

2925-2934). Smith et al. have described engineered T cells with B7H4-specific chimeric antigen receptors (CARs) that displayed anti-tumor activity against B7H4-positive human ovarian tumor xenografts, but which also showed multi-organ lymphocytic infiltration and lethal toxicity (Smith et al. 2016, Molecular Therapy, Vol.24 Iss. 11 pp 1987-99) in mice.

- 5 While there has been some progress made, there is a need for the development of antibody-based cancer therapy targeting B7H4 that is efficacious and/or safe for human use.

Summary

- In one aspect, the present disclosure refers to an antibody comprising a first antigen-binding region which binds to human B7H4, wherein the first antigen-binding region comprises a variable heavy chain (VH) region comprising: a CDR1 comprising the sequence of SEQ ID NO: 26, a CDR2 comprising the sequence of SEQ ID NO: 30, and a CDR3 comprising the sequence of SEQ ID NO: 28; and a variable light chain (VL) region comprising: a CDR1 comprising the sequence of SEQ ID NO: 34, a CDR2 comprising the sequence GAS, and a CDR3 comprising the sequence of SEQ ID NO: 35; and said antibody further comprising a second antigen-binding region which binds to human CD3.
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- 15 In another aspect, the present disclosure refers to an antibody comprising a first antigen-binding region which binds to human B7H4, wherein the first antigen-binding region comprises a variable heavy chain (VH) region comprising the sequence of SEQ ID NO. 29 and a variable light chain (VL) region comprising the sequence of SEQ ID NO. 33, said antibody further comprising a second antigen-binding region which binds to human CD3.
- 20 In another aspect, the present disclosure refers to a bispecific antibody comprising a first antigen-binding region which binds to human B7H4, wherein the first antigen-binding region comprises a variable heavy chain (VH) region comprising: a CDR1 comprising the sequence of SEQ ID NOs.: 26, a CDR2 comprising the sequence of SEQ ID NOs.: 30, and a CDR3 comprising the sequence of SEQ ID NO: 28; and a variable light chain (VL) region comprising: a CDR1 comprising the sequence of SEQ ID NO. 34,
- 25 a CDR2 comprising the sequence GAS; and a CDR3 comprising the sequence of SEQ ID NO. 35, and said bispecific antibody further comprising a second antigen-binding region which binds to human CD3, wherein the second antigen-binding region comprises a VH region comprising: a CDR1 comprising the sequence of SEQ ID NO: 18, a CDR2 comprising the sequence of SEQ ID NO: 19, and a CDR3 comprising the sequence of SEQ ID NO: 21; and a VL region comprising: a CDR1 comprising the sequence of SEQ ID
- 30 NO: 23, a CDR2 comprising the sequence GTN, and a CDR3 comprising the sequence of SEQ ID NO: 24.

In another aspect, the present disclosure refers to a bispecific antibody comprising a first antigen-binding region which binds to human B7H4, wherein the first antigen-binding region comprises a variable heavy chain region comprising the sequence of SEQ ID NO. 29 and a variable light chain region comprising the sequence of SEQ ID NO. 33, said bispecific antibody further comprising a second antigen-binding region which binds to human CD3, wherein the second antigen-binding region comprises a variable heavy chain region comprising the sequence of SEQ ID NO. 17 and a variable light chain region comprising the sequence of SEQ ID NO. 22.

In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising an antibody as defined in the disclosure and a pharmaceutically acceptable carrier.

10 In yet another aspect, the present disclosure refers to use of the antibody according to the disclosure in the manufacture of a medicament for treating cancer.

In yet another aspect, the present disclosure refers to a nucleic acid encoding for the antibody according to the disclosure.

15 In yet another aspect, the present disclosure refers to a cell comprising a nucleic acid according to the disclosure.

In yet another aspect, the present disclosure refers to a method for producing an antibody which binds to B7H4 and CD3 according the disclosure comprising the steps of: a) providing an antibody which binds to B7H4 according the disclosure; b) providing an antibody which binds to CD3 according to the disclosure; c) incubating said antibody which binds to B7H4 together with said antibody which binds to CD3 under reducing conditions sufficient to allow cysteines in the hinge region to undergo disulfide-bond isomerization, and d) obtaining said antibody which binds to human B7H4 and human CD3.

20 In yet another aspect, the present disclosure refers to an antibody comprising an antigen-binding region capable of binding to human B7H4, wherein said antigen-binding region capable of binding to human B7H4 comprises: a) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions of SEQ ID NO. 29 : and a variable light chain region comprising the CDR1, CDR2 and CDR3 regions respectively of SEQ ID NO. 33; b) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions respectively of SEQ ID NOs.: 26, 30 and 28, and a variable light chain region comprising the CDR1, CDR2 and CDR3 respectively of SEQ ID NO. 34, GAS and SEQ ID NO. 35; and c) a variable heavy chain (VH) region of SEQ ID NO. 29 : and a variable light chain region of SEQ ID NO. 33.

25 In yet another aspect, the present disclosure refers to antibody in the disclosure, wherein said antibody is a monospecific antibody.

30 It is an object of the present invention to provide for antibodies comprising an antigen-binding region capable of binding to human B7H4 and an antigen-binding region that binds to CD3, such as human CD3ε (epsilon). The antigen-binding regions of such antibodies comprise at least human framework regions, such as e.g FR1, FR2, FR3 and FR4. Most preferred is that all framework regions are human.

Such antigen-binding regions are humanized and/or human antigen-binding regions. These antibodies are useful in the treatment of conditions wherein specific targeting and T cell mediated killing of B7H4 expressing cells is desired, e.g. in conditions such as cancer. Preferably, such an antibody is suitable for human use, e.g. in a medical treatment. Cancers that may be suitable for treatment are solid tumors.

- 5 Said B7H4 expression, and T cell mediated killing, e.g. in cancer cells, may range in accordance with the invention from relatively low expression of B7H4, such as in MCF-7 cells, to relatively high expression of B7H4, such as in SK-BR3 cells, as shown e.g. in example 12. More preferably such bispecific antibodies have substitutions within the constant region that renders the Fc region, if present, inert.

- 10 In one example, a bispecific antibody is provided comprising an antigen-binding region capable of binding to human B7H4 and an antigen-binding region that is capable of binding to CD3, such as human CD3 ϵ (epsilon), wherein the antigen-binding region capable of binding to human B7H4 comprises a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions of SEQ ID NO. 25, 29 or 31, and a variable light chain region comprising the CDR1, CDR2 and CDR3 of SEQ ID NO. 33 and wherein the antigen-binding region that is capable of binding to CD3 comprises a heavy chain variable region (VH) comprising the CDR1, CDR2, and CDR3 sequences of 18, 19 and 21 respectively; and, a light chain variable region (VL) comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 23, 24, and 25, respectively.

In another example, nucleic acids, such as DNA or RNA, are provided encoding antibodies as defined herein, as well as methods of producing the antibodies, or components thereof, as defined herein.

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In a further example, said antibodies, or nucleic acids, in accordance with the invention are for use in a medical treatment.

Brief description of the figures

Figure 1. Determination of B7H4 domain involved in binding using B7H4-B7H3 chimeric molecules. The B7H4 domain specificity of the B7H4 antibodies was determined using a panel of cells transfected to express human B7H4 (I), human B7H4-B7H3 chimeric molecules B7H3-IgV/B7H4-IgC (II) or B7H4-IgV/B7H3-IgC (III), or human B7H3 (IV). Binding was determined by flow cytometry. A = bsIgG1-huCD3-FEALxB7H4-C4-FEAR; B = bsIgG1-huCD3-FEALxB7H4-C3-FEAR; C = bsIgG1-huCD3-FEALxB7H4-C2-FEAR; D = bsIgG1-huCD3-FEALxB7H4-C1-FEAR; E = IgG1-B7H3-BRCA84D.

Figure 2. Binding of B7H4 antibodies to B7H4, B7H3 or B7H4-B7H3 chimeric molecules. Binding of bsIgG1-huCD3-H101G-FEALxB7H4-C1-N52S-FEAR, bsIgG1-huCD3-H101G-FEALxB7H4-C2-FEAR, bsIgG1-huCD3-H101G-FEALxB7H4-C3-FEAR, bsIgG1-huCD3-H101G-FEALxB7H4-C4-FEAR and bsIgG1-huCD3-H101G-FEALxB7H4-C5-FEAR to HEK cells transiently transfected to express human B7H4 or the B7H4-B7H3 chimeric molecules B7H3-IgV/B7H4-IgC or B7H4-IgV/B7H3-IgC was assessed using flow cytometry.

Figure 3. Binding of B7H4 antibodies to B7H4 variants with alanine mutations in the ECD. Binding was expressed as fold change compared to a reference antibody. Fold change was defined as $\text{Log}_{10}(\text{Normalized gMFI}[\text{ala mutant}]/\text{Normalized gMFI}[\text{wt}])$. Residues where the Fold Change in binding was lower than mean Fold Change $-1.5 \times \text{SD}$ were considered 'loss of binding mutants'. Residues with a positive Fold Change in binding are loss of binding residues for the reference antibody. Numbers below the x-axis refer to amino acid positions. (A) Results for C1-N52S, with C2 as reference antibody. (B) Results for C2, with C1-N52S as reference antibody. (C) Results for C3, with C2 as reference antibody.

Figure 4. Binding of B7H4 antibody and CD3xB7H4 bispecific antibody to human and cynomolgus monkey B7H4. Binding of IgG1-B7H4-C1-N52S-FEAR (A) and bsIgG1-huCD3-H101G-FEALxB7H4-C1-N52S-FEAR (B) to HEK-293F cells transiently transfected with human B7H4 or cynomolgus monkey B7H4 was determined by flow cytometry. Non-transfected HEK-293F cells (C) were used as negative control; for these binding of bsIgG1-huCD3-H101G-FEALxB7H4-C1-N52S-FEAR is shown.

Figure 5. Binding of B7H4 antibody and CD3xB7H4 bispecific antibody to B7H4 from rabbit, rat, mouse, dog and pig. Binding of IgG1-B7H4-C1-N52S-FEAR (A) and bsIgG1-huCD3-H101G-FEALxB7H4-C1-N52S-FEAR (B) to HEK-293F cells transiently transfected with B7H4 from rabbit, rat, mouse, dog or pig was

Claims

1. An antibody comprising a first antigen-binding region which binds to human B7H4, wherein the first antigen-binding region comprises a variable heavy chain (VH) region comprising:
 - a CDR1 comprising the sequence of SEQ ID NO: 26,
 - a CDR2 comprising the sequence of SEQ ID NO: 30, and
 - a CDR3 comprising the sequence of SEQ ID NO: 28
 and a variable light chain (VL) region comprising:
 - a CDR1 comprising the sequence of SEQ ID NO: 34,
 - a CDR2 comprising the sequence GAS, and
 - a CDR3 comprising the sequence of SEQ ID NO: 35, and
 said antibody further comprising a second antigen-binding region which binds to human CD3.

2. An antibody comprising a first antigen-binding region which binds to human B7H4, wherein the first antigen-binding region comprises a variable heavy chain (VH) region comprising the sequence of SEQ ID NO: 29 and a variable light chain (VL) region comprising the sequence of SEQ ID NO: 33, said antibody further comprising a second antigen-binding region which binds to human CD3.

3. The antibody according to claim 1, wherein the second antigen-binding region binds to human CD3ε.

4. The antibody according to claim 1, wherein the second antigen-binding region comprises a variable heavy chain (VH) region comprising:
 - a CDR1 comprising the sequence of SEQ ID NOs.: 18,
 - a CDR2 comprising the sequence of SEQ ID NO: 19, and
 - a CDR3 comprising the sequence of SEQ ID NO: 20 or 21,
 and,
 - a variable light chain (VL) region comprising:
 - a CDR1 comprising the sequence of SEQ ID NO: 23,
 - a CDR2 comprising the sequence GTN, and
 - a CDR3 comprising the sequence of SEQ ID NO: 24.

5. The antibody according to claim 4, wherein the CDR3 of the VH region of the second antigen-binding region comprises the sequence of SEQ ID NO: 21.

6. The antibody according to claim 2, wherein the second antigen-binding region comprises a heavy chain variable (VH) region comprising the sequence of SEQ ID NO: 16 or 17, and a light chain variable (VL) region comprising the sequence of SEQ ID NO: 22.

7. The antibody according to claim 6, wherein the second antigen-binding region comprises a VH region comprising the sequence of SEQ ID NO: 17.

8. The antibody according to claim 5, wherein the antibody is a full-length antibody.

9. The antibody according to claim 7, wherein the antibody is a full-length antibody.

10. The antibody according to claim 5, wherein the antibody comprises a human IgG1 heavy chain

constant region.

11. The antibody according to claim 7, wherein the antibody comprises a human IgG1 heavy chain constant region.

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12. The antibody according to claim 5, wherein said antibody comprises a first heavy chain and a second heavy chain, wherein the amino acid in the positions corresponding to K409 in a human IgG1 heavy chain is R in said first heavy chain, and the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is L in said second heavy chain, or *vice versa*, and wherein the amino acid positions are numbered according to Eu numbering.

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13. The antibody according to claim 7, wherein said antibody comprises a first heavy chain and a second heavy chain, wherein the amino acid in the position corresponding to K409 in a human IgG1 heavy chain is R in said first heavy chain, and the amino acid in the position corresponding to F405 in a human IgG1 heavy chain is L in said second heavy chain, or *vice versa*, and wherein the amino acid positions are numbered according to Eu numbering.

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14. The antibody according to claim 5, wherein the antibody comprises a first heavy chain and a second heavy chain, and wherein in both the first heavy chain and the second heavy chain, the amino acid residues at the positions corresponding to positions L234, L235 and D265 in a human IgG1 heavy chain according to Eu numbering are F, E and A, respectively.

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15. The antibody according to claim 7, wherein the antibody comprises a first heavy chain and a second heavy chain, and wherein in both the first heavy chain and the second heavy chain, the amino acid residues at the positions corresponding to positions L234, L235 and D265 in a human IgG1 heavy chain according to Eu numbering are F, E and A, respectively.

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16. The antibody according to claim 5, wherein said antibody comprises a human kappa (κ) light chain and/or a human lambda (λ) light chain.

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17. The antibody according to claim 7, wherein said antibody comprises a human kappa (κ) light chain and/or a human lambda (λ) light chain.

18. The antibody according to claim 5, wherein said first antigen binding region which binds to human B7H4 is comprised in a first heavy chain and a first light chain, said first heavy chain comprising said VH region and a human IgG1 heavy chain constant region and said first light chain comprising said VL region and a human kappa light chain constant region; and wherein said antigen binding region which binds to human CD3 is comprised in a second heavy chain and a second light chain, said second heavy chain comprising said VH region and a human IgG1 heavy chain constant region and said second light chain comprising said VL region and a human lambda light chain constant region.

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19. The antibody according to claim 7, wherein said first antigen binding region which binds to human B7H4 is comprised in a first heavy chain and a first light chain, said first heavy chain comprising said VH region and a human IgG1 heavy chain constant region and said first light chain comprising said VL region and a human kappa light chain constant region; and wherein said second antigen binding region which binds to human CD3 is comprised in a second heavy chain and a second light chain, said second heavy chain comprising said VH region and a human IgG1 heavy chain constant region and said second light chain comprising said VL region and a human lambda light chain constant region.

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20. The antibody according to claim 18, wherein said first IgG1 heavy chain constant region comprises the sequence of SEQ ID NO. 61, and said second IgG1 heavy chain constant region comprises the sequence of SEQ ID NO. 60, and wherein said kappa light chain constant region comprises the sequence of SEQ ID NO. 63 and said lambda light chain constant region comprises the sequence of SEQ ID NO. 64.
- 5 21. The antibody according to claim 19, wherein said first IgG1 heavy chain constant region comprises the sequence of SEQ ID NO. 61 and said second IgG1 heavy chain constant region comprises the sequence of SEQ ID NO. 60, and wherein said kappa light chain constant region comprises the sequence of SEQ ID NO. 63 and said lambda light chain constant region comprises the sequence of SEQ ID NO. 64.
- 10 22. The antibody according to claim 20, wherein at least one of said IgG1 heavy chain constant regions lacks the C-terminal lysine.
- 15 23. The antibody according to claim 21, wherein at least one of said IgG1 heavy chain constant regions lacks the C-terminal lysine.
24. A bispecific antibody comprising a first antigen-binding region which binds to human B7H4, wherein the first antigen-binding region comprises a variable heavy chain (VH) region comprising:
- a CDR1 comprising the sequence of SEQ ID NOs.: 26,
 - a CDR2 comprising the sequence of SEQ ID NOs.: 30, and
 - a CDR3 comprising the sequence of SEQ ID NO: 28
- 20 and a variable light chain (VL) region comprising:
- a CDR1 comprising the sequence of SEQ ID NO. 34,
 - a CDR2 comprising the sequence GAS, and
 - a CDR3 comprising the sequence of SEQ ID NO. 35, and
- 25 said bispecific antibody further comprising a second antigen-binding region which binds to human CD3, wherein the second antigen-binding region comprises a VH region comprising:
- a CDR1 comprising the sequence of SEQ ID NO: 18,
 - a CDR2 comprising the sequence of SEQ ID NO: 19, and
 - a CDR3 comprising the sequence of SEQ ID NO: 21
- 30 and a VL region comprising:
- a CDR1 comprising the sequence of SEQ ID NO: 23,
 - a CDR2 comprising the sequence GTN, and
 - a CDR3 comprising the sequence of SEQ ID NO: 24.
- 35 25. A bispecific antibody comprising a first antigen-binding region which binds to human B7H4, wherein the first antigen-binding region comprises a variable heavy chain region comprising the sequence of SEQ ID NO. 29 and a variable light chain region comprising the sequence of SEQ ID NO. 33, said bispecific antibody further comprising a second antigen-binding region which binds to human CD3, wherein the second antigen-binding region comprises a variable heavy chain region comprising the sequence of SEQ
- 40 ID NO. 17 and a variable light chain region comprising the sequence of SEQ ID NO. 22.
26. The bispecific antibody of claim 24, wherein the antibody further comprises a first heavy chain constant region and a second heavy chain constant region and a first light chain constant region and a

- second light chain constant region, wherein said first heavy chain constant region comprises the sequence of SEQ ID NO. 61 and said second heavy chain constant region comprises the sequence of SEQ ID NO. 60, and wherein said first light chain constant region comprises the sequence of SEQ ID NO. 63 and said second light chain constant region comprises the sequence of SEQ ID NO. 64.
- 5** 27. The bispecific antibody of claim 25, wherein the antibody further comprises a first heavy chain constant region and a second heavy chain constant region and a first light chain constant region and a second light chain constant region, wherein said first heavy chain constant region comprises the sequence of SEQ ID NO. 61 and said second heavy chain constant region comprises the sequence of SEQ
- 10** ID NO. 60, and wherein said first light chain constant region comprises the sequence of SEQ ID NO. 63 and said second light chain constant region comprises the sequence of SEQ ID NO. 64.
28. The bispecific antibody of claim 26, wherein at least one of the heavy chain constant regions lacks the C-terminal lysine.
- 15** 29. The bispecific antibody of claim 27, wherein at least one of the heavy chain constant regions lacks the C-terminal lysine.
30. The bispecific antibody of claim 29, wherein both heavy chain constant regions lack the C-terminal lysine.
- 20** 31. A pharmaceutical composition comprising an antibody as defined in any one of claims 1 to 30 and a pharmaceutically acceptable carrier.
32. The antibody as defined in any of claims 1 to 30 for use as a medicament.
- 25** 33. Use of the antibody according to any one of claims 1 to 30 in the manufacture of a medicament for treating cancer.
34. The use according to claim 33, wherein the cancer is characterized by expression of B7H4 in cancer cells.
- 30** 35. The use according to claim 33 wherein the cancer is a solid tumor.
36. The use according to claim 33, wherein the cancer is selected from the group consisting of lung cancer, NSCLC (ADC or SQCC), stomach cancer, pancreas cancer, cholangiocarcinoma, bladder cancer, cervical cancer, head and neck cancer, breast cancer, ovarian cancer and uterine cancer.
- 35** 37. A nucleic acid encoding for the antibody according to any one of claims 1 to 30.
38. The nucleic acid as defined in claim 37, wherein said nucleic acid is RNA or DNA.
- 40** 39. The nucleic acid according to claim 37 for use in expression in mammalian cells.
40. A cell comprising a nucleic acid according to claim 37.
- 45** 41. The cell according to claim 40, wherein said cell is of human origin, such as a human embryonic kidney (HEK) cell, such as a HEK/Expi cell, or is of rodent origin, such as a Chinese hamster ovary cell, such as a CHO/N50 cell.

42. A method for producing an antibody which binds to B7H4 and CD3 according to any one of claims 1 to 30, comprising the steps of:
- 5 a) providing an antibody which binds to B7H4 according to any one of claims 1 to 30;
 - b) providing an antibody which binds to CD3 according to any one of claims 1 to 30;
 - c) incubating said antibody which binds to B7H4 together with said antibody which binds to CD3 under reducing conditions sufficient to allow cysteines in the hinge region to undergo disulfide-bond isomerization, and
 - d) obtaining said antibody which binds to human B7H4 and human CD3.
- 10 43. An antibody comprising an antigen-binding region capable of binding to human B7H4, wherein said antigen-binding region capable of binding to human B7H4 comprises:
- 15 a) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions of SEQ ID NO. 29 : and a variable light chain region comprising the CDR1, CDR2 and CDR3 regions respectively of SEQ ID NO. 33;
 - b) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions respectively of SEQ ID NOs.: 26, 30 and 28, and a variable light chain region comprising the CDR1, CDR2 and CDR3 respectively of SEQ ID NO. 34, GAS and SEQ ID NO. 35; and
 - c) a variable heavy chain (VH) region of SEQ ID NO. 29 : and a variable light chain region of SEQ ID NO. 33.
- 20 44. An antibody in accordance with claim 43, wherein said antibody is a monospecific antibody.