

Inhibition of the PDL1/PD-1 interaction allows for potent anti-tumor activity. Various antibodies against PDL1 are already known (see, for example, WO 2013/079174 and WO 2017/118321), and a number of antibodies that disrupt the PD-1 signaling have entered clinical development. These antibodies belong to the following two main categories: those that target PD-1 (nivolumab, Bristol-Myers Squibb; pembrolizumab, Merck, Whitehouse Station, NJ; pidilizumab, CureTech, Yavne, Israel) and those that target PDL1 (MPDL3280A, Genentech, South San Francisco, CA; MEDI4736, MedImmune/AstraZeneca; BMS-936559, Bristol-Myers Squibb; MSB0010718C, EMD Serono, Rockland, MA) (for review see Postow MA et al., J Clin Oncol. Jun 10;33(17):1974-82 (2015)). Targeting PDL1 versus targeting PD-1 may result in different biologic effects. PD-1 antibodies prevent interaction of PD-1 with both its ligands, PDL1 and PDL2. PDL1 antibodies do not prevent PD-1 from interacting with PDL2, although the effect of this interaction remains unknown. PDL1 antibodies however prevent interaction of PDL1 with not only PD-1, but also B7-1 (Butte MJ, et al., Immunity 27:111-122, (2007)), which is believed to exert negative signals on T cells. Blocking PDL1 has demonstrated promising early data, and currently, four clinical anti-PDL1 mAbs are in the testing: atezolizumab and MEDI4736 (both are Fc null variants of human IgG1), MSB001078C (IgG1), and BMS-936559 (IgG4) (Chester C., et al., Cancer Immunol Immunother Oct;65(10):1243-8 (2016)).

To date, no satisfactory approach has been proven to induce potent immune responses in cancer patients. There is a need in the field to generate improved therapeutic modulators of the PDL1/PD-1 interaction and methods to overcome the immunosuppressive mechanisms observed in cancer patients.

SUMMARY

It is an object of the present invention to provide antibodies that specifically bind to human PDL1 protein, and which have beneficial properties for use in therapies, such as higher affinity, improved efficacy and improved biophysical properties, such as solubility, developability, and stability.

In one aspect, the present disclosure provides for an isolated antibody having a binding specificity for human PDL1 comprising an HCDR1, HCDR2, HCDR3 sequences of SEQ ID

NOs: 1, 2 and 3, respectively, and an LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 17, 18 and 19, respectively.

In another aspect, the present disclosure provides for a pharmaceutical composition comprising the isolated antibody disclosed herein, and a pharmaceutically acceptable carrier.

5 In yet another aspect, the present disclosure provides for the antibody disclosed herein, or the composition disclosed herein for use as a medicament.

In yet another aspect, the present disclosure provides for use of the antibody disclosed herein, or the pharmaceutical composition disclosed herein in the manufacture of a medicament for the treatment of a cancer.

10 In yet another aspect, the present disclosure provides for a nucleic acid encoding the antibody disclosed herein.

In yet another aspect, the present disclosure provides for a method of producing the antibody disclosed herein, the method comprising the step of culturing a host cell comprising the nucleic acid disclosed herein.

15 In one example, the present disclosure relates to a novel PDL1 antibody.

In one example, the present disclosure relates to a pharmaceutical composition comprising the antibody of the disclosure, and a pharmaceutically acceptable carrier.

In another example, the present disclosure relates to the antibody of the disclosure, or the composition of the disclosure for use as a medicament

5 In one example, the present disclosure relates to the antibody of the disclosure, or the composition of the disclosure for use in the treatment of a cancer in a subject in need thereof.

In one example, the present disclosure relates to use of the antibody of the disclosure, or the composition of the disclosure in the manufacture of a medicament for the treatment of a cancer in a subject in need thereof.

10 In another example, the present disclosure relates to a method of treating a cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the antibody of the disclosure, or the composition of the disclosure.

In yet another example, the present disclosure relates to a nucleic acid encoding the antibody of the disclosure. In a further example, the present disclosure relates to a vector
15 comprising said nucleic acid. In a further example, the present disclosure relates to a host cell comprising said nucleic acid or said vector.

In another example, the present disclosure relates to a method of producing the antibody of the disclosure, the method comprising the step of culturing a host cell comprising the nucleic acid or the vector of the disclosure.

20 The examples, advantageous features and preferred examples of the present disclosure, summarized in the following items, respectively alone or in combination, further contribute to solving the object of the disclosure:

1. An isolated antibody having a binding specificity for human PDL1, which comprises:
(a) a heavy chain variable region CDR1 comprising, preferably consisting of, an
25 amino acid sequence selected from any one of SEQ ID NOs: 1, 4, 5, 8, 11, 32, 35, 36, 39 and 42, preferably SEQ ID NO: 1 or 32, more preferably SEQ ID NO: 1; (b) a heavy chain variable region CDR2 comprising, preferably consisting of, an amino acid sequence selected from any of SEQ ID NOs: 2, 6, 9, 12, 33, 37, 40 and 43, preferably SEQ ID NO: 2 or 33, more preferably SEQ ID NO: 2; (c) a heavy chain variable
30 region CDR3 comprising, preferably consisting of, an amino acid sequence selected

(ii) optionally, has the ability to neutralize PDL1/PD-1 interaction with a potency relative to that of avelumab (relative potency), determined in NFAT reporter gene assay, greater than 1.5, e.g. greater than 2, greater than 2.5, preferably greater than 3, more preferably greater than 4, and wherein said relative potency is the ratio of the IC₅₀ value in ng/mL of avelumab as measured in the NFAT reporter gene assay to the IC₅₀ value in ng/mL of said antibody as measured in the NFAT reporter gene assay, in particular wherein said antibody is an scFv; and/or

(iii) has the ability to neutralize PDL1/B7-1 interaction with a potency relative to that of avelumab (relative potency), determined in ELISA assay, greater than 1.5, e.g. greater than 2, greater than 2.5, preferably greater than 3, more preferably greater than 4 and wherein said relative potency is the ratio of the IC₅₀ value in ng/mL of avelumab as measured in the ELISA assay to the IC₅₀ value in ng/mL of said antibody as measured in the ELISA assay, in particular wherein said antibody is an scFv.

15. The antibody of any of the preceding items, wherein said antibody:

(i) when in scFv format, has a melting temperature (T_m), determined by differential scanning fluorimetry, of at least 60°C, preferably at least 65°C, more preferably at least 70°C, in particular wherein said antibody is formulated in 50 mM phosphate-citrate buffer at pH 6.4, 150 mM NaCl;

(ii) when in scFv format, has a loss in monomer content, after five consecutive freeze-thaw cycles, of less than 5%, preferably less than 3%, more preferably less than 1%, when the antibody of the disclosure is at a starting concentration of 10 mg/ml, and in particular wherein the antibody is formulated 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4; and/or

(iii) when in scFv format, has a loss in monomer content, after storage for at least two weeks, particularly for at least four weeks, at 4°C, of less than 15%, e.g. less than 12%, less than 10%, less than 7%, less than 5%, less than 4%, less than 3%, less than 2%, preferably less than 1%, when the antibody of the disclosure is at a starting concentration of 10 mg/ml, and in particular wherein

the antibody of the disclosure is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4.

- 5 16. The antibody of any of the previous items, wherein the antibody is selected from the group consisting of: a monoclonal antibody, a chimeric antibody, a Fab, an Fv, an scFv, dsFv, a scAb, and binding domains based on alternative scaffolds including but limited to ankyrin-based domains, fynomers, avimers, anticalins, fibronectins, and binding sites being built into constant regions of antibodies (e.g. F-star's Modular Antibody TechnologyTM).
- 10 17. The antibody of any one of the preceding items, wherein said antibody is a single-chain variable fragment (scFv) or Fv.
18. The antibody of item 17, wherein said scFv has the amino acid sequence selected from the group consisting of SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 60, SEQ ID NO: 61 and SEQ ID NO: 62, preferably SEQ ID NO: 29 and SEQ ID NO: 31, more preferably SEQ ID NO: 31.
- 15 19. The antibody of item 16, wherein the antibody is an IgG selected from the group consisting of an IgG1, an IgG2, an IgG3 and an IgG4, preferably wherein the antibody is an IgG1.
- 20 20. The antibody of any of the previous items, wherein the antibody is chimeric or humanized.
21. An antibody binding to essentially the same epitope as the antibody of any one of items 1 to 20.
22. The antibody of any one of the preceding items which is a multispecific molecule, in particular a multispecific molecule having at least a second functional molecule.
- 25 23. The antibody of item 22, wherein said 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,
- 30 tetrabody, scFv-Fc-scFv fusion, scFv-HSA-scFv fusion, di-diabody, DVD-Ig, COVD,

CLAIMS

1. An isolated antibody having a binding specificity for human PDL1 comprising an HCDR1, HCDR2, HCDR3 sequences of SEQ ID NOs: 1, 2 and 3, respectively, and an LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 17, 18 and 19, respectively.
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2. The antibody of claim 1, wherein the antibody comprises a heavy chain variable region (VH), wherein said VH is VH3 or VH4, preferably VH3.
3. The antibody of claim 1 or claim 2, wherein the antibody comprises a light chain variable region (VL), wherein said VL comprises V κ frameworks FR1, FR2 and FR3, particularly V κ 1 or V κ 3 FR1 to FR3, preferably V κ 1 FR1 to FR3, and a framework FR4, which is selected from a V κ FR4, particularly V κ 1 FR4, V κ 3 FR4, and V λ FR4, particularly V λ FR4 comprising the amino acid sequence having at least 60, 70, 80, 90 percent identity to an amino acid sequence selected from any of SEQ ID NO: 64 to SEQ ID NO: 70, preferably V λ FR4 as set forth in any of SEQ ID NO: 64 to SEQ ID NO: 70, preferably V λ FR4 as set forth in SEQ ID NO: 64 or 65, more preferably V λ FR4 as set forth in SEQ ID NO: 64.
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4. The antibody of any one of the preceding claims, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence that is at least 90 percent identical to the amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 15, 16, preferably SEQ ID NO: 14 or 16, more preferably SEQ ID NO: 16; and a light chain variable region comprising an amino acid sequence that is at least 90 percent identical to the amino acid sequence selected from the group consisting of SEQ ID NOs: 26 and 27, preferably SEQ ID NO: 27.
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5. The antibody of any of the preceding claims, comprising: (a) a VH sequence of SEQ ID NO: 14 and a VL sequence of SEQ ID NO: 26; or (b) a VH sequence of SEQ ID NO: 15 and a VL sequence of SEQ ID NO: 26; or (c) a VH sequence of SEQ ID NO: 16 and a VL sequence of SEQ ID NO: 27.
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6. The antibody of any of the preceding claims, wherein said antibody:

(i) binds to human PDL1 with a dissociation constant (KD) of less than 5 nM, particularly less than 1 nM, particularly less than 500 pM, more particularly less than 100 pM, preferably less than 50 pM, more preferably less than 10 pM as measured by surface plasmon resonance (SPR);

(ii) binds to human PDL1 with a K_{off} rate of 10^{-3} s^{-1} or less, or 10^{-4} s^{-1} or less, or 10^{-5} s^{-1} or less as measured by SPR;

(iii) binds to human PDL1 with a K_{on} rate of at least $10^3 \text{ M}^{-1}\text{s}^{-1}$ or greater, at least $10^4 \text{ M}^{-1}\text{s}^{-1}$ or greater, at least $10^5 \text{ M}^{-1}\text{s}^{-1}$ or greater, at least $10^6 \text{ M}^{-1}\text{s}^{-1}$ or greater as measured by SPR;

(iv) is cross-reactive with *Macaca fascicularis* (Cynomolgus) PDL1, in particular binds to Cynomolgus PDL1 with a KD of less than 5 nM, particularly less than 1 nM, particularly less than 500 pM, more particularly less than 100 pM, preferably less than 50 pM, more preferably less than 10 pM as measured by surface plasmon resonance;

(v) is non-cross-reactive to *Mus musculus* PDL1, in particular as measured by SPR;

(vi) does not bind to human PDL2, in particular as measured by SPR;

(vii) has the ability to neutralize PDL1/PD-1 interaction with a potency relative to that of avelumab (relative potency), determined in ELISA assay, greater than 2, preferably greater than 3, more preferably greater than 4, and wherein said relative potency is the ratio of the IC_{50} value in ng/mL of avelumab as measured in the ELISA assay to the IC_{50} value in ng/mL of said antibody, in particular wherein said antibody is an scFv, as measured in the ELISA assay;

(viii) has the ability to neutralize PDL1/PD-1 interaction with a potency relative to that of avelumab (relative potency), determined in NFAT reporter gene assay, greater 2, preferably greater than 3, more preferably greater than 4, and wherein said relative potency is the ratio of the IC_{50} value in ng/mL of avelumab as measured in the NFAT reporter gene assay to the IC_{50} value in

ng/mL of said antibody, in particular wherein said antibody is an scFv, as measured in the NFAT reporter gene assay; and/or

(ix) has the ability to neutralize PDL1/B7-1 interaction with a potency relative to that of avelumab (relative potency), determined in ELISA assay, greater than 2, preferably greater than 3, more preferably greater than 4, and wherein said relative potency is the ratio of the IC₅₀ value in ng/mL of avelumab as measured in the ELISA assay to the IC₅₀ value in ng/mL of said antibody, in particular wherein said antibody is an scFv, as measured in the ELISA assay.

7. The antibody of any of the preceding items, wherein said antibody:
- (i) when in scFv format, has a melting temperature (T_m), determined by differential scanning fluorimetry, of at least 60°C, preferably at least 65°C, more preferably at least 70°C, in particular wherein said antibody is formulated in 50 mM phosphate-citrate buffer at pH 6.4, 150 mM NaCl;
 - (ii) when in scFv format, has a loss in monomer content, after five consecutive freeze-thaw cycles, of less than 5%, preferably less than 3%, more preferably less than 1%, when the antibody of the invention is at a starting concentration of 10 mg/ml, and in particular wherein the antibody of the invention is formulated 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4; and/or
 - (iii) when in scFv format, has a loss in monomer content, after storage for at least two weeks, particularly for at least four weeks, at 4°C, of less than 15%, e.g. less than 12%, less than 10%, less than 7%, less than 5%, less than 4%, less than 3%, less than 2%, preferably less than 1%, when the antibody of the invention is at a starting concentration of 10 mg/ml, and in particular wherein said antibody is formulated in 50 mM phosphate citrate buffer with 150 mM NaCl at pH 6.4.
8. The antibody of any one of the preceding claims, wherein the antibody is selected from the group consisting of: a monoclonal antibody, a chimeric antibody, a Fab, an

Fv, an scFv, dsFv, a scAb, STAB, and binding domains based on alternative scaffolds including but limited to ankyrin-based domains, fynomers, avimers, anticalins, fibronectins, and binding sites being built into constant regions of antibodies (e.g. F-star's Modular Antibody Technology), preferably scFv.

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9. The antibody of claim 8, which is an scFv, wherein said scFv has an amino acid sequence selected from the group consisting of SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, preferably SEQ ID NO: 31.

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10. The antibody of any one of claims 1 to 7, wherein said antibody is a multispecific molecule, in particular wherein said multispecific molecule comprises at least a second functional molecule.

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11. A pharmaceutical composition comprising the isolated antibody of any one of claims 1 to 10, and a pharmaceutically acceptable carrier.

12. The antibody of any one of claims 1 to 10, or the composition of claim 11 for use as a medicament.

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13. Use of the antibody of any one of claims 1 to 10, or the pharmaceutical composition of claim 11 in the manufacture of a medicament for the treatment of a cancer.

14. A nucleic acid encoding the antibody of any one of claims 1-10.

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15. A method of producing the antibody of any one of claims 1- 10, the method comprising the step of culturing a host cell comprising the nucleic acid of claim 14.