

CLAIMS

1. A multispecific antigen-binding protein comprising at least one immunoglobulin single variable domain (ISV) specifically binding to 4-1BB, wherein the at least one ISV specifically binding to 4-1BB has pure agonist activity.
2. The multispecific antigen-binding protein according to claims 1, wherein the at least one ISV specifically binding to 4-1BB comprises three complementary determining regions CDR1, CDR2 and CDR3;
 wherein CDR3 comprises or consists of the amino acid sequence ARGTRYKLST (SEQ ID NO: 14), ARGTRYKMST (SEQ ID NO: 15), or ARGTRYKIFA (SEQ ID NO: 62),
 wherein CDR1 comprises or consists of the amino acid sequence GFTFSDHT (SEQ ID NO: 16), GFAFRDFT (SEQ ID NO: 66), GDTFSSYA (SEQ ID NO: 67), or GFTFANYR (SEQ ID NO: 68), and
 wherein CDR2 comprises or consists of the amino acid sequence ISSGGSRI (SEQ ID NO: 17), INPSGGSQ (SEQ ID NO: 77), or IKKSGNRT (SEQ ID NO: 78).
3. The multispecific antigen-binding protein according to claim 1 or 2, wherein the at least one ISV specifically binding to 4-1BB comprises or consists of:
 - (i) an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 3, 58, 59, 60 and 61; or
 - (ii) an amino acid sequence sharing at least 70 % of sequence identity over the non-CDR regions of SEQ ID NOs: 2, 3, 58, 59, 60 or 61.
4. The multispecific antigen-binding protein according to any one of claims 1 to 3, comprising at least two ISVs specifically binding to 4-1BB, wherein said at least two ISVs specifically binding to 4-1BB are different and bind (i) to the same epitope, (ii) to overlapping epitopes, or (iii) to distinct epitopes of 4-1BB, preferably the at least two ISVs specifically binding to 4-1BB are different and bind to distinct epitopes of 4-1BB.
5. The multispecific antigen-binding protein according to claim 4, wherein at least a second ISV specifically binding to 4-1BB comprises or consists of:

- (i) an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 4,
or
 - (ii) an amino acid sequence sharing at least 70 % sequence identity over the non-CDR regions of SEQ ID NOs: 1 or 4.
6. The multispecific antigen-binding protein according to any one of claims 1 to 5, comprising at least four ISVs specifically binding to 4-1BB.
 7. The multispecific antigen-binding protein according to claim 6, wherein the at least four ISVs comprises:
 - (i) a first set of at least two identical ISVs specifically binding to 4-1BB, and (ii) a second set of at least two other identical ISVs specifically binding to 4-1BB; or
 - (i') a first set of at least two ISVs specifically binding to a first epitope of 4-1BB, and (ii') a second set of at least two other ISVs specifically binding to a second epitope of 4-1BB.
 8. The multispecific antigen-binding protein according to any one of claims 1 to 7, further comprising an antibody Fc region or a fragment thereof;
preferably wherein the Fc region or fragment thereof is ADCC- and/or ADCP-silenced.
 9. The multispecific antigen-binding protein according to any one of claims 1 to 8, further comprising at least one Fab fragment.
 10. The multispecific antigen-binding protein according to any one of claims 1 to 9, wherein the multispecific antigen-binding protein comprises:
 - a) a first polypeptide comprising, preferably from N-terminus to C-terminus:
 - i. a first ISV specifically binding to 4-1BB;
 - ii. a second ISV specifically binding to 4-1BB, preferably wherein the second ISV is different from the first ISV;
 - iii. at least one C_H domain of a Fc region; and
 - iv. a variable and constant domain of a Fab fragment;
 - b) a second polypeptide comprising a variable and constant domain of a Fab fragment;
wherein the variable and constant domains of the first and second polypeptide form a Fab fragment.

11. The multispecific antigen-binding protein according to claim **10**, further comprising a third and fourth polypeptide identical to the first and second polypeptide, respectively, wherein the at least one C_H domain of the first and third polypeptides form an Fc region.
12. The multispecific antigen-binding protein according to claim **10** or **11**, wherein the at least one C_H domain of the first polypeptide comprises C_{H2} and C_{H3} domains of IgG; preferably wherein IgG is IgG1 or IgG4; more preferably wherein IgG is IgG1.
13. The multispecific antigen-binding protein according to any one of claims **10** to **12**, wherein the first polypeptide comprises, preferably from N-terminus to C-terminus:
 - a first ISV specifically binding to 4-1BB;
 - a first linker;
 - a second ISV specifically binding to 4-1BB, preferably wherein the second ISV is different from the first ISV;
 - a second linker;
 - an IgG hinge region;
 - an IgG C_{H2} domain;
 - and IgG C_{H3} domain;
 - a third linker;
 - a V_H domain of a Fab fragment; and
 - a C_{H1} domain of a Fab fragment.
14. The multispecific antigen-binding protein according to any one of claims **10** to **13**, wherein the second polypeptide comprises, preferably from N-terminus to C-terminus:
 - a V_L domain of a Fab fragment; and
 - a C_L domain of a Fab fragment.
15. The multispecific antigen-binding protein according to any one of claims **9** to **14**, wherein the at least one Fab fragment is a PD-1 antagonist.

- 16.** The multispecific antigen-binding protein according to any one of claims **9** to **15**, wherein the at least one Fab fragment is an antigen-binding protein that specifically binds to PD-1, and which comprises:
- (i) a light chain variable region comprising the three following CDR sequences:
 - a. V_L-CDR1: QSVPINF (SEQ ID NO: 18) or QSVSINF (SEQ ID NO: 19);
 - b. V_L-CDR2: EAS; and
 - c. V_L-CDR3: GQYGSSPYT (SEQ ID NO: 20) or QQYGSSPYT (SEQ ID NO: 21); and
 - (ii) a heavy chain variable region comprising the three following CDR sequences:
 - a. V_H-CDR1: GGSISSSSYF (SEQ ID NO: 22) or GGSISTSSYF (SEQ ID NO: 23);
 - b. V_H-CDR2: IYRSGST (SEQ ID NO: 24); and
 - c. V_H-CDR3: ARGITGDPGDY (SEQ ID NO: 25).
- 17.** The multispecific antigen-binding protein according to any one of claims **9** to **16**, wherein the at least one Fab fragment is an antigen-binding protein that specifically binds to PD-1, and which comprises:
- (i) a light chain variable region with SEQ ID NO: 7 or 5, or a light chain variable region sharing at least 70 % of sequence identity over the non-CDR regions of SEQ ID NO: 7 or 5; and
 - (ii) a heavy chain variable region with SEQ ID NO: 8 or 6, or a heavy chain variable region sharing at least 70 % of sequence identity over the non-CDR regions of SEQ ID NO: 8 or 6.
- 18.** The multispecific antigen-binding protein according to any one of claims **1** to **17**, comprising at least a first polypeptide with SEQ ID NO: 11 or 9, and at least a second polypeptide with SEQ ID NO: 12 or 10; or comprising at least a first polypeptide sharing at least 70 % of sequence identity over the non-CDR regions of SEQ ID NO: 11 or 9, and at least a second polypeptide sharing at least 70 % of sequence identity over the non-CDR regions of SEQ ID NO: 12 or 10.

- 19.** The multispecific antigen-binding protein according to any one of claims **1** to **18**, comprising (i) at least a first polypeptide with SEQ ID NO: 11 and at least a second polypeptide with SEQ ID NO: 12 or (ii) at least a first polypeptide with SEQ ID NO: 9 and at least a second polypeptide with SEQ ID NO: 10.
- 20.** A composition comprising the (i) multispecific antigen-binding protein according to any one of claims **1** to **19**, and a (ii) pharmaceutically acceptable carrier or excipient.
- 21.** The composition according to claims **20**, for use as medicament.
- 22.** The composition according to claim **20**, for use in treating cancer in a subject in need thereof.
- 23.** An isolated polynucleotide encoding the multispecific antigen-binding protein according to any one of claims **1** to **19**.
- 24.** A vector or a host cell comprising the polynucleotide according to claim **25**.
- 25.** A method of making the multispecific antigen-binding protein according to any one of claims **1** to **19**, comprising expressing the polynucleotide of claim **25** in a cell.