

Given the tremendous therapeutic potential of antibodies, particularly bispecific antibodies for the activation of T cells, there is a need for bispecific CD3/FolR1 antibodies with optimized properties.

SUMMARY

5 The present disclosure provides antibodies, including multispecific (e.g. bispecific) antibodies, that bind to CD3 and are resistant to degradation by e.g. asparagine deamidation and thus particularly stable as required for therapeutic purposes. The (multispecific) antibodies provided further combine good efficacy and produceability with low toxicity and favorable pharmacokinetic properties.

10 As is shown herein, the antibodies, including multispecific antibodies, that bind to CD3, provided by the present disclosure, retain more than about 90% binding activity to CD3 after 2 weeks at pH 7.4, 37°C, relative to the binding activity after 2 weeks at pH 6, -80°C, as determined by surface plasmon resonance (SPR).

In particular, the present disclosure provides bispecific antibodies that bind to CD3 and Folate
15 receptor 1 (FolR1) that retain more than about 90% binding activity to CD3 after 2 weeks at pH 7.4, 37°C, relative to the binding activity after 2 weeks at pH 6, -80°C, as determined by surface plasmon resonance (SPR).

In one aspect, provided is bispecific antibody that binds to CD3 and Folate receptor 1 (FolR1), wherein the bispecific antibody comprises

- 20 (i) a first antigen binding domain capable of specific binding to CD3, comprising a heavy chain variable region (VH) comprising a heavy chain complementarity determining region (HCDR) 1 of SEQ ID NO: 2, a HCDR 2 of SEQ ID NO: 3, and a HCDR 3 of SEQ ID NO: 5, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 8, a LCDR 2 of SEQ ID NO: 9 and a LCDR 3 of SEQ ID NO: 10, and
25 (ii) a second antigen binding domain capable of specific binding to FolR1.

In one example, provided is a bispecific antibody, 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 the amino acid sequence of SEQ ID NO: 7, and/or the VL comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino
30 acid sequence of SEQ ID NO: 11.

In one example, the bispecific antibody binds to CD3 and FolR1, wherein the bispecific antibody comprises

(i) a first antigen binding domain capable of specific binding to CD3, comprising a VH sequence of SEQ ID NO: 7 and a VL sequence of SEQ ID NO: 11, and

(ii) a second antigen binding domain capable of specific binding to FolR1.

Thus, in another aspect, the present disclosure provides a bispecific antibody that binds to CD3
5 and FolR1, wherein the bispecific antibody comprises

(i) a first antigen binding domain capable of specific binding to CD3, comprising a VH sequence of SEQ ID NO: 7 and a VL sequence of SEQ ID NO: 11, and

(ii) a second antigen binding domain capable of specific binding to FolR1.

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

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

In one example, the bispecific antibody, comprises a third antigen binding domain capable of specific binding to FolR1.

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

15 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.

20 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
25 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
5 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 bispecific 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 IgG₁, Fc domain.

In one example, the Fc domain is a human Fc domain.

10 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.

15 In one example, the second and, where present, the third antigen binding domain comprises a VH comprising a HCDR 1 of SEQ ID NO: 124, a HCDR 2 of SEQ ID NO: 125, and a HCDR 3 of SEQ ID NO: 126, and a VL comprising a LCDR 1 of SEQ ID NO: 8, a LCDR 2 of SEQ ID NO: 9 and a LCDR 3 of SEQ ID NO: 10.

20 In one example, provided is the bispecific antibody as described herein above, 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: 123, 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: 11.

25 In another aspect, provided is an isolated polynucleotide encoding the bispecific antibody of the disclosure.

In another aspect, provided is a host cell comprising the isolated polynucleotide of the disclosure.

In another aspect, provided is a method of producing a bispecific antibody that binds to CD3 and FolR1, comprising the steps of (a) culturing the host cell of the disclosure under conditions suitable for the expression of the bispecific antibody and optionally (b) recovering the bispecific antibody.

30 In another aspect, provided is a bispecific antibody that binds to CD3 and FolR1 produced by the method of as described herein above.

In another aspect, provided is a pharmaceutical composition comprising the bispecific antibody of the disclosure and a pharmaceutically acceptable carrier.

In another aspect, provided is the bispecific antibody of the disclosure or the pharmaceutical composition of the disclosure for use as a medicament.

In another aspect, provided is the bispecific antibody of the disclosure or the pharmaceutical composition of the disclosure for use in the treatment of cancer.

- 5 In another aspect, provided is the use of the bispecific antibody of the disclosure or the pharmaceutical composition of the disclosure in the manufacture of a medicament.

In another aspect, provided is use of the bispecific antibody of the disclosure or the pharmaceutical composition of the disclosure in the manufacture of a medicament for the treatment of cancer.

- 10 The present disclosure provides a method of treating a disease in an individual, comprising administering to said individual an effective amount of the bispecific antibody of the disclosure or the pharmaceutical composition of the disclosure.

In one example, the disease is cancer.

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” 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 optionally introduced in the CH1 and CL domains. Crossfab molecules are depicted as comprising an exchange of VH and VL regions, but may – in aspects wherein no charge modifications are introduced in CH1 and CL domains – alternatively comprise an exchange of the CH1 and CL domains.
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- 30 **Figure 2.** Relative binding activity of original and optimized CD3 binders, CD3_{orig} and CD3_{opt}, to recombinant CD3 as measured by SPR in unstressed condition, after 14 d at 40°C pH 6, or after 14 d at 37°C pH 7.4 (IgG format).

CLAIMS

1. A bispecific antibody that binds to CD3 and Folate receptor 1 (FolR1), wherein the bispecific antibody comprises
 - 5 (i) a first antigen binding domain capable of specific binding to CD3, comprising a heavy chain variable region (VH) comprising a heavy chain complementarity determining region (HCDR) 1 of SEQ ID NO: 2, a HCDR 2 of SEQ ID NO: 3, and a HCDR 3 of SEQ ID NO: 5, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 8, a LCDR 2 of SEQ ID NO: 9 and a LCDR 3 of SEQ ID NO: 10, and
 - 10 (ii) a second antigen binding domain capable of specific binding to FolR1.
2. The bispecific 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 the amino acid sequence of SEQ ID NO: 7, and/or the VL comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid
15 sequence of SEQ ID NO: 11.
3. A bispecific antibody that binds to CD3 and FolR1, wherein the bispecific antibody comprises
 - (i) a first antigen binding domain capable of specific binding to CD3, comprising a VH sequence of SEQ ID NO: 7 and a VL sequence of SEQ ID NO: 11, and
 - (ii) a second antigen binding domain capable of specific binding to FolR1.
- 20 4. The bispecific antibody of any one of claims 1-3, wherein the first antigen binding domain is a Fab molecule.
5. The bispecific antibody of any one of claims 1-4, comprising an Fc domain composed of a first and a second subunit.
6. The bispecific antibody of any one of claims 1-5, comprising a third antigen binding domain
25 capable of specific binding to FolR1.
7. The bispecific antibody of claim 6, wherein the second and/or, where present, the third antigen binding domain is a Fab molecule.
8. The bispecific 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,
30 particularly the variable domains VL and VH, of the Fab light chain and the Fab heavy chain are replaced by each other.

9. The bispecific 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 bispecific 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 bispecific 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 bispecific 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 bispecific 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 bispecific 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 bispecific antibody of any one of claims 5-13, wherein the Fc domain is an IgG, particularly an IgG₁, Fc domain.

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

16. The bispecific 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 bispecific 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.

5 18. The bispecific antibody of any one of claims 6-17, wherein the second and, where present, the third antigen binding domain comprises a VH comprising a HCDR 1 of SEQ ID NO: 124, a HCDR 2 of SEQ ID NO: 125, and a HCDR 3 of SEQ ID NO: 126, and a VL comprising a LCDR 1 of SEQ ID NO: 8, a LCDR 2 of SEQ ID NO: 9 and a LCDR 3 of SEQ ID NO: 10.

10 19. The bispecific 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: 123, 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: 11.

20. An isolated polynucleotide encoding the bispecific antibody of any one of claims 1-19.

15 21. A host cell comprising the isolated polynucleotide of claim 20.

22. A method of producing a bispecific antibody that binds to CD3 and FolR1, comprising the steps of (a) culturing the host cell of claim 21 under conditions suitable for the expression of the bispecific antibody and optionally (b) recovering the bispecific antibody.

23. A bispecific antibody that binds to CD3 and FolR1 produced by the method of claim 22.

20 24. A pharmaceutical composition comprising the bispecific antibody of any one of claims 1-19 or 23 and a pharmaceutically acceptable carrier.

25. The bispecific antibody of any one of claims 1-19 or 23 or the pharmaceutical composition of claim 24 for use as a medicament.

25 26. The bispecific antibody of any one of claims 1-19 or 23 or the pharmaceutical composition of claim 24 for use in the treatment of cancer.

27. Use of the bispecific antibody of any one of claims 1-19 or 23 or the pharmaceutical composition of claim 24 in the manufacture of a medicament.

28. Use of the bispecific antibody of any one of claims 1-19 or 23 or the pharmaceutical composition of claim 24 in the manufacture of a medicament for the treatment of cancer.