

SEQ ID NO:136 (CDR1), SEQ ID NO:137 (CDR2) and SEQ ID NO:138 (CDR3);
and

xxiv) an antigen binding unit comprising light chain CDRs comprising the amino acid
sequences of SEQ ID NO:139 (CDR1), SEQ ID NO:140 (CDR2) and SEQ ID
5 NO:141 (CDR3) and heavy chain CDRs comprising the amino acid sequences of
SEQ ID NO:142 (CDR1), SEQ ID NO:143 (CDR2) and SEQ ID NO:144 (CDR3).

In one aspect, the present disclosure provides a protein comprising a first antigen binding
unit specifically binding to B7H6 and a second antigen binding unit specifically binding to
CD3, wherein said first antigen binding unit specifically binding to B7H6 is selected from
10 the group consisting of i) to iii):

- i) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:79 (CDR1), SEQ ID NO:80 (CDR2) and SEQ ID NO:81 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:82 (CDR1), SEQ ID NO:83 (CDR2) and SEQ ID NO:84 (CDR3);
- 5 ii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:85 (CDR1), SEQ ID NO:86 (CDR2) and SEQ ID NO:87 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:88 (CDR1), SEQ ID NO:89 (CDR2) and SEQ ID NO:90 (CDR3); and
- 10 iii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:91 (CDR1), SEQ ID NO:92 (CDR2) and SEQ ID NO:93 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:94 (CDR1), SEQ ID NO:95 (CDR2) and SEQ ID NO:96 (CDR3); and

wherein said second antigen binding unit specifically binding to CD3 comprises light chain CDRs comprising the amino acid sequences of SEQ ID NO:257 (CDR1), SEQ ID NO:258 (CDR2) and SEQ ID NO:259 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:260 (CDR1), SEQ ID NO:261 (CDR2) and SEQ ID NO:262 (CDR3).

In some examples of the binding protein of the disclosure, the first antigen binding unit specifically binding to B7H6 is selected from the group consisting of i) to xxiv):

- 20 i) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:145 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:146;
- ii) a light chain variable domain comprising the amino acid sequences of SEQ ID NO:147 and heavy chain variable domain comprising the amino acid sequences of SEQ ID NO:148;
- 25 iii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:149 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:150;
- iv) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:151 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:152;
- 30

- v) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:153 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:154;
- 5 vi) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:155 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:156;
- vii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:157 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:158;

In another aspect, the present disclosure provides a protein comprising a first polypeptide chain specifically binding to B7H6 comprising an amino acid sequence selected from the group consisting of SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:231, and SEQ ID NO:232, and a second polypeptide chain specifically binding to CD3 comprising the amino acid sequence of SEQ ID NO:311.

The disclosure provides an isolated nucleic acid molecule i) encoding a first antigen binding unit and/or a second antigen binding unit of a binding protein of the disclosure, optionally further encoding a first and/or a second Fc domain, or ii) encoding the first and/or the second polypeptide chain of binding protein of the disclosure. The disclosure provides expression
5 vectors comprising the nucleic acid molecule of the disclosure, host cells transfected with such expression vectors, and methods of manufacturing a protein of the disclosure. In a further aspect, the disclosure provides a host cell transfected with two expression vectors, one expression vector encoding a first polypeptide chain of any one of SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:231 or SEQ ID NO:232 and another expression vector
10 encoding a second polypeptide chain of SEQ ID NO:311. In a further aspect, the disclosure provides a method of manufacturing a protein of the disclosure comprising

- i) cultivating the host cell of the disclosure under conditions allowing expression of the protein of the disclosure; and,
- ii) recovering the protein.

15 The disclosure provides a multi-specific binding protein comprising a first polypeptide chain specifically binding to B7H6 and a second polypeptide chain specifically binding to CD3, where the first polypeptide chain comprises a first light chain, a first linker, and a first heavy chain and the second polypeptide chain comprises a second light chain, a second linker, and a second heavy chain, preferably the C-terminus of the first light chain is covalently bound
20 to the N-terminus of the first heavy chain via the first peptide linker and the C-terminus of the second light chain is covalently bound to the N-terminus of the second heavy chain via the second peptide linker. The skilled person would understand that any reference herein to a “light chain” or “heavy chain” refers to an antibody light chain or antibody heavy chain, respectively.

The disclosure provides for novel binding proteins that allow a more efficient treatment of B7H6 expressing cancers, such as (metastatic) colorectal cancer ((m)CRC), non-small cell lung cancer (NSCLC), or head and neck squamous cell carcinoma (HNSCC).

In a further aspect, the disclosure provides the protein of the disclosure for use in medicine.

5 In a further aspect, the disclosure provides a pharmaceutical composition comprising the protein of the disclosure and a pharmaceutically acceptable carrier.

In a further aspect, the disclosure provides use of the protein of the disclosure in the manufacture of a pharmaceutical composition for treating a B7H6 expressing cancer.

10 In a further aspect, the disclosure provides the protein of the disclosure for use in treating a B7H6 expressing cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Schematic representation of a bispecific binding protein of the invention

15 **Figure 2:** Schematic representation of the extracellular B7H6 protein expressed on the cell surface of CHO-K1 cells.

Figure 3: Binding of 34 exemplary B7H6/CD3 binding proteins to recombinant human B7H6 extracellular protein.

Figure 4: Binding of 34 exemplary B7H6/CD3 binding proteins to recombinant human Alanine-mutated B7H6 extracellular protein.

20 **Figure 5:** Binding of 34 exemplary B7H6/CD3 binding proteins to HCT-15 cells expressing endogenous human B7H6.

Figure 6: Binding of 23 exemplary B7H6/CD3 binding proteins to recombinant CHO-K1 cells expressing cynomolgus monkey B7H6.

25 **Figure 7:** Binding of 23 exemplary B7H6/CD3 binding proteins to human T-cells expressing CD3.

CLAIMS

1. A protein comprising a first antigen binding unit specifically binding to B7H6 and a second antigen binding unit specifically binding to CD3, wherein said first antigen binding unit specifically binding to B7H6 is selected from the group consisting of i) to iii):

- i) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:79 (CDR1), SEQ ID NO:80 (CDR2) and SEQ ID NO:81 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:82 (CDR1), SEQ ID NO:83 (CDR2) and SEQ ID NO:84 (CDR3);
- ii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:85 (CDR1), SEQ ID NO:86 (CDR2) and SEQ ID NO:87 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:88 (CDR1), SEQ ID NO:89 (CDR2) and SEQ ID NO:90 (CDR3); and
- iii) an antigen binding unit comprising light chain CDRs comprising the amino acid sequences of SEQ ID NO:91 (CDR1), SEQ ID NO:92 (CDR2) and SEQ ID NO:93 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:94 (CDR1), SEQ ID NO:95 (CDR2) and SEQ ID NO:96 (CDR3); and

wherein said second antigen binding unit specifically binding to CD3 comprises light chain CDRs comprising the amino acid sequences of SEQ ID NO:257 (CDR1), SEQ ID NO:258 (CDR2) and SEQ ID NO:259 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:260 (CDR1), SEQ ID NO:261 (CDR2) and SEQ ID NO:262 (CDR3).

2. The protein of claim 1, wherein said first antigen binding unit specifically binding to B7H6 is selected from the group consisting of i) to iv):

- i) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:167 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:168;
- ii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:171 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:172;

- iii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:173 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:174; and
 - iv) a light chain variable domain comprising the amino acid sequence of SEQ ID NO:175 and heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:176.
- 3. The protein of claim 1 or 2, wherein said second antigen binding unit specifically binding to CD3 comprises a light chain variable domain comprising the amino acid sequence of SEQ ID NO:293 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:294.
- 4. The protein of any one of claims 1 to 3, wherein
 - i) said first antigen binding unit specifically binding to B7H6 comprises from its N- to C-terminus a first light chain variable domain, a first light chain constant domain, a first peptide linker, a first heavy chain variable domain and a first heavy chain constant CH1 domain; and
 - ii) said second antigen binding unit specifically binding to CD3 comprises from its N- to C-terminus a second light chain variable domain, a second light chain constant domain, a second peptide linker, a second heavy chain variable domain and a second heavy chain constant CH1 domain.
- 5. The protein of claim 4, wherein said first antigen binding unit comprises light chain CDRs comprising the amino acid sequences of SEQ ID NO:79 (CDR1), SEQ ID NO:80 (CDR2) and SEQ ID NO:81 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:82 (CDR1), SEQ ID NO:83 (CDR2) and SEQ ID NO:84 (CDR3) and said second antigen binding unit comprises light chain CDRs comprising the amino acid sequences of SEQ ID NO:257 (CDR1), SEQ ID NO:258 (CDR2) and SEQ ID NO:259 (CDR3) and heavy chain CDRs comprising the amino acid sequences of SEQ ID NO:260 (CDR1), SEQ ID NO:261 (CDR2) and SEQ ID NO:262 (CDR3).
- 6. The protein of claim 4 or 5, wherein said first antigen binding unit comprises a light chain variable domain comprising the amino acid sequence of SEQ ID NO:171 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:172, and said second antigen binding unit comprises a light chain variable domain comprising the

amino acid sequence of SEQ ID NO:293 and a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:294.

7. The protein of any one of claims 4 to 6, wherein said first and/or second peptide linker comprises 26 to 42 amino acids, preferably any one of 30 to 40 amino acids, 34 to 40 amino acids, or 36 to 39 amino acids.
8. The protein of claim 7, wherein said first and/or second peptide linker comprises 38 amino acids.
9. The protein of any one of claims 4 to 8, wherein said first peptide linker and/or second peptide linker is a Gly-Ser linker.
10. The protein of claim 9, wherein said first and/or said second peptide linker comprises the amino acid sequence of SEQ ID NO:250.
11. The protein of any one of claims 4 to 10, wherein the first light chain constant domain and the second light chain constant domain comprise a human kappa or lambda domain.
12. The protein of any one of claims 4 to 11, wherein said first antigen binding unit specific for B7H6 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:204, SEQ ID NO:206, SEQ ID NO:207, and SEQ ID NO:208, and said second antigen binding unit specific for CD3 comprises the amino acid sequence of SEQ ID NO:305.
13. The protein of claim 12, wherein said first antigen binding unit specific for B7H6 comprises the amino acid sequence of SEQ ID NO: 206 and said second antigen binding unit specific for CD3 comprises the amino acid sequence of SEQ ID NO.305.
14. The protein of any one of claims 4 to 13 further comprising a first and a second Fc domain, said first Fc domain being covalently linked to said first antigen binding unit, and said second Fc domain being covalently linked to said second antigen binding unit.
15. The protein of claim 14, wherein said first Fc domain is covalently linked to the C-terminus of said first antigen binding unit and said second Fc domain is covalently linked to the C-terminus of said second antigen binding unit.
16. The protein of claim 15, wherein
 - i) said first Fc domain comprises a tyrosine (Y) at position 366 [T366Y], and said second Fc domain comprises a threonine (T) at position 407 [Y407T], or

- ii) said first Fc domain comprises a tryptophan (W) at position 366 [T366W], and said second Fc domain comprises a serine (S) at position 366 [T366S], an alanine (A) at position 368 [L368A] and a valine (V) at position 407 [Y407V], or
 - iii) said second Fc domain comprises a tyrosine (Y) at position 366 [T366Y], and said first Fc domain comprises a threonine (T) at position 407 [Y407T], or
 - iv) said second Fc domain comprises a tryptophan (W) at position 366 [T366W], and said first Fc domain comprises a serine (S) at position 366 [T366S], an alanine (A) at position 368 [L368A] and a valine (V) at position 407 [Y407V].
17. The protein of claim 16, wherein said first or said second Fc domain further comprises an arginine at position 435 [H435R] and a phenylalanine at position 436 [Y436F].
 18. The protein of any one of claims 14 to 17, wherein said first and/or said second Fc domain comprises an alanine at position 234[L234A] and at position 235 [L235A].
 19. The protein of claim 18, wherein said first Fc domain comprises an amino acid sequence of SEQ ID NO:242 and said second Fc domain comprises an amino acid sequence of SEQ ID NO:243.
 20. A protein comprising a first polypeptide chain specifically binding to B7H6 comprising an amino acid sequence selected from the group consisting of SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:231, and SEQ ID NO:232, and a second polypeptide chain specifically binding to CD3 comprising the amino acid sequence of SEQ ID NO:311.
 21. The protein of claim 20, wherein said first polypeptide chain specifically binding to B7H6 comprises the amino acid sequence of SEQ ID NO: 230 and said second polypeptide chain specifically binding to CD3 comprises the amino acid sequence of SEQ ID NO:311.
 22. The protein of claim 20 or 21, wherein said first and said second polypeptide chain are linked via disulfide bonds forming a bispecific, bivalent and heterodimeric protein.
 23. A host cell transfected with two expression vectors, one expression vector encoding a first polypeptide chain of any one of SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:231 or SEQ ID NO:232 and another expression vector encoding a second polypeptide chain of SEQ ID NO:311.
 24. A method of manufacturing a protein of claim 20 comprising

- i) cultivating the host cell of claim 23 under conditions allowing expression of the protein of claim 20; and,
 - ii) recovering the protein.
25. The method of claim 24 further comprising purifying and/or modifying and/or formulating the protein.
26. The protein of any one of claims 1 to 22 for use in medicine.
27. A pharmaceutical composition comprising the protein of any one of claims 1 to 22 and a pharmaceutically acceptable carrier.
28. Use of the protein of any one of claims 1 to 22 in the manufacture of a pharmaceutical composition for treating a B7H6 expressing cancer.
29. The protein of any one of claims 1 to 22 for use in treating a B7H6 expressing cancer.
30. The use of claim 28 or the protein for use of claim 29, wherein the cancer is (metastatic) colorectal cancer ((m)CRC), non-small cell lung cancer (NSCLC), or head and neck squamous cell carcinoma (HNSCC).
31. The use of claim 28 or the protein for use of claim 29, wherein the protein is to be used in combination with an anti-PD-1 antibody or an anti-PD-L1 antibody.