

Bispecific antibodies that bind to a surface antigen on target cells and an activating T cell antigen such as CD3 on T-cells (also called herein T cell bispecific antibodies or “TCBs”) hold great promise for the treatment of various cancers. The simultaneous binding of such an antibody to both of its targets will force a temporary interaction between target cell and T cell, causing crosslinking of the T cell receptor and subsequent activation of any cytotoxic T cell and subsequent lysis of the target cell. Given their potency in target cell killing, the choice of target and the specificity of the targeting antibody is of utmost importance for T cell bispecific antibodies to avoid on- and off-target toxicities. Intracellular proteins such as WT1 represent attractive targets, but are only accessible to T cell receptor (TCR)-like antibodies that bind major histocompatibility complex (MHC) presenting peptide antigens derived from the intracellular protein on the cell surface. An inherent issue of TCR-like antibodies is potential cross-reactivity with MHC molecules per se, or MHC molecules presenting peptides other than the desired one, which could compromise organ or tissue selectivity.

Summary

The disclosure provides a multispecific antibody that binds to human HLA-G and to a T cell activating antigen (particularly human CD3), comprising a first antigen binding moiety that binds to human HLA-G and a second antigen binding moiety that binds to a T cell activating antigen (particularly human CD3).

In one aspect the multispecific antibody that binds to human HLA-G and to human CD3, comprising a first antigen binding moiety that binds to human HLA-G and a second antigen binding moiety that binds to human CD3, does not crossreact with a modified human HLA-G β 2M MHC I complex (wherein the HLA-G specific amino acids have been replaced by HLA-A consensus amino acids) comprising SEQ ID NO:44.

In one example of the disclosure the multispecific antibody is bispecific; and

the first antigen binding moiety antibody that binds to human HLA-G comprises

A) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:3; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; (ii) HVR-L2

comprising the amino acid sequence of SEQ ID NO:5 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:6; or

5 B) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:9, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:10, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:11; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:12; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:13 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:14; or

10 C) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:17, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:18, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:19; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:20; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:21 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:22; or

20 D) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:27; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:28; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:29 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30;

25 and the second antigen binding moiety, that binds to a T cell activating antigen binds to human CD3, and comprises

30 E) (a) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:56, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:57, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:58; and (b) a VL domain comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:59; (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:60 and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:61.

In one example of the disclosure the first antigen binding moiety

A)

i) comprises a VH sequence of SEQ ID NO:7 and a VL sequence of SEQ ID NO:8;

ii) or humanized variant of the VH and VL of the antibody under i); or

5 iii) comprises a VH sequence of SEQ ID NO:33 and a VL sequence of SEQ ID NO:34; or

B)

comprises a VH sequence of SEQ ID NO:15 and a VL sequence of SEQ ID NO:16; or

10 C)

comprises a VH sequence of SEQ ID NO:23 and a VL sequence of SEQ ID NO:24; or

D)

15 comprises a VH sequence of SEQ ID NO:31 and a VL sequence of SEQ ID NO:32;

and the second antigen binding moiety

E)

comprises a VH sequence of SEQ ID NO:62 and a VL sequence of SEQ ID NO:63.

20 In one example of the disclosure the

the first antigen binding moiety comprises i) a VH sequence of SEQ ID NO:31 and a VL sequence of SEQ ID NO:32; or ii) a VH sequence of SEQ ID NO:33 and a VL sequence of SEQ ID NO:34;

and the second antigen binding moiety

25 comprises a VH sequence of SEQ ID NO:62 and a VL sequence of SEQ ID NO:63.

In one example of the disclosure the multispecific antibody

- a) does not crossreact with a modified human HLA-G β 2M MHC I complex comprising SEQ ID NO:44; and/ or
- b) does not crossreact with human HLA-A2 β 2M MHC I complex comprising SEQ ID NO:39 and SEQ ID NO: 37; and/ or
- c) does not crossreact with a mouse H2Kd β 2M MHC I complex comprising SEQ ID NO:45; and/ or
- d) does not crossreact with rat RT1A β 2M MHC I complex comprising SEQ ID NO:47; and/ or
- e) inhibits ILT2 binding to monomeric HLA-G β 2M MHC I complex (comprising SEQ ID NO: 43); and/or
- f) inhibits ILT2 binding to trimeric HLA-G β 2M MHC I complex (comprising SEQ ID NO: 43), by more than 50% (in one embodiment by more than 60 %) (when compared to the binding without antibody) (see Example 4b); and/or
- g) inhibits ILT2 binding to monomeric and/or dimeric and/or trimeric HLA-G β 2M MHC I complex (comprising SEQ ID NO: 43), by more than 50% (in on embodiment by more than 80 %) (when compared to the binding without antibody) (see Example 4b); and/ or
- h) inhibits ILT2 binding to (HLA-G on) JEG3 cells (ATCC No. HTB36) (by more than 50 % (in one embodiment by more than 80%)) (when compared to the binding without antibody) (see Example 6); and/or
- i) binds to (HLA-G on) JEG3 cells (ATCC No. HTB36) (see Example 5), and inhibits ILT2 binding to (HLA-G on) JEG-3 cells (ATCC No. HTB36) (by more than 50 % (in one embodiment by more than 80%)) (when compared to the binding without antibody) (see Example 6); and/or
- j) inhibits CD8a binding to HLAG by more than 80% (when compared to the binding without antibody) (see e.g Example 4c); and/or

k) restores HLA-G specific suppressed immune response (e.g., suppressed Tumor necrose factor (TNF) alpha release) by monocytes co-cultured with JEG-3 cells (ATCC HTB36); and/or

5 l) induces T cell mediated cytotoxicity in the presence of HLAG expressing tumor cells (e.g. JEG-3 cells (ATCC HTB36) (see Example 12).

In one example of the disclosure the first and the second antigen binding moiety is a Fab molecule (are each a Fab molecule).

10 In one example of the disclosure the second antigen binding moiety is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1, particularly the variable domains VL and VH, of the Fab light chain and the Fab heavy chain are replaced by each other.

15 In one example of the disclosure the first antigen binding moiety is a Fab molecule wherein in the constant domain the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU
20 index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In one example of the disclosure the first and the second antigen binding moiety are fused to each other, optionally via a peptide linker.

25 In one example of the disclosure the first and the second antigen binding moiety are each a Fab molecule and wherein either (i) the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety, or (ii) the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety.

30 In one example of the disclosure the multispecific antibody comprises a third antigen binding moiety.

In one example of the disclosure such third antigen moiety is identical to the first antigen binding moiety.

In one example of the disclosure the multispecific antibody comprise an Fc domain composed of a first and a second subunit.

5 In one example of the disclosure the first, the second and, where present, the third antigen binding moiety are each a Fab molecule; and wherein either (i) the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, or (ii) the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety and the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain; and wherein the third antigen binding moiety, where present, is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

In another aspect, the disclosure provides an isolated nucleic acid encoding the multispecific antibody described herein.

The disclosure provides a host cell comprising such nucleic acid.

20 The disclosure provides a method of producing an antibody comprising culturing the host cell so that the antibody is produced.

The disclosure provides such method of producing an antibody, further comprising recovering the antibody from the host cell.

25 In another aspect, the disclosure provides a pharmaceutical formulation comprising the multispecific antibody described herein and a pharmaceutically acceptable carrier.

The disclosure provides the antibody described herein for use as a medicament.

In another aspect, the disclosure provides the multispecific antibody described herein for use in treating cancer.

30 The disclosure provides the use of the antibody described herein in the manufacture of a medicament. In one example the medicament is for treatment of cancer.

The disclosure provides a method of treating an individual having cancer comprising administering to the individual an effective amount of the antibody described herein.

5 With the screening methods described herein new anti-HLA-G antibodies could be selected. These antibodies show highly valuable properties like strong inhibition of ILT2 binding to HLA-G expressed on JEG3 cells or inhibition of ILT2 binding to monomeric and/or dimeric and/or trimeric HLA-G β 2M MHC I complex.

10 Furthermore, the antibodies according to the disclosure are able to restore a HLA-G specific suppressed immune response, i.e. restoration of LPS-induced TNF α production by monocytes in co-culture with HLA-G-expressing cells.

In addition, the antibodies are highly specific and do not show cross reactivity with HLA-A MHC I complexes or MHC I complexes from mouse or rat origin.

Description of the Figures

Figure 1: Different isoforms of HLA-G

15 **Figure 2:** **Fig. 2A:** Schematic representation of HLA-G with molecule in association with β 2M

20 **Fig. 2B:** Structure of HLA-G molecule in association with certain receptors : HLA-G structure in complex with given receptors such as ILT4 and KIR2DL1. ILT4 structure (PDB code: 2DYP). The KIR2DL1 structure is taken from PDB code 1IM9 (KIR2DL1: HLA-Cw4 complex structure) and was positioned on HLA-G by superposition of the HLA-Cw4 and HLA-G structures. Receptors are shown in a ribbon representation, HLA-G is shown in a molecular surface representation. HLA-G residues that are unique or conserved in other HLA paralogs are colored in white and gray, respectively. Unique surface residues were replaced by a HLA consensus sequence in the chimeric counter antigen.

25 **Figure 3:** HLA-G antibodies which inhibit (or stimulate) HLA-G interaction/binding with ILT2 and ILT4 as well as CD8:

30 **Figure 3A:** ILT2 inhibition