

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, 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).

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 CD20, wherein the first Fab molecule comprises a heavy chain complementarity determining region (CDR) 1 comprising the amino acid sequence of SEQ ID NO: 46, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 47, a heavy chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 48, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 49, a light chain CDR 2

comprising the amino acid sequence of SEQ ID NO: 50 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 51;

(b) a second Fab molecule which specifically binds to CD3, wherein the second Fab molecule comprises a heavy chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 4, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 5, a heavy chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 6, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 8, a light chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 9 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 10, wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain of the second Fab molecule, respectively, are replaced by each other, and

(c) a third Fab molecule which specifically binds to CD20, wherein the third Fab molecule comprises a heavy chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 46, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 47, a heavy chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 48, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 49, a light chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 50 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 51; and wherein:

i) in the constant domain CL of the first Fab molecule and the third molecule, the amino acid at position 124, according to Kabat, is substituted by lysine (K), arginine (R), or histidine (H), and, in the constant domain CH1 of the first Fab molecule and the third Fab molecule, one or both of the amino acids at positions 147 and 213, according to Kabat EU index, are substituted by glutamic acid (E), or aspartic acid (D); or

ii) in the constant domain CL of the second Fab molecule, the amino acid at position 124, according to Kabat, is substituted by lysine (K), arginine (R) or histidine (H), and, in the constant domain CH1 of the second Fab molecule, one or both of the amino acids at positions 147 and 213, according to the Kabat EU index, are substituted by glutamic acid (E), or aspartic acid (D).

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 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

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

The disclosure also 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 another aspect, the present disclosure provides a T cell activating bispecific antigen binding molecule comprising:

(a) a first Fab molecule which specifically binds to CD20, wherein the first Fab molecule comprises a heavy chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 46, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 47, a heavy chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 48, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 49, a light chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 50 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 51;

(b) a second Fab molecule which specifically binds to CD3, wherein the second Fab molecule comprises a heavy chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 4, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 5, a heavy chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 6, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 8, a light chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 9 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 10, and wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain of the second Fab molecule, respectively, are replaced by each other;

(c) a third Fab molecule which specifically binds to CD20, wherein the third Fab molecule comprises a heavy chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 46, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 47, a heavy chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 48, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 49, a light chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 50 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 51; and

(d) an Fc domain comprising a first and a second subunit capable of stable association, wherein, in the constant domain CL of the first Fab molecule and the third Fab molecule, the amino acid at position 124, according to Kabat, is substituted by lysine (K), and the amino acid at position 123, according to Kabat, is substituted by arginine (R) or lysine (K), and, in the constant domain CH1 of the first Fab molecule and the third Fab molecule, the amino acid at position 147, according to the Kabat EU index, is substituted by glutamic acid (E), and the amino acid at position 213, according to the Kabat EU index, is substituted by glutamic acid (E), and wherein:

(i) the C-terminus of the Fab heavy chain of the first Fab molecule is fused to the N-terminus of the Fab heavy chain of the second Fab molecule, and the C-terminus of the Fab heavy chain of the second Fab molecule and the C-terminus of the Fab heavy chain of the third Fab molecule are each fused to the N-terminus of one of the subunits of the Fc domain, or

(ii) the C-terminus of the Fab heavy chain of the second Fab molecule is fused to the N-terminus of the Fab heavy chain of the first Fab molecule, and the C-terminus of the Fab heavy chain of the first Fab molecule and the C-terminus of the Fab heavy chain of the third Fab molecule are each fused to the N-terminus of one of the subunits of the Fc domain.

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

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

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

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).

The disclosure also 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

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 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.

The disclosure also 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 another aspect, the present disclosure provides a T cell activating bispecific antigen binding molecule comprising:

(a) a first Fab molecule which specifically binds to CD20, wherein the first Fab molecule comprises a heavy chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 46, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 47, a heavy chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 48, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 49, a light chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 50 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 51;

b) a second Fab molecule which specifically binds to CD3, wherein the second Fab molecule comprises a heavy chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 4, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 5, a heavy chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 6, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 8, a light chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 9 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 10, and wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain of the second Fab molecule, respectively, are replaced by each other;

(c) a third Fab molecule which specifically binds to CD20, wherein the third Fab molecule comprises a heavy chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 46, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 47, a heavy chain CDR 3

comprising the amino acid sequence of SEQ ID NO: 48, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 49, a light chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 50 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 51; and

(d) an Fc domain comprising a first and a second subunit capable of stable association, wherein,

in the constant domain CL of the first Fab molecule and the third Fab molecule, the amino acid at position 124, according to Kabat, is substituted by lysine (K) and the amino acid at position 123, according to Kabat, is substituted by lysine (K) or arginine (R), and in the constant domain CH1 of the first Fab molecule and the third Fab molecule, the amino acids at positions 147 and 213, according to the Kabat EU index, are each substituted by glutamic acid (E); and wherein the C-terminus of the Fab heavy chain of the first Fab molecule is fused to the N-terminus of the Fab heavy chain of the second Fab molecule, and the C-terminus of the Fab heavy chain of the second Fab molecule and the third Fab molecule are each fused to the N-terminus of one of the subunits of the Fc domain.

The disclosure also 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;

Also provided is the use of a T cell activating bispecific antigen binding molecule of the disclosure for the manufacture of a medicament for the treatment of a disease in an individual in need thereof. In another aspect, the disclosure provides for use of the the T cell activating bispecific antigen binding molecule of the disclosure in the manufacture of a medicament for the treatment of cancer 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 a specific embodiment the disease is cancer. In any of the above embodiments the individual preferably is a mammal, particularly a human.

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

Claims

1. A T cell activating bispecific antigen binding molecule comprising:

(a) a first Fab molecule which specifically binds to CD20, wherein the first Fab molecule comprises a heavy chain complementarity determining region (CDR) 1 comprising the amino acid sequence of SEQ ID NO: 46, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 47, a heavy chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 48, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 49, a light chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 50 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 51;

(b) a second Fab molecule which specifically binds to CD3, wherein the second Fab molecule comprises a heavy chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 4, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 5, a heavy chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 6, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 8, a light chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 9 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 10, wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain of the second Fab molecule, respectively, are replaced by each other, and

(c) a third Fab molecule which specifically binds to CD20, wherein the third Fab molecule comprises a heavy chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 46, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 47, a heavy chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 48, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 49, a light chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 50 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 51; and wherein:

- i) in the constant domain CL of the first Fab molecule and the third molecule, the amino acid at position 124, according to Kabat, is substituted by lysine (K), arginine (R), or histidine (H), and, in the constant domain CH1 of the first Fab molecule and the third Fab molecule, one or both of the amino acids at positions 147 and 213, according to Kabat EU index, are substituted by glutamic acid (E), or aspartic acid (D); or
 - ii) in the constant domain CL of the second Fab molecule, the amino acid at position 124, according to Kabat, is substituted by lysine (K), arginine (R) or histidine (H), and, in the constant domain CH1 of the second Fab molecule, one or both of the amino acids at positions 147 and 213, according to the Kabat EU index, are substituted by glutamic acid (E), or aspartic acid (D).
2. The T cell activating bispecific antigen binding molecule according to claim 1, wherein, in the constant domain CL of the first Fab molecule, the amino acid at position 124, according to Kabat, is substituted by lysine (K), arginine (R) or histidine (H); and

in the constant domain CH1 of the first Fab molecule, one or both of the amino acids at positions 147 and 213, according to Kabat EU index, are substituted by glutamic acid (E), or aspartic acid (D).

3. The T cell activating bispecific antigen binding molecule according to claim 1 or 2, wherein, in the constant domain CH1 of the first Fab molecule, the amino acid at position 147, according to Kabat EU index, is substituted by glutamic acid (E), or aspartic acid (D).

4. The T cell activating bispecific antigen binding molecule according to any one of claims 1-3, wherein, the amino acid at position 123, according to Kabat, is substituted by lysine (K), arginine (R) or histidine (H) in the constant domain CH1 of the first Fab molecule, and the amino acid at position 213, according to the Kabat EU index, is substituted by glutamic acid (E), or aspartic acid (D).

5. The T cell activating bispecific antigen binding molecule according to claim 4, wherein, in the constant domain CL of the first Fab molecule, the amino acid at position 124, according to Kabat, is substituted by lysine (K) and the amino acid at position 123, according to Kabat, is substituted by arginine (R), and in the constant domain CH1 of the first Fab molecule, the amino acid at position 147, according to the Kabat EU index, is substituted by glutamic acid (E) and the amino acid at position 213, according to the Kabat EU index, is substituted by glutamic acid (E).

6. The T cell activating bispecific antigen binding molecule according to claim 4, wherein, in the constant domain CL of the first Fab molecule, the amino acid at position 124, according to Kabat, is substituted by lysine (K), and the amino acid at position 123, according to Kabat, is substituted by lysine (K), and in the constant domain CH1 of the first Fab molecule, the amino acid at position 147, according to the Kabat EU index, is substituted by glutamic

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

7. The T cell activating bispecific antigen binding molecule according to any one of claims 1 to 6, further comprising:

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

8. The T cell activating bispecific antigen binding molecule according to any one of claims 1 to 7, wherein the first and the second Fab molecule are fused to each other.

9. The T cell activating bispecific antigen binding molecule according to any one of claims 1 to 8, wherein the C-terminus of the Fab heavy chain of the second Fab molecule is fused to the N-terminus of the Fab heavy chain of the first Fab molecule.

10. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 8, wherein the C-terminus of the Fab heavy chain of the first Fab molecule is fused to the N-terminus of the Fab heavy chain of the second Fab molecule.

11. The T cell activating bispecific antigen binding molecule of claim 9 or 10, 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.
12. The T cell activating bispecific antigen binding molecule according to claim 7, wherein the C-terminus of the Fab heavy chain of the second Fab molecule is fused to the N-terminus of the first or the second subunit of the Fc domain.
13. The T cell activating bispecific antigen binding molecule according to claim 7, wherein the C-terminus of the Fab heavy chain of the first Fab molecule is fused to the N-terminus of the first or the second subunit of the Fc domain.
14. The T cell activating bispecific antigen binding molecule according to claim 7, wherein the C-terminus of the Fab heavy chain of the first and the second Fab molecule are each fused to the N-terminus of one of the subunits of the Fc domain.
15. The T cell activating bispecific antigen binding molecule according to any one of claims 7, 12 or 13, wherein the C-terminus of the Fab heavy chain of the third Fab molecule is fused to the N-terminus of the first or second subunit of the Fc domain.
16. The T cell activating bispecific antigen binding molecule of claim 7, wherein the C-terminus of the Fab heavy chains of the second and the third Fab molecules are each fused to the N-terminus of one of the subunits of the Fc domain, and the C-terminus of the Fab heavy chain of the first Fab molecule is fused to the N-terminus of the Fab heavy chain of the second Fab molecule.
17. The T cell activating bispecific antigen binding molecule according to claim 7, wherein the C-terminus of the Fab heavy chain of the first and the third Fab molecules are each fused to the N-terminus of one of the subunits of the Fc domain, and the C-terminus of the Fab heavy chain of the second Fab molecule is fused to the N-terminus of the Fab heavy chain of the first Fab molecule.
18. The T cell activating bispecific antigen binding molecule according to claim 17, wherein the first and the third Fab molecules and the Fc domain are part of an immunoglobulin molecule.
19. A T cell activating bispecific antigen binding molecule comprising:

(a) a first Fab molecule which specifically binds to CD20, wherein the first Fab molecule comprises a heavy chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 46, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 47, a heavy chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 48, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 49, a light chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 50 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 51;

(b) a second Fab molecule which specifically binds to CD3, wherein the second Fab molecule comprises a heavy chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 4, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 5, a heavy chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 6, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 8, a light chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 9 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 10, and wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain of the second Fab molecule, respectively, are replaced by each other;

(c) a third Fab molecule which specifically binds to CD20, wherein the third Fab molecule comprises a heavy chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 46, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 47, a heavy chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 48, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 49, a light chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 50 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 51; and

(d) an Fc domain comprising a first and a second subunit capable of stable association,

wherein, in the constant domain CL of the first Fab molecule and the third Fab molecule, the amino acid at position 124, according to Kabat, is substituted by lysine (K),

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

wherein:

(i) the C-terminus of the Fab heavy chain of the first Fab molecule is fused to the N-terminus of the Fab heavy chain of the second Fab molecule, and the C-terminus of the Fab heavy chain of the second Fab molecule and the C-terminus of the Fab heavy chain of the third Fab molecule are each fused to the N-terminus of one of the subunits of the Fc domain, or

(ii) the C-terminus of the Fab heavy chain of the second Fab molecule is fused to the N-terminus of the Fab heavy chain of the first Fab molecule, and the C-terminus of the Fab heavy chain of the first Fab molecule and the C-terminus of the Fab heavy chain of the third Fab molecule are each fused to the N-terminus of one of the subunits of the Fc domain.

20. The T cell activating bispecific antigen binding molecule according to any one of the preceding claims, wherein the 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.

21. The T cell activating bispecific antigen binding molecule according to claim 19 or 20, wherein the first Fab molecule and the third Fab molecule each comprise 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.

22. A T cell activating bispecific antigen binding molecule comprising:

- (a) a first Fab molecule which specifically binds to CD20, wherein the first Fab molecule comprises a heavy chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 46, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 47, a heavy chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 48, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 49, a light chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 50 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 51;
- b) a second Fab molecule which specifically binds to CD3, wherein the second Fab molecule comprises a heavy chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 4, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 5, a heavy chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 6, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 8, a light chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 9 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 10, and wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain of the second Fab molecule, respectively, are replaced by each other;

(c) a third Fab molecule which specifically binds to CD20, wherein the third Fab molecule comprises a heavy chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 46, a heavy chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 47, a heavy chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 48, a light chain CDR 1 comprising the amino acid sequence of SEQ ID NO: 49, a light chain CDR 2 comprising the amino acid sequence of SEQ ID NO: 50 and a light chain CDR 3 comprising the amino acid sequence of SEQ ID NO: 51; and

(d) an Fc domain comprising a first and a second subunit capable of stable association,

wherein,

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

the C-terminus of the Fab heavy chain of the first Fab molecule is fused to the N-terminus of the Fab heavy chain of the second Fab molecule, and the C-terminus of the Fab heavy chain of the second Fab molecule and the third Fab molecule are each fused to the N-terminus of one of the subunits of the Fc domain.

23. The T cell activating bispecific antigen binding molecule of claim 22, wherein the first Fab molecule and the third Fab molecule each comprise 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.

24. The T cell activating bispecific antigen binding molecule of claim 22, wherein the 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.

25. The T cell activating bispecific antigen binding molecule according to any one of claims 7-24, wherein the Fc domain is an IgG.

26. The T cell activating bispecific antigen binding molecule according to any one of claims 7-25, wherein the Fc domain is a human Fc domain.

27. The T cell activating bispecific antigen binding molecule according to any one of claims 7-24, wherein the Fc domain comprises a modification promoting the association of the first and the second subunit of the Fc domain.

28. The T cell activating bispecific antigen binding molecule of claim 25, wherein, in the CH3 domain of the first subunit of the Fc domain, an amino acid residue 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 in the CH3 domain of the second subunit of the Fc domain an amino acid residue 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.

29. The T cell activating bispecific antigen binding molecule of claim 28, wherein the amino acid residue having a larger side chain volume is selected from the group consisting of arginine (R), phenylalanine (F), tyrosine (Y), and tryptophan (W), and the amino acid residue having a smaller side chain volume is selected from the group consisting of alanine (A), serine (S), threonine (T), and valine (V).

30. The T cell activating bispecific antigen binding molecule of claim 28 or 29, wherein, in the CH3 domain of the first subunit of the Fc domain, the threonine residue at position 366, according to the Kabat EU index, is replaced with a tryptophan residue (T366W), and in the CH3 domain of the second subunit of the Fc domain, the tyrosine residue at position 407, according to the Kabat EU index, is replaced with a valine residue (Y407).

31. The T cell activating bispecific antigen binding molecule of any one of claims 28-30, wherein, in the first subunit of the Fc domain, the serine residue at position 354, according to the Kabat EU index, is replaced with a cysteine residue (S354C), or the glutamic acid residue at position 356, according to the Kabat EU index, is replaced with a cysteine residue (E356C), and, in the second subunit of the Fc domain, the tyrosine residue at position 349, according to the Kabat EU index, is replaced by a cysteine residue (Y349C).

32. The T cell activating bispecific antigen binding molecule of any one of claims 28-31, wherein the first subunit of the Fc domain comprises amino acid substitutions S354C and T366W, according to the Kabat EU index, and the second subunit of the Fc domain comprises amino acid substitutions Y349C, T366S, L368A and Y407V.

33. The T cell activating bispecific antigen binding molecule according to any one of claims 7-32, wherein the Fc domain exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG₁ Fc domain.

34. The T cell activating bispecific antigen binding molecule according to any one of claims 7-33, wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.
35. The T cell activating bispecific antigen binding molecule according to claim 34, wherein the one or more amino acid substitution is at one or more position selected from the group of L234, L235, and P329, according to the Kabat EU index.
36. The T cell activating bispecific antigen binding molecule according to any one of claims 7-35, wherein each subunit of the Fc domain comprises three amino acid substitutions that reduce binding to an activating Fc receptor and/or reduce effector function, wherein the amino acid substitutions are L234A, L235A and P329G, according to the Kabat EU index.
36. The T cell activating bispecific antigen binding molecule of any one of claims 33 to 36, wherein the Fc receptor is an Fc γ receptor.
37. The T cell activating bispecific antigen binding molecule of any one of claims 33 to 36, wherein the effector function is antibody-dependent cell-mediated cytotoxicity (ADCC).
38. The T cell activating bispecific antigen binding molecule according to any one of claims 1 to 37, wherein the CD3 is CD3 epsilon.
39. The T cell activating bispecific antigen binding molecule according to claim 8, wherein the first and the second Fab molecule are fused to each other via a peptide linker.
40. The T cell activating bispecific antigen binding molecule according to claim 11, 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 via a peptide linker.
41. The T cell activating bispecific antigen binding molecule according to claim 18, wherein the first and the third Fab molecules and the Fc domain are part of an IgG class immunoglobulin.
42. The T cell activating bispecific antigen binding molecule according to claim 22, wherein, in the constant domain CL of the first Fab molecule and the third Fab molecule, the amino acid at position 123, according to Kabat, is substituted by arginine (R).

43. The T cell activating bispecific antigen binding molecule according to claim 25, wherein the Fc domain is an IgG₁ Fc domain or an IgG₄ Fc domain.
44. The T cell activating bispecific antigen binding molecule according to claim 36, wherein, in the second subunit of the Fc domain, the threonine residue at position 366, according to the Kabat EU index, is replaced with a serine residue (T366S), and the leucine residue at position 368, according to the Kabat EU index, is replaced with an alanine residue (L368A).
45. One or more isolated polynucleotide encoding the T cell activating bispecific antigen binding molecule of any one of claims 1 to 44.
46. One or more vector, particularly expression vector, comprising the polynucleotide(s) of claim 45.
47. A host cell comprising the polynucleotide(s) of claim 45 or the vector(s) of claim 46.
48. 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 of claim 47 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.
49. A T cell activating bispecific antigen binding molecule produced by the method of claim 48.
50. A pharmaceutical composition comprising the T cell activating bispecific antigen binding molecule of any one of claims 1 to 44 or 49 and a pharmaceutically acceptable carrier.

51. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 44 or 49 or the pharmaceutical composition of claim 50 for use as a medicament.
52. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 44 or 49 or the pharmaceutical composition of claim 50 for use in the treatment of a disease in an individual in need thereof.
53. The T cell activating bispecific antigen binding molecule or the pharmaceutical composition for use according to claim 52, wherein the disease is cancer.
54. Use of the T cell activating bispecific antigen binding molecule of any one of claims 1 to 44 or 49 in the manufacture of a medicament for the treatment of cancer in an individual in need thereof.
55. 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 1-44 or 49 in the presence of a T cell.