

[0006] HLA-G mediates immune function through at least three ITIM-containing inhibitory receptors, ILT2, ILT4, and KIR2DL4. On lymphoid and myeloid cells, for example, HLA-G mediates function through ILT2. On myeloid cells, HLA-G mediates function through ILT4. On decidual NK cells, HLA-G mediates immune function through KIR2DL4 and ILT2.

[0007] HLA-G is an immune checkpoint target. HLA-G can directly inhibit immune cell function through receptor binding and/or trogocytosis and impairment of chemotaxis. HLA-G can lend tumor cells a higher invasive and metastatic potential. HLA-G promotes evasion of tumor immune surveillance, and enhances metastasis and the progression of malignancies. During tumor progression HLA-G has other effects, such as, inhibition of immune cell cytotoxicity, induction of immune cell apoptosis, and/or the generation of regulatory cells through receptor binding and/or trogocytosis.

[0008] HLA-G expression is upregulated on a broad spectrum of tumors and is associated with poor prognosis and disease progression. Serum HLA-G levels are elevated in breast, lung, colorectal cancer (CRC), gastric, esophageal, neuroblastoma, cervical, and hematological cancers. HLA-G has also been found to be correlated with clinical parameters in advanced disease, such as, tumor metastasis, poor prognosis, immune escape, and tumor invasiveness.

[0009] HLA-G is an attractive target for diseases, such as, for example, cancer.

SUMMARY

[0010] Provided herein are antibodies that selectively bind HLA-G. In some examples, the antibodies bind human HLA-G. In some examples, the antibodies comprise at least one CDR sequence defined by a consensus sequence provided in this disclosure. In some examples, the antibodies comprise one or more of an illustrative CDR, V_H, or V_L sequence provided in this disclosure, or a variant thereof. In some examples, the variant is a variant with one or more conservative amino acid substitutions.

[0010a] In one aspect, provided herein is an antibody that specifically binds to human HLA-G (hHLA-G), comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein a) the VH comprises: i) a VH-complementarity determining region (CDR) 1 comprising the amino acid sequence set forth in SEQ ID NO: 7; a VHCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 44; and a VHCDR3 comprising

the amino acid sequence set forth in SEQ ID NO: 93, wherein the CDRs are according to Chothia; or ii) a VHCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 25; a VHCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 65; and a VHCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 93, wherein the CDRs are according to Kabat; and b) the VL comprises: i) a VLCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 118; a VLCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 138; and a VLCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 155, wherein the CDRs are according to Chothia and Kabat.

[0011] Also provided are compositions and kits comprising the antibodies. In some examples, the compositions are pharmaceutical compositions. In another aspect, provided herein is a pharmaceutical composition comprising the antibody as provided herein and a pharmaceutically acceptable carrier or excipient. Any suitable pharmaceutical composition may be used. In some examples, the pharmaceutical composition is a composition for parenteral administration.

[0011a] In another aspect, provided herein is one or more isolated nucleic acid molecules encoding the antibody as provided herein.

[0011b] In another aspect, provided herein is an expression vector comprising the nucleic acid molecule as provided herein.

[0011c] In another aspect, provided herein is a prokaryotic or eukaryotic host cell comprising the vector as provided herein.

[0011d] In another aspect, provided herein is a kit comprising the antibody as provided herein.

[0011e] In another aspect, provided herein is a method for the production of an antibody that specifically binds to hHLA-G, comprising providing nucleic acids encoding the antibody as provided herein, or vectors or host cells comprising said nucleic acids.

[0012] This disclosure also provides methods of using the anti-HLA-G antibodies provided herein. In some embodiments, the method is a method of treatment. In some

examples, the method is an analytical method. In some examples, the method is a method of purifying and/or quantifying HLA-G.

[0013] In some examples, the method is a diagnostic method. In some embodiments, the diagnostic method comprises or consists of detecting tumor expressed HLA-G. In some examples, the diagnostic method comprises or consists of detecting soluble HLA-G. In some examples, the detection method comprises or consists of detecting HLA-G expression on immune cells.

[0014] In some examples, the antibodies are used to treat a disease or condition. In some examples, the disease or condition is selected from a cancer, autoimmune disease, and infection. Some examples provide for the use of any of the antibodies or pharmaceutical compositions provided herein to treat a disease or condition selected from a cancer, autoimmune disease, and infection.

[0014a] In another aspect, provided herein is use of a pharmaceutical composition comprising an effective amount of the antibody as provided herein in the manufacture of a medicament for the treatment of a subject suffering from cancer, autoimmune disease, infection, a chronic infection, an inflammatory disease, or for modulating immune system function in a subject in need thereof.

[0014b] In another aspect, provided herein is a pharmaceutical composition comprising an effective amount of the antibody as provided herein for use as a medicament.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] **FIG. 1** provides a table showing avid and monomeric affinities of anti-HLA-G antibodies to recombinant HLA-G protein.

[0016] **FIG. 2A and FIG. 2B** provide biolayer interferometry sensorgrams showing anti-HLA-G antibodies that bind to HLA-G and can be separated into three biochemical bins based on their ability to cross-block each other when tested for binding in pairwise fashion.

[0017] **FIG. 3** provide biolayer interferometry sensorgrams showing antibodies that bind and block HLA-G interaction with ILT2 and ILT4 at varying levels of effectiveness.

[0018] **FIG. 4** shows evaluation of anti-HLA-G antibodies binding to naturally expressed HLA-G found on JEG-3 tumor cells.

[0019] **FIG. 5A, FIG. 5B, FIG. 5C, and FIG. 5D** provide data showing anti-HLA-G Fabs and antibodies that restore NKL killing activity by blocking suppression mediated through interaction of HLA-G with ILT2 or ILT4.

[0020] **FIG. 6** provides evaluation of anti-HLA-G antibodies to reverse HLA-G mediated suppression of primary human cells. **FIG. 6A** and **FIG. 6B** provide evaluation of phagocytosis in a human macrophage assay. **FIG. 6C** provides evaluation of primary human NK cell cytotoxic activity. **FIG. 6D** provides evaluation of primary human CD8⁺ T cell function.

[0021] **FIG. 7A and FIG. 7B** shows results representing values for binding of anti-

WHAT IS CLAIMED IS:

1. An antibody that specifically binds to human HLA-G (hHLA-G), comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein
 - a) the VH comprises:
 - i) a VH-complementarity determining region (CDR) 1 comprising the amino acid sequence set forth in SEQ ID NO: 7; a VHCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 44; and a VHCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 93, wherein the CDRs are according to Chothia; or
 - ii) a VHCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 25; a VHCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 65; and a VHCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 93, wherein the CDRs are according to Kabat; and
 - b) the VL comprises:
 - i) a VLCDR1 comprising the amino acid sequence set forth in SEQ ID NO: 118; a VLCDR2 comprising the amino acid sequence set forth in SEQ ID NO: 138; and a VLCDR3 comprising the amino acid sequence set forth in SEQ ID NO: 155, wherein the CDRs are according to Chothia and Kabat.
2. The antibody of claim 1, comprising a VH sequence that is at least 85% identical to the amino acid sequence set forth in SEQ ID NO: 192 and a VL sequence that is at least 85% identical to the amino acid sequence set forth in SEQ ID NO: 220.
3. The antibody of claim 1, comprising a VH sequence that is at least 95% identical to the amino acid sequence set forth in SEQ ID NO: 192 and a VL sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 220.
4. The antibody of claim 1, wherein the antibody comprises a heavy chain of SEQ ID NO: 254, and a light chain of SEQ ID NO: 322.
5. The antibody of claim 1, wherein the antibody comprises a heavy chain of SEQ ID NO: 288, and a light chain of SEQ ID NO: 322.

6. The antibody of claim 1, wherein the antibody is a monoclonal antibody.
7. A pharmaceutical composition comprising the antibody of claim 1 and a pharmaceutically acceptable carrier or excipient.
8. The pharmaceutical composition of claim 7, further comprising an effective amount of at least one of the following:
 - a) an anti-ILT2 antibody;
 - b) an anti-ILT4 antibody;
 - c) an anti-KIR2DL4 antibody;
 - d) an anti-HLA-E antibody;
 - e) an anti-NKG2A antibody
 - f) an anti-HLA-F antibody
 - g) an anti-PD-L1 antibody;
 - h) an anti-PD-l antibody;
 - i) an anti-CD38 antibody;
 - j) an anti-CD39 antibody;
 - k) an anti-CD73 antibody;
 - l) an anti-A2A receptor antibody;
 - m) an anti-A2B receptor antibody;
 - n) an anti-A2A/A2B dual receptor antibody or a combination thereof;
 - o) an anti-CD47 antibody;
 - p) an anti-CTLA-4 antibody;
 - q) an anti-TIM-3 antibody;
 - r) an anti-TIGIT antibody;
 - s) an anti-VISTA antibody;
 - t) an anti-CD94 antibody;
 - u) a small molecule inhibitor;
 - v) a bi-specific T cell engager, CAR-T therapy, CAR-NK therapy, CAR-Macrophage therapy, engineered cell therapy, and/or adaptive T cell therapy
 - w) an oncolytic virus;
 - x) a chemotherapy; and/or

- y) ADCC capable therapies using effector competent antibodies such as anti-CD19, anti-CD20, anti-EGFR, anti-Her2, anti-SLAMF7, anti-CD52, anti-BCMA, anti-GD2, and/or anti-CCR4.
9. The pharmaceutical composition of claim 7 or 8, further comprising one or both of
 - a) an antibody to an immune inhibitory receptor or ligand and/or
 - b) an antibody to an immune stimulatory receptor or ligand.
 10. One or more isolated nucleic acid molecules encoding the antibody of claim 1.
 11. An expression vector comprising the nucleic acid molecule of claim 10.
 12. A prokaryotic or eukaryotic host cell comprising the vector of claim 11.
 13. A kit comprising the antibody of claim 1.
 14. A method for the production of an antibody that specifically binds to hHLA-G, comprising providing nucleic acids encoding the antibody of claim 1, or vectors or host cells comprising said nucleic acids.
 15. Use of a pharmaceutical composition comprising an effective amount of the antibody of claim 1 in the manufacture of a medicament for the treatment of a subject suffering from cancer, autoimmune disease, infection, a chronic infection, an inflammatory disease, or for modulating immune system function in a subject in need thereof.
 16. The use of claim 15, wherein said antibody comprises a bispecific antibody or a complexing antibody.
 17. The use of claim 15, wherein the use further comprises one or more of the following:
 - a) an anti-ILT2 antibody;
 - b) an anti-ILT4 antibody;
 - c) an anti-KIR2DL4 antibody;
 - d) an anti-HLA-E antibody;

- e) an anti-NKG2A antibody
 - f) an anti-HLA-F antibody
 - g) an anti-PD-L1 antibody;
 - h) an anti-PD-l antibody;
 - i) an anti-CD38 antibody;
 - j) an anti-CD39 antibody;
 - k) an anti-CD73 antibody;
 - l) an anti-A2A receptor antibody;
 - m) an anti-A2B receptor antibody;
 - n) an anti-A2A/A2B dual receptor antibody or a combination thereof;
 - o) an anti-CD47 antibody;
 - p) an anti-CTLA-4 antibody;
 - q) an anti-TIM-3 antibody;
 - r) an anti-TIGIT antibody;
 - s) an anti-VISTA antibody;
 - t) an anti-CD94 antibody;
 - u) a small molecule inhibitor;
 - v) a bi-specific T cell engager, CAR-T therapy, CAR-NK therapy, CAR-Macrophage therapy, engineered cell therapy, and/or adaptive T cell therapy
 - w) an oncolytic virus;
 - x) a chemotherapy; and/or
 - y) ADCC capable therapies using effector competent antibodies such as anti-CD19, anti-CD20, anti-EGFR, anti-Her2, anti-SLAMF7, anti-CD52, anti-BCMA, anti-GD2, and/or anti-CCR4;
- to be administered in the subject.

18. The use of claim 15, wherein the use further comprises one or more of the following:

- i) chemotherapy;
 - ii) radiation therapy; and/or
 - iii) one or more additional therapeutic agents;
- to be administered in the subject.

19. The use of claim 18, wherein the one or more additional therapeutic agents comprises one or more immunostimulatory agents.
20. A pharmaceutical composition comprising an effective amount of the antibody of claim 1 for use as a medicament.