SUMMARY

The present inventors aimed at generating antibodies that bind to CD3, with optimized properties for therapeutic use, e.g. as bispecific antibodies in cancer immunotherapy.

High affinity binding to CD3 was not considered a priority. Rather to the contrary, the inventors aimed at modulating binding affinity to be in an intermediate to low range, in order to reduce the potential for adverse events such as excessive cytokine release induced by the antibodies (cytokine release syndrome (CRS) being a severe and common side effect of CD3 antibodies, in particular CD3 bispecific antibodies as used in cancer immunotherapy).

The inventors also aimed at minimizing immunogenicity of the antibodies, in order to minimize the risk of anti-drug antibodies (ADA) being formed against them, which can interfere with therapeutic efficacy or even pose a safety risk in patients.

Further, the inventors aimed at antibodies that bind to CD3-expressing cells with high specificity, to prevent undesired effects on non-target cells. The reduction of non-specific binding to cells and/or cellular accumulation (e.g. due to a reduction in positively charged patches and hydrophobic, surface-exposed residues) also serves to reduce non-specific clearance and thereby improve pharmacokinetic properties and may also contribute to reducing the risk of ADA formation.

At the same time, of course, the CD3 antibodies should show sufficient functional activity for therapeutic efficacy, and preserve desirable properties like produceability and stability.

The disclosure accordingly provides antibodies that bind to CD3 which combine the above-mentioned favorable properties for therapeutic use.

In one aspect, the disclosure provides an antibody that binds to CD3, wherein the antibody comprises a first antigen binding domain, comprising

(i) a heavy chain variable region (VH) comprising a heavy chain complementarity determining region (HCDR) 1 of SEQ ID NO: 1, a HCDR 2 of SEQ ID NO: 9, SEQ ID NO: 13 or SEQ ID NO: 15, and a HCDR 3 of SEQ ID NO: 3,

and

(ii) a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 5, a LCDR 2 of SEQ ID NO: 11 and a LCDR 3 of SEQ ID NO: 7.

In one example, the VH of the first antigen binding domain comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 17,

SEQ ID NO: 18 and SEQ ID NO: 19, and/or the VL of the first antigen binding domain comprises 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: 12.

In one example, the VH of the first antigen binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 14 and SEQ ID NO: 16, and/or the VL sequence of the first antigen binding domain comprises the amino acid sequence of SEQ ID NO: 12.

In one example, the VH of the first antigen binding domain comprises amino acid substitutions L108S and S113G (Kabat numbering). Accordingly, in one example, the VH of the first antigen binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, and/or the VL sequence of the first antigen binding domain comprises the amino acid sequence of SEQ ID NO: 12.

In one example, the disclosure provides an antibody that binds to CD3, wherein the antibody comprises a first antigen binding domain comprising a VH sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, and/or a VL sequence of SEQ ID NO: 12. In another aspect, the present disclosure provides an antibody that binds to CD3, wherein the antibody comprises a first antigen binding domain comprising a VH sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, and a VL sequence of SEQ ID NO: 12. In one such example, the disclosure provides an antibody that binds to CD3, wherein the antibody comprises a first antigen binding domain comprising a VH sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 14 and SEQ ID NO: 16, and/or a VL sequence of SEQ ID NO: 12. In a further such example, the disclosure provides an antibody that binds to CD3, wherein the antibody comprises a first antigen binding domain comprising a VH sequence selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, and/or a VL sequence of SEQ ID NO: 12.

In one example, the first antigen binding domain is a Fab molecule.

In one example, the antibody comprises an Fc domain composed of a first and a second subunit.

In one example, the antibody comprises a second and optionally a third antigen binding domain that binds to a second antigen.

In one example, the second and/or, where present, the third antigen binding domain is a Fab molecule.

In one example, the first antigen binding domain is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1, particularly the variable domains VL and VH, of the Fab light chain and the Fab heavy chain are replaced by each other.

In one example, the second and, where present, the third antigen binding domain is a conventional Fab molecule.

In one example, the second and, where present, the third antigen binding domain is a Fab molecule wherein in the constant domain CL the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In one example, the first and the second antigen binding domain are fused to each other, optionally via a peptide linker.

In one example, the first and the second antigen binding domain are each a Fab molecule and either (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain.

In one example, the first, the second and, where present, the third antigen binding domain are each a Fab molecule and the antibody comprises an Fc domain composed of a first and a second subunit; and wherein either (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain and the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain and the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain; and the third antigen binding domain, where present, is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

In one example, the Fc domain is an IgG, particularly an IgG_1, Fc domain. In one example the Fc domain is a human Fc domain. In one example, the Fc comprises a modification promoting the association of the first and the second subunit of the Fc domain. In one example, the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

In one example, the second antigen is a target cell antigen, particularly a tumor cell antigen.

In one example, the second antigen is HER2. In one example, the second and, where present, the third antigen binding domain comprises a VH comprising a HCDR 1 of SEQ ID NO: 20, a HCDR 2 of SEQ ID NO: 21, and a HCDR 3 of SEQ ID NO: 22, and a VL comprising a LCDR 1 of SEQ ID NO: 24, a LCDR 2 of SEQ ID NO: 25 and a LCDR 3 of SEQ ID NO: 26. In one example, the second and, where present, the third antigen binding domain comprises a VH comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 23, and/or a VL 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: 27.

According to a further aspect of the disclosure there is provided an isolated polynucleotide encoding the antibody of the disclosure. In another aspect, there is provided a host cell comprising the isolated polynucleotide of the disclosure.

In another aspect, there is provided a method of producing an antibody that binds to CD3, comprising the steps of (a) culturing the host cell of the disclosure under conditions suitable for the expression of the antibody and optionally (b) recovering the antibody. In another aspect, the disclosure also encompasses an antibody that binds to CD3 produced by the method of the disclosure.

In another aspect, the disclosure further provides a pharmaceutical composition comprising the antibody of the disclosure and a pharmaceutically acceptable carrier.

Also encompassed by the disclosure are methods of using the antibody and pharmaceutical composition of the disclosure. In another aspect, the disclosure provides the antibody according to the disclosure or the pharmaceutical composition according to the disclosure for use as a medicament. In another aspect, there is provided the antibody according to the disclosure or the pharmaceutical composition according to the disclosure for use in the treatment of a disease. In a specific example, the disease is cancer.

Also provided is the use of an antibody or pharmaceutical composition according to the disclosure in the manufacture of a medicament, the use of an antibody or pharmaceutical composition according to the disclosure in the manufacture of a medicament for the treatment of a disease, particularly cancer. The disclosure also provides a method of treating a disease in an individual, comprising administering to said individual an effective amount of the antibody or pharmaceutical composition according to the disclosure.

In another aspect, there is provided use of the antibody according to the disclosure or the pharmaceutical composition according to the disclosure in the manufacture of a medicament. In another aspect, there is provided use of the antibody according to the disclosure or the pharmaceutical composition according to the disclosure in the manufacture of a medicament for the treatment of a disease.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Exemplary configurations of the (multispecific) antibodies of the invention. (A, D) Illustration of the "1+1 CrossMab" molecule. (B, E) Illustration of the "2+1 IgG Crossfab"

CLAIMS

1. An antibody that binds to CD3, wherein the antibody comprises a first antigen binding domain, comprising

(i) a heavy chain variable region (VH) comprising a heavy chain complementarity determining region (HCDR) 1 of SEQ ID NO: 1, a HCDR 2 of SEQ ID NO: 9, SEQ ID NO: 13 or SEQ ID NO: 15, and a HCDR 3 of SEQ ID NO: 3,

and

(ii) a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 5, a LCDR 2 of SEQ ID NO: 11 and a LCDR 3 of SEQ ID NO: 7.

2. The antibody of claim 1, wherein the VH of the first antigen binding domain comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, and/or the VL of the first antigen binding domain comprises 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: 12.

3. An antibody that binds to CD3, wherein the antibody comprises a first antigen binding domain comprising a VH sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, and a VL sequence of SEQ ID NO: 12.

4. The antibody of any one of claims 1-3, wherein the first antigen binding domain is a Fab molecule.

5. The antibody of any one of claims 1-4, comprising an Fc domain composed of a first and a second subunit.

6. The antibody of any one of claims 1-5, comprising a second and optionally a third antigen binding domain that binds to a second antigen.

7. The antibody of claim 6, wherein the second and/or, where present, the third antigen binding domain is a Fab molecule.

8. The antibody of any one of claims 1-7, wherein the first antigen binding domain is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1, particularly the variable domains VL and VH, of the Fab light chain and the Fab heavy chain are replaced by each other.

9. The antibody of any one of claims 6-8, wherein the second and, where present, the third antigen binding domain is a conventional Fab molecule.

10. The antibody of any one of claims 6-9, wherein the second and, where present, the third antigen binding domain is a Fab molecule wherein in the constant domain CL the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 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).

11. The antibody of any one of claims 6-10, wherein the first and the second antigen binding domain are fused to each other, optionally via a peptide linker.

12. The antibody of any one of claims 6-11, wherein the first and the second antigen binding domain are each a Fab molecule and either (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain.

13. The antibody of any one of claims 6-12, wherein the first, the second and, where present, the third antigen binding domain are each a Fab molecule and the antibody comprises an Fc domain composed of a first and a second subunit; and wherein either (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain and the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain and the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain; and the third antigen binding domain, where present, is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

14. The antibody of any one of claims 5-13, wherein the Fc domain is an IgG, particularly an IgG_1, Fc domain.

15. The antibody of any one of claims 5-14, wherein the Fc domain is a human Fc domain.

16. The antibody of any one of claims 5-15, wherein the Fc comprises a modification promoting the association of the first and the second subunit of the Fc domain.

17. The antibody of any one of claims 5-16, wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

18. The antibody of any one of claims 6-17, wherein the second antigen is a target cell antigen, particularly a tumor cell antigen.

19. The antibody of any one of claims 6-18, wherein the second antigen is HER2.

20. The antibody of claim 19, wherein the second and, where present, the third antigen binding domain comprises a VH comprising a HCDR 1 of SEQ ID NO: 20, a HCDR 2 of SEQ ID NO: 21, and a HCDR 3 of SEQ ID NO: 22, and a VL comprising a LCDR 1 of SEQ ID NO: 24, a LCDR 2 of SEQ ID NO: 25 and a LCDR 3 of SEQ ID NO: 26.

21. The antibody of claims 19 or 20, wherein the second and, where present, the third antigen binding domain comprises a VH comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 23, and/or a VL 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: 27.

22. An isolated polynucleotide encoding the antibody of any one of claims 1-21.

23. A host cell comprising the isolated polynucleotide of claim 22.

24. A method of producing an antibody that binds to CD3, comprising the steps of (a) culturing the host cell of claim 23 under conditions suitable for the expression of the antibody and optionally (b) recovering the antibody.

25. An antibody that binds to CD3 produced by the method of claim 24.

26. A pharmaceutical composition comprising the antibody of any one of claims 1-21 or 25 and a pharmaceutically acceptable carrier.

27. The antibody of any one of claims 1-21 or 25 or the pharmaceutical composition of claim 26 for use as a medicament.

28. The antibody of any one of claims 1-21 or 25 or the pharmaceutical composition of claim 26 for use in the treatment of a disease.

29. The antibody or pharmaceutical composition for use of claim 28, wherein the disease is cancer.

30. Use of the antibody of any one of claims 1-21 or 25 or the pharmaceutical composition of claim 26 in the manufacture of a medicament.

31. Use of the antibody of any one of claims 1-21 or 25 or the pharmaceutical composition of claim 26 in the manufacture of a medicament for the treatment of a disease.

32. The use of claim 31, wherein the disease is cancer.