

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:

10 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;
 15 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;
 20 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.

25 SUMMARY

According to an 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 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
 30 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 aspect, 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:

- (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 disclosure, the SIRP α amino acid sequence is at least
5 115 amino acids in length.

According to some examples of the disclosure, the SIRP α amino acid sequence is 116 amino acids in length.

According to some examples of the disclosure, the SIRP α amino acid sequence comprises a mutation at an amino acid residue selected from the group consisting of L4, A27, E47 and V92
10 corresponding to SEQ ID NO: 2.

According to some examples of the disclosure, the mutation is selected from the group consisting of L4I, A27I, E47V and V92I corresponding to SEQ ID NO: 2.

According to some examples of the disclosure, the SIRP α amino acid sequence does not comprise any of amino acid residues K117 – Y343 corresponding to SEQ ID NO: 2.

15 According to some examples of the disclosure, the SIRP α amino acid sequence comprises the SEQ ID NO: 24 or 26.

According to some examples of the disclosure, the SIRP α amino acid sequence consists of the SEQ ID NO: 24 or 26.

According to some examples of the disclosure, the 4-1BBL amino acid sequence has at
20 least 95 % identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 22-23.

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
25 the SEQ ID NO: 22, 23, 27 or 28.

According to some examples of the disclosure, the 4-1BBL amino acid sequence consists of the SEQ ID NO: 22, 23, 27 or 28.

According to some examples of the disclosure:

- (i) the SIRP α amino acid sequence is as set forth in SEQ ID NO: 2 or 25 and the 4-
30 1BBL amino acid sequence is as set forth in SEQ ID NO: 22, 23, 27 or 28; or
- (ii) the SIRP α amino acid sequence is as set forth in SEQ ID NO: 24 or 26 and the 4-1BBL amino acid sequence is as set forth in SEQ ID NO: 3, 22, 23, 27 or 28.

According to some examples of the disclosure:

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

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

5 According to some examples of the disclosure, the 4-1BBL amino acid sequence comprises the SEQ ID NO: 72, 74 or 76.

According to some examples of the disclosure, the 4-1BBL amino acid sequence consists of the SEQ ID NO: 72, 74 or 76.

10 According to some examples of the disclosure, the 4-1BBL amino acid sequence comprises the SEQ ID NO: 70.

According to some examples of the disclosure, the 4-1BBL amino acid sequence consists of the SEQ ID NO: 70.

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

15 According to some examples of the disclosure, the SIRP α -4-1BBL fusion protein 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.

20 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 or a fragment thereof.

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

25 According to some examples of the disclosure, the SIRP α -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.

30 According to some examples of the disclosure, the SIRP α -4-1BBL fusion protein or the isolated 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.

According to some examples of the disclosure, the SIRP α -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: 11, 13, 15, 16, 18-21 and 45-49.

5 According to some examples of the disclosure, the SIRP α -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: 11, 13 and 16.

According to some examples of the disclosure, the 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, 15, 16, 18-21 and 45-49.

10 According to some examples of the disclosure, the 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.

According to some examples of the disclosure, the 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, 15, 16, 18-21 and 45-49.

15 According to some examples of the disclosure, the 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.

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

According to an example 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.

25 According to some examples of the disclosure, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 55 – 66 and 68.

According to some examples of the disclosure, the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 55, 56 and 58.

30 According to an example of the present disclosure there is provided a host cell comprising the SIRP α -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 SIRP α -4-1BBL fusion protein or the polypeptide or the polynucleotide as disclosed herein.

35 According to an example of the present disclosure there is provided a method of producing a SIRP α -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 SIRP α -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 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 a disease that can benefit from activating immune cells.

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.

According to an example 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 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 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, the polynucleotide or the host cell as disclosed herein.

According to some examples of the disclosure, the activating is in the presence of cells expressing CD47 or exogenous CD47.

According to some examples of the disclosure, the cells expressing the CD47 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.

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, Dinutuximab, Elotuzumab, Ertumaxomab, Etaracizumab, Gemtuzumab ozogamicin, Girentuximab, Necitumumab, Obinutuzumab, Ofatumumab, Pertuzumab, Ramucirumab, Siltuximab, Tositumomab, Trastuzumab,

Nivolumab, Pembrolizumab, Durvalumab, Atezolizumab, Avelumab and ipilimumab.

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

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.

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

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,
 5 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

10 Some embodiments of the invention are herein described, by way of example only, with 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.

15 In the drawings:

FIG. 1 is a schematic representation of the SIRP α -4-1BBL fusion protein referred to herein as “DSP107” (SEQ ID NO: 5) comprising an N-terminal signal peptide and a C-terminal his-tag (SEQ ID NO 43). Shown the signal peptide (underlined, SEQ ID NO: 4), the SIRP α domain (red, SEQ ID NO: 2), the glycine linker (black), the 4-1BBL domain (blue, SEQ ID NO: 3) and the C-
 20 terminal His-tag (black bold).

FIGs. 2A-C demonstrate the predicted 3D structure of DSP107 (SEQ ID NO: 5). Figure 2A is a schematic 3D model. SIRP α is shown in grey ribbons, CD47 (SIRP α receptor) is shown in green ribbons, 4-1BBL is shown in blue ribbons and 2 additional copies of 4-1BBL (forming the trimer) are shown in light blue. Figure 2B is a schematic, full atomic 3D model. Figure 2C is
 25 a schematic 3D model. The X-ray resolved domains are represented by its surface and colored by a hydrophobicity scale - blue (most hydrophilic) to Brown (Hydrophobic). 4-1BBL shows a higher level of exposed hydrophobic areas.

FIG. 3 is a schematic representation of the domain and segments identified in DSP107 (SEQ ID NO: 5). An Ig-like V type domain is highlighted in light blue, an Ig-like C1-type1 domain is highlighted in yellow, an Ig-like C1-type2 domain is highlighted in green, an X-ray resolved part is highlighted in grey and flanking/unstructured regions are marked with red boxes.
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FIG. 4 demonstrates the predicted 3D structure of SIRP α -4-1BBL variant fusion protein referred to herein as” DSP107_var1” (SEQ ID NO: 11). The upper panel shows the 3D structure in surface representation and colored by hydrophobicity scale: hydrophilic in blue and

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 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 said 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 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. An isolated polypeptide comprising a SIRP α amino acid sequence, wherein said 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 said polypeptide is capable of binding CD47.

3. The SIRP α -4-1BBL fusion protein of claim 1 or the isolated polypeptide of claim 2, wherein said SIRP α amino acid sequence is at least 115 amino acids in length and/or wherein said SIRP α amino acid sequence is 116 amino acids in length.

4. The SIRP α -4-1BBL fusion protein or the isolated polypeptide of any one of claims 2-3, 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 1-4, wherein said SIRP α amino acid sequence does not comprise any of amino acid residues K117 – Y343 corresponding to SEQ ID NO: 2.

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

7. The SIRP α -4-1BBL fusion protein of any one of claims 1 and 3-6, 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.

8. The SIRP α -4-1BBL fusion protein of any one of claims 1 and 3-7, wherein said 4-1BBL amino acid sequence comprises or 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: 3, 22, 23, 27 or 28.

10. 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, 15, 16, 18-21 and 45-49.

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

12. A host cell comprising the SIRP α -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 SIRP α -4-1BBL fusion protein or a polypeptide, the method comprising expressing in a host cell the polynucleotide of claim 11.

14. Use of the SIRP α -4-1BBL fusion protein 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 SIRP α -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.