

tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Activated T cells transiently express PD1, but sustained hyperexpression of PD1 and its ligand PDL1 promote immune exhaustion, leading to persistence of viral infections, tumor evasion, increased infections and mortality. PD1 expression is induced by antigen recognition via the T-cell receptor and its expression is maintained primarily through continuous T-cell receptor signaling. After prolonged antigen exposure, the PD1 locus fails to be remethylated, which promotes continuous hyperexpression. Blocking the PD1 pathway can restore the exhausted T-cell functionality in cancer and chronic viral infections (Sheridan, *Nature Biotechnology* 30 (2012), 729-730). Monoclonal antibodies to PD-1 have been described, for example, in WO 2003/042402, WO 2004/004771, WO 2004/056875, WO 2004/072286, WO 2004/087196, WO 2006/121168, WO 2006/133396, WO 2007/005874, WO 2008/083174, WO 2008/156712, WO 2009/024531, WO 2009/014708, WO 2009/101611, WO 2009/114335, WO 2009/154335, WO 2010/027828, WO 2010/027423, WO 2010/029434, WO 2010/029435, WO 2010/036959, WO 2010/063011, WO 2010/089411, WO 2011/066342, WO 2011/110604, WO 2011/110621, WO 2012/145493, WO 2013/014668, WO 2014/179664, and WO 2015/112900.

It has also been shown that blocking both PD1 and TIM3 can restore the antibacterial immune responses, for instance in patients with acute alcoholic hepatitis (AAH). Lymphocytes from these patients express high levels of immune inhibitory receptors, produce lower levels of interferon gamma, and have increased IL10 production due to chronic endotoxin exposure. These effects can be reversed by blocking PD1 and TIM3, which increase the antimicrobial activities of T cells and neutrophils (Markwick et al, *Gastroenterology* 148 (2015), 590–602).

Bispecific antibodies against TIM3 and PD1 for immunotherapy in chronic immune conditions have already been described in WO 2011/159877. However, there is a need of providing new bispecific antibodies that not only simultaneously bind to PD1 and TIM3 and thus selectively target T cells expressing both PD1 and TIM3, but that also avoid blocking of TIM3 on other cells such as innate immune cells, for example naive dendritic cells (DCs) and monocytes. The bispecific antibodies of the present invention do not only effectively block PD1 and Tim3 on T cells overexpressing both PD1 and TIM3, they are very selective for these cells and thereby side effects by administering highly active TIM3 antibodies may be avoided.

SUMMARY

In one aspect, the disclosure provides a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, wherein

In one embodiment, the bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3 is bivalent.

5 In another embodiment, provided is a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, wherein the bispecific antibody binds to TIM3 with low affinity and binds to PD1 with high affinity. In a particular embodiment, the disclosure provides a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, wherein the bispecific antibody binds to TIM3 with
10 an at least 50fold lower binding affinity when compared to the binding to PD1, more particularly with an at least 100fold lower binding affinity when compared to the binding to PD1. In one preferred embodiment the binding affinity (KD) is determined with Surface Plasmon Resonance Assay (as described e.g. in Example 12.)

15 In a further embodiment, provided is a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, wherein

said first antigen-binding site specifically binding to PD1 comprises

- (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 43 and a VL domain comprising the amino acid sequence of SEQ ID NO: 44, or
- 20 (b) a VH domain comprising the amino acid sequence of SEQ ID NO: 45 and a VL domain comprising the amino acid sequence of SEQ ID NO: 46, or
- (c) a VH domain comprising the amino acid sequence of SEQ ID NO: 45 and a VL domain comprising the amino acid sequence of SEQ ID NO: 47, or
- (d) a VH domain comprising the amino acid sequence of SEQ ID NO: 45 and a VL domain
25 comprising the amino acid sequence of SEQ ID NO: 48, or
- (e) a VH domain comprising the amino acid sequence of SEQ ID NO: 45 and a VL domain comprising the amino acid sequence of SEQ ID NO: 49,

and said second antigen-binding site specifically binding to TIM3 comprises

- (a) a VH domain comprising the amino acid sequence of SEQ ID NO: 7 and a VL domain
30 comprising the amino acid sequence of SEQ ID NO: 8, or
- (b) a VH domain comprising the amino acid sequence of SEQ ID NO: 9 and a VL domain comprising the amino acid sequence of SEQ ID NO: 10, or

(c) a VH domain comprising the amino acid sequence of SEQ ID NO: 13 and a VL domain comprising the amino acid sequence of SEQ ID NO: 14, or

(d) a VH domain comprising the amino acid sequence of SEQ ID NO: 15 and a VL domain comprising the amino acid sequence of SEQ ID NO: 16, or

5 (e) a VH domain comprising the amino acid sequence of SEQ ID NO: 23 and a VL domain comprising the amino acid sequence of SEQ ID NO: 24, or

(f) a VH domain comprising the amino acid sequence of SEQ ID NO: 25 and a VL domain comprising the amino acid sequence of SEQ ID NO: 26, or

10 (g) a VH domain comprising the amino acid sequence of SEQ ID NO: 27 and a VL domain comprising the amino acid sequence of SEQ ID NO: 28, or

(h) a VH domain comprising the amino acid sequence of SEQ ID NO: 35 and a VL domain comprising the amino acid sequence of SEQ ID NO: 36.

15 In a particular embodiment, provided is a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, wherein

said first antigen-binding site specifically binding to PD1 comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 45 and a VL domain comprising the amino acid sequence of SEQ ID NO: 46,

20 and said second antigen-binding site specifically binding to TIM3 comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 15 and a VL domain comprising the amino acid sequence of SEQ ID NO: 16 or a VH domain comprising the amino acid sequence of SEQ ID NO: 25 and a VL domain comprising the amino acid sequence of SEQ ID NO: 26.

25 Particularly, the disclosure provides a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, wherein

said first antigen-binding site specifically binding to PD1 comprises
a VH domain comprising

- 30 (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:37,
(ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:38, and
(iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO:39; and
a VL domain comprising
(i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:40;

- (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:41, and
- (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:42; and

said second antigen-binding site specifically binding to TIM3 comprises a VH domain comprising

- 5 (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 of SEQ ID NO:19; and
- a VL domain comprising
- (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:20,
 - 10 (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.

More particularly, provided is a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, wherein said first antigen-binding site specifically binding to PD1 comprises a VH domain

15 comprising the amino acid sequence of SEQ ID NO: 45 and a VL domain comprising the amino acid sequence of SEQ ID NO: 46, and said second antigen-binding site specifically binding to TIM3 comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 25 and a VL domain comprising the amino acid sequence of SEQ ID NO: 26.

In a particular embodiment, the disclosure provides a bispecific antibody comprising a first

20 antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, wherein the bispecific antibody binds to TIM3 with an at least 50fold lower binding affinity when compared to the binding to PD1, more particularly with an at least 100fold lower binding affinity when compared to the binding to PD1.

In a further embodiment, the bispecific antibody comprising a first antigen-binding site

25 that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3 is a human, humanized or chimeric antibody. In particular, it is a humanized or chimeric antibody.

In another embodiment, the disclosure relates to a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that

30 specifically binds to TIM3, wherein the bispecific antibody comprises an Fc domain, a first Fab fragment comprising the antigen-binding site that specifically binds to PD1 and a second Fab fragment comprising the antigen-binding site that specifically binds to TIM3.

In particular, the Fc domain is an IgG domain, more particularly an IgG1 Fc domain or an IgG4 Fc domain.

In one embodiment, the disclosure relates to a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor, in particular towards Fcγ receptor. In particular, the Fc domain is of human IgG1 subclass with the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

In another embodiment, the disclosure relates to a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, wherein the Fc domain comprises a modification promoting the association of the first and second subunit of the Fc domain.

In one embodiment, the disclosure relates to a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, wherein the first subunit of the Fc domain comprises knobs and the second subunit of the Fc domain comprises holes according to the knobs into holes method. In a particular embodiment, the first subunit of the Fc domain comprises the amino acid substitutions S354C and T366W (EU numbering) and the second subunit of the Fc domain comprises the amino acid substitutions Y349C, T366S and Y407V (numbering according to Kabat EU index).

In an additional embodiment, the disclosure relates to a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, wherein in one of the Fab fragments the the variable domains VL and VH are replaced by each other so that the VH domain is part of the light chain and the VL domain is part of the heavy chain. In a particular embodiment, the bispecific antibody is one, wherein in the first Fab fragment comprising the antigen-binding site that specifically binds to PD1 the variable domains VL and VH are replaced by each other.

In a further embodiment, the disclosure is concerned with a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, wherein in one of the Fab fragments in the constant domain CL the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat EU Index), and in the constant domain CH1 the amino acids at positions 147 and 213 are substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index). In a particular embodiment, the bispecific antibody is one, wherein in the second Fab fragment comprising the antigen-binding site that specifically binds to TIM3 the constant domain CL the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat EU Index), and in the

constant domain CH1 the amino acids at positions 147 and 213 are substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

In another embodiment, the disclosure provides a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, comprising

(a) a first heavy chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 50, a first light chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 52, a second heavy chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 51, and a second light chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO:53, or

(b) a first heavy chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 54, a first light chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 56, a second heavy chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 55, and a second light chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO:57, or

(c) a first heavy chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 58, a first light chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 60, a second heavy chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 59, and a second light chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO:61, or

(d) a first heavy chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 62, a first light chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 64, a second heavy chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 63, and a second light chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO:65, or

(e) a first heavy chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 66, a first light chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 68, a second heavy chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO: 67, and a second light chain comprising an amino acid sequence with at least 95% sequence identity to the sequence of SEQ ID NO:69.

In a particular embodiment, the disclosure provides a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, comprising

- 5 (a) a first heavy chain comprising the amino acid sequence of SEQ ID NO: 50, a first light chain comprising the amino acid sequence of SEQ ID NO: 52, a second heavy chain comprising the amino acid sequence of SEQ ID NO: 51, and a second light chain comprising the amino acid sequence of SEQ ID NO:53, or
- 10 (b) a first heavy chain comprising the amino acid sequence of SEQ ID NO: 54, a first light chain comprising the amino acid sequence of SEQ ID NO: 56, a second heavy chain comprising the amino acid sequence of SEQ ID NO: 55, and a second light chain comprising the amino acid sequence of SEQ ID NO:57, or
- 15 (c) a first heavy chain comprising the amino acid sequence of SEQ ID NO: 58, a first light chain comprising the amino acid sequence of SEQ ID NO: 60, a second heavy chain comprising the amino acid sequence of SEQ ID NO: 59, and a second light chain comprising the amino acid sequence of SEQ ID NO:61, or
- (d) a first heavy chain comprising the amino acid sequence of SEQ ID NO: 62, a first light chain comprising the amino acid sequence of SEQ ID NO: 64, a second heavy chain comprising the amino acid sequence of SEQ ID NO: 63, and a second light chain comprising the amino acid sequence of SEQ ID NO:65, or
- 20 (e) a first heavy chain comprising the amino acid sequence of SEQ ID NO: 66, a first light chain comprising the amino acid sequence of SEQ ID NO: 68, a second heavy chain comprising the amino acid sequence of SEQ ID NO: 67, and a second light chain comprising the amino acid sequence of SEQ ID NO:69.

25 According to another aspect of the disclosure, there is provided a polynucleotide encoding the bispecific antibody as described herein before. The disclosure, in another aspect, provides a vector, particularly an expression vector, comprising a polynucleotide of the disclosure. The disclosure, in another aspect, provides a prokaryotic or eukaryotic host cell comprising the polynucleotide or the vector of the disclosure. In some embodiments the host cell is a eukaryotic cell, particularly a mammalian cell.

30 In another aspect, provided is a method for producing a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3 as described herein, comprising the steps of a) transforming a host cell with vectors comprising polynucleotides encoding said bispecific antibody, b) culturing the host cell according under conditions suitable for the expression of the bispecific antibody and c)
35 recovering the bispecific antibody from the culture. The disclosure also encompasses a bispecific antibody produced by the method of the disclosure.

The disclosure, in another aspect, provides a pharmaceutical composition comprising a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3 as described herein, and at least one pharmaceutically acceptable excipient.

- 5 The disclosure, in another aspect, provides the bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3 as described herein, or the pharmaceutical composition comprising the bispecific antibody, for use as a medicament.

10 In another aspect, the disclosure provides a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3 as described herein, or the pharmaceutical composition comprising the bispecific antibody, for use

- i) in the modulation of immune responses, such as restoring T cell activity,
- ii) in stimulating an immune response or function,
- 15 iii) in the treatment of infections,
- iv) in the treatment of cancer,
- v) in delaying progression of cancer,
- vi) in prolonging the survival of a patient suffering from cancer.

20 In one embodiment provided is the bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3 as described herein, or the pharmaceutical composition comprising the bispecific antibody, for use in the treatment of a disease in an individual in need thereof. In a specific aspect, the disclosure provides a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, or the
25 pharmaceutical composition comprising the bispecific antibody, for use in the treatment of cancer. In a further specific embodiment, a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, or the pharmaceutical composition comprising the bispecific antibody, for use in the modulation of immune responses is provided. In another aspect, a bispecific antibody comprising
30 a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3, or a pharmaceutical composition comprising the bispecific antibody for use in the treatment of a chronic viral infection is provided.

Also provided is the use of the the bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to
35 TIM3 as described herein for the manufacture of a medicament for the treatment of a disease in

an individual in need thereof, in particular for the manufacture of a medicament for the treatment of cancer, as well as a method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second
5 antigen-binding site that specifically binds to TIM3 as described herein in a pharmaceutically acceptable form. In a specific embodiment, the disease is cancer. In another specific embodiment, the disease is a chronic viral infection. In another embodiment, a method of modulating of immune responses in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the bispecific antibody comprising a first antigen-
10 binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3 as described herein in a pharmaceutically acceptable form is provided. In any of the above aspects or embodiments the individual is preferably a mammal, particularly a human.

The disclosure, in another aspect, provides use of a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that
15 specifically binds to TIM3 as described herein in the manufacture of a medicament for the inhibition of growth of tumor cells, for the prevention or treatment of cancer or for the treatment of a chronic viral infection.

The disclosure, in another aspect, provides a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically
20 binds to TIM3 as described herein, or a pharmaceutical composition comprising the bispecific antibody for use in the prevention or treatment of cancer, wherein the bispecific antibody is to be administered in combination with a chemotherapeutic agent, radiation and/or other agents for use in cancer immunotherapy.

Furthermore, provided is a method of inhibiting the growth of tumor cells in an individual
25 comprising administering to the individual an effective amount of a bispecific antibody comprising a first antigen-binding site that specifically binds to PD1 and a second antigen-binding site that specifically binds to TIM3 as described herein to inhibit the growth of the tumor cells. The individual is preferably a mammal, particularly a human.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Blockade of PD1 with chimeric PD1-0103 strongly enhances IFN-gamma secretion by allogenic stimulated primary human T cells.

Figure 2: Blockade of PD1 with chimeric PD1-0103 strongly increases interferon-gamma (IFN- γ) secretion by allogenic stimulated primary human T cells.

Figure 3: Blockade of PD1 with chimeric PD1-0103 strongly increases tumor necrosis factor alpha (TNF) secretion by allogenic stimulated primary human T cells.

Figure 4: 4A) frequency of CD4 T cells producing Granzyme B and 4B) Amount of IFN- γ detected by absorbance (Optical Density, O.D.) in the supernatant of

25. The bispecific antibody according to any one of claims 1 to 19 or the pharmaceutical composition according to claim 24 for use as a medicament.
26. The bispecific antibody according to any one of claims 1 to 19 or the pharmaceutical composition according to claim 24 for use
- i) in the modulation of immune responses, such as restoring T cell activity,
 - ii) in stimulating an immune response or function,
 - iii) in the treatment of infections,
 - iv) in the treatment of cancer,
 - v) in delaying progression of cancer,
 - vi) in prolonging the survival of a patient suffering from cancer.
27. The bispecific antibody according to any one of claims 1 to 19 or the pharmaceutical composition according to claim 24 for use in the prevention or treatment of cancer.
28. The bispecific antibody according to any one of claims 1 to 19 or the pharmaceutical composition according to claim 24 for use in the treatment of a chronic viral infection.
29. The bispecific antibody according to any one of claims 1 to 19 or the pharmaceutical composition according to claim 24 for use in the prevention or treatment of cancer, wherein the bispecific antibody is to be administered in combination with a chemotherapeutic agent, radiation and/ or other agents for use in cancer immunotherapy.
30. Use of bispecific antibody according to any one of claims 1 to 19 in the manufacture of a medicament for the inhibition of growth of tumor cells, for the prevention or treatment of cancer or for the treatment of a chronic viral infection.
