

4-1BBL is the activating ligand of the 4-1BB receptor (CD137), a member of the TNF receptor superfamily and a potent activation-induced T cell costimulatory molecule. 4-1BBL naturally forms a homo-trimer but signaling via 4-1BB requires significant oligomerization of 4-1BBL. 4-1BBL is present on a variety of antigen presenting cells (APCs), including dendritic cells (DCs), B cells, and macrophages. The 4-1BB receptor is not detected (<3%) on resting T cells or T cell lines, however, 4-1BB is stably upregulated when T cells are activated. 4-1BB activation upregulates survival genes, enhances cell division, induces cytokine production and prevents activation induced cell death in T-cells.

Additional background art includes:

Weiskopf K et al. Science. (2013); 341(6141):88-91;
 International Patent Application Publication No. WO 2017027422;
 International Patent Application Publication No. WO 2001086003;
 International Patent Application Publication No. WO 2001075067;
 International Patent Application Publication No. WO 2017194641;
 International Patent Application Publication No. WO 2014180288;
 International Patent Application Publication No. WO2017059168;
 International Patent Application Publication No. WO2001/049318;
 International Patent Application Publication No. WO2016/139668;
 International Patent Application Publication No. WO2014/106839;
 International Patent Application Publication No. WO2012/042480;
 US Patent Application Publication No. 20150183881;
 US Patent Application Publication No. US20070110746;
 US Patent Application Publication No. US20070036783; and
 US Patent No. US9562087.

SUMMARY

In one aspect, the present disclosure refers to a SIRP α -4-1BBL fusion protein comprising a SIRP α amino acid sequence and a 4-1BBL amino acid sequence, 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, 23, 27 and 28; and

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

- (i) binding CD47 and 4-1BB;
- (ii) activating said 4-1BB signaling pathway in a cell expressing said 4-1BB;
- (iii) co-stimulating immune cells expressing said 4-1BB; and/or
- (iv) enhancing phagocytosis of pathologic cells expressing said CD47 by phagocytes

compared to same in the absence of said SIRP α -4-1BBL fusion protein.

According to an example, the present disclosure refers to a SIRP α -4-1BBL fusion protein comprising a SIRP α amino acid sequence and a 4-1BBL amino acid sequence, wherein the SIRP α amino acid sequence is 100 - 119 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 24 and 26, and/or wherein the 4-1BBL amino acid sequence:

- (a) 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, 23, 27 and 28, 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: 74 and 76 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: 72 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: 70; and/or

(b) comprises three repeats of a 4-1BBL amino acid sequence; and wherein the fusion protein is capable of at least one of:

- (i) binding CD47 and 4-1BB;
- (ii) activating the 4-1BB signaling pathway in a cell expressing the 4-1BB;
- (iii) co-stimulating immune cells expressing the 4-1BB; and/or
- (iv) enhancing phagocytosis of pathologic cells expressing the CD47 by phagocytes compared to same in the absence of the SIRP α -4-1BBL fusion protein.

According to an example of the present disclosure there is provided a SIRP α -4-1BBL fusion protein comprising a SIRP α amino acid sequence and a 4-1BBL amino acid sequence, wherein the SIRP α amino acid sequence is 100 - 119 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 24 and 26, 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, 23, 27 and 28; and wherein the fusion protein is capable of at least one of:

- (i) binding CD47 and 4-1BB;
- (ii) activating the 4-1BB signaling pathway in a cell expressing the 4-1BB;
- (iii) co-stimulating immune cells expressing the 4-1BB; and/or
- (iv) enhancing phagocytosis of pathologic cells expressing the CD47 by phagocytes compared to same in the absence of the SIRP α -4-1BBL fusion protein.

According to an example, the present disclosure refers to an isolated polypeptide comprising a SIRP α amino acid sequence, wherein the SIRP α amino acid sequence is 100 - 119 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 24 and 26; and wherein the polypeptide is capable of binding CD47.

According to an example 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 - 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, 23, 27 and 28; and wherein the polypeptide is capable of at least one of:

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

According to an example 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 SIRP α -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 use of the SIRP α -4-1BBL fusion protein, the polynucleotide or the host cell as disclosed herein, in the manufacture of a medicament for treating cancer.

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

According to an example of the present disclosure there is provided the SIRP α -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 some examples of the disclosure, the composition further comprising a therapeutic agent for treating the disease.

According to an example 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 SIRP α -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 cells of the disease express CD47.

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 or 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 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.

WHAT IS CLAIMED IS:

1. A SIRP α -4-1BBL fusion protein comprising a SIRP α amino acid sequence and a 4-1BBL amino acid sequence, 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, 23, 27 and 28; and

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

- (i) binding CD47 and 4-1BB;
- (ii) activating said 4-1BB signaling pathway in a cell expressing said 4-1BB;
- (iii) co-stimulating immune cells expressing said 4-1BB; and/or
- (iv) enhancing phagocytosis of pathologic cells expressing said CD47 by phagocytes compared to same in the absence of said SIRP α -4-1BBL fusion protein.

2. The SIRP α -4-1BBL fusion protein of claim 1, wherein said SIRP α amino acid sequence comprises a functional fragment of an extracellular domain of SIRP α capable binding CD47, wherein said functional fragment does not comprise an amino acid segment K117 – Y343 corresponding to SEQ ID NO: 2 and any amino acid residue thereof.

3. The SIRP α -4-1BBL fusion protein of any one of claims 1 and 2, wherein said SIRP α amino acid sequence is 116 amino acids in length having at least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 24 and 26.

4. The SIRP α -4-1BBL fusion protein of any one of claims 1-3, wherein said SIRP α amino acid sequence comprises a mutation at an amino acid residue selected from the group consisting of L4, A27, E47 and V92 corresponding to SEQ ID NO: 2 and optionally wherein said mutation is selected from the group consisting of L4I, A27I, E47V and V92I corresponding to SEQ ID NO: 2.

5. The SIRP α -4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-3, wherein said SIRP α amino acid sequence consists of said SEQ ID NO: 24 or 26.

6. The SIRP α -4-1BBL fusion protein of claim 1, wherein said SIRP α amino acid sequence comprises SEQ ID NO: 2.

7. The SIRP α -4-1BBL fusion protein of any one of claims 1-6, wherein said 4-1BBL amino acid sequence comprises said SEQ ID NO: 22, 23, 27 or 28.

8. The SIRP α -4-1BBL fusion protein of any one of claims 1-6, wherein said 4-1BBL amino acid sequence consists of said SEQ ID NO: 22, 23, 27 or 28.

9. The SIRP α -4-1BBL fusion protein of claim 1, wherein:

(i) said SIRP α amino acid sequence is as set forth in SEQ ID NO: 2 or 25 and said 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 27 or 28; or

(ii) said SIRP α amino acid sequence is as set forth in SEQ ID NO: 24 or 26 and said 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 27 or 28.

10. The SIRP α -4-1BBL fusion protein of claim 1, wherein:

(i) said SIRP α amino acid sequence is as set forth in SEQ ID NO: 2 and said 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 23;

(ii) said SIRP α amino acid sequence is as set forth in SEQ ID NO: 24 and said 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22; or

(iii) said SIRP α amino acid sequence is as set forth in SEQ ID NO: 2 and said 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 22.

11. The SIRP α -4-1BBL fusion protein of claim 1, wherein said SIRP α -4-1BBL fusion protein amino acid sequence comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 11, 13, 16, 18-19, 21 and 45-49.

12. The SIRP α -4-1BBL fusion protein of claim 1, wherein said SIRP α -4-1BBL fusion protein amino acid sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11, 13 and 16.

13. The SIRP α -4-1BBL fusion protein of claim 1, wherein said SIRP α -4-1BBL fusion protein amino acid sequence consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 11, 13 and 16.

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

15. A host cell comprising the SIRP α -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 SIRP α -4-1BBL fusion protein or a polypeptide, the method comprising expressing in a host cell the polynucleotide of claim 14.

17. Use of the SIRP α -4-1BBL fusion protein of any one of claims 1-13, the polynucleotide of claim 14 or the host cell of claim 15, 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 the SIRP α -4-1BBL fusion protein or the isolated polypeptide of any one of claims 1-13, the polynucleotide of claim 14 or the host cell of claim 15.