

- (i) a first antigen binding moiety which is a Fab molecule capable of specific binding to CD3, and which comprises at least one heavy chain complementarity determining region (CDR) amino acid sequence selected from the group consisting of SEQ ID NO: 37, SEQ ID NO: 38 and SEQ ID NO: 39 and at least one light chain CDR selected from the group of SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34; and
- (ii) a second antigen binding moiety capable of specific binding to Folate Receptor 1 (FolR1).

In one aspect, the disclosure provides a T cell activating bispecific antigen binding molecule comprising

- (i) a first antigen binding moiety which is a Fab molecule capable of specific binding to CD3;
- (ii) a second antigen binding moiety capable of specific binding to Folate Receptor 1 (FolR1) which comprises:

- a) the heavy chain CDR1 of SEQ ID NO: 16, the heavy chain CDR2 of SEQ ID NO: 17, the heavy chain CDR3 of SEQ ID NO: 18, the light chain CDR1 of SEQ ID NO: 32, the light chain CDR2 of SEQ ID NO: 33, and the light chain CDR3 of SEQ ID NO: 34;

- b) the heavy chain CDR1 of SEQ ID NO: 8, the heavy chain CDR2 of SEQ ID NO: 56, the heavy chain CDR3 of SEQ ID NO: 57, the light chain CDR1 of SEQ ID NO: 59, the light chain CDR2 of SEQ ID NO: 60, and the light chain CDR3 of SEQ ID NO: 65; or

- c) a complementarity determining region heavy chain 1 (CDR-H1) amino acid sequences of SEQ ID NO: 16, a CDR-H2 amino acid sequence of SEQ ID NO: 275, a CDR-H3 amino acid sequence of SEQ ID NO: 315, a complementarity determining region light chain 1 (CDR-L1) amino acid sequence of SEQ ID NO: 32, a CDR-L2 amino acid sequence of SEQ ID NO: 33, and, a CDR-L3 amino acid sequence of SEQ ID NO: 34.

In one embodiment, the T cell activating bispecific antigen binding molecule comprises a first antigen binding moiety that comprises a variable heavy chain comprising an amino acid

sequence of SEQ ID NO: 36 and a variable light chain comprising an amino acid sequence of SEQ ID NO: 31. In one embodiment, the T cell activating bispecific antigen binding molecule additionally comprises (iii) a third antigen binding moiety capable of specific binding to FolR1. In one embodiment, the second and third antigen binding moiety capable of specific binding to
5 FolR1 comprise identical heavy chain complementarity determining region (CDR) and light chain CDR sequences. In one embodiment, the third antigen binding moiety is identical to the second antigen binding moiety.

In one embodiment of the T cell activating bispecific antigen binding molecule of the above embodiments, at least one of the second and third antigen binding moiety is a Fab molecule. In
10 one embodiment, the T cell activating bispecific antigen binding molecule of the above embodiments, additionally comprises an Fc domain composed of a first and a second subunit capable of stable association. In some embodiments, the first antigen binding moiety and the second antigen binding moiety are each connected at the C-terminus of the Fab heavy chain to the N-terminus of the first or second subunit of the Fc domain. In some embodiments, a third
15 antigen binding moiety 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 moiety, optionally via a peptide linker.

In one embodiment of the T cell activating bispecific antigen binding molecule of the above embodiments, the antigen binding moiety capable of specific binding to Folate Receptor 1 (FolR1) comprises at least one heavy chain complementarity determining region (CDR) amino
20 acid sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18 and at least one light chain CDR selected from the group of SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34. In one embodiment, the antigen binding moiety capable of specific binding to Folate Receptor 1 (FolR1) comprises a variable heavy chain comprising an amino

the T cell activating bispecific antigen binding molecule of any of the above embodiments has an affinity (monovalent binding) of 1 μ M or greater. In one embodiment, the affinity is around 1.4 μ M for human FolR1. In one embodiment, the T cell activating bispecific antigen binding molecule of any of the above embodiments has an avidity (bivalent binding) of about 1-100nM or lower. In one embodiment, the avidity is about 10 nM or less. In one embodiment, the avidity is 10 nM.

In one embodiment, the T cell activating bispecific antigen binding molecule of any of the above embodiments binds to FolR1 expressed on a human tumor cell. In one embodiment, the T cell activating bispecific antigen binding molecule of any of the above embodiments binds to a conformational epitope on human FolR1. In one embodiment, the T cell activating bispecific antigen binding molecule of any of the above embodiments does not bind to human Folate Receptor 2 (FolR2) or to human Folate Receptor 3 (FolR3). In one embodiment of the T cell activating bispecific antigen binding molecule of any of the above embodiments, the antigen binding moiety binds to a FolR1 polypeptide comprising the amino acids 25 to 234 of human FolR1 (SEQ ID NO:227). In one embodiment of the T cell activating bispecific antigen binding molecule of any of the above embodiments, the FolR1 antigen binding moiety binds to a FolR1 polypeptide comprising the amino acid sequence of SEQ ID NOs:227, 230 and 231, and wherein the FolR1 antigen binding moiety does not bind to a FolR polypeptide comprising the amino acid sequence of SEQ ID NOs:228 and 229.

The disclosure provides for a bispecific antibody comprising a) a first antigen-binding site that competes for binding to human FolR1 with a reference antibody comprising a variable heavy chain domain (VH) of SEQ ID NO: 49 and a variable light chain domain of SEQ ID NO: 51; and b) a second antigen-binding site that competes for binding to human CD3 with a reference antibody comprising a variable heavy chain domain (VH) of SEQ ID NO: 36 and a variable light chain domain of SEQ ID NO: 31, wherein binding competition is measured using a surface plasmon resonance assay.

The disclosure provides for a bispecific antibody comprising a) a first antigen-binding site that competes for binding to human FolR1 with a reference antibody comprising a variable heavy chain domain (VH) of SEQ ID NO: 274 and a variable light chain domain of SEQ ID NO: 31; and b) a second antigen-binding site that competes for binding to human CD3 with a reference antibody comprising a variable heavy chain domain (VH) of SEQ ID NO: 36 and a variable light chain domain of SEQ ID NO: 31, wherein binding competition is measured using a surface plasmon resonance assay.

chain comprises the amino acid sequence of SEQ ID NO:309. In one of the above embodiments of the T cell activating bispecific antigen binding molecule, the fifth polypeptide chain comprises the amino acid sequence of SEQ ID NO:308. In one of the above embodiments of the T cell activating bispecific antigen binding molecule, the first and second polypeptide chain
5 comprise the amino acid sequence of SEQ ID NO:230; the third polypeptide chain comprises the amino acid sequence of SEQ ID NO:86; the fourth polypeptide chain comprises the amino acid sequence of SEQ ID NO:309; and the fifth polypeptide chain comprise the amino acid sequence of SEQ ID NO:308.

The disclosure provides for a T cell activating bispecific antigen binding molecule comprising
10 the amino acid sequence of SEQ ID NO:308. In one embodiment, the T cell activating bispecific antigen binding molecule of the above embodiment further comprises the amino acid sequence of SEQ ID NO:230 and of SEQ ID NO:86.

The disclosure provides for an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:308. The disclosure provides for an isolated polypeptide comprising the amino acid
15 sequence of SEQ ID NO:309.

The disclosure provides for a T cell activating bispecific antigen binding molecule comprising the amino acid sequence of SEQ ID NO:276. The T cell activating bispecific antigen binding molecule of the disclosure further comprises the amino acid sequence of SEQ ID NO:277 and of SEQ ID NO:35. Thus, in one aspect, the disclosure provides for a T cell activating bispecific antigen binding molecule
20 comprising the amino acid sequence of SEQ ID NO:276 and further comprising the amino acid sequence of SEQ ID NO: 277 and of SEQ ID NO: 35.

The disclosure provides for an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:277. The disclosure provides for an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:276.

In one aspect, the disclosure provides for an isolated polynucleotide encoding the T cell activating bispecific antigen binding molecule as disclosed herein or a fragment thereof. In one embodiment, the disclosure provides for an isolated polynucleotide encoding a T cell activating bispecific antigen binding molecule comprising the nucleotide sequence of SEQ ID NO:169. In one embodiment, the disclosure provides for an isolated polynucleotide encoding a T cell activating bispecific antigen binding molecule
30 comprising the nucleotide sequence of SEQ ID NO:246. In one embodiment, the disclosure provides for an isolated polynucleotide encoding a T cell activating bispecific antigen binding molecule comprising the nucleotide sequence of SEQ ID NO:247. In one embodiment, the disclosure provides for an isolated polynucleotide encoding a T cell activating bispecific antigen binding molecule comprising the nucleotide sequence of SEQ ID NO:97. In one embodiment, the disclosure provides for an isolated
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The disclosure provides for 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 of any one of the above embodiments in a pharmaceutically acceptable form. In some embodiments, said disease is a cancer.

- 5 The disclosure provides for a 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 the above embodiments in the presence of a T cell. In one aspect, the disclosure provides for an *in vitro* method for inducing lysis of a FolR1⁺ target cell, comprising contacting a target cell with the T cell activating bispecific antigen binding molecule as disclosed herein in the presence of a CD3⁺
- 10 T cell.

Brief Description of the Drawings

Figures 1A-I illustrate exemplary configurations of the T cell activating bispecific antigen binding molecules (TCBs) of the invention. All constructs except the kappa-lambda format in (Fig. 1I) have P329G LALA mutations and comprise knob-into-hole Fc fragments with knob-into-hole modifications. (Fig. 1A) Illustration of the “FolR1 TCB 2+1 inverted (common light chain)”. The FolR1 binder is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain comprising the knob modification. These constructs are not

15 crossed and have three times the same VLCL light chain. (Fig. 1B) Illustration of the “FolR1 TCB 1+1 head-to-tail (common light chain)”. These constructs are not crossed and have two times the same VLCL light chain. (Fig. 1C) Illustration of the “FolR1 TCB 1+1 classical (common light chain)”. These constructs are not crossed and have two times the same VLCL light chain. (Fig. 1D) Illustration of the “FolR1 TCB 2+1 classical (common light chain)”. The

20 CD3 binder is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain comprising the knob modification. These constructs are not crossed and have three times the same VLCL light chain. (Fig. 1E) Illustration of the “FolR1 TCB 2+1 crossfab classical”. These constructs comprise a Ck-VH chain for the CD3 binder instead of the conventional CH1-VH chain. The CD3 binder is fused at the C-terminus of the Fab heavy chain

25 to the N-terminus of the first subunit of the Fc domain comprising the knob modification. (Fig. 1F) Illustration of the “FolR1 TCB 2+1 crossfab inverted”. These constructs comprise a Ck-VH chain for the CD3 binder instead of the conventional CH1-VH chain. The FolR1 binder is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain comprising the knob modification. (Fig. 1G) Illustration of the “FolR1 TCB 1+1 crossfab head-

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Claims

1. A T cell activating bispecific antigen binding molecule comprising
 - (i) a first antigen binding moiety which is a Fab molecule capable of specific binding to CD3;
 - (ii) a second antigen binding moiety capable of specific binding to Folate Receptor 1 (FolR1) which comprises:
 - a) the heavy chain CDR1 of SEQ ID NO: 16, the heavy chain CDR2 of SEQ ID NO: 17, the heavy chain CDR3 of SEQ ID NO:18, the light chain CDR1 of SEQ ID NO: 32, the light chain CDR2 of SEQ ID NO: 33, and the light chain CDR3 of SEQ ID NO:34;
 - b) the heavy chain CDR1 of SEQ ID NO: 8, the heavy chain CDR2 of SEQ ID NO: 56, the heavy chain CDR3 of SEQ ID NO:57, the light chain CDR1 of SEQ ID NO: 59, the light chain CDR2 of SEQ ID NO: 60, and the light chain CDR3 of SEQ ID NO:65; or
 - c) a complementarity determining region heavy chain 1 (CDR-H1) amino acid sequences of SEQ ID NO: 16, a CDR-H2 amino acid sequence of SEQ ID NO: 275, a CDR-H3 amino acid sequence of SEQ ID NO: 315, a complementarity determining region light chain 1 (CDR-L1) amino acid sequence of SEQ ID NO: 32, a CDR-L2 amino acid sequence of SEQ ID NO: 33, and, a CDR-L3 amino acid sequence of SEQ ID NO: 34.
2. The T cell activating bispecific antigen binding molecule of claim 1, wherein the first antigen binding moiety comprises the heavy chain CDR1 of SEQ ID NO: 37, the heavy chain CDR2 of SEQ ID NO: 38, the heavy chain CDR3 of SEQ ID NO:39, the light chain CDR1 of SEQ ID NO: 32, the light chain CDR2 of SEQ ID NO: 33, and the light chain CDR3 of SEQ ID NO:34.
3. The T cell activating bispecific antigen binding molecule of claim 1 or 2, wherein the first antigen binding moiety comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO: 36 and a variable light chain comprising an amino acid sequence of SEQ ID NO: 31.

4. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 3, additionally comprising
 - (iii) a third antigen binding moiety capable of specific binding to FolR1.

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5. The T cell activating bispecific antigen binding molecule of claim 4, wherein at least one of the second and third antigen binding moiety is a Fab molecule.
6. The T cell activating bispecific antigen binding molecule of any of claims 1 to 5, additionally comprising

(iv) an Fc domain composed of a first and a second subunit capable of stable association.

- 5 7. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 6, wherein the antigen binding moiety capable of specific binding to FolR1 comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO: 15 and a variable light chain comprising an amino acid sequence of SEQ ID NO: 31.
- 10 8. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 6, wherein the antigen binding moiety capable of specific binding to FolR1

comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO: 55 and a variable light chain comprising an amino acid sequence of SEQ ID NO: 64.

9. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 6, wherein the antigen binding moiety capable of specific binding to FolR1 comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO: 274 and a variable light chain comprising an amino acid sequence of SEQ ID NO: 31.

5 10. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 6 or 8, wherein the first antigen binding moiety is a crossover Fab molecule wherein either the variable or the constant regions of the Fab light chain and the Fab heavy chain are exchanged.

11. The T cell activating bispecific antigen binding molecule of any one of claims 6 to 10, wherein the Fc domain is an IgG class immunoglobulin, specifically an IgG₁ or IgG₄, Fc domain.
- 5 12. The T cell activating bispecific antigen binding molecule of any one of claims 6 to 11, wherein the Fc domain is a human Fc domain.
13. The T cell activating bispecific antigen binding molecule of any one of claims 6 to 12, wherein the Fc domain comprises a modification promoting the association of the first and the second subunit of the Fc domain.
- 10 14. The T cell activating bispecific antigen binding molecule of claim 13, 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
15 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.
- 15 15. The T cell activating bispecific antigen binding molecule of any one of claims 6 to 14, wherein the Fc domain comprises at least one amino acid substitution that reduces
20 binding to an Fc receptor and/or effector function, as compared to a native IgG₁ Fc domain.
- 25 16. The T cell activating bispecific antigen binding molecule of claim 15, wherein each subunit of the Fc domain comprises three amino acid substitutions that reduce binding to an activating Fc receptor and/or effector function wherein said amino acid substitutions are L234A, L235A and P329G (Kabat numbering).

17. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 16, wherein the T cell activating bispecific antigen binding molecule does not bind to human Folate Receptor 2 (FolR2) or to human Folate Receptor 3 (FolR3).

18. A T cell activating bispecific antigen binding molecule comprising the amino acid sequence of SEQ ID NO:276 and further comprising the amino acid sequence of SEQ ID NO: 277 and of SEQ ID NO: 35.

19. An isolated polynucleotide encoding the T cell activating bispecific antigen binding molecule of any one of claims 1 to 18 or a fragment thereof.
20. A polypeptide encoded by the polynucleotide of claim 19.
21. A vector, particularly an expression vector, comprising the polynucleotide of claim 19.
- 5 22. A host cell comprising the polynucleotide of claim 19 or the vector of claim 21.
23. A method of producing the 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 22 under conditions suitable for the expression of the T cell activating bispecific antigen binding molecule and b) recovering the T cell activating
10 bispecific antigen binding molecule.

24. A T cell activating bispecific antigen binding molecule produced by the method of claim 23.
25. A pharmaceutical composition comprising the T cell activating bispecific antigen binding molecule of any one of claims 1 to 18 and a pharmaceutically acceptable carrier.
- 5 26. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 18 or the pharmaceutical composition of claim 25 for use as a medicament.
27. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 18 or the pharmaceutical composition of claim 25 for use in the treatment of cancer.
- 10 28. Use of the T cell activating bispecific antigen binding molecule of any one of claims 1 to 18 for the manufacture of a medicament for the treatment of cancer.
29. An *in vitro* method for inducing lysis of a FolR1⁺ target cell, comprising contacting a target cell with the T cell activating bispecific antigen binding molecule of any one of claims 1 to 18 in the presence of a CD3⁺ T cell.