

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 a target cell antigen;
- b) a second Fab molecule which specifically binds to CD3, 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 target cell antigen; and
- d) an Fc domain composed of a first and a second subunit capable of stable association;

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 the Fc domain is an IgG Fc domain, and 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; 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).

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

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 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 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 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; 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

(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 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 another aspect, there is provided one or more vector comprising the polynucleotide(s) of the disclosure. In another aspect, there is provided a host cell comprising the polynucleotide(s) of the disclosure or one or more vector comprising the polynucleotide(s). In some examples the host cell is a eukaryotic cell, particularly a mammalian cell.

In another example there 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. In another aspect, there is provided a method of producing a T cell activating bispecific antigen binding molecule according to the disclosure and capable of specific binding to CD3 and a target cell antigen, 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. In another aspect, there is provided a pharmaceutical composition for the treatment of cancer 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 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. In another aspect, there is provided use of the T cell activating bispecific antigen binding molecule of the disclosure in the manufacture of a medicament for the treatment of a proliferative disorder. There is also provides 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 the T cell activating bispecific antigen binding molecule of the disclosure for use in therapy. In a specific example the disease is cancer. In any of the above examples the individual preferably is a mammal, particularly a human.

The disclosure also provides 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.

Claims

1. A T cell activating bispecific antigen binding molecule comprising

- a) a first Fab molecule which specifically binds to a target cell antigen;
- b) a second Fab molecule which specifically binds to CD3, 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 target cell antigen; and
- d) an Fc domain composed of a first and a second subunit capable of stable association;

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 the Fc domain is an IgG Fc domain , and 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; 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).

2. The T cell activating bispecific antigen binding molecule according to claim 1, wherein the Fab molecule which specifically binds to CD3 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.

3. The T cell activating bispecific antigen binding molecule of claim 1, wherein the Fab molecule which specifically binds to CD3 comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 3 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 7.

4. The T cell activating bispecific antigen binding molecule according to claim 1, wherein the target cell antigen is CD20 and the Fab molecule which specifically binds to the target cell antigen 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.

5. The T cell activating bispecific antigen binding molecule according to claim 1, 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 of SEQ ID NO: 30 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 31.

6. The T cell activating bispecific antigen binding molecule of claim 1, wherein the first Fab molecule under a) and the third Fab molecule under c) 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.

7. The T cell activating bispecific antigen binding molecule of claim 1, wherein the second Fab molecule under b) 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.

8. The T cell activating bispecific antigen binding molecule according to claim 1, wherein the Fc domain is an IgG₁ or IgG₄, Fc domain.

9. The T cell activating bispecific antigen binding molecule according to claim 1, wherein the Fc domain is a human Fc domain.

10. The T cell activating bispecific antigen binding molecule according to claim 1, wherein said 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 said 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).

11. The T cell activating bispecific antigen binding molecule according to claim 1, wherein in the CH3 domain of the first subunit of the Fc domain the threonine residue at position 366 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 is replaced with a valine residue (Y407V).

12. The T cell activating bispecific antigen binding molecule according to claim 11, wherein in the second subunit of the Fc domain additionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A) (numberings according to Kabat EU index).

13. The T cell activating bispecific antigen binding molecule according to claim 1, wherein in the first subunit of the Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C) or the glutamic acid residue at position 356 is replaced with a cysteine residue (E356C), and in the second subunit of the Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C) (numberings according to Kabat EU index).

14. The T cell activating bispecific antigen binding molecule according to claim 1, wherein the first subunit of the Fc domain comprises amino acid substitutions S354C and T366W, and the second subunit of the Fc domain comprises amino acid substitutions Y349C, T366S, L368A and Y407V (numbering according to Kabat EU index).
15. The T cell activating bispecific antigen binding molecule according to claim 1, wherein the Fc domain exhibits reduced binding affinity to an Fc receptor, reduced effector function, or reduced binding affinity to an Fc receptor and reduced effector function, as compared to a native IgG₁ Fc domain.
16. The T cell activating bispecific antigen binding molecule according to claim 1, wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor, an effector function, or binding to an Fc receptor and an effector function.
17. The T cell activating bispecific antigen binding molecule according to claim 1, wherein said one or more amino acid substitution is at one or more position selected from the group of L234, L235, and P329 (Kabat EU index numbering).
18. The T cell activating bispecific antigen binding molecule according to claim 1, wherein each subunit of the Fc domain comprises three amino acid substitutions that reduce binding to an activating Fc receptor, an effector function, or binding to an activating Fc receptor and an effector function wherein said amino acid substitutions are L234A, L235A and P329G (Kabat EU index numbering).
19. The T cell activating bispecific antigen binding molecule according to claim 1, wherein the Fc receptor is an Fc γ receptor.
20. The T cell activating bispecific antigen binding molecule according to claim 1, wherein the effector function is antibody-dependent cell-mediated cytotoxicity (ADCC).
21. One or more isolated polynucleotide(s) encoding the T cell activating bispecific antigen binding molecule of any one of claims 1 to 20.
22. One or more vector comprising the polynucleotide(s) of claim 21.
23. A host cell comprising the polynucleotide(s) of claim 21 or one or more vector comprising the polynucleotide(s).

24. A method of producing a T cell activating bispecific antigen binding molecule according to claim 1 and capable of specific binding to CD3 and a target cell antigen, comprising the steps of a) culturing the host cell of claim 23 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.
25. A pharmaceutical composition for the treatment of cancer comprising the T cell activating bispecific antigen binding molecule of any one of claims 1 to 20 and a pharmaceutically acceptable carrier.
26. Use of the T cell activating bispecific antigen binding molecule of any one of claims 1 to 20 in the manufacture of a medicament for the treatment of a proliferative disorder.
27. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 20 for use in therapy.