

SUMMARY

The disclosure provides a TNF family ligand trimer-containing antigen binding molecule comprising

(a) at least one moiety capable of specific binding to PD1 and

- 5 (b) a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises two ectodomains of a TNF ligand family member or a fragment thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises only one ectodomain of said TNF ligand family member or a fragment thereof.

10 In a particular embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule comprising

(a) at least one moiety capable of specific binding to PD1,

(b) a first and a second polypeptide that are linked to each other by a disulfide bond,

- 15 wherein the antigen binding molecule is characterized in that the first polypeptide comprises two ectodomains of a TNF ligand family member or a fragment thereof that are connected to each other by a peptide linker and in that the second polypeptide comprises only one ectodomain of said TNF ligand family member or a fragment thereof, and

(c) an Fc domain composed of a first and a second subunit capable of stable association.

20 In a further embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule, comprising

(a) at least one moiety capable of specific binding to PD1 and

(b) a first and a second polypeptide that are linked to each other by a disulfide bond,

wherein the antigen binding molecule is characterized in that

- 25 (i) the first polypeptide contains a CH1 or CL domain and the second polypeptide contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of a TNF ligand family member or two fragments thereof that are connected to each other and to the CH1 or CL domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of said
- 30 TNF ligand family member or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, or

- (ii) the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises two
- 35 ectodomains of a TNF ligand family member or two fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker and wherein the second polypeptide comprises only one ectodomain of said TNF ligand

each other and to the CH1 or CL domain by a peptide linker and in that the second polypeptide comprises only one ectodomain of said TNF ligand family member or a fragment thereof connected by a peptide linker to the CL or CH1 domain of said polypeptide.

In one aspect, provided is a 4-1BB ligand (4-1BBL) trimer-containing antigen binding molecule comprising

(a) at least one Fab molecule capable of specific binding to PD1 comprising a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:13, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO:16, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO:18, and (b) a first polypeptide containing a first heavy chain constant domain (CH1) or a constant light chain domain (CL) and a second polypeptide containing a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain,

wherein the antigen binding molecule is characterized in that the first polypeptide comprises two ectodomains of 4-1BBL comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO: 6, SEQ ID NO:7 and SEQ ID NO:8 that are connected to each other and to the CH1 or CL domain by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO: 6, SEQ ID NO:7 and SEQ ID NO:8 connected via a peptide linker to the CL or CH1 domain of said polypeptide, and (c) an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G (numbering according to Kabat EU index).

In one embodiment, provided is a TNF family ligand trimer-containing antigen binding molecule comprising

(a) at least one moiety capable of specific binding to PD1,

(b) a first polypeptide containing a CH1 domain and a second polypeptide containing a CL domain, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain,

and wherein the antigen binding molecule is characterized in that the first polypeptide comprises two ectodomains of a TNF ligand family member or fragments thereof that are connected to each other and to the CH1 domain by a peptide linker and in that the second polypeptide comprises one ectodomain of said TNF ligand family member or a fragment thereof connected via a peptide linker to the CL domain of said polypeptide.

In another embodiment, provided is a TNF family ligand trimer-containing antigen binding molecule comprising

(a) at least one moiety capable of specific binding to PD1,

(b) a first polypeptide containing a CL domain and a second polypeptide containing a CH1

5 domain, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain,

and wherein the antigen binding molecule is characterized in that the first polypeptide comprises two ectodomains of a TNF ligand family member or fragments thereof that are connected to each other and to the CL domain by a peptide linker and in that the second polypeptide comprises one
10 ectodomain of said TNF ligand family member or a fragment thereof connected via a peptide linker to the CH1 domain of said polypeptide.

In a further embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule as defined herein before, wherein the moiety capable of specific binding PD1 is selected from the group consisting of an antibody, an antibody fragment and a
15 scaffold antigen binding protein. In particular, the moiety capable of specific binding to PD1 is selected from the group consisting of an antibody fragment, a Fab molecule, a crossover Fab molecule, a single chain Fab molecule, a Fv molecule, a scFv molecule, a single domain antibody, an aVH and a scaffold antigen binding protein.

In one embodiment, the disclosure provides a TNF family ligand trimer-containing antigen
20 binding molecule as defined herein before, wherein the moiety capable of specific binding to PD1 is an antibody fragment. In particular, the moiety capable of specific binding to PD1 is

In another particular embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule as described herein before, wherein the TNF family ligand trimer-containing antigen binding molecule comprises one Fab domain capable of specific binding to PD1.

5 In such embodiment, provided is a TNF family ligand trimer-containing antigen binding molecule, wherein the antigen binding molecule comprises

(i) a fusion polypeptide comprising a first subunit of a Fc domain and two ectodomains of a TNF ligand family member or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain by a peptide linker, (ii) a heavy chain comprising the VH domain of
10 the Fab domain capable of specific binding to PD1, a second subunit of the Fc domain and one ectodomain of said TNF ligand family member or a fragment thereof connected via a peptide linker to C-terminus of the CH3 domain, and (iii) a light chain comprising the VL domain of the Fab domain capable of specific binding to PD1, or

(i) a fusion polypeptide comprising a first subunit of a Fc domain and one ectodomain of a TNF
15 ligand family member or a fragment thereof that is connected to the C-terminus of the CH3 domain by a peptide linker, (ii) a heavy chain comprising the VH domain of the Fab domain capable of specific binding to PD1, a second subunit of the Fc domain and two ectodomains of said TNF ligand family member or fragments thereof that are connected to each other and to the C-terminus of the CH3 domain via a peptide linker, and (iii) a light chain comprising the VL
20 domain of the Fab domain capable of specific binding to PD1.

In a particular embodiment, provided is a TNF family ligand trimer-containing antigen binding molecule, wherein the antigen binding molecule comprises

(a) a fusion polypeptide comprising the amino acid sequence of SEQ ID NO:88, a heavy chain comprising the amino acid sequence of SEQ ID NO:87 and a light chain comprising the amino
25 acid sequence of SEQ ID NO:83 or SEQ ID NO:171,

(b) a fusion polypeptide comprising the amino acid sequence of SEQ ID NO:89, a heavy chain comprising the amino acid sequence of SEQ ID NO:90 and a light chain comprising the amino acid sequence of SEQ ID NO:83 or SEQ ID NO:171,

(c) a fusion polypeptide comprising the amino acid sequence of SEQ ID NO:91, a heavy chain
30 comprising the amino acid sequence of SEQ ID NO:86 and a light chain comprising the amino acid sequence of SEQ ID NO:83 or SEQ ID NO:171,

(b) a fusion polypeptide comprising the amino acid sequence of SEQ ID NO:93, a heavy chain comprising the amino acid sequence of SEQ ID NO:92 and a light chain comprising the amino acid sequence of SEQ ID NO:83 or SEQ ID NO:171.

35 The disclosure also provides an isolated polynucleotide encoding a TNF family ligand trimer-containing antigen binding molecule as defined herein

before. In another aspect, provided is an isolated polynucleotide encoding the 4-1BBL trimer-containing antigen binding molecule of the disclosure. In another aspect, provided is a vector, particularly an expression vector, comprising the isolated polynucleotide of the disclosure. In another aspect, provided is a host cell comprising the isolated polynucleotide or the vector of the disclosure. In some embodiments the host cell is an eukaryotic cell, particularly a mammalian cell.

There is also provided a method for producing the TNF family ligand trimer-containing antigen binding molecule of the disclosure, comprising culturing the host cell of the disclosure under conditions suitable for expression of the TNF family ligand trimer-containing antigen binding molecule, and isolating the antigen TNF family ligand trimer-containing binding molecule. The disclosure also encompasses a TNF family ligand trimer-containing antigen binding molecule produced by the method of the disclosure.

In another aspect, provided is a method of producing the 4-1BBL trimer-containing antigen binding molecule of the disclosure, comprising culturing the host cell of the disclosure under conditions suitable for expression of the 4-1BBL trimer-containing antigen binding molecule, and isolating the 4-1BBL trimer-containing antigen binding molecule.

There is also provided a pharmaceutical composition comprising the TNF family ligand trimer-containing antigen binding molecule of the disclosure and at least one pharmaceutically acceptable excipient. In another aspect, provided is a pharmaceutical composition comprising the 4-1BBL trimer-containing antigen binding molecule of the disclosure and at least one pharmaceutically acceptable excipient.

There is also provided the TNF family ligand trimer-containing antigen binding molecule of the disclosure, or the pharmaceutical composition of the disclosure, for use as a medicament. In another aspect, provided is the 4-1BBL trimer-containing antigen binding molecule of the disclosure, or the pharmaceutical composition of the disclosure, for use as a medicament. There is provided the TNF family ligand trimer-containing antigen binding molecule of the disclosure, or the pharmaceutical composition of the disclosure, for use in the treatment of a disease in an individual in need thereof. There is also provided the TNF family ligand trimer-containing antigen binding molecule of the disclosure, or the pharmaceutical composition of the disclosure, for use in the treatment of cancer. In another aspect, provided is the 4-1BBL trimer-containing antigen binding molecule of the disclosure for use in the treatment of cancer. There is also provided the TNF family ligand trimer-containing antigen binding molecule of the disclosure, or the pharmaceutical composition of the disclosure, for use in up-regulating or prolonging cytotoxic T cell activity.

Also provided is the use of the TNF family ligand trimer-containing antigen binding molecule of the disclosure 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
5 said individual a therapeutically effective amount of a composition comprising the TNF family ligand trimer-containing antigen binding molecule of the disclosure in a pharmaceutically acceptable form. In a specific embodiment, the disease is cancer. In another aspect, there is provided use of the 4-1BBL trimer-containing antigen binding molecule of the disclosure for the manufacture of a medicament for the treatment of cancer. Also provided is a method of up-
10 regulating or prolonging cytotoxic T cell activity in an individual having cancer, comprising administering to the individual an effective amount of the TNF family ligand trimer-containing antigen binding molecule of the disclosure, or the pharmaceutical composition of the disclosure. In any of the above embodiments the individual is preferably a mammal, particularly a human.

Claims

1. A 4-1BB ligand (4-1BBL) trimer-containing antigen binding molecule comprising
(a) at least one Fab molecule capable of specific binding to PD1 comprising a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:13, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO:16, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO:18, and
(b) a first polypeptide containing a first heavy chain constant domain (CH1) or a constant light chain domain (CL) and a second polypeptide containing a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain,
wherein the antigen binding molecule is characterized in that the first polypeptide comprises two ectodomains of 4-1BBL comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO: 6, SEQ ID NO:7 and SEQ ID NO:8 that are connected to each other and to the CH1 or CL domain by a peptide linker and in that the second polypeptide comprises one ectodomain of 4-1BBL comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO: 6, SEQ ID NO:7 and SEQ ID NO:8 connected via a peptide linker to the CL or CH1 domain of said polypeptide, and
(c) an IgG1 Fc domain comprising the amino acid substitutions L234A, L235A and P329G (numbering according to Kabat EU index).
2. The 4-1BBL trimer-containing antigen binding molecule of claim 1, wherein the ectodomain of a TNF ligand family member comprises the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:5.
3. The 4-1BBL trimer-containing antigen binding molecule of claim 1 or 2, comprising
(a) at least one Fab molecule capable of specific binding to PD1 comprising a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:13, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:14, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:15, and a VL domain comprising (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO:16, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO:17, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO:18, and
(b) a first and a second polypeptide that are linked to each other by a disulfide bond,

wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12 and SEQ ID NO:10 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4 and SEQ ID NO:5.

4. The 4-1BBL trimer-containing antigen binding molecule of any one of claims 1 to 3, wherein the moiety capable of specific binding to PD1 comprises
- (a) a VH domain comprising an amino acid sequence of SEQ ID NO:19 and a VL domain comprising an amino acid sequence of SEQ ID NO:20,
 - (b) a VH domain comprising an amino acid sequence of SEQ ID NO:21 and a VL domain comprising an amino acid sequence of SEQ ID NO:22,
 - (c) a VH domain comprising an amino acid sequence of SEQ ID NO:21 and a VL domain comprising an amino acid sequence of SEQ ID NO:23,
 - (d) a VH domain comprising an amino acid sequence of SEQ ID NO:21 and a VL domain comprising an amino acid sequence of SEQ ID NO:24, or
 - (e) a VH domain comprising an amino acid sequence of SEQ ID NO:21 and a VL domain comprising an amino acid sequence of SEQ ID NO:25.

5. The 4-1BBL trimer-containing antigen binding molecule of any one of claims 1 to 4, wherein the antigen binding molecule comprises
- a first heavy chain and a first light chain, wherein the first heavy chain comprises the VH domain of the Fab molecule capable of specific binding to PD1 and wherein the first light chain comprises the VL domain of the Fab molecule capable of specific binding to PD1, a first peptide comprising two ectodomains of 4-1BBL comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO: 6, SEQ ID NO:7 and SEQ ID NO:8 connected to each other by a first peptide linker fused at its C-terminus by a second peptide linker to a second heavy or light chain,
- and a second peptide comprising one ectodomain of said 4-1BBL comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO: 6, SEQ ID NO:7 and SEQ ID NO:8 fused at its C-terminus by a third peptide linker to a second light or heavy chain, respectively.

6. The 4-1BBL trimer-containing antigen binding molecule of any one of claims 1 to 4, wherein the first peptide comprising two ectodomains of 4-1BBL comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO: 6, SEQ ID NO:7 and SEQ ID NO:8

connected to each other by a first peptide linker is fused at its C-terminus by a second peptide linker to a CL domain that is part of a heavy chain, and the second peptide comprising one ectodomain of said 4-1BBL comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO: 6, SEQ ID NO:7 and SEQ ID NO:8 is fused at its C-terminus by a third peptide linker to a CH1 domain that is part of a light chain.

7. The TNF family ligand trimer-containing antigen binding molecule of any one of claims 1 to 6, wherein the Fc domain comprises knob-into-hole modifications promoting association of the first and the second subunit of the Fc domain.

8. The TNF family ligand trimer-containing antigen binding molecule of any one of claims 1 to 7, wherein the antigen binding molecule comprises

(i) a first heavy chain comprising the VH domain comprising the amino acid sequence of SEQ ID NO:19 and a first light chain comprising the VL domain comprising the amino acid sequence of SEQ ID NO:20 or

a first heavy chain comprising the VH domain comprising the amino acid sequence of SEQ ID NO:21 and a first light chain comprising the VL domain comprising the amino acid sequence selected from the group consisting of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24 and SEQ ID NO:25,

(ii) a second heavy chain comprising the amino acid sequence selected from the group consisting of SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78 and SEQ ID NO:80, and

(iii) a second light chain comprising the amino acid sequence selected from the group consisting of SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79 and SEQ ID NO:81.

9. The TNF family ligand trimer-containing antigen binding molecule of any one of claims 1 to 8, wherein the antigen binding molecule comprises

(a) a first heavy chain comprising the amino acid sequence of SEQ ID NO:82, a first light chain comprising the amino acid sequence of SEQ ID NO:83 or SEQ ID NO:171, a second heavy chain comprising the amino acid sequence of SEQ ID NO:74 and a second light chain comprising the amino acid sequence of SEQ ID NO:75,

(b) a first heavy chain comprising the amino acid sequence of SEQ ID NO:82, a first light chain comprising the amino acid sequence of SEQ ID NO:83 or SEQ ID NO:171, a second heavy chain comprising the amino acid sequence of SEQ ID NO:76 and a second light chain comprising the amino acid sequence of SEQ ID NO:77,

(c) a first heavy chain comprising the amino acid sequence of SEQ ID NO:82, a first light chain comprising the amino acid sequence of SEQ ID NO:83 or SEQ ID NO:171, a second heavy chain comprising the amino acid sequence of SEQ ID NO:78 and a second light chain

comprising the amino acid sequence of SEQ ID NO:79, or

(d) a first heavy chain comprising the amino acid sequence of SEQ ID NO:82, a first light chain comprising the amino acid sequence of SEQ ID NO:83 or SEQ ID NO:171, a second heavy chain comprising the amino acid sequence of SEQ ID NO:80 and a second light chain comprising the amino acid sequence of SEQ ID NO:81.

10. An isolated polynucleotide encoding the 4-1BBL trimer-containing antigen binding molecule of any one of claims 1 to 9.

11. A vector, particularly an expression vector, comprising the isolated polynucleotide of claim 10.

12. A host cell comprising the isolated polynucleotide of claim 10 or the vector of claim 11.

13. A method of producing the 4-1BBL trimer-containing antigen binding molecule of any one of claims 1 to 9, comprising culturing the host cell of claim 12 under conditions suitable for expression of the 4-1BBL trimer-containing antigen binding molecule, and isolating the 4-1BBL trimer-containing antigen binding molecule.

14. A pharmaceutical composition comprising the 4-1BBL trimer-containing antigen binding molecule of any one of claims 1 to 9 and at least one pharmaceutically acceptable excipient.

15. The 4-1BBL trimer-containing antigen binding molecule of any one of claims 1 to 9, or the pharmaceutical composition of claim 14, for use as a medicament.

16. The 4-1BBL trimer-containing antigen binding molecule of any one of claims 1 to 9 for use in the treatment of cancer.

17. Use of the 4-1BBL trimer-containing antigen binding molecule of any one of claims 1 to 9 in the manufacture of a medicament for the treatment of cancer.
