

CLAIMS

1. A binding agent comprising a first antigen-binding region binding to human CD137 and a second antigen-binding region binding to human PD-L1, wherein the second antigen-binding region inhibits the binding of human PD-L1 to human PD-1.
2. The binding agent according claim 1, wherein said second antigen-binding region binding to human PD-L1 comprises a heavy chain variable region (VH) comprising a HCDR1, HCDR2, and HCDR3 sequence and a light chain variable region (VL) comprising a LCDR1, LCDR2, and LCDR3 sequence, wherein the HCDR1, HCDR2 and HCDR3 sequences are the sequences as set forth in: SEQ ID NO: 18, 19, 20 respectively and the LCDR1, LCDR2 and LCDR3 sequences are the sequences as set forth in: SEQ ID NO: 22, DDN, 23 respectively.
3. The binding agent according to claim 1 or 2, wherein said second antigen-binding region binding to human PD-L1 comprises a heavy chain variable region (VH) comprising a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identity to the amino acid sequence of the VH sequence as set forth in: SEQ ID NO: 17.
4. The binding agent according to any one of the preceding claims, wherein said second antigen-binding region binding to human PD-L1 comprises a heavy chain variable region (VH), wherein the VH comprises the sequence as set forth in: SEQ ID NO: 17.
5. The binding agent according to any one of the preceding claims, wherein said second antigen-binding region binding to human PD-L1 comprises a light chain variable region (VL) comprising a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identity to the amino acid sequence of the VL sequence as set forth in: SEQ ID NO: 21.
6. The binding agent according to any one of the preceding claims, wherein said second antigen-binding region binding to human PD-L1 comprises a light chain variable region (VL), wherein the VL comprises the sequence as set forth in: SEQ ID NO: 21.

7. The binding agent according to any one of the preceding claims, wherein said second antigen-binding region binding to human PD-L1 comprises a heavy chain variable region (VH) and a variable region (VL), wherein the VH comprises the sequence as set forth in: SEQ ID NO: 17 and the VL comprises the sequence as set forth in: SEQ ID NO: 21.
8. The binding agent according to any one of the preceding claims, wherein said first antigen-binding region binding to human CD137 comprises:
 - a. a heavy chain variable region (VH) comprising a HCDR1, HCDR2, and HCDR3 sequence and a light chain variable region (VL) comprising a LCDR1, LCDR2, and LCDR3 sequence, wherein the HCDR1, HCDR2 and HCDR3 sequences are the sequences as set forth in: SEQ ID NO: 9, 10, 11, respectively and the LCDR1, LCDR2 and LCDR3 sequences are the sequences as set forth in: SEQ ID NO: 13, GAS, 14, respectively; or
 - b. a heavy chain variable region (VH) comprising a HCDR1, HCDR2, and HCDR3 sequence and a light chain variable region (VL) comprising a LCDR1, LCDR2, and LCDR3 sequence, wherein the HCDR1, HCDR2, and HCDR3 sequences are the sequences as set forth in: SEQ ID NO: 50, 51 and 52 respectively, and the LCDR1, LCDR2, and LCDR3 sequences are the sequences as set forth in: SEQ ID NO: 54, SAS, 55 respectively.
9. The binding agent according to any one of the preceding claims, wherein said first antigen-binding region binding to human CD137 comprises:
 - a. a heavy chain variable region (VH) comprising a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identity to the amino acid sequence of the VH sequence as set forth in: SEQ ID NO: 15; or
 - b. a heavy chain variable region (VH) comprising a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identity to the amino acid sequence of the VH sequence as set forth in: SEQ ID NO: 49.
10. The binding agent according to any one of the preceding claims, wherein said first antigen-binding region binding to human CD137 comprises:
 - a. a heavy chain variable region (VH), wherein the VH comprises the sequence as set forth in: SEQ ID NO: 15; or

- b. a heavy chain variable region (VH), wherein the VH comprises the sequence as set forth in: SEQ ID NO: 49.
- 11. The binding agent according to any one of the preceding claims, wherein said first antigen-binding region binding to human CD137 comprises:
 - a. a light chain variable region (VL) comprising a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identity to the amino acid sequence of the VL sequence as set forth in: SEQ ID NO: 16; or
 - b. a light chain variable region (VL) comprising a sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% identity to the amino acid sequence of the VL sequence as set forth in: SEQ ID NO: 53.
- 12. The binding agent according to any one of the preceding claims, wherein said first antigen-binding region binding to human CD137 comprises:
 - a. a light chain variable region (VL), wherein the VL comprises the sequence as set forth in: SEQ ID NO: 16; or
 - b. a light chain variable region (VL), wherein the VL comprises the sequence as set forth in: SEQ ID NO: 53.
- 13. The binding agent according to any one of the preceding claims, wherein said first antigen-binding region binding to human CD137 comprises:
 - a. a heavy chain variable region (VH) and a variable region (VL), wherein the VH sequence comprises the sequence as set forth in: SEQ ID NO: 15 and the VL sequence comprises the sequence as set forth in: SEQ ID NO: 16; or
 - b. a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH sequence comprises the sequence as set forth in: SEQ ID NO: 49 and the VL sequence comprises the sequence as set forth in: SEQ ID NO: 53.
- 14. The binding agent according to any one of the preceding claims, comprising a first antigen-binding region binding to human CD137 and a second antigen-binding region binding to human PD-L1, wherein
 - a. the first antigen-binding region comprises a heavy chain variable region (VH) comprising the CDR1, CDR2, and CDR3 sequences, as set forth in: SEQ ID NO: 9, 10, 11 respectively, or a heavy chain variable region (VH)

comprising the HCDR1, HCDR2, and HCDR3 sequences, as set forth in: SEQ ID NO:50, 51, 52 respectively; and

- b. the second antigen-binding region comprises a heavy chain variable region (VH) comprising the CDR1, CDR2, and CDR3 sequences, as set forth in: SEQ ID NO: 18, 19, 20, respectively.

15. The binding agent according to any one of the preceding claims, comprising a first antigen-binding region binding to human CD137 and a second antigen-binding region binding to human PD-L1, wherein

- a. the first antigen-binding region comprises
 - a heavy chain variable region (VH) comprising a CDR1, CDR2, and CDR3 sequence, as set forth in: SEQ ID NO: 9, 10, 11, respectively, and a light chain variable region (VL) comprising a CDR1, CDR2, and CDR3 sequence, as set forth in: SEQ ID NO:13, 14, 15, respectively, or
 - a heavy chain variable region (VH) comprising a HCDR1, HCDR2, and HCDR3 sequence, as set forth in: SEQ ID NO: 50, 51 and 52, respectively, and a light chain variable region (VL) comprising a LCDR1, LCDR2, and LCDR3 sequence, as set forth in: SEQ ID NO: 54, 55 and 56, respectively;

and

- b. the second antigen-binding region comprises a heavy chain variable region (VH) comprising a CDR1, CDR2, and CDR3 sequence, as set forth in: SEQ ID NO:18, 19, 20 respectively, and a light chain variable region (VL) comprising a CDR1, CDR2, and CDR3 sequence, as set forth in: SEQ ID NO:22, 23, 24, respectively.

16. The binding agent according to any one of the preceding claims, comprising a first antigen-binding region binding to human CD137 and a second antigen-binding region binding to human PD-L1, wherein

- a. the first antigen-binding region comprises
 - a heavy chain variable region (VH) comprising the sequence as set forth in: SEQ ID NO:15, or
 - a heavy chain variable region (VH) comprising the sequence as set forth in: SEQ ID NO:49;

and

- b. the second antigen-binding region comprises a heavy chain variable region (VH) comprising the sequence as set forth in: SEQ ID NO:17.
17. The binding agent according to any one of the preceding claims comprising a first antigen-binding region binding to human CD137 and a second antigen-binding region binding to human PD-L1, wherein
- a. the first antigen-binding region comprises
 - a heavy chain variable region (VH) comprising the sequence as set forth in: SEQ ID NO:15 and a light chain variable region (VL) comprising the sequence as set forth in: SEQ ID NO: 16, or
 - a heavy chain variable region (VH) comprising the sequence as set forth in: SEQ ID NO:49 and a light chain variable region (VL) comprising the sequence as set forth in: SEQ ID NO: 53;
 - and
 - b. the second antigen-binding region comprises a heavy chain variable region (VH) comprising the sequence as set forth in: SEQ ID NO:17 and a light chain variable region (VL) comprising the sequence as set forth in: SEQ ID NO:21.
18. The binding agent according to any one of the preceding claims, wherein the binding agent is in the format of a full-length antibody or an antibody fragment.
19. The binding agent according to any one of the preceding claims, wherein said first antigen-binding region comprises a first heavy chain variable region (VH) and a first light chain variable region (VL) and said second antigen-binding region comprises a second heavy chain variable region (VH) and a second light chain variable region (VL).
20. The binding agent according to any one of the preceding claims, wherein each variable region comprises three complementarity determining regions (CDR1, CDR2, and CDR3) and four framework regions (FR1, FR2, FR3, and FR4).
21. The binding agent according to claim 20, wherein said complementarity determining regions and said framework regions are arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

22. The binding agent according to any one of the preceding claims, which comprises (i) a polypeptide comprising said first heavy chain variable region (VH) and further comprising a first heavy chain constant region (CH) and (ii) a polypeptide comprising said second heavy chain variable region (VH) and further comprising a second heavy chain constant region (CH).
23. The binding agent according to any one of the preceding claims, which comprises (i) a polypeptide comprising said first light chain variable region (VL) and further comprising a first light chain constant region (CL) and (ii) a polypeptide comprising said second light chain variable region (VL) and further comprising a second light chain constant region (CL).
24. The binding agent according to claims 22 to 23, which is an antibody comprising a first binding arm and a second binding arm, wherein
- a. the first binding arm comprises i) a polypeptide comprising said first heavy chain variable region (VH) and said first heavy chain constant region (CH) and ii) a polypeptide comprising said first light chain variable region (VL) and said first light chain constant region (CL) and;
 - b. the second binding arm comprises iii) a polypeptide comprising said second heavy chain variable region (VH) and said second heavy chain constant region (CH) and iv) a polypeptide comprising said second light chain variable region (VL) and said second light chain constant region (CL).
25. The binding agent according to any one of claims 22 to 24, wherein each of the first and second heavy chain constant region (CH) comprises one or more of a constant region domain 1 region (CH1 region), a hinge region, a CH2 region and a CH3 region, preferably at least a hinge region, a CH2 region and a CH3 region.
26. The binding agent according to any one of the preceding claims, wherein the binding agent is of an isotype selected from the group consisting of IgG1, IgG2, IgG3, and IgG4.
27. The binding agent according to any one of the preceding claims, wherein the binding agent is a full-length IgG1 antibody.

28. The binding agent according to any one of the preceding claims, wherein
- a. the first antigen-binding region binding to CD137 is derived from a chimeric antibody, and/or
 - b. the second antigen-binding region binding to human PD-L1 is derived from a chimeric antibody.
29. The binding agent according to any one of the preceding claims, wherein
- a. the first antigen-binding region binding to CD137 is derived from a humanized antibody, and/or
 - b. the second antigen-binding region binding to human PD-L1 is derived from a humanized antibody.
30. The binding agent according to any one of the preceding claims, wherein
- a. the first antigen-binding region binding to human CD137 is derived from a human antibody, and/or
 - b. the second antigen-binding region binding to human PD-L1 is derived from a human antibody.
31. The binding agent according to any one of the preceding claims, wherein
- a. the first antigen-binding region binding to human CD137 is derived from a humanized antibody, and/or
 - b. the second antigen-binding region binding to human PD-L1 is derived from a human antibody.
32. The binding agent according to any one of the preceding claims, wherein each of the first and second heavy chain constant regions (CHs) comprises a CH3 region and wherein the two CH3 regions comprise asymmetrical mutations.
33. The binding agent according to any one of the preceding claims, wherein in said first heavy chain constant region (CH) at least one of the amino acids in a position corresponding to a position selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 in a human IgG1 heavy chain according to EU numbering has been substituted, and in said second heavy chain constant region (CH) at least one of the amino acids in a position corresponding to a position selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 in a human IgG1 heavy chain according to EU numbering has been substituted, and wherein said first and said second heavy chains are not substituted in the same positions.

34. The binding agent according to claim 33, wherein (i) the amino acid in the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering is L in said first heavy chain constant region (CH), and the amino acid in the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering is R in said second heavy chain constant region (CH), or (ii) the amino acid in the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering is R in said first heavy chain, and the amino acid in the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering is L in said second heavy chain.
35. The binding agent according to any of the preceding claims, wherein said antibody induces Fc-mediated effector function to a lesser extent compared to another antibody comprising the same first and second antigen binding regions and two heavy chain constant regions (CHs) comprising human IgG1 hinge, CH2 and CH3 regions.
36. The binding agent according to claim 35, wherein said first and second heavy chain constant regions (CHs) are modified so that the antibody induces Fc-mediated effector function to a lesser extent compared to an antibody which is identical except for comprising non-modified first and second heavy chain constant regions (CHs).
37. The binding agent according to any of claims 35 to 36, wherein said Fc-mediated effector function is measured by binding to Fcγ receptors, binding to C1q, or induction of Fc-mediated cross-linking of Fcγ receptors.
38. The binding agent according to claim 37, wherein said Fc-mediated effector function is measured by binding to C1q.
39. The binding agent according to claim 38, wherein said first and second heavy chain constant regions have been modified so that binding of C1q to said antibody is reduced compared to a wild-type antibody, preferably reduced by at least 70%, at least 80%, at least 90%, at least 95%, at least 97%, or 100%, wherein C1q binding is preferably determined by ELISA.
40. The binding agent according to any one of the preceding claims, wherein in at least one of said first and second heavy chain constant region (CH) one or more

amino acids in the positions corresponding to positions L234, L235, D265, N297, and P331 in a human IgG1 heavy chain according to EU numbering, are not L, L, D, N, and P, respectively.

41. The binding agent according to claim 40, wherein the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain according to EU numbering are F and E, respectively, in said first and second heavy chains.
42. The binding agent according to claim 40, wherein the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain according to EU numbering are F, E, and A, respectively, in said first and second heavy chain constant regions (HCs).
43. The binding agent according to claim 42, wherein the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain according to EU numbering of both the first and second heavy chain constant regions are F, E, and A, respectively, and wherein (i) the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering of the first heavy chain constant region is L, and the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering of the second heavy chain constant region is R, or (ii) the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering of the first heavy chain is R, and the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering of the second heavy chain is L.
44. The binding agent according to claim 41, wherein the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain according to EU numbering of both the first and second heavy chain constant regions are F and E, respectively, and wherein (i) the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering of the first heavy chain constant region is L, and the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering of the second heavy chain is R, or (ii) the position corresponding to K409 in a human IgG1 heavy chain according to EU numbering of the first heavy chain constant region is R, and the position corresponding to F405 in a human IgG1 heavy chain according to EU numbering of the second heavy chain is L.

45. The binding agent according to any one of the preceding claims, wherein the binding agent is a multispecific antibody such as a bispecific antibody.
46. A nucleic acid encoding a binding agent according to any one of claims 1 to 45 or a polypeptide chain thereof.
47. An expression vector comprising a nucleic acid according to claim 46.
48. A cell comprising a nucleic acid according to claim 46 or an expression vector according to claim 47.
49. The cell according to claim 48, wherein said cell is a mammalian cell, such as a Chinese hamster ovary cell.
50. A composition comprising a binding agent according to any one of claims 1 to 45, a nucleic acid according to claim 46, an expression vector according to claim 47, or a cell according to claim 48 or 49.
51. The composition according to claim 50, which is a pharmaceutical composition.
52. The composition according to claim 51, further comprising a pharmaceutically acceptable carrier and/or excipient.
53. A binding agent according to any one of claims 1 to 45, a nucleic acid according to claim 46, an expression vector according to claim 47, a cell according to claim 48 or 49, or a composition according to any one of claims 50 to 52 for use as a medicament.
54. The binding agent, nucleic acid, expression vector, cell, or composition according to claim 53 for use in the treatment of cancer.
55. The binding agent, nucleic acid, expression vector, cell, or composition for the use according to claim 54, wherein the cancer is characterized by the presence of solid tumors or is selected from the group consisting of: melanoma, ovarian cancer, lung cancer, colon cancer and head and neck cancer.

56. The binding agent, nucleic acid, expression vector, cell, or composition for the use according to claim 55, wherein the cancer is non-small cell lung cancer (NSCLC).
57. Use of a binding agent according to any one of claims 1 to 45, a nucleic acid according to claim 46, an expression vector according to claim 47, a cell according to claim 48 or 49, or a composition according to any one of claims 50 to 52 for the manufacture of a medicament, such as a medicament for the treatment of cancer, e.g. a cancer characterized by the presence of solid tumors or a cancer selected from the group consisting of: melanoma, ovarian cancer, lung cancer, colon cancer and head and neck cancer.
58. The use according to claim 57, wherein the lung cancer is non-small cell lung cancer (NSCLC).
59. The use according to claim 57, wherein the method or use comprises combination with one or more further therapeutic agents, such as a chemotherapeutic agent.
60. A method for producing a bispecific antibody according to claim 45, comprising the steps of:
- culturing a host cell producing a first antibody comprising an antigen-binding region binding to human CD137 as defined in any one of claims 1, 8 to 17 and 28-31, and optionally purifying said first antibody from the culture;
 - culturing a host cell producing a second antibody comprising an antigen-binding region binding to human PD-L1 as defined in any one of claims 1 to 7 and 28 to 51 and optionally purifying said second antibody from the culture;
 - incubating said first antibody together with said second antibody under reducing conditions sufficient to allow the cysteines in the hinge region to undergo disulfide-bond isomerization, and
 - obtaining said CD137xPD-L1 bispecific antibody.
61. An anti-idiotypic antibody which binds to the first and/or second antigen-binding region as defined in any one of claims 1 to 45.