

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 of Invention

- 10 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.
- 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.

5 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 an 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 embodimentexample, 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 CD3ε (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;
- 15 and, a light chain variable region (VL) comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO: 23, GTN and 24, respectively.

- In another aspectexample, 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
- 20 herein.

In a further aspectexample, 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 ~~capable of binding~~ 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~~~~and an said antibody further comprising a second antigen-binding region capable of binding which binds to human CD3, wherein said antigen-binding regions comprise heavy and light chain variable regions, wherein said heavy and light chain variable regions are humanized and/or human.~~
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.~~
2. ~~An antibody according to claim 1, which is a bispecific antibody.~~
3. ~~An antibody in accordance with claim 1 or 2, wherein said antibody is capable of binding cancer cells and T cells.~~
4. ~~An antibody in accordance with any one of claims 1-3, wherein said cancer cells express said human B7H4.~~
5. ~~An antibody in accordance with claim 3 or claim 4, wherein said cancer cells are of a solid tumor.~~
6. ~~An antibody in accordance with any one of claims 1-5, wherein said antibody is capable of inducing T cell mediated cell killing.~~
7. ~~An The antibody according to any one of the preceding claims claim 1, wherein the second antigen-binding region capable of binding CD3, is capable of binding binds to human CD3ε (epsilon), such as human CD3ε (epsilon) as specified in SEQ ID NO: 13.~~
8. ~~An antibody according to any one of claims 1-7, wherein the antigen-binding region that binds to CD3 comprises~~
 - ~~a heavy chain variable region (VH) comprising the CDR1, CDR2, and CDR3 regions of SEQ ID NO: 16 or of SEQ ID NO. 17,~~~~and,~~
 - ~~a light chain variable region (VL) comprising the CDR1, CDR2, and CDR3 regions of SEQ ID NO: 22.~~
9. ~~An The antibody according to any one of claims 1-7 claim 1, wherein the second antigen-binding region that binds to CD3 comprises~~
 - ~~a variable heavy chain variable region (VH) region comprising: the~~
 - ~~a CDR1, CDR2, and CDR3 comprising the sequences 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 18, 19 and 21 respectively,~~~~and,~~
 - ~~a variable light chain variable region (VL) region comprising:~~
 - ~~a CDR1, CDR2, and CDR3 comprising the sequences of SEQ ID NO: 23,~~

- a CDR2 comprising the sequence GTN₁ and
- a CDR3 comprising the sequence of SEQ ID NO: 24, respectively.

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.

~~106. An~~ The antibody according ~~any one of claims 1-7~~ to claim 2, wherein the second antigen-binding region ~~that binds to CD3~~ comprises a heavy chain variable (VH) region (~~VH~~) comprising the sequence of SEQ ID NO: 16 or 17, ~~or a sequence having at least 90%, at least 95%, at least 97%, or at least 99% amino acid sequence identity to the sequence of SEQ ID NO: 16;~~

and,

a light chain variable (VL) region (~~VL~~) comprising the sequence of SEQ ID NO: 22 ~~or a sequence having at least 90%, at least 95%, at least 97%, or at least 99% amino acid sequence identity to 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.

11. ~~An antibody according to any one of claims 7 to 10, wherein the dissociation equilibrium constant K_D between the antigen-binding region that binds to CD3 and CD3 is within the range of 1—100 nM, such as within the range of 5—100 nM, within the range of 10—100 nM, within the range of 1—80 nM, within the range of 1—60 nM within the range of 1—40 nM, within the range of 1—20 nM, within the range of 5—80 nM, within the range of 5—60 nM, within the range of 5—40 nM, within the range of 5—20 nM, within the range of 10—80 nM, within the range of 10—60 nM, within the range of 10—40 nM, or such as within the range of 10—20 nM.~~

12. ~~An antibody according to any one of claims 7 to 10, wherein said antibody has a lower binding affinity for human CD3 ϵ than an antibody having an antigen-binding region comprising a VH sequence as set forth in SEQ ID NO: 16, and a VL sequence as set forth in SEQ ID NO: 22, preferably wherein said affinity is at least 5-fold lower, such as at least 10-fold lower, e.g. at least 20-fold lower, at least 30-fold lower, at least 40-fold lower, at least 45-fold lower or such as at least 50-fold lower.~~

13. ~~An antibody according to any one of claims 7 to 10, wherein said antigen-binding region that binds to CD3 has an equilibrium dissociation constant K_D which is within the range of 200—1000 nM, such as within the range of 300—1000 nM, within the range of 400—1000 nM, within the range of 500—1000 nM, within the range of 300—900 nM within the range of 400—900 nM, within the range of 400—700 nM, within the range of 500—900 nM, within the range of 500—800 nM, within the range of 500—700 nM, within the range of 600—1000 nM, within the range of 600—900 nM, within the range of 600—800 nM, or such as within the range of 600—700 nM.~~

- 14.—An antibody according to any one of claims 11 to 13, wherein
- 5 the antigen-binding region that binds to CD3 comprises a heavy chain variable (VH) region comprising a CDR1 sequence, a CDR2 sequence and a CDR3 sequence, the heavy chain variable (VH) region, when compared to a heavy chain variable (VH) region comprising the sequence set forth in SEQ ID NO: 16 has an amino acid substitution being at a position selected from the group consisting of: T31, N57, H101, G105, S110 and Y114, the positions being numbered according to the sequence of SEQ ID NO: 16; and the wild type light chain variable (VL) region comprises the CDR1, CDR2 and CDR3 sequences set forth in SEQ ID NO: 23, GTN and SEQ ID NO: 24, respectively.
- 10 15.—An antibody according to claim 14, wherein the antigen-binding region that binds to CD3 comprises in the heavy chain variable (VH) region a substitution selected from the group consisting of: T31M, T31P, N57E, H101G, H101N, G105P, S110A, S110G, Y114M, Y114R, Y114V.
- 15 16.—An antibody according to any one of claims 8-10, wherein the CDR1, CDR2 and CDR3 of the heavy chain variable (VH) region of the antigen-binding region that binds to CD3 comprises, in total, at the most 1, 2, 3, 4 or 5 amino acid substitutions, when compared with the CDR1, CDR2 and CDR3 of the sequences of SEQ ID NO: 16, said amino acid substitutions comprising preferably amino acid substitutions as defined in claim 14 or claim 15.
- 17.—The antibody according to any one of the preceding claims, wherein said human B7H4 is human B7H4 of SEQ ID NO. 1.
- 20 18.—The antibody according to any one of the preceding claims, wherein said antigen-binding region capable of binding to human B7H4 is capable of binding to the extracellular domain of human B7H4.
- 19.—The antibody according to claim 17 or claim 18, wherein said antigen-binding region capable of binding to human B7H4 is capable of binding to the IgC-like constant region of human B7H4.
- 25 20.—An antibody according to any one of the preceding claims, wherein said antigen-binding region capable of binding to human B7H4 is capable of binding to B7H3-IgV/B7H4-IgC having the sequence of SEQ ID NO. 11.
- 21.—An antibody according to claim 20, wherein said antigen-binding region capable of binding to human B7H4 is not capable of binding to B7H4-IgV/B7H3-IgC having the sequence of SEQ ID NO. 10.
- 22.—An antibody in accordance with any one of claims 17-20, wherein said antigen-binding region
- 30 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. 25; and a variable light chain region comprising the CDR1, CDR2 and CDR3 regions respectively of SEQ ID NO. 33;
- 35 b) 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;
- c) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions of SEQ ID NO. 36; and a variable light chain region comprising the CDR1, CDR2 and CDR3 regions respectively of SEQ ID NO. 40;
- 40 d) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions of SEQ ID NO. 43; and a variable light chain region comprising the CDR1, CDR2 and CDR3 regions respectively of SEQ ID NO. 47;
- e) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions of SEQ ID NO. 50; and a variable light chain region comprising the CDR1, CDR2 and CDR3 regions respectively of SEQ ID NO. 54; or
- 45 f) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions of SEQ ID NO. 31

- 5 : and a variable light chain region comprising the CDR1, CDR2 and CDR3 regions respectively of SEQ ID NO. 33
- g) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions of SEQ ID NO. 65 : and a variable light chain region comprising the CDR1, CDR2 and CDR3 regions respectively of SEQ ID NO. 69.
- 23.—An antibody in accordance with claim 17-20, wherein said antigen-binding region capable of binding to human B7H4 comprises:
- 10 a) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions respectively of SEQ ID NOs.: 26, 27 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;
- 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;
- 15 c) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions respectively of SEQ ID NOs.: 37, 38 and 39, and a variable light chain region comprising the CDR1, CDR2 and CDR3 respectively of SEQ ID NO. 41, DTS and SEQ ID NO. 42;
- d) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions respectively of SEQ ID NOs.: 44, 45 and 46, and a variable light chain region comprising the CDR1, CDR2 and CDR3 respectively of SEQ ID NO. 48, YTS and SEQ ID NO. 49;
- 20 e) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions respectively of SEQ ID NOs.: 51, 52 and 53, and a variable light chain region comprising the CDR1, CDR2 and CDR3 respectively of SEQ ID NO. 55, GAS and SEQ ID NO. 56; or
- f) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions respectively of SEQ ID NOs.: 26, 32 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
- 25 g) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions respectively of SEQ ID NOs.: 66, 67 and 68, and a variable light chain region comprising the CDR1, CDR2 and CDR3 respectively of SEQ ID NO. 70, GAS and SEQ ID NO. 71.
- 24.—An antibody in accordance with any one of claims 17-20, wherein said antigen-binding region
- 30 capable of binding to human B7H4 comprises:
- a) a variable heavy chain (VH) region of SEQ ID NO. 25 : and a variable light chain region of SEQ ID NO. 33;
- b) a variable heavy chain (VH) region of SEQ ID NO. 29 : and a variable light chain region of SEQ ID NO. 33;
- 35 c) a variable heavy chain (VH) region of SEQ ID NO. 36 : and a variable light chain region of SEQ ID NO. 40;
- d) a variable heavy chain (VH) region of SEQ ID NO. 43 : and a variable light chain region of SEQ ID NO. 47;
- e) a variable heavy chain (VH) region of SEQ ID NO. 50 : and a variable light chain region of SEQ ID NO. 54; or
- 40 f) a variable heavy chain (VH) region of SEQ ID NO. 29 : and a variable light chain region of SEQ ID NO. 33
- g) a variable heavy chain (VH) region of SEQ ID NO. 65 : and a variable light chain region of SEQ ID NO. 69.
- 45 25.—The antibody according to any one of the preceding claims, wherein said antigen-binding region capable of binding to human B7H4 has a binding affinity that corresponds to a K_D value of $5E-7$ M or less, such as $1E-7$ M or less, such as with a binding affinity corresponding to a K_D value which is within the range of $5E-7$ to $2E-10$ M, such as within the range of $2E-7$ to $1E-10$ M or $1E-7$ to $5E-9$ M.
- 26.—The antibody according to claim 25, wherein the binding affinity is determined by biolayer

interferometry, optionally as set forth in Example 3 herein.

27.—The antibody according to any one of claims 25 and 26, wherein the binding affinity is determined using a biolayer interferometry comprising the steps of:

- I) —immobilizing the antibody at an amount of 1 µg/mL for 600 seconds on an anti-human IgG Fc Capture biosensor;
- II) —determining association over a time period of 300 seconds and dissociation over a time period of 1000 seconds of human recombinant His tagged B7H4 protein (Sino Biological cat no 10738-H08H; a protein expressed from a construct of DNA sequence encoding the human VTCN1(Uniprot accession no. Q7Z7D3) (Phe29-Ala258) with a C-terminal polyhistidine tag) using a 2-fold dilution series ranging from 1.56 nM to 100 nM;
- III) —referencing the data to a buffer control (0 nM).

28.—The antibody according to any one of claims 25 to 27, wherein the binding affinity is determined using an antibody as defined in any one of the preceding claims, which is a monospecific, bivalent antibody, such as an antibody which is a full length IgG1.

29.—An antibody in accordance with any one of claims 1-28, comprising an antigen region capable of binding to human B7H4, said antigen-binding region capable of crossblocking

an antibody comprising a variable heavy chain (VH) region of SEQ ID NO. 29 and a variable light chain region of SEQ ID NO. 33; and

an antibody comprising a variable heavy chain (VH) region of SEQ ID NO. 36 : and a variable light chain region of SEQ ID NO. 40; and

wherein said antigen-binding region is not capable of crossblocking

an antibody comprising a variable heavy chain (VH) region of SEQ ID NO. 43 : and a variable light chain region of SEQ ID NO. 47;

an antibody comprising a variable heavy chain (VH) region of SEQ ID NO. 50 : and a variable light chain region of SEQ ID NO.54; and

an antibody comprising a variable heavy chain (VH) region of SEQ ID NO. 65 and a variable light chain region of SEQ ID NO. 69.

30.—An antibody in accordance with any one of claims 1-28, comprising an antigen region capable of binding to human B7H4, said antigen-binding region capable of crossblocking

an antibody comprising a variable heavy chain (VH) region of SEQ ID NO. 43 : and a variable light chain region of SEQ ID NO. 47;

an antibody comprising a variable heavy chain (VH) region of SEQ ID NO. 50 : and a variable light chain region of SEQ ID NO.54; and

an antibody comprising a variable heavy chain (VH) region of SEQ ID NO. 65 and a variable light chain region of SEQ ID NO. 69;

and wherein said antigen-binding region is not capable of crossblocking an antibody comprising

an antibody comprising a variable heavy chain (VH) region of SEQ ID NO. 29 and a variable light chain region of SEQ ID NO. 33 and

an antibody comprising a variable heavy chain (VH) region of SEQ ID NO. 36 : and a variable light chain region of SEQ ID NO. 40.

31.—An antibody in accordance with claim 29 or claim 30, wherein said antigen-binding region capable of binding to human B7H4 is capable of binding to B7H3-IgV/B7H4-IgC of SEQ ID NO. 11, and optionally is not capable of binding to B7H4-IgV/B7H3-IgC of SEQ ID NO. 10.

32.—The antibody according to any one of the preceding claims, wherein each antigen-binding region comprises a heavy chain variable region (VH) and a light chain variable region (VL), and wherein said variable regions each comprise three CDR sequences, CDR1, CDR2 and CDR3, respectively, and four framework sequences, FR1, FR2, FR3 and FR4, respectively.

33. ~~The antibody according to any one of the preceding claims, wherein~~
a) ~~the antigen-binding region capable of binding to B7H4 is human, and~~
b) ~~the antigen-binding region capable of binding to CD3, is humanized.~~
- 5 34. ~~The antibody according to any one of claims 1-32 wherein~~
a) ~~the antigen-binding region capable of binding to B7H4 is human, and/or~~
b) ~~the antigen-binding region capable of binding to CD3, is humanized.~~
35. ~~The antibody according to any one of the preceding claims, wherein the antibody comprises two heavy chain constant regions (CH), and two light chain constant regions (CL).~~
- 10 36. ~~The antibody according to claim 35, wherein the two heavy chain constant domains, and two light chain constant regions are derived from human.~~
37. ~~The antibody according to any one of the preceding claims, wherein the antibody is a full-length antibody.~~
38. ~~The antibody according to any one of the preceding claims, wherein the antibody is of the IgG1 isotype.~~
- 15 3912. ~~The antibody according to any one of the preceding claims~~claim 5, wherein said antibody comprises a first heavy chain and a second heavy chain, ~~each of said first and second heavy chain comprises at least a hinge region, a CH2 and CH3 region, wherein in said first heavy chain at least one of~~wherein the amino acids in the positions corresponding to ~~positions selected from the group consisting of T366, L368, K370, D399, F405, Y407 and K409 in a human IgG1 heavy chain has been~~
20 ~~substituted, and in said second heavy chain at least one of the amino acids in the positions corresponding to a position selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 in a human IgG1 heavy chain has been substituted, wherein said substitutions of said first and said second heavy chains are not in the same positions, and wherein the amino acid positions are numbered according to Eu numbering.~~
- 25 40. ~~The antibody according to claim 39, 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.
- 30 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.
- 35 41. ~~The antibody according to any one of the preceding claims, wherein said antibody comprises a first and, optionally, a second heavy chain and wherein the first heavy chain, and the second heavy chain if present, is/are modified so that the antibody induces Fc-mediated effector function to a lesser extent relative to an identical non-modified antibody.~~
- 40 4214. ~~The antibody according to claim 41~~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, ~~and~~ L235 and D265 in a human IgG1 heavy chain according to Eu numbering are F, ~~and~~ E and A, respectively.
- 45 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.

43. The antibody according to claim 41 or claim 42 wherein antibody comprises a first and a second heavy chain, and wherein in both the first and the second heavy chain, the amino acid residue at the position corresponding to position D265 in a human IgG1 heavy chain according to Eu numbering is A. 4416. The antibody according to ~~any one of the preceding claims~~claim 5, wherein said antibody comprises a human kappa (κ) light chain and/or a human lambda (λ) light chain.

4517. The antibody according to ~~any one of the preceding claims~~claim 7, wherein said antibody comprises a human kappa (κ) light chain and/or a human lambda (λ) light chain.

46. The antibody according to any of the preceding claims, wherein said antibody comprises a lambda (λ) light chain and a kappa (κ) light chain; e.g. an antibody with a heavy chain and a lambda light chain which comprise the binding region capable of binding to CD3, and a heavy chain and a kappa light chain which comprise the binding region capable of binding to B7H4.

4718. The antibody according to ~~any of the preceding claims~~claim 5, wherein said first antigen binding region ~~capable of binding~~which binds to human B7H4 is comprised in an 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 ~~capable of binding~~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.

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.

4820. The antibody according to claim 4718, wherein ~~one said first~~ IgG1 heavy chain constant region ~~is as defined in~~comprises the sequence of SEQ ID NO. 60 and the other is as defined in 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 ~~is as defined in~~comprises the sequence of SEQ ID NO. 63 and said lambda light chain constant region ~~is as defined in~~comprises the sequence of SEQ ID NO. 64.

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.

4922. The antibody according to claim 4820, wherein ~~said at least one of said~~ IgG1 heavy chain constant regions ~~as defined in SEQ ID NO. 60 and 61 have their~~lacks the C-terminal lysines ~~deleted.~~

23. The antibody according to claim 21, wherein at least one of said IgG1 heavy chain constant regions lacks the C-terminal lysine.

50. The antibody according to any one of the preceding claims, wherein said antibody is devoid of, or

has reduced Fc-mediated effector function, and the antibody:

- a) is capable of binding to B7H4-expressing human tumor cells such as described in Examples 9 and 10 herein,
- b) mediating concentration-dependent cytotoxicity in B7H4-expressing human tumor cells when using e.g. PBMCs or T cells as effector cells when assayed as described in Examples 11 and 12 herein,
- c) is capable of mediating concentration-dependent cytotoxicity in one or more human B7H4-expressing tumor cell lines selected from the group consisting of MCF-7, MDA-MB-468, SK-BR3, NIH-OVCAR-3, HCC1954, and NCI-H1650 when using e.g. PBMCs or T cells as effector cells when assayed as described in Examples 11 and 12 herein,
- d) is capable of activating T cells *in vitro* in the presence of B7H4-expressing human tumor cells; e.g. when assayed as described in Example 13 herein,
- e) is capable of activating T cells *in vitro* in the presence of one or more B7H4-expressing human tumor cell lines selected from the group consisting of MCF-7, MDA-MB-468, SK-BR3, NIH-OVCAR-3, HCC1954, and NCI-H1650; e.g. when assayed as described in Example 13 herein,
- f) is capable of inducing cytotoxicity of B7H4-expressing human tumor cells; e.g. when assayed as described in Examples 11 and 12 herein, and/or
- g) is capable of inducing T cell mediated cytotoxicity in one or more B7H4-expressing human tumor cell lines selected from the group consisting of MCF-7, MDA-MB-468, SK-BR3, NIH-OVCAR-3, HCC1954, and NCI-H1650; e.g. when assayed as described in Examples 11 and 12 herein.

51. The antibody according to claim 47, wherein the antibody has an IC50 in the range of 0.001-5 microgram/ml, wherein the IC50 is determined in an *in vitro* cytotoxicity assay comprising the steps of:

- i) providing isolated peripheral blood mononuclear cells (PBMCs) or purified T cells from healthy human donor buffy coats,
- ii) providing B7H4-expressing tumor cells;
- iii) combining PBMCs or purified T cells and a human B7H4-expressing tumor cell line selected from the group consisting of MCF-7, MDA-MB-468, SK-BR3, NIH-OVCAR-3, HCC1954, and NCI-H1650, in a plurality of samples, wherein the ratio of the number of T cells from said PBMCs, or purified T cells, to the selected tumor cell is 8:1;
- iv) providing said antibody in a dilution series to said samples, ranging e.g. from 0.0128 ng/mL to 10,000 ng/mL and
- v) incubating the samples, e.g. for 72 hours at 37°C; and subsequently,
- vi) assessing the viability of the B7H4-expressing tumor cells,
- vii) determining the percentage of viable cells for each dilution sample, and
- viii) determining the IC50.

52. The antibody according to claim 48, wherein the IC50 is in the range of 0.001-0.03 microgram/ml.

53. The antibody according to claim 48, wherein the IC50 is in the range of 0.05-5 microgram/ml.

5424. An bispecific antibody comprising an first antigen-binding region capable of binding which binds to human B7H4, wherein said the first antigen-binding region capable of binding to human B7H4 comprises a variable heavy chain (VH) region comprising:

- a) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions of SEQ ID NO. 25; 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 of SEQ ID NO. 29; and a variable light chain region comprising the CDR1, CDR2 and CDR3 regions respectively of SEQ ID NO. 33;
- c) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions respectively comprising the sequence of SEQ ID NOs.: 26, 27 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;
- d) a variable heavy chain (VH) region comprising the CDR1, CDR2 and CDR3 regions respectively of comprising the sequence of SEQ ID NOs.: 26, 30, and
- a CDR3 comprising the sequence of SEQ ID NO: 28,
- and a variable light chain (VL) region comprising:
- the a CDR1, CDR2 and CDR3 respectively 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 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 NO: 23,
- a CDR2 comprising the sequence GTN, and
- a CDR3 comprising the sequence of SEQ ID NO: 24.
- e) a variable heavy chain (VH) region of SEQ ID NO. 25; and a variable light chain region of SEQ ID NO. 33; or
- f) a variable heavy chain (VH) region of SEQ ID NO. 29; and a variable light chain region of SEQ ID NO. 33;
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 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.
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 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 28. The bispecific antibody of claim 26, wherein at least one of the heavy chain constant regions lacks the C-terminal lysine.
29. The bispecific antibody of claim 27, wherein at least one of the heavy chain constant regions lacks the C-terminal lysine.
- 10 30. The bispecific antibody of claim 29, wherein both heavy chain constant regions lack the C-terminal lysine.
- ~~55.—An antibody in accordance with claim 54, wherein said antibody is a monospecific antibody.~~
- ~~56.—An antibody in accordance with claim 54 or claim 55, wherein said antibody is a bivalent antibody.~~
- 15 ~~57.—An antibody in accordance with claim 56, wherein the antibody is a bispecific antibody comprising a further antigen-binding region capable of binding to human CD3, wherein preferably said further antigen-binding region capable of binding to CD3 is an antigen-binding region capable of binding to human CD3 as defined in any one of claims 7-16.~~
- ~~58.—A composition comprising an antibody as defined in any one of claims 1 to 57.~~
- 20 ~~5931.~~ A pharmaceutical composition comprising an antibody as defined in any one of claims 1 to ~~5830~~. and a pharmaceutically acceptable carrier.
- ~~6032.~~ The antibody as defined in any of claims 1 to ~~5730~~ for use as a medicament.
- 25 ~~61.—The antibody for use as a medicament according to claim 60 for use in the treatment of a disease.~~
- ~~6233.~~ Use of ~~the antibody for use as a medicament according to any one of claims 1 to 3061 in the manufacture of a medicament for treating, wherein the disease is cancer.~~
- ~~6334.~~ The ~~antibody for use as a medicament~~ according to claim ~~6233~~, wherein the cancer is characterized by expression of B7H4 in cancer cells.
- 30 ~~64.—The antibody for use as a medicament for use according to claim 63, wherein said expression of B7H4 is determined in cancer cells obtained from a patient.~~
- ~~6535.~~ The ~~antibody for use as a medicament~~ according to ~~any one of claims 62 to 64~~claim 33 wherein the cancer is a solid tumor.
- 35 ~~6636.~~ The ~~antibody for~~ use according to ~~any one of claims 62 to 65~~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.
- 40 ~~67.—A method of treating a disease, the method comprising administering an antibody as defined in any one of claims 1 to 57, the composition as defined in claim 58, or the pharmaceutical composition defined in claim 59, to a subject in need thereof.~~
- ~~68.—The method according to claim 67, said method being for treatment of a cancer.~~
- 45 ~~69.—The method according to claim 68, wherein the cancer is selected from the group consisting of uterine carcinosarcoma (UCS), bladder urothelial carcinoma (BLCA), pancreatic adenocarcinoma (PAAD), lung squamous cell carcinoma (LUSC), breast invasive carcinoma (BRCA), uterine corpus endometrial carcinoma (UCEC), ovarian serous cystadenocarcinoma (OV) and cholangiocarcinoma (CHOL).~~

5 ~~7037.~~ A nucleic acid encoding for the antibody according to any one of claims 1 to 30 comprising:
a nucleic acid sequence encoding a heavy chain variable region sequence of an antigen-binding region
capable of binding to B7H4 as defined in claim 54, and/or
a nucleic acid sequence encoding the corresponding light chain variable region sequence of said antigen-
binding region capable of binding to B7H4 as defined in claim 54.

10 ~~71.~~ One or more nucleic acids comprising

15 a) ~~a nucleic acid sequence encoding a heavy chain sequence of an antibody comprising an~~
~~antigen-binding region capable of binding to B7H4 as defined in any one of claims 22-24,~~
b) ~~a nucleic acid sequence encoding the corresponding light chain sequence of an antibody~~
~~comprising said antigen-binding region capable of binding to B7H4 as defined in any one of~~
~~claims 22-24,~~
c) ~~a nucleic acid sequence encoding a heavy chain sequence of an antibody comprising an~~
~~antigen-binding region capable of binding to CD3 as defined in any one of claims 8-10; and~~
d) ~~a nucleic acid sequence encoding the corresponding light chain sequence of an antibody~~
~~comprising said antigen-binding region capable of binding to CD3 as defined in any one of~~
~~claims 8-10.~~

20 ~~7238.~~ ~~A~~The nucleic acid, ~~or one or more nucleic acids,~~ as defined in claim ~~7037~~ or 71, wherein said
nucleic acid is RNA or DNA.

~~7339.~~ ~~A~~The nucleic acid, ~~or one or more nucleic acids,~~ according to ~~any one of claims 70 to 72~~ claim 37
for use in expression in mammalian cells.

25 ~~7440.~~ A cell comprising a nucleic acid, ~~or comprising one or more nucleic acids,~~ as defined in according
to any one of claims 70-73 claim 37.

30 ~~7541.~~ ~~A~~The cell according to claim ~~7440~~, 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.

~~7642.~~ A method for producing an antibody which binds ~~capable of binding to both~~ B7H4 and CD3 ~~in~~
~~accordance with any one of claims 1 to 53~~ according to any one of claims 1 to 30, comprising the steps
of:

35 a) providing an antibody ~~capable of binding~~ which binds to B7H4 according to any one of claims 1
to 30, ~~said antibody comprising an antigen-binding region capable of binding to B7H4 as~~
~~defined in any one of claims 1 to 6 and 17 to 34;~~
b) providing an antibody ~~capable of binding~~ which binds to CD3 according to any one of claims 1 to
30, ~~said antibody comprising an antigen-binding region capable of binding to CD3 as defined in~~
~~any one of claims 1 to 16 and 32 to 34;~~
40 c) incubating said antibody ~~capable of binding~~ which binds to B7H4 together with said antibody
~~capable of binding~~ 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 ~~capable of binding~~ which binds to human B7H4 and human CD3.

45 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:

- 5 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;
c) a variable heavy chain (VH) region of SEQ ID NO. 29 : and a variable light chain region of SEQ ID NO. 33;
44. An antibody in accordance with claim 43, wherein said antibody is a monospecific antibody.
- 10 ~~77.—A method for producing an antibody capable of binding to both B7H4 and CD3, in accordance with claim 76, wherein the steps a) and/or b) comprise:~~
 ~~—providing cells containing expression vectors for producing said antibody or said antibodies; and~~
 ~~—allowing the cells to produce said antibody or said antibodies and subsequently,~~
15 ~~—obtaining said antibody or said antibodies, thereby providing said antibody or said antibodies.~~
- 20 ~~78.—A kit of parts, such as a kit for use as a companion diagnostic/for identifying within a population of patients those patients which have a propensity to respond to treatment with an antibody as defined in any one of claims 1 to 57, comprising an antibody as defined in any one of claims 1 to 57; and instructions for use of said kit.~~