

expression of stimulatory receptor TMIGD2 is gradually lost, allowing expression of the inhibitory receptor KIR3DL3 to become dominant.

[0006] While HHLA2 binding agents have been investigated as immunotherapies, the present disclosure encompasses, *inter alia*, the discovery of HHLA2 binding agents described herein with novel activity that result in: (i) inhibition of HHLA2 binding to KIR3DL3; and/or (ii) enhancement of HHLA2 binding to TMIGD2. In some examples, HHLA2 binding agents are capable of: (i) inhibiting HHLA2 binding to KIR3DL3; and (ii) enhancing HHLA2 binding to TMIGD2. In some examples, HHLA2 binding agents described herein cause allosteric changes in HHLA2, thereby resulting in conformational changes in HHLA2 that enhance HHLA2 binding to TMIGD2. In some examples, HHLA2 binding agents described herein directly compete with at least one binding site for TMIGD2 on HHLA2. In some examples, a HHLA2 binding agent described herein enhances an early stage immune response and/or a later stage immune response. In some examples, a HHLA2 binding agent described herein enhances HHLA2 binding to TMIGD2 in naïve immune effector cells. In some examples, a HHLA2 binding agent described herein blocks HHLA2 binding to KIR3DL3 in exhausted immune effector cells. In some examples, exhausted immune effector cells comprise or express certain cell surface markers, such as PD-1, CTLA-4, LAG-3, TIM-3, 2B4/CD244/SLAMF4, CD160, and/or TIGIT.

[0007] Accordingly, the present disclosure provides several examples of such HHLA2 binding agents that are particularly useful for treating a variety of cancers, including solid tumors, such as non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), cholangiocarcinoma, or breast cancer, and hematological tumors, as well as modulating an immune response in a subject.

[0008] In some examples, an HHLA2 binding agent described herein is used for tumor targeting of at least one cytotoxic agent. In some examples, an HHLA2 binding agent described herein is administered or co-formulated with a cytotoxic agent. In some examples, an HHLA2 binding agent described herein is used for delivery of at least one radionuclide to a tumor (e.g., a tumor described herein). In some examples, an HHLA2 binding agent described herein is administered or co-formulated with a radionuclide. In some examples, an HHLA2 binding agent described herein is used for targeting a tumor (e.g., a tumor described herein) in combination with a monoclonal antibody that binds Fc receptor (FcR), thereby mediating antibody dependent cellular cytotoxicity (ADCC). In some

examples, an HHLA2 binding agent described herein is administered or co-formulated with a monoclonal antibody that binds FcR.

[0009] In one aspect, the disclosure provides HHLA2 binding agent capable of: (i) inhibiting HHLA2 binding to KIR3DL3; and/or (ii) enhancing HHLA2 binding to TMIGD2.

[0010] In some examples, an HHLA2 binding agent is or comprises an antibody or antigen-binding fragment thereof, a small molecule, a polypeptide, or an aptamer.

[0011] In some examples, an antibody or antigen-binding fragment thereof is or comprises: (i) a chimeric antibody, a human antibody, or a humanized antibody, or antigen-binding fragment thereof; (ii) a monospecific antibody or a bispecific antibody, or antigen-binding fragment thereof; and/or (iii) a monoclonal antibody, or antigen-binding fragment thereof. In some examples, an antigen-binding fragment comprises an scFv, Fab, Fab', F(ab')₂, Fc, nanobody, or camelid antibody. In some examples, an antibody or antigen-binding fragment thereof is or comprises: (i) a heavy chain constant region chosen from IgG1, IgG2, IgG3, or IgG4, and/or (ii) a light chain constant region chosen from the light chain constant regions of kappa or lambda.

[0012] In some examples, an antibody or antigen-binding fragment thereof is or comprises: (a) a heavy chain variable region (VH) comprising one, two, or three VH CDR sequences each with at least about 90% identity to a VH CDR of Table 1; and/or (b) a light chain variable region (VL) comprising one, two, or three VL CDR sequences each with at least about 90% identity to a VL CDR of Table 1. In some examples, an antibody or antigen-binding fragment thereof is or comprises: (a) a VH comprising one, two, or three VH CDR sequences each with at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or higher identity to a VH CDR of Table 1; and/or (b) a VL comprising one, two, or three VL CDR sequences each with at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or higher identity to a VL CDR of Table 1. In some examples, an antibody or antigen-binding fragment thereof is or comprises: (a) a VH comprising one, two, or three VH CDR sequences each comprising or consisting of a VH CDR of Table 1; and/or (b) a VL comprising one, two, or three VL CDR sequences each comprising or consisting of a VL CDR of Table 1.

[0013] In some examples, an antibody or antigen-binding fragment thereof is or comprises: (a) a VH with at least about 90% or more identity to a VH of Table 1; and/or (b) a VL with at least about 90% or more identity to a VL of Table 1. In some examples, an antibody or antigen-binding fragment thereof is or comprises: (a) a VH with at least about 95%, 96%, 97%, 98%, 99%, 99.5% or higher identity to a VH of Table 1; and/or (b) a VL with at least about 95%, 96%, 97%, 98%, 99%, 99.5% or higher identity to a VL of Table 1. In some examples, an antibody or antigen-binding fragment thereof is or comprises: (a) a VH comprising or consisting of a VH of Table 1; and/or (b) a VL comprising or consisting of a VL of Table 1.

[0014] In some examples, an antibody or antigen-binding fragment thereof is or comprises: (a) a heavy chain with at least about 90% or more identity to a heavy chain of Table 1; and/or (b) a light chain with at least about 90% or more identity to a light chain of Table 1. In some examples, an antibody or antigen-binding fragment thereof is or comprises: (a) a heavy chain with at least about 95%, 96%, 97%, 98%, 99%, 99.5% or higher identity to a heavy chain of Table 1; and/or (b) a light chain with at least about 95%, 96%, 97%, 98%, 99%, 99.5% or higher identity to a light chain of Table 1. In some examples, an antibody or antigen-binding fragment thereof is or comprises: (a) a heavy chain comprising or consisting of a heavy chain of Table 1; and/or (b) a light chain comprising or consisting of a light chain of Table 1.

[0015] In another aspect, the disclosure provides agents that bind and/or compete for binding with the same epitope on HHLA2 as an HHLA2 binding agent of any aspect or embodiment described herein.

[0016] In some examples, an HHLA2 binding agent enhances HHLA2 binding to TMIGD2 in naïve immune effector cells. In some examples, an HHLA2 binding agent blocks HHLA2 binding to KIR3DL3 in exhausted immune effector cells. In some examples, immune effector cells comprise or are T cells and/or NK cells. In some examples, T cells comprise or are CD4+ T cells and/or CD8+ T cells.

[0017] In some examples, an HHLA2 binding agent binds HHLA2 with a KD of about 5 nM or less. In some examples, an HHLA2 binding agent binds HHLA2 with a KD of about 15 nM or less. In some examples, an HHLA2 binding agent binds human HHLA2 with an affinity of at least about 50-fold to about 800-fold over background. In

some examples, an HHLA2 binding agent enhances HHLA2 binding to TMIGD2 at a ratio of greater than about 2.

[0018] In another aspect, the disclosure provides pharmaceutical compositions comprising at least one HHLA2 binding agent of any aspect or example described herein, and a pharmaceutically acceptable carrier. In yet another aspect, the disclosure provides a use of at least one HHLA2 binding agent as disclosed herein, or a pharmaceutical composition as disclosed herein in the manufacture of a medicament for modulating an immune response or treating a subject having or is at risk of developing cancer.

[0019] In another aspect, the disclosure provides methods of treating a subject having a disease, disorder, or condition comprising: administering a therapeutically effective amount of at least one HHLA2 binding agent of any aspect or example described herein, or a pharmaceutical composition of any aspect or example described herein.

[0020] In another aspect, the disclosure provides methods of modulating an immune response in a subject comprising: administering a therapeutically effective amount of at least one HHLA2 binding agent of any aspect or example described herein, or a pharmaceutical composition of any aspect or example described herein.

[0021] In some examples, a subject has or is at risk of developing a cancer. In some examples, a subject has a solid tumor or a hematological cancer. In some examples, a solid tumor is or comprises one or more of: a renal cancer, a bone cancer, a skin cancer, a breast cancer, a cervical cancer, a colorectal cancer, an endometrial cancer, a lung cancer, an ovarian cancer, a liver cancer, cholangiocarcinoma, or a thyroid cancer. In some examples, a hematological cancer comprises or is a leukemia or lymphoma. In some examples, a leukemia comprises or is acute lymphocytic leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic leukemia, or acute leukemia. In some examples, a lymphoma comprises or is Hodgkin lymphoma (HL), non-Hodgkin's lymphoma, lymphocytic lymphoma, or diffuse large B cell lymphoma (DLBCL).

[0022] In some examples, a disease, disorder, or condition is associated with aberrant HHLA2 expression.

[0023] In some examples, an HHLA2 binding agent is administered parenterally. In some examples, parenteral administration is or comprises subcutaneous, intravenous, intramuscular, or intrasternal injection or infusion.

[0024] In some examples, an HHLA2 binding agent is administered in combination with a second agent.

[0025] In another aspect, the disclosure provides nucleic acids encoding at least one HHLA2 binding agent of any aspect or example described herein, or an antigen-binding fragment thereof.

[0026] In another aspect, the disclosure provides expression vectors comprising at least one nucleic acid of any aspect or example described herein.

[0027] In another aspect, the disclosure provides host cells comprising or expressing at least one HHLA2 binding agent of any aspect or example described herein, comprising at least one nucleic acid of aspect or example described herein, or comprising at least one expression vector of aspect or example described herein.

[0028] In another aspect, the disclosure provides methods of making an HHLA2 binding agent, comprising: (i) culturing a host cell comprising at least one nucleic acid of any aspect or example described herein or at least one expression vector of any aspect or example described herein under conditions suitable for expression of the HHLA2 binding agent, and (ii) recovering the HHLA2 binding agent.

[0029] In another aspect, the disclosure provides methods of detecting the presence or level of an HHLA2 polypeptide in a sample comprising: detecting an HHLA2 polypeptide in a sample using at least one HHLA2 binding agent of any aspect or example described herein.

[0030] In another aspect, the disclosure provides kits comprising at least one HHLA2 binding agent of any aspect or example described herein, and instructions for use and/or administration.

[0031] In some examples, an HHLA2 binding agent forms a complex with an HHLA2 polypeptide. In some examples, a complex is detected by an assay comprising an enzyme linked immunosorbent assay (ELISA), radioimmune assay (RIA), and/or Western blot. In some examples, a HHLA2 binding agent is directly labeled.

[0032] Other features, objects, and advantages of the present disclosure are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating examples of the present disclosure, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the disclosure will become apparent to those skilled in the art from the detailed description.

BRIEF DESCRIPTION OF THE DRAWING

[0033] The Figures described below, which together make up the Drawing, are for illustration purposes only, not for limitation.

[0034] **FIGS. 1A-1B** are schematics showing an alignment of heavy chain variable domains (**FIG. 1A**) and light chain variable domains (**FIG. 1A**) of exemplary anti-HHLA2 antibodies Ab-60638 and Ab-60665.

[0035] **FIGS. 2A-2B** are graphs showing binding affinity of exemplary anti-HHLA2 antibodies Ab-60638 (**FIG. 2A**) and Ab-60665 (**FIG. 2B**) for recombinant HHLA2-Fc using the Octet system from ForteBio.

[0036] **FIGS. 3A-3C** are graphs of flow cytometry histograms showing binding of exemplary anti-HHLA2 antibodies Ab-60638 (**FIG. 3A**), Ab-60665 (**FIG. 3B**), and Ab-65885 / Ab-65886 / Ab-65887 / Ab-65889 / Ab-65890 (**FIG. 3C**) to 300.19 cells over-expressing human HHLA2 relative to an isotope control.

[0037] **FIGS. 4A-4B** are graphs of flow cytometry data showing the ability of exemplary anti-HHLA2 antibodies Ab-60638 and Ab-60665 to block binding of human HHLA2-Fc to 300.19 cells over-expressing human KIR3DL3 (**FIG. 4A**) and enhance binding of human TMIGD2-Fc to 300.19 cells over-expressing human HHLA2 (**FIG. 4B**), both relative to an isotype control.

[0038] **FIGS. 5A-5B** are graphs of flow cytometry data showing the ability of exemplary anti-HHLA2 antibodies Ab-65885, Ab-65886, Ab-65887, Ab-65889, and Ab-65890 to block binding of human HHLA2-Fc to 300.19 cells over-expressing human KIR3DL3 (**FIG. 5A**) and enhance binding of human TMIGD2-Fc to 300.19 cells over-expressing human HHLA2 (**FIG. 5B**), both relative to an isotype control.

CLAIMS

1. An HHLA2 binding agent capable of:
 - (i) inhibiting HHLA2 binding to KIR3DL3; and
 - (ii) enhancing HHLA2 binding to TMIGD2.
2. The HHLA2 binding agent of claim 1, wherein the HHLA2 binding agent is or comprises an antibody or antigen-binding fragment thereof, a small molecule, a polypeptide, or an aptamer.
3. The HHLA2 binding agent claim 2, wherein the antibody or antigen-binding fragment thereof is or comprises:
 - (i) a chimeric antibody, a human antibody, or a humanized antibody, or antigen-binding fragment thereof;
 - (ii) a monospecific antibody or a bispecific antibody, or antigen-binding fragment thereof;
 - (iii) a monoclonal antibody, or antigen-binding fragment thereof;
 - (iv) an scFv, Fab, Fab', F(ab')₂, Fc, nanobody, or camelid antibody;
 - (v) a heavy chain constant region chosen from IgG1, IgG2, IgG3, or IgG4; and/or
 - (vi) a light chain constant region chosen from the light chain constant regions of kappa or lambda.
4. The HHLA2 binding agent of claim 2 or 3, wherein the antibody or antigen-binding fragment thereof is or comprises:
 - (a) a VH comprising a VH CDR1 amino acid sequence of SEQ ID NO: 105, a VH CDR2 amino acid sequence of SEQ ID NO: 106, and a VH CDR3 amino acid sequence of SEQ ID NO: 107;
 - (b) a VL comprising a VL CDR1 amino acid sequence of SEQ ID NO: 118, a VL CDR2 amino acid sequence of SEQ ID NO: 119, and a VL CDR3 amino acid sequence of SEQ ID NO: 120;
 - (c) a VH comprising a VH CDR1 amino acid sequence of SEQ ID NO: 108, a VH CDR2 amino acid sequence of SEQ ID NO: 109, and a VH CDR3 amino acid sequence of SEQ ID NO: 110;
 - (d) a VL comprising a VL CDR1 amino acid sequence of SEQ ID NO: 121, a VL CDR2 amino acid sequence of SEQ ID NO: 122, and a VL CDR3 amino acid sequence of SEQ ID NO: 123;
 - (e) a VH comprising a VH CDR1 amino acid sequence of SEQ ID NO: 111, a VH CDR2 amino acid sequence of SEQ ID NO: 112, and a VH CDR3 amino acid sequence of SEQ ID NO: 113; and/or
 - (f) a VL comprising a VL CDR1 amino acid sequence of SEQ ID NO: 124, a VL CDR2 amino acid sequence of SEQ ID NO: 125, and a VL CDR3 amino acid sequence of SEQ ID NO: 126.

5. The HHLA2 binding agent of any one of claims 2-4, wherein the antibody or antigen-binding fragment thereof is or comprises:

a VH with at least 90% or more identity to SEQ ID NO: 114.

6. The HHLA2 binding agent of any one of claims 2-5, wherein the antibody or antigen-binding fragment thereof is or comprises:

a VL with at least 90% or more identity to SEQ ID NO: 127.

7. The HHLA2 binding agent of any one of claims 2-6, wherein the antibody or antigen-binding fragment thereof is or comprises:

(a) a VH comprising SEQ ID NO: 114; and

(b) a VL comprising SEQ ID NO: 127.

8. The HHLA2 binding agent of any one of claims 2-7, wherein the antibody or antigen-binding fragment thereof is or comprises:

a heavy chain with at least 90% or more identity to SEQ ID NO: 116.

9. The HHLA2 binding agent of any one of claims 2-8, wherein the antibody or antigen-binding fragment thereof is or comprises:

a light chain with at least 90% or more identity to SEQ ID NO: 129.

10. The HHLA2 binding agent of any one of claims 2-9, wherein the antibody or antigen-binding fragment thereof is or comprises:

(a) a heavy chain comprising SEQ ID NO: 116; and

(b) a light chain comprising SEQ ID NO: 129.

11. An agent that binds and/or competes for binding with the same epitope on HHLA2 as an HHLA2 binding agent of any one of claims 1-10.

12. The HHLA2 binding agent of any one of claims 1-11, wherein the binding agent enhances HHLA2 binding to TMIGD2 in naïve immune effector cells; and/or

blocks HHLA2 binding to KIR3DL3 in exhausted immune effector cells, optionally

wherein the immune effector cells comprise or are T cells and/or NK cells; and/or wherein the T cells comprise or are CD4+ T cells and/or CD8+ T cells.

13. The HHLA2 binding agent of any one of claims 1-12, wherein the binding agent binds HHLA2 with a K_D of 15 nM or less;

binds human HHLA2 with an affinity of at least 50-fold to 800-fold over background; and/or enhances HHLA2 binding to TMIGD2 at a ratio of greater than 2.

14. A pharmaceutical composition comprising at least one HHLA2 binding agent of any of claims 1-13, and a pharmaceutically acceptable carrier.

15. Use of at least one HHLA2 binding agent of any one of claims 1-13, or a pharmaceutical composition of claim 14 in the manufacture of a medicament for modulating an immune response or treating a subject having or is at risk of developing cancer .

16. The use of claim 15, wherein the cancer is a solid tumor or a hematological cancer; optionally wherein the solid tumor is or comprises one or more of:

a renal cancer, a bone cancer, a skin cancer, a breast cancer, a cervical cancer, a colorectal cancer, an endometrial cancer, a lung cancer, an ovarian cancer, a liver cancer, cholangiocarcinoma, or a thyroid cancer; optionally wherein the hematological cancer comprises or is a leukemia or lymphoma;

optionally wherein the leukemia comprises or is acute lymphocytic leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic leukemia, or acute leukemia;

optionally wherein the lymphoma comprises or is Hodgkin lymphoma (HL), non-Hodgkin's lymphoma, lymphocytic lymphoma, or diffuse large B cell lymphoma (DLBCL).

17. The use of any one of claims 15-16, wherein the HHLA2 binding agent is to be administered parenterally.

18. The use of claim 17, wherein the parenteral administration is or comprises subcutaneous, intravenous, intramuscular, or intrasternal injection or infusion.

19. The use of any one of claims 15-18, wherein the HHLA2 binding agent is to be administered in combination with a second agent.

20. A nucleic acid encoding at least one HHLA2 binding agent of any one of claims 1-13, or an antigen-binding fragment thereof.

21. An expression vector comprising at least one nucleic acid of claim 20.

22. A host cell comprising or expressing at least one HHLA2 binding agent of any one of claims 1-13, comprising at least one nucleic acid of claim 20, or comprising at least one expression vector of claim 21.

23. A method of making an HHLA2 binding agent, comprising:

(i) culturing a host cell comprising at least one nucleic acid of claim 23 or at least one expression vector of claim 21 under conditions suitable for expression of the HHLA2 binding agent, and

(ii) recovering the HHLA2 binding agent.

24. A method of detecting the presence or level of an HHLA2 polypeptide in a sample comprising:
detecting the HHLA2 polypeptide in the sample using at least one HHLA2 binding agent of any one of claims 1-13.

25. A kit comprising at least one HHLA2 binding agent of any one of claims 1-13, and instructions for use and/or administration.

26. The kit of claim 25, wherein the HHLA2 binding agent forms a complex with an HHLA2 polypeptide;

wherein the complex is detected by an assay comprising an enzyme linked immunosorbent assay (ELISA), radioimmune assay (RIA), and/or Western blot; and/or

wherein the HHLA2 binding agent is directly labeled.

27. The at least one HHLA2 binding agent of any one of claims 1-13, or a pharmaceutical composition of claim 14 for use in therapy.