

SUMMARY

In one aspect, 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

family member or a fragment thereof connected via a peptide linker to C-terminus of the CH3 domain of said polypeptide, or

- (iii) the first polypeptide contains a VH-CL or a VL-CH1 domain and the second polypeptide contains a VL-CH1 domain or a VH-CL 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 fragments thereof that are connected to each other and to VH or VL by a peptide linker and wherein the second polypeptide comprises one ectodomain of said TNF ligand family member or a fragment thereof connected via a peptide linker to VL or VH of said polypeptide.

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 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

- (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 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 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 family member or a fragment thereof connected via a peptide linker to C-terminus of the CH3 domain of said polypeptide.

In one embodiment, the TNF ligand family member is one that costimulates human T-cell activation. Thus, the TNF family ligand trimer-containing antigen binding molecule comprises (a) at least one moiety capable of specific binding to a target cell antigen 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 two ectodomains of a TNF ligand family member or two fragments 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, wherein the TNF ligand family member costimulates human T-cell activation. More particularly, the TNF ligand family member is selected from 4-1BBL and OX40L.

5 In a particular embodiment, the TNF ligand family member is 4-1BBL.

In a further embodiment, the ectodomain of a TNF ligand family member comprises 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, particularly the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:5. More particularly, the
10 ectodomain of a TNF ligand family member comprises the amino acid sequence of SEQ ID NO:5.

In a further embodiment, the TNF family ligand trimer-containing antigen binding molecule of the disclosure comprises

(a) at least one moiety capable of specific binding to PD1 and
15 (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
20 SEQ ID NO:5.

In one embodiment, the TNF family ligand trimer-containing antigen binding molecule of the disclosure comprises

(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,
25 wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence of SEQ ID NO:10 and in that the second polypeptide comprises the amino acid sequence of SEQ ID NO:5.

In another embodiment, the TNF family ligand trimer-containing antigen binding molecule of the disclosure comprises

30 (a) at least one moiety capable of specific binding to PD1,
(b) a first polypeptide containing a CH1 or CL domain 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,
and wherein the antigen binding molecule is characterized in that the first polypeptide comprises
35 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 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.

5 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,
10 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.

15 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 domain, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond
20 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 ectodomain of said TNF ligand family member or a fragment thereof connected via a peptide
25 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 scaffold antigen binding protein. In particular, the moiety capable of specific binding to PD1 is
30 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 binding molecule as defined herein before, wherein the moiety capable of specific binding to
35 PD1 is an antibody fragment. In particular, the moiety capable of specific binding to PD1 is

selected from the group consisting of a Fab molecule, a crossover Fab molecule, a single chain Fab molecule, a Fv molecule, a scFv molecule, a single domain antibody and an aVH.

In one embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule as defined herein before, wherein the moiety capable of specific binding to

5 PD1 is a scaffold antigen binding protein.

In a particular embodiment, the disclosure is concerned with a TNF family ligand trimer-containing antigen binding molecule as defined above, wherein the moiety capable of specific binding to PD1 is a Fab molecule or a scFV molecule capable of specific binding to a target cell antigen. More particularly, the moiety capable of specific binding to PD1 is a Fab molecule.

10 The disclosure provides a TNF family ligand trimer-containing antigen binding molecule that comprises at least one moiety capable of specific binding to PD1. In a particular embodiment, the TNF family ligand trimer-containing antigen binding molecule comprises one moiety capable of specific binding to PD1, meaning the TNF family ligand trimer-containing antigen binding molecule is monovalent. In another embodiment, the disclosure provides a TNF
15 family ligand trimer-containing antigen binding molecule comprising two moieties capable of specific binding to PD1, meaning the TNF family ligand trimer-containing antigen binding molecule is bivalent.

In one embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule, wherein the moiety capable of specific binding to PD1 comprises

- 20 (a) 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,
- 25 (b) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:26, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:27, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:28, and a VL domain comprising (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO:29 (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO:30, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO:31,
- 30 (c) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:34, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:35, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:36, and a VL domain comprising (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO:37 (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO:38, and (vi) HVR-L3 comprising the amino acid sequence of SEQ

- ID NO:39,
- (d) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:42, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:43, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:44, and a VL domain comprising (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO:45 (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO:46, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO:47,
- (e) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:50, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:51, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:52, and a VL domain comprising (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO:53 (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO:54, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO:55,
- (f) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:58, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:59, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:60, and a VL domain comprising (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO:61 (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO:62, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO:63, or
- (g) a VH domain comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:66, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:67, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:68, and a VL domain comprising (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO:69 (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO:70, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO:71.

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

- (f) a VH domain comprising an amino acid sequence of SEQ ID NO:32 and a VL domain comprising an amino acid sequence of SEQ ID NO:33,
- (g) a VH domain comprising an amino acid sequence of SEQ ID NO:40 and a VL domain comprising an amino acid sequence of SEQ ID NO:41,
- 5 (h) a VH domain comprising an amino acid sequence of SEQ ID NO:48 and a VL domain comprising an amino acid sequence of SEQ ID NO:49,
- (i) a VH domain comprising an amino acid sequence of SEQ ID NO:56 and a VL domain comprising an amino acid sequence of SEQ ID NO:57,
- (k) a VH domain comprising an amino acid sequence of SEQ ID NO:64 and a VL domain
- 10 comprising an amino acid sequence of SEQ ID NO:65, or
- (l) a VH domain comprising an amino acid sequence of SEQ ID NO:72 and a VL domain comprising an amino acid sequence of SEQ ID NO:73.

- In one embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule, wherein the moiety capable of specific binding to PD1 comprises
- 15 (a) 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
 - 20 ID NO:18.

- In a particular embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule, 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,
 - 25 (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
 - 30 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.

- In one embodiment, provided is a TNF family ligand trimer-containing antigen binding molecule as defined herein before, wherein the moiety capable of specific binding to PD1 comprises
- 35 a variable heavy chain comprising an amino acid sequence of SEQ ID NO:21 and a variable light chain comprising an amino acid sequence of SEQ ID NO:22 or wherein the moiety capable of

specific binding to PD1 comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO:21 and a variable light chain comprising an amino acid sequence of SEQ ID NO:24.

In a particular embodiment, the disclosure relates to a TNF family ligand trimer-containing antigen binding molecule as defined above, wherein the peptide linker is (G4S)₂, i.e. a peptide
5 linker of SEQ ID NO:117. In one embodiment, the peptide linker in all instances is (G4S)₂.

The disclosure is further concerned with a TNF family ligand trimer-containing antigen binding molecule as defined herein before, comprising an Fc domain composed of a first and a second subunit capable of stable association.

In one embodiment, the TNF family ligand trimer-containing antigen binding molecule of
10 the disclosure comprising (c) an Fc domain composed of a first and a second subunit capable of stable association further comprises (a) a Fab molecule capable of specific binding to a target cell antigen, wherein the Fab heavy chain is fused at the C-terminus to the N-terminus of a CH2 domain in the Fc domain.

In a further embodiment, the Fc domain is an IgG, particularly an IgG1 Fc domain or an
15 IgG4 Fc domain.

More particularly, the Fc domain is an IgG1 Fc domain. In a particular embodiment, the Fc domain comprises a modification promoting the association of the first and second subunit of the Fc domain. In a specific embodiment, the disclosure provides a TNF family ligand trimer-
20 containing antigen binding molecule, wherein the first subunit of the Fc domain comprises the amino acid substitutions S354C and T366W (numbering according to Kabat EU index) and the second subunit of the Fc domain comprises the amino acid substitutions Y349C, T366S, L368A and Y407V (numbering according to Kabat EU index).

In another embodiment, the disclosure is concerned with a TNF family ligand trimer-
25 containing antigen binding molecule as defined herein before, comprising (c) an Fc domain composed of a first and a second subunit capable of stable association, 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 comprises amino acid substitutions at positions 234 and 235 (EU numbering) and/or 329 (EU numbering) of the IgG heavy chains. More particularly,
30 provided is a trimeric TNF family ligand-containing antigen binding molecule according to the disclosure which comprises an IgG1 Fc domain comprising the amino acid substitutions at positions 234 and 235 (EU numbering) and/or 329 (EU numbering), in particular the amino acid substitutions L234A, L235A and P329G (EU numbering).

In a further embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule, wherein the antigen binding molecule comprises a first heavy chain and a first light chain, both comprising a Fab molecule capable of specific binding to PD1,

- 5 a first peptide comprising two ectodomains of a TNF ligand family member or fragments thereof 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 TNF ligand family member fused at its C-terminus by a third peptide linker to a second light or heavy chain, respectively.

- 10 In another embodiment, provided is a TNF family ligand trimer-containing antigen binding molecule, wherein the first peptide comprising two ectodomains of a TNF ligand family member or fragments thereof connected to each other by a first peptide linker is fused at its C-terminus by a second peptide linker to a CH1 domain that is part of a heavy chain,
and the second peptide comprising one ectodomain of said TNF ligand family member or a
15 fragment thereof is fused at its C-terminus by a third peptide linker to a CL domain that is part of a light chain.

- In yet another embodiment, provided is a TNF family ligand trimer-containing antigen binding molecule, wherein the first peptide comprising two ectodomains of a TNF ligand family member or fragments thereof connected to each other by a first peptide linker is fused at its C-terminus by a
20 second peptide linker to a CL domain that is part of a heavy chain,
and the second peptide comprising one ectodomain of said TNF ligand family member or a fragment thereof is fused at its C-terminus by a third peptide linker to a CH1 domain that is part of a light chain.

- In a further embodiment, the disclosure provides a TNF family ligand trimer-containing
25 antigen binding molecule, wherein the first peptide comprising two ectodomains of a TNF ligand family member or fragments thereof connected to each other by a first peptide linker is fused at its C-terminus by a second peptide linker to a VH domain that is part of a heavy chain,
and the second peptide comprising one ectodomain of said TNF ligand family member or a fragment thereof is fused at its C-terminus by a third peptide linker to a VL domain that is part of a light chain.

- 30 In a particular embodiment, the disclosure relates to a TNF family ligand trimer-containing antigen binding molecule as defined above, wherein the peptide linker is (G4S)₂, i.e. a peptide linker of SEQ ID NO:117. In one embodiment, the first, second and third peptide linker is (G4S)₂.

Provided is further a TNF family ligand trimer-containing antigen binding molecule, wherein in the CL domain adjacent to the TNF ligand family member the amino acid at position

123 (EU numbering) has been replaced by arginine (R) and the amino acid at position 124 (EU numbering) has been substituted by lysine (K), and wherein in the CH1 domain adjacent to the TNF ligand family member the amino acids at position 147 (EU numbering) and at position 213 (EU numbering) have been substituted by glutamic acid (E).

- 5 In a further embodiment, provided is a TNF family ligand trimer-containing antigen binding molecule as described herein before, wherein the antigen binding molecule comprises
- (a) a first heavy chain and a first light chain, both comprising a Fab molecule capable of specific binding to PD1,
 - (b) a second heavy chain comprising an amino acid sequence selected from the group consisting
- 10 of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:12 and SEQ ID NO:10, and
- a second light chain comprising an 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.

- In one embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule, wherein the antigen binding molecule comprises
- 15 (a) a Fab molecule capable of specific binding to PD1, and
- (b) a second heavy chain comprising an 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
 - a second light chain comprising an 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.

- 20 In a particular embodiment, provided is a TNF family ligand trimer-containing antigen binding molecule, 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
- 25 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
- 30 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..

- In another embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule, wherein the antigen binding molecule comprises
- 35 (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
5 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
10 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
15 comprising the amino acid sequence of SEQ ID NO:81.

In yet another 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,
20 wherein the antigen binding molecule is characterized in that the first polypeptide contains a CH3 domain and the second polypeptide contains a CH3 domain, respectively, and wherein the first polypeptide comprises 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 and wherein the second polypeptide comprises one ectodomain of said TNF ligand family
25 member or a fragment thereof connected via a peptide linker to the C-terminus of the CH3 domain of said polypeptide.

In particular, such a TNF family ligand trimer-containing antigen binding molecule comprises two Fab domains capable of specific binding to PD1.

In particular, provided is a TNF family ligand trimer-containing antigen binding molecule
30 as described herein before comprises

- (a) a first heavy chain comprising the amino acid sequence of SEQ ID NO:84, a second heavy chain comprising the amino acid sequence of SEQ ID NO:85, and two light chains comprising the amino acid sequence of SEQ ID NO:83 or SEQ ID NO:171, or
(b) a first heavy chain comprising the amino acid sequence of SEQ ID NO:86, a second heavy
35 chain comprising the amino acid sequence of SEQ ID NO:87, and two light chains comprising the amino acid sequence of SEQ ID NO:83 or SEQ ID NO:171.

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 According to another aspect of the disclosure, there is provided an isolated polynucleotide encoding a TNF family ligand trimer-containing antigen binding molecule as defined herein

before. 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.

5 In another aspect, provided is 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
10 binding molecule produced by the method of the disclosure.

 In another aspect, provided is 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 the TNF family ligand trimer-containing antigen binding
15 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. In an aspect, provided is the TNF family ligand trimer-containing antigen binding molecule of the disclosure, or the pharmaceutical
20 composition of the disclosure, for use in the treatment of cancer. In another aspect, provided is 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
25 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 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
30 acceptable form. In a specific embodiment, the disease is cancer. In another aspect, there is provided use of the TNF family ligand 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-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
35 disclosure. In any of the above embodiments the individual is preferably a mammal, particularly a human.

suitable for expression of the TNF family ligand trimer-containing antigen binding molecule, and isolating the TNF family ligand trimer-containing antigen binding molecule.

5 30. A pharmaceutical composition comprising the TNF family ligand trimer-containing antigen binding molecule of any one of claims 1 to 25 and at least one pharmaceutically acceptable excipient.

31. The TNF family ligand trimer-containing antigen binding molecule of any one of claims 1 to 25, or the pharmaceutical composition of claim 30, for use as a medicament.

10 32. The TNF family ligand trimer-containing antigen binding molecule of any one of claims 1 to 25, or the pharmaceutical composition of claim 30, for use in up-regulating or prolonging cytotoxic T cell activity.

33. The TNF family ligand trimer-containing antigen binding molecule of any one of claims 1 to 25 for use in the treatment of cancer.

34. Use of the TNF family ligand trimer-containing antigen binding molecule of any one of claims 1 to 25 for the manufacture of a medicament for the treatment of cancer.

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