

CLAIMS

1. A multispecific antibody comprising:
 - a) two antibody-based binding domains, which specifically bind to mesothelin (MSLN-BDs); and
 - b) at least one antibody-based binding domain, which specifically binds to CD3 (CD3-BD);wherein said multispecific antibody does not comprise an immunoglobulin Fc region polypeptide, and wherein each of said MSLN-BDs binds to mesothelin (MSLN) with a monovalent dissociation constant (K_D) in the range of from 0.5 to 20 nM, in particular in the range of from 0.6 to 10 nM when measured by SPR.
2. The multispecific antibody of claim 1, wherein said MSLN-BD comprises
 - (i) the HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2 (or 10) and 3, respectively, and the LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 4, 5 and 6, respectively; or the HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 11, 12 and 13, respectively, and the LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 14, 15 and 16, respectively; and
 - (ii) VH3 or VH4 domain framework sequences FR1 to FR4; particularly VH3 domain framework sequences FR1 to FR4; and
 - (iii) a VL domain comprising a VL framework comprising V κ frameworks FR1, FR2 and FR3, particularly V κ 1 or V κ 3 FR1 to FR3, particularly V κ 1 FR1 to FR3, and a framework FR4, which is selected from a V κ FR4, and a V λ FR4, particularly a V λ FR4 comprising an amino acid sequence having at least 70, 80, or 90 percent identity to any of SEQ ID NO: 132 to SEQ ID NO: 139, more particularly V λ FR4 selected from any of SEQ ID NO: 132 to SEQ ID NO: 139, particularly V λ FR4 according to SEQ ID NO: 132 or 139.
3. The multispecific antibody of claim 1 or 2, wherein said MSLN-BD comprises
 - a.1) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2 (or 10) and 3, respectively,

- b.1) LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 4, 5 and 6, respectively,
- c.1) a VH sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 percent identical to the amino acid sequence SEQ ID NO: 7, and
- 5 d.1) a VL sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 percent identical to the amino acid sequence SEQ ID NO: 9;
- or
- a.2) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 1, 2 (or 10) and 3, respectively,
- 10 b.2) LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 4, 5 and 6, respectively,
- c.2) a VH sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 percent identical to the amino acid sequence SEQ ID NO: 8, and
- d.2) a VL sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 percent identical to the amino acid sequence SEQ ID NO: 9;
- 15 or
- a.3) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 11, 12 and 13, respectively,
- b.3) LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 14, 15 and 16, respectively,
- 20 c.3) a VH sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 percent identical to the amino acid sequence SEQ ID NO: 17, and
- d.3) a VL sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 percent identical to the amino acid sequence SEQ ID NO: 18;
- 25 or
- a.4) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 11, 12 and 13, respectively,
- b.4) LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 14, 15 and 16, respectively,
- 30 c.4) a VH sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 percent identical to the amino acid sequence SEQ ID NO: 19, and
- d.4) a VL sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 percent identical to the amino acid sequence SEQ ID NO: 21;
- or

- a.5) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 11, 12 and 13, respectively,
- b.5) LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 14, 15 and 16, respectively,
- 5 c.5) a VH sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 percent identical to the amino acid sequence SEQ ID NO: 20, and
- d.5) a VL sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 percent identical to the amino acid sequence SEQ ID NO: 21;
- or
- 10 a.6) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 11, 12 and 13, respectively,
- b.6) LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 14, 15 and 16, respectively,
- c.6) a VH sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 percent identical to the amino acid sequence SEQ ID NO: 22, and
- 15 d.6) a VL sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 percent identical to the amino acid sequence SEQ ID NO: 24;
- or
- a.7) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 11, 12 and 13, respectively,
- 20 b.7) LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 14, 15 and 16, respectively,
- c.7) a VH sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 percent identical to the amino acid sequence SEQ ID NO: 23, and
- 25 d.7) a VL sequence at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 percent identical to the amino acid sequence SEQ ID NO: 24.
4. The multispecific antibody of any one of claims 1 to 3, wherein said CD3-BD is binding to CD3ε.

5. The multispecific antibody of any one of the preceding claims, wherein said antibody comprises one CD3-BD, wherein said CD3-BD binds CD3 ϵ with a monovalent K_D of 0.5 to 50 nM, particularly of 1 to 40 nM, particularly of 2 to 35 nM, as measured by SPR.
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6. The multispecific antibody of claim 4 or 5, wherein said CD3-BD comprises
- (i) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 45, 46 and 47, respectively in a human antibody VH framework, particularly a VH3 framework, and
- 10 (ii) LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 48, 49 and 50, respectively in a human antibody VL framework, wherein the VL framework comprises V κ frameworks FR1, FR2 and FR3, particularly V κ 1 frameworks, and a framework FR4, which is selected from a V κ FR4, and a V λ framework 4,
- 15 particularly, wherein said CD3-BD comprises
- (i) a VH domain comprising the amino acid sequence of SEQ ID NO: 51, 140, 141 or 142, and
- (ii) a VL domain comprising the amino acid sequence of SEQ ID NO: 52.
- 20 7. The multispecific antibody of any one of the preceding claims, wherein said antibody further comprises at least one human serum albumin binding domain (hSA-BD), particularly one hSA-BD, particularly wherein said hSA-BD comprises
- (i) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 53, 54 and 55, respectively in a human antibody VH framework, particularly a VH3
- 25 framework, and
- (ii) LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 56, 57 and 58, respectively in a human antibody VL framework, wherein the VL framework comprises V κ frameworks FR1, FR2 and FR3, particularly V κ 1 frameworks, and a framework FR4, which is selected from a V κ FR4, particularly V κ 1
- 30 FR4, and a V λ framework 4;
- or

- (i) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 63, 64 and 65, respectively in a human antibody VH framework, particularly a VH3 framework, and
- (ii) LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 66, 67 and 68, respectively in a human antibody VL framework, wherein the VL framework comprises Vk frameworks FR1, FR2 and FR3, particularly Vk1 frameworks, and a framework FR4, which is selected from a Vk FR4, particularly Vk1 FR4, and a Vλ framework 4;
- or
- (i) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 73, 74 and 75, respectively in a human antibody VH framework, particularly a VH3 framework, and
- (ii) LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 76, 77 and 78, respectively in a human antibody VL framework, wherein the VL framework comprises Vk frameworks FR1, FR2 and FR3, particularly Vk1 frameworks, and a framework FR4, which is selected from a Vk FR4, particularly Vk1 FR4, and a Vλ framework 4;
- particularly, wherein said hSA-BD comprises
- (i) a VH domain comprising the amino acid sequence of SEQ ID NO: 59, and
- (ii) a VL domain comprising the amino acid sequence of SEQ ID NO: 60;
- or
- (i) a VH domain comprising the amino acid sequence of SEQ ID NO: 69, and
- (ii) a VL domain comprising the amino acid sequence of SEQ ID NO: 70;
- or
- (i) a VH domain comprising the amino acid sequence of SEQ ID NO: 79 or 143, and
- (ii) a VL domain comprising the amino acid sequence of SEQ ID NO: 80 or 144;
- or
- (i) a VH domain comprising the amino acid sequence of SEQ ID NO: 81 or 145, and
- (ii) a VL domain comprising the amino acid sequence of SEQ ID NO: 82 or 146.

8. The multispecific antibody of any one of the preceding claims, wherein said antibody does not comprise CH1 and/or CL regions.

5 9. The multispecific antibody of any one of the preceding claims, wherein
said first single-chain protein comprises the amino acid sequence of SEQ ID NO:
101, especially consists of the amino acid sequence SEQ ID NO: 101 and
said second single-chain protein comprises the amino acid sequence of SEQ ID
NO: 102, especially consists of the amino acid sequence SEQ ID NO: 102; or
10 said first single-chain protein comprises the amino acid sequence of SEQ ID NO:
109, especially consists of the amino acid sequence SEQ ID NO: 109 and
said second single-chain protein comprises the amino acid sequence of SEQ ID
NO: 110, especially consists of the amino acid sequence SEQ ID NO: 110; or
said first single-chain protein comprises the amino acid sequence of SEQ ID NO:
15 111, especially consists of the amino acid sequence SEQ ID NO: 111 and
said second single-chain protein comprises the amino acid sequence of SEQ ID
NO: 112, especially consists of the amino acid sequence SEQ ID NO: 112; or
said first single-chain protein comprises the amino acid sequence of SEQ ID NO:
119, especially consists of the amino acid sequence SEQ ID NO: 119 and
20 said second single-chain protein comprises the amino acid sequence of SEQ ID
NO: 120, especially consists of the amino acid sequence SEQ ID NO: 120; or
said first single-chain protein comprises the amino acid sequence of SEQ ID NO:
121, especially consists of the amino acid sequence SEQ ID NO: 121 and
said second single-chain protein comprises the amino acid sequence of SEQ ID
25 NO: 122, especially consists of the amino acid sequence SEQ ID NO: 122; or
said first single-chain protein comprises the amino acid sequence of SEQ ID NO:
123, especially consists of the amino acid sequence SEQ ID NO: 123 and
said second single-chain protein comprises the amino acid sequence of SEQ ID
NO: 124, especially consists of the amino acid sequence SEQ ID NO: 124.

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10. A nucleic acid sequence or two nucleic acid sequences encoding the multispecific antibody of any one of claims 1 to 9.
11. A vector or two vectors comprising the nucleic acid sequence or the two nucleic acid sequences of claim 10.
12. A host cell or host cells comprising the vector or the two vectors of claim 11.
13. A method for producing the multispecific antibody of any one of claims 1 to 9, comprising (i) providing the nucleic acid sequence or the two nucleic acid sequences of claim 10, or the vector or the two vectors of claim 11, expressing said nucleic acid sequence or nucleic acid sequences, or said vector or vectors, and collecting said multispecific antibody from the expression system, or (ii) providing a host cell or host cells according to claim 12, culturing said host cell or said host cells; and collecting said multispecific antibody from the cell culture.
14. A pharmaceutical composition comprising the multispecific antibody of any one of claims 1 to 9 and a pharmaceutically acceptable carrier.
15. The multispecific antibody of any one of claims 1 to 9 for use in the treatment of a disease, particularly a human disease, more particularly a human disease selected from cancer, particularly a cancer selected from mesothelioma, pancreatic cancer, and ovarian cancer, an inflammatory and an autoimmune disease, particularly wherein said multispecific antibody is either a single-chain protein comprising three or four binding domains, or a hetero-dimeric protein comprising three or four binding domains.