

CLAIMS

What is claimed is:

1. A bispecific antibody, comprising IgG domains having heavy chains and light chains, and two scFv components being connected to either C terminal of the heavy chains or N terminal of the light chains, wherein the IgG domains have a first binding specificity to a first antigen α -CTLA4, wherein the scFv components have a second binding specificity to a second antigen α -PD-1 or α -PD-L1, and wherein the first antigen and the second antigen are different and are independently selected from α -CTLA4, α -PD-1, and α -PD-L1 the bispecific antibody comprises:

- i. the 3 complementarity determining regions (CDRs) of SEQ ID No. 94, the 3 CDRs of SEQ ID No. 92, the 3 CDRs of SEQ ID No: 96 and the 3 CDRs of SEQ ID No: 98; or
- ii. the 3 complementarity determining regions (CDRs) of SEQ ID No. 94, the 3 CDRs of SEQ ID No. 92, the 3 CDRs of SEQ ID No: 120 and the 3 CDRs of SEQ ID No: 122.

2. The bispecific antibody of claim 1, wherein:

- i. the scFv components comprise an amino acid sequence having at least 98% sequence identity to SEQ ID No. 96 and 98, and the IgG domains comprise an amino acid sequence having at least 98% identity to SEQ ID No. 92 and 94; or
- ii. the scFv components comprise an amino acid sequence having at least 98% sequence identity to SEQ ID No. 120 and 122, and the IgG variable domains comprise an amino acid sequence having at least 98% identity to SEQ ID No. 92 and 94.

3. The bispecific antibody of claim 1, comprising:

- i. an amino acid sequence having at least 98% sequence identity to SEQ ID No. 2 and an amino acid sequence having at least 98% sequence identity to SEQ ID No. 4; or
- ii. an amino acid sequence having at least 98% sequence identity to SEQ ID No. 8 and an amino acid sequence having at least 98% sequence identity to SEQ ID No. 4.

42. The bispecific antibody of any one of cClaims 1 to 3, wherein the two scFv components are connected to the C terminal of the heavy chain.

3. ~~The bispecific antibody of Claim 2, wherein the first antigen comprises α -CTLA4 and the second antigen comprises α -PD-1 or α -PD-L1.~~

4. ~~The bispecific antibody of Claim 2, wherein the first antigen comprises α -PD-1 or α -PD-L1 and the second antigen comprises α -CTLA4.~~

55. The bispecific antibody of any one of cClaims 1 to 3, wherein the two scFv components are connected to the N terminal of the light chain.

~~6.~~ The bispecific antibody of Claim 5, wherein the first antigen comprises α -PD-1 or α -PD-L1 and the second antigen comprises α -CTLA4.

~~7.~~ The bispecific antibody of Claim 5, wherein the first antigen comprises α -CTLA4 and the second antigen comprises α -PD-1 or α -PD-L1.

68. The bispecific antibody of any one of cClaims 1 to 5, wherein the bispecific antibody is an isolated monoclonal antibody.

~~9.~~ The bispecific antibody of Claim 1, comprising an antigen-binding fragment having a sequence having at least 98% similarity with SEQ ID No. 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, or 134.

710. The bispecific antibody of any one of cClaims 1 to 6, having a first binding specificity to α -CTLA4 and a second binding specificity to α -PD-1 or α -PD-L1, wherein neither the first nor the second binding specificity has a Kd greater than 70nM.

~~11.~~ The bispecific antibody of Claim 1, having a first binding specificity to α -CTLA4 and a second specificity to α -PD-1, wherein neither the first nor the second has a Kd greater than 70nM.

~~12.~~ The bispecific antibody of Claim 1, having a first binding specificity to α -CTLA4 and a second binding specificity to α -PD-L1, wherein neither the first nor the second has a Kd greater than 70nM.

813. The bispecific antibody of any one of cClaims 1 to 7, comprising a human framework region.

914. The bispecific antibody of any one of cClaims 1 to 8, wherein the antibody is a humanized antibody, a chimeric antibody, or a recombinant antibody.

1015. The bispecific antibody of any one of cClaims 1 to 9, wherein the IgG domain comprises an IgG1 constant region, wherein the IgG1 constant region comprises an amino acid sequence having at least 98% similarity with SEQ ID No. 136.

~~16.~~ An IgG1 heavy chain, comprising an amino acid sequence having at least 98% similarity with SEQ ID No. 02, 06, 08, 10, 12, 14, 16, 18, 20, 22, 26, 30, 34, 38, 42, 46, 50, 54, 58, 62, 72, 92, 96, 100, 104, 108, 112, 116, 120, 124, 128, or 132.

~~17.~~ A kappa light chain, comprising an amino acid sequence having at least 98% similarity with SEQ ID No. 04, 28, 32, 36, 40, 44, 48, 52, 56, 60, and 64.

~~18. — A variable light chain, comprising an amino acid sequence having at least 98% similarity with SEQ ID No. 94, 98, 102, 106, 110, 114, 118, 122, 126, 130, or 134.~~

~~19. — A variable heavy chain, comprising an amino acid sequence having at least 98% similarity with SEQ ID No. 92, 96, 100, 104, 108, 112, 116, 120, 124, 128, or 132.~~

1120. An isolated nucleic acid encoding the bispecific antibody of any one of Claims 1 to 10, the IgG1 heavy chain of Claim 16, the kappa light chain of Claim 17, the variable light chain of Claim 18, or the variable heavy chain of Claim 19.

1221. An expression vector comprising the isolated nucleic acid of Claim 1120.

1322. The expression vector of Claim 1221, wherein the vector is expressible in a cell.

1423. A host cell comprising the nucleic acid of Claim 1120.

1524. A host cell comprising the expression vector of Claim 1221.

1625. The host cell of Claim 1423 or 1524, wherein the host cell is a prokaryotic cell or a eukaryotic cell.

1726. A method of producing a bispecific antibody, comprising culturing the host cell of any one of Claims 1423 to 1625 so that the bispecific antibody is produced.

1827. An immunoconjugate comprising the bispecific antibody of any one of Claims 1 to 10 and a cytotoxic agent.

1928. The immunoconjugate ~~according to~~ claim 1827, wherein the cytotoxic agent comprises a chemotherapeutic agent, a growth inhibitory agent, a toxin, or a radioactive isotope.

2029. A pharmaceutical composition, comprising the bispecific antibody of any one of Claims 1 to 10 and a pharmaceutically acceptable carrier.

2130. The pharmaceutical composition of Claim 2029, further comprising radioisotope, radionuclide, a toxin, a therapeutic agent, a chemotherapeutic agent or a combination thereof.

2231. A pharmaceutical composition, comprising the immunoconjugate of Claim 2130 and a pharmaceutically acceptable carrier.

~~32. — A method of treating a subject with a cancer, comprising administering to the subject an effective amount of the bispecific antibody of Claim 1~~

23. Use of the bispecific antibody of any one of claims 1 to 10 in the manufacture of a medicament for the treatment of a subject with a cancer that is responsive to regulation of CTLA4 and PD-1 or PD-L1 checkpoint antigens.

2433. The method use of cClaim 2332, wherein the cancer comprises cells expressing PD-L1.

2534. The method use of cClaim 2332, wherein the cancer comprises breast cancer, colorectal cancer, pancreatic cancer, head and neck cancer, melanoma, ovarian cancer, prostate cancer, non-small lung cell cancer, small cell lung cancer, glioma, esophageal cancer, nasopharyngeal cancer, kidney cancer, gastric cancer, liver cancer, bladder cancer, cervical cancer, brain cancer, lymphoma, leukaemia, myeloma.

2638. The method use of cClaim 2332, wherein the subject is a human.

39. — A solution comprising an effective concentration of the bispecific antibody of Claim 1, wherein the solution is blood plasma in a subject.

40. — A bispecific antibody, having a first binding specificity to a first antigen and a second binding specificity to a second antigen, wherein both the first and the second antigen are check point antigens, and wherein neither the first specificity nor the second specificity has a Kd greater than 70nM.

41. — A bispecific antibody, comprising IgG domains having heavy chains and light chains, and two scFv components being connected to either C terminal of the heavy chains or N terminal of the light chains, wherein the IgG domains have a first binding specificity to a first antigen, wherein the scFv components have a second binding specificity to a second antigen, and wherein both the first antigen and the second antigen are checkpoint antigens and wherein the first antigen differs from the second antigen.