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5 SUMMARY

In one aspect, the present disclosure provides a PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence and a 4-1BBL amino acid sequence, wherein

(a) said PD1 amino acid sequence comprises a functional fragment of an extracellular domain of PD1 capable binding PDL1, wherein said functional fragment does not comprise amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2 and any amino acid residue thereof, and is 127 – 140 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89, 93 and 115 ; and

(b) wherein said 4-1BBL amino acid sequence comprises a functional fragment of an extracellular domain of 4-1BBL capable binding 4-1BB, wherein said functional fragment does not comprise an amino acid segment A1 – V6 corresponding to SEQ ID NO: 3 and any amino acid residue thereof, and is 185 - 199 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25, is 182 amino acids in length having at least 95 % identity to SEQ ID NO: 125 and does not comprise an amino acid segment A1-E23 corresponding to SEQ ID NO: 3 or is 184 amino acids in length having at least 95 % identity to SEQ ID NO: 123;

and wherein said fusion protein is capable of at least one of:

(i) binding PDL1 and 4-1BB;

(ii) activating said 4-1BB signaling pathway in a cell expressing said 4-1BB; and/or

(iii) co-stimulating immune cells expressing said 4-1BB.

According to an aspectexample, the present refers to a PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence and a 4-1BBL amino acid sequence, wherein the PD1 amino acid sequence:

(a) is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89, 93 and 115 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2, or is 138-145 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 117 and 119 and does not comprise an amino acid segment F146 – V150 corresponding to SEQ ID NO: 2; and/or

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(b) comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and/or

wherein the 4-1BBL amino acid sequence:

(aa) is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25, is 170 – 197 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 127 and 129 and does not comprise an amino acid segment G198-E205 corresponding to SEQ ID NO: 3, is 170 – 182 amino acids in length having at least 80 % identity to SEQ ID NO: 125 and does not comprise an amino acid segment A1-E23 corresponding to SEQ ID NO: 3 or is 184 amino acids in length having at least 80 % identity to SEQ ID NO: 123; and/or

(bb) comprises three repeats of a 4-1BBL amino acid sequence;

and wherein the fusion protein is capable of at least one of:

- (i) binding PDL1 and 4-1BB;
- (ii) activating the 4-1BB signaling pathway in a cell expressing the 4-1BB; and/or
- (iii) co-stimulating immune cells expressing the 4-1BB.

According to some examples of the present disclosure there is provided a PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence and a 4-1BBL amino acid sequence, wherein the PD1 amino acid sequence:

(a) is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89 and 93 and does not

According to an [aspectexample](#), the present disclosure refers to an isolated polypeptide comprising a PD1 amino acid sequence, wherein the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89, 93 and 115 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2, or is 138-145 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 117 and 119 and does not comprise an amino acid segment F146 – V150 corresponding to SEQ ID NO: 2; and optionally comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and wherein the polypeptide is capable of binding PDL1.

According to some examples of the present disclosure there is provided an isolated polypeptide comprising a PD1 amino acid sequence, wherein the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89 and 93 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and optionally comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and wherein the polypeptide is capable of binding PDL1.

According to some examples of the present disclosure there is provided an isolated polypeptide comprising a PD1 amino acid sequence, wherein the PD1 amino acid sequence is 123 – 148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and wherein the polypeptide is capable of binding PDL1.

According to an [aspectexample](#), the present disclosure refers to an isolated polypeptide comprising a 4-1BBL amino acid sequence, wherein the 4-1BBL amino acid sequence is 185 - 202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25, is 170 – 197 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 127 and 129 and does not comprise an amino acid segment G198-E205 corresponding to SEQ ID NO: 3, or is 170 – 182 amino acids in length having at least 80 % identity to SEQ ID NO: 125 and does not comprise an amino acid segment A1-E23 corresponding to SEQ ID NO: 3; and optionally comprises three repeats of the 4-1BBL amino acid sequence; and wherein the polypeptide is capable of at least one of:

- (i) binding 4-1BB,

construct or the host cell, for use in treating a disease that can benefit from activating immune cells. According to an aspect, the present disclosure refers to use of the PD1-4-1BBL fusion protein or the isolated polypeptide as disclosed herein, the polynucleotide as disclosed herein or the host cell as disclosed herein, in the manufacture of a medicament for treating ~~a disease that can benefit from activating immune cells~~ cancer.

According to some examples of the disclosure, the composition further comprising a therapeutic agent for treating the disease.

According to some examples of the present disclosure there is provided an article of manufacture comprising a packaging material packaging a therapeutic agent for treating a disease that can benefit from activating immune cells; and the PD1-4-1BBL fusion protein or the isolated polypeptide, the polynucleotide or the nucleic acid construct or the host cell.

According to some examples of the disclosure, cells of the disease express PDL1.

According to some examples of the disclosure, the disease comprises a hyper-proliferative disease.

According to some examples of the disclosure, the hyper-proliferative disease comprises sclerosis, fibrosis, Idiopathic pulmonary fibrosis, psoriasis, systemic sclerosis/scleroderma, primary biliary cholangitis, primary sclerosing cholangitis, liver fibrosis, prevention of radiation-induced pulmonary fibrosis, myelofibrosis or retroperitoneal fibrosis.

According to some examples of the disclosure, the hyper-proliferative disease comprises cancer.

According to some examples of the disclosure, the cancer is selected from the group consisting of lymphoma, leukemia and carcinoma.

According to some examples of the disclosure, the disease comprises a disease associated with immune suppression or medication induced immunosuppression.

According to some examples of the disclosure, the disease comprises HIV, Measles, influenza, LCCM, RSV, Human Rhinoviruses, EBV, CMV or Parvo viruses.

According to some examples of the disclosure, the disease comprises an infection.

According to some examples of the present disclosure there is provided a method of activating immune cells, the method comprising in-vitro activating immune cells in the presence of the PD1-4-1BBL fusion protein or the isolated polypeptide, the polynucleotide or the nucleic acid construct or the host cell. According to an aspect, the present disclosure refers to a method of activating immune cells, the method comprising in-vitro activating immune cells in the presence of the PD1-4-1BBL fusion protein or the isolated polypeptide as disclosed herein, the polynucleotide as disclosed herein or the host cell as disclosed herein.

According to some examples of the disclosure, the activating is in the presence of cells expressing PDL1 or exogenous PDL1.

According to some examples of the disclosure, the cells expressing the PDL1 comprise pathologic cells.

According to some examples of the disclosure, the pathologic cells comprise cancer cells.

WHAT IS CLAIMED IS:

1. A PD1-4-1BBL fusion protein comprising a PD1 amino acid sequence and a 4-1BBL amino acid sequence, wherein

(a) said PD1 amino acid sequence comprises a functional fragment of an extracellular domain of PD1 capable binding PDL1, wherein said functional fragment does not comprise amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2 and any amino acid residue thereof, and:

(a) ~~is 123-127 – 148-140 amino acids in length having at least 80-95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89, 93 and 115 and does not comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2, or is 138-145 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 117 and 119 and does not comprise an amino acid segment F146 – V150 corresponding to SEQ ID NO: 2; and/or~~

(b) ~~comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and/or~~

(b) wherein said 4-1BBL amino acid sequence comprises a functional fragment of an extracellular domain of 4-1BBL capable binding 4-1BB, wherein said functional fragment does not comprise an amino acid segment A1 – V6 corresponding to SEQ ID NO: 3 and any amino acid residue thereof, and:

(aa) ~~is 185 - 202-199 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25, is 170 – 197 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 127 and 129 and does not comprise an amino acid segment G198-E205 corresponding to SEQ ID NO: 3, is 170 – 182 amino acids in length having at least 80-95 % identity to SEQ ID NO: 125 and does not comprise an amino acid segment A1-E23 corresponding to SEQ ID NO: 3 or is 184 amino acids in length having at least 80-95 % identity to SEQ ID NO: 123; and/or~~

(bb) ~~comprises three repeats of a 4-1BBL amino acid sequence;~~

and wherein said fusion protein is capable of at least one of:

- (i) binding PDL1 and 4-1BB;
- (ii) activating said 4-1BB signaling pathway in a cell expressing said 4-1BB; and/or
- (iii) co-stimulating immune cells expressing said 4-1BB.

~~2. — An isolated polypeptide comprising a PD1 amino acid sequence, wherein said PD1 amino acid sequence is 123—148 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18-21, 85, 89, 93 and 115 and does not comprise any of amino acid segments P1—L5 and F146—V150 corresponding to SEQ ID NO: 2, or is 138-145 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 117 and 119 and does not comprise an amino acid segment F146—V150 corresponding to SEQ ID NO: 2; and optionally comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2; and wherein said polypeptide is capable of binding PDL1.~~

~~3. — An isolated polypeptide comprising a 4-1BBL amino acid sequence, wherein said 4-1BBL amino acid sequence is 185—202 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25, is 170—197 amino acids in length having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 127 and 129 and does not comprise an amino acid segment G198—E205 corresponding to SEQ ID NO: 3, or is 170—182 amino acids in length having at least 80 % identity to SEQ ID NO: 125 and does not comprise an amino acid segment A1—E23 corresponding to SEQ ID NO: 3; and optionally comprises three repeats of said 4-1BBL amino acid sequence; and wherein said polypeptide is capable of at least one of:~~

- ~~—— (i) — binding 4-1BB,~~
 - ~~—— (ii) — activating said 4-1BB signaling pathway in a cell expressing said 4-1BB;~~
- ~~and/or~~
- ~~—— (iii) — co-stimulating immune cells expressing said 4-1BB.~~

~~4. — The PD1-4-1BBL fusion protein of claim 1 or the isolated polypeptide of claim 2, wherein said PD1 amino acid sequence is no more than 140 amino acids long and/or wherein said PD1 amino acid sequence is at least 126 amino acids long.~~

52. The PD1-4-1BBL fusion protein ~~or the isolated polypeptide of any one of claims 1-2 and 4 of claim 1~~, wherein said PD1 amino acid sequence comprises or consists of said SEQ ID NO: 18, 19, 20, 21, 85, 89, 93 or 115. ~~or~~

3. The PD1-4-1BBL fusion of claim 1, wherein said PD1 amino acid sequence comprises a C to S amino acid modification in a position corresponding to amino acid 73 of SEQ ID NO: 2.

4. The PD1-4-1BBL fusion of any one of claims 1 and 3, wherein said PD1 amino acid sequence comprises or consists of SEQ ID NO: 75, 83, 87, 91 or 95.

~~6. The PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1 and 3-5, wherein said 4-1BBL amino acid sequence does not comprise any of amino acid residues A1-V6 or A1-G14 corresponding to SEQ ID NO: 3.~~

75. The PD1-4-1BBL fusion protein ~~or the isolated polypeptide~~ of any one of claims 1-4 and 3-6, wherein said 4-1BBL amino acid sequence comprises or consists of said SEQ ID NO: 22, 23, 24 or 25.

6. The PD1-4-1BBL fusion protein of any one of claims 1-4, wherein said 4-1BBL amino acid sequence comprises or consists of said SEQ ID NO: 123 or 125.

7. The PD1-4-1BBL fusion protein of claim 1, wherein:

(i) said PD1 amino acid sequence is as set forth in SEQ ID NO: 75, 83, 87, 91 or 93 and said 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22;

(ii) said PD1 amino acid sequence is as set forth in SEQ ID NO: 115 and said 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 123 or 125.

~~8. The PD1-4-1BBL fusion protein of any one of claims 1 and 3-7, wherein production yield of said fusion protein is at least 1.5 fold higher than the production yield of SEQ ID NO: 5 under the same production conditions, or wherein the amount of aggregates of said fusion protein is at least 20 % lower than the amount of aggregates of SEQ ID NO: 5 under the same production conditions, said aggregates are of at least 300 kDa in molecular weight and said production conditions comprise expression in a mammalian cell and culturing at 32-37 °C, 5-10 % CO₂ for 5-13 days.~~

98. The PD1-4-1BBL fusion protein of ~~any one of claims 1 and 8~~, wherein said PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least ~~80~~

95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: ~~12, 14, 16, 44-56,~~ 97, 101, 103, 105, ~~107, 111,~~ 113, 133 and, 135, ~~137, 139, 141, 143 and 147.~~

~~109.~~ The PD1-4-1BBL fusion protein of ~~any one of~~ claims 1 ~~and 8~~, wherein said PD1-4-1BBL fusion protein amino acid sequence consists of an amino acid sequence selected from the group consisting of SEQ ID NO: ~~12, 14, 16, 44-56,~~ 97, 101, 103, 105, ~~107, 111,~~ 113, 133 and, 135, ~~137, 139, 141, 143 and 147.~~

~~110.~~ A polynucleotide encoding the PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-~~109~~.

~~111.~~ A host cell comprising the PD1-4-1BBL fusion protein or the polypeptide of any one of claims 1-~~109~~ or the polynucleotide of claim ~~110~~.

~~112.~~ A method of producing a PD1-4-1BBL fusion protein or a polypeptide, the method comprising expressing in a host cell the polynucleotide of claim ~~110~~.

~~113.~~ Use of the PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-~~109~~, the polynucleotide of claim ~~110~~ or the host cell of claim ~~111~~, in the manufacture of a medicament for treating ~~a disease that can benefit from activating immune cells~~ cancer.

~~114.~~ A method of activating immune cells, the method comprising in-vitro activating immune cells in the presence of the PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-~~109~~, the polynucleotide of claim ~~110~~ or the host cell of claim ~~111~~.