

(WO 2005/086875). WO 2007/071426 or WO 2014/131712 describe bispecific antibodies targeting CD3 on T cells and carcinoembryonic antigen (CEA) on target cells.

The present invention provides novel, improved bispecific antigen binding molecules designed for T cell activation and re-direction, targeting CD3 and CEA, that combine good efficacy and produceability with low toxicity and favorable pharmacokinetic properties.

Summary

The present inventors have developed a novel T cell activating bispecific antigen binding molecule with unexpected, improved properties using a novel humanized anti-CEA antibody.

Thus, in a first aspect the present disclosure provides a T cell activating bispecific antigen binding molecule comprising

- (a) a first antigen binding moiety which specifically binds to a first antigen;
- (b) a second antigen binding moiety which specifically binds to a second antigen;

wherein the first antigen is an activating T cell antigen and the second antigen is CEA, or the first antigen is CEA and the second antigen is an activating T cell antigen; and

wherein the antigen binding moiety which specifically binds to CEA comprises a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the heavy chain complementarity determining region (HCDR) 1 of SEQ ID NO: 14, the HCDR 2 of SEQ ID NO: 15 and the HCDR 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 17, the LCDR 2 of SEQ ID NO: 18 and the LCDR 3 of SEQ ID NO: 19.

In one embodiment, the antigen binding moiety which specifically binds to CEA comprises a heavy chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 22 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 23.

In particular embodiments, the first and/or the second antigen binding moiety is a Fab molecule. In a particular embodiment, the second antigen binding moiety is a Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other (i.e. according to such embodiment, the second Fab molecule is a crossover Fab

molecule wherein the variable or constant domains of the Fab light chain and the Fab heavy chain are exchanged).

In particular embodiments, the first (and the third, if any) Fab molecule is a conventional Fab molecule. In a further particular embodiment, not more than one Fab molecule capable of
5 specific binding to an activating T cell antigen is present in the T cell activating bispecific antigen binding molecule (i.e. the T cell activating bispecific antigen binding molecule provides monovalent binding to the activating T cell antigen).

In one embodiment, the first antigen is CEA and the second antigen is an activating T cell antigen. In a more specific embodiment, the activating T cell antigen is CD3, particularly CD3
10 epsilon.

In a particular embodiment, the T cell activating bispecific antigen binding molecule of the disclosure comprises

- (a) a first Fab molecule which specifically binds to a first antigen;
- (b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable
15 domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other;

wherein the first antigen is CEA and the second antigen is an activating T cell antigen;
wherein the first Fab molecule under (a) comprises a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the heavy chain complementarity

20 determining region (HCDR) 1 of SEQ ID NO: 14, the HCDR 2 of SEQ ID NO: 15 and the HCDR 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 17, the LCDR 2 of SEQ ID NO: 18 and the LCDR 3 of SEQ ID NO: 19.

According to the disclosure, the ratio of a desired bispecific antibody compared to undesired side
25 products, in particular Bence Jones-type side products occurring in bispecific antibodies with a VH/VL domain exchange in one of their binding arms, can be improved by the introduction of charged amino acids with opposite charges at specific amino acid positions in the CH1 and CL domains (sometimes referred to herein as "charge modifications").

Thus, in some embodiments the first antigen binding moiety under (a) is a first Fab molecule
30 which specifically binds to a first antigen, the second antigen binding moiety under (b) is a second Fab molecule which specifically binds to a second antigen wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other;

213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In one embodiment, in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) (numbering according to Kabat), and in the constant domain CH1 of the second Fab molecule under b) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

In another embodiment, in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by arginine (R) (numbering according to Kabat), and in the constant domain CH1 of the second Fab molecule under b) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

In a particular embodiment, the T cell activating bispecific antigen binding molecule of the disclosure comprises

(a) a first Fab molecule which specifically binds to a first antigen;

(b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other;

wherein the first antigen is CEA and the second antigen is an activating T cell antigen;

wherein the first Fab molecule under (a) comprises a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the heavy chain complementarity

determining region (HCDR) 1 of SEQ ID NO: 14, the HCDR 2 of SEQ ID NO: 15 and the

HCDR 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light

chain variable region, comprising the light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 17, the LCDR 2 of SEQ ID NO: 18 and the LCDR 3 of SEQ ID NO: 19; and

wherein in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R))

and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid

(D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In some embodiments, the T cell activating bispecific antigen binding molecule according to the disclosure further comprises a third antigen binding moiety which specifically binds to the first antigen. In particular embodiments, the third antigen binding moiety is identical to the first antigen binding moiety. In one embodiment, the third antigen binding moiety is a Fab molecule.

In particular embodiments, the third and the first antigen binding moiety are each a Fab molecule and the third Fab molecule is identical to the first Fab molecule. In these embodiments, the third

10 Fab molecule thus comprises the same amino acid substitutions, if any, as the first Fab molecule. Like the first Fab molecule, the third Fab molecule particularly is a conventional Fab molecule.

If a third antigen binding moiety is present, in a particular embodiment the first and the third antigen moiety specifically bind to CEA, and the second antigen binding moiety specifically binds to an activating T cell antigen, particularly CD3, more particularly CD3 epsilon.

15 In some embodiments of the T cell activating bispecific antigen binding molecule according to the disclosure the first antigen binding moiety under a) and the second antigen binding moiety under b) are fused to each other, optionally via a peptide linker. In particular embodiments, the first and the second antigen binding moiety are each a Fab molecule. In a specific such embodiment, the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the

20 N-terminus of the Fab heavy chain of the first Fab molecule. In an alternative such embodiment, the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule. In embodiments wherein either (i) the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule or (ii) the first Fab molecule is fused at the C-terminus of the Fab

25 heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule, additionally the Fab light chain of the Fab molecule and the Fab light chain of the second Fab molecule may be fused to each other, optionally via a peptide linker.

In particular embodiments, the T cell activating bispecific antigen binding molecule according to the disclosure additionally comprises an Fc domain composed of a first and a second subunit capable of

30 stable association.

The T cell activating bispecific antigen binding molecule according to the disclosure can have different configurations, i.e. the first, second (and optionally third) antigen binding moiety may be fused to

molecule essentially comprises an immunoglobulin molecule with an additional Fab molecule N-terminally fused to one of the immunoglobulin Fab arms, wherein in said additional Fab molecule the heavy and light chain variable regions VH and VL (or the constant regions CH1 and CL in embodiments wherein no charge modifications as described herein are introduced in CH1 and CL domains) are exchanged/replaced by each other (see Figure 1C, F).

In a particular embodiment, the immunoglobulin molecule comprised in the T cell activating bispecific antigen binding molecule according to the disclosure is an IgG class immunoglobulin. In an even more particular embodiment the immunoglobulin is an IgG₁ subclass immunoglobulin. In another embodiment, the immunoglobulin is an IgG₄ subclass immunoglobulin.

10 In a particular embodiment, the disclosure provides a T cell activating bispecific antigen binding molecule comprising

- a) a first Fab molecule which specifically binds to a first antigen;
- b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other;
- c) a third Fab molecule which specifically binds to the first antigen; and
- d) an Fc domain composed of a first and a second subunit capable of stable association; wherein the first antigen is CEA and the second antigen is an activating T cell antigen, particularly CD3, more particularly CD3 epsilon;

20 wherein the third Fab molecule under c) is identical to the first Fab molecule under a); wherein

- (i) the first Fab molecule under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule under b), and the second Fab molecule under b) and the third Fab molecule under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d), or
- (ii) the second Fab molecule under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule under a), and the first Fab molecule under a) and the third Fab molecule under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d); and

30 wherein the first Fab molecule under a) and the third Fab molecule under c) comprise a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the heavy chain complementarity determining region (HCDR) 1 of SEQ ID NO: 14, the HCDR 2 of SEQ ID NO: 15 and the HCDR 3 of SEQ ID NO: 16, and a light chain variable region,

particularly a humanized light chain variable region, comprising the light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 17, the LCDR 2 of SEQ ID NO: 18 and the LCDR 3 of SEQ ID NO: 19.

In another embodiment, the disclosure provides a T cell activating bispecific antigen binding molecule comprising

a) a first Fab molecule which specifically binds to a first antigen;
b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other;

c) an Fc domain composed of a first and a second subunit capable of stable association; wherein the first antigen is CEA and the second antigen is an activating T cell antigen, particularly CD3, more particularly CD3 epsilon;

wherein

(i) the first Fab molecule under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule under b), and the second Fab molecule under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c), or

(ii) the second Fab molecule under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule under a), and the first Fab molecule under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c); and

wherein the first Fab molecule under a) comprises a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the heavy chain complementarity determining region (HCDR) 1 of SEQ ID NO: 14, the HCDR 2 of SEQ ID NO: 15 and the HCDR 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 17, the LCDR 2 of SEQ ID NO: 18 and the LCDR 3 of SEQ ID NO: 19.

In a further embodiment, the disclosure provides a T cell activating bispecific antigen binding molecule comprising

a) a first Fab molecule which specifically binds to a first antigen;
b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other; and

c) an Fc domain composed of a first and a second subunit capable of stable association;
wherein

(i) the first antigen is CEA and the second antigen is an activating T cell antigen, particularly CD3, more particularly CD3 epsilon; or

5 (ii) the second antigen is CEA and the first antigen is an activating T cell antigen, particularly CD3, more particularly CD3 epsilon;

wherein the first Fab molecule under a) and the second Fab molecule under b) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c); and

10 wherein the Fab molecule which specifically binds to CEA comprises a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the heavy chain complementarity determining region (HCDR) 1 of SEQ ID NO: 14, the HCDR 2 of SEQ ID NO: 15 and the HCDR 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the light chain complementarity determining
15 region (LCDR) 1 of SEQ ID NO: 17, the LCDR 2 of SEQ ID NO: 18 and the LCDR 3 of SEQ ID NO: 19.

In all of the different configurations of the T cell activating bispecific antigen binding molecule according to the disclosure, the amino acid substitutions described herein, if present, may either be in the CH1 and CL domains of the first and (if present) the third Fab molecule, or in the CH1
20 and CL domains of the second Fab molecule. Preferably, they are in the CH1 and CL domains of the first and (if present) the third Fab molecule. In accordance with the concept of the disclosure, if amino acid substitutions as described herein are made in the first (and, if present, the third) Fab molecule, no such amino acid substitutions are made in the second Fab molecule. Conversely, if amino acid substitutions as described herein are made in the second Fab molecule, no such
25 amino acid substitutions are made in the first (and, if present, the third) Fab molecule. No amino acid substitutions are made in T cell activating bispecific antigen binding molecules comprising a Fab molecule wherein the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other.

In particular embodiments of the T cell activating bispecific antigen binding molecule according to
30 the disclosure, particularly wherein amino acid substitutions as described herein are made in the first (and, if present, the third) Fab molecule, the constant domain CL of the first (and, if present, the third) Fab molecule is of kappa isotype. In other embodiments of the T cell activating bispecific antigen binding molecule according to the disclosure, particularly wherein

amino acid substitutions as described herein are made in the second Fab molecule, the constant domain CL of the second Fab molecule is of kappa isotype. In some embodiments, the constant domain CL of the first (and, if present, the third) Fab molecule and the constant domain CL of the second Fab molecule are of kappa isotype.

- 5 In a particular embodiment, the disclosure provides a T cell activating bispecific antigen binding molecule comprising
- a) a first Fab molecule which specifically binds to a first antigen;
 - b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other;
 - 10 c) a third Fab molecule which specifically binds to the first antigen; and
 - d) an Fc domain composed of a first and a second subunit capable of stable association;
- wherein the first antigen is CEA and the second antigen is an activating T cell antigen, particularly CD3, more particularly CD3 epsilon;
- wherein the third Fab molecule under c) is identical to the first Fab molecule under a);
- 15 wherein in the constant domain CL of the first Fab molecule under a) and the third Fab molecule under c) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat), and wherein in the constant domain CH1 of the first Fab molecule under a) and the third Fab molecule under c) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is
- 20 substituted by glutamic acid (E) (numbering according to Kabat EU index);
- wherein
- (i) the first Fab molecule under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule under b), and the second Fab molecule under b) and the third Fab molecule under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d), or
 - 25 (ii) the second Fab molecule under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule under a), and the first Fab molecule under a) and the third Fab molecule under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d); and
- 30 wherein the first Fab molecule under a) and the third Fab molecule under c) comprise a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the heavy chain complementarity determining region (HCDR) 1 of SEQ ID NO: 14, the HCDR 2 of

SEQ ID NO: 15 and the HCDR 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 17, the LCDR 2 of SEQ ID NO: 18 and the LCDR 3 of SEQ ID NO: 19.

- 5 In an even more particular embodiment, the disclosure provides a T cell activating bispecific antigen binding molecule comprising
- a) a first Fab molecule which specifically binds to a first antigen;
 - b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other;
 - 10 c) a third Fab molecule which specifically binds to the first antigen; and
 - d) an Fc domain composed of a first and a second subunit capable of stable association;
- wherein the first antigen is CEA and the second antigen is an activating T cell antigen, particularly CD3, more particularly CD3 epsilon;
- wherein the third Fab molecule under c) is identical to the first Fab molecule under a);
- 15 wherein in the constant domain CL of the first Fab molecule under a) and the third Fab molecule under c) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by arginine (R) (numbering according to Kabat), and wherein in the constant domain CH1 of the first Fab molecule under a) and the third Fab molecule under c) the amino acid at position 147 is substituted by glutamic acid (E)
- 20 (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index);
- wherein the first Fab molecule under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule under b), and the second Fab molecule under b) and the third Fab molecule under c) are each fused at the C-terminus of the
- 25 Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d); and
- wherein the first Fab molecule under a) and the third Fab molecule under c) comprise a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the heavy chain complementarity determining region (HCDR) 1 of SEQ ID NO: 14, the HCDR 2 of SEQ ID NO: 15 and the HCDR 3 of SEQ ID NO: 16, and a light chain variable region,
- 30 particularly a humanized light chain variable region, comprising the light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 17, the LCDR 2 of SEQ ID NO: 18 and the LCDR 3 of SEQ ID NO: 19.

In another embodiment, the disclosure provides a T cell activating bispecific antigen binding molecule comprising

- a) a first Fab molecule which specifically binds to a first antigen;
 - b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other;
 - c) an Fc domain composed of a first and a second subunit capable of stable association;
- wherein the first antigen is CEA and the second antigen is an activating T cell antigen, particularly CD3, more particularly CD3 epsilon;

wherein in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat), and wherein in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index);

wherein

(i) the first Fab molecule under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule under b), and the second Fab molecule under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c), or

(ii) the second Fab molecule under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule under a), and the first Fab molecule under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c); and

wherein the first Fab molecule under a) comprises a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the heavy chain complementarity determining region (HCDR) 1 of SEQ ID NO: 14, the HCDR 2 of SEQ ID NO: 15 and the HCDR 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 17, the LCDR 2 of SEQ ID NO: 18 and the LCDR 3 of SEQ ID NO: 19.

In a further embodiment, the disclosure provides a T cell activating bispecific antigen binding molecule comprising

- a) a first Fab molecule which specifically binds to a first antigen;

having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second subunit, and an amino acid residue in the CH3 domain of the second subunit of the Fc domain is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable.

In a particular embodiment the Fc domain exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG₁ Fc domain. In certain embodiments the Fc domain is engineered to have reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a non-engineered Fc domain. In one embodiment, the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function. In one embodiment, the one or more amino acid substitution in the Fc domain that reduces binding to an Fc receptor and/or effector function is at one or more position selected from the group of L234, L235, and P329 (Kabat EU index numbering). In particular embodiments, each subunit of the Fc domain comprises three amino acid substitutions that reduce binding to an Fc receptor and/or effector function wherein said amino acid substitutions are L234A, L235A and P329G (Kabat EU index numbering). In one such embodiment, the Fc domain is an IgG₁ Fc domain, particularly a human IgG₁ Fc domain. In other embodiments, each subunit of the Fc domain comprises two amino acid substitutions that reduce binding to an Fc receptor and/or effector function wherein said amino acid substitutions are L235E and P329G (Kabat EU index numbering). In one such embodiment, the Fc domain is an IgG₄ Fc domain, particularly a human IgG₄ Fc domain. In one embodiment, the Fc domain of the T cell activating bispecific antigen binding molecule is an IgG₄ Fc domain and comprises the amino acid substitutions L235E and S228P (SPLE) (Kabat EU index numbering).

In one embodiment the Fc receptor is an Fcγ receptor. In one embodiment the Fc receptor is a human Fc receptor. In one embodiment, the Fc receptor is an activating Fc receptor. In a specific embodiment, the Fc receptor is human FcγRIIa, FcγRI, and/or FcγRIIIa. In one embodiment, the effector function is antibody-dependent cell-mediated cytotoxicity (ADCC).

In a specific embodiment of the T cell activating bispecific antigen binding molecule according to the disclosure, the antigen binding moiety which specifically binds to an activating T cell antigen, particularly CD3, more particularly CD3 epsilon, comprises a heavy chain variable region comprising the heavy chain complementarity determining region (HCDR) 1 of SEQ ID NO: 4, the HCDR 2 of SEQ ID NO: 5, the HCDR 3 of SEQ ID NO: 6, and a light chain variable

region comprising the light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 8, the LCDR 2 of SEQ ID NO: 9 and the LCDR 3 of SEQ ID NO: 10. In an even more specific embodiment, the antigen binding moiety which specifically binds to an activating T cell antigen, particularly CD3, more particularly CD3 epsilon, comprises a heavy chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 3 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 7. In some embodiments, the antigen binding moiety which specifically binds to an activating T cell antigen is a Fab molecule. In one specific embodiment, the second antigen binding moiety, particularly Fab molecule, comprised in the T cell activating bispecific antigen binding molecule according to the disclosure specifically binds to CD3, more particularly CD3 epsilon, and comprises the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 4, the heavy chain CDR 2 of SEQ ID NO: 5, the heavy chain CDR 3 of SEQ ID NO: 6, the light chain CDR 1 of SEQ ID NO: 8, the light chain CDR 2 of SEQ ID NO: 9 and the light chain CDR 3 of SEQ ID NO: 10. In an even more specific embodiment, said second antigen binding moiety, particularly Fab molecule, comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 3 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 7.

In a further specific embodiment of the T cell activating bispecific antigen binding molecule according to the disclosure, the antigen binding moiety, particularly Fab molecule, which specifically binds to CEA comprises the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 14, the heavy chain CDR 2 of SEQ ID NO: 15, the heavy chain CDR 3 of SEQ ID NO: 16, the light chain CDR 1 of SEQ ID NO: 17, the light chain CDR 2 of SEQ ID NO: 18 and the light chain CDR 3 of SEQ ID NO: 19. In an even more specific embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to CEA comprises a heavy chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 22 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 23. In one specific embodiment, the first (and, if present, the third) antigen binding moiety, particularly Fab molecule, comprised in the T cell activating bispecific antigen binding molecule according to the disclosure specifically binds to CEA, and comprises the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 14, the heavy chain CDR 2 of SEQ ID NO: 15, the

heavy chain CDR 3 of SEQ ID NO: 16, the light chain CDR 1 of SEQ ID NO: 17, the light chain CDR 2 of SEQ ID NO: 18 and the light chain CDR 3 of SEQ ID NO: 19. In an even more specific embodiment, said first (and, if present, said third) antigen binding moiety, particularly Fab molecule, comprises a heavy chain variable region comprising the amino acid sequence of
5 SEQ ID NO: 22 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 23.

In a particular embodiment, the disclosure provides a T cell activating bispecific antigen binding molecule comprising

- a) a first Fab molecule which specifically binds to a first antigen;
- 10 b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other;
- c) a third Fab molecule which specifically binds to the first antigen; and
- d) an Fc domain composed of a first and a second subunit capable of stable association;

15 wherein

- (i) the first antigen is CEA and the second antigen is CD3, particularly CD3 epsilon;
- (ii) the first Fab molecule under a) and the third Fab molecule under c) each comprise the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 14, the heavy chain CDR 2 of SEQ ID NO: 15, the heavy chain CDR 3 of SEQ ID NO: 16, the light chain CDR 1 of SEQ ID
20 NO: 17, the light chain CDR 2 of SEQ ID NO: 18 and the light chain CDR 3 of SEQ ID NO: 19, and the second Fab molecule under b) comprises the heavy chain CDR 1 of SEQ ID NO: 4, the heavy chain CDR 2 of SEQ ID NO: 5, the heavy chain CDR 3 of SEQ ID NO: 6, the light chain CDR 1 of SEQ ID NO: 8, the light chain CDR 2 of SEQ ID NO: 9 and the light chain CDR 3 of SEQ ID NO: 10; and
- 25 (iii) the first Fab molecule under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule under b), and the second Fab molecule under b) and the third Fab molecule under c) are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d).

In one embodiment, in the second Fab molecule under b) the variable domains VL and VH are
30 replaced by each other and further (iv) in the constant domain CL of the first Fab molecule under a) and the third Fab molecule under c) the amino acid at position 124 is substituted by lysine (K)

(numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R), particularly by arginine (R) (numbering according to Kabat), and in the constant domain CH1 of the first Fab molecule under a) and the third Fab molecule under c) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

According to another aspect of the disclosure there is provided one or more isolated polynucleotide(s) encoding a T cell activating bispecific antigen binding molecule of the disclosure. The disclosure further provides one or more expression vector(s) comprising the isolated polynucleotide(s) of the disclosure, and a host cell comprising the isolated polynucleotide(s) or the expression vector(s) of the disclosure. According to another aspect of the disclosure there is provided one or more vector, particularly expression vector, comprising the polynucleotide(s) of the disclosure. According to another aspect of the disclosure there is provided a host cell comprising the polynucleotide(s) of the disclosure or the vector(s) of the disclosure. In some embodiments the host cell is a eukaryotic cell, particularly a mammalian cell.

In another aspect is provided a method of producing the T cell activating bispecific antigen binding molecule of the disclosure, comprising the steps of a) culturing the host cell of the disclosure under conditions suitable for the expression of the T cell activating bispecific antigen binding molecule and optionally b) recovering the T cell activating bispecific antigen binding molecule. In another aspect is provided a T cell activating bispecific antigen binding molecule produced by the method of the disclosure.

In another aspect is provided a pharmaceutical composition comprising the T cell activating bispecific antigen binding molecule of the disclosure and a pharmaceutically acceptable carrier.

Also encompassed by the disclosure are methods of using the T cell activating bispecific antigen binding molecule and pharmaceutical composition of the disclosure. In one aspect the disclosure provides a T cell activating bispecific antigen binding molecule or a pharmaceutical composition of the disclosure for use as a medicament. In one aspect is provided a T cell activating bispecific antigen binding molecule or a pharmaceutical composition according to the disclosure for use in the treatment of a disease in an individual in need thereof. In a specific embodiment the disease is cancer.

In one aspect is provided the use of a T cell activating bispecific antigen binding molecule of the disclosure for the manufacture of a medicament for the treatment of a disease in an individual in need thereof. Also provided is a method of treating a disease in an individual, comprising
5 administering to said individual a therapeutically effective amount of a composition comprising the T cell activating bispecific antigen binding molecule according to the disclosure in a pharmaceutically

acceptable form. In a specific embodiment the disease is cancer. In any of the above embodiments the individual preferably is a mammal, particularly a human.

The disclosure also provides a method for inducing lysis of a target cell, particularly a tumor cell, comprising contacting a target cell with a T cell activating bispecific antigen binding molecule of the disclosure in the presence of a T cell, particularly a cytotoxic T cell. In one aspect is provided a method for inducing lysis of a target cell, comprising contacting a target cell with the T cell activating bispecific antigen binding molecule of the disclosure in the presence of a T cell.

Brief Description of the Drawings

FIGURE 1. Exemplary configurations of the T cell activating bispecific antigen binding molecules (TCBs) of the invention. (A, D) Illustration of the “1+1 CrossMab” molecule. (B, E) Illustration of the “2+1 IgG Crossfab” molecule with alternative order of Crossfab and Fab components (“inverted”). (C, F) Illustration of the “2+1 IgG Crossfab” molecule. (G, K) Illustration of the “1+1 IgG Crossfab” molecule with alternative order of Crossfab and Fab components (“inverted”). (H, L) Illustration of the “1+1 IgG Crossfab” molecule. (I, M) Illustration of the “2+1 IgG Crossfab” molecule with two CrossFabs. (J, N) Illustration of the “2+1 IgG Crossfab” molecule with two CrossFabs and alternative order of Crossfab and Fab components (“inverted”). (O, S) Illustration of the “Fab-Crossfab” molecule. (P, T) Illustration of the “Crossfab-Fab” molecule. (Q, U) Illustration of the “(Fab)₂-Crossfab” molecule. (R, V) Illustration of the “Crossfab-(Fab)₂” molecule. (W, Y) Illustration of the “Fab-(Crossfab)₂” molecule. (X, Z) Illustration of the “(Crossfab)₂-Fab” molecule. Black dot: optional modification in the Fc domain promoting heterodimerization. ++, --: amino acids of opposite charges optionally introduced in the CH1 and CL domains. Crossfab molecules are depicted as comprising an exchange of VH and VL regions, but may – in embodiments wherein no charge modifications are introduced in CH1 and CL domains – alternatively comprise an exchange of the CH1 and CL domains.

FIGURE 2. Binding of different humanized variants of T84.66 IgGs to cells. EC50 values, based on binding curves, were calculated by Graph Pad Prism and are presented in Table 1.

FIGURE 3. Illustration of the TCBs prepared in the Examples. (A, B) Illustration of “2+1 IgG CrossFab, inverted” anti-CEA/anti-CD3 TCB molecules with charge modifications (VH/VL exchange in CD3 binder, charge modification in CEA binder, molecule A and B). (C) Illustration of “1+1 IgG CrossFab, inverted” anti-CEA/anti-CD3 TCB molecule with charge modifications (VH/VL exchange in CD3 binder, charge modification in CEA binder, molecule C). (D) Illustration of “1+1 IgG CrossMab” anti-CEA/anti-CD3 TCB molecule with charge

57. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 49 or 54 or the pharmaceutical composition of claim 55 for use in the treatment of a disease in an individual in need thereof.

58. The T cell activating bispecific antigen binding molecule or the pharmaceutical composition of claim 57, wherein the disease is cancer.

59. Use of the T cell activating bispecific antigen binding molecule of any one of claims 1 to 49 or 54 for the manufacture of a medicament for the treatment of a disease in an individual in need thereof.

60. The use of claim 59, wherein said disease is cancer.

61. A method for inducing lysis of a target cell, comprising contacting a target cell with the T cell activating bispecific antigen binding molecule of any one of claims 1-49 or 54 in the presence of a T cell.