

Hence there is the need to generate and/or select further improved, truly HLA-G specific antibodies with receptor inhibition properties.

Summary

5 The disclosure provides an antibody that bind to human HLA-G (and that inhibits
ILT2 binding to HLAG on JEG-3 cells (ATCC HTB36) and restores HLA-G
specific suppressed TNF alpha release by monocytes co-cultured with JEG-3
cells. In one aspect, the present disclosure provides an isolated antibody that
bind to human HLA-G; and that inhibits ILT2 binding to HLAG on JEG-3 cells
10 (ATCC HTB36) and restores HLA-G specific suppressed TNF alpha release
by monocytes co-cultured with JEG-3 cells

In another aspect, the present disclosure provides an isolated antibody that binds to
human HLA-G (in one embodiment the antibody binds to HLA-G β 2M MHC
I complex comprising SEQ ID NO: 43), wherein the antibody comprises

- 15 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
20 sequence of SEQ ID NO:6; or
- 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
25 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
- 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
30 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

- 5 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.

5 One example of the disclosure is an isolated antibody that binds to human HLA-G(in one embodiment the antibody binds to HLA-G β 2M MHC I complex comprising SEQ ID NO: 43), wherein the antibody

A)

- i) comprises a VH sequence of SEQ ID NO:7 and a VL sequence of SEQ ID NO:8;
- 10 ii) or humanized variant of the VH and VL of the antibody under i);or

B)

- i) comprises a VH sequence of SEQ ID NO:15 and a VL sequence of SEQ ID NO:16;
- ii) or humanized variant of the VH and VL of the antibody under i);or

15 C)

- i) comprises a VH sequence of SEQ ID NO:23 and a VL sequence of SEQ ID NO:24;
- ii) or humanized variant of the VH and VL of the antibody under i); or

D)

- 20 i) comprises a VH sequence of SEQ ID NO:31 and a VL sequence of SEQ ID NO:32;
- ii) or humanized variant of the VH and VL of the antibody under i).

In another aspect, the present disclosure provides an isolated antibody that binds to human HLA-G, wherein the antibody

- 25 a) binds to the same epitope as an antibody which comprises a VH sequence of SEQ ID NO:7 and a VL sequence of SEQ ID NO:8;

or b) binds to the same epitope as an antibody which comprises a VH sequence of SEQ ID NO:31 and a VL sequence of SEQ ID NO:32.

In one example the anti-HLA-G antibody described herein

- 5 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
- 5 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
- 10 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
- 15 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
- 20 i) binds to (HLA-G on) JEG3 cells (ATCC No. HTB36) (see Example 5), and 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
- 25 j) inhibits CD8a binding to HLAG by more than 80% (when compared to the binding without antibody) (see e.g Example 4c).

In one example the anti-HLA-G antibody is of IgG1 isotype.

In one example the anti-HLA-G antibody is of IgG1 isotype with mutations L234A, L235A and P329G (numbering according to the EU index of Kabat).

In one preferred example the anti-HLA-G antibody inhibits ILT2 binding to monomeric HLA-G β 2M MHC I complex.

In one example the anti-HLA-G antibody according to the disclosure is a monoclonal antibody.

5 In one example the anti-HLA-G antibody according to the disclosure is a human, humanized, or chimeric antibody.

In one example the anti-HLA-G antibody according to the disclosure which is an antibody fragment that binds to HLA-G.

10 In one example the anti-HLA-G antibody according to the disclosure which is Fab fragment.

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

In another aspect, the present disclosure provides a host cell comprising the nucleic acid described herein.

15 In another aspect, the present disclosure provides a method of producing an antibody comprising culturing the host cell described herein so that the antibody is produced.

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

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

In another aspect, the present disclosure provides the antibody described herein for use as a medicament.

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

In another aspect, the present 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.

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

The present disclosure uses tailor-made chimeric antigens and/or stringent screening assays to identify HLA-G specific antibodies among numerous candidates (avoiding cross-reactivity to other MHC class I complex molecules and at the same time

selecting HLA-G receptor (such as ILT2) blocking antibodies) which show HLA-G-specific induction (restoration) of TNF alpha in co-cultures of HLA-G expressin JEG-3 cells and monocytes. With these 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.

The disclosure provides antibodies that specifically binds to human HLA-G, inhibit ILT2 binding to HLAG, and restore a HLA-G specific suppressed immune response, restoration of Lipopolysaccharide (LPS) -induced TNFalpha production/secretion by monocytes in co-culture with HLA-G-expressing cells (as e.g. JEG-3 cells). The restoration of a truly HLA-G specific immune suppression of monocytes by HLA-G expressing cells like JEG-3 cells can be evaluated in comparison to JEG-3 cells with a HLA-G knock-out.

Thus the antibodies of the disclosure restore a HLAG specific release of TNF alpha in Lipopolysaccharide (LPS) stimulated co-cultures of HLA-G expressin JEG-3 cells and monocytes compared to untreated co-cultred JEG-3 cells (untreated cells are taken 0% negative reference; monocyte only cultures are taken as 100% positive reference, in which TNF alpha secretion is not suppressed by any HLA-G /IL-T2 specific effects((see Example 7).

In addition, the antibodies are highly specific and to 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

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

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