

CLAIMS:

1. 1. A monoclonal antibody or antigen binding fragment comprising three heavy chain CDRs (CDR-H) and three light chain CDRs (CDR-L), wherein:

- a. ~~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), 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), wherein said X is selected from A, C and S;~~
 - b. ~~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 (QGSNEDPYT); or~~
 - c. ~~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 (KASDHINNWLA), 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).~~
- a) CDR-H1, CDR-H2, and CDR-H3 comprise SEQ ID NOs: 13, 14, and 15, respectively, wherein X in SEQ ID NO: 15 is selected from A, C and S, and

CDR-L1, CDR-L2, and CDR-L3 comprise SEQ ID NOs: 16, 17, and 18, respectively;

- b) CDR-H1, CDR-H2, and CDR-H3 comprise SEQ ID NOs: 1, 2, and 3, respectively, and CDR-L1, CDR-L2, and CDR-L3 comprise SEQ ID NOs: 4, 5, and 6, respectively; or
- c) CDR-H1, CDR-H2, and CDR-H3 comprise SEQ ID NOs: 7, 8, and 9, respectively, and CDR-L1, CDR-L2, and CDR-L3 comprise SEQ ID NOs: 10, 11, and 12, respectively.

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

- ~~2.~~ a) a heavy chain comprising ~~an amino acid sequence selected from:~~ SEQ ID NO: 19

~~(QVQLQQSDAELVKPGASVKISCKVSGYTFTDHTIHWMKQRPEQGLEWIGYI YPRDGSTKYNEKFKGKATLTADKSSSTAYMQLNSLTSEDSAVYFCARTWDY FDYWGQGTTTLTVSS), SEQ ID NO: 21~~

~~(QVQLQQSGAELARPGASVKLSCKASGYTFTSYGISWVKQRTGQGLEWVGEI YPGSGNSYYNEKFKGKATLTADKSSSTAYMELRSLTSEDSAVYFCARSNDGY PDYWGQGTTTLTVSS) and SEQ ID NO: , or 23~~

~~(DVQLQGSGLVVKPSETLSLTCSVTGYTSITSGYYWNWIRQFPQKLEWMGYI SYDGSNNYNPSLKNRITISRDTSKNQFSLKLSVTAADTATYYCAHGYSYYY AMDXWGQGTSTVTVSS), wherein said X in SEQ ID NO: 23 is selected from A, C and S;~~

- b) a light chain comprising SEQ ID NO: 20, 22, 24, or 45; or
- c) both a) and b).

~~3.~~ 3. The antibody or antigen binding fragment of claim 1 ~~or 2, comprising a light chain comprising an amino acid sequence selected from: SEQ ID NO: 20~~

~~(DIVLTQSPASLAVSLGQRATISCRASESVDSYGNSFMHWYQQKPGQPPKLLIYRASN LESGIPARFSGSGSRDFTLTINPVEADDVATYYCQQSNEDPYTFGGGTKLEIK), SEQ ID NO: 22~~

~~(DIQMTQSSSYLSVSLGGRVTITCKASDHINNWLAWYQQKPGNAPRLISGATSLET GVPSRFSGSGSGKDYTLSTSLQTEDVATYYCQQYWSTPWTFGGGTKLEIK), SEQ ID NO: 24~~

~~(DIQMTQSPSSLSASVGDRVTITCRTSQDISNYLNWYQQKPGKAVKLLISYTSRLHSGV
PSRFGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPTFGQGTKLEIK)~~ and SEQ ID NO:
45

~~(DIQMTQTSSLSASLGDRVTISCRISQDISNYLNWYQQKPDGTVKLLISYTSRLHSGV
PSRFGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPTFGSGTKLEIK).~~

4. ~~The antibody or antigen binding fragment of any one of claims 1 to 3~~, wherein said antibody or antigen binding fragment is humanized and said X is ~~selected from~~ A ~~and/or~~ S.

5. ~~4.~~ 4. The antibody or antigen binding fragment of ~~any one of~~ claim ~~43~~, wherein said X is A, SEQ ID NO: 15 is ~~GYSYYYAMDA~~ (identical to SEQ ID NO: 25), and SEQ ID NO: 23 is

~~DVQLQGSGPGLVKPSETLSLTCSVTGYSITSGYYWNWIRQFPGKKLEWMGYI
SYDGSNNYNPSLKNRITISRDTSKNQFSLKLNSVTAADTATYYCAHGYSYYY
AMDAWGQGTSTVTVSS (SEQ ID NO: identical to SEQ ID NO: 28).~~

5. A monoclonal antibody or antigen-binding fragment comprising a heavy chain that comprises SEQ ID NO: 28 and a light chain that comprises SEQ ID NO: 24.

6. A monoclonal antibody comprising a heavy chain that comprises SEQ ID NO: 28 and a light chain that comprises SEQ ID NO: 24, wherein said antibody is of a human IgG4 isotype.

7. A monoclonal antibody or antigen binding fragment that:

6. a) binds a human leukocyte immunoglobulin-like receptor subfamily B member 1 (ILT2) epitope within a sequence of human ILT2 selected from ~~VKKGQFPISITWEH~~ (SEQ ID NO: 41), ~~LELVVTGAYIKPTLS~~ (SEQ ID NO: 42), ~~VILQCDSQVAFDGF~~ (SEQ ID NO: 43), and ~~WYRCYAYDSNSPYEW~~ (SEQ ID NO: 44), optionally wherein said epitope is a three-dimensional epitope comprising residues within SEQ ID NOs: 41, 42, 43 and 44;

7. ~~The antibody or antigen binding fragment of claim 6, wherein said epitope is a 3-dimensional epitope comprising SEQ ID NO: 41, 42, 43 and 44.~~

~~8. A monoclonal antibody or antigen binding fragment that b)~~ binds ILT2 and inhibits direct interaction between said ILT2 and beta-2-microglobulin (B2M);

~~9. The antibody or antigen binding fragment of claim 8, optionally~~ 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; ~~optionally wherein said HLA is HLA-G; or~~

~~10. The antibody or antigen binding fragment of claim 9, wherein said HLA is HLA-G.~~

~~A c) both a) and b).~~

~~11. 8. The~~ monoclonal antibody or antigen binding fragment ~~that of any one claims 1 to 7, wherein said antibody or antigen-binding fragment~~ binds ILT2 and induces in a subject suffering from cancer at least three of:

~~a. a)~~ increased natural killer (NK) cell cytotoxicity;

~~b. b)~~ increased T cell cytotoxicity, proliferation, or both;

~~c. c)~~ increased macrophage phagocytosis, increased generation of M1 inflammatory macrophages, decreased generation of M2 suppressor macrophages or a combination thereof; and

~~d. d)~~ increased dendritic cell homing to a tumor of said cancer, increased dendritic cell activation or a combination thereof;

~~12. The antibody or antigen binding fragment of claim 11, optionally~~ wherein said cancer is ~~an~~ HLA-G or MHC-I expressing cancer.

The

~~9. An~~ antibody or antigen binding fragment of any one of claims 1 to ~~12~~8, 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 or~~

~~13.~~ treating ~~an~~ MHC-I expressing cancer.

~~Thein a patient in need thereof.~~

~~14.—10.~~ An antibody or antigen binding fragment of any one of claims 1 to ~~13~~8, for use in combination with an opsonizing agent for treating an HLA-G or MHC-I expressing cancer.

~~15.—The antibody or antigen binding fragment of any one of claims 1 to 14, for use in combination with an anti PD-L1/PD-1 based therapy for treating an HLA-G or MHC-I expressing cancer.~~

~~A method of treating an HLA-G or MHC-I expressing cancer in a subject in need thereof, the method comprising administering to,~~

~~16.—11.~~ The antibody or antigen binding fragment for use of claim 10, wherein said subject a pharmaceutical composition comprising an antibody or antigen binding fragment of any one of claims 1 to 15.

~~17.~~ The method of claim 16, treatment further comprisingcomprises administering to said subject an opsonizing agent.

~~18.~~ 12. The ~~method~~antibody or antigen binding fragment for use of claim ~~14 or 17~~11, wherein said opsonizing agent is an EGFR inhibitor, optionally wherein said EGFR inhibitor is cetuximab.

~~19.~~ 13. The ~~method~~antibody or antigen binding fragment for use of claim ~~17~~11 or ~~18, 12~~, wherein said treatment further comprisingcomprises administering to said subject an anti-PD-L1/PD-1 based immunotherapy.

~~A method of treating an HLA-G or MHC-I expressing cancer in~~

14. The antibody or antigen binding fragment for use of claim 10, wherein said treatment comprises:

~~20.—a subject in need thereof, the method comprising:~~

~~a.~~) confirming ~~that~~ expression of ILT2 or soluble HLA-G in said subject is above a predetermined threshold; and

- b. ~~b)~~ administering to said subject ~~an agent that inhibits ILT2 based immune suppression; thereby treating a cancer in a subject~~ said antibody or antigen binding fragment.

~~The method~~

- ~~21.~~ 15. The antibody or antigen binding fragment for use of claim ~~20~~14, wherein said confirming comprises measuring expression of ~~said~~ ILT2 or soluble HLA-G in said subject before said administering.

~~The method of claim 20 or 21, comprising~~

- ~~22.~~ 16. The antibody or antigen binding fragment for use of claim 14 or 15, wherein said treatment comprises confirming expression of ILT2 and wherein said expression of ILT2 is in an immune cell of said subject.

- ~~23.~~ 17. The ~~method~~ antibody or antigen binding fragment for use of claim ~~22~~16, wherein said immune cell is ~~selected from~~ a peripheral blood immune cell ~~and/or~~ an intratumor immune cell.

- ~~24.~~ 18. The ~~method~~ antibody or antigen binding fragment for use of claim ~~22~~16 or ~~23~~17, wherein said immune cell is selected from a CD8 positive T cell, a macrophage, an NK cell and a TEMRA cell~~-, optionally wherein said immune cell is a peripheral blood CD8 positive T cell.~~

- ~~25.~~ ~~19.~~ The ~~method of any one of claims 22 to 24~~ antibody or antigen binding fragment for use of claim 14 or 15, wherein said ~~immune cell is a peripheral blood CD8 positive T cell.~~

- ~~26.~~ The ~~method of any one of claims 20 to 25, comprising~~ treatment comprises confirming expression of soluble HLA-G.

~~The method~~

- ~~27.~~ ~~20.~~ An antibody or antigen binding fragment of any one of claims ~~20~~1 to ~~26~~, ~~further comprising administering to said subject an anti PD-L1/PD-1 based therapy.~~

- ~~28.~~ A method of treating an HLA-G or MHC-I expressing cancer⁸, for use in a subject in need thereof, the method comprising:

- a. ~~administering to said subject an agent that inhibits ILT2 based immune suppression; and~~

~~b. administering to said subject an anti PD-L1/PD-1 based therapy;
thereby treating a cancer in a subject.~~

~~29. A method of increasing the efficacy of an anti-PD-L1/PD-1 based therapy against a cancer cell expressing HLA-G, MHC-I₂ or both, ~~the method comprising contacting said cancer cell with an ILT2 antagonist.~~~~

~~30. The method of any one of claims 20 to 28, wherein said agent that inhibits ILT2-based immune suppression is an ILT2 antagonist.~~

~~31. The method of claim 29 or 30, wherein said ILT2 antagonist is an antibody or antigen binding fragment that specifically binds to ILT2 and inhibits ILT2-mediated immune cell suppression.~~

~~The method of claim 31, wherein said antibody or antigen binding fragment is the~~

~~32. 21. The antibody or antigen binding fragment ~~of any one of claims 1 to 15.~~~~

~~33. The method of any one of claims 27 to 32 for use of claim 13, wherein said anti-PD-L1/PD-1 based immunotherapy is an anti-PD-1 blocking antibody.~~

~~34. 22. The ~~method~~ antibody or antigen binding fragment for use of any one of claims 209 to 3321, wherein said cancer is refractory to anti-PD-L1/PD-1 based therapy.~~

~~35. The method of any one of claims 20 to 34, further comprising administering to said subject an opsonizing agent.~~

~~36. The method of claim 35, wherein said opsonizing agent is an EGFR inhibitor, optionally wherein said EGFR inhibitor is cetuximab.~~

~~37. A pharmaceutical composition comprising an agent that binds to ILT2 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.~~

~~38. 23. A pharmaceutical composition comprising an antibody or antigen binding fragment of any one of claims 1 to 458.~~

~~39. 24. A method for producing an agent, the method comprising:~~

a) obtaining an agent that binds to an ILT2 extracellular domain or fragment thereof, testing an ability of said agent to induce at least two 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 a cancer cell and selecting at least one agent that induces at least two of said increased phagocytosis, said increased activity, said increased generation, said reduced generation, said recruitment, said increased activation, said decreased activity and said increased cytotoxicity; or

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 induce at least two 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 a cancer cell; and
 - iii) selecting at least one agent that increases at least two of said increased phagocytosis, said increased activity, said increased generation, said reduced generation, said recruitment, said increased activation, said decreased activity and said increased cytotoxicity;
- thereby producing an agent.

~~40. The method of claim 39, comprising 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.~~

~~41.~~ 25. 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 increase efficacy of an anti-PD-L1/PD-1 based therapy against a cancer cell and selecting at least one agent that increases the efficacy of an anti-PD-L1/PD-1 based therapy; or 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.~~ i) obtaining an agent that binds to an ILT2 extracellular domain or fragment thereof;

~~ii.~~ ii) testing an ability of said agent to increase efficacy of an anti-PD-L1/PD-1 based therapy against a cancer cell; and

~~iii.~~ iii) selecting at least one agent that increases efficacy of an anti-PD-L1/PD-1 based therapy against a cancer cell;

thereby producing an agent.

~~42. The method of any one of claims 39 to 41, wherein said increasing efficacy comprises a synergistic increase in pro-inflammatory cytokine secretion or wherein said increased cytotoxicity comprises an increase in pro-inflammatory cytokine secretion.~~

~~43. The method of claim 42, wherein said pro-inflammatory cytokine is selected from GM-CSF, TNF α and IFN γ .~~

~~44. The method of any one of claims 41 to 43, wherein said increasing efficacy comprises a synergistic increase in T cell activation, cytotoxicity or both.~~

~~45. The method of any one of claims 39, 40 and 44, wherein said increase in T cell activation, cytotoxicity or both comprises increased membranial CD107a expression.~~

~~46. The method of any one of claims 41 to 45, wherein said increasing efficacy comprises converting a cancer refractory to anti-PD-L1/PD-1 based therapy to a cancer that responds to anti-PD-L1/PD-1 based therapy.~~

~~47. The method of any one of claims 39 to 46, wherein said cancer cell is an HLA-G or MHC-I expressing cancer.~~

~~48.~~ 26. 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; or 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.~~ i) obtaining an agent that binds to an ILT2 extracellular domain or fragment thereof;
 - ~~ii.~~ ii) testing an ability of said agent to inhibit interaction between ILT2 and B2M; and
 - ~~iii.~~ iii) selecting at least one agent that inhibits interaction between ILT2 and B2M;
- thereby producing an agent.

~~49.~~ 27. A method for producing an agent, the method comprising:

obtaining an agent that binds to an ILT2 epitope within a sequence of human ILT2 selected from SEQ ID NO: 41, 42, 43 and 44, or

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 an ILT2 epitope within a sequence of human ILT2 selected from SEQ ID NO: 41, 42, 43 and 44; thereby producing an agent.

~~50.~~ 28. A nucleic acid molecule encoding an antibody or antigen binding fragment of any one of claims 1 to ~~45~~28.

29. The nucleic acid molecule of claim ~~50~~28, wherein said nucleic acid molecule is an expression vector.

~~51.~~