

maturation/activation of myeloid DCs, and the induction of tolerogenic DCs by HLA-G was through disrupting the MHC class II presentation pathway (Ristich *et al.* Eur J Immunol. 2005).

An unmet medical need exists for patients that do not respond to T-cell therapy but may benefit from relief of tissue associated-macrophage/ MDSC-mediated tumor tolerance (e.g. myeloid “rich” tumors). ILT4 blockade would fill this need and would differentiate from current T-cell-targeted antibodies (e.g. anti-PD1, anti-TIGIT) by relieving suppression of tolerogenic myeloid cells in the tumor microenvironment.

Summary

The present disclosure provides antibodies or antigen-binding fragments thereof that bind to human ILT4. In certain examples, the antibody or antigen-binding fragment thereof binds to one or more amino acid residues in a human ILT4 epitope selected from the group consisting of LYREKKSASW (SEQ ID NO:59), TRIRPEL (SEQ ID NO:60), NGQF (SEQ ID NO:61), and HTGRYGCQ (SEQ ID NO:62). In certain examples, the antibody or antigen-binding fragment thereof protects the epitope from deuterium exchange with a deuterium source, such as D₂O. In one example, the antibody or antigen-binding fragment thereof binds to one or more amino acid residues in the epitope LYREKKSASW (SEQ ID NO:59). In another example, the antibody or antigen-binding fragment thereof binds to one or more amino acid residues in the epitope TRIRPEL (SEQ ID NO:60). In yet another example, the antibody or antigen-binding fragment thereof binds to one or more amino acid residues in the epitope NGQF (SEQ ID NO:61). In still another example, the antibody or antigen-binding fragment thereof binds to one or more amino acid residues in the epitope HTGRYGCQ (SEQ ID NO:62). In yet still another example, the antibody or antigen-binding fragment thereof binds to one or more amino acid residues in two, three, or four ILT4 epitopes selected from the group consisting of LYREKKSASW (SEQ ID NO:59), TRIRPEL (SEQ ID NO:60), NGQF (SEQ ID NO:61), and HTGRYGCQ (SEQ ID NO:62). In one example, the antibody or antigen-binding fragment thereof binds to one or more amino acid residues in the epitope LYREKKSASW (SEQ ID NO:59) and protects the epitope from deuterium exchange with a deuterium source such as D₂O. In another

binding fragment thereof binds to one or more amino acid residues in the epitope HTGRYGCQ (SEQ ID NO:62) and protects the epitope from deuterium exchange with a deuterium source such as D₂O. In yet still another example, the antibody or antigen-binding fragment thereof binds to one or more amino acid residues in two, three, or four ILT4 epitopes selected from the group consisting of LYREKKSASW (SEQ ID NO:59), TRIRPEL (SEQ ID NO:60), NGQF (SEQ ID NO:61), and HTGRYGCQ (SEQ ID NO:62) and protects the epitopes from deuterium exchange with a deuterium source such as D₂O.

The present disclosure also provides an antibody or antigen-binding fragment thereof that binds to the same epitope of human ILT4 as any antibody or antigen-binding fragment thereof disclosed herein. In certain examples, the antibody or antigen-binding fragment thereof binds to the same epitope of human ILT4 as an antibody or antigen-binding fragment thereof comprising the heavy chain and light chain amino acid sequences set forth in SEQ ID NOs:1 and 3; 2 and 4; 2 and 5; 2 and 6; 2 and 7; 2 and 3; 8 and 11; 9 and 11; 10 and 11; 12 and 13; 14 and 15; 79 and 3; 80 and 4; 80 and 5; 80 and 6; 80 and 7; 80 and 3; 82 and 11; 83 and 11; 84 and 11; 85 and 13; and 86 and 15; respectively. In some examples, the antibody or antigen-binding fragment thereof binds to the same epitope of human ILT4 as an antibody or antigen-binding fragment thereof comprising the heavy chain variable domain and light chain variable domain amino acid sequences set forth in SEQ ID NOs:63 and 70; 57 and 71; 57 and 72; 57 and 73; 57 and 58; 57 and 70; 64 and 74; 65 and 74; 66 and 74; 67 and 75; 68 and 76; respectively.

The present disclosure further provides an antibody or antigen-binding fragment thereof that competes for binding to human ILT4 with an antibody or antigen-binding fragment thereof disclosed herein. In certain examples, the antibody or antigen-binding fragment thereof competes for binding to human ILT4 with an antibody or fragment comprising the heavy chain and light chain amino acid sequences set forth in SEQ ID NOs:1 and 3; 2 and 4; 2 and 5; 2 and 6; 2 and 7; 2 and 3; 8 and 11; 9 and 11; 10 and 11; 12 and 13; 14 and 15; 79 and 3; 80 and 4; 80 and 5; 80 and 6; 80 and 7; 80 and 3; 82 and 11; 83 and 11; 84 and 11; 85 and 13; and 86 and 15; respectively. In some

determining region-L3 (CDR-L3) of a light chain variable (V_L) domain of an immunoglobulin chain that comprises the amino acid sequence set forth in SEQ ID NO: 3-7, 11, 13, 15, or 45; and/or (b) the complementarity determining region-H1 (CDR-H1), complementarity determining region-H2 (CDR-H2), and complementarity determining region-H3 (CDR-H3) of a heavy chain variable (V_H) domain of an immunoglobulin chain that comprises the amino acid sequence set forth in SEQ ID NO: 1, 2, 8-10, 12, 14, 44, or 79-86.

In an example of the disclosure, the antibody or antigen-binding fragment thereof comprises: (1) a V_H domain comprising: CDR-H1: GYYWS (SEQ ID NO: 16), CDR-H2: EINHGXGSTNYNPSLKS wherein X is S or A (SEQ ID NO: 17); and CDR-H3:

LPTRWVTTRYFDL (SEQ ID NO: 18); and/or, a V_L domain comprising: CDR-L1: TGSSSNIGAGYDVH (SEQ ID NO: 19), CDR-L2: GX_1X_2 NRPS; wherein X_1 is S or A and X_2 is N, Q, E or D (SEQ ID NO: 20); and CDR-L3: QSFDNSLSAYV (SEQ ID NO: 21); (2) a V_H domain comprising: CDR-H1: SYAIS (SEQ ID NO: 22); CDR-H2: GIPIFGTANYAQKFQG (SEQ ID NO: 23); and CDR-H3: YFX_1X_2 SGWYKGGAFDI; wherein X_1 is D or S and X_2 is S or A (SEQ ID NO: 24); and/or, a V_L domain comprising: CDR-L1: TLRSGINVDITYRIH (SEQ ID NO: 25); CDR-L2: YKSDSDKHQGS (SEQ ID NO: 26); and CDR-L3: AIWYSSTWV (SEQ ID NO: 27); (3) a V_H domain comprising: CDR-H1: SYAMH (SEQ ID NO: 28); CDR-H2: VISYDGSNKYYADSVKG (SEQ ID NO: 29); and CDR-H3: VGEWIQLWSPFDY (SEQ ID NO: 30); and/or, a V_L domain comprising: CDR-L1: RASQGISSWLA (SEQ ID NO: 31); CDR-L2: AASSLQS (SEQ ID NO: 32); and CDR-L3: QQYNSYPPT (SEQ ID NO: 33); and/or (4) a V_H domain comprising: CDR-H1: ELSMH (SEQ ID NO: 34); CDR-H2: GFDPEDGETIYAQKFQG (SEQ ID NO: 35); and CDR-H3: AGPLYTIFGVVIIPDNWFDP (SEQ ID NO: 36); and/or, a V_L domain comprising: CDR-L1: TGSSSNIGAGYDVH (SEQ ID NO: 37); CDR-L2: GNSNRPS (SEQ ID NO: 38); and CDR-L3: QSYDSSLSGSGVV (SEQ ID NO: 39).

In another aspect, there is provided an antibody or antigen-binding fragment thereof that binds human ILT4, comprising:

a heavy chain variable domain comprising:

CDR-H1: GYYWS (SEQ ID NO: 16),

CDR-H2: EINHGXGSTNYNPSLKS wherein X is S or A (SEQ ID NO: 17), and
CDR-H3: LPTRWVTTRYFDL (SEQ ID NO: 18); and,

a light chain variable domain comprising:

CDR-L1: TGSSSNIGAGYDVH (SEQ ID NO: 19),

CDR-L2: GX_1X_2 NRPS, wherein X_1 is N, Q, E or D and X_2 is S or A (SEQ ID NO: 20),

and
CDR-L3: QSFDNSLSAYV (SEQ ID NO: 21).

In one example, the antibody or antigen-binding fragment comprises: a V_H domain comprising: CDR-H1: GYYWS (SEQ ID NO: 16), CDR-H2: EINHSGSTNYNPSLKS (SEQ ID NO: 47), and CDR-H3: LPTRWVTTRYFDL (SEQ ID NO: 18); and/or, a V_L domain
5 comprising: CDR-L1: TGSSSNIGAGYDVH (SEQ ID NO: 19), CDR-L2: GNSNRPS (SEQ ID NO: 49), and CDR-L3: QSFDNSLSAYV (SEQ ID NO: 21).

In another example, the antibody or antigen-binding fragment comprises: a V_H domain comprising: CDR-H1: GYYWS (SEQ ID NO: 16), CDR-H2: EINHSGSTNYNPSLKS (SEQ ID NO: 47), and CDR-H3: LPTRWVTTRYFDL (SEQ ID NO: 18); and/or, a V_L domain
10 comprising: CDR-L1: TGSSSNIGAGYDVH (SEQ ID NO: 19), CDR-L2: GQSNRPS (SEQ ID NO: 50), and CDR-L3: QSFDNSLSAYV (SEQ ID NO: 21).

The present disclosure also provides a method of treating a cancer in a subject (e.g., a human subject), comprising administering to the subject an effective amount of the antibody or antigen-binding fragment thereof disclosed herein. In certain examples, the method of treating a cancer further comprises performing a therapeutic procedure (e.g., anti-cancer radiation therapy or surgical tumorectomy) to the subject. In some examples, the method of treating a cancer further comprises administering a therapeutic agent (e.g., pembrolizumab) to the subject. In other examples, the method of treating a cancer further comprises performing a therapeutic procedure (e.g., anti-cancer radiation therapy or surgical tumorectomy) and administering a therapeutic agent (e.g., pembrolizumab) to the subject.

The present disclosure also provides a method of producing the antibody or antigen-binding fragment thereof of the present disclosure or an immunoglobulin chain thereof (e.g., a V_H and/or V_L thereof), comprising culturing a host cell (e.g., a CHO cell) comprising a polynucleotide (e.g., wherein the polynucleotide is in a vector and/or is integrated into one or more chromosomes of the host cell) encoding the antibody or antigen-binding fragment thereof or an immunoglobulin chain thereof to express the antibody or antigen-binding fragment thereof or an immunoglobulin chain thereof.

In another aspect, there is provided a method of producing the antibody or antigen-binding fragment thereof disclosed herein, comprising culturing the host cell disclosed herein to express the antibody or antigen-binding fragment thereof.

Also provided is a method of producing the antibody or antigen-binding fragment thereof of the present disclosure or an immunoglobulin chain thereof (e.g., a V_H and/or V_L thereof), comprising: expressing a polynucleotide encoding the antibody or antigen-binding fragment thereof or an immunoglobulin chain thereof.

In another aspect, there is provided a method of producing the antibody or antigen-binding fragment thereof disclosed herein, comprising expressing the polynucleotide disclosed herein.

An antibody or antigen-binding fragment thereof that binds human ILT4 or an immunoglobulin chain thereof which is a product of said method is also part of the present disclosure.

A method for detecting the presence of an ILT4 peptide or a fragment thereof in a sample also forms part of the present disclosure. The method comprises contacting the sample with an antibody or antigen-binding fragment of the present disclosure and detecting the presence of a complex between the antibody or

antigen-binding fragment and the ILT4 peptide or fragment thereof, wherein detection of the complex indicates the presence of the ILT4 peptide or fragment thereof. In another aspect, there is provided a method for detecting an ILT4 peptide or fragment thereof in a sample, comprising contacting the sample with the antibody or antigen-binding fragment thereof disclosed herein and detecting the presence of a complex between the antibody or antigen-binding fragment thereof and the ILT4 peptide or fragment thereof; wherein detection of the complex indicates the presence of the ILT4 peptide or fragment thereof. In an example of the disclosure, the method is performed *in vitro*, e.g., in a biological sample, e.g., surgical section or blood sample, of a subject. In another example, the method is performed *in vivo*, e.g., in the body of a subject. In yet another example, the subject is a human being.

In another aspect, there is provided use of the antibody or antigen-binding fragment thereof disclosed herein in the manufacture of a medicament for the treatment of cancer.

In another aspect, there is provided an antibody or antigen-binding fragment thereof disclosed herein for use in treating cancer.

In another aspect, there is provided an antibody that consists of two heavy chains and two light chains, wherein each light chain comprises the amino acid sequence set forth in SEQ ID NO:58 and each heavy chain comprises the amino acid sequence set forth in SEQ ID NO:57.

In another aspect, there is provided an antibody that consists of two heavy chains and two light chains, wherein each light chain comprises the amino acid sequence set forth in SEQ ID NO:7 and each heavy chain comprises the amino acid sequence set forth in SEQ ID NO:2.

In another aspect, there is provided a pharmaceutical composition comprising: (i) an antibody that consists of two heavy chains and two light chains, wherein each light chain consists of the amino acid sequence set forth in SEQ ID NO:7 and each heavy chain consists of the amino acid sequence set forth in SEQ ID NO:2, and (ii) pembrolizumab.

Brief Description of the Figures

Figure 1. Deuterium labeling Heatmap of p1E1(G1) binding to ILT4-His.

We claim:

1. An antibody or antigen-binding fragment thereof that binds human ILT4, comprising:
a heavy chain variable domain comprising:
5 CDR-H1: GYYWS (SEQ ID NO: 16),
CDR-H2: EINHGXGSTNYNPSLKS wherein X is S or A (SEQ ID NO: 17), and
CDR-H3: LPTRWVTTRYFDL (SEQ ID NO: 18); and,
a light chain variable domain comprising:
CDR-L1: TGSSSNIGAGYDVH (SEQ ID NO: 19),
10 CDR-L2: GX₁X₂NRPS, wherein X₁ is N, Q, E or D and X₂ is S or A (SEQ ID NO: 20),
and
CDR-L3: QSFDNSLSAYV (SEQ ID NO: 21).
2. The antibody or antigen-binding fragment thereof of claim 1, comprising:
15 a heavy chain variable domain comprising:
CDR-H1: GYYWS (SEQ ID NO: 16),
CDR-H2: EINHAGSTNYNPSLKS (SEQ ID NO: 48), and
CDR-H3: LPTRWVTTRYFDL (SEQ ID NO: 18); and,
a light chain variable domain comprising:
20 CDR-L1: TGSSSNIGAGYDVH (SEQ ID NO: 19),
CDR-L2: GDSNRPS(SEQ ID NO: 52), and
CDR-L3: QSFDNSLSAYV (SEQ ID NO: 21).
3. The antibody or antigen-binding fragment thereof of claim 1, comprising:
25 a light chain immunoglobulin having at least 90% amino acid sequence identity to the
amino acid sequence set forth in SEQ ID NO:3, 4, 5, 6 ,7, or 45, and
a heavy chain immunoglobulin having at least 90% amino acid sequence identity to the
amino acid sequence set forth in SEQ ID NO:1, 2, 44, 79, 80, or 81.
- 30 4. The antibody or antigen-binding fragment thereof of claim 1, comprising:
a light chain immunoglobulin comprising a light chain variable domain having at least
90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID
NO:70, 71, 72, 73, 58, or 77, and
a heavy chain immunoglobulin comprising a heavy chain variable domain having at least
35 90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID
NO:63, 57, or 69.

5. The antibody or antigen-binding fragment thereof of claim 1, comprising:

(1)

5 a heavy chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 1 or 79; and

a light chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 3;

(2)

10 a heavy chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 2 or 80; and

a light chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 4;

(3)

15 a heavy chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 2 or 80; and

a light chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 5;

(4)

20 a heavy chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 2 or 80; and

a light chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 6;

(5)

25 a heavy chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 2 or 80; and

a light chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 7; or

(6)

30 a heavy chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 2 or 80; and

a light chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 3.

35 6. The antibody or antigen-binding fragment thereof of claim 1, comprising:

(1)

a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:63; and

a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:70;

5 (2)

a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:57; and

a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:71;

10 (3)

a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:57; and

a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:72;

15 (4)

a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:57; and

a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:73;

20 (5)

a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:57; and

a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:58; or

25 (6)

a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:57; and

a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:70.

30

7. The antibody or antigen-binding fragment thereof of claim 1, comprising:

a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:57; and

a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO:58.

35

8. The antibody or antigen-binding fragment thereof of claim 1, comprising:
a heavy chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID
NO:2; and
a light chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID
5 NO:7.
9. The antibody or antigen-binding fragment thereof of claim 1, comprising:
a heavy chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID
NO:80; and
10 a light chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID
NO:7.
10. The antibody or antigen-binding fragment thereof of any one of claims 1-9, wherein the
antibody or antigen-binding fragment thereof is glycosylated with engineered yeast N-
15 linked glycans or CHO N-linked glycans.
11. A pharmaceutical composition comprising the antibody or antigen-binding fragment
thereof of any of claims 1-10.
- 20 12. The pharmaceutical composition of claim 11, further comprises a therapeutic agent.
13. The pharmaceutical composition of claim 11 or 12, further comprises a pharmaceutically
acceptable carrier.
- 25 14. A polynucleotide comprising nucleotide sequence encoding a VH, a VL, both a VH and
a VL, a heavy chain immunoglobulin, a light chain immunoglobulin, or both a heavy
chain and a light chain immunoglobulin of the antibody or antigen-binding fragment
according to any one of the claims 1-9.
- 30 15. A vector comprising the polynucleotide of claim 14.
16. A host cell comprising the polynucleotide of claim 14 or the vector of claim 15.
17. An antibody or antigen-binding fragment thereof of any one of claims 1-10, for use in
35 blocking the binding of ILT4 to HLA-G, HLA-A, HLA-B and/or HLA-F in a human subject.

18. The antibody or antigen-binding fragment thereof of any one of claims 1-10 for use according to claim 17, wherein the antibody or antigen-binding fragment thereof is to be administered with a therapeutic procedure and/or a therapeutic agent.
- 5 19. A method of producing the antibody or antigen-binding fragment thereof of any one of claims 1-10, comprising culturing the host cell of claim 16 to express the antibody or antigen-binding fragment thereof.
- 10 20. A method of producing the antibody or antigen-binding fragment thereof of any one of claims 1-10, comprising expressing the polynucleotide of claim 14.
- 15 21. A method for detecting an ILT4 peptide or fragment thereof in a sample, comprising contacting the sample with the antibody or antigen-binding fragment thereof of any one of claims 1-10 and detecting the presence of a complex between the antibody or antigen-binding fragment thereof and the ILT4 peptide or fragment thereof; wherein detection of the complex indicates the presence of the ILT4 peptide or fragment thereof.
- 20 22. Use of the antibody or antigen-binding fragment thereof of any one of claims 1-10 in the manufacture of a medicament for the treatment of cancer.
23. An antibody or antigen-binding fragment thereof of any one of claims 1-10 for use in treating cancer.
- 25 24. An antibody that consists of two heavy chains and two light chains, wherein each light chain comprises the amino acid sequence set forth in SEQ ID NO:58 and each heavy chain comprises the amino acid sequence set forth in SEQ ID NO:57.
- 30 25. The antibody of claim 24, wherein the light chain further comprises the amino acid sequence set forth in SEQ ID NO:90 and/or the heavy chain further comprises the amino acid sequence set forth in SEQ ID NO:89.
- 35 26. An antibody that consists of two heavy chains and two light chains, wherein each light chain comprises the amino acid sequence set forth in SEQ ID NO:7 and each heavy chain comprises the amino acid sequence set forth in SEQ ID NO:2.

27. A pharmaceutical composition comprising: (i) an antibody that consists of two heavy chains and two light chains, wherein each light chain consists of the amino acid sequence set forth in SEQ ID NO:7 and each heavy chain consists of the amino acid sequence set forth in SEQ ID NO:2, and (ii) pembrolizumab.