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

In one example, the second antigen is TYRP-1. In one example, the second and, where present, the third antigen binding domain comprises a VH comprising a HCDR 1 of SEQ ID NO: 15, a HCDR 2 of SEQ ID NO: 16, and a HCDR 3 of SEQ ID NO: 17, and a VL comprising a LCDR 1 of SEQ ID NO: 19, a LCDR 2 of SEQ ID NO: 20 and a LCDR 3 of SEQ ID NO: 21. 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: 18, 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: 22.

In one example, the second antigen is EGFRvIII. In one example, the second and, where present, the third antigen binding domain comprises a VH comprising a HCDR 1 of SEQ ID NO: 85, a HCDR 2 of SEQ ID NO: 86, and a HCDR 3 of SEQ ID NO: 87, and a VL comprising a LCDR 1 of SEQ ID NO: 89, a LCDR 2 of SEQ ID NO: 90 and a LCDR 3 of SEQ ID NO: 91. 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: 88, 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: 92.

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

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 provides a method of producing an antibody that binds to CD3 and EGFRvIII, 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. 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 one aspect the disclosure provides an antibody or pharmaceutical composition according to the disclosure for use as a medicament. In one example is provided an antibody or pharmaceutical composition according to the disclosure for use in the treatment of a disease. In a specific example the disease is cancer. In another aspect the disclosure provides an antibody or pharmaceutical composition according to the disclosure for use in the treatment of cancer, wherein the cancer is a cancer expressing EGFRvIII.

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. In another aspect the disclosure provides a use of the antibody of the disclosure or the pharmaceutical composition of the disclosure in the manufacture of a medicament. In another aspect the disclosure provides a use of the antibody of the disclosure or the pharmaceutical composition of the disclosure in the manufacture of a medicament for the treatment of cancer, wherein the cancer is a cancer expressing EGFRvIII. 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.

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.

CLAIMS

1. An antibody that binds to CD3 and EGFRvIII, wherein the antibody comprises

(a) a first antigen binding domain that binds to CD3, comprising a heavy chain variable region (VH) comprising a heavy chain complementary 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

(b) a second and optionally a third antigen binding domain that binds to EGFRvIII.

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 the amino acid sequence of SEQ ID NO: 7, 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: 11.

3. An antibody that binds to CD3 and EGFRvIII, wherein the antibody comprises

(a) a first antigen binding domain that binds to CD3, comprising a VH sequence of SEQ ID NO: 7 and a VL sequence of SEQ ID NO: 11; and

(b) a second and optionally a third antigen binding domain that binds to EGFRvIII.

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

7. The antibody of any one of claims 1-6, 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.

8. The antibody of any one of claims 1-7, wherein the second and, where present, the third antigen binding domain is a conventional Fab molecule.

9. The antibody of any one of claims 1-8, 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).

10. The antibody of any one of claims 1-9, wherein the first and the second antigen binding domain are fused to each other, optionally via a peptide linker.

11. The antibody of any one of claims 1-10, 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.

12. The antibody of any one of claims 1-11, 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.

13. The antibody of claim 12, wherein the antibody consists of the first, the second and the third antigen binding domain, the Fc domain, and optionally one or more peptide linkers, wherein 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, and wherein the third antigen binding domain 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 domain is a human IgG1 Fc domain.

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

18. The antibody of any one of claims 5-17, 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.

19. The antibody of any one of claims 5-18, wherein in the first subunit of the Fc domain the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the second subunit of the Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V); and wherein optionally

(a) 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); and/or

(b) 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).

20. The antibody of any one of claims 5-19, wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

21. The antibody of any one of claims 5-20, wherein the Fc domain comprises an amino acid substitution at a position selected from the group of E233, L234, L235, N297, P331 and P329, particularly a position selected from the group of L234, L235 and P329 (numberings according to Kabat EU index).

22. The antibody of any one of claims 5-21, wherein each subunit of the Fc domain comprises the amino acid substitutions L234A, L235A and P329G (numberings according to Kabat EU index).

23. The antibody of any one of claims 1-22, wherein the second and, where present, the third antigen binding domain comprises a VH comprising a HCDR 1 of SEQ ID NO: 85, a HCDR 2 of

SEQ ID NO: 86, and a HCDR 3 of SEQ ID NO: 87, and a VL comprising a LCDR 1 of SEQ ID NO: 89, a LCDR 2 of SEQ ID NO: 90 and a LCDR 3 of SEQ ID NO: 91.

24. The antibody of any one of claims 1-23, 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: 88, 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: 92.

25. The antibody of any one of claims 1-24, wherein the antibody comprises a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 109, a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 110, a polypeptide (particularly two polypeptides) comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 111, and a polypeptide comprising an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 27.

26. An isolated polynucleotide encoding the antibody of any one of claims 1-25.

27. A host cell comprising the isolated polynucleotide of claim 26.

28. A method of producing an antibody that binds to CD3 and EGFRvIII, comprising the steps of (a) culturing the host cell of claim 27 under conditions suitable for the expression of the antibody and optionally (b) recovering the antibody.

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

30. The antibody of any one of claims 1-25 or the pharmaceutical composition of claim 29 for use as a medicament.

31. The antibody of any one of claims 1-25 or the pharmaceutical composition of claim 29 for use in the treatment of cancer, wherein the cancer is a cancer expressing EGFRvIII.

32. The antibody of claim 31, wherein the cancer is a brain cancer, in particular a glioblastoma.

33. Use of the antibody of any one of claims 1-25 or the pharmaceutical composition of claim 29 in the manufacture of a medicament.

34. Use of the antibody of any one of claims 1-25 or the pharmaceutical composition of claim 29 in the manufacture of a medicament for the treatment of cancer, wherein the cancer is a cancer expressing EGFRvIII.

35. The use of claim 34, wherein the cancer is a brain cancer, in particular a glioblastoma.