

Summary

According to the disclosure, the ratio of a desired bispecific antibody compared to undesired side 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.

Thus, in one aspect, the present 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;

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

wherein

- i) 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), and wherein in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index); or
- ii) in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the second Fab molecule under b) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

According to the disclosure, the second Fab molecule is a crossover Fab molecule wherein the variable regions of the Fab light chain and the Fab heavy chain are exchanged. In particular examples, the first (and the third, if any) Fab molecule is a conventional Fab molecule. In a further particular example, not more than one Fab molecule capable of 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 a particular example, the first antigen is a target cell antigen and the second antigen is an activating T cell antigen. In a more specific example, the activating T cell antigen is CD3, particularly CD3 epsilon. In one example, the target cell antigen is CD20.

In one example of the T cell activating bispecific antigen binding molecule according to the disclosure, 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 example 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 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In a further example, 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), 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).

In yet another example, 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 example 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 example 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 a particular example, 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) (numbering according to Kabat), and 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).

In another particular example, 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 arginine (R) (numbering according to Kabat), and 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).

In one example, 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 a target cell antigen and the second antigen is an activating T cell antigen; 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 example 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 example 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 an alternative example of the T cell activating bispecific antigen binding molecule according to the disclosure, in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred example independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the second Fab molecule under b) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In a further example, in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (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 independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In still another example, in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred example 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 example independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the second Fab molecule under b) 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 one example, 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 example, 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 some examples, the T cell activating bispecific antigen binding molecule according to the disclosure further comprises a third Fab molecule which specifically binds to the first antigen.

In particular examples, the third Fab molecule is identical to the first Fab molecule. In these examples, the third Fab molecule thus comprises the same amino acid substitutions as the first Fab molecule. Like the first Fab molecule, the third Fab molecule particularly is a conventional Fab molecule.

If a third Fab molecule is present, in a particular example the first and the third Fab molecule specifically bind to a target cell antigen, and the second Fab molecule specifically binds to an activating T cell antigen, particularly CD3, more particularly CD3 epsilon.

In some examples of the T cell activating bispecific antigen binding molecule according to the disclosure the first Fab molecule under a) and the second Fab molecule under b) are fused to each other, optionally via a peptide linker. In a specific example, 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. In an alternative example, 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 examples 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 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 examples, 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 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) Fab molecule may be fused to each other and to the Fc domain in different ways. The components may be fused to each other directly or, preferably, via one or more suitable peptide linkers. Where fusion is to the N-terminus of a subunit of the Fc domain, it is typically via an immunoglobulin hinge region.

In one example, the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or the second subunit of the Fc domain. In such example, the first Fab molecule may be fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule or to the N-terminus of the other one of the subunits of the Fc domain.

In one example, the first and the second Fab molecule 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. In this example, the T cell activating bispecific antigen binding molecule essentially comprises an immunoglobulin molecule, wherein in one of the Fab arms the heavy and light chain variable regions VH and VL are exchanged/replaced by each other (see Figure 1A, D).

In alternative examples, the third Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or second subunit of the Fc domain. In a particular such example, the second and the third Fab molecule 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, and 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 this example, the T cell activating bispecific antigen binding molecule essentially comprises an immunoglobulin molecule, wherein in one of the Fab arms the heavy and light chain variable regions VH and VL are exchanged/replaced by each other, and wherein an additional (conventional) Fab molecule is N-terminally fused to said Fab arm (see Figure 1B, E). In another such example, the first and the third Fab molecule 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, and 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. In this example, the T cell activating bispecific antigen binding 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 are exchanged/replaced by each other (see Figure 1C, F).

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 may either be in the CH1 and CL domains of the first and (if present) the third Fab molecule, or in the CH1 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 amino acid substitutions are made in the first (and, if present, the third) Fab molecule.

In particular examples 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 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 examples 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 examples, 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.

In a particular example, 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 example the immunoglobulin is an IgG₁ subclass immunoglobulin. In another example, the immunoglobulin is an IgG₄ subclass immunoglobulin.

In another aspect, 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) 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 a target cell antigen 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);

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 substituted by glutamic acid (E) (numbering according to Kabat EU index); and

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).

In an even more particular example, 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) 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 a target cell antigen 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);

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) (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); and

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 Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under d).

In another aspect, 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; and

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

wherein

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

(ii) the second antigen is a target cell antigen and the first 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); and

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).

In particular examples of the T cell activating bispecific antigen binding molecule, the Fc domain is an IgG Fc domain. In a specific example, the Fc domain is an IgG₁ Fc domain. In another specific example, the Fc domain is an IgG₄ Fc domain. In an even more specific example, the Fc domain is an IgG₄ Fc domain comprising the amino acid substitution S228P (Kabat numbering). In particular examples the Fc domain is a human Fc domain.

In particular examples, the Fc domain comprises a modification promoting the association of the first and the second Fc domain subunit. In a specific such example, an amino acid residue in the CH3 domain of the first subunit of the Fc domain is replaced with an amino acid residue 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 example 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 examples 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 example, the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function. In one example, 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 examples, 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 example, the Fc domain is an IgG₁ Fc domain, particularly a human IgG₁ Fc domain. In other examples, 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 example, the Fc domain is an IgG₄ Fc domain, particularly a human IgG₄ Fc domain. In one example, 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 example the Fc receptor is an Fcγ receptor. In one example the Fc receptor is a human Fc receptor. In one example, the Fc receptor is an activating Fc receptor. In a specific example, the Fc receptor is human FcγRIIa, FcγRI, and/or FcγRIIIa. In one example, the effector function is antibody-dependent cell-mediated cytotoxicity (ADCC).

In a specific example of the T cell activating bispecific antigen binding molecule according to the disclosure, the Fab molecule which specifically binds to an activating T cell antigen, particularly CD3, more particularly CD3 epsilon, 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 example, the Fab molecule 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 one specific example, the second 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 example, said second 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 example of the T cell activating bispecific antigen binding molecule according to the disclosure, the Fab molecule which specifically binds to a target cell antigen, particularly CD20, comprises the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 46, the heavy chain CDR 2 of SEQ ID NO: 47, the heavy chain CDR 3 of SEQ ID

NO: 48, the light chain CDR 1 of SEQ ID NO: 49, the light chain CDR 2 of SEQ ID NO: 50 and the light chain CDR 3 of SEQ ID NO: 51. In an even more specific example, the Fab molecule which specifically binds to a target cell antigen, particularly CD20, 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: 30 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: 31. In one specific example, the first (and, if present, the third) Fab molecule comprised in the T cell activating bispecific antigen binding molecule according to the disclosure specifically binds to CD20, and comprises the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 46, the heavy chain CDR 2 of SEQ ID NO: 47, the heavy chain CDR 3 of SEQ ID NO: 48, the light chain CDR 1 of SEQ ID NO: 49, the light chain CDR 2 of SEQ ID NO: 50 and the light chain CDR 3 of SEQ ID NO: 51. In an even more specific example, said first (and, if present, said third) Fab molecule comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 30 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 31.

In a particular example, 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) 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

- (i) the first antigen is CD20 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: 46, the heavy chain CDR 2 of SEQ ID NO: 47, the heavy chain CDR 3 of SEQ ID NO: 48, the light chain CDR 1 of SEQ ID NO: 49, the light chain CDR 2 of SEQ ID NO: 50 and the light chain CDR 3 of SEQ ID NO: 51, 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;

(iii) 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 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 substituted by glutamic acid (E) (numbering according to Kabat EU index); and

(iv) 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 a further example, 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) 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

- (i) the first antigen is CD20 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: 46, the heavy chain CDR 2 of SEQ ID NO: 47, the heavy chain CDR 3 of SEQ ID NO: 48, the light chain CDR 1 of SEQ ID NO: 49, the light chain CDR 2 of SEQ ID NO: 50 and the light chain CDR 3 of SEQ ID NO: 51, 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: 67, the heavy chain CDR 3 of SEQ ID NO: 6, the light chain CDR 1 of SEQ ID NO: 68, the light chain CDR 2 of SEQ ID NO: 9 and the light chain CDR 3 of SEQ ID NO: 10;

(iii) 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 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 substituted by glutamic acid (E) (numbering according to Kabat EU index); and

(iv) 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).

According to another example 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. In some examples the host cell is a eukaryotic cell, particularly a mammalian cell.

In another example 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 b) recovering the T cell activating bispecific antigen binding molecule. The disclosure also encompasses a T cell activating bispecific antigen binding molecule produced by the method of the disclosure.

The disclosure further provides 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 example 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 example 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 example the disease is cancer.

Also provided in another aspect is the use of a T cell activating bispecific antigen binding molecule of the disclosure in the manufacture of a medicament for the treatment of a disease in an individual in need thereof. There is also provided a method of treating a disease in an individual, comprising 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 another aspect, there is provided a composition comprising the T cell activating bispecific antigen binding molecule of the disclosure in a pharmaceutically acceptable form for use in treating a disease. In a specific example the disease is cancer. In any of the above examples the individual preferably is a mammal, particularly a human.

In another aspect, there is provided a method for inducing lysis of a target 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. The target cell may particularly be a tumor cell and the T cell may particularly be a cytotoxic 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 introduced in the CH and CL domains.

FIGURE 2. Illustration of the TCBs prepared in Example 1. (A) “2+1 IgG CrossFab, inverted” without charge modifications (CH1/CL exchange in CD3 binder), (B) “2+1 IgG CrossFab, inverted” with charge modifications (VH/VL exchange in CD3 binder, charge modification in CD20 binders, EE = 147E, 213E; RK = 123R, 124K), (C) “2+1 IgG CrossFab” with charge modifications (VH/VL exchange in CD3 binder, charge modification in CD20 binders, EE = 147E, 213E; RK = 123R, 124K), (D) “2+1 IgG CrossFab, inverted” without charge

Claims

1. 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,

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

wherein

- i) 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), and wherein in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index); or
- ii) in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the second Fab molecule under b) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

2. The T cell activating bispecific antigen binding molecule of claim 1, wherein the first antigen is a target cell antigen and the second antigen is an activating T cell antigen.

3. The T cell activating bispecific antigen binding molecule of claim 1 or 2, wherein the activating T cell antigen is CD3, particularly CD3 epsilon.

4. The T cell activating bispecific antigen binding molecule according to any one of claims 1-3, 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), and wherein in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

5. The T cell activating bispecific antigen binding molecule according to any one of claims 1-4, 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), and wherein 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).

6. The T cell activating bispecific antigen binding molecule according to any one of claims 1-5, 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) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (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 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).

7. The T cell activating bispecific antigen binding molecule according to claim 6, 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 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).

8. The T cell activating bispecific antigen binding molecule according to claim 6, 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) (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).

9. The T cell activating bispecific antigen binding molecule according to any one of claims 1-3, wherein in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the second Fab molecule under b) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

10. The T cell activating bispecific antigen binding molecule according to any one of claims 1-3 or 9, wherein in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the second Fab molecule under b) the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

11. The T cell activating bispecific antigen binding molecule according to any one of claims 1-3, 9 or 10, wherein in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the second Fab molecule under b) 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).

12. The T cell activating bispecific antigen binding molecule according to claim 11, wherein 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 wherein 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).

13. The T cell activating bispecific antigen binding molecule according to claim 11, wherein 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 wherein 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).

14. The T cell activating bispecific antigen binding molecule according to any one of claims 1-13, further comprising

c) a third Fab molecule which specifically binds to the first antigen.

15. The T cell activating bispecific antigen binding molecule according to claim 14, wherein the third Fab molecule is identical to the first Fab molecule.

16. The T cell activating bispecific antigen binding molecule according to claims 14 or 15, wherein the first and the third Fab molecule specifically bind to a target cell antigen, and the second Fab molecule specifically binds to an activating T cell antigen, particularly CD3, more particularly CD3 epsilon.

17. The T cell activating bispecific antigen binding molecule according to any one of claims 1 to 16, additionally comprising

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

18. The T cell activating bispecific antigen binding molecule according to any one of claims 1 to 17, wherein the first and the second Fab molecule are fused to each other, optionally via a peptide linker.

19. The T cell activating bispecific antigen binding molecule according to any one of claims 1 to 18, wherein 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.

20. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 18, wherein 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.

21. The T cell activating bispecific antigen binding molecule of claim 19 or 20, wherein the Fab light chain of the first Fab molecule and the Fab light chain of the second Fab molecule are fused to each other, optionally via a peptide linker.
22. The T cell activating bispecific antigen binding molecule according to claim 17, wherein the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or the second subunit of the Fc domain.
23. The T cell activating bispecific antigen binding molecule according to claim 17, wherein the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or the second subunit of the Fc domain.
24. The T cell activating bispecific antigen binding molecule according to claim 17, wherein the first and the second Fab molecule 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.
25. The T cell activating bispecific antigen binding molecule according to any one of claims 17, 22 or 23, wherein the third Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or second subunit of the Fc domain.
26. The T cell activating bispecific antigen binding molecule of claim 17, wherein the second and the third Fab molecule 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, and 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.
27. The T cell activating bispecific antigen binding molecule according to claim 17, wherein the first and the third Fab molecule 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, and 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.
28. The T cell activating bispecific antigen binding molecule according to claim 27, wherein the first and the third Fab molecule and the Fc domain are part of an immunoglobulin molecule, particularly an IgG class immunoglobulin.
29. 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) 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 a target cell antigen 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);

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) or lysine (K) (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 substituted by glutamic acid (E) (numbering according to Kabat EU index); and

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).

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

wherein

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

(ii) the second antigen is a target cell antigen and the first 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 arginine (R) or lysine (K) (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); and

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).

31. The T cell activating bispecific antigen binding molecule according to any one of the preceding claims, wherein the activating T cell antigen is CD3, particularly CD3 epsilon, and the Fab molecule which specifically binds to the activating T cell antigen 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.

32. The T cell activating bispecific antigen binding molecule according to any one of the preceding claims, wherein the activating T cell antigen is CD3, particularly CD3 epsilon, and the Fab molecule which specifically binds to the activating T cell antigen 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.

33. The T cell activating bispecific antigen binding molecule according to any one of the preceding claims, wherein the target cell antigen is CD20 and the Fab molecule which specifically binds to the target cell antigen 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: 30 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: 31.

34. Use of the T cell activating bispecific antigen binding molecule of any one of claims 1 to 33 in the manufacture of a medicament for the treatment of a disease in an individual in need thereof.
35. A composition comprising the T cell activating bispecific antigen binding molecule of any one of claims 1 to 33 in a pharmaceutically acceptable form for use in treating a disease.
36. 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 1-33 in the presence of a T cell.