure further comprises a stop codon. In some embodiments, a stop codon is a plurality of stop codons. In some embodiments, the stop codon is selected from tga, tag, and taa.

[0152] In some embodiments, a nucleic acid sequence encoding a light chain of an antibody or antigen-binding fragment of the present disclosure comprises or consists of
gacatccagatgaccagctccatcctctgtctgcctctgtggcgacagagtgaccatcacctgtcggacctctcaggacatctccaact
acctgaactgggtatcagcagaaacccggcaaggccgtgaagctgctgatctctacacctccagactgcactctggcgtgccctccagattt
ctggctctggatctggcaccgactacacctgacctcagttctgtcagcctgaggacttcgccactactactgtcagcagggcaaacacct
gcctacctttggccagggcaccaagctggaatcaagagaacctgtggctgccccttcctgttcatcttcccacctgtacgagcagctgaa
gtccggcacagcttctgtcgtgtgcctgtgaacaacttctacctcgggaagccaaggtgcagtggagggtggacaatgccctgcagtcgg
gcaactccaagagtctgtgaccgagcaggactccaaggactctacctacagcctgtcctccactgacctgtctaaggccgactacgag
aagcacaaggtgtacgcctgtgaagtgaccaccaggagctgttagccccgtgaccaagtctttcaacagaggcagtg (SEQ ID
NO: 111). In some embodiments, the nucleic acid sequence encoding a light chain of an antibody
or antigen-binding fragment of the present disclosure further comprises a sequence encoding a
signal peptide. In some embodiments, the sequence encoding a light chain of an antibody or
antigen-binding fragment of the present disclosure further comprises a stop codon.

[0153] In some embodiments, the antibody or antigen-binding fragment is murine and the
sequence encoding a heavy chain is selected from SEQ ID NOs: 32, 33, and 35. In some
embodiments, the antibody or antigen-binding fragment is murine and the sequence encoding a
light chain is selected from SEQ ID NOs: 36, 37, and 39. In some embodiments, the antibody or
antigen-binding fragment is humanized and the sequence encoding a heavy chain is SEQ ID NO:
34. In some embodiments, the antibody or antigen-binding fragment is humanized and the
sequence encoding a light chain is SEQ ID NO: 38.

[0154] “Polynucleotide,” or “nucleic acid,” as used interchangeably herein, refer to polymers
of nucleotides of any length, and include DNA and RNA.

[0155] Polynucleotides encoding polypeptides may be obtained from any source including, but
not limited to, a cDNA library prepared from tissue believed to possess the polypeptide mRNA
and to express it at a detectable level. Accordingly, polynucleotides encoding a polypeptide can be
conveniently obtained from a cDNA library prepared from human tissue. The polypeptide-
encoding gene may also be obtained from a genomic library or by known synthetic procedures
(e.g., automated nucleic acid synthesis).

[0156] For example, the polynucleotide may encode an entire immunoglobulin molecule
chain, such as a light chain or a heavy chain. A complete heavy chain includes not only a heavy
chain variable region (VH) but also a heavy chain constant region (CH), which typically will

comprise three constant domains: CH1, CH2 and CH3; and a “hinge” region. In some situations, the presence of a constant region is desirable.

[0157] Other polypeptides which may be encoded by the polynucleotide include antigen-binding antibody fragments such as single domain antibodies (“dAbs”), Fv, scFv, Fab’ and CHI and CK or CL domain has been excised. As minibodies are smaller than conventional antibodies they may achieve better tissue penetration in clinical/diagnostic use, but being bivalent they may retain higher binding affinity than monovalent antibody fragments, such as dAbs. Accordingly, unless the context dictates otherwise, the term “antibody” as used herein encompasses not only whole antibody molecules, but also antigen-binding antibody fragments of the type discussed above. Each framework region present in the encoded polypeptide may comprise at least one amino acid substitution relative to the corresponding human acceptor framework. Thus, for example, the framework regions may comprise, in total, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen amino acid substitutions relative to the acceptor framework regions. Given the properties of the individual amino acids comprising the disclosed protein products, some rational substitutions will be recognized by the skilled worker. Amino acid substitutions, i.e., “conservative substitutions,” may be made, for instance, on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved.

[0158] Suitably, the polynucleotides described herein may be isolated and/or purified. In some embodiments, the polynucleotides are isolated polynucleotides.

[0159] As used herein, the term “non-naturally occurring” substance, composition, entity, and/or any combination of substances, compositions, or entities, or any grammatical variants thereof, is a conditional term that explicitly excludes, but only excludes, those forms of the substance, composition, entity, and/or any combination of substances, compositions, or entities that are well-understood by persons of ordinary skill in the art as being “naturally-occurring,” or that are, or might be at any time, determined or interpreted by a judge or an administrative or judicial body to be, “naturally-occurring.”

Methods for treatment and diagnosis

[0160] By another aspect, there is provided a method of treating an HLA, MHC-I or both expressing cancer in a subject in need thereof, the method comprising administering to the subject an antibody or antigen-binding fragment of the present disclosure.

[0161] By another aspect, there is provided a method of treating a cancer in a subject in need thereof, the method comprising confirming expression of ILT2 in the subject is above a

predetermined threshold and administering to the subject an agent that inhibits ILT2 based immune suppression, thereby treating a cancer in a subject.

[0162] By another aspect, there is provided a method of treating a cancer in a subject in need thereof, the method comprising: administering to the subject an agent that inhibits ILT2-mediated immune suppression; and administering to the subject a PD-1/PD-L1 based therapy; thereby treating a cancer in a subject.

[0163] By another aspect, there is provided a method of increasing efficacy of a PD-1/PD-L1 based therapy against a cancer cell, the method comprising contacting the cancer cell with an agent that inhibits ILT2-mediated immune suppression.

[0164] By another aspect, there is provided an agent that binds and inhibits ILT2 mediated immune cell suppression for use in combination with an anti-PD-L1/PD-1 based therapy to treat a subject suffering from cancer.

[0165] As used herein, the terms “treatment” or “treating” of a disease, disorder, or condition encompasses alleviation of at least one symptom thereof, a reduction in the severity thereof, or inhibition of the progression (e.g., cancer metastasis) thereof. Treatment need not mean that the disease, disorder, or condition is totally cured. To be an effective treatment, a useful composition herein needs only to reduce the severity of a disease, disorder, or condition, reduce the severity of symptoms associated therewith, or provide improvement to a patient or subject’s quality of life.

[0166] As used herein the term “treatment” refers to clinical intervention in an attempt to alter the course of disease in the individual being treated and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include preventing occurrence or recurrence of the disease, alleviation of symptoms, reducing a pathological consequence of the disease, reducing the rate of disease progression, amelioration of the disease state, remission or improved prognosis. The term “treatment” may also encompass *ex vivo* procedures affecting cells or tissues in culture.

[0167] In some embodiments, the antibody or antigen-binding fragment is administered as a monotherapy. In some embodiments, the antibody or antigen-binding fragment is administered with PD-1/PD-L1 therapy. In some embodiments, the antibody or antigen-binding fragment is administered with an opsonizing agent. In some embodiments, the opsonizing agent is not an anti-CD47 agent. In some embodiments, an anti-CD47 agent is an anti-CD47 antibody. In some embodiments, the antibody or antigen-binding fragment is not administered with anti-CD47 agent or therapy. In some embodiments, the antibody or antigen-binding fragment is not combined with an anti-CD47 agent or therapy.

[0168] In some embodiments, treating comprises increasing immune surveillance. In some embodiments, treating comprises increasing an immune response. In some embodiments, treating comprises decreasing tumor burden. In some embodiments, treating comprises reducing cancer metastasis. In some embodiments, treating comprises increasing cytotoxicity against the cancer. In some embodiments, treating comprises increasing inflammatory response against the cancer. In some embodiments, treating comprises increased phagocytosis of the cancer.

[0169] As used herein the term “subject” refers to an individual, or a patient, which is a vertebrate, e.g., a mammal, including especially a human. In some embodiments, the subject is a human. In some embodiments, the subject is a mammal (e.g., a mouse, a rat, a dog, a rabbit, or a non-human primate). In some embodiments, the subject has cancer.

[0170] In some embodiments, the cancer is an HLA expressing cancer. In some embodiments, HLA is HLA-G such as HLA-G1 and other isoforms of HLA-G. In some embodiments, the cancer is an MHC-I expressing cancer. In some embodiments, the cancer is a PD-L1 expressing cancer. In some embodiments, the cancer is a solid cancer. In some embodiments, the cancer is a blood cancer. In some embodiments, the cancer is refractory to PD-1 and/or PD-L1 based therapy. In some embodiments, the cancer never responded to PD-1 and/or PD-L1 based therapy. In some embodiments, the cancer was responsive to a PD-1 and/or PD-L1 based therapy but became refractory. In some embodiments, the method of the present disclosure converts a refractory cancer to a responsive cancer.

[0171] In some embodiments, the cancer is unresectable, metastatic, or refractory to or not a candidate for standard approved therapy, or any combination of the above.

[0172] In some embodiments, the method comprises confirming the cancer expresses HLA, MHC-I or both. In some embodiments, the method comprises confirming the cancer expresses HLA. In some embodiments, the method comprises confirming the cancer expresses MHC-I. In some embodiments, the method comprises confirming the cancer expresses MHC-I (i.e., Class I HLA, in humans). In some embodiments, the confirming comprises measuring expression in the cancer. In some embodiments, the confirming comprises measuring expression on the surface of the cancer. In some embodiments, in and/or on the cancer is in and/or on a cancer cell. In some embodiments, the confirming comprises measuring HLA-G secreted by the cancer. In some embodiments, the confirming comprises measuring soluble HLA-G. In some embodiments, the soluble HLA-G is in a bodily fluid. In some embodiments, the bodily fluid is blood.

[0173] In some embodiments, the method comprises confirming expression of ILT2 in the subject. In some embodiments, the method comprises confirming expression of ILT2 in the subject is above a predetermined threshold. In some embodiments, the confirming comprises

measuring expression of ILT2 in the subject. In some embodiments, the confirming is before the administering. In some embodiments, the measuring is before the administering. In some embodiments, expression of ILT2 is expression in an immune cell. In some embodiments, expression of ILT2 is expression in an immune cell of the subject. In some embodiments, the immune cell is a peripheral blood immune cell. In some embodiments, the immune cell is a peripheral blood mononuclear cell (PBMC). In some embodiments, the immune cell is an intratumor immune cell. In some embodiments, the immune cell is an immune cell in the tumor microenvironment (TME). In some embodiments, the immune cell is selected from a CD8-positive T cell, a macrophage, an NK cell, and a T_{EMRA} cell. In some embodiments, the immune cell is CD8-positive T cell. In some embodiments, the immune cell is a peripheral blood CD8-positive T cell.

[0174] In some embodiments, administering an antibody or antigen-binding fragment of the present disclosure comprises administering a pharmaceutical composition comprising an antibody or antigen-binding fragment of the present disclosure. In some embodiments, a therapeutically effective amount of antibody or antigen-binding fragment is administered. In some embodiments, the pharmaceutical composition further comprises a carrier, excipient, or adjuvant. In some embodiments, the carrier is a pharmaceutically acceptable carrier.

[0175] As used herein, the term “carrier,” “excipient,” or “adjuvant” refers to any component of a pharmaceutical composition that is not the active agent. As used herein, the term “pharmaceutically acceptable carrier” refers to non-toxic, inert solid, semi-solid liquid filler, diluent, encapsulating material, formulation auxiliary of any type, or simply a sterile aqueous medium, such as saline. Some examples of the materials that can serve as pharmaceutically acceptable carriers are sugars, such as lactose, glucose and sucrose, glycols, such as propylene glycol, polyols such as glycerin, sorbitol, mannitol, and polyethylene glycol; esters such as ethyl oleate and ethyl laurate, pyrogen-free water; isotonic saline, Ringer’s solution; ethyl alcohol and phosphate buffer solutions, as well as other non-toxic compatible substances used in pharmaceutical formulations. Some non-limiting examples of substances which can serve as a carrier herein include sugar, stearic acid, magnesium stearate, calcium sulfate, polyols, pyrogen-free water, isotonic saline, phosphate buffer solutions, as well as other non-toxic pharmaceutically compatible substances used in other pharmaceutical formulations. Wetting agents and lubricants such as sodium lauryl sulfate, as well excipients, stabilizers, antioxidants, and preservatives may also be present. Any non-toxic, inert, and effective carrier may be used to formulate the compositions contemplated herein.

[0176] The carrier may comprise, in total, from about 0.1% to about 99.99999% by weight of the pharmaceutical compositions presented herein.

[0177] The term “therapeutically effective amount” refers to an amount of a drug effective to treat a disease or disorder in a mammal. The term “a therapeutically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. The exact dosage form and regimen would be determined by the physician according to the patient’s condition.

[0178] In some embodiments, the method further comprises administering to the subject an opsonizing agent. In some embodiments, the method further comprises contacting the cell with an opsonizing agent. In some embodiments, the opsonizing agent is an epidermal growth factor receptor (EGFR) inhibitor. In some embodiments, the EGFR inhibitor is cetuximab. In some embodiments, the opsonizing agent is not an anti-CD47 agent. In some embodiments, the method further comprises administering to the subject a PD-1/PD-L1 based therapy. In some embodiments, the method further comprises contacting the cell with a PD-1/PD-L1 based therapy. In some embodiments, the method further comprises growing the cell in the presence of a PD-1/PD-L1 based therapy. In some embodiments, the PD-1/PD-L1 based therapy is a PD-1 or PD-L1 blocking antibody (e.g., pembrolizumab). In some embodiments, the method does not comprise administering an anti-CD47 agent or therapy. In some embodiments, the method is devoid of administration of an anti-CD47 agent or therapy. In some embodiments, the method further comprises administering an anti-CD47 agent or therapy.

[0179] In some embodiments, the agent that inhibits ILT2 based immune suppression binds to ILT2. In some embodiments, the agent binds the ILT2 extracellular domain. In some embodiments, the agent is an ILT2 antagonist. In some embodiments, the agent is an ILT2 blocking antibody. In some embodiments, the agent inhibits ILT2 interaction with B2M. In some embodiments, the agent is an antibody of the present disclosure.

[0180] In some embodiments, the agent that inhibits ILT2 based immune suppression is administered before, after or concomitantly with the opsonizing agent. In some embodiments, the agent that inhibits ILT2 based immune suppression and the opsonizing agent are administered in a single composition. In some embodiments, the agent that inhibits ILT2 based immune suppression and the opsonizing agent are administered in separate compositions.

[0181] In some embodiments, the agent that inhibits ILT2 based immune suppression is administered before, after or concomitantly with the PD-1/PD-L1 therapy. In some embodiments, the agent that inhibits ILT2 based immune suppression and the PD-1/PD-L1 therapy are administered in a single composition. In some embodiments, the agent that inhibits ILT2 based

immune suppression and the PD-1/PD-L1 therapy are administered in separate compositions. In some embodiments, at least one of the agents or therapies is adapted for co-administration.

[0182] The term “adapted for co-administration” as used herein refers to the antibodies being present in a form such they can be safely and easily administered to a subject. Co-administration, in some non-limiting embodiments, can be done by injection, i.e., intratumoral injection, intravenous injection or infusion, or subcutaneous injection, or by other known methods such as oral administration or inhalation. In some embodiments, the antibodies will be comprised within a pharmaceutical composition such as can be safely and easily administered to a subject. In some embodiments, the pharmaceutical composition comprises the antibodies and a pharmaceutically acceptable carrier or excipient.

[0183] In some embodiments, HLA is HLA-G. In some embodiments, HLA is a non-canonical HLA. In some embodiments, the HLA is a canonical HLA. In some embodiments, mRNA expression is confirmed. In some embodiments, protein expression is confirmed. In some embodiments, surface expression of the protein is confirmed. Methods of measuring expression are well known in the art and include, PCR, Q-PCR, Northern blot, immunoblot, in situ hybridization, immunostaining, and FACS. In some embodiments, the method comprises FACS analysis of the cancer to confirm surface expression.

[0184] It is understood that the antibodies or antigen-binding fragments and pharmaceutical compositions of the present disclosure may be used in a method of treatment as described herein, may be for use in a treatment as described herein, and/or may be for use in the manufacture of a medicament for a treatment as described herein. The present disclosure also provides kits and articles of manufacture comprising the antibodies or antigen-binding fragments or pharmaceutical compositions described herein.

Formulations

[0185] The present disclosure also contemplates pharmaceutical formulations for human medical use, which comprise as the active agent at least one antibody which recognizes ILT2, for the manufacture of a therapeutic composition for the treatment, diagnosis or prophylaxis of the conditions variously described herein.

[0186] In such pharmaceutical and medicament formulations, the active agent is preferably utilized together with one or more pharmaceutically acceptable carrier(s) and optionally any other therapeutic ingredients. The carrier(s) must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and not unduly deleterious to the recipient thereof. The active agent is provided in an amount effective to achieve the desired

pharmacological effect, as described above, and in a quantity appropriate to achieve the desired daily dose.

[0187] Typically, the molecules of the present disclosure comprising the antigen-binding portion of an antibody will be suspended in a sterile saline solution for therapeutic uses. The pharmaceutical compositions may alternatively be formulated to control release of active ingredient (molecule comprising the antigen-binding portion of an antibody) or to prolong its presence in a patient's system. Numerous suitable drug delivery systems are known and include, e.g., implantable drug release systems, hydrogels, hydroxymethylcellulose, microcapsules, liposomes, microemulsions, microspheres, and the like. Controlled release preparations can be prepared through the use of polymers to complex or adsorb the molecule according to the present disclosure. For example, biocompatible polymers include matrices of poly(ethylene-co-vinyl acetate) and matrices of a polyanhydride copolymer of a stearic acid dimer and sebacic acid. The rate of release of the molecule according to the present disclosure, i.e., of an antibody or antibody fragment, from such a matrix depends upon the molecular weight of the molecule, the amount of the molecule within the matrix, and the size of dispersed particles.

[0188] The pharmaceutical composition of this invention may be administered by any suitable means, such as orally, topically, intranasally, subcutaneously, intramuscularly, intravenously, intraarterially, intraarticularly, intralesionally, or parenterally. Ordinarily, intravenous (IV) or intraarticular administration will be preferred.

[0189] It will be apparent to those of ordinary skill in the art that the therapeutically effective amount of the molecule according to the present disclosure will depend, inter alia, upon the administration schedule, the unit dose of molecule administered, whether the molecule is administered in combination with other therapeutic agents, the immune status and health of the patient, the therapeutic activity of the molecule administered and the judgment of the treating physician.

[0190] Although an appropriate dosage of a molecule (an antibody or a fragment thereof) of the present disclosure varies depending on the administration route, type of molecule (polypeptide, polynucleotide, organic molecule etc.) age, body weight, sex, or conditions of the patient, and should be determined by the physician in the end, in the case of oral administration, the daily dosage can generally be between about 0.01 mg to about 500 mg, preferably about 0.01 mg to about 50 mg, more preferably about 0.1 mg to about 10 mg, per kg body weight. In the case of parenteral administration, the daily dosage can generally be between about 0.001 mg to about 100 mg, preferably about 0.001 mg to about 10 mg, more preferably about 0.01 mg to about 1 mg, per kg body weight. The daily dosage can be administered, for example in regimens typical of 1-4

individual administration daily. Other preferred methods of administration include intraarticular administration of about 0.01 mg to about 100 mg per kg body weight. Various considerations in arriving at an effective amount are described, e.g., in Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th ed., Pergamon Press, 1990; and Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pa., 1990.

[0191] Suitable dosing regimens of combination chemotherapies are known in the art and described in, for example, Saltz et al., *Proc ASCO* (1999) 18:233a and Douillard et al., *Lancet* (2000) 355:1041-7.

[0192] The molecules of the present disclosure as active ingredients are dissolved, dispersed, or admixed in an excipient that is pharmaceutically acceptable and compatible with the active ingredient as is well known. Suitable excipients are, for example, water, saline, phosphate buffered saline (PBS), dextrose, glycerol, or the like and combinations thereof. Other suitable carriers are well known to those skilled in the art. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents.

Methods of production

[0193] By another aspect, there is provided a method for producing an agent, the method comprising:

obtaining an agent that binds to an ILT2 extracellular domain or fragment thereof, testing an ability of said agent to inhibit interaction between ILT2 and B2M and selecting at least one agent that inhibits interaction between ILT2 and B2M; thereby producing an agent. As used herein, an agent may be, e.g., a molecule or protein.

[0194] By another aspect, there is provided a method for producing an agent, the method comprising:

culturing a host cell comprising one or more vectors comprising a nucleic acid sequence encoding an agent, wherein the nucleic acid sequence is that of an agent that was selected by:

- i. obtaining an agent that binds to an ILT2 extracellular domain or fragment thereof;
- ii. testing an ability of said agent to inhibit interaction between ILT2 and B2M; and
- iii. selecting at least one agent that inhibits interaction between ILT2 and B2M; thereby producing an agent.

[0195] By another aspect, there is provided a method for producing an agent, the method comprising: obtaining an agent that binds to a sequence of human ILT2 selected from SEQ ID NOs: 41-44 and 68-70; thereby producing an agent.

[0196] By another aspect, there is provided a method of identifying an antibody that competes for binding to ILT2 with a reference antibody whose heavy and light chains comprise SEQ ID NOs: 48 and 49, respectively, the method comprising contacting a library of antibodies with a polypeptide sequence comprising an ILT2 sequence selected from SEQ ID NOs: 41-44 and 68-70, and selecting from the library an antibody that binds to the ILT2 sequence, thereby obtaining an antibody that competes for binding to ILT2 with the reference antibody.

[0197] By another aspect, there is provided a method for producing an agent, the method comprising: culturing a host cell comprising one or more vectors comprising a nucleic acid sequence encoding an agent, wherein the nucleic acid sequence is that of an agent that was selected by obtaining an agent that binds to a sequence of human ILT2 selected from SEQ ID NOs: 41-44 and 68-70; thereby producing an agent.

[0198] In some embodiments, the method comprises obtaining an agent that binds to a sequence selected from SEQ ID NOs: 41-44 and 68-70. In some embodiments, the nucleic acid sequence is that of an agent that was selected by obtaining an agent that binds to a sequence selected from SEQ ID NOs: 41-44 and 68-70. In some embodiments, the method comprises obtaining an agent that binds to a sequence selected from SEQ ID NOs: 68-70. In some embodiments, the nucleic acid sequence is that of an agent that was selected by obtaining an agent that binds to a sequence selected from SEQ ID NOs: 68-70. In some embodiments, the method comprises obtaining an agent that binds to a sequence selected from SEQ ID NOs: 71 and 72. In some embodiments, the nucleic acid sequence is that of an agent that was selected by obtaining an agent that binds to a sequence selected from SEQ ID NOs: 71 and 72. In some embodiments, the method comprises obtaining an agent that binds to the sequences of SEQ ID NOs: 71 and 72. In some embodiments, the nucleic acid sequence is that of an agent that was selected by obtaining an agent that binds to the sequences of SEQ ID NOs: 71 and 72. In some embodiments, the reference antibody is 15G8. In some embodiments, the reference antibody is 15G8-13.

[0199] In some embodiments, the method further comprises testing an ability of the agent to inhibit ILT2 mediated immune suppression and selecting at least one agent that inhibits ILT2 mediated immune suppression. In some embodiments, the nucleic acid sequence is of an agent selected by testing an ability of the agent to inhibit ILT2 mediated immune suppression and selecting an agent that inhibits ILT2 mediated immune suppression. In some embodiments, the method comprises testing an ability of said agent to induce at least three of: increased phagocytosis of a cancer cell by macrophages, increased T cell activity against a cancer cell, increased generation of M1 macrophages, reduced generation of M2 macrophages, increased recruitment of dendritic cells to a tumor microenvironment, increased dendritic cell activation, and

increased natural killer (NK) cell cytotoxicity against and selecting at least one agent that induces at least three. In some embodiments, the method comprises testing the ability of the agent to induce an effect in at least three of: T cells, NK cells, dendritic cells and macrophages. In some embodiments, the method comprises testing the ability of the agent to induce an effect in T cells, NK cells, dendritic cells and macrophages.

[0200] In some embodiments, increasing efficacy comprises a synergistic increase in an anti-cancer effect. In some embodiments, the anti-cancer effect is pro-inflammatory cytokine secretion. In some embodiments, the pro-inflammatory cytokine is selected from GM-CSF, IL-6, and IFN γ . In some embodiments, the pro inflammatory cytokine is GM-CSF, IL-6, or IFN γ . Each possibility represents a separate embodiment of the present disclosure. In some embodiments, the pro inflammatory cytokine is GM-CSF. In some embodiments, the increased efficacy comprises a synergistic increase in T cell activation. In some embodiments, the increased efficacy comprises a synergistic increase in T cell cytotoxicity. In some embodiments, the increased efficacy comprises a synergistic increase in both T cell activation and cytotoxicity. In some embodiments, the increase comprises increased membranous CD107a expression. In some embodiments, the increase is characterized by increased membranous CD107a expression. In some embodiments, the increase is as compared to efficacy when the agent is not administered or contacted. In some embodiments, increasing efficacy comprises converting a cancer refractory to the PD-1/PD-L1 based therapy to a cancer that responds to the therapy. In some embodiments, the cancer expresses HLA. In some embodiments, the cancer expresses MHC-I.

[0201] In some embodiments, increased macrophage inflammatory activity comprises increased phagocytosis of a cancer cell by a macrophage. In some embodiments, increased macrophage inflammatory activity comprises increasing generation of M1 macrophages. In some embodiments, increased macrophage inflammatory activity comprises decreasing generation of M2 macrophages. In some embodiments, increased macrophage inflammatory activity comprises increasing M1 phenotype on macrophages. In some embodiments, increased macrophage inflammatory activity comprises decreasing M2 phenotype on macrophages.

[0202] In some embodiments, dendritic cell activity comprises dendritic cell activation. In some embodiments, dendritic cell activity comprises dendritic cell recruitment to a tumor. In some embodiments, dendritic cell activity is activity against a cancer cell. In some embodiments, activity against a cancer cell is activity in the TME. In some embodiments, a tumor is the TME. In some embodiments, a tumor comprises the TME. In some embodiments, a tumor includes the tumor and its TME. In some embodiments, dendritic cell activity comprises antigen presentation.

[0203] In some embodiments, testing an ability of the agent comprises the ability of the agent to increase at least 1, 2, 3, 4, 5 or all of T cell activity against a cancer cell, macrophage inflammatory activity, dendritic cell activity, and natural killer (NK) cell cytotoxicity against a cancer cell. Each possibility represents a separate embodiment of the present disclosure. In some embodiments, selecting at least one agent comprises selecting an agent that increases at least 1, 2, 3, 4, 5 or all of T cell activity against a cancer cell, macrophage inflammatory activity, dendritic cell activity, and natural killer (NK) cell cytotoxicity against a cancer cell. In some embodiments, increasing macrophage inflammatory activity is increasing generation of M1 macrophages and/or increasing phagocytosis of a cancer cell by macrophages. In some embodiments, increasing macrophage inflammatory activity is decreasing generation of M2 macrophages. In some embodiments, testing an ability of an agent comprises the ability of the agent to increase macrophage inflammatory activity. In some embodiments, testing an ability of an agent comprises the ability of the agent to increase dendritic cell activity. In some embodiments, to a tumor is to a TME. In some embodiments, testing an ability of an agent comprises the ability of the agent to increase NK cell cytotoxicity against a cancer cell.

[0204] In some embodiments, the method further comprises testing an ability of the agent to inhibit interaction of ILT2 and B2M. In some embodiments, the interaction is direct interaction. In some embodiments, the method further comprises testing an ability of the agent to inhibit contact of ILT2 and B2M. In some embodiments, interaction is binding. In some embodiments, contact is binding. In some embodiments, the method further comprises testing an ability of the agent to bind the epitope.

[0205] The following examples are intended to illustrate how to make and use the compounds and methods of this invention and are in no way to be construed as a limitation. Although the present disclosure will now be described in conjunction with specific embodiments thereof, it is evident that many modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such modifications and variations that fall within the spirit and broad scope of the appended claims.

EXAMPLES

[0206] Generally, the nomenclature used herein and the laboratory procedures utilized in the present disclosure include molecular, biochemical, microbiological, and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols

in Molecular Biology,” John Wiley and Sons, Baltimore, Maryland (1989); Perbal, “A Practical Guide to Molecular Cloning,” John Wiley & Sons, New York (1988); Watson et al., “Recombinant DNA,” Scientific American Books, New York; Birren et al. (eds) “Genome Analysis: A Laboratory Manual Series,” Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; “Cell Biology: A Laboratory Handbook,” Volumes I-III Cellis, J. E., ed. (1994); “Culture of Animal Cells - A Manual of Basic Technique” by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; “Current Protocols in Immunology” Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), “Basic and Clinical Immunology” (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), “Strategies for Protein Purification and Characterization – A Laboratory Course Manual” CSHL Press (1996); “Monoclonal Antibodies: Methods and Protocols.” Vincent Ossipow, Nicolas Fischer. Humana Press (2014); “Monoclonal Antibodies: Methods and Protocols.” Maher Albitar. Springer Science & Business Media (2007), all of which are incorporated by reference. Other general references are provided throughout this document.

Materials and Methods

[0207] Antibodies – Commercial anti-ILT2 mAbs are: Clone #1 – GHI/75 (BioLegend, Cat. No. 333704), Clone #2 – HP-F1 (eBioscience, Cat. No. 16-5129). Additional mAbs used: HLA-G (MEM-G/9; Abcam, Cat. No. ab7758; G-0031.), ILT4 (42D1, Biolegend, Cat. No. 338704), ILT6 (Sino Biological, Cat No. 13549-MM06), LILRA1 (R&D systems, Cat. No. MAB30851), pan-HLA (W6/22; eBioscience, Cat. No. 16-9983-85) and His (Proteintech, Cat. No. 10001-0-AP).

[0208] Flow Cytometry – In general, cells were kept on ice or at 4°C during all steps. Prior to staining, 5×10^5 cells were blocked with 50 µg/mL human IgG (Sigma, Cat. No. I4506) in FACS buffer (PBS with 0.1 % BSA) for 15 min. Antibodies were used at concentrations recommended by the manufacturer and incubated for 30 min. in the dark. Incubation was done in 100 µL in 96-well U bottom plates, cells were washed twice with 200 µL FACS buffer and transferred to FACS tubes in 150 µL FACS buffer for analysis. Cells were analyzed on Gallios Flow Cytometer (Beckman coulter) using the Kaluza for Gallios Flow Cytometry Acquisition Software.

[0209] Myeloid cell differentiation – Monocytes were isolated from fresh blood samples from healthy donors using EasySep™ Human Monocyte Enrichment Kit (STEMCELL, Cat. No. 19059) by negative selection method. The different cell populations were tested for the indicated phenotypes by FACS analysis of relevant markers and by analysis of secretion of characteristic

cytokines. For maturation, monocytes were cultured at a density of $0.8 \times 10^6/\text{mL}$ in RPMI media with growth factors that was refreshed at day 3 and at day 6. Inflammatory M1 macrophages were matured in the presence of 50 ng/mL GM-CSF (M1 phenotype) for 6 days and then 20 ng/mL IFN-gamma and 50 ng/mL LPS for 48 hr. Suppressive M2 macrophages were differentiated using 50 ng/mL of M-CSF for 6 days and then 10 ng/mL M-CSF and 20 ng/mL IL-4 and IL-10 for 48 hr. Dendritic cells were induced by 50 ng/mL GM-CSF and 20 ng/mL IL-4 for 6 days and further differentiated into mature (100 ng/mL LPS) or tolerogenic (IL-10 100 U/mL and IFN- α 2b (1000 U/mL) dendritic cells.

[0210] Transfection – HLA-G1 (encoding the full-length HLA-G transcript) plasmids were generated by cloning HLA-G1 cDNA into a pCDNA3.1 vector. Transfection was done using jetPEI® Transfection reagent (PolyPlus Transfections). ILT2/CD3z plasmid was generated by combining in frame the extra-cellular portion of human ILT2 protein with the trans-membrane and cytoplasmic residues of the mouse CD3 gene. The plasmid was nucleofected into mouse BW5417.3 T cell line using Nucleofector II (Lonza) as described by the manufacturer. Stable transfectants were selected in G418-containing medium.

[0211] NK and cancer cell line co-culture assay - NK cells were incubated with the indicated cell lines in the presence of anti-ILT2 antibodies and matching isotype controls for 5 hours at 37°C. Cytotoxicity levels were measured using a fluorometric LDH detection kit (Promega).

[0212] Flow cytometry blocking assay – Recombinant human ILT2 protein fused with the Fc portion of human IgG1 at the N terminal was conjugated with biotin (Innova bioscience). A total of 5×10^5 A375/HLA-G1 cells were incubated in a volume of 100 μL in the presence of anti-ILT2 clone #1 or isotype matched control mAb and ILT2-Fc conjugated with biotin (10 $\mu\text{g}/\text{mL}$) for 30 minutes at room temperature. After several washing steps, streptavidin-PE was added at a final concentration of 0.2 $\mu\text{g}/\text{mL}$ and incubated for 30 min on ice followed by FACS analysis.

[0213] BW ILT2/CD3z-chain chimera assay - 3×10^4 BW/ILT2z were mixed with equivalent number of A375/WT or A375/HLA-G1 cells for 24 hr. Functional mAbs were used at indicated concentrations and the matching isotype controls. The amount of secreted mouse IL2 was evaluated by commercial ELISA kit (BioLegend).

[0214] Phagocytosis assay - Monocytes were isolated from buffy coat samples obtained from healthy blood bank donors using a human monocyte enrichment kit. The monocytes were grown in RPMI medium supplement with 10% human serum and M-CSF (50 ng/ml) for 6-7 days in order to generate macrophages. Mature Macrophages were detached and re-seeded in 96 well plates (15K cells/ well) and were incubated O.N in 37°C with 5% CO₂. Target primary cancer cells or cell lines from various indications were labeled with pHrodo Red Cell Labeling Dye, washed

and added to macrophages (75K cells/well to achieve Effector:Target ratio of 1:5). The assay plate was assayed with an IncuCyte S3 instrument.

[0215] The fluorescence of the IncuCyte pHrodo Red Cell Labeling Dye is increased in an acidic environment such as the one that is resident in the phagosome, thus enabling the quantitation of phagocytosis events by measurement of fluorescence. The IncuCyte instrument sampled the assay plate (4 images/ well, X10 magnification) every 30min for fluorescent red signal intensity and phase images. Phagocytosis events were reflected as accumulation of red fluorescent signal and the phagocytosis rate was reflected from the kinetics of red fluorescent signal accumulation.

Example 1: ILT2 and HLA-G are found on cancer cells and cancer relevant immune cells

[0216] ILT2, is a known immunosuppressive molecule found on the surface of healthy immune cells as well as many tumor cells. ILT2 has been shown to bind MHC-1 as well as HLA class molecules (HLA-G, as well as HLA-F and HLA-B27), and competes with CD8 and thereby inhibits T cell activation. In order to further understand the breadth of cells that express ILT2, flow cytometric analysis was performed using a commercial antibody (antibody #1) on a variety of immune cells. As reported in the literature, cytotoxic T cells (CTLs) derived from a melanoma patient, as well as natural killer (NK) cells, were positive for surface expression of ILT2 (**Fig. 1**). Monocytes from the blood of healthy donors were also examined and found to highly express ILT2 (**Fig. 2**, leftmost panels). Upon differentiation of the monocytes into different myeloid cell populations (dendritic cells and macrophages), whether immature, inflammatory or tolerogenic, ILT2 expression was retained (**Fig. 2**, right panels).

[0217] ILT2 expression in different cancer indications was examined by bioinformatic analysis of the TCGA database (**Fig. 3A**). Interestingly, a correlation between ILT2 RNA expression levels and the presence of myeloid derived suppressor cells (MDSC) and suppressive M2 tumor associated macrophages (TAM) in the tumors of samples represented in the TCGA was observed (**Fig. 3B**). An analysis of fresh tumor samples from different solid tumors by flow cytometry demonstrated the expression of ILT2 by innate and adaptive immune cells in the tumor microenvironment (TME). Tumor samples from non-small cell lung cancer (NSCLC), kidney cancer (RCC), head and neck cancer, esophageal cancer and colon cancer patients were collected and single cell suspensions were generated by enzymatic digestion. The percent of ILT2 positive cells is presented in **Fig. 3C** for total immune cells, tumor associated macrophages (TAM), CD4-positive T cells, CD8-positive T cells and natural killer cells (NK). Thus, it is apparent that ILT2

is expressed both on cells with anti-cancer activity (inflammatory cells) as well as on cells with cancer-promoting and immunosuppressing activity (tolerogenic and MDSCs).

[0218] HLA-G expression was also investigated in various cancers. Tissue microarrays (TMA) of cancer samples from the different indications were stained with a commercial polyclonal HLA-G antibody by immuno-histochemistry. The percent of positive cases for each cancer type are indicated (**Fig. 4A**). In addition, for several indications, extended TMAs were examined. A score of HLA-G staining was calculated by the multiplication of staining intensity and the percent of positive cells. A high score of HLA-G staining of above 100 was detected in a high percentage of esophagus, gastric, head and neck and kidney cancer (**Fig. 4B**). Percent of positive cases in each indication are shown in **Table 1**.

Table 1: Percent of cases positive for HLA-G by tumor type

Cancer	Tumor Types	N	Positive Cases (%)
Male urogenital system	Prostate adenocarcinoma and testis seminoma	6	0
Thyroid	Thyroid carcinoma	6	0
Ovary	Ovary - adenocarcinoma and granular cell tumor	8	11
CNS	Cerebrum, Cerebellum, Eye	15	17
Lung	Lung - adenocarcinoma, large cell, small cell and squamous cell carcinoma	12	17
Sarcoma	Bone, Abdominal cavity, Retroperitoneum, soft tissue	15	20
Pancreas	Pancreatic adenocarcinoma	9	22
Bladder	Bladder transitional cell carcinoma	3	33
Skin	Squamous cell carcinoma and melanoma	6	33
Large intestine	Colon adenocarcinoma and rectal adenocarcinoma	5	40
Kidney	Kidney - clear cell carcinoma, nephroblastoma, chromophobe adenoma, sarcomatoid carcinoma	12	42
Upper gastrointestinal tract	Esophagus carcinoma and stomach adenocarcinoma	9	56
Breast	Breast - invasive ductal carcinoma	3	67

H&N	H&N - laryngeal squamous cell carcinoma	3	67
Lymphomas	Hodgkin's lymphoma, Diffuse small B and T cell lymphoma	9	78

[0219] HLA-G has a soluble secreted form as well as the more common membranal form. In order to examine the expression levels of soluble HLA-G in cancer patients, plasma samples were examined for the presence of HLA-G using a commercial ELISA. HLA-G was found to be overexpressed in several cancer indications as compared to normal (healthy) controls (**Fig. 5**). Further, in certain cancer types a population of patients with significantly higher levels could be detected.

Example 2: Generation of ILT2 blocking antibodies

[0220] Hybridoma technology was employed to generate monoclonal ILT2 antagonist antibodies. 69 ILT2-specific hybridomas were originally generated. 3 lead antibodies were selected according to their preferable binding, cross reactivity profile and functional activity in the various assays examined. The selected antibodies were 19E3, 15G8 and 17F2. These antibodies were sequenced using common methods. The sequences of the variable regions of the selected antibodies are indicated in **Fig. 6A**. The CDRs were determined by the KABAT system. 15G8 and 19E3 were humanized using a common CDR-graftment approach. Briefly, the essential CDR and framework residues from the original hybridoma-derived antibodies were identified and grafted into the variable and constant regions of germline human antibodies. The final humanized antibodies are IgG4 antibodies.

[0221] The IgG4 heavy chain constant region used for graftment contained two point-mutations known to reduce binding to FcγR. These mutations are conventionally known as S228P and L235E, although their exact position depends on the length of the variable region of the heavy chain. In the case of 15G8 antibody the serine at position 227 was mutated to proline and the leucine at position 234 was mutated to glutamic acid. In the case of 19E3 the serine at position 225 was mutated to proline and the leucine at position 232 was mutated to glutamic acid. The final humanized 15G8 also contained a single amino acid change, removing the cysteine in CDR-H3 and replace it with alanine or serine. This change was made in order to improve developability. The binding of both resulting antibodies was confirmed, and the 15G8 antibody with an alanine was selected for further testing. All future references to humanized 15G8 refer to the alanine variant.

[0222] During grafting of the 15G8 CDRs, five heavy chains and four kappa light chains were generated. These chains were designated VH1-5 and Vk1-4. The sequences of these chains are provided in **Table 2**. By combining each heavy chain with each light chain twenty different resultant antibodies are possible. All twenty possible antibodies were transiently expressed in HEK EBNA cells, and the supernatants were tested for binding to recombinant ILT2 peptide using a Biacore T200. The chimeric 15G8 antibody was used as control. The binding results are summarized in **Table 3**.

Table 2: Humanized 15G8 chains

Description	Sequence	SEQ
VH1	DVQLQGSGPGLVKPSETLSLTCSVTGYSITSGYYWNWIRQFPGK KLEWMGYISYDGSNNYNPSLKNRITISRDTSKNQFSLKLNSVTA ADTATYYCAHGYSYYYAMDAWGQGTSTTVSS	28
VH2	DVQLQGSGPGLVKPSETLSLTCSVTGYSITSGYYWNWIRQFPGK KLEWMGYISYDGSNNYNPSLKNRITISRDTSKNQFSLKLSSVTA ADTATYYCAHGYSYYYAMDAWGQGTSTTVSS	56
VH3	DVQLQGSGPGLVKPSETLSLTCSVTGYSITSGYYWNWIRQPPGK GLEWMGYISYDGSNNYNPSLKNRITISRDTSKNQFSLKLSSVTA ADTATYYCAHGYSYYYAMDAWGQGTSTTVSS	57
VH4	QVQLQGSGPGLVKPSETLSLTCSVTGYSITSGYYWNWIRQPPGK GLEWMGYISYDGSNNYNPSLKNRVTISRDTSKNQFSLKLSSVTA ADTATYYCAHGYSYYYAMDAWGQGTSTTVSS	58
VH5	QVQLQGSGPGLVKPSETLSLTCTVTGYSITSGYYWNWIRQPPGK GLEWIGYISYDGSNNYNPSLKNRVTISRDTSKNQFSLKLSSVTA ADTATYYCAHGYSYYYAMDAWGQGTSTTVSS	59
Vk1	DIQMTQSTSSLSASVGDRVTITCRTSQDISNYLNWYQQKPGKAV KLLISYTSRLHSGVPSRFSGSGSDYTLTISSLQPEDFATYFC QQGNTLPTFGQGTKLEIK	60
Vk2	DIQMTQSPSSLSASVGDRVTITCRTSQDISNYLNWYQQKPGKAV KLLISYTSRLHSGVPSRFSGSGSDYTLTISSLQPEDFATYFC QQGNTLPTFGQGTKLEIK	61
Vk3	DIQMTQSPSSLSASVGDRVTITCRTSQDISNYLNWYQQKPGKAV KLLISYTSRLHSGVPSRFSGSGSDYTLTISSLQPEDFATYYC QQGNTLPTFGQGTKLEIK	24
Vk4	DIQMTQSPSSLSASVGDRVTITCRTSQDISNYLNWYQQKPGKAP KLLISYTSRLHSGVPSRFSGSGSDYTLTISSLQPEDFATYFC QQGNTLPTFGQGTKLEIK	62

SEQ: SEQ ID NO.

Table 3: Results of binding assay for all twenty antibody combinations

Antibody	K _a (1/Ms)	K _d (1/s)	K _D (/M)	Relative KD	Expression (ug/mL)	Designation of selected antibodies
Control	8.08x10 ⁵	1.02x10 ⁻²	1.26x10 ⁻⁸	1	3.65	-
VH1/Vk1	1.06x10 ⁶	9.31x10 ⁻³	8.77x10 ⁻⁹	0.7	1.27	-

VH1/Vk2	7.69x10 ⁵	6.78x10 ⁻³	8.82x10 ⁻⁹	0.7	5.3	-
VH1/Vk3	1.30x10 ⁶	9.05x10 ⁻³	6.94x10 ⁻⁹	0.55	6.81	15G8-13
VH1/Vk4	4.57x10 ⁵	8.54x10 ⁻³	1.87x10 ⁻⁸	1.48	23.5	-
VH2/Vk1	7.60x10 ⁵	7.28x10 ⁻³	9.58x10 ⁻⁹	0.76	1.7	-
VH2/Vk2	7.52x10 ⁵	6.60x10 ⁻³	8.79x10 ⁻⁹	0.7	6.38	-
VH2/Vk3	9.15x10 ⁵	7.60x10 ⁻³	8.31x10 ⁻⁹	0.66	7.69	15G8-23
VH2/Vk4	5.54x10 ⁵	7.89x10 ⁻³	1.42x10 ⁻⁸	1.13	26.6	-
VH3/Vk1	7.99x10 ⁵	6.81x10 ⁻³	8.52x10 ⁻⁹	0.68	3.49	-
VH3/Vk2	8.11x10 ⁵	6.64x10 ⁻³	8.19x10 ⁻⁹	0.65	11.2	15G8-32
VH3/Vk3	6.77x10 ⁵	6.11x10 ⁻³	9.03x10 ⁻⁹	0.72	10.3	-
VH3/Vk4	6.61x10 ⁵	8.32x10 ⁻³	1.26x10 ⁻⁸	1	29	15G8-34
VH4/Vk1	9.15x10 ⁵	7.92x10 ⁻³	8.66x10 ⁻⁹	0.69	1.74	-
VH4/Vk2	6.90x10 ⁵	6.85x10 ⁻³	9.93x10 ⁻⁹	0.79	6.38	-
VH4/Vk3	8.86x10 ⁵	8.43x10 ⁻³	9.51x10 ⁻⁹	0.75	7.87	-
VH4/Vk4	7.24x10 ⁵	9.77x10 ⁻³	1.35x10 ⁻⁸	1.07	24.8	15G8-44
VH5/Vk1	1.16x10 ⁶	9.94x10 ⁻³	8.55x10 ⁻⁹	0.68	3.09	-
VH5/Vk2	1.26x10 ⁶	9.54x10 ⁻³	7.57x10 ⁻⁹	0.6	6.77	-
VH5/Vk3	6.82x10 ⁵	7.20x10 ⁻³	1.06x10 ⁻⁸	0.84	12.4	15G8-53
VH5/Vk4	1.14x10 ⁶	1.17x10 ⁻²	1.03x10 ⁻⁸	0.82	27.9	15G8-54

[0223] Seven of the twenty combinations were selected from further study. Antibodies containing light chain Vk1 consistently produced the worst yields of antibodies regardless of the heavy chain and thus no antibodies containing Vk1 were selected. Vk4 consistently produced the highest yields of antibodies and so combinations of VH3, VH4 and VH5 were selected. Though combination of Vk4 with VH1 and VH2 also yielded high expression, the KD of these antibodies was found to be worse than the control antibody and the worse for all antibodies containing VH1 and VH2. VH1/Vk3, VH2/Vk3 and VH3/Vk2 were all selected as they had the lowest relative KD values. VH5/Vk3 was also selected for a combination of high expression and low relative KD values.

[0224] These seven antibodies were purified by Protein A chromatography and their concentrations were calculated. Several assays were performed to characterize the selected antibodies functional capabilities.

[0225] First, the seven 15G8 antibodies were tested for binding to membranal ILT2. BW cells were transfected with human ILT2 and 5x10⁵ BW-ILT2 were incubated with the seven antibodies or with a control IgG at 10 µg/ml in staining buffer (0.05% BSA in PBS) for 30 minutes followed by washing in staining buffer and incubation with a secondary PE-conjugated donkey anti mouse antibody. Cells were then washed twice using staining buffer and analyzed using the Cytotflex flow cytometer (Beckman Coulter) and the data were analyzed using CytExpert software (Ver

2.3). As can be seen in **Fig. 6B**, the overall binding kinetics were similar between the seven antibodies, but when EC50 values were calculated (**Table 4**) four of the antibodies were superior to the other three. These four antibodies were further tested for their functionality.

Table 4: EC50 values for binding surface ILT2

Antibody	EC50
Control	0.16
15G8-13	0.06
15G8-23	0.01
15G8-32	0.06
15G8-34	0.15
15G8-44	0.14
15G8-53	0.04
15G8-54	0.39

[0226] The ability of the humanized 15G8 antibodies to enhance the phagocytosis of tumor cells was tested for several cancer types. Real time monitoring of the phagocytosis of tumor cell lines by macrophages was performed (see Materials and Methods). As can be seen in **Figs. 6C-6D**, the different humanized ILT2 blocking antibodies tested could enhance the phagocytosis of both HLA-G positive tumor cells (**Fig. 6C**) and MHC-I only positive tumor cells (**Fig. 6D**). Variants 15G8-13, 15G8-23 and 15G8-34 showed somewhat higher efficacy than the other variants in the phagocytosis of A375-HLA-G (**Fig. 6C**). 15G8-13 and 15G8-23 were therefore further evaluated in additional phagocytosis experiments. As displayed in **Figs. 6E-6F**, both antibodies could enhance the phagocytosis of the tested cell lines, yet 15G8-13 showed slightly superior activity especially at low concentration.

[0227] Next, the ability of the humanized 15G8 variants to enhance NK cells effector activity was tested in a system in which NK cells were incubated with target cancer cell lines followed by evaluation of cytotoxicity by measuring LDH levels. As can be seen in **Figs. 6G-6H**, the different variants could all significantly enhance the cytotoxicity of NK cells against both HLA-G-positive (**Fig. 6G**) and MHC-I only cells (**Fig. 6H**) in a dose-dependent manner. Once again variant 15G8-13 demonstrated somewhat superior activity, this time in enhancement of NK cell cytotoxicity. Due to its slight, but consistent, superiority, 15G8-13 was selected for all future analysis and is hereinafter referred to simply as the 15G8 humanized antibody.

Example 3: Comparison of ILT2 blocking antibodies

[0228] The ability of the three CDR-distinct anti-ILT2 antibodies to bind to ILT2 was tested using three different systems. Binding to recombinant ILT2 was tested using ELISA (**Fig. 7A**), and binding to membranous ILT2 was tested using BW cells transfected with ILT2 (**Figs. 7B-7C**). The chimeric and humanized antibodies showed similar binding (**Figs. 7C-7D**). A commercial mouse anti-human ILT2 antibody (Biolegend; Clone GHI/75) was used as a positive control. The three tested antibodies successfully bound ILT2 whether in solution (**Fig. 7A**) or on the surface of cells (**Fig. 7B**). Cross-reactivity to several similar ILT family members –PIRB, ILT6 and LILRA1 was examined using binding ELISA as well (**Fig. 7A**). Antibodies to these proteins were used as positive control. None of the antibodies cross-reacted with PIRB, ILT6 and LILRA1. The antibodies were also effective for immunostaining (**Fig. 7D**). Interestingly, when PBMCs were isolated from the blood of cancer patients, it was found that ILT2 was expressed on more T cells and NK cells in the cancer patients than in healthy controls (**Fig. 7E**).

Example 4: ILT2 antibodies block ILT2-HLA-G interaction

[0229] The ability of the generated anti-ILT2 antibodies to block the interaction between HLA-G and ILT2 was tested using four different assays. First, a blocking flow cytometry assay was performed. HLA-G transfected A375 cells were incubated with biotinylated ILT2 in the presence of the antibodies of the present disclosure and a positive control antibody. The commercially available anti-ILT2 antibody GHI/75 (BioLegend, Cat. No. 333704) was used as the positive control. The binding of ILT2-biotin to the cells was determined using Streptavidin-PE by flow cytometry analysis (**Fig. 8A**). The percent of blocking was determined by normalizing to a negative control (ILT2 binding in the presence of control IgG). A representative FACS analysis showing ILT2 binding without antibody (grey line), in the presence of 15G8 (light grey line), and the isotype control (black line) is presented in **Fig. 8B**. The percentage of blocking was calculated at various concentrations of antibody (**Fig. 8C**). The chimeric murine and humanized antibodies showed similar blocking ability (**Fig. 8D**).

[0230] The ability of ILT2 antibodies to functionally block the interaction between HLA-G and ILT2 was also examined in a BW ILT2/mouse Z-chain chimera reporter assay. BW cells were transfected with human ILT2 fused to a mouse T cell zeta chain (BW-ILT2). The cells were then incubated with A375-HLA-G cells in the presence of the selected ILT2 antibodies. Upon a functional ILT2- HLA-G interaction the BW cells secrete a reporter cytokine, mouse IL-2. Blocking of the interaction would reduce secretion of the reporter cytokine. The secretion of a mouse IL-2 was determined by ELISA after 24 hours of incubation. The results represent an

average of mIL-2 levels $\pm$ SE from triplicate wells per treatment (**Fig. 8E**). A commercial mouse anti-human ILT2 antibody (Biolegend; clone GHI/75) was used as a positive control (PC) for both assays. The percentage of blocking was calculated at various concentrations of antibody (**Fig. 8F**). This same BW ILT2/mouse Z-chain chimera reporter assay was used to rule out the possibility that the new antibodies might have an ILT2 activating effect on their own. The cells were incubated with the ILT2 antibodies without the cancer cells and mouse IL-2 secretion was again measured (**Fig. 8G**). The new ILT2 antibodies were found to have no agonistic effect, though other antibodies generated by the same hybridoma process (1G7) can bind ILT2 and induce its activity.

[0231] Functional blocking was also examined in human Jurkat cells (T cells). Jurkat cells were incubated with or without A375 cancer cells exogenously expressing HLA-G and a single chain anti-CD3 (OKT3). Secretion of pro-inflammatory human IL-2 was measured. When unmodified Jurkat cells were used (cells which are ILT2 negative) high levels of IL-2 were secreted when the Jurkat cells were cocultured with the cancer cells (**Fig. 8H**). Not surprisingly, the addition of the 15G8 antibody had no effect on IL-2 secretion as there was no ILT2 to block. Jurkat cells were therefore transfected to express human ILT2. First, the ILT2-positive Jurkat cells were cultured with and without A375 cancer cell exogenously expressing OKT3. These cancer cells are naturally MHC-I positive. The MHC-I from the cancer cells strongly inhibited IL-2 secretion (**Fig. 8I**). In this case, addition of the 15G8 antibody blocked the ILT2/MHC-I interaction and increased IL-2 secretion in a dose dependent manner. A pan-HLA antibody was used as a positive control, and at equal concentrations the 15G8 antibody was comparable to the pan-HLA antibody (**Fig. 8I**). In order to enhance the inhibitory effect, the A375 cells were also transfected with HLA-G, making them MHC-I and HLA-G positive. These cells produced an even stronger inhibitory effect on the ILT2 positive cells, reducing IL-2 secretion to that of Jurkat cells cultured alone (**Fig. 8J**). A dose dependent effect was again observed when the 15G8 antibody was administered, and again at an equal dosage the 15G8 antibody and pan-HLA antibody were comparably effective (**Fig. 8J**). Notably, when only an HLA-G specific antibody was used instead of the pan-HLA the effect was greatly reduced and was comparable to the 15G8 antibody used at 1/100th of the concentration (**Fig. 8K**).

[0232] This Jurkat system was also used to compare the 15G8 antibody to two commercially available antibodies: GHI/75 and HP-F1. Jurkat cells expressing human ILT2 were cultured with A375 cells expression HLA-G/OKT3 in the presence and absence of various concentrations of 15G8, GHI/75 and HP-F1. As already observed, 15G8 caused a statistically significant, dose dependent, increase in IL-2 secretion (**Fig. 8L**). GHI/75 had no effect on IL2 secretion as

compared to medium alone but resulted in a small increase as compared to the IgG control (**Fig. 8M**). HP-F1 produced a small but significant increase that plateaued and did not increase with increased dosing (**Fig. 8N**). Even at 20 µg/ml HP-F1 was inferior as compared to only 4 µg/ml of 15G8.

[0233] Lastly, activation was directly measured in TILs, and NK cells. TILs were incubated with A375-HLA-G-OKT3 cells for 5 minutes followed by detection of the T cell activation marker, phosphorylated ZAP70. NK cells were incubated with A253-HLA-G cells for 2 minutes followed by detection of the NK cell activation marker, phosphorylated Syk. Activation was observed in both cell types when cocultured with cancer cells, however this activation was enhanced in the presence of ILT2 antibody (**Figs. 8O-8P**). These results demonstrate that the ILT2 antibodies can efficiently block the ILT2-HLA-G interaction which results in enhanced T cell and NK cell activation.

Example 5: ILT2 antibodies enhance the phagocytosis of HLA-G and MHC-I- positive tumor cells

[0234] The ability of the generated anti-ILT2 antibodies to enhance the phagocytosis of tumor cells was tested using two different systems. Monocytes were isolated from the blood of healthy donors and incubated for 6-7 days in the presence of M-CSF to generate macrophages. First, a flow cytometry-based assay was employed. Different cancer cell lines stained with PKH67-FITC were incubated with the macrophages which were stained with eFluor 670-APC in the presence of the indicated antibodies. Phagocytosis levels were determined by the percent of macrophages which were double stained indicating the engulfment of the target cells. Phagocytosis levels are presented as percent from control (medium only). As demonstrated in **Fig. 9A**, the different ILT2 blocking antibodies could enhance the phagocytosis of HLA-G positive A375 cells by macrophages. In addition, the ability of macrophages to enhance the phagocytosis of tumor cells was examined using a real-time IncuCyte® analysis system. Target cell lines were labeled with pHrodo™ Red Cell Labeling Dye, washed and added to macrophages along with various treatments in replicates. The fluorescence of the IncuCyte® pHrodo™ Red Cell Labeling Dye is increased in an acidic environment such as the one that is resident in the phagosome, thus enabling the quantitation of phagocytosis events by measurement of fluorescence. The IncuCyte® instrument sampled the assay plate every 30min for fluorescent red signal intensity and phase images. Phagocytosis events are reflected as accumulation of red fluorescent signal and the phagocytosis rate was reflected from the kinetics of red fluorescent signal accumulation. Using this real-time system, the ability of a humanized anti-ILT2 antibody to enhance the phagocytosis

of HLA-G positive A375 cells was confirmed (**Fig. 9B**). In addition, using the IncuCyte® system, it was demonstrated that the generated blocking ILT2 antibodies can enhance the phagocytosis of both HLA-G positive as well as various MHC-I positive (WT) cancer cell lines (**Fig. 9C**).

[0235] The effect of combining the generated ILT2 antibodies with the antibody-dependent cellular phagocytosis (ADCP) inducing antibody, Erbitux, on phagocytosis of cancer cells was examined using the IncuCyte® real-time system described above. The combination of the ILT2 blocking antibody with Erbitux significantly increased the phagocytosis of a cancer cell line overexpressing HLA-G (**Fig. 9D**) in comparison to the activity of each antibody alone. Indeed, the combination of Erbitux and the 15G8 humanized antibody had a synergistic effect, with the increase in phagocytosis of the combination treatment being larger than merely additive.

Example 6: Selected ILT2 antibodies can restore T cell activity which is inhibited by HLA-G

[0236] In order to examine the ability of the generated anti-ILT2 antibodies to restore T cell activity which was inhibited by HLA-G, human CD8 T cells were co-incubated with either wild type 721.221 cells (221 WT) or 721.221 cells which overexpress the soluble HLA-G5 (221-HLA-G). IFN γ secretion levels from the T cells were measured following 5 days using a standard ELISA. The results are demonstrated as percent of fold above the effect of 221-HLA-G only and represent an average of 4 independent experiments. The results displayed in **Fig. 10A** demonstrate that several ILT2 antibodies can restore HLA-G-inhibited T cell activity. This was also tested with incubation with A375-HLA-G-OKT3 cells. After 72 hours secretion of human granzyme B was also measured and was found to be increased in the presence of 15G8 antibody in a dose dependent manner (**Fig. 10B**).

Example 7: Selected ILT2 antibodies can enhance NK cytotoxicity against HLA-G and MHC-I- positive tumor cells

[0237] The ability of the generated anti-ILT2 antibodies to enhance NK cells effector activity was tested in a system in which NK cells were incubated with various target cancer cell lines. The cells were co-incubated for 5 hours at effector-to-target ratio of 7.5:1, followed by the detection of cytotoxicity levels using a fluorometric LDH detection kit. Percent of specific cytotoxicity was calculated as follows:

$$\% \text{ Cytotoxicity} = \frac{100 \times (\text{Test sample} - \text{Low control (target cells only)})}{(\text{High control (target cells in lysis buffer)} - \text{Low control - effectors only})}$$

[0238] As demonstrated in **Fig. 11A**, the ILT2 antibodies of the present disclosure could significantly enhance the cytotoxicity of NK cells against both HLA-G positive cells and various MHC-I-positive cancer cell lines (**Fig. 11B**) in a dose-dependent manner. Granzyme B (**Fig. 11C**) and interferon gamma (**Fig. 11D**) secretion was also measured and found to increase in a dose-dependent fashion. Primary NK cells were co-cultured with target HLA-G+ melanoma cells followed by analysis by FACS for expression of IFN γ , ILT2, CD56, and CD107A. The ILT2 positive, CD56 positive, NK cell population was specifically analyzed and the dose dependent increase in IFN γ expression and membranal CD107A expression was observed (**Figs. 11E-11F**). When each experiment was plotted separately, the correlation between % ILT2 positive cells and increased expression of IFN γ and CD107A was clearly apparent (**Figs. 11G-11H**).

Example 8: ILT2 antibodies increase the generation of inflammatory macrophages

[0239] The effect of blocking ILT2 on the maturation of macrophages was examined *in vitro*. Monocytes isolated from healthy donors were differentiated in the presence of M-CSF (50 mg/mL) for 5 days to generate mature macrophages (M0) in the presence of a humanized blocking ILT2 antibody or control IgG. The macrophages were further differentiated in the presence of LPS (50 ng/mL) to generate M1 macrophages or with IL-4 (25 ng/mL) to generate M2 macrophages. As demonstrated in **Fig. 12**, the presence of ILT2 blocking antibodies during the maturation process of macrophages increased the expression of HLA-DR (a marker of M1 inflammatory macrophages) on the macrophages of most of the donors tested, whether they were differentiated into M0, M1 or M2 macrophages. In addition, macrophages differentiated into M1 macrophages also had increased CD80 levels in most of the donors tested. Taken together, these results demonstrate that the selected ILT2 antagonist antibodies can induce macrophages that display higher levels of HLA-DR and CD80, which represent macrophages with a more inflammatory M1 phenotype.

Example 9: ILT2 blocking antibodies enhance the activity of immune cells against tumor cells from patients

[0240] The activity of the generated anti-ILT2 antibodies was examined in *ex vivo* systems with tumor samples from cancer patients (RCC and H&N). In order to test the ability of the antibodies to increase phagocytosis of tumor cells from patients, macrophages generated from monocytes were incubated with tumor cells isolated from tumor samples. Phagocytosis levels were examined using the IncuCyte[®] real-time analysis system as detailed above. As demonstrated

in **Fig. 13A**, ILT2 antibodies could enhance the phagocytosis of tumor cells from patients from different cancer indications. Further, the effect was dose dependent, and present even with autologous macrophages and was seen both for RCC (**Fig. 13B**) and squamous cell carcinoma from H&N (**13C**). In addition, the effect of ILT2 antibodies to enhance the activity of PBMCs was examined. Single cell suspensions of tumor samples from patients were incubated with PBMCs isolated from the same patients in the presence of IL-2 (activated PBMCs). As demonstrated in **Fig. 14G**, PBMC secretion of the pro-inflammatory TNF- α cytokine in the presence of the tumor cells was elevated in the presence of the ILT2 antibodies. Taken together, these results demonstrate the ability of blocking ILT2 antibodies to increase the activity of immune cells against tumor cells from various cancer indications.

Example 10: ILT2 blocking antibodies can be combined with PD-1/PD-L1 therapy

[0241] ILT2 and PD-1 are, for the most part, expressed on different immune cells that comprise both the peripheral blood cells and the tumor microenvironment resident immune cells (**Fig. 14A**). Analysis of ILT2 and PD-1 expression in intra-tumoral CD8-positive T cells from CRC patients found that T central memory cells (T_{cm}) and exhausted T cells (T_{ex}) both expressed high levels of PD-1 (**Fig. 14B**), but low levels of ILT2 (**Fig. 14C**). CD45RA re-expressing T cells (T_{EMRA}) showed the exact opposite pattern, expressing high levels of ILT2 and low levels of PD-1. This dichotomy was not a cancer specific phenomenon, a large percentage (83%) of T_{EMRA} cells from the blood of healthy donors were found to be ILT2 positive while only a small percentage (17%) of total CD8-positive T cells were positive (**Fig. 14D**). Nevertheless, ILT2 expression was enhanced in T cells in the TME. A single cell suspension was generated by enzymatic digestion of a tumor isolated from an esophageal cancer patient. FACS analysis showed that a large proportion of CD8-positive tumor infiltrating lymphocytes (TILs) were T_{EMRA} cells (50%) and that these T_{EMRA} cells were 100% ILT2 positive, but almost completely PD-1 negative (95%) (**Fig. 14E**).

[0242] The effect of the combination of an anti-ILT2 antibody of the present disclosure and anti-PD-1 was tested in SEB-activated (10 ng/ml) PBMCs from 10 healthy donors. Expression of membranial CD107a was used as a marker for increased cytotoxicity. Overall, the 15G8 antibody produced on average a small increase in surface CD107a, while anti-PD-1 produced a somewhat larger response which was donor-dependent (**Fig. 14F**). The combination of the two antibodies produced increased CD107a levels on average; however, these changes were variable based on the specific donor sample. **Fig. 14G** presents three exemplary samples. The first donor saw an additive effect when anti-PD-1 was combined with 15G8, with the total CD107a level being approximately equal to the sum of the effects of each antibody alone. The second donor had a

stronger response to anti-PD-1 than to anti-ILT2, but unexpectedly the combination of the two antibodies had a more than additive effect. Anti-PD-1 produced 19% increase in expression, anti-ILT2 produced 3.7% increase, but the combined treatment resulted in 33.2% increase. This synergistic effect was even more pronounced in the cells of donor #3. In donor #3 15G8 was more effective than anti-PD-1 (13.1% increase vs. 9.3% increase) and the combined therapy was vastly more effective (41%) producing almost twice the effect of what would be predicted from a merely additive combination.

[0243] A combination treatment of patient tumor cells with PD-1 blocking antibody and the generated ILT2 antibodies was assessed next. Various patient cancer cells were incubated with autologous PBMCs in the presence of anti-PD1 antibody, antibodies of the present disclosure and combinations thereof. IgG was used as a control and secretion of pro-inflammatory molecules was measured as a readout. An enhanced secretion of pro-inflammatory cytokines was observed in the combination treatments (**Figs. 14H-14J**). Treatment of colon adenocarcinoma cells from a first patient by humanized antibody 15G8 did not enhance IFN γ secretion at all as compared to IgG control, while anti-PD-1 produced a robust increase in cytokine secretion (**Fig. 14H**).

Unexpectedly however, the combination of anti-PD-1 with the ILT2 antibody increased secretion by more than 50%. A second patient showed a similar trend with small increases induced by ILT2 antibody or anti-PD-1 and with an enhanced synergistic increase present when the two antibodies were used in combination (**Fig. 14I**). GM-CSF expression was not altered by either antibody alone as compared to control, however, surprisingly, the combination of the two antibodies produced a robust increase of nearly 100% of the control GM-CSF levels (**Fig. 14J**).

[0244] Next a mixed lymphocyte reaction was used to assess combined therapy. Dendritic cells and CD8-positive T cells were isolated from different healthy donors and macrophages were generated from monocytes isolated from a H&N cancer patient. The cells were combined in an effector cell to target ratio of 5:1, with the indicated treatments (20 ug/mg of each). IFN γ secretion by the T cells was enhanced when either anti-ILT2 antibodies or anti-PD-1 antibodies were present and this effect was increased with the use of both antibodies in combination (**Figs. 14K-14L**). A greater cumulative effect was observed in the macrophage culture (**Fig. 14L**) as compared to the dendritic cell culture (**Fig. 14K**). These results clearly show that anti-ILT2 and anti-PD-1 therapy have a synergistic and de novo effect on enhancing immune cell inflammatory response.

Example 11: ILT2 blocking antibodies reduce tumor burden *in vivo*

[0245] The efficacy of the anti-ILT2 antibodies was examined in a xenograft *in vivo* model. Immune compromised SCID-NOD or NSG mice were inoculated with cancer cell lines (A375-HLA-G, A375-WT, COLO-320-HLA-G) and human macrophages generated from the blood of healthy donors were injected into the mice in the presence of ILT2 antibodies. As demonstrated in **Fig. 15A**, the administration of the generated ILT2 antibodies led to significant tumor inhibition in this model which was most likely mediated by the activity of the human macrophages in this system. In addition, anti-tumor efficacy was observed in HLA-G-positive as well as MHC-I-positive tumor cells.

[0246] The efficacy of the anti-ILT2 antibodies was also examined in a lung lesion melanoma xenograft *in vivo* model. Immune compromised SCID-NOD mice were inoculated with melanoma cells (MEL526-HLA-G). Human PBMC, isolated from the blood of healthy donors, were injected into the mice in the presence of select ILT2 antibodies starting one day after the inoculations and repeated at days 2, 10, 18 (**Fig. 15B**). ILT2 antibodies were administered at days 1, 4, 8, 11, 15, 18, 22 and 25. As demonstrated in **Fig. 15C**, the administration of the generated ILT2 antibody led to a significant reduction in the metastasis of the tumor cells, which is represented by the formation of black lesions in the lungs of the mice. The lungs of the mice that were treated with the ILT2 antibody have very few such lesions compared to the mice that were treated with the control IgG. This effect is also demonstrated by the reduction of the weight of the lungs in these mice (**Fig. 15D**) and was most likely mediated by the human lymphocytes that were administered to the mice in combination with the inhibition of ILT2 by the administered antibody. Thus, the anti-ILT2 antibodies were effective at preventing metastasis and tumor formation.

[0247] Next, the effectiveness of the new antibodies in treating an already formed tumor was tested in the same *in vivo* mouse model. SCID-NOD mice were engrafted by IV administration with MEL526-HLA-G cells as before. After 15 days, human PBMCs isolated from healthy donors were administered to the relevant groups of mice and this administration was repeated at days 25, 35 and 51 (see **Fig. 15E**). Antibodies, (ILT2 antibodies, anti-PD-1 antibodies or a combination of the two) were administered at days 14, 17, 20, 24, 27, 30, 34, 37 and 50 (see **Fig. 15E**). At day 53 the mice were sacrificed, and the lungs weighed. Tumor weight was calculated by subtracting naïve mice lung weight from the lung weight of the test mice. Anti-PD-1 antibody decreased tumor weight, though not significantly, while the ILT2 antibody and the combination treatment had a significant effect (**Fig. 15F**).

[0248] Tumor derived CD8 T cells, TEMRA cells and NK cells were tested for CD107A and CD69 expression. In total CD8 T cells, anti-PD-1 antibody induced a non-significant increase in

CD107A expression, while the ILT2 antibody, but not the combination therapy, induced a significant change (**Fig. 16A**). In T_{EMRA} cells, both the ILT2 antibody and the combination therapy induced a significant increase (**Fig. 16B**). In NK cells, both anti-PD1 and anti-ILT2 antibodies significantly increased the percentage of CD69 positive cells, but surprisingly the combined therapy had a greatly enhanced effect with the total percentage of CD69 positive cells being more than the combination of either therapy alone (**Fig. 16C**). Surprisingly, when CD69 expression was examined in CD8 T cells neither anti-PD1 nor anti-ILT2 increase expression, however, the combined treatment induced a highly significant increase in CD69 expression (**Fig. 16D**). Further, it was determined that the effect of the ILT2 antibody was correlated with ILT2 expression. When the experiments were broken down into mice that received PBMCs with low or high ILT2 expression a significant difference in activation markers was observed. In T_{EMRA} cells the high ILT2 expressing PBMCs included more than doubling of CD107A expression as compared to the low ILT2 expressing PBMCs (**Fig. 16E**). Similarly, when NK cells were examined, the high ILT2 expressing PBMCs induced nearly 90% of cells to express CD69 when the combination treatment was administered; while the low ILT2 expressing PBMCs induced less than 40% of NK cells to express CD69 (**Fig. 16F**). Thus, the expression level of ILT2 in the PBMCs is essential for the most potent effects of the antibodies.

Example 12: *In vivo* humanized H&N model

[0249] In a second *in vivo* model, humanized mice (human CD34+ engrafted mice) were inoculated with A253-HLA-G cells. When tumors reached a size of 80 cubic millimeters mice were treated with control IgG or ILT2 antibody (15G8, 10 mg/kg for both). The treatment was repeated twice a week (**Fig. 17A**) until day 43 and the tumors were measured by a caliper at various time points to determine tumor size. The ILT2 antibodies completely retarded tumor growth in 2 of the 4 of the mice (mice # 23 and 28), with the tumor being eradicated by day 43 (**Fig. 17B**). In order to determine if the different responses to the treatment were due to different levels of expression of ILT2 in the immune cells of the mice, CD8 T cells from peripheral blood were assayed for ILT2 expression at baseline. Indeed, both mice that had a complete response had T cells with high expression of ILT2, the other two mice had significantly lower expression levels (**Fig. 17C**). Further, by examining the TME post treatment, three other pharmacodynamic markers of response which differentiate responders from non-responders, CD107A expression in T cells (**Fig. 17D**), M1/M2 macrophage ratio (**Fig. 17E**), and total CD80 positive dendritic cells (**Fig. 17F**) were demonstrated. These results point to the fact that anti-ILT2 generates a shift in the

myeloid and lymphoid compartments of the tumor microenvironment and can also increase the capability of dendritic cells to present antigens and recruit more T cells to the tumor.

Example 13: Epitope mapping of the 15G8 humanized antibody

[0250] The 15G8 antibody was sent for epitope mapping to determine the location on ILT2 to which it binds. Mapping was performed by the MAbSilico company. The structure of ILT2 used was modelled using the structures: 6AEE (four Ig-like domains, some loops missing), 1VDG (unpublished, domains 1 and 2), 1G0X (domains 1 and 2) and 4LL9 (domains 3 and 4). The 6AEE and 1G0X structures were taken from Wang et al., *Cell Mol Immunol.* (2020) 17(9):966-75, and the 4LL9 structure was taken from Chapman et al., *Immunity* (2000) 13(5):727-36. Region D1 was defined as residues 24-121 of ILT2. Region D2 was defined as residues 122-222 of ILT2. Region D3 was defined as residues 223-321 of ILT2. Region D4 was defined as residues 322-409 of ILT2. 3D model of the antibody was built using MODELLER.

[0251] Based on the top 30 ranking docking poses, the residues of the target were scored for their probability to belong to the epitope. The residues that probably belong to the epitope are shown on the sequence in **Fig. 18A** and on the structure of the target in **Fig. 18B**. From these residues, four main interaction regions are defined on the target (**Fig.18C**). All four of these interaction regions are found in the interdomain section of ILT2, that is the hinge section between D1 and D2.

[0252] The binding epitopes of most ILT2 antibodies are not known, however, International Patent Publication WO2020/136145 does disclose epitope information for a variety of antibodies. Two general binding regions were found, one within the D1 region and one within the D4 region. In particular, three antibodies designated 3H5, 12D12 and 27H5 were characterized by loss of binding to a mutant with substitutions at E34, R36, Y76, A82 and R84 in D1. One of those antibodies, 3H5, showed diminished binding to a mutation with substitutions at G29, Q30, T32, Q33 and D80 of D1. These residues are exclusively in the D1 region and are all outside of the 4 regions (all within the interdomain) defined as the binding epitope of the 15G8 antibody (note that in **Fig. 18A** the sequence starts one amino acid later, so that E34 of WO2020/136145 for example, is E33 in **18A**). Thus, antibody 15G8 binds to a different 3-dimensional epitope than that of the antibodies of the WO2020/136145 Publication (**Fig. 18D**).

[0253] Next, the specific epitopes bound by the 15G8 antibody were empirically tested. Empirical mapping was performed by Neoproteomics Inc. using hydroxyl radical foot-printing (HRF) and mass spectrometry techniques (see Materials and Methods). The overall sequence coverage of ILT2 protein obtained from both trypsin and dual trypsin and Asp-N digestions, was

~ 90.7%. For ILT2 protein digested by trypsin, a total of 23 peptides were detected by LCMS and MS/MS analysis. Out of these 23 peptides, 20 were observed to be labeled (**Table 5**; the highest normalized protection ratio (NR) values are bolded. Peptide location and the corresponding sequence are shown in columns 1 and 2). For ILT2 protein digested by trypsin and Asp-N, a total of 15 peptides were detected by LCMS and MS/MS analysis. Out of these 15 peptides, 14 were observed to be labeled (**Table 6**; the highest normalized protection ratio (NR) values are in bold. Residues in bold are confirmed to be modified.).

Table 5: Rate constants for the modified peptides of ILT2 derived from trypsin digestion.

Position in ILT2	Sequence of ILTS (SEQ ID NO)	Free ILT2, $K_{\text{free}}, \text{S}^{-1}$	ILT2-BND-22com, $K_{\text{com}}, \text{S}^{-1}$	Ratio, $K_{\text{free}}/K_{\text{com}}$	NR Ratio/1.44
5-24	PTLWAEFGSVITQGSPVTLR (73)	8.93 ± 1.86	7.04 ± 1.24	1.27	0.88
25-35	CQGGQETQEYR (74)	1.18 ± 0.11	0.77 ± 0.070	1.53	1.06
42-48	TALWITR (75)	2.62 ± 0.50	1.56 ± 0.26	1.68	1.17
49-55	IPQELVK (76)	2.41 ± 0.42	1.5 ± 0.24	1.61	1.12
56-71	KGQFPIPSITWEHAGR (68)	9.09 ± 0.87	2.31 ± 0.17	3.94	2.74
57-71	GQFPIPSITWEHAGR (69)	6.93 ± 0.68	1.97 ± 0.38	3.52	2.44
74-83	CYYGSDTAGR (77)	0.58 ± 0.057	0.32 ± 0.078	1.81	1.26
84-100	SESSDPLELVVTGAYIK (70)	1.26 ± 0.15	0.37 ± 0.055	3.41	2.35
101-134	PTLSAQFSPVNVNNGGNVILQC DSQVAFDGFSLCK (78)	5.46 ± 0.85	11.08 ± 1.94	0.49	0.34
135-151	EGEDEHPQCLNSQPHAR (79)	5.62 ± 0.81	6.81 ± 1.08	0.83	0.58
156-167	AIFSVGPVSPSR (80)	1.51 ± 0.20	1.0 ± 0.13	1.51	1.05
156-168	AIFSVGPVSPSRR (81)	1.6 ± 0.17	0.94 ± 0.013	1.7	1.18
168-172	RWWYR (82)	0	0	-	-
173-200	CYAYDSNSPYEWSLPSDLLEL LVLGVSK (83)	7.67 ± 0.73	5.39 ± 0.57	1.42	0.99

Position in ILT2	Sequence of ILTS (SEQ ID NO)	Free ILT2, K_{free}, S^{-1}	ILT2-BND-22com, K_{com}, S^{-1}	Ratio, K_{free}/K_{com}	NR Ratio/1.44
201-230	KPSLSVQPGPIVAPEETLTQLQCGSDAGYNR (84)	4.24 ± 0.37	2.64 ± 0.19	1.61	1.12
236-265	DGERDFLQLAGAPQAGLSQAN*FTLGPVSR (85)	4.55 ± 0.84	3.21 ± 0.40	1.41	0.98
240-265	DFLQLAGAPQAGLSQAN*FTLGPVSR (86)	3.55 ± 0.70	2.37 ± 0.11	1.5	1.04
240-265	DFLQLAGAPQAGLSQANFTLGPVSR (87)	1.96 ± 0.24	1.55 ± 0.17	1.26	0.88
266-272	SYGGQYR (88)	0	0		-
273-301	CYGAHNLSSEWSAPSDPLDIL IAGQFYDR (89)	5.83 ± 0.83	5.16 ± 0.82	1.13	0.78
336-344	EGAADDPWR (90)	5.37 ± 0.69	5.33 ± 0.98	1	0.69
354-372	YQAEFFPMGPVTSAHAGTYR (91)	23.01 ± 6.49	15.19 ± 1.98	1.51	1.05
373-380	CYGSQSSK (92)	0	0	-	-

Mean-1.37, Median-1, Normalized ratio- $(1.37+1.50)/2=1.44$, no labeling-'0', '*'-potential glycosylation site.

Table 6: Rate constants for the modified peptides of the ILT2 derived from trypsin/Asp-N digestion

Position	Sequence (SEQ ID NO)	Free ILT2, K_{free}, S^{-1}	ILT2-15G8com, K_{com}, S^{-1}	Ratio, K_{free}/K_{com}	NR Ratio/1.53
5-24	PTLWAEFGSVITQGSPVTLR (93)	15.07 ± 1.16	19.66 ± 1.7	0.78	0.51
10-24	EPGSVITQGSPVTLR (94)	1.91 ± 0.97	1.05 ± 0.030	1.82	1.19
42-48	TALWITR (95)	2.84 ± 0.49	1.74 ± 0.31	1.63	1.07
49-55	IPQELVK (96)	2.69 ± 0.42	2.02 ± 0.32	1.33	0.85
57-66	GQFPIPSITW (71)	4.32 ± 0.51	0.72 ± 0.082	6	3.92
91-100	ELVVTGAYIK (72)	0.70 ± 0.082	0.14 ± 0.0071	5	3.27
138-151	EDEHPQCLNSQPHAR (97)	0.82 ± 0.11	0.77 ± 0.14	1.06	0.69
156-167	AIFSVGPVSPSR (98)	1.8 ± 0.21	1.15 ± 0.088	1.57	1.03

183-193	EWSLPS (99)	0.52 ± 0.11	0.33 ± 0.065	1.58	1.03
189-200	DLLELLVLGVSK (100)	2.92 ± 0.27	1.81 ± 0.017	1.61	1.05
192-200	ELLVLGVSK (101)	1.16 ± 0.21	0.84 ± 0.13	1.38	0.9
266-272	SYGGQYR (102)	0	0	-	-
291-299	DILIAGQFY (103)	0.89 ± 0.092	0.44 ± 0.066	2.02	1.32
354-372	YQAEFPMGPVTSAHAGTYR (104)	5.98 ± 0.90	3.35 ± 0.27	1.79	1.17
357-372	EFPMGPVTSAHAGTYR (105)	18.19 ± 2.27	15.01 ± 1.98	1.21	0.79

Mean-1.48, Median-1.58, Normalized ratio- $(1.48+1.58)/2=1.53$, no labeling- $^{\circ}0$

[0254] The HRF process introduces stable side chain oxidative modifications resulting in specific mass shifts, which were identified from the tandem mass spectrometry data. The selected ion chromatograms (SIC) were extracted and integrated for the un-oxidized and all oxidized forms of peptide ion (with particular m/z). These peak area values were used to characterize reaction kinetics in the form of dose response (DR) plots, which measure the loss of unmodified peptide as a function of the hydroxyl radical exposure. The solvent protected regions in the complex exhibit decreased oxidation reaction compared to the same regions in the free protein. Differences in the rate of oxidation (called rate constant, K) indicate the potential locations of the binding interface.

[0255] MS data from one replicate experiments were used to calculate K values for each peptide and specific residues. The overall fit results for all detected peptides with errors are shown in **Tables 5** and **6**. The third and fourth columns denote the K values for the free ILT2 and its complex respectively. Error bars representing the fitting error are shown next to each K value. The fifth column shows the ratio, R ($=K_{\text{Free}}/K_{\text{Complex}}$). The sixth column shows normalized ratio (NR) calculated as $R/((\text{mean} + \text{median})/2)$. If the R value for a given peptide is less than 1, it suggests that the corresponding region experienced gain in solvent accessibility due to structural changes introduced during complex formation. A R value close to 1 indicates that the solvent accessibility of the region remains unchanged, while a $R > 1$ suggests that the corresponding region exhibits protection from the solvent as a function of the complex formation. However, the R values for most of the peptides (column five in **Table 5**) fall between 0.49 and 3.94, with the mean value of 1.37 and a median value of 1.5 (3.41, 3.52 and 3.94 values were excluded from the statistical analysis). The R values for most of the peptides (column five in **Table 6**) fall between

0.78 and 6, with the mean value of 1.48 and a median value of 1.58 (5 and 6 values were excluded from the statistical analysis). Furthermore, **Figs. 19A** and **19B** showing the distribution of R values for all tryptic peptides and tryptic/Asp-N peptides within ILT2 respectively, also indicate that the majority of the peptides within ILT2 exhibit changes in modification upon complex formation greater than 1. Using a strategy similar to one used in metabolomics to correct for non-biological variations between samples (mean scaling, division by central tendency) the average of the mean and the median were used to normalize the ratios to a value of 1. A normalization factor of 1.44 and 1.53 was used to normalize ratio (NR) for R values derived from trypsin and trypsin/Asp-N experiments respectively. The NR of 2+ considered to be a significant protection in oxidation upon complex formation in these studies.

[0256] Overall, **Table 5** shows 3 peptides from ILT2 that cover the ILT2 region from 56-71, 57-71 and 84-100 and exhibit the highest protections of 2.74, 2.44 and 2.35 respectively in ILT2-15G8 complex vs. the ILT2 alone. **Table 6** found two peptides covering amino acids 57-66 and 91-100 which show the highest protections of 3.92 and 3.27, respectively. These regions are a part of the binding interface with the 15G8 mAb.

[0257] Individual DR plots for the five significant peptides are shown in **Figs. 19C-19G**. These illustrate the comparative DR plots for the five most protected peptides as a result of complex formation. DR plots for free ILT2 form and for the ILT2-15G8 complex are shown in blue and red, respectively. The red and blue solid lines show the best fit to the theoretical first order equation. **Tables 5** and **6** and **Figs. 19C-G** show the most reductions/protections in solvent accessibility for five peptides covering residues 56-71 (SEQ ID NO: 3), 57-71 (SEQ ID NO: 4), 84-100 (SEQ ID NO: 5), 57-66 (SEQ ID NO: 6) and 91-100 (SEQ ID NO: 7) of the ILT2. The overall protection levels for 57-66 (NR=3.92), 91-100 (NR=3.27), 56-71 (NR=2.74), 57-71 (NR=2.44) and 84-100 (NR=2.35) peptides demonstrate that these regions are part of the binding interface with 15G8.

[0258] Interestingly, the region defined as the 15G8 epitope, that is the interdomain between D1 and D2, has been identified as the main interaction region of ILT2 that binds with beta-2-microglobulin (B2M) when it is in complex with HLA (see Kuroki et al., *J Immuno.* (2019) 203(12):3386-94.) (**Figs. 18E-18F**). Indeed, residues G97, A98, Y99, I100, Q125 and V126 were specifically identified by Kuroki et al., (Supplementary Figure S2 in Kuroki) as interacting with B2M. Residues 97-100 of Kuroki, which correspond to residues 96-99 in **Tables 5** and **6**, fall within two of the interacting peptides for 15G8 and thus are residues of the 15G8 epitope. This strongly suggests that 15G8 inhibits the binding of ILT2 to HLA in a B2M-dependent manner, and indeed blocks ILT2 binding directly to B2M. In contrast, other reported antibodies (3H5,

12D12 and 27H5) bind to the N-terminal D1 region of ILT2 that interacts with the $\alpha 3$ domain of HLA-G (see Supplementary Figure S2 in Kuroki). This is highly significant as Kuroki et al. found that the main interaction site for ILT2 is the B2M site and binding to the $\alpha 3$ domain is additional and flexible. This may explain 15G8's unique ability to effect T cell, NK cell and macrophage/dendritic cell function: it is blocking the main interaction site of ILT2 and not a secondary site.

[0259] To test whether 15G8 can block the interaction between ILT2 and B2M, a B2M blocking ELISA was performed. 96 well nickel plates were coated with recombinant human B2M-His tag (3 μ g/ml; Sino Biologics, 11976-H08H) overnight. Biotinylated ILT2-Fc (20 μ g/ml; Sino Biologics, 1614-H02H; Innova Biosciences, 370-0010) was added for 2 hours at 37°C, which allowed ILT2 binding to B2M, in the presence or absence of titrated BND-22 or an irrelevant antibody (anti human CD28, Biolegend, 302934) (Ab range of 80- 0.1 μ g/ml, X3 fold). ILT2 binding to B2M was detected by HRP conjugated streptavidin (R&D Systems, DY998). The absorbance was measured using an ELISA Reader (Biotek Synergy-H1 plate reader).

[0260] As shown in **Fig. 20**, 15G8 blocked the ILT2-B2M interaction, specifically at higher antibody concentrations. By contrast, a non-relevant antibody, which had no effect in this system, did not block this interaction. By directly demonstrating that 15G8 can disrupt binding between ILT2 and B2M, these results substantiate previous observations that the 15G8 epitope includes residues known to be involved specifically in the interaction of ILT2 with B2M.

[0261] The only ILT2 antibody identified to have any effect on phagocytosis is GHI/75, which was shown to enhance anti-CD47 blockade mediated cancer cell phagocytosis but was not shown to have an effect on its own (see Barkal et al., *Nat Immunol.* (2018) 19(1):76-84). The combined GHI/75 and anti-CD47 effect was found not to be B2M dependent as deletion of B2M had no effect on the increased phagocytosis. Thus, it may be that the effect of 15G8 alone on phagocytosis (**Figs. 13A-13C**) is B2M dependent, which would explain the unique capabilities of this antibody. The superiority of the 15G8 antibody in this regard was directly tested. A375 or SKMEL28 cancer cells expressing exogenous HLA-G were cocultured with macrophages in the presence of IgG control, 15G8, or GHI/75. HP-F1 antibody was also tested in A375 cells. The cancer cell lines stained with PKH67-FITC were incubated with the macrophages which were stained with eFluor 670-APC in the presence of the indicated antibodies. Phagocytosis levels were determined by the percent of macrophages which were double stained, indicating the engulfment of the target cells. The % increase in phagocytosis compared to IgG control was calculated. 15G8 increased phagocytosis as compared to control in both cell types (**Figs. 21A-21B**). As expected,

neither GHI/75 nor HP-F1 had any effect on phagocytosis. This confirms that 15G8 is the first anti-ILT2 antibody that can enhance phagocytosis as a monotherapy.

[0262] This raises the question of the epitope of GHI/75 and other commercial antibodies. Though the epitopes of these antibodies are not published, a competition ELISA assay was performed to see if 15G8 and commercially available antibodies GHI/75, HP-F1 and MAB20172 (R&D Systems, clone 292319) could bind ILT2 at the same time. Biotinylated 15G8 antibody was used at a constant concentration (1 µg/mL) in an ILT2 binding ELISA. GHI/75, HP-F1 or MAB20172 were added in increasing concentrations and competition was assessed. Regardless of the amount of these three antibodies added, none of them competed with 15G8 for binding to ILT2 (**Figs. 21C-21D**). In contrast, when naked (unbiotinylated) 15G8 was added, the binding decreased in a dose dependent manner as expected. This indicates that GHI/75, HP-F1 and MAB20172 bind to different epitopes than 15G8. This makes 15G8 the first anti-ILT2 antibody ever identified to bind this epitope, to specifically block interaction with B2M and to be able to simultaneously activate/recruit T cells, NK cells and macrophages/dendritic cells against cancer.

Example 14: Screening methods for identifying antibodies capable of inhibiting the interaction between ILT2 and B2M

[0263] B2M is a protein that forms part of HLA-G as well as other ILT2 ligands. As shown in Example 13, 15G8 has the unique property of binding to ILT2 in a specific region responsible for the interaction between ILT2 and B2M. This binding enables 15G8 to inhibit or block binding of ILT2 to its ligands (e.g., HLA-G), resulting in an immune activation effect. As described in Example 13 above, the specific binding region was characterized as residing within the interface of the ILT2 D1 and D2 domains, and was further determined using an empirical method to include residues within SEQ ID NOs: 68, 69, 70, 71 and 72. The 15G8 binding region was also corroborated using *in silico* methods (**Fig. 18A**).

[0264] It is contemplated that other antibodies characterized by binding to a similar epitope and with the ability to specifically block the interaction between ILT2 and B2M can be produced. Such antibodies can be elicited by methods including immunization of animals with full recombinant ILT2, or with just the D1 and D2 domains including the D1-D2 interface region, or with linear peptides similar to (e.g., comprising, within, or identical to) SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71 and/or SEQ ID NO: 72. In all cases, elicited antibodies are further screened for their ability to block ILT2-B2M binding. The elicited antibodies may be screened for specific binding to the B2M binding region in ILT2, e.g., using empirical techniques such as hydroxyl radical foot-printing (HRF) and mass spectrometry techniques, as described

above (“Materials and Methods”), or other similar known techniques for epitope binding determination such as Hydrogen-Deuterium Exchange (HDX) further coupled to mass spectrometry.

[0265] Similarly, naive antibody libraries can be screened for antibodies that bind to either recombinant ILT2 or to ILT2 expressed on cells. Screening can be carried out, for example, for binding to an ILT2 fragment composed of the D1 and D2 domains including the D1-D2 interface region. Screening can also be carried out for binding to linear peptides similar to (e.g., comprising, within, or identical to) SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71 and/or SEQ ID NO: 72, or to a polypeptide comprised of these two peptides and the amino acids that reside between them in the ILT2 sequence. Antibodies exhibiting binding to these sequences can be further screened, as described above, using different empirical epitope mapping techniques.

[0266] In some cases, screening may involve the evaluation of candidate antibodies for the ability to specifically block the ILT2-B2M interaction using assays to detect blocking of ILT2 binding to different proteins, either recombinant or cell-expressed, that include B2M or a B2M moiety. In some cases, screening may involve the evaluation of candidate antibodies for the ability to specifically compete with the 15G8 antibody, which has been shown to block the binding of ILT2 to B2M.

[0267] The foregoing description of the specific embodiments will so fully reveal the general nature of the present disclosure that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. The means, materials, and steps for carrying out various disclosed functions may take a variety of alternative forms without departing from the present disclosure.

[0268] Throughout this specification and embodiments, the words “have” and “comprise,” or variations such as “has,” “having,” “comprises,” or “comprising,” will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. “About” when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 20\%$, or in some instances $\pm 10\%$, or in some instances $\pm 5\%$, or in some instances $\pm 1\%$, or in some instances $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods. Further, unless

otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

Table 7: Antibody Sequences (SEQ ID NOs)

Ab	H-CDR1	H-CDR2	H-CDR3	L-CDR1	L-CDR2	L-CDR3	VH	VL	VH nt	VL nt	HC	LC
17F2 (mu)	1	2	3	4	5	6	19	20	35	36	53	54
19E3 (mu)	7	8	9	10	11	12	21	22	32	39	53	54
15G8 (X108) (hu)	13	14	15	16	17	18	23	24	N/A	38	N/A	49
15G8-13 (A108) (hu)			25				28		34		48	
15G8-13 (S108) (hu)			26				29		116		51	
15G8-13 (C108) (mu)			27				30	45	33	37	52	49
15G8-23 (hu)			25				56	24	112	38	64	49
15G8-32 (hu)	13	14	25	16	17	18	57	61	113	115	65	66
15G8-53 (hu)			25				59	24	114	38	67	49

*Sequences are amino acid sequences unless marked otherwise (nt: nucleotide)

CLAIMS

1. A monoclonal antibody or an antigen-binding fragment thereof that binds to a sequence of human ILT2 selected from SEQ ID NOs: 41-44 and 68-70.
2. The antibody or antigen-binding fragment of claim 1, wherein the sequence is selected from SEQ ID NOs: 68-70.
3. A monoclonal antibody or an antigen-binding fragment thereof that binds to a sequence of human ILT2 selected from SEQ ID NOs: 71 and 72.
4. The antibody or antigen-binding fragment of claim 3, wherein the antibody or antigen-binding fragment binds to SEQ ID NOs: 71 and 72.
5. The antibody or antigen-binding fragment of any one of claims 1 to 4, wherein said antibody or antigen-binding fragment binds ILT2 and inhibits direct interaction between said ILT2 and beta-2-microglobulin (B2M).
6. The antibody or antigen-binding fragment of claim 5, wherein said antibody or antigen-binding fragment inhibits interaction of said ILT2 and an HLA protein or MHC-I protein via said inhibition of ILT2 direct interaction with B2M.
7. The antibody or antigen-binding fragment of claim 6, wherein said HLA is HLA-G.
8. The antibody of any one of claims 1 to 7, wherein said antibody is an IgG4 antibody and comprises a heavy chain constant region of a human IgG4 antibody comprising the S228P and L235E mutations (Eu numbering).
9. A monoclonal anti-ILT2 IgG4 antibody comprising three heavy chain CDRs (CDR-H1-3) and three light chain CDRs (CDR-L1-3), wherein the CDR-H1-3 and CDR-L1-3 comprise
 - a. SEQ ID NOs: 13-18, respectively,
 - b. SEQ ID NOs: 1-6, respectively, or
 - c. SEQ ID NOs: 7-12, respectively, and

wherein the antibody comprises a heavy chain constant region of a human IgG4 antibody and comprises one or both of the S228P and L235E mutations in the heavy chain constant region (Eu numbering).

10. The IgG4 antibody of claim 9, comprising a heavy chain variable domain comprising an amino acid sequence selected from SEQ ID NOs: 19, 21, and 23, or an amino acid sequence at least 95% identical thereto.
11. The IgG4 antibody of claim 9 or 10, comprising a light chain variable domain comprising an amino acid sequence selected from SEQ ID NOs: 20, 22, 24, and 45, or an amino acid sequence at least 95% identical thereto.
12. The IgG4 antibody of any one of claims 9 to 11, wherein
the CDR-H1-3 and CDR-L1-3 comprise SEQ ID NOs: 13-18, respectively,
the X in SEQ ID NO: 15 is A, and
said heavy chain comprises a variable domain sequence selected from SEQ ID NOs: 28 and 56-59, or an amino acid sequence at least 95% identical thereto.
13. The IgG4 antibody of claim 12, wherein said light chain comprises a variable domain sequence selected from SEQ ID NOs: 24 and 60-62, or an amino acid sequence at least 95% identical thereto.
14. The IgG4 antibody of any one of claims 9 to 13, wherein said heavy chain constant region comprises SEQ ID NO: 55, or an amino acid sequence at least 95% identical thereto.
15. The IgG4 antibody of claim 14, wherein said heavy chain comprises SEQ ID NO: 48 or an amino acid sequence at least 95% identical thereto and said light chain comprises SEQ ID NO: 49 or an amino acid sequence at least 95% identical thereto.
16. The IgG4 antibody of claim 14, wherein said heavy chain comprises SEQ ID NO: 51 or an amino acid sequence at least 95% identical thereto and said light chain comprises SEQ ID NO: 49 or an amino acid sequence at least 95% identical thereto.

17. The IgG4 antibody of claim 14, wherein said heavy chain comprises SEQ ID NO: 52 or an amino acid sequence at least 95% identical thereto and said light chain comprises the amino acid sequence of SEQ ID NO: 49 or an amino acid sequence at least 95% identical thereto.
18. The IgG4 antibody of claim 14, wherein said heavy chain comprises SEQ ID NO: 64 or an amino acid sequence at least 95% identical thereto and said light chain comprises SEQ ID NO: 49 or an amino acid sequence at least 95% identical thereto.
19. The IgG4 antibody of claim 14, wherein said heavy chain comprises the amino acid sequence SEQ ID NO: 65 or an amino acid sequence at least 95% identical thereto and said light chain comprises SEQ ID NO: 66 or an amino acid sequence at least 95% identical thereto.
20. The IgG4 antibody of claim 14, wherein said heavy chain comprises SEQ ID NO: 67 or an amino acid sequence at least 95% identical thereto and said light chain comprises SEQ ID NO: 49 or an amino acid sequence at least 95% identical thereto.
21. The IgG4 antibody of claim 14, wherein said heavy chain comprises SEQ ID NO: 53 or an amino acid sequence at least 95% identical thereto and said light chain comprises SEQ ID NO: 54 or an amino acid sequence at least 95% identical thereto.
22. A monoclonal antibody comprising a heavy chain and a light chain, wherein the heavy and light chains comprise SEQ ID NOs: 48 and 49, respectively.
23. The antibody or antigen-binding fragment of any one of claims 1 to 22 for use in at least one of binding ILT2, inducing/enhancing an anti-tumor T cell response, increasing T cell proliferation, reducing cancer-induced suppressor myeloid activity, increasing natural killer cell cytotoxicity, increasing macrophage phagocytosis, increasing generation of M1 inflammatory macrophages, decreasing generation of M2 suppressor macrophages, increasing dendritic cell number in a tumor microenvironment, increasing dendritic cell activation, treating an HLA-G expressing cancer, and treating a MHC-I expressing cancer.
24. The antibody or antigen-binding fragment of any one of claims 1 to 23 for use in combination with an opsonizing agent for treating an HLA-G or MHC-I expressing cancer.

25. The antibody or antigen-binding fragment of any one of claims 1 to 24 for use in combination with an anti-PD-L1/PD-1 based immunotherapy for treating an HLA-G or MHC-I expressing cancer, optionally wherein the anti-PD-L1/PD-1 based immunotherapy is pembrolizumab immunotherapy.
26. A pharmaceutical composition comprising an antibody or antigen-binding fragment of any one of claims 1 to 25 and a pharmaceutically acceptable excipient.
27. A method of treating an HLA-G or MHC-I expressing cancer in a subject in need thereof, the method comprising administering to said subject a pharmaceutical composition of claim 26 or an antibody or antigen-binding fragment of any one of claims 1 to 25.
28. The method of claim 27, further comprising administering to said subject an anti-PD-L1/PD-1 based immunotherapy, optionally wherein the anti-PD-L1/PD-1 based immunotherapy is pembrolizumab immunotherapy.
29. A method of increasing efficacy of an anti-PD-L1/PD-1 based therapy against a cancer cell expressing HLA-G or MHC-I in a subject in need thereof, the method comprising administering to said subject a pharmaceutical composition of claim 26 or an antibody or antigen-binding fragment of any one of claims 1 to 25 to a subject receiving an anti-PD-L1/PD-1 based therapy.
30. The method of any one of claims 27 to 29, further comprising administering to said subject an opsonizing agent.
31. The method of claim 30, wherein said opsonizing agent is an EGFR inhibitor, optionally wherein said EGFR inhibitor is cetuximab.
32. Use of the antibody or antigen-binding fragment of any one of claims 1 to 22 for the manufacture of a medicament for the use of any one of claims 23 to 25 or for use in the method of any one of claims 27 to 31.
33. The antibody or antigen-binding fragment of any one of claims 1 to 22 for use in the method of any one of claims 27 to 31.

34. A method of identifying an antibody that competes for binding to ILT2 with a reference antibody whose heavy and light chains comprise SEQ ID NOs: 48 and 49, respectively, the method comprising:
- contacting a library of antibodies with a polypeptide sequence comprising an ILT2 sequence selected from SEQ ID NOs: 41-44 and 68-70, and
 - selecting from the library an antibody that binds the ILT2 sequence, thereby obtaining an antibody that competes for binding to ILT2 with said reference antibody.
35. A method for producing an ILT2-binding molecule, the method comprising:
- obtaining a host cell comprising one or more nucleotide sequences encoding a molecule that binds to a sequence of human ILT2 selected from SEQ ID NOs: 41-44 and 68-70,
 - culturing the host cell under conditions that allow expression of the molecule, thereby producing an ILT2-binding molecule.
36. The method of claim 35, wherein the molecule binds to a sequence selected from SEQ ID NOs: 68-70.
37. The method of any one of claims 34 to 36, wherein the molecule binds to one or both of SEQ ID NOs: 71 and 72.
38. The method of claim 37, wherein the molecule binds to SEQ ID NOs: 71 and 72.
39. An ILT2-binding molecule produced by a method of any one of claims 35 to 38.
40. An isolated nucleic acid molecule encoding an antibody or antigen-binding fragment of any one of claims 1 to 25.
41. The isolated nucleic acid molecule of claim 40, wherein said nucleic acid molecule is an expression vector.
42. A host cell comprising the isolated nucleic acid molecule of claim 40 or 41.