

Summary of the ~~Invention~~

According to the ~~invention~~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 a first aspect the present ~~disclosure invention~~ 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 invention~~, 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 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 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 embodiment, the first antigen is a target cell antigen and the second antigen is an activating T cell antigen. In a more specific embodiment, the activating T cell antigen is CD3, particularly CD3 epsilon. In one embodiment, the target cell antigen is CD20.

In one embodiment of the T cell activating bispecific antigen binding molecule according to the disclosure~~invention~~, 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 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 embodiment, 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 embodiment, 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 a particular embodiment, 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 embodiment, 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 embodiment, the T cell activating bispecific antigen binding molecule of the disclosure ~~invention~~ 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 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 an alternative embodiment of the T cell activating bispecific antigen binding molecule according to the

disclosure ~~invention~~, 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 embodiment 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 embodiment, 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 embodiment, 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 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 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 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 some embodiments, the T cell activating bispecific antigen binding molecule according to the disclosure invention further comprises a third Fab molecule which specifically binds to the first antigen.

In particular embodiments, the third Fab molecule is identical to the first Fab molecule. In these embodiments, 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 embodiment 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 embodiments of the T cell activating bispecific antigen binding molecule according to the ~~disclosure invention~~ 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 embodiment, 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 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 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 invention~~ 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 invention~~ 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 embodiment, 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 embodiment, 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 embodiment, 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 embodiment, 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 embodiments, 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 embodiment, 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 embodiment, 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 embodiment, 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 embodiment, 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~~invention, 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~~invention, 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 embodiments of the T cell activating bispecific antigen binding molecule according to the ~~disclosure~~invention, 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~~invention, 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.

In a particular embodiment, the immunoglobulin molecule comprised in the T cell activating bispecific antigen binding molecule according to the ~~disclosure invention~~ 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.

In ~~another aspect~~ ~~particular embodiment~~, the ~~disclosure invention~~ 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 embodiment, the ~~disclosure invention~~ 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—further embodiment, the disclosure invention—~~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

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 (SPLF) (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~~*invention*, 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 embodiment, 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 embodiment, the second Fab molecule comprised in the T cell activating bispecific antigen binding molecule according to the ~~disclosure~~*invention* 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 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~~*invention*, 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 embodiment, 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 embodiment, the first (and, if present, the third) Fab molecule comprised in the T cell activating bispecific antigen binding molecule according to the ~~disclosure invention~~ 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 embodiment, 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 aspect, the ~~disclosure invention~~ 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 aspect, the ~~disclosure invention~~ 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 aspect of the ~~disclosure invention~~—there is provided one or more isolated polynucleotide(s) encoding a T cell activating bispecific antigen binding molecule of the ~~disclosure invention~~. The ~~disclosure invention~~ further provides one or more expression vector(s) comprising the isolated polynucleotide(s) of the ~~disclosure invention~~, and a host cell comprising the isolated polynucleotide(s) or the expression vector(s) of the ~~disclosure invention~~. According to another aspect of the disclosure, there is provided one or more vector, particularly expression vector, comprising the polynucleotide(s) as defined herein. According to another aspect of the disclosure, there is provided a host cell comprising the polynucleotide(s) as defined herein or the vector(s) as defined herein. In some embodiments the host cell is a eukaryotic cell, particularly a mammalian cell.

~~In another aspect—There is also is—~~provided a method of producing the T cell activating bispecific antigen binding molecule of the ~~disclosure invention~~, comprising the steps of a) culturing the host cell of the ~~disclosure invention~~ 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. In another aspect, there is provided a method of producing a T cell activating bispecific antigen binding molecule capable of specific binding to CD3 and a target cell antigen, comprising the steps of a) culturing the host cell as defined herein 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. Another aspect of the disclosure provides for ~~The invention also encompasses a T cell activating bispecific antigen binding molecule produced by the method of the disclosure invention.~~

The ~~disclosure invention~~ further provides, in another aspect, a pharmaceutical composition comprising the T cell activating bispecific antigen binding molecule of the ~~disclosure invention~~ and a pharmaceutically acceptable carrier.

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

Also provided, in another aspect, is the use of a T cell activating bispecific antigen binding molecule of the disclosure invention for the manufacture of a medicament for the treatment of a disease in an individual in need thereof. There is also provided, ~~as well as a~~ method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the T cell activating bispecific antigen binding molecule according to the disclosure invention 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 invention 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 invention in the presence of a T cell, particularly a cytotoxic T cell. In another aspect, there is provided an *in vitro* method for inducing lysis of a target cell, comprising contacting a target cell with the T cell activating bispecific antigen binding molecule as defined herein 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 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 modifications

58. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 51 or 56 or the pharmaceutical composition of claim 57 for use as a medicament.

59. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 51 or 56 or the pharmaceutical composition of claim 57 for use in the treatment of a disease in an individual in need thereof.

60. The T cell activating bispecific antigen binding molecule or the pharmaceutical composition of claim 59, wherein the disease is cancer.

61. Use of the T cell activating bispecific antigen binding molecule of any one of claims 1 to 51 or 56 for the manufacture of a medicament for the treatment of a disease in an individual in need thereof.

~~62. 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 of any one of claims 1 to 51 or 56 in a pharmaceutically acceptable form.~~

~~63~~62. The use of claim 61 ~~or the method of claim 62~~, wherein said disease is cancer.

~~64~~63. An *in vitro* 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 1-51 or 56 in the presence of a T cell.