SUMMARY

[0006] The present disclosure provides proteins, such as antibodies, that include an antigen binding portion that specifically binds to Programmed Death 1 Ligand 1 (PD-L1). Also provided are nucleic acids encoding the proteins, and cells (*e.g.*, genetically modified cytotoxic lymphocytes) that include such nucleic acids. In some embodiments, a subject method includes reducing the interaction between PD-L1 on a first-cell and PD-1 on a second cell. For example, the methods and compositions provided can be used in the treatment of viral infection and cancer, such as the treatment of solid tumors via ACT or via administration of a subject protein that specifically binds to PD-L1.

[0007] The compositions and methods of the present disclosure can allow genetically modified cytotoxic lymphocytes, *e.g.*, activated T-cells, TILs, and NK cells, to be propagated and infused as a cell therapy which allows for the secretion of a PD-L1 binding protein (*e.g.*, an scFV, a maxibody) in the genetically modified cytotoxic lymphocytes to be administered to the subject. In this way, the cytotoxic lymphocytes of the present disclosure are able to "relieve" themselves of the inhibitory effect of the PD-1 checkpoint, providing for an improved anti-cancer effect in the subject to which the genetically modified cytotoxic lymphocytes are administered. Specific binding proteins of the disclosure (*e.g.*, anti-PD-L1 antibodies) can also be administered to an individual directly, *e.g.*, intravenous (i.v.) or intratumoral injection.

[0008] In one aspect, there is provided a protein that specifically binds to PD-L1 and comprises an antigen binding portion that comprises: (a) a first polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs:2-4, and a second polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs:6-8; or (b) a first polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs: 10-12, and a second polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs:14-16, with the exception that each of the three CDR amino acid sequences of the first and/or second polypeptide comprises two or less conservative amino acid substitutions relative to the specified SEQ ID number.

[0009] In some embodiments, the antigen binding portion comprises a first polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs:2-4, and a second polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs:6-8.

[0010] In some embodiments, the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO:1, and the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO:5.

[0011] In some embodiments, the antigen binding portion comprises a first polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs:10-12, and a second polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs:14-16.

[0012] In some embodiments, the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO:9, and the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO:13.

[0013] In some embodiments, the first polypeptide is a light chain, and the second polypeptide is a heavy chain.

[0014] In some embodiments, the protein that specifically binds to PD-L1 is a single-chain antibody (scFv) and the first and second polypeptides are fused directly or via a linker to one another. In some embodiments, the scFv comprises the amino acid sequence set forth in SEQ ID NO:17 or SEQ ID NO:19.

[0015] In some embodiments, the protein that specifically binds to PD-L1 is a maxibody comprising an immunoglobulin Fc domain fused directly or via a linker to the antigen binding portion. In some embodiments, the immunoglobulin Fc domain is an IgG1 Fc domain. In some embodiments, the protein comprises the amino acid sequence set forth in SEQ ID NO:18 or SEQ ID NO:20. In some embodiments, the immunoglobulin Fc domain is an IgG4 Fc domain.

[0016] In some embodiments, the protein that specifically binds to PD-L1 is a humanized antibody.

[0017] In another aspect, there is also provided a nucleic acid comprising a nucleotide sequence encoding the protein that specifically binds to PD-L1, as discussed herein. In some embodiments, the nucleic acid comprises a promoter that is operably linked to the nucleotide sequence encoding the protein. In some embodiments, the promoter is a constitutive promoter. In some embodiments, the promoter is an inducible promoter.

[0018] In another aspect, there is also provided a cell comprising the nucleic acid that encodes the protein that specifically binds to PD-L1. In some embodiments, the nucleic acid is integrated into the cell's genome. In some embodiments, the cell is a cytotoxic lymphocyte genetically modified to express and secrete the protein. In some embodiments, the cytotoxic lymphocyte is a T-cell. In some embodiments, the T-cell is a CD8+ T-cell. In some embodiments, the T-cell is a CD4+ T-helper cell. In some embodiments, the T-cell is derived from peripheral blood. In some embodiments, the cytotoxic lymphocyte is a natural killer (NK) cell. In some embodiments, the NK is derived from peripheral blood.

[0019] In some embodiments, the cytotoxic lymphocyte is a tumor infiltrating lymphocyte (TIL) derived from a tumor from a subject.

[0020] In some embodiments, the TIL comprises a receptor specific for an antigen from the tumor.

[0021] In some embodiments, the cytotoxic lymphocyte exhibits an increased level of expression of one or more activation antigens relative to a naïve T-cell. In some embodiments, the one or more activation antigens are selected from CD25, CD26, CD27, CD28, CD38, CD40L, CD69, CD134, CD137, BTLA, PD-1, HVEM, LIGHT, and HLA-DR.

[0022] In some embodiments, the cytotoxic lymphocyte comprises a T-cell receptor specific for a tumor associated antigen.

[0023] The present disclosure also provides a method comprising: genetically modifying a cytotoxic lymphocyte isolated from a tumor of a subject by introducing into the cytotoxic lymphocyte the nucleic acid encoding a protein that specifically binds to PD-L1, wherein the genetically modified cytotoxic lymphocyte expresses and secretes the protein that specifically binds to PD-L1; expanding the genetically modified cytotoxic lymphocyte to generate a population of genetically modified cytotoxic lymphocytes; and administering the population of genetically modified cytotoxic lymphocytes to the subject to treat the tumor.

[0023a] In another aspect, there is provided use of a population of genetically modified cytotoxic lymphocytes in the manufacture of a medicament for treating a tumor in a subject, wherein the population of genetically modified cytotoxic lymphocytes is generated by genetically modifying a cytotoxic lymphocyte that has been isolated from the tumor of the subject by introducing into the cytotoxic lymphocyte the nucleic acid described herein, wherein the genetically modified cytotoxic lymphocyte expresses and secretes the protein that specifically binds to PD-L1; and expanding the genetically modified cytotoxic lymphocyte to generate the population of genetically modified cytotoxic lymphocytes.

[0024] In some embodiments, the genetically modified cytotoxic lymphocyte constitutively expresses the protein that specifically binds to PD-L1. In some embodiments, the genetically modified cytotoxic lymphocyte inducibly expresses the protein that specifically binds to PD-L1. In some embodiments, the nucleic acid integrates into the cytotoxic lymphocyte's genome.

[0025] In some embodiments, the cytotoxic lymphocyte is a T-cell. In some embodiments, the T-cell is a CD8+ T-cell. In some embodiments, the T-cell is a CD4+ T-helper cell. In some embodiments, the cytotoxic lymphocyte is a natural killer (NK) cell.

[0026] In some embodiments, the genetically modified cytotoxic lymphocyte comprises a receptor specific for an antigen from the tumor.

[0027] In some embodiments, the method comprises isolating the cytotoxic lymphocyte from the subject prior to genetically modifying.

[0028] In some embodiments of the method, the protein that specifically binds to PD-L1 and comprises an antigen binding portion that comprises: (a) a first polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs:2-4, and a second polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs:6-8; or (b) a first polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs: 10-12, and a second polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs:14-16, with the exception that each of the three CDR amino acid sequences of the first and/or second

embodiments, the genetically modified cytotoxic lymphocyte inducibly expresses the protein that specifically binds to PD-L1.

[0039a] In another aspect, there is provided a method of making a genetically modified cytotoxic lymphocyte, the method comprising: genetically modifying a cytotoxic lymphocyte that has been isolated from a subject having or suspected of having cancer by introducing into the cytotoxic lymphocyte the nucleic acid described herein, wherein the genetically modified cytotoxic lymphocyte expresses and secretes the protein that specifically binds to PD-L1.

[0040] In some embodiments, the method comprises expanding the cytotoxic lymphocyte in vitro to provide an expanded population of genetically modified cytotoxic lymphocytes.

[0041] In some embodiments, the method comprises isolating the cytotoxic lymphocyte from the subject prior to the genetically modifying.

[0042] In some embodiments, the isolating comprises isolating the cytotoxic lymphocyte from a tumor of the subject. In some embodiments, the isolating comprises isolating the cytotoxic lymphocyte from peripheral blood of the subject.

[0043] In some embodiments of the method, the cytotoxic lymphocyte is a T-cell. In some embodiments of the method, the T-cell is a CD8+ T-cell. In some embodiments of the method, the T-cell is a CD4+ T-helper cell. In some embodiments of the method, the cytotoxic lymphocyte is a natural killer (NK) cell.

[0044] In some embodiments of the method, the nucleic acid integrates into the cytotoxic lymphocyte's genome.

[0045] In some embodiments of the method, the cytotoxic lymphocyte exhibits an increased level of expression of one or more activation antigens relative to a naïve T-cell. In some embodiments of the method, the one or more activation antigens are selected from CD25, CD26, CD27, CD28, CD38, CD40L, CD69, CD134, CD137, BTLA, PD-1, HVEM, LIGHT, and HLA-DR.

[0046] In some embodiments of the method, the genetically modified cytotoxic lymphocyte comprises a T-cell receptor specific for an antigen from a tumor of the subject.

[0047] The present disclosure also provides a method of treating an individual who has or is suspected of having cancer, the method comprising: administering the protein that specifically binds to PD-L1 to the individual (*e.g.*, subject or patient, including for example a mammal such as a human).

[0047a] In another aspect, there is provided use of the protein that specifically binds to PD-L1 described herein in the manufacture of a medicament for treating cancer in an individual who has or is suspected of having cancer.

[0048] In some embodiments of the method, the administering comprises introducing into the subject a nucleic acid encoding the protein that specifically binds to PD-L1.

[0049] In some embodiments of the method, administering comprises introducing into the subject a genetically modified cytotoxic lymphocyte that expresses and secretes the protein that specifically binds to PD-L1.

[0050] In some embodiments of the method, the genetically modified cytotoxic lymphocyte constitutively expresses the protein that specifically binds to PD-L1. In some

embodiments of the method, the genetically modified cytotoxic lymphocyte inducibly expresses the protein that specifically binds to PD-L1. In some embodiments, the method, comprises inducing expression of the protein that specifically binds to PD-L1.

[0051] In some embodiments of the method, the cytotoxic lymphocyte is a T-cell. In some embodiments of the method, the T-cell is a CD8+ T-cell. In some embodiments of the method, the T-cell is a CD4+ T-helper cell. In some embodiments of the method, the cytotoxic lymphocyte is a natural killer (NK) cell.

[0052] In some embodiments of the method, the cytotoxic lymphocyte exhibits an increased level of expression of one or more activation antigens relative to a naïve T-cell. In some embodiments of the method, the one or more activation antigens are selected from CD25, CD26, CD27, CD28, CD38, CD40L, CD69, CD134, CD137, BTLA, PD-1, HVEM, LIGHT, and HLA-DR.

[0053] In some embodiments of the method, the genetically modified cytotoxic lymphocyte comprises a T-cell receptor specific for an antigen from a tumor of the subject.

[0054] The present disclosure also provides a method of reducing the interaction between PD-L1 on a first-cell and PD-1 on a second cell, the method comprising: contacting PD-L1 on the first-cell with the protein that specifically binds to PD-L1.

[0054a] In another aspect, there is provided a method of reducing the interaction between PD-L1 on a first-cell and PD-1 on a second cell, the method comprising: contacting PD-L1 on the first-cell with the protein described herein.

[0055] In some embodiments of the method, the first and second cells are introduced to an individual, and the contacting comprises administering the protein that specifically binds to PD-L1. In some embodiments of the method, introducing comprises systemic administration. In some embodiments of the method, the introducing comprises local administration. In some embodiments of the method, the local administration comprises intratumoral administration. In some embodiments of the method, the individual has cancer. In some embodiments of the method, the individual has a solid tumor.

BRIEF DESCRIPTION OF THE DRAWINGS

[0056] The invention may be best understood from the following detailed description when read in conjunction with the accompanying drawings. Included in the drawings are the following figures:

[0057] **Figure 1** provides a schematic representation of the lentiviral plasmid used to produce 38A1-Fc, 19H9-Fc and FMC63-Fc. Anti PD-L1 maxibodies are encoded downstream of the U3 promoter from MSCV promoter and upstream of an IRES-eGFP cassette.

19. The cell of claim 18, wherein the nucleic acid is integrated into the cell's genome.

20. The cell of claim 18 or claim 19, wherein the cell is a cytotoxic lymphocyte genetically modified to express and secrete the protein.

21. The cell of claim 20, wherein the cytotoxic lymphocyte is a T-cell.

22. The cell of claim 21, wherein the T-cell is a CD8+ T-cell.

23. The cell of claim 21, wherein the T-cell is a CD4+ T-helper cell.

24. The cell of claim 21, wherein the T-cell is derived from peripheral blood

25. The cell of claim 20, wherein the cytotoxic lymphocyte is a natural killer (NK) cell.

26. The cell of claim 25, wherein the NK is derived from peripheral blood.

27. The cell of claim 20, wherein the cytotoxic lymphocyte is a tumor infiltrating lymphocyte (TIL) derived from a tumor that has been obtained from a subject.

28. The cell of claim 27, wherein the TIL comprises a receptor specific for an antigen from the tumor.

29. The cell of claim of any of claims 20-28, wherein the cytotoxic lymphocyte exhibits an increased level of expression of one or more activation antigens relative to a naïve T-cell.

30. The cell of claim 29, wherein the one or more activation antigens are selected from CD25, CD26, CD27, CD28, CD38, CD40L, CD69, CD134, CD137, BTLA, PD-1, HVEM, LIGHT, and HLA-DR.

31. The cell of claim 29 or claim 30, wherein the cytotoxic lymphocyte comprises a T-cell receptor specific for a tumor associated antigen.

32. Use of a population of genetically modified cytotoxic lymphocytes in the manufacture of a medicament for treating a tumor in a subject, wherein the population of genetically modified cytotoxic lymphocytes is generated by genetically modifying a cytotoxic lymphocyte that has been isolated from the tumor of the subject by introducing into the cytotoxic lymphocyte the nucleic acid of any of claims 14-17, wherein the genetically modified cytotoxic lymphocyte expresses and secretes the protein that specifically binds to PD-L1; and

expanding the genetically modified cytotoxic lymphocyte to generate the population of genetically modified cytotoxic lymphocytes.

33. The use of claim 32, wherein the genetically modified cytotoxic lymphocyte constitutively expresses the protein that specifically binds to PD-L1.

34. The use of claim 32, wherein the genetically modified cytotoxic lymphocyte inducibly expresses the protein that specifically binds to PD-L1.

35. The use of any one of claims 32-34, wherein the nucleic acid integrates into the cytotoxic lymphocyte's genome.

36. The use of any one of claims 32-35, wherein the cytotoxic lymphocyte is a T-cell.

37. The use of claim 36, wherein the T-cell is a CD8+ T-cell.

38. The use of claim 36, wherein the T-cell is a CD4+ T-helper cell.

39. The use of any one of claims 32-35, wherein the cytotoxic lymphocyte is a natural killer (NK) cell.

40. The use of any one of claims 32-39, wherein the genetically modified cytotoxic lymphocyte comprises a receptor specific for an antigen from the tumor.

41. The use of any one of claims 32-40, wherein the cytotoxic lymphocyte is to be isolated from the subject prior to the genetically modifying.

42. The use of any one of claims 32-41, wherein said protein that specifically binds to PD-L1 and comprises an antigen binding portion that comprises:

(a) a first polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs:2-4, and a second polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs:6-8; or

(b) a first polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs: 10-12, and a second polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs:14-16,

with the exception that each of the three CDR amino acid sequences of the first and/or second polypeptide comprises two or less conservative amino acid substitutions relative to the specified SEQ ID number.

43. The use of claim 42, wherein said wherein the antigen binding portion comprises a first polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs:2-4, and a second polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs:6-8.

44. The use of claim 43, wherein the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO:1, and the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO:5.

45. The use of claim 42, wherein the antigen binding portion comprises a first polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs:10-12, and a second polypeptide comprising the 3 CDR amino acid sequences set forth in SEQ ID NOs:14-16.

46. The use of claim 45, wherein the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO:9, and the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO:13.

47. The use of any of claims 42-46, wherein the first polypeptide is a light chain, and the second polypeptide is a heavy chain.

48. The use of any of claims 42-47, wherein the protein is a single-chain antibody (scFv) and the first and second polypeptides are fused directly or via a linker to one another.

49. The use of claim 48, wherein the scFv comprises the amino acid sequence set forth in SEQ ID NO:17 or SEQ ID NO:19.

50. The use of any of claims 42-49, wherein the protein is a maxibody comprising an immunoglobulin Fc domain fused directly or via a linker to the antigen binding portion.

51. The use of claim 50, wherein the immunoglobulin Fc domain is an IgG1 Fc domain.

52. The use of claim 51, wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:18 or SEQ ID NO:20.

53. A method of making a genetically modified cytotoxic lymphocyte, the method comprising:

genetically modifying a cytotoxic lymphocyte that has been isolated from a subject having or suspected of having cancer by introducing into the cytotoxic lymphocyte the nucleic acid of any of claims 14-17, wherein the genetically modified cytotoxic lymphocyte expresses and secretes the protein that specifically binds to PD-L1.

54. The method of claim 53, wherein the genetically modified cytotoxic lymphocyte constitutively expresses the protein that specifically binds to PD-L1.

55. The method of claim 53, wherein the genetically modified cytotoxic lymphocyte inducibly expresses the protein that specifically binds to PD-L1.

56. The method of any one of claims 53-55, comprising expanding the cytotoxic lymphocyte *in vitro* to provide an expanded population of genetically modified cytotoxic lymphocytes.

57. The method of any one of claims 53-56, wherein the cytotoxic lymphocyte is to be isolated from the subject prior to the genetically modifying.

58. The method of claim 57, wherein the cytotoxic lymphocyte is to be isolated from a tumor of the subject.

59. The method of any one of claims 57, wherein the cytotoxic lymphocyte is to be isolated from peripheral blood of the subject.

60. The method of any one of claims 53-59, wherein the cytotoxic lymphocyte is a T-cell.

61. The method of claim 60, wherein the T-cell is a CD8+ T-cell.

62. The method of claim 60, wherein the T-cell is a CD4+ T-helper cell.

63. The method of any one of claims 53-59, wherein the cytotoxic lymphocyte is a natural killer (NK) cell.

64. The method of any one of claims 53-63, wherein the nucleic acid integrates into the cytotoxic lymphocyte's genome.

65. The method of any one of claims 53-64, wherein the cytotoxic lymphocyte exhibits an increased level of expression of one or more activation antigens relative to a naïve T-cell.

66. The method of claim 65, wherein the one or more activation antigens are selected from CD25, CD26, CD27, CD28, CD38, CD40L, CD69, CD134, CD137, BTLA, PD-1, HVEM, LIGHT, and HLA-DR.

67. The method of claim 65 or claim 66, wherein the genetically modified cytotoxic lymphocyte comprises a T-cell receptor specific for an antigen from a tumor of the subject.

68. Use of the protein that specifically binds to PD-L1 according to any of claims 1-13 in the manufacture of a medicament for treating cancer in an individual who has or is suspected of having cancer.

69. The use of claim 68, wherein a nucleic acid encoding the protein is to be introduced into the individual.

70. The use of claim 68, wherein a genetically modified cytotoxic lymphocyte that expresses and secretes the protein is to be introduced into the individual.

71. The use of claim 70, wherein the genetically modified cytotoxic lymphocyte constitutively expresses the protein.

72. The use of claim 71, wherein the genetically modified cytotoxic lymphocyte inducibly expresses the protein.

73. The use of claim 72, comprising inducing expression of the protein.

74. The use of any one of claims 70-73, wherein the cytotoxic lymphocyte is a T-cell.

75. The use of claim 74, wherein the T-cell is a CD8+ T-cell.

76. The use of claim 74, wherein the T-cell is a CD4+ T-helper cell.

77. The use of any one of claims 70-73, wherein the cytotoxic lymphocyte is a natural killer (NK) cell.

78. The use of any one of claims 70-77, wherein the cytotoxic lymphocyte exhibits an increased level of expression of one or more activation antigens relative to a naïve T-cell.

79. The use of claim 78, wherein the one or more activation antigens are selected from CD25, CD26, CD27, CD28, CD38, CD40L, CD69, CD134, CD137, BTLA, PD-1, HVEM, LIGHT, and HLA-DR.

80. The use of claim 78 or claim 79, wherein the genetically modified cytotoxic lymphocyte comprises a T-cell receptor specific for an antigen from a tumor of the subject.

81. A method of reducing the interaction between PD-L1 on a first-cell and PD-1 on a second cell, the method comprising:

contacting PD-L1 on the first-cell with the protein of any one of claims 1-13.

82. The method of claim 81, wherein the protein of any of claims 1-13 is to be administered to an individual having the first and second cells.

83. The method of claim 82, wherein the administration comprises systemic administration.

84. The method of claim 82, wherein the administration comprises local administration.

85. The method of claim 84, wherein the local administration comprises intratumoral administration.

86. The method of any of claims 81-85, wherein the individual has cancer.

87. The method of claim 87, wherein the individual has a solid tumor.