

Claims:

1. An isolated monoclonal antibody which binds to human ILT4, or antigen-binding fragment thereof, comprising heavy and light chain variable region CDR1, CDR2 and CDR3 amino acid sequences selected from the group consisting of:
- (i) a heavy chain variable region CDR1 amino acid sequence selected from the consensus sequence: G Y T (I,M) H (SEQ ID NO: 21), or conservative sequence modifications thereof;
- (ii) a heavy chain variable region CDR2 amino acid sequence as set forth in SEQ ID NO:3, or conservative sequence modifications thereof;
- (iii) a heavy chain variable region CDR3 amino acid sequence selected from the consensus sequence: E R P G G S Q F I Y Y Y (P,A) (M,L) D Y (SEQ ID NO:22) , or conservative sequence modifications thereof;
- (iv) a light chain variable region CDR1 amino acid sequence selected from the consensus sequence: R A S (A,E) N I Y S Y L A (SEQ ID NO: 23), or conservative sequence modifications thereof;
- (v) a light chain variable region CDR2 amino acid sequence selected from the consensus sequence: N A (I,D) T L A E (SEQ ID NO: 24), or conservative sequence modifications thereof,;
- (vi) a light chain variable region CDR3 amino acid sequence as set forth in SEQ ID NO:8, or conservative sequence modifications thereof.
2. An isolated monoclonal antibody which binds to human ILT4, or antigen-binding fragment thereof, comprising:
- (a) heavy chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs:1, 3, and 5, respectively, or conservative sequence modifications thereof, and light chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs:6, 7, and 8, respectively, or conservative sequence modifications thereof; ~~or~~
- (b) heavy chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs:11, 13, and 15, respectively, or conservative sequence modifications thereof, and light chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set

forth in SEQ ID NOs:16, 17, and 18, respectively, or conservative sequence modifications thereof;

(c) a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:9, or a sequence at least 80% identical thereto, and a light chain variable region amino acid sequence as set forth in SEQ ID NO:10, or a sequence at least 80% identical thereto;

(d) a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:19, or a sequence at least 80% identical thereto, and a light chain variable region amino acid sequence as set forth in SEQ ID NO:20, or a sequence at least 80% identical thereto;

(e) a heavy chain amino acid sequence as set forth in SEQ ID NO:25, or a sequence at least 80% identical thereto, and a light chain amino acid sequence as set forth in SEQ ID NO:26, or a sequence at least 80% identical thereto;

(f) a heavy chain amino acid sequence as set forth in SEQ ID NO:27, or a sequence at least 80% identical thereto, and a light chain amino acid sequence as set forth in SEQ ID NO:28, or a sequence at least 80% identical thereto;

(g) a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO:9, 19, 97, 98, 99, 103, 104, 105, or a sequence at least 80% identical thereto; or

(h) a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO:10, 20, 100, 101, 102, 106, 107, 108, or a sequence at least 80% identical thereto.

3. The antibody, or antigen-binding fragment thereof, of claim 1 or 2, wherein the antibody, or antigen-binding fragment thereof, exhibits one or more of the following properties:

- a. blocking ILT4 ligand (*e.g.*, HLA-G ligand) binding to human ILT4;
- b. enhancing or increasing cytokine or chemokine release by human macrophages;
- c. potentiating the activation effects of LPS and IFN γ on macrophages;
- d. promoting M1 macrophage polarization;
- e. binding to human ILT4 with an equilibrium dissociation constant K_d of 10^{-9} M or less, or alternatively, an equilibrium association constant K_a of 10^{+9} M $^{-1}$ or greater;
- f. lack of cross-reactivity with other ILT family members;
- g. cross-reactivity with cynomolgus ILT4; and / or
- h. inhibiting tumor cells that express ILT4.

4. The antigen-binding fragment thereof of any one of claims 1 to 3, wherein the fragment is an Fab, Fab', F(ab')₂, Fv, or a single chain Fv.

5 5. A bispecific construct comprising the ILT4 antibody, or antigen binding fragment thereof, of any one of claims 1 to 4 linked to a second binding agent.

6. The bispecific construct of claim 5, wherein the second binding agent binds to an immune checkpoint molecule, an immune costimulatory molecule, or a tumor antigen.

10 7. The bispecific construct of claim 6, wherein:

(a) the immune checkpoint molecule is PD-1, PD-L1 CTLA-4, LAG-3, TIGIT, TIM-3, VISTA, AXL, ILT2, or ILT3;

(b) the immune costimulatory molecule is CD27, CD40, 4-1BB, OX40, or GITR; or

(c) the tumor antigen is HER2, EGFR, ErbB3, or CD24.

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8. The bispecific construct of claim 6, wherein the binding agent binds to PD-L1 and comprises heavy and light chain CDR1, CDR2, and CDR3 amino acid sequences selected from the group consisting of:

(a) heavy chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs: 59, 60, and 61, respectively, and light chain variable region CDR1, CDR2
20 and CDR3 amino acid sequences as set forth in SEQ ID NOs:62, 63, and 64, respectively;

(b) heavy chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs: 35, 36, and 37, respectively, and light chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs:38, 39, and 40, respectively;

(c) heavy chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs: 41, 42, and 43, respectively, and light chain variable region CDR1, CDR2
25 and CDR3 amino acid sequences as set forth in SEQ ID NOs:44, 45, and 46, respectively;

(d) heavy chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs: 47, 48, and 49, respectively, and light chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs:50, 51, and 52, respectively;

(e) heavy chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs: 53, 54, and 55, respectively, and light chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs:56, 57, and 58, respectively; and

5 (f) heavy chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs: 29, 30, and 31, respectively, and light chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs:32, 33, and 34, respectively.

9. The bispecific construct of claim 8, wherein the PD-L1 binding agent comprises heavy and light chain variable region sequences selected from the group consisting of:

10 (a) a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:87 and a light chain variable region amino acid sequence as set forth in SEQ ID NO:88;

(b) a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:79 and a light chain variable region amino acid sequence as set forth in SEQ ID NO:80;

15 (c) a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:81 and a light chain variable region amino acid sequence as set forth in SEQ ID NO:82;

(d) a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:83 and a light chain variable region amino acid sequence as set forth in SEQ ID NO:84;

(e) a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:85 and a light chain variable region amino acid sequence as set forth in SEQ ID NO:86; and

20 (f) a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:77 and a light chain variable region amino acid sequence as set forth in SEQ ID NO:78.

10. The bispecific construct of any one of claims 5 to 9, wherein;

(a) the ILT4 antibody, or antigen binding fragment thereof, is an scFv; or

25 (b) the second antibody, or antigen binding fragment thereof, is an scFv.

11. The bispecific construct of any one of claims 5 to 10, wherein the ILT4 antibody, or antigen binding fragment thereof, and the second binding agent comprise a human IgG1 constant domain.

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12. The bispecific construct of any one of claims 5 to 11, wherein (a) the ILT4 antibody, or antigen binding fragment thereof, is linked to the C-terminus of the heavy chain of the second binding agent or (b) the second binding agent is linked to the C-terminus of the heavy chain of the ILT4 antibody, or antigen binding fragment thereof.

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13. The bispecific construct of any one of claims 5 to 12, wherein the ILT4 antibody, or antigen binding fragment thereof, and the second binding agent are:

- (a) genetically fused; or
- (b) chemically conjugated.

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14. A bispecific construct comprising an antibody which binds to human PD-L1, or antigen-binding fragment thereof, linked to an ILT4 scFv, wherein:

(a) the PD-L1 antibody, or antigen binding fragment thereof, comprises heavy chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs: 59, 60, and 61, respectively, and light chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs:62, 63, and 64, respectively; and

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(b) the ILT4 scFv comprises:

(i) heavy chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs:1, 3, and 5, respectively, and light chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs:6, 7, and 8, respectively; or

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(ii) heavy chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs:11, 13, and 15, respectively, and light chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs:16, 17, and 18, respectively.

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15. The bispecific construct of claim 14, wherein

(a) the PD-L1 antibody, or antigen binding fragment thereof, comprises a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:87 and a light chain variable region amino acid sequence as set forth in SEQ ID NO:88; and

(b) the ILT4 scFv comprises:

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(i) a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:9 and a light chain variable region amino acid sequence as set forth in SEQ ID NO:10; or

(ii) a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:19 and a light chain variable region amino acid sequence as set forth in SEQ ID NO:20.

16. The bispecific construct of claim 14 or 15, wherein the ILT4 scFv further
5 comprises disulfide stabilization modifications with Cys substitutions at VH44 and VL100.

17. The bispecific construct of any one of claims 14 to 16, wherein the PD-L1 antibody, or binding fragment thereof, comprises a human IgG1 constant domain.

10 18. A bispecific construct comprising an antibody which binds to human ILT4, or antigen-binding fragment thereof, linked to a PD-L1 scFv, wherein:

(a) the ILT4 antibody, or antigen binding fragment thereof, comprises:

(i) heavy chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs:1, 3, and 5, respectively, and light chain variable region CDR1, CDR2
15 and CDR3 amino acid sequences as set forth in SEQ ID NOs:6, 7, and 8, respectively; or

(ii) heavy chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs:11, 13, and 15, respectively, and light chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs:16, 17, and 18, respectively.

(b) the PD-L1 scFv comprises heavy chain variable region CDR1, CDR2 and CDR3
20 amino acid sequences as set forth in SEQ ID NOs: 59, 60, and 61, respectively, and light chain variable region CDR1, CDR2 and CDR3 amino acid sequences as set forth in SEQ ID NOs:62, 63, and 64, respectively.

19. The bispecific construct of claim 18, wherein

25 (a) the ILT4 antibody, or antigen binding fragment thereof, comprises:

(i) a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:9 and a light chain variable region amino acid sequence as set forth in SEQ ID NO:10; or

(ii) a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:19 and a light chain variable region amino acid sequence as set forth in SEQ ID NO:20; and

(b) the PD-L1 scFv comprises a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:87 and a light chain variable region amino acid sequence as set forth in SEQ ID NO:88.

5 20. The bispecific construct of claim 18 or 19, wherein the ILT4 antibody, or binding fragment thereof, comprises a human IgG1 constant domain.

 21. A multispecific construct comprising the bispecific construct of any one of claims 5 to 20 and a third binding agent.

10 22. The bispecific or multispecific construct of any one of claims claim 5 to 21 which comprises a modified IgG1 domain with (i) a mutated human IgG1 Fc domain which comprises non-naturally occurring amino acids 234A, 235Q and 322Q as numbered by the EU index as set forth in Kabat.

15 23. The bispecific or multispecific construct of any one of claims claim 5 to 22 wherein the modified human IgG1 Fc domain further comprises non-naturally occurring amino acids 252Y, 254T and 256E as numbered by the EU index as set forth in Kabat.

20 24. A bispecific or multispecific antibody construct which comprises a modified human IgG1 Fc domain which comprises;

 (a) non-naturally occurring amino acids 234A, 235Q and 322Q as numbered by the EU index as set forth in Kabat; and /or

 (b) non-naturally occurring amino acids 252Y, 254T and 256E as numbered by the EU
25 index as set forth in Kabat.

 25. A composition comprising the antibody, or antigen binding fragment thereof, of any one of claims 1 to 4, or the bispecific or multispecific construct of any one of claims 5 to 24, and a pharmaceutically acceptable carrier.

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26. A kit comprising the antibody, or antigen binding fragment thereof, of any one of claims 1 to 4, the bispecific bispecific or multispecific construct of any one of claims 5 to 24, or the composition of claim 25, and instructions for use.

27. An isolated nucleic acid molecule comprising a nucleotide sequence encoding:
(a) the heavy and/or light chain variable regions of the antibody, or antigen binding fragment thereof, of any one of claims 1 to 4; or
(b) the bispecific or multispecific construct of any one of claims 5 to 24.

28. A vector comprising at least one nucleic acid molecule of claim 27.

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

30. An *in vitro* method of activating macrophages comprising contacting macrophages with the antibody, or antigen binding fragment thereof, of any one of claims 1 to 4, the bispecific or multispecific construct of any one of claims 5 to 24, or the composition of claim 25.

31. The antibody, or antigen binding fragment thereof, of any one of claims 1 to 4, the bispecific or multispecific construct of any one of claims 5 to 24, or the composition of claim 25, for use in a method of inducing or enhancing an immune response in the subject.

32. The antibody, or antigen binding fragment thereof, of any one of claims 1 to 4, the bispecific or multispecific construct of any one of claims 5 to 24, or the composition of claim 25, for use in a method of treating cancer in a subject.

33. The antibody, or antigen binding fragment thereof, the bispecific or multispecific construct, or the composition for the use of claim 32, wherein the cancer is selected from the group consisting of colorectal cancer, ovarian cancer, renal cell carcinoma, head and neck squamous cell carcinoma, breast cancer, lung cancer, bladder cancer, prostate cancer, melanoma, gynecological cancers, sarcoma, lymphoma, and glioblastoma.

34. The antibody, or antigen binding fragment thereof, of any one of claims 1 to 4, the bispecific or multispecific construct of any one of claims 5 to 24, or the composition of claim 25, for use in a method of treating a tumor in a subject.

5 35. Use of the antibody, or antigen binding fragment thereof, of any one of claims 1 to 4, the bispecific or multispecific construct of any one of claims 5 to 24, or the composition of claim 25, in the manufacture of a medicament for inducing or enhancing an immune response in a subject.

10 36. Use of the antibody, or antigen binding fragment thereof, of any one of claims 1 to 4, the bispecific or multispecific construct of any one of claims 5 to 24, or the composition of claim 25, in the manufacture of a medicament for treating cancer in a subject.

15 37. The antibody, or antigen binding fragment thereof, the bispecific or multispecific construct, or the composition for the use of claim 33, wherein the cancer is selected from the group consisting of colorectal cancer, ovarian cancer, renal cell carcinoma, head and neck squamous cell carcinoma, breast cancer, lung cancer, bladder cancer, prostate cancer, melanoma, gynecological cancers, sarcoma, lymphoma, and glioblastoma.

20 38. Use of the antibody, or antigen binding fragment thereof, of any one of claims 1 to 4, the bispecific or multispecific construct of any one of claims 5 to 24, or the composition of claim 25, in the manufacture of a medicament for treating a tumor in a subject.