

The present invention provides novel antibodies, including bispecific antibodies, that bind STEAP-1 and are resistant to degradation by e.g. succinimide formation and thus show good stability. The (bispecific) antibodies provided further combine good efficacy and produceability with low toxicity and favorable pharmacokinetic properties.

5

Summary

The present inventors have developed a novel antibody with unexpected, improved properties, that binds to STEAP-1. In particular, the antibody is resistant to degradation e.g. by succinimide formation, and thus particularly stable as required for therapeutic purposes. Furthermore, the
10 inventors have developed bispecific antigen binding molecules that bind to STEAP-1 and an activating T cell antigen, incorporating the novel STEAP-1 antibody.

The present disclosure provides an antibody that binds to STEAP-1, wherein the antibody shows less than about 5% succinimide degradation after 4 weeks at pH 7.4, 37°C, and/or less than about 10% succinimide degradation after 4 weeks at pH 6.0, 40°C, as determined by mass
15 spectrometry.

In one aspect the present disclosure provides an antibody that binds to STEAP-1, wherein the antibody comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 1, a HCDR 2 of SEQ ID NO: 2, and a HCDR 3 selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID
20 NO: 6, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 7, a LCDR 2 of SEQ ID NO: 8 and a LCDR 3 of SEQ ID NO: 9. In one example, the VH 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 of SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13, and the VL comprises an amino
25 acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 14. In one example, the antibody is an IgG, particularly an IgG₁, antibody. In one example, the antibody is a full-length antibody. In another example, the antibody is an antibody fragment selected from the group of an Fv molecule, a scFv molecule, a Fab molecule, and a F(ab')₂ molecule. In one example, the antibody is a multispecific antibody.

30 In a further aspect, the disclosure also provides a bispecific antigen binding molecule, comprising (a) a first antigen binding moiety that binds to a first antigen, wherein the first antigen is STEAP-1 and the first

antigen binding moiety comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 1, a HCDR 2 of SEQ ID NO: 2, and a HCDR 3 selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 7, a LCDR 2 of SEQ ID NO: 8 and a LCDR 3 of SEQ ID NO: 9, and (b) a second antigen binding moiety which specifically binds to a second antigen. In one example, the VH of the first antigen binding moiety 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 of SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13, and the VL of the first antigen binding moiety 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: 14. In one example, the second antigen is CD3, particularly CD3ε. In one example, the second antigen binding moiety 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: 18, a LCDR 2 of SEQ ID NO: 19 and a LCDR 3 of SEQ ID NO: 20. In one example, the VH of the second antigen binding moiety 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: 21, and the VL of the second antigen binding moiety 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: 22. In one example, the first and/or the second antigen binding moiety is a Fab molecule. In one example, the second antigen binding moiety 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 first antigen binding moiety is a Fab molecule wherein in the constant domain 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 moiety are fused to each other, optionally via a peptide linker. In one example, the first and the second antigen binding moiety are each a Fab

molecule and either (i) the second 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, or (ii) the first 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 second antigen binding moiety. In one example, the bispecific antigen binding molecule comprises a third antigen binding moiety. In one example, the third antigen moiety is identical to the first antigen binding moiety. In one example, the bispecific antigen binding molecule comprises an Fc domain composed of a first and a second subunit. In one example, the first, the second and, where present, the third antigen binding moiety are each a Fab molecule; and either (i) the second 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 and the first antigen binding moiety 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 moiety 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 moiety and the second antigen binding moiety 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 moiety, 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. In one example, an amino acid residue in the CH3 domain of the first subunit of the Fc domain 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 an amino acid residue in the CH3 domain of the second subunit of the Fc domain 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. In one example, the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

According to another aspect of the disclosure there is provided one or more isolated polynucleotide(s) encoding an antibody or 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 invention. According to another

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

In another aspect is provided a method of producing an antibody that binds to STEAP-1, comprising the steps of a) culturing the host cell of the disclosure under conditions suitable for the expression of the antibody and b) recovering the antibody. In another aspect the disclosure encompasses an antibody that binds to STEAP-1 produced by the method of the disclosure.

- 5 In another aspect the disclosure provides a pharmaceutical composition comprising the antibody or bispecific antigen binding molecule of the disclosure and a pharmaceutically acceptable carrier.

Also encompassed by the disclosure are methods of using the antibody, bispecific antigen binding molecule and pharmaceutical composition of the disclosure. In another aspect the
10 disclosure provides an antibody, bispecific antigen binding molecule or pharmaceutical composition according to the disclosure for use as a medicament. In another aspect is provided an antibody, bispecific antigen binding molecule or pharmaceutical composition according to the disclosure for use in the treatment of a disease. In a specific embodiment the disease is cancer.

In another aspect there is provided use of an antibody or bispecific antigen binding molecule
15 according to the disclosure in the manufacture of a medicament for the treatment of a disease. There is also provided a method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the antibody or bispecific antigen binding molecule according to the disclosure in a pharmaceutically acceptable form. In a specific example the disease is cancer. In any of the above examples the individual
20 preferably is a mammal, particularly a human.

Brief Description of the Drawings

Figure 1. Exemplary configurations of the bispecific antigen binding molecules 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
25 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-
30 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”

Claims

1. An antibody that binds to STEAP-1, wherein the antibody comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 1, a HCDR 2 of SEQ ID NO: 2, and a HCDR 3 selected from the group consisting
5 of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 7, a LCDR 2 of SEQ ID NO: 8 and a LCDR 3 of SEQ ID NO: 9.
2. The antibody of claim 1, wherein the VH comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected
10 from the group of SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13, and the VL 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: 14.
3. The antibody of claim 1 or 2, wherein the antibody is an IgG, particularly an IgG₁, antibody.
- 15 4. The antibody of any one of claims 1 to 3, wherein the antibody is a full-length antibody.
5. The antibody of any one of claims 1 to 3, wherein the antibody is an antibody fragment selected from the group of an Fv molecule, a scFv molecule, a Fab molecule, and a F(ab')₂ molecule.
6. The antibody of any one of claims 1 to 5, wherein the antibody is a multispecific
20 antibody.
7. A bispecific antigen binding molecule, comprising
(a) a first antigen binding moiety that binds to a first antigen,
wherein the first antigen is STEAP-1 and the first antigen binding moiety comprises a heavy chain variable region (VH) comprising a heavy chain complementary determining region
25 (HCDR) 1 of SEQ ID NO: 1, a HCDR 2 of SEQ ID NO: 2, and a HCDR 3 selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, and a light chain variable region (VL) comprising a light chain complementarity determining region (LCDR) 1 of SEQ ID NO: 7, a LCDR 2 of SEQ ID NO: 8 and a LCDR 3 of SEQ ID NO: 9, and
(b) a second antigen binding moiety which specifically binds to a second antigen.

8. The bispecific antigen binding molecule of claim 7, wherein the VH of the first antigen binding moiety 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 of SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13, and the VL of the first antigen binding moiety comprises
5 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: 14.
9. The bispecific antigen binding molecule of claim 8, wherein the second antigen is CD3, particularly CD3ε.
10. The bispecific antigen binding molecule of claim 9, wherein the second antigen binding
10 moiety 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: 18, a LCDR 2 of SEQ ID NO: 19 and a LCDR 3 of SEQ ID NO: 20.
11. The bispecific antigen binding molecule of claim 10, wherein the VH of the second
15 antigen binding moiety 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: 21, and the VL of the second antigen binding moiety 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: 22.
12. The bispecific antigen binding molecule of any one of claims 7 to 11, wherein the first and/or the second antigen binding moiety is a Fab molecule.
- 20 13. The bispecific antigen binding molecule of any one of claims 7 to 12, wherein the second antigen binding moiety 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.
14. The bispecific antigen binding molecule of any one of claims 7 to 13, wherein the first
25 antigen binding moiety is a Fab molecule wherein in the constant domain 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
30 (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).

15. The bispecific antigen binding molecule of any one of claims 7 to 14, wherein the first and the second antigen binding moiety are fused to each other, optionally via a peptide linker.

5 16. The bispecific antigen binding molecule of any one of claims 7 to 15, wherein the first and the second antigen binding moiety are each a Fab molecule and wherein either (i) the second 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, or (ii) the first 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
10 second antigen binding moiety.

17. The bispecific antigen binding molecule of any one of claims 7 to 16, comprising a third antigen binding moiety.

18. The bispecific antigen binding molecule of claim 17, wherein the third antigen moiety is identical to the first antigen binding moiety.

15 19. The bispecific antigen binding molecule of any one of claims 7 to 18, comprising an Fc domain composed of a first and a second subunit.

20. The bispecific antigen binding molecule of claim 19, wherein the first, the second and, where present, the third antigen binding moiety are each a Fab molecule;
and wherein either (i) the second antigen binding moiety is fused at the C-terminus of the Fab
20 heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety and the first antigen binding moiety 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 moiety 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 moiety and the second antigen binding moiety is fused at the C-terminus of the Fab
25 heavy chain to the N-terminus of the first subunit of the Fc domain;
and wherein the third antigen binding moiety, 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.

21. The bispecific antigen binding molecule of claim 19 or 20, wherein the Fc domain is an IgG, particularly an IgG₁, Fc domain.

22. The bispecific antigen binding molecule of any one of claims 19 to 21, wherein the Fc domain is a human Fc domain.

23. The bispecific antigen binding molecule of any one of claims 19 to 22, wherein an amino acid residue in the CH3 domain of the first subunit of the Fc domain 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 an amino acid residue in the CH3 domain of the second subunit of the Fc domain 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.

24. The bispecific antigen binding molecule of any one of claims 19 to 22, wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

25. One or more isolated polynucleotide encoding the antibody or bispecific antigen binding molecule of any one of claims 1 to 24.

26. One or more vector, particularly expression vector, comprising the polynucleotide(s) of claim 25.

27. A host cell comprising the polynucleotide(s) of claim 25 or the vector(s) of claim 26.

28. A method of producing an antibody that binds to STEAP-1, comprising the steps of a) culturing the host cell of claim 27 under conditions suitable for the expression of the antibody and b) optionally recovering the antibody.

29. An antibody that binds to STEAP-1, produced by the method of claim 28.

30. A pharmaceutical composition comprising the antibody or bispecific antigen binding molecule of any one of claims 1 to 24 or 29 and a pharmaceutically acceptable carrier.

31. The antibody or bispecific antigen binding molecule of any one of claims 1 to 24 or 29 or the pharmaceutical composition of claim 30 for use as a medicament.

32. The antibody or bispecific antigen binding molecule of any one of claims 1 to 24 or 29 or the pharmaceutical composition of claim 30 for use in the treatment of a disease.

33. The antibody, bispecific antigen binding molecule or pharmaceutical composition of claim 32, wherein the disease is cancer.

34. Use of the antibody or bispecific antigen binding molecule of any one of claims 1 to 24 or 29 in the manufacture of a medicament for the treatment of a disease.

5 35. The use of claim 34, wherein said disease is cancer.