

CLAIMS

1. ~~A multispecific antibody comprising~~
 - (a) ~~a first domain specifically binding to PD-L1 comprising a VH sequence of SEQ ID NO: 11 and a VL sequence of SEQ ID NO: 16, and~~
 - (b) ~~a second domain specifically binding to a tumor-associated antigen (TAA).~~
2. ~~The multispecific antibody of claim 1, wherein said TAA is selected from the group consisting of EGFRvIII, 5T4, CD19, CD20, CD22, CD38, BCMA, IL4RA, mesothelin, GD2, Tn antigen, sTn antigen, Tn-O-Glycopeptides, sTn-O-Glycopeptides, PSMA, CD97, TAG72, CD44v6, CEA, EPCAM, KIT, IL-13Ra2, leguman, GD3, CD171, IL-11Ra, IL-13RA2, ROR1, PSCA, MAD-CT-1, MAD-CT-2, VEGFR2, CLEC12A, LewisY, CD24, PDGFR-beta, SSEA-4, folate receptor alpha, ERBBs (e.g., ERBB2), Her2/neu (HER2), MUC1, MUC16, EGFR, NCAM, Ephrin B2, CAIX, LMP2, sLe, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, CD33, CD123, CD133, CD135, TEM7R, FAP, Legumain, HPV E6 or E7, ML-IAP, CLDN6, TSHR, GPRC5D, ALK, Polysialic acid, Fos-related antigen, neutrophil elastase, TRP-2, CYP1B1, sperm protein 17, beta human chorionic gonadotropin, AFP, thyroglobulin, PLAC1, globoH, RAGE1, MN-CA-IX, human telomerase reverse transcriptase, intestinal carboxyl esterase, mut hsp 70-2, NA-17, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, NY-ESO-1, GPR20, Ly6k, OR51E2, TARP, GFRalpha4, GPC3, CDH3, B7H3, FGFR1, SSTR2, CECAM6, GA733, and gp120; particularly, wherein said TAA is selected from HER2 and mesothelin, more particularly HER2.~~
3. ~~The multispecific antibody of claim 2, wherein said second domain comprises the VH sequence of SEQ ID NO: 34 and the VL sequence of SEQ ID NO: 36.~~
4. ~~The multispecific antibody of any one of claims 1 to 3 further comprising a third domain specifically binding to an immune cell antigen, in particular wherein said immune cell antigen is present on T cell or NK cell.~~

5. ~~The multispecific antibody of claim 4, wherein said third domain is an agonist and said second immune checkpoint antigen is a stimulatory immune cell antigen, particularly wherein said stimulatory immune cell antigen is selected from the group consisting of CD3, and CD16, more particularly CD3.~~
6. ~~The multispecific antibody of claim 5, wherein said third domain comprises the VH sequence of SEQ ID NO: 43 and the VL sequence of SEQ ID NO: 44.~~
7. ~~The multispecific antibody of claim 4, wherein said third domain is an agonist and said second immune checkpoint antigen is a co-stimulatory immune cell antigen, particularly wherein said co-stimulatory immune cell antigen is selected from the group consisting of CD137, CD28, ICOS, HVEM, CD27, OX40, DR3, GITR, CD30, SLAM, CD2, 2B4, TIM1, TIM2, CD226, more particularly CD137.~~
8. ~~The multispecific antibody of claim 7, wherein said third domain specifically binds to CD137 at an epitope comprised in the distal part of the extracellular domain of CD137, particularly within the cysteine-rich domains CRD1 and/or CRD2, more particularly within amino acid residues 24-86 of SEQ ID NO: 153, provided that amino acid residue Asn42 of CD137 is not a critical residue for binding.~~
9. ~~The multispecific antibody of claim 8, wherein said third domain comprises the VH sequence of SEQ ID NO: 77 and the VL sequence of SEQ ID NO: 78.~~
10. ~~The multispecific antibody of claim 4, wherein said third domain is an inhibitor and said second immune checkpoint antigen is an inhibitory immune checkpoint antigen, particularly wherein said inhibitory immune checkpoint antigen is selected from the group consisting of cytotoxic T-lymphocyte-associated protein 4 (CTLA4), PD-1, lymphocyte-activation gene 3, and T-cell immunoglobulin mucin-3, BTLA, TIM3, TIGIT, CD160, LAIR1, B7-1, and B7-H1.~~
11. ~~The multispecific antibody of any one of the preceding claims further comprising a domain specifically binding to human serum albumin (HSA).~~

1. An antibody or antigen-binding fragment thereof specifically binding to HSA that comprises (i) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 45, 46 and 47, respectively, and LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 48, 49 and 50, respectively, (ii) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 53, 54 and 55, respectively, and LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 56, 57 and 58, respectively, or (iii) HCDR1, HCDR2, and HCDR3 sequences of SEQ ID NOs: 61, 62 and 63, respectively, and LCDR1, LCDR2, and LCDR3 sequences of SEQ ID NOs: 64, 65 and 66, respectively.
2. The antibody or antigen-binding fragment thereof of claim 1, wherein one or more of said CDR sequences optionally comprises one or two mutations.
3. The antibody or antigen-binding fragment thereof of claim 2, wherein said one or two mutations are mutations to alanine residues.
4. The antibody or antigen-binding fragment thereof of claim 1, wherein said antibody or antigen-binding fragment thereof comprises (i) a VH domain having an amino acid sequence that is in the framework sequences at least 90 percent, in particular at least 95 percent identical to the amino acid sequence of SEQ ID NO: 51; and a VL domain having an amino acid sequence that is in the framework sequences at least 90 percent, in particular at least 95 percent identical to the amino acid sequence of SEQ ID NO: 52; (ii) a VH domain having an amino acid sequence that is in the framework sequences at least 90 percent, in particular at least 95 percent identical to the amino acid sequence of SEQ ID NO: 59; and a VL domain having an amino acid sequence that is in the framework sequences at least 90 percent, in particular at least 95 percent identical to the amino acid sequence of SEQ ID NO: 60; (iii) a VH domain having an amino acid sequence that is in the framework sequences at least 90 percent, in particular at least 95 percent identical to the amino acid sequence of SEQ ID NO: 67; and a VL domain having an amino acid sequence that is in the framework sequences at least 90 percent, in particular at least 95 percent identical to the amino acid sequence of SEQ ID NO: 69; or (iv) a VH domain having an amino acid sequence that is in the framework sequences at least 90 percent, in particular at least 95 percent identical to the amino acid sequence of SEQ ID NO: 68; and a VL domain having an amino

acid sequence that is in the framework sequences at least 90 percent, in particular at least 95 percent identical to the amino acid sequence of SEQ ID NO: 70.

~~12.5. The antibody or antigen-binding fragment thereof multispecific antibody of claim 114, wherein said domain specifically binding to HSA comprises~~comprising:

- ~~(ai) the a VH sequence domain of SEQ ID NO: 51 and the a VL sequence domain of SEQ ID NO: 52,~~
- ~~(bij) the a VH sequence domain of SEQ ID NO: 59 and the a VL sequence domain of SEQ ID NO: 60,~~
- ~~(eiii) the a VH domain sequence of SEQ ID NO: 67 and the a VL domain sequence of SEQ ID NO: 69, or~~
- ~~(div) the a VH domain sequence of SEQ ID NO: 68 and the a VL domain sequence of SEQ ID NO: 70.~~

~~13. The multispecific antibody of any one of the preceding claims, wherein said multispecific antibody is in a format selected from the group consisting of a single-chain diabody (scDb), a tandem scDb (Tandab), a linear dimeric scDb (LD-scDb), a circular dimeric scDb (CD-scDb), a bispecific T-cell engager (BiTE; tandem di-scFv), a tandem tri-scFv, a tribody (Fab-(scFv)₂) or bibody (Fab-(scFv)₁), Fab, Fab-Fv₂, Morrison (IgG-CH₃-scFv fusion (Morrison L) or IgG-CL-scFv fusion (Morrison H)), triabody, scDb-scFv, bispecific Fab₂, di-miniantibody, tetrabody, scFv-Fc-scFv fusion, scFv-HSA-scFv fusion, di-diabody, DVD-Ig, COVD, IgG-scFab, scFab-dsscFv, Fv₂-Fc, IgG-scFv fusions, such as bsAb (scFv linked to C-terminus of light chain), Bs1Ab (scFv linked to N-terminus of light chain), Bs2Ab (scFv linked to N-terminus of heavy chain), Bs3Ab (scFv linked to C-terminus of heavy chain), Ts1Ab (scFv linked to N-terminus of both heavy chain and light chain), Ts2Ab (dsscFv linked to C-terminus of heavy chain), Bispecific antibodies based on heterodimeric Fc domains, such as Knob-into-Hole antibodies (KiHs); an scDb, tandem-di-scFv, tandem tri-scFv, Fab-(scFv)₂, Fab-(scFv)₁, Fab, Fab-Fv₂, COVD fused to the N- and/or the C-terminus of either chain of a heterodimeric Fc domain or any other heterodimerization domain, a MATCH and DuoBodies, particularly wherein said antibody is a scDb-scFv, tribody, DVD-tribody, MATCH, in particular wherein said multispecific antibody is in a MATCH or tribody format,~~

~~more particularly wherein said multispecific antibody is in a MATCH format, more particularly wherein said multispecific antibody is a MATCH3 or a MATCH4.~~

~~14. The multispecific antibody of any one of the preceding claims, wherein said multispecific antibody is in the MATCH4 format and comprises the two chains according to SEQ ID NOs: 123 and 124, or SEQ ID NOs: 127 and 128.~~

6. A multispecific antibody comprising (i) at least one antibody or antigen-binding fragment thereof of any one of claims 1 to 5, and (ii) at least one antibody or antigen-binding fragments thereof with specificity for a target different from HSA.

7. The multispecific antibody of claim 6, wherein said target different from HSA is a tumor-associated immune checkpoint antigen.

8. The multispecific antibody of claim 7, wherein said tumor-associated immune checkpoint antigen is selected from the group consisting of PD-L1, PD-L2, CD80, CD86, CD276 (B7-H3), and VTCN1 (B7-H4)

9. The multispecific antibody of claim 7, wherein said tumor-associated immune checkpoint antigen is an inhibitory immune cell antigen selected from group consisting of CTLA4, PD-1, lymphocyte-activation gene 3, T-cell immunoglobulin mucin-3, BTLA, TIM3, TIGIT, CD160, LAG3, LAIR1, B7-1, and B7-H1.

10. The multispecific antibody of any one of claims 6 to 9, wherein said target different from HSA is a tumor-associated antigen (TAA).

11. The multispecific antibody of claim 10, wherein said TAA is selected from of the group of EGFRvIII, 5T4, CD19, CD20, CD22, CD38, BCMA, IL4RA, mesothelin, GD2, Tn antigen, sTn antigen, Tn-O-Glycopeptides, sTn-O-Glycopeptides, PSMA, CD97, TAG72, CD44v6, CEA, EPCAM, KIT, IL-13Ra2, leguman, GD3, CD171, IL-11Ra, IL-13RA2, ROR1, PSCA, MAD-CT-1, MAD-CT-2, VEGFR2, CLEC12A, LewisY, CD24, PDGFR-beta, SSEA-4, folate receptor alpha, ERBBs (e.g., ERBB2), Her2/neu (HER2), MUC1, MUC16, EGFR, NCAM, Ephrin B2, CAIX, LMP2, sLe, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, CD33, CD123, CD133, CD135, TEM7R, FAP, Legumain, HPV E6

or E7, ML-IAP, CLDN6, TSHR, GPRC5D, ALK, Polysialic acid, Fos-related antigen, neutrophil elastase, TRP-2, CYP1B1, sperm protein 17, beta human chorionic gonadotropin, AFP, thyroglobulin, PLAC1, globoH, RAGE1, MN-CA IX, human telomerase reverse transcriptase, intestinal carboxyl esterase, mut hsp 70-2, NA-17, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, NY-ESO-1, GPR20, Ly6k, OR51E2, TARP, GFRalpha4; GPC3, CDH3, B7H3; FGFR1, SSTR2, CECAM6, GA733, and gp120.

12. The multispecific antibody of any one of claims 6 to 11, further comprising (iii) at least one antibody or antigen-binding fragments thereof with specificity for an immune cell antigen.

13. The multispecific antibody of claim 12, wherein said immune cell antigen is a stimulatory immune cell antigen selected from the group consisting of CD3 and CD16.

14. The multispecific antibody of claim 12, wherein said immune cell antigen is a co-stimulatory immune cell antigen selected from the group consisting of is CD137, CD28, ICOS, HVEM, CD27, OX40, DR3, GITR, CD30, SLAM, CD2, 2B4, TIM1, TIM2, and CD226.

15. A pharmaceutical composition comprising the multispecific antibody of any one of claim 6 to 14, and a pharmaceutically acceptable carrier.

16. The multispecific antibody of any one of claims 6 to 14 or the pharmaceutical composition of claim 15, for use in treatment of a cancer in a subject in need thereof.

17. A nucleic acid encoding the antibody or antigen-binding fragment thereof of claim 1 to 5 or the multispecific antibody of any one of claims 6 to 14.

18. A vector comprising the nucleic acid of claim 17.

19. A host cell comprising the nucleic acid of claim 17 or the vector of claim 18.

20. A method of producing the antibody or antigen-binding fragment thereof of claim 1 to 5 or the multispecific antibody of any one of claims 6 to 14, the method comprising the steps of (i) culturing the host cell of claim 19, and (ii) expressing the antibody or antigen-binding fragment thereof of claim 1 to 5 or the multispecific antibody of any one of claims 6 to 14.