

Claims

1. A bispecific antibody construct comprising a first binding domain which binds to human and macaque MSLN on the surface of a target cell and a second binding domain which binds to human CD3 on the surface of a T cell, wherein the first binding domain binds to an epitope of MSLN which is comprised within the MSLN variants as depicted in SEQ ID NOs: 231, 232, and 233 and further binds to *Macaca fascicularis* MSLN as depicted in SEQ ID NO: 234.
2. The antibody construct according to claim 1, wherein the second binding domain binds to human CD3 epsilon and to *Callithrix jacchus*, *Saguinus Oedipus* or *Saimiri sciureus* CD3 epsilon.
3. The antibody construct according to any one of the preceding claims, wherein the antibody construct is in a format selected from the group consisting of (scFv)₂, scFv-single domain mAb, diabodies and oligomers of those formats.
4. The antibody construct according to any one of the preceding claims, wherein the first binding domain binds to an epitope of MSLN which is comprised within the region of the human MSLN having a sequence as depicted in SEQ ID NO selected from the group consisting of SEQ ID NO: 244, SEQ ID NO: 245 and SEQ ID NO: 241 .
5. The antibody construct according to claim 4, wherein the first binding domain comprises a VH region comprising CDR-H1, CDR-H2 and CDR-H3 and a VL region comprising CDR-L1, CDR-L2 and CDR-L3 selected from the group consisting of:
 - a) CDR-H1 as depicted in SEQ ID NO: 151, CDR-H2 as depicted in SEQ ID NO: 152, CDR-H3 as depicted in SEQ ID NO: 153, CDR-L1 as depicted in SEQ ID NO: 154, CDR-L2 as depicted in SEQ ID NO: 155 and CDR-L3 as depicted in SEQ ID NO: 156;
 - b) CDR-H1 as depicted in SEQ ID NO: 161, CDR-H2 as depicted in SEQ ID NO: 162, CDR-H3 as depicted in SEQ ID NO: 163, CDR-L1 as depicted in SEQ ID NO: 164, CDR-L2 as depicted in SEQ ID NO: 165 and CDR-L3 as depicted in SEQ ID NO: 166;
 - c) CDR-H1 as depicted in SEQ ID NO: 171, CDR-H2 as depicted in SEQ ID NO: 172, CDR-H3 as depicted in SEQ ID NO: 173, CDR-L1 as depicted in SEQ ID NO: 174, CDR-L2 as depicted in SEQ ID NO: 175 and CDR-L3 as depicted in SEQ ID NO: 176;

- d) CDR-H1 as depicted in SEQ ID NO: 181, CDR-H2 as depicted in SEQ ID NO: 182, CDR-H3 as depicted in SEQ ID NO: 183, CDR-L1 as depicted in SEQ ID NO: 184, CDR-L2 as depicted in SEQ ID NO: 185 and CDR-L3 as depicted in SEQ ID NO: 186;
 - e) CDR-H1 as depicted in SEQ ID NO: 191, CDR-H2 as depicted in SEQ ID NO: 192, CDR-H3 as depicted in SEQ ID NO: 193, CDR-L1 as depicted in SEQ ID NO: 194, CDR-L2 as depicted in SEQ ID NO: 195 and CDR-L3 as depicted in SEQ ID NO: 196;
 - f) CDR-H1 as depicted in SEQ ID NO: 201, CDR-H2 as depicted in SEQ ID NO: 202, CDR-H3 as depicted in SEQ ID NO: 203, CDR-L1 as depicted in SEQ ID NO: 204, CDR-L2 as depicted in SEQ ID NO: 205 and CDR-L3 as depicted in SEQ ID NO: 206;
 - g) CDR-H1 as depicted in SEQ ID NO: 211, CDR-H2 as depicted in SEQ ID NO: 212, CDR-H3 as depicted in SEQ ID NO: 213, CDR-L1 as depicted in SEQ ID NO: 214, CDR-L2 as depicted in SEQ ID NO: 215 and CDR-L3 as depicted in SEQ ID NO: 216;
and
 - h) CDR-H1 as depicted in SEQ ID NO: 221, CDR-H2 as depicted in SEQ ID NO: 222, CDR-H3 as depicted in SEQ ID NO: 223, CDR-L1 as depicted in SEQ ID NO: 224, CDR-L2 as depicted in SEQ ID NO: 225 and CDR-L3 as depicted in SEQ ID NO: 226.
6. The antibody construct according to claim 4 or 5, wherein the first binding domain comprises a VH region selected from the group consisting of those depicted in SEQ ID NO: 157, SEQ ID NO: 167, SEQ ID NO: 177, SEQ ID NO: 187, SEQ ID NO: 197, SEQ ID NO: 207, SEQ ID NO: 217, and SEQ ID NO: 227.
7. The antibody construct according to any one of claims 4 to 6, wherein the first binding domain comprises a VL region selected from the group consisting of those depicted in SEQ ID NO: 158, SEQ ID NO: 168, SEQ ID NO: 178, SEQ ID NO: 188, SEQ ID NO: 198, SEQ ID NO: 208, SEQ ID NO: 218, and SEQ ID NO: 228.
8. The antibody construct according to any one of claims 4 to 7, wherein the first binding domain comprises a VH region and a VL region selected from the group consisting of pairs of a VH region and a VL region as depicted in SEQ ID NO: 157+158, SEQ ID NO: 167+168, SEQ ID NO: 177+178, SEQ ID NO: 187+188, SEQ ID NO: 197+198, SEQ ID NO: 207+208, SEQ ID NO: 217+218, and SEQ ID NO: 227+228.
9. The antibody construct according to any one of claims 4 to 8, wherein the first binding domain comprises a polypeptide selected from the group consisting of those depicted in

SEQ ID NO: 159, SEQ ID NO: 169, SEQ ID NO: 179, SEQ ID NO: 189, SEQ ID NO: 199, SEQ ID NO: 209, SEQ ID NO: 219, and SEQ ID NO: 229.

10. The antibody construct according to any one of claims 1 to 7, comprising:
- (a) a polypeptide comprising in the following order starting from the N-terminus:
- a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 159, SEQ ID NO: 169, SEQ ID NO: 179, SEQ ID NO: 189, SEQ ID NO: 199, SEQ ID NO: 209, SEQ ID NO: 219, and SEQ ID NO: 229;
 - a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-9; and
 - a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 46, SEQ ID NO: 55, SEQ ID NO: 64, SEQ ID NO: 73, SEQ ID NO: 82, SEQ ID NO: 91, SEQ ID NO: 100, and SEQ ID NO: 103; and
 - optionally a His-tag, such as the one depicted in SEQ ID NO 10;
- (b) a polypeptide comprising in following order starting from the N-terminus:
- a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 159, SEQ ID NO: 169, SEQ ID NO: 179, SEQ ID NO: 189, SEQ ID NO: 199, SEQ ID NO: 209, SEQ ID NO: 219, and SEQ ID NO: 229;
 - a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-9;
 - a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 46, SEQ ID NO: 55, SEQ ID NO: 64, SEQ ID NO: 73, SEQ ID NO: 82, SEQ ID NO: 91, SEQ ID NO: 100, and SEQ ID NO: 103;
 - optionally a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-9;
 - a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 104-134; and
 - optionally a His-tag, such as the one depicted in SEQ ID NO 10;
- (c) a polypeptide comprising in the following order starting from the N-terminus:
- a polypeptide having the amino acid sequence QRFVTGHFGGLX₁PANG (SEQ ID NO: 135) whereas X₁ is Y or H; and

- a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 159, SEQ ID NO: 169, SEQ ID NO: 179, SEQ ID NO: 189, SEQ ID NO: 199, SEQ ID NO: 209, SEQ ID NO: 219, and SEQ ID NO: 229;
 - a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-9;
 - a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 46, SEQ ID NO: 55, SEQ ID NO: 64, SEQ ID NO: 73, SEQ ID NO: 82, SEQ ID NO: 91, SEQ ID NO: 100, and SEQ ID NO: 103;
 - a polypeptide having the amino acid sequence QRFVTGHFGGLHPANG (SEQ ID NO: 137) or QRFCTGHFGGLHPCNG (SEQ ID NO: 139); and
 - optionally a His-tag, such as the one depicted in SEQ ID NO 10;
- (d) a polypeptide comprising in the following order starting from the N-terminus
- a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 26, SEQ ID NO: 35, SEQ ID NO: 44, SEQ ID NO: 53, SEQ ID NO: 62, SEQ ID NO: 71, SEQ ID NO: 80, SEQ ID NO: 89, SEQ ID NO: 98, and SEQ ID NO: 101;
 - a peptide linker having the amino acid sequence depicted in SEQ ID NO: 8;
 - a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 158, SEQ ID NO: 168, SEQ ID NO: 178, SEQ ID NO: 188, SEQ ID NO: 198, SEQ ID NO: 208, SEQ ID NO: 218, and SEQ ID NO: 228 and a serine residue at the C-terminus;
 - a polypeptide having the amino acid sequence depicted in SEQ ID NO: 140; and
- a polypeptide comprising in the following order starting from the N-terminus:
- a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 157, SEQ ID NO: 167, SEQ ID NO: 177, SEQ ID NO: 187, SEQ ID NO: 197, SEQ ID NO: 207, SEQ ID NO: 217, and SEQ ID NO: 227;
 - a peptide linker having the amino acid sequence depicted in SEQ ID NO: 8;
 - a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 18, SEQ ID NO: 27, SEQ ID NO: 36, SEQ ID NO: 45, SEQ ID NO: 54, SEQ ID NO: 63, SEQ ID NO: 72, SEQ ID NO: 81, SEQ ID NO: 90, SEQ ID NO: 99, and SEQ ID NO: 102 and a serine residue at the C-terminus;
 - a polypeptide having the amino acid sequence depicted in SEQ ID NO: 141;
- (e) a polypeptide comprising in the following order starting from the N-terminus:

- a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 26, SEQ ID NO: 35, SEQ ID NO: 44, SEQ ID NO: 53, SEQ ID NO: 62, SEQ ID NO: 71, SEQ ID NO: 80, SEQ ID NO: 89, SEQ ID NO: 98, and SEQ ID NO: 101;
 - a peptide linker having the amino acid sequence depicted in SEQ ID NO: 8;
 - a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 158, SEQ ID NO: 168, SEQ ID NO: 178, SEQ ID NO: 188, SEQ ID NO: 198, SEQ ID NO: 208, SEQ ID NO: 218, and SEQ ID NO: 228;
 - a polypeptide having the amino acid sequence depicted in SEQ ID NO: 142; and
- a polypeptide comprising in the following order starting from the N-terminus:
- a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 157, SEQ ID NO: 167, SEQ ID NO: 177, SEQ ID NO: 187, SEQ ID NO: 197, SEQ ID NO: 207, SEQ ID NO: 217, and SEQ ID NO: 227;
 - a peptide linker having an amino acid sequence depicted in SEQ ID NO: 8;
 - a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 18, SEQ ID NO: 27, SEQ ID NO: 36, SEQ ID NO: 45, SEQ ID NO: 54, SEQ ID NO: 63, SEQ ID NO: 72, SEQ ID NO: 81, SEQ ID NO: 90, SEQ ID NO: 99, and SEQ ID NO: 102 and a serine residue at the C-terminus;
 - a polypeptide having the amino acid sequence depicted in SEQ ID NO: 143;
- (f) a polypeptide comprising in the following order starting from the N-terminus:
- a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 159, SEQ ID NO: 169, SEQ ID NO: 179, SEQ ID NO: 189, SEQ ID NO: 199, SEQ ID NO: 209, SEQ ID NO: 219, and SEQ ID NO: 229;
 - a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-9; and
 - a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 46, SEQ ID NO: 55, SEQ ID NO: 64, SEQ ID NO: 73, SEQ ID NO: 82, SEQ ID NO: 91, SEQ ID NO: 100, and SEQ ID NO: 103; and
 - a polypeptide having the amino acid sequence depicted in SEQ ID NO: 144; and
- a polypeptide having the amino acid sequence depicted in SEQ ID NO: 145;
- (g) a polypeptide comprising in the following order starting from the N-terminus:
- a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 159, SEQ ID NO: 169, SEQ ID NO: 179, SEQ ID NO: 189, SEQ ID NO: 199, SEQ ID NO: 209, SEQ ID NO: 219, and SEQ ID NO: 229; and

- a polypeptide having the amino acid sequence depicted in SEQ ID NO: 146; and a polypeptide comprising in the following order starting from the N-terminus:
 - a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 46, SEQ ID NO: 55, SEQ ID NO: 64, SEQ ID NO: 73, SEQ ID NO: 82, SEQ ID NO: 91, SEQ ID NO: 100, and SEQ ID NO: 103; and
 - a polypeptide having the amino acid sequence depicted in SEQ ID NO: 147;
- (h) a polypeptide comprising in the following order starting from the N-terminus:
- a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 159, SEQ ID NO: 169, SEQ ID NO: 179, SEQ ID NO: 189, SEQ ID NO: 199, SEQ ID NO: 209, SEQ ID NO: 219, and SEQ ID NO: 229; and
 - a polypeptide having the amino acid sequence depicted in SEQ ID NO: 148; and a polypeptide comprising in the following order starting from the N-terminus:
 - a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 46, SEQ ID NO: 55, SEQ ID NO: 64, SEQ ID NO: 73, SEQ ID NO: 82, SEQ ID NO: 91, SEQ ID NO: 100, and SEQ ID NO: 103; and
 - a polypeptide having the amino acid sequence depicted in SEQ ID NO: 149; or
- (i) a polypeptide comprising in the following order starting from the N-terminus:
- a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 159, SEQ ID NO: 169, SEQ ID NO: 179, SEQ ID NO: 189, SEQ ID NO: 199, SEQ ID NO: 209, SEQ ID NO: 219, and SEQ ID NO: 229;
 - a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-9; and
 - a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 46, SEQ ID NO: 55, SEQ ID NO: 64, SEQ ID NO: 73, SEQ ID NO: 82, SEQ ID NO: 91, SEQ ID NO: 100, and SEQ ID NO: 103; and
 - a polypeptide having the amino acid sequence depicted in SEQ ID NO: 150.
11. The antibody construct according to any one of claims 1 to 9 comprising in an N- to C-terminal order:
- the first binding domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 159, SEQ ID NO: 169, SEQ ID NO: 179, SEQ ID

NO: 189, SEQ ID NO: 199, SEQ ID NO: 209, SEQ ID NO: 219, and SEQ ID NO: 229;

- a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 8 and 9;
 - the second binding domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 46, SEQ ID NO: 55, SEQ ID NO: 64, SEQ ID NO: 73, SEQ ID NO: 82, SEQ ID NO: 91, SEQ ID NO: 100, and SEQ ID NO: 103 (see also SEQ ID NOs: 23, 25, 41, 43, 59, 61, 77, 79, 95, 97, 113, 115, 131, 133, 149, 151, 167, 169, 185 or 187 of WO 2008/119567);
 - a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 4, 5, 6, 8 and 9; and
 - the third domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 260-267.
12. The antibody construct according to claim 11, comprising a sequence selected from the group consisting of SEQ ID NOs: 276 to 287
13. The antibody construct according to any one of claims 1 to 12, wherein the ratio of the binding affinity of the first binding domain for macaque MSLN/human MSLN (measured in a Scatchard analysis) is <100, preferably <20, more preferably <15, further preferably <10, even more preferably <8, more preferably <6 and most preferably <2.
14. A polynucleotide encoding an antibody construct as defined in any one of the preceding claims.
15. A vector comprising a polynucleotide as defined in claim 14.
16. A host cell transformed or transfected with the polynucleotide as defined in claim 14 or with the vector as defined in claim 15.
17. A process for the production of an antibody construct according to any one of claims 1 to 13, said process comprising culturing a host cell as defined in claim 16 under conditions allowing the expression of the antibody construct as defined in any one of claims 1 to 13 and recovering the produced antibody construct from the culture.

18. A pharmaceutical composition comprising an antibody construct according to any one of claims 1 to 14, or produced according to the process of claim 17.
19. The antibody construct according to any one of claims 1 to 13, or produced according to the process of claim 17, for use in the prevention, treatment or amelioration of a *solid tumor* disease or a metastatic cancer disease.
20. A method for the treatment or amelioration of a *solid tumor* disease or a metastatic cancer disease, comprising the step of administering to a subject in need thereof the antibody construct according to any one of claims 1 to 13, or produced according to the process of claim 17.
21. The method according to claim 20 or the antibody construct according to claim 19, wherein the *solid tumor* disease is selected from the group consisting of ovarian cancer, pancreatic cancer, mesothelioma, lung cancer, gastric cancer and triple negative breast cancer disease or a metastatic cancer disease derived from any of the forgoing.
22. A kit comprising an antibody construct according to any one of claims 1 to 13, an antibody construct produced according to the process of claim 17, a polynucleotide as defined in claim 14, a vector as defined in claim 15, and/or a host cell as defined in claim 16.