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5 SUMMARY

According to an aspect, 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:

10 (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

15 (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

25 (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.

30 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

comprise any of amino acid segments P1 – L5 and 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

5 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; 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
- 10 (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 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
15 comprise any of amino acid segments P1 – L5 and F146 – V150 corresponding to SEQ ID NO: 2; and/or 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; and wherein the fusion protein is capable of at least one of:

- (i) binding PDL1 and 4-1BB;
- 20 (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 (GGGGS)_n(n=1-4) linker between the PD1 and the 4-1BBL, wherein the fusion protein is capable of at least one of:

- 25 (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 disclosure, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 % identity to SEQ ID NO: 145.

30 According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence comprises SEQ ID NO: 145.

According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence consists of SEQ ID NO: 145.

According to an aspect, 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 aspect, 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,

- (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 an isolated polypeptide comprising a 4-1BBL amino acid sequence, wherein the 4-1BBL amino acid sequence is 185 -
 5 202 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; and wherein the polypeptide is capable of at least one of:

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

According to some examples of the disclosure, the PD1 amino acid sequence is no more than 140 amino acids long.

According to some examples of the disclosure, the PD1 amino acid sequence is at least 126 amino acids long.

15 According to some examples of the disclosure, the PD1 amino acid sequence comprises the SEQ ID NO: 18, 19, 20 or 21.

According to some examples of the disclosure, the PD1 amino acid sequence comprises the SEQ ID NO: 18, 19, 20, 21, 85, 89 or 93.

20 According to some examples of the disclosure, the PD1 amino acid sequence comprises the SEQ ID NO: 18, 19, 20, 21, 85, 89, 93 or 115.

According to some examples of the disclosure, the PD1 amino acid sequence comprises the SEQ ID NO: 117 or 119.

According to some examples of the disclosure, the PD1 amino acid sequence comprises SEQ ID NO: 75, 79, 81, 83, 87, 91 or 95.

25 According to some examples of the disclosure, the PD1 amino acid sequence comprises SEQ ID NO: 75, 83, 87, 91 or 95.

According to some examples of the disclosure, the PD1 amino acid sequence comprises SEQ ID NO: 73.

30 According to some examples of the disclosure, the PD1 amino acid sequence comprises the SEQ ID NO: 18.

According to some examples of the disclosure, the PD1 amino acid sequence consists of the SEQ ID NO: 18, 19, 20 or 21.

According to some examples of the disclosure, the PD1 amino acid sequence consists of the SEQ ID NO: 18, 19, 20, 21, 85, 89 or 93.

According to some examples of the disclosure, the PD1 amino acid sequence consists of the SEQ ID NO: 18, 19, 20, 21, 85, 89, 93 or 115.

According to some examples of the disclosure, the PD1 amino acid sequence consists of the SEQ ID NO: 117 or 119.

5 According to some examples of the disclosure, the PD1 amino acid sequence consists of SEQ ID NO: 75, 79, 81, 83, 87, 91 or 95.

According to some examples of the disclosure, the PD1 amino acid sequence consists of SEQ ID NO: 75, 83, 87, 91 or 95.

10 According to some examples of the disclosure, the PD1 amino acid sequence consists of SEQ ID NO: 73.

According to some examples of the disclosure, the PD1 amino acid sequence consists of the SEQ ID NO: 18.

15 According to some examples of the disclosure, the 4-1BBL amino acid sequence has at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 22-24.

According to some examples of the disclosure, the 4-1BBL amino acid sequence does not comprise any of amino acid residues A1 – V6 or A1 – G14 corresponding to SEQ ID NO: 3.

According to some examples of the disclosure, the 4-1BBL amino acid sequence comprises the SEQ ID NO: 22, 23, 24 or 25.

20 According to some examples of the disclosure, the 4-1BBL amino acid sequence consists of the SEQ ID NO: 22, 23, 24 or 25.

According to some examples of the disclosure, the 4-1BBL amino acid sequence comprises the SEQ ID NO: 125, 127 or 129.

25 According to some examples of the disclosure, the 4-1BBL amino acid sequence consists of the SEQ ID NO: 125, 127 or 129.

According to some examples of the disclosure, the 4-1BBL amino acid sequence comprises the SEQ ID NO: 123.

According to some examples of the disclosure, the 4-1BBL amino acid sequence consists of the SEQ ID NO: 123.

30 According to some examples of the disclosure, the PD1-4-1BBL fusion protein comprises a linker between the PD1 and the 4-1BBL.

According to some examples of the disclosure, the PD1-4-1BBL fusion protein or the isolated polypeptide comprising a linker between each of the three repeats of the 4-1BBL amino acid sequence.

According to some examples of the disclosure, the linker has a length of one to six amino acids.

According to some examples of the disclosure, the linker is a single amino acid linker.

According to some examples of the disclosure, the linker is glycine.

According to some examples of the disclosure, the linker is not an Fc domain of an antibody
5 or a fragment thereof.

According to some examples of the disclosure, the linker is an Fc domain of an antibody
or a fragment thereof.

According to some examples of the disclosure, the linker is a (GGGGS)_n(n=1-4) (SEQ ID
NO: 149) linker.

10 According to some examples of the disclosure, the PD1-4-1BBL fusion protein being in a form
of at least a homo-trimer.

According to some examples of the disclosure, the at least homo-trimer is at least 100 kD in
molecular weight as determined by SEC-MALS.

According to some examples of the disclosure, the PD1-4-1BBL fusion protein or the isolated
15 polypeptide being soluble.

According to some examples of the disclosure, the production yield of the fusion protein is at
least 1.5 fold higher than the production yield of SEQ ID NO: 5 under the same production
conditions, the production conditions comprise expression in a mammalian cell and culturing at
32 – 37 °C, 5 – 10 % CO₂ for 5 - 13 days.

20 According to some examples of the disclosure, the amount of aggregates of the fusion protein
is at least 20 % lower than the amount of aggregates of SEQ ID NO: 5 under the same production
conditions, the aggregates are of at least 300 kDa in molecular weight and the production
conditions comprise expression in a mammalian cell and culturing at 32 – 37 °C, 5 – 10 % CO₂ for
5 - 13 days.

25 According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid
sequence comprises an amino acid sequence having at least 80 % 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, 135, 137, 139, 141, 143 and 147.

According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid
30 sequence comprises an amino acid sequence having at least 80 % identity to an amino acid
sequence selected from the group consisting of SEQ ID NO: 133, 135, 137, 139, 141, 143 and 147.

According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid
sequence comprises an amino acid sequence having at least 80 % 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 and 111.

According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105, 107 and 111.

According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105 and 107.

According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16 and 44-56.

According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 80 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14 and 16.

According to some examples of the disclosure, the at least 80 % identity comprises at least 90 % identity.

According to some examples of the disclosure, the at least 80 % identity comprises at least 95 % identity.

According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence comprises 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, 135, 137, 139, 141, 143 and 147.

According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 133, 135, 137, 139, 141, 143 and 147.

According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 44-56, 97, 101, 103, 105, 107 and 111.

According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105, 107 and 111.

According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105 and 107.

According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16 and 44-56.

5 According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14 and 16.

According to some examples of the disclosure, the 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, 135, 137, 139, 141, 143 and 147.

10 According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 133, 135, 137, 139, 141, 143 and 147.

According to some examples of the disclosure, the 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 and 111.

15 According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105, 107 and 111.

According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101, 103, 105 and 107.

20 According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence consist of an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14, 16 and 44-56.

25 According to some examples of the disclosure, the PD1-4-1BBL fusion protein amino acid sequence consist of an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 14 and 16.

According to an aspect, the present disclosure refers to a polynucleotide encoding the PD1-4-1BBL fusion protein or the isolated polypeptide as disclosed herein.

30 According to some examples of the present disclosure there is provided a nucleic acid construct comprising the polynucleotide, and a regulatory element for directing expression of the polynucleotide in a host cell.

According to some examples of the disclosure, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 57 – 72, 98, 102, 104, 106, 108, 112, 114, 134, 136, 138, 140, 142, 144 and 148.

According to some examples of the disclosure, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 57 – 72, 98, 102, 104, 106, 108 and 112.

According to some examples of the disclosure, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 98, 102, 104, 106, 108 and 112.

According to some examples of the disclosure, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 98, 102, 104, 106 and 108.

According to some examples of the disclosure, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 57 – 72.

According to some examples of the disclosure, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 70 – 72.

According to some examples of the disclosure, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 134, 136, 138, 140, 142, 144 and 148.

According to some examples of the disclosure, the polynucleotide comprises SEQ ID NO: 146.

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

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

According to some examples of the disclosure, the method comprising isolating the fusion protein or the polypeptide.

According to some examples of the present disclosure there is provided a method of treating a disease that can benefit from activating immune cells comprising administering to a subject in need thereof 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, the method further comprising administering to the subject a therapeutic agent for treating the disease.

According to some examples of the present disclosure there is provided the PD1-4-1BBL fusion protein or the isolated polypeptide, the polynucleotide or the nucleic acid

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.

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.

According to some examples of the disclosure, the activating is in the presence of an anti-cancer agent.

According to some examples of the disclosure, the therapeutic agent for treating the disease or the anti-cancer agent comprises an antibody.

5 According to some examples of the disclosure, the antibody is selected from the group consisting rituximab, cetuximab, trastuzumab, edrecolomab, almetuzumab, gemtuzumab, ibritumomab, panitumumab, Belimumab, Bevacizumab, Bivatuzumab mertansine, Blinatumomab, Blontuvetmab, Brentuximab vedotin, Catumaxomab, Cixutumumab, Daclizumab, Adalimumab, Bezlotoxumab, Certolizumab pegol, Citatuzumab bogatox, Daratumumab,
10 Dinutuximab, Elotuzumab, Ertumaxomab, Etaracizumab, Gemtuzumab ozogamicin, Girentuximab, Necitumumab, Obinutuzumab, Ofatumumab, Pertuzumab, Ramucirumab, Siltuximab, Tositumomab, Trastuzumab and ipilimumab.

According to some examples of the disclosure, the antibody is selected from the group consisting of rituximab and cetuximab.

15 According to some examples of the disclosure, the therapeutic agent for treating the disease or the anti-cancer agent comprises an IMiD (e.g. Thalidomide, Lenalidomide, Pomalidomide).

According to some examples of the disclosure, the method comprising adoptively transferring the immune cells following the activating to a subject in need thereof.

According to some examples of the disclosure, the immune cells comprise T cells.

20 Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of examples of the disclosure, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition,
25 the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with
30 reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

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 said 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

(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 said 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 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.

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

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

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.

9. 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 % 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, 135, 137, 139, 141, 143 and 147.

10. 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, 135, 137, 139, 141, 143 and 147.

11. A polynucleotide encoding the PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-10.

12. A host cell comprising the PD1-4-1BBL fusion protein or the polypeptide of any one of claims 1-10 or the polynucleotide of claim 11.

13. 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 11.

14. Use of the PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-10, the polynucleotide of claim 11 or the host cell of claim 12, in the manufacture of a medicament for treating a disease that can benefit from activating immune cells.

15. 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 1-10, the polynucleotide of claim 11 or the host cell of claim 12.