

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

(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
15 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.

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

20 (a) at least one moiety capable of specific binding to a target cell antigen,

(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

25 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.

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

(a) at least one moiety capable of specific binding to a target cell antigen and

- 30 (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

- 5 (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 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
- 10 (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 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 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 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.

In one 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:96, SEQ ID NO: 373, SEQ ID NO:374 and SEQ ID NO:375, particularly the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:96.

In another embodiment, the ectodomain of a TNF ligand family member or fragment thereof 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 and SEQ ID NO:96, particularly the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:96. More particularly, the ectodomain of a TNF ligand family member comprises the amino acid sequence of SEQ ID NO:96.

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 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 the amino acid sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:97, SEQ ID NO:98 and SEQ ID NO:99 and in that the second polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:96, SEQ ID NO:3 and SEQ ID NO:4.

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 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 the amino acid sequence of SEQ ID NO:5 and in that the second polypeptide comprises the amino acid sequence of SEQ ID NO:6.

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 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 the amino acid sequence of SEQ ID NO:5 and in that the second polypeptide comprises the amino acid sequence of SEQ ID NO:183.

In yet 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 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 the amino acid sequence of SEQ ID NO:97 and in that the second polypeptide comprises the amino acid sequence of SEQ ID NO:184 or SEQ ID NO:185.

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

- (a) at least one moiety capable of specific binding to a target cell antigen,
 - (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 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.

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

- 5 (a) at least one moiety capable of specific binding to a target cell antigen,
- (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
- 10 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

- 15 molecule comprising
- (a) at least one moiety capable of specific binding to a target cell antigen,
- (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 between the CH1 and CL domain,
- 20 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 linker to the CH1 domain of said polypeptide.

25 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 to a target cell antigen is selected from the group consisting of an antibody, an antibody fragment and a scaffold antigen binding protein.

In one embodiment, the disclosure provides a TNF family ligand trimer-containing antigen

- 30 binding molecule as defined herein before, wherein the moiety capable of specific binding to a target cell antigen is an antibody fragment.

In particular, the moiety capable of specific binding to a target cell antigen 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

- 35 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 a target cell antigen is a scaffold antigen binding protein.

5 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 a target cell antigen is a Fab molecule capable of specific binding to a target cell antigen.

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

15 In another embodiment, provided is a TNF family ligand trimer-containing antigen binding molecule of the disclosure, wherein the target cell antigen is selected from the group consisting of Fibroblast Activation Protein (FAP), Carcinoembryonic Antigen (CEA), Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP), Epidermal Growth Factor Receptor (EGFR), CD19, CD20 and CD33.

In a particular embodiment, the target cell antigen is Fibroblast Activation Protein (FAP).

20 In one embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule, wherein the moiety capable of specific binding to FAP comprises a VH domain comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:7 or SEQ ID NO:100, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:101, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:102, and a VL domain comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:10 or SEQ ID NO:103, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:11 or SEQ ID NO:104, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:12 or SEQ ID NO:105.

30 In one embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule, wherein the moiety capable of specific binding to FAP comprises a VH domain comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:7, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:8 and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:9, and a VL domain comprising (iv) CDR-L1 comprising

the amino acid sequence of SEQ ID NO:10, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:11 and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:12.

In a particular embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule, wherein the moiety capable of specific binding to FAP comprises a
5 VH domain comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:100, (ii) CDR-H2 comprising the amino acid sequence SEQ ID NO:101, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:102, and a VL domain comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:103, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:104, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID
10 NO:105.

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 FAP comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO:16 and a variable light chain comprising an amino acid sequence of SEQ ID NO:17 or wherein the moiety
15 capable of specific binding to FAP comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO:106 and a variable light chain comprising an amino acid sequence of SEQ ID NO:107.

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

In a particular embodiment, the disclosure relates to a TNF family ligand trimer-containing
25 antigen binding molecule as defined above, wherein the peptide linker is (G4S)₂, i.e. a peptide linker of SEQ ID NO:13. In one embodiment, the first peptide linker is (G4S)₂, the second peptide linker is GSPGSSSSGS (SEQ ID NO:57) and the third peptide linker is (G4S)₂. In another embodiment, the first, the second and the third peptide linker is (G4S)₂.

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

In particular, the TNF family ligand trimer-containing antigen binding molecule of 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 IgG4 Fc domain. More particularly, the Fc domain is an IgG1 Fc domain. In a particular
5 embodiment, the Fc domain comprises a modification promoting the association of the first and second subunit of the Fc domain.

In another embodiment, the disclosure is concerned with a TNF family ligand trimer-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
10 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, provided is a trimeric TNF family ligand-containing antigen binding molecule according to the
15 disclosure which comprises an IgG1 Fc domain with 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
20 binding to a target cell antigen,
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
25 C-terminus by a third peptide linker to a second light or heavy chain, respectively.

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,
30 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 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
35 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 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
5 of a light chain.

In a further embodiment, the disclosure provides 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 VH domain that is part of a heavy chain,
10 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.

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
15 123 (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).

In a further embodiment, provided is a TNF family ligand trimer-containing antigen
20 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 a target cell antigen,
(b) a second heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:97, SEQ ID NO:98 and SEQ ID NO:99, and
25 a second light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:96, SEQ ID NO:3 and SEQ ID NO:4.

In one embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule, wherein the antigen binding molecule comprises
(a) a Fab molecule capable of specific binding to FAP, and
30 (b) a second heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:97, SEQ ID NO:98 and SEQ ID NO:99, and
a second light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:96, SEQ ID NO:3 and SEQ ID NO:4.

In a particular embodiment, the disclosure provides a TNF family ligand trimer-containing antigen
35 binding molecule comprising a moiety capable of specific binding to FAP. In one 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:16 and a first light chain comprising the VL domain comprising the amino acid sequence of SEQ ID NO:17 or

a first heavy chain comprising the VH domain comprising the amino acid sequence of SEQ ID NO:106 and a first light chain comprising the VL domain comprising the amino acid sequence of SEQ ID NO:107,

(ii) a second heavy chain comprising the amino acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:108, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:139 and SEQ ID NO:148, and

(iii) a second light chain comprising the amino acid sequence of SEQ ID NO:15, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114 and SEQ ID NO:115.

In another embodiment, the disclosure provides 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:16 and a first light chain comprising the VL domain comprising the amino acid sequence of SEQ ID NO:17 or

a first heavy chain comprising the VH domain comprising the amino acid sequence of SEQ ID NO:106 and a first light chain comprising the VL domain comprising the amino acid sequence of SEQ ID NO:107,

(ii) a second heavy chain comprising the amino acid sequence selected from the group consisting of SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119 and SEQ ID NO:173, and

(iii) a second light chain comprising the amino acid sequence selected from the group consisting of SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120 and SEQ ID NO:174.

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

In particular, such a TNF family ligand trimer-containing antigen binding molecule comprises two moieties capable of specific binding to a target cell antigen.

In one embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule comprising two moieties capable of specific binding to FAP. In particular,
5 provided is a TNF family ligand trimer-containing antigen binding molecule as described herein before comprises

- (i) a first heavy chain comprising the amino acid sequence of SEQ ID NO:121, a second heavy chain comprising the amino acid sequence of SEQ ID NO:122, and two light chains comprising the amino acid sequence of SEQ ID NO:19, or
- 10 (ii) a first heavy chain comprising the amino acid sequence of SEQ ID NO:123, a second heavy chain comprising the amino acid sequence of SEQ ID NO:124, and two light chains comprising the amino acid sequence of SEQ ID NO:125, or
- (iii) a first heavy chain comprising the amino acid sequence of SEQ ID NO:126, a second heavy chain comprising the amino acid sequence of SEQ ID NO:127, and two light chains comprising
15 the amino acid sequence of SEQ ID NO:125.

In another particular embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule as described herein before, wherein the target cell antigen is CD19.

- In one embodiment, provided is a TNF family ligand trimer-containing antigen binding
20 molecule, wherein the moiety capable of specific binding to CD19 comprises a VH domain comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:195 or SEQ ID NO:252, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:196 or SEQ ID NO:253, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:197 or SEQ ID NO:254, and a VL domain comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ
25 ID NO:198 or SEQ ID NO:249, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:199 or SEQ ID NO:250, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:200 or SEQ ID NO:251.

- In a further embodiment, provided is a TNF family ligand trimer-containing antigen binding molecule, wherein the moiety capable of specific binding to CD19 comprises a variable
30 heavy chain comprising an amino acid sequence of SEQ ID NO:201 and a variable light chain comprising an amino acid sequence of SEQ ID NO:202 or wherein the moiety capable of specific binding to FAP comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO:357 and a variable light chain comprising an amino acid sequence of SEQ ID NO:358.

- 35 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:201 and a first light chain comprising the VL domain comprising the amino acid sequence of SEQ ID NO:202 or
a first heavy chain comprising the VH domain comprising the amino acid sequence of SEQ ID NO:357 and a first light chain comprising the VL domain comprising the amino acid sequence of SEQ ID NO:358,
- (ii) a second heavy chain comprising the amino acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:108, SEQ ID NO:111 and SEQ ID NO:113, and
- (iii) a second light chain comprising the amino acid sequence of SEQ ID NO:15, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:112 and SEQ ID NO:114.

In another 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:201 and a first light chain comprising the VL domain comprising the amino acid sequence of SEQ ID NO:202 or
a first heavy chain comprising the VH domain comprising the amino acid sequence of SEQ ID NO:357 and a first light chain comprising the VL domain comprising the amino acid sequence of SEQ ID NO:358,
- (ii) a second heavy chain comprising the amino acid sequence selected from the group consisting of SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119 and SEQ ID NO:173, and
- (iii) a second light chain comprising the amino acid sequence selected from the group consisting of SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120 and SEQ ID NO:174.

In one embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule comprising two moieties capable of specific binding to CD19. In particular, provided is a TNF family ligand trimer-containing antigen binding molecule of any one of claims 1 to 14, 29, 30 and 32 to 34, wherein the antigen binding molecule comprises

- (i) a first heavy chain comprising the amino acid sequence of SEQ ID NO:209, a second heavy chain comprising the amino acid sequence of SEQ ID NO:210, and two light chains comprising the amino acid sequence of SEQ ID NO:206, or
- (ii) a first heavy chain comprising the amino acid sequence of SEQ ID NO:213, a second heavy chain comprising the amino acid sequence of SEQ ID NO:214, and two light chains comprising the amino acid sequence of SEQ ID NO:206, or
- (iii) a first heavy chain comprising the amino acid sequence of SEQ ID NO:309, a second heavy chain comprising the amino acid sequence of SEQ ID NO:310, and two light chains comprising the amino acid sequence of SEQ ID NO:279, or
- (iv) a first heavy chain comprising the amino acid sequence of SEQ ID NO:313, a second heavy

chain comprising the amino acid sequence of SEQ ID NO:314, and two light chains comprising the amino acid sequence of SEQ ID NO:279.

In another particular embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule as described herein before, wherein the target cell antigen is CEA.

In one embodiment, provided is a TNF family ligand trimer-containing antigen binding molecule, wherein the moiety capable of specific binding to CEA comprises a VH domain comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:321, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:322, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:323, and a VL domain comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO:324, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:325, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:326.

In another embodiment, provided is a TNF family ligand trimer-containing antigen binding molecule, wherein the moiety capable of specific binding to CEA comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO:329 and a variable light chain comprising an amino acid sequence of SEQ ID NO:330.

In one embodiment, provided is a TNF family ligand trimer-containing antigen binding molecule as described herein before, wherein the antigen binding molecule comprises (i) a first heavy chain comprising the VH domain comprising the amino acid sequence of SEQ ID NO:329 and a first light chain comprising the VL domain comprising the amino acid sequence of SEQ ID NO:330, (ii) a second heavy chain comprising the amino acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:108, SEQ ID NO:111 and SEQ ID NO:113, and (iii) a second light chain comprising the amino acid sequence of SEQ ID NO:15, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:112 and SEQ ID NO:114.

In another 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:329 and a first light chain comprising the VL domain comprising the amino acid sequence of SEQ ID NO:330, (ii) a second heavy chain comprising the amino acid sequence selected from the group consisting of SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119 and SEQ ID NO:173, and (iii) a second light chain comprising the amino acid sequence selected from the group consisting of SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120 and SEQ ID NO:174.

In one embodiment, the disclosure provides a TNF family ligand trimer-containing antigen binding molecule comprising two moieties capable of specific binding to CEA. Particularly, provided is a TNF family ligand trimer-containing antigen binding molecule, wherein the antigen binding molecule comprises

- 5 (i) a first heavy chain comprising the amino acid sequence of SEQ ID NO:337, a second heavy chain comprising the amino acid sequence of SEQ ID NO:338, and two light chains comprising the amino acid sequence of SEQ ID NO:334, or
(ii) a first heavy chain comprising the amino acid sequence of SEQ ID NO:341, a second heavy chain comprising the amino acid sequence of SEQ ID NO:342, and two light chains comprising
10 the amino acid sequence of SEQ ID NO:334.

In a further embodiment, provided is a TNF family ligand trimer-containing antigen binding molecule as described herein before, wherein the TNF ligand family member is OX40L. In one embodiment, provided is a TNF family ligand trimer-containing antigen binding molecule, wherein the ectodomain of a TNF ligand family member comprises the amino acid sequence of
15 SEQ ID NO:53 or SEQ ID NO:54, particularly the amino acid sequence of SEQ ID NO:53.

In a further embodiment, provided is a TNF family ligand trimer-containing antigen binding molecule of any one of claims 1 to 5, 10 to 24, 29, 30, 32 to 34, 38 to 40, 44 and 45, comprising

- (a) at least one moiety capable of specific binding to a target cell antigen and
20 (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 of SEQ ID NO:371 or SEQ ID:372 and in that the second polypeptide comprises the amino acid sequence of SEQ ID NO:53 or SEQ ID NO:54.

In another embodiment, provided is a TNF family ligand trimer-containing antigen binding
25 molecule, wherein the target cell antigen is Fibroblast Activation Protein (FAP) and the moiety capable of specific binding to FAP comprises a VH domain comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:7 or SEQ ID NO:100, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:8 or SEQ ID NO:101, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:102, and a VL domain comprising (iv)
30 CDR-L1 comprising the amino acid sequence of SEQ ID NO:10 or SEQ ID NO:103, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO:11 or SEQ ID NO:104, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO:12 or SEQ ID NO:105.

Particularly, provided is a TNF family ligand trimer-containing antigen binding molecule as described herein, wherein the antigen binding molecule comprises

(i) a first heavy chain comprising the VH domain comprising the amino acid sequence of SEQ

ID NO:16 and a first light chain comprising the VL domain comprising the amino acid sequence of SEQ ID NO:17 or

a first heavy chain comprising the VH domain comprising the amino acid sequence of SEQ ID NO:106 and a first light chain comprising the VL domain comprising the amino acid sequence of

5 SEQ ID NO:107,

(ii) a second heavy chain comprising the amino acid sequence selected from the group consisting of SEQ ID NO:355, and

(iii) a second light chain comprising the amino acid sequence of SEQ ID NO:356.

10 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. According to another aspect of the disclosure, there is provided a vector, particularly an expression vector, comprising the isolated polynucleotide of the disclosure. According to another aspect of the disclosure, there is provided a host cell comprising the isolated polynucleotide or the vector of the disclosure. In some embodiments the host cell is a eukaryotic cell, particularly a
15 mammalian cell.

In another aspect, provided is a method for producing the TNF family ligand trimer-containing antigen binding molecule of the disclosure, comprising the steps of (i) culturing the host cell of the disclosure under conditions suitable for expression of the antigen binding molecule, and (ii) recovering the antigen binding molecule. The disclosure also encompasses a
20 TNF family ligand trimer-containing antigen binding molecule produced by the method of the disclosure.

In another aspect, the disclosure further provides a pharmaceutical composition comprising the TNF family ligand trimer-containing antigen binding molecule of the disclosure and at least one pharmaceutically acceptable excipient.

25 In another aspect, the disclosure also provides for the TNF family ligand trimer-containing antigen binding molecule of the disclosure, or the pharmaceutical composition of the disclosure, for use as a medicament. The disclosure provides for 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 another aspect, provided
30 is 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.

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

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. Another aspect of the disclosure provides for 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. The individual is preferably a mammal, particularly a human.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the components for the assembly of split trimeric human 4-1BB ligands. Figure (1A) shows the dimeric ligand that is fused at the C-terminus to a human CH1 or CL domain or a VL or VH domain and Figure (1B) shows the monomeric ligand fused to human CL or CH1 domain or a VL or VH domain. Figure (1C) shows the dimeric ligand that is fused at the N-terminus to a human CH3 domain and Figure (1D) shows the monomeric ligand fused at the N-terminus to a human CH3 domain.

Figure 2 shows the 4-1BBL-trimer-containing antigen binding molecules Constructs 1.1 to 1.10 of the invention. The preparation and production of these constructs is described in Example 1. The VH and VL domains are those of anti-FAP antibody 28H1, the thick black point stands for the knob-into-hole modification. * symbolizes amino acid modifications in the CH1 and CL domain (so-called charged residues).

Figure 3 shows the components for the assembly of split trimeric murine 4-1BB ligands. Figure (3A) shows the dimeric ligand that is fused at the C-terminus to murine CL domain and Figure (3B) shows the monomeric ligand fused at the C-terminus to murine CH1 domain. Components for the assembly of FAP targeted split trimeric murine 4-1BB ligand. Figure (3C) shows the assembled murine 4-1BBL-trimer-containing antigen binding molecules as described in more detail in Example 1.3.

Figure 4 shows the 4-1BBL-trimer-containing antigen binding molecules Constructs 2.1 to 2.6 of the invention. The preparation and production of these constructs is described in Example 2. The VH and VL domains are those of anti-FAP antibody 4B9, the thick black point stands for the knob-into-hole modification. * symbolizes amino acid modifications in the CH1 and CL domain (so-called charged residues).

Figure 5A and **Figure 5B** show the “untargeted” variants of Constructs 1.1 and 1.2 comprising a DP47 Fab molecule instead of the anti-FAP Fab molecule. The molecules are named Control A and Control B, respectively. The preparation is described in Example 1.4. **Figure 5C** is a drawing of the monomeric 4-1BB Fc(kih) construct as prepared in Example 3.

Figure 6 relates to the binding of FAP-targeted 4-1BB ligand trimer-containing Fc(kih) fusion antigen binding molecule (FAP split 4-1BBL trimer, filled circles) or DP-47 untargeted 4-