US Patent Application Publication No. US20070110746;

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US Patent No. US9562087.

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 the amino acid segments set forth in SEQ ID Nos: 8 and 9 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, 93 and 115 ; and

(b) wherein said 4-1BBL amino acid sequence comprises a functional fragment of an extracellular domain of 4-1BBL capable of binding 4-1BB, wherein said functional fragment does not comprise the amino acid segment set forth in SEQ ID NO: 11 and is 185 - 199 amino acids in length having at least 95 % identity to an amino acid sequence selected form 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 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 example, 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

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 a PD1-4-1BBL fusion protein as set forth in SEQ ID NO: 97 or 113 in the manufacture of a medicament for treating 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 a PD1-4-1BBL fusion protein as set forth in SEQ ID NO: 97 or 113.

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 the amino acid segments set forth in SEQ ID Nos: 8 and 9 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, 93 and 115 ; and

(b) wherein said 4-1BBL amino acid sequence comprises a functional fragment of an extracellular domain of 4-1BBL capable of binding 4-1BB, wherein said functional fragment does not comprise the amino acid segment set forth in SEQ ID NO: 11 and is 185 - 199 amino acids in length having at least 95 % identity to an amino acid sequence selected form 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 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.

2. The PD1-4-1BBL fusion protein of claim 1, wherein said PD1 amino acid sequence comprises or consists of said SEQ ID NO: 18, 19, 20, 21, 93 or 115.

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 claim 3, wherein said PD1 amino acid sequence comprises or consists of SEQ ID NO: 75, 83, 87, 91 or 95.

5. 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: 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: 93 and said 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22; or

(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 claim 3, wherein said PD1 amino acid sequence is as set forth in SEQ ID NO: 75, 83, 87 or 91 and said 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22.

9. The PD1-4-1BBL fusion protein of anyone of claims 1-8, being characterized by a single glycine linker between said PD1 and said 4-1BBL.

10. The PD1-4-1BBL fusion protein of claim 1, wherein said PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 105, 113, 133 and 135.

11. The PD1-4-1BBL fusion protein of claim 1, 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: 113, 133 and 135.

12. The PD1-4-1BBL fusion protein of claim 3, wherein said PD1-4-1BBL fusion protein amino acid sequence comprises an amino acid sequence having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 97, 101 and 103.

13. The PD1-4-1BBL fusion protein of claim 3, 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: 105, 113, 133 and 135.

14. A polynucleotide encoding the PD1-4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-13.

15. A host cell comprising the PD1-4-1BBL fusion protein or the polypeptide of any one of claims 1-13 or the polynucleotide of claim 14.

16. 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 14.

17. Use of a PD1-4-1BBL fusion protein as set forth in SEQ ID NO: 97 or 113 in the manufacture of a medicament for treating cancer.

18. A method of activating immune cells, the method comprising in-vitro activating immune cells in the presence of a PD1-4-1BBL fusion protein as set forth in SEQ ID NO: 97 or 113.