PROGRAMMED DEATH 1 LIGAND 1 (PD-L1) BINDING PROTEINS AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/359,612, filed on July 7, 2016, all of which is expressly incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 7, 2017, is named 116983-5024_ST25.txt and is 101,943 bytes in size.

INTRODUCTION

[0003] Adoptive Cell Transfer (ACT) is a treatment approach in which a patient's autologous T-cells are expanded, manipulated *ex vivo*, and then re-introduced into the patient to exert a response, *e.g.*, an anti-tumor response. Tumor Infiltrating Lymphocytes (TILs) generally refers to a heterogeneous population of lymphocytes which can be found in the tumor microenvironment. The general rationale of ACT therapy using TILs is that the anti-tumor immune response can be enhanced by removing cells with anti-tumor potential from the immunosuppressive tumor microenvironment, expanding the cells *in vitro*, and then returning the expanded population of cells to tumor sites to kill tumor cells and possibly other cell targets that sustain the tumor, such as vascular endothelial cells. Lee and Margolin, *Curr. Oncol. Rep.* 2012; 14(5) 468-474.

[0004] Programmed Death 1 (PD-1) is a well described inhibitory receptor expressed on activated human T-cells that, in cooperation with its ligands, Programmed Death 1 Ligand 1 (PD-L1) and Programmed Death 1 Ligand 2 (PD-L2), acts as checkpoint factor limiting T-cell mediated anti-tumor activity in a variety human cancers including melanoma. PD-1 is expressed by TILs and as such, expression of PD-L1 in the tumor microenvironment is inhibitory to cancer disease progression, including, for example, tumor growth.

[0005] Accordingly, there is a need in the art for new compositions and methods which interfere with the inhibitory effect of the PD-1–PD-L1 interaction, for example, in the context of ACT therapy using TILs.

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] The disclosure provides 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] The present disclosure also provides 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] The present disclosure also provides 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.

[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

polypeptide comprises two or less conservative amino acid substitutions relative to the specified SEQ ID number.

[0029] In some embodiments of the method, 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.

[0030] In some embodiments of the method, 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.

[0031] In some embodiments of the method, 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.

[0032] In some embodiments of the method, 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.

[0033] In some embodiments of the method, the first polypeptide is a light chain, and the second polypeptide is a heavy chain.

[0034] In some embodiments of the method, the protein is a single-chain antibody (scFv) and the first and second polypeptides are fused directly or via a linker to one another.

[0035] In some embodiments of the method, the scFv comprises the amino acid sequence set forth in SEQ ID NO:17 or SEQ ID NO:19.

[0036] In some embodiments of the method, the protein is a maxibody comprising an immunoglobulin Fc domain fused directly or via a linker to the antigen binding portion.

[0037] In some embodiments of the method, the immunoglobulin Fc domain is an IgG1 Fc domain.

[0038] In some embodiments of the method, the protein comprises the amino acid sequence set forth in SEQ ID NO:18 or SEQ ID NO:20.

[0039] The present disclosure also provides a method of making a genetically modified cytotoxic lymphocyte, the method comprising: genetically modifying a cytotoxic lymphocyte isolated from a subject having or suspected of having cancer by introducing into the cytotoxic lymphocyte the nucleic acid encoding for the protein that specifically binds to PD-L1, wherein the genetically modified cytotoxic lymphocyte expresses and secretes the protein that specifically binds to PD-L1. 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.

[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).

[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.

[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.

[0058] **Figure 2** provides a schematic representation of the retroviral 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.

[0059] **Figure 3A-3B** provides data from cell based ELISA to determine affinity of 38A1-scFV-Fc and 19H9-scFV-Fc for PD-L1. 293-PD-L1 were stained with 19H9-scFV-Fc (panel **A**) or 38A1-scFV-Fc (panel **B**). Maxibodies were titrated from 100 nM to 0.01 nM on 293-PD-L1, washed and stained with HRP labelled anti-human Fcγ specific antibody. Binding was determined by optical density at 450 nm following addition of TMB (3,3',5,5'-tetramethylbenzidine). 12D10-scFV-Fc was used as the negative control.

[0060] **Figure 4A-4C** provides data showing that 293-PD-L1 cells bind anti-PD-L1 maxibodies secreted by Jurkat T-cells. Jurkat T-cells stably expressing FMC63-scFV-Fc (panel **A**), 38A1-scFV-Fc (panel **B**) or 19H9-scFV-Fc (panel **C**) were co-cultured at a ratio of 1:1 for 16 hour. The cells were harvested and stained with Alexa 647 labelled anti-human Fcγ specific antibody. Compared to the negative control maxibody (FMC63-scFV-Fc), between a 100 fold and 300 fold increase mean fluorescent intensity (binding of maxibody) was observed for 19H9-scFV-Fc and 38A1-scFV-Fc, respectively.

[0061] **Figure 5A-5D** provides data showing that 293-PD-L1 cells bind anti-PD-L1 maxibodies secreted by tumor infiltrating lymphocytes (TIL). Supernatant from TIL lines M1034 (panel **A**, panel **B**) and M1015 (panel **C**, panel **D**) stably expressing FMC63-scFV-Fc (panel A, panel **C**) or 38A1-scFV-Fc (panel **B**, panel **D**) were concentrated 10 fold and used to stain 293-PD-L1. The maxibodies were detected using Alexa 647 labelled anti-human Fcγ specific antibody. Compared to the negative control maxibody (FMC63-scFV-Fc), we observed greater than a 50 fold in increase in mean fluorescent intensity (binding of maxibody) for 38A1-scFV-Fc.

[0062] **Figure 6** provides data showing the capacity of anti-PD-L1 maxibodies to limit PD-L1 mediated inhibition of NFAT activity. Jurkat T-cells expressing PD1 and firefly luciferase downstream of the NFAT promoter were co-cultured at a 2:1 ratio with CHO cells expressing PD-L1 and a proprietary T-cell agonistic protein (PD1/PD-L1 Blockade Bioassay Kit, Promega catalog number CS187111) and one of 12D10-scFV-Fc (negative control; grey), 19H9-scFV-Fc (red), 38A1-scFV-Fc (blue) or the positive control PD1 specific antibody EH12.2H7 (Biolegend; green). The maxibodies were titrated from 25 µg/ml to 0.016 µg/ml. Jukat T-cell activation (de-inhibition of PD-L1) was measured as bioluminescent activity (Relative Luminescent Units; RLU) following addition of 5'-Fluoroluciferin.

[0063] **Figure 7** provides sequences of the antigen binding regions of the newly generated 38A1 and 19H9 antibodies, as well as examples of scFV proteins (*e.g.*, scFV and scFV-FC fusions) that include antigen binding regions 38A1 or 19H9.

[0064] **Figure 8** depicts a schematic of an example of an expression vector (*e.g.*, lentivector) encoding an inducible promoter

[0065] **Figure 9** depicts a schematic of an example of an expression vector (*e.g.*, lentivector) encoding an inducible promoter.

[0066] **Figure 10** provides the empty vector sequence for the pLEV vector used as an exemplary vector in the present methods. pLV430G (Lentiviral Vector) (SEQ ID NO:35) (**A**) and pCIGO-VSV.G (VSVG) (SEQ ID NO:36) (**B**).

[0067] **Figure 11A-11B** provides the complete vector nucleotide sequences for the pLV4301G PDLV scFV 38A1 (SEQ NO: 37) (**A**) and pLV4301G PDLV scFV 19H9 (SEQ ID NO:38) (**B**).

[0068] **Figure 12** provides data showing that 19H9 exhibits greater secretion capacity. A comparison of the secretion capacity of Jurkat cells overexpressing anti-PD-L1 ScFV clone 19H9 versus 38A1 is provided. Supernatant from Jurkat cells overexpressing anti-PD-L1 ScFV clone 19H9 and 38A1 were harvested and concentrated for IgG ELISA assay to determine the anti-PD-L1 ScFV concentration. It was found that Jurkat cells clone 19H9 had greater secretion capacity as compared with 38A1 (9.82 versus 6.75 μg/cells).

[0069] **Figure 13A-13B** provides data showing that 38A1 exhibits greater binding capacity in Jurkat cells overexpressing PD-L1. The binding affinity of anti-PD-L1 ScFV clone 19H9 and 38A1 using EL4 PD-L1 cells was examined. EL4 PD-L1 were incubated with either anti-PD-L1 ScFV clone 19H9 or 38A1 at concentration of 1, 3, 10, 30, 100, and 300 ng/ml, and stained with Amcyan and goat anti-human FITC. The cells were washed with FACs buffer and analyzed by flow cytometry. Clone 38A1 exhibited greater binding capacity in Jurkat cells overexpressing PD-L1 in both the percentage of PD-L1 positive cells (**A**) and Mean of Fluorescence Intensity (MFI) (**B**).

[0070] **Figure 14A-14B** provides data showing that 38A1 exhibits greater binding capacity in melanoma tumor cells. A comparison of the binding affinity of anti-PD-L1 ScFV clone 19H9 and 38A1 using EL4 PD-L1 cells is provided. EL4 PD-L1 were incubated with either anti-PD-L1 ScFV clone 19H9 or 38A1 at concentration of 1, 3, 10, 30, 100, and 300 ng/ml, and stained with Amcyan and goat anti-human FITC. The cells were washed with FACs buffer and analyzed by flow cytometry. Clone 38A1 exhibited greater binding capacity in Jurkat cells overexpressing PD-L1 in both the percentage of PD-L1 positive cells (**A**) and Mean of

Fluorescence Intensity (MFI) (**B**). To validate the binding of anti-PD-L1 ScFV in tumor cells, three melanoma tumor cells were treated with IFN-gamma (100 ng/ml) to enhance PD-L1 expression. After three days, the cells were harvested with cell dissociation buffer, incubated with either anti-PD-L1 ScFV clone 19H9 or 38A1 at concentration of 100 ng/ml, and stained with goat anti-human FITC. The cells were washed with FACs buffer and analyzed by flow cytometry. We found that clone 38A1 exhibited greater binding affinity in both the percentage of PD-L1 positive cells and MFI. Overall, clone 38A1 has greater binding affinity, but slightly less secretion capacity when compared to Clone 19H9.

[0071] **Figure 15A-15B** provides data showing that 38A1 exhibits greater biological function than 19H9. To further determine the biological function of two clones of anti-PD-L1 ScFV (19H9 and 38A1), a PD-L1 blockade assay was conducted, which can be used to determine the potency of either anti PD-1 or PD-L1 antibody that block the engagement of PD-1 and PD-L1 interaction. The assay consists of two genetically engineered cell lines: PD-1 effector cell (Jurkat T-cells stably expressing human PD-1 and NFAT-induced luciferase) and PD-L1 aAPC/CHO-K1 Cells stably expressing human PD-L1 with a cell surface protein activating cognate TCRs. When two cell types were co-cultured, the engagement of PD-1 and PD-L1 inhibited TCR signaling and decreased luciferase activity. Addition of either anti-PD-1 or PD-L1 blockade antibody helped release the inhibitory signal, resulting in enhanced TCR signaling and NFAT-mediated luciferase activity. Luciferase signal (RLU) was found to be greater when blocked with anti-PD-L1 as compared to anti-PD1, suggesting that blocking PD-L1 seemed to be more effective than PD-1 in this context (**A**). Expectedly, anti-PD-L1 ScFV clone 38A1, which previously shown to have greater binding affinity, provided greater biological function due to highly increased in luciferase signal in both purified ScFV (P) and non-purified (NP) settings (**B**).

[0072] **Figure 16** provides the vector maps for pLV4301G PDLV scFV 38A1 (**A**) and pLV4301G PDLV scFV 19H9 (**B**).

[0073] **Figure 17** provides examples of IgG1 (SEQ ID NO:39), IgG2 (SEQ ID NO:40), IgG3 (SEQ ID NO:41), and IgG4 (SEQ ID NO:42) sequences.

DETAILED DESCRIPTION

[0074] 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 infections and cancer, such as the treatment of solid tumors via ACT or via administration of a subject protein that specifically binds to PD-L1.

[0075] The compositions and methods of the present disclosure provide genetically modified cytotoxic lymphocytes, *e.g.*, activated T-cells, TILs, and NK cells, that can be propagated and infused as a cell therapy, resulting in the secretion of a PD-L1 binding protein (*e.g.* an scFV, a maxibody, and the like) in the genetically modified cytotoxic lymphocytes infused into 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. 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.

[0076] Before the present invention is described in greater detail, it is to be understood that this invention is not limited to particular embodiments described, and as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0077] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0078] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Such terms are found defined and used in context in various standard references illustratively including J. Sambrook and D. W. Russell, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press; 4th Ed., 2012; F. M. Ausubel, Ed., Short Protocols in Molecular Biology, Current Protocols; 5th Ed., 2002; B. Alberts et al.,

Molecular Biology of the Cell, 4th Ed., Garland, 2002; D. L. Nelson and M. M. Cox, Lehninger Principles of Biochemistry, 4th Ed., W.H. Freeman & Company, 2004; and Herdewijn, P. (Ed.), Oligonucleotide Synthesis: Methods and Applications, Methods in Molecular Biology, Humana Press, 2004. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

[0079] As used herein, each of the following terms has the meaning associated with it in this section.

[0080] The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0081] "About" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or ±10%, more preferably ±5%, even more preferably ±1%, and still more preferably ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

[0082] The term "anti-tumor effect" as used herein, refers to a biological effect which can be manifested by a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in the number of metastases, an increase in life expectancy, or amelioration of various physiological symptoms associated with the cancerous condition. An "anti-tumor effect" can also be manifested by the ability of the disclosed compositions and methods to prevent the occurrence of tumor in the first place.

[0083] The term "antibody" is used in the broadest sense and includes, for example, an intact immunoglobulin or an antigen binding portion thereof that competes with the intact antibody for specific binding, unless otherwise specified. Antigen binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen binding portions include Fab, Fab', F(ab')2, Fd, Fv, and domain antibodies (dAbs), and complementarity determining region (CDR) fragments, single-chain antibodies (single-chain variable domain fragment; scFv), diabodies, triabodies, tetrabodies, and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide. Antibody includes a human antibody, a humanized antibody, chimeric antibody, a monoclonal antibody, a polyclonal antibody, a recombinant antibody, an antigen-binding antibody fragment, a single chain antibody, a maxibody (scFv fused by a linker or direct attachment to an Fc or an Fc fragment), a diabody, a triabody, a tetrabody, a Fab fragment, an F(fa')x fragment, a domain antibody, an IgD antibody, an IgE

antibody, and IgM antibody, and IgG1 antibody, and IgG2 antibody, and IgG3 antibody, and IgG4 antibody, and IgG4 antibody having at least one mutation in the hinge region that alleviates a tendency to for intra H-chain disulfide bonds.

[0084] A "Fab fragment" is a monovalent fragment having the VL, VH, CL and CH1 domains; a F(ab')2 fragment is a bivalent fragment having two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment has the VH and CH1 domains; an Fv fragment has the VL and VH domains of a single arm of an antibody; and a dAb fragment has a VH domain, a VL domain, or an antigen-binding fragment of a VH or VL domain.

[0085] A "single-chain antibody" (scFv) is an antibody in which a VL and a VH region are fused directly or joined via a linker (*e.g.*, a synthetic sequence of amino acid residues) to form a continuous protein chain wherein the linker is long enough to allow the protein chain to fold back on itself and form a monovalent antigen binding site (see, *e.g.*, Bird et al., 1988, Science 242:423-26 and Huston et al, 1988, Proc. Natl. Acad. Sci. USA 85:5879-83). Diabodies are bivalent antibodies comprising two polypeptide chains, wherein each polypeptide chain comprises VH and VL domains joined by a linker that is too short to allow for pairing between two domains on the same chain, thus allowing each domain to pair with a complementary domain on another polypeptide chain (see, *e.g.*, Holliger et al., 1993, Proc. Natl. Acad. Sci. USA 90:6444-48, and Poljak et al., 1994, Structure 2:1121-23). If the two polypeptide chains of a diabody are identical, then a diabody resulting from their pairing will have two identical antigen binding sites. Polypeptide chains having different sequences can be used to make a diabody with two different antigen binding sites. Similarly, tribodies and tetrabodies are antibodies comprising three and four polypeptide chains, respectively, and forming three and four antigen binding sites, respectively, which can be the same or different.

[0086] Complementarity determining regions (CDRs) and framework regions (FR) of a given antibody may be identified using the system described by Kabat et al. in Sequences of Proteins of Immunological Interest, 5th Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication no. 91-3242, 1991. One or more CDRs may be incorporated into a molecule either covalently or noncovalently to make it an antigen binding protein. An antigen binding protein may incorporate the CDR(s) as part of a larger polypeptide chain, may covalently link the CDR(s) to another polypeptide chain, or may incorporate the CDR(s) noncovalently. The CDRs permit the antigen binding protein to specifically bind to a particular antigen of interest.

[0087] The term "human antibody" includes all antibodies that have one or more variable and constant regions derived from human immunoglobulin sequences. In one

embodiment, all of the variable and constant domains are derived from human immunoglobulin sequences (a fully human antibody). Human antibodies may be prepared in a variety of ways, including immunization of a mouse that is genetically modified to express human antibodies. One can engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci in anticipation that such mice would produce human antibodies in the absence of mouse antibodies. Large human Ig fragments may preserve the large variable gene diversity as well as the proper regulation of antibody production and expression. By exploiting the mouse machinery for antibody diversification and selection and the lack of immunological tolerance to human proteins, the reproduced human antibody repertoire in these mouse strains may yield high affinity fully human antibodies against any antigen of interest, including human antigens. Using the hybridoma technology, antigen-specific human MAbs with the desired specificity may be produced and selected. Human antibodies can also be prepared by panning human antibody libraries expressed on phage, phagemids, ribosomes, or other particles.

[0088] A "humanized antibody" has a sequence that differs from the sequence of an antibody derived from a non-human species by one or more amino acid substitutions, deletions, and/or additions, such that the humanized antibody is less likely to induce an immune response, and/or induces a less severe immune response, as compared to the non-human species antibody, when it is administered to a human subject. In one embodiment, certain amino acids in the framework and constant domains of the heavy and/or light chains of the non-human species antibody are mutated to produce the humanized antibody. In another embodiment, the constant domain(s) from a human antibody are fused to the variable domain(s) of a non-human species. In another embodiment, one or more amino acid residues in one or more CDR sequences of a non-human antibody are changed to reduce the likely immunogenicity of the non-human antibody when it is administered to a human subject, wherein the changed amino acid residues either are not critical for immunospecific binding of the antibody to its antigen, or the changes to the amino acid sequence that are made are conservative changes, such that the binding of the humanized antibody to the antigen is not significantly worse than the binding of the non-human antibody to the antigen. Examples of methods for making humanized antibodies may be found in U.S. Pat. Nos. 6,054,297, 5,886,152 and 5,877,293, the disclosures of each of which are incorporated by reference herein.

[0089] The term "chimeric antibody" refers to an antibody that contains one or more regions from one antibody and one or more regions from one or more other antibodies. A "CDR grafted antibody" is an antibody comprising one or more CDRs derived from an antibody of a

particular species or isotype and the framework of another antibody of the same or different species or isotype.

[0090] The term "K_{assoc}" or "K_a", as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term "K_{dis}" or "K_d," as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term "K_D", as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of K_d to K_a (*i.e.*, K_d/K_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. In some embodiments, the method for determining the K_D of an antibody is by using surface plasmon resonance, for example, by using a biosensor system such as a Biacore® system.

[0091] As used herein, the term "high affinity" for an IgG antibody refers to an antibody having a K_D of 10^{-8} M or less, in some embodiments, 10^{-9} M or less, and in some embodiments, 10^{-10} M or less for a target antigen. However, "high affinity" binding can vary for other antibody isotypes. For example, "high affinity" binding for an IgM isotype refers to an antibody having a K_D of 10^{-7} M or less, in some embodiments, 10^{-8} M or less, and in some embodiments, 10^{-9} M or less.

[0092] "Constitutive" expression includes a state in which a gene product is produced in a living cell under most or all physiological conditions of the cell.

[0093] A "coding region" of a gene includes the nucleotide residues of the coding strand of the gene and the nucleotides of the non-coding strand of the gene which are homologous with or complementary to, respectively, the coding region of an mRNA molecule which is produced by transcription of the gene. A "coding region" of a mRNA molecule also includes the nucleotide residues of the mRNA molecule which are matched with an anti-codon region of a transfer RNA molecule during translation of the mRNA molecule or which encode a stop codon. The coding region may thus include nucleotide residues comprising codons for amino acid residues which are not present in the mature protein encoded by the mRNA molecule (*e.g.*, amino acid residues in a protein export signal sequence).

[0094] By "tumor infiltrating lymphocytes" or "TILs" herein is meant a population of cells originally obtained as white blood cells that have left the bloodstream of a subject and migrated into a tumor. TILs include, but are not limited to, CD8+ cytotoxic T-cells (lymphocytes), Th1 and Th17 CD4+ T-cells, natural killer cells, dendritic cells, and M1 macrophages. TILs include both primary and secondary TILs. "Primary TILs" are those that are obtained from patient tissue samples as outlined herein (sometimes referred to as "freshly harvested"), and "secondary TILs" are any TIL cell populations that have been expanded or

proliferated as discussed herein, including, but not limited to bulk TILs and expanded TILs ("REP TILs") as discussed herein.

[0095] TILs can generally be defined either biochemically, using cell surface markers, or functionally, by their ability to infiltrate tumors and effect treatment. TILs can be generally categorized by expressing one or more of the following biomarkers: CD4, CD8, TCR αβ, CD27, CD28, CD56, CCR7, CD45Ra, CD95, PD-1, and CD25. Additionally and alternatively, TILs can be functionally defined by their ability to infiltrate solid tumors upon reintroduction into a patient.

[0096] The term "cytotoxic lymphocyte" includes cytotoxic T (CTL) cells (including $CD8^+$ cytotoxic T lymphocytes and $CD4^+$ T-helper lymphocytes), natural killer T (NKT) cells and natural killer (NK) cells. Cytotoxic lymphocytes can include, for example, peripheral blood-derived αβ TCR-positive or γδ TCR-positive T-cells activated by tumor associated antigens and/or transduced with tumor specific chimeric antigen receptors or T-cell receptors, and tumor-infiltrating lymphocytes (TILs). Cytotoxic lymphocyte generally kill cancer cells, cells that are infected (particularly with viruses), or cells that are otherwise damaged or defective. A cytotoxic lymphocyte can also be referred to as a cytotoxic T-cell, TC, cytotoxic T lymphocyte, CTL, T-killer cell, cytolytic T-cell, CD8+ T-cell or killer T-cell is a T lymphocyte (a type of white blood cell).

[0097] The terms "fragmenting," "fragment," and "fragmented," as used herein to describe processes for disrupting a tumor, includes mechanical fragmentation methods such as crushing, slicing, dividing, and morcellating tumor tissue as well as any other method for disrupting the physical structure of tumor tissue.

[0098] The term "*in vivo*" refers to an event that takes place in a subject's body.

[0099] The term "*in vitro*" refers to an event that takes places outside of a subject's body. In vitro assays encompass cell-based assays in which cells alive or dead are employed and may also encompass a cell-free assay in which no intact-cells are employed.

[00100] The term "anti-CD3 antibody" refers to an antibody or variant thereof, e.g., a monoclonal antibody and including human, humanized, chimeric or murine antibodies which are directed against the CD3 receptor in the T-cell antigen receptor of mature T-cells. Anti-CD3 antibodies include OKT-3, also known as muromonab. Other anti-CD3 antibodies include, for example, otelixizumab, teplizumab, and visilizumab.

[00101] The term "OKT-3" (also referred to herein as "OKT3") refers to a monoclonal antibody or biosimilar or variant thereof, including human, humanized, chimeric, or murine antibodies, directed against the CD3 receptor in the T-cell antigen receptor of mature T-cells,

and includes commercially-available forms such as OKT-3 (30 ng/mL, MACS GMP CD3 pure, Miltenyi Biotech, Inc., San Diego, CA, USA) and muromonab or variants, conservative amino acid substitutions, glycoforms, or biosimilars thereof. The amino acid sequences of the heavy and light chains of muromonab are given in Table 1 (SEQ ID NO:27 and SEQ ID NO:28). A hybridoma capable of producing OKT-3 is deposited with the American Type Culture Collection and assigned the ATCC accession number CRL 8001. A hybridoma capable of producing OKT-3 is also deposited with European Collection of Authenticated Cell Cultures (ECACC) and assigned Catalogue No. 86022706.

TABLE 1. Amino acid sequences of muromonab.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:27	QVQLQQSGAE LARPGASVKM SCKASGYTFT RYTMHWVKQR PGQGLEWIGY INPSRGYTNY	60
Muromonab	NQKFKDKATL TTDKSSSTAY MQLSSLTSED SAVYYCARYY DDHYCLDYWG QGTTLTVSSA	120
heavy chain	KTTAPSVYPL APVCGGTTGS SVTLGCLVKG YFPEPVTLTW NSGSLSSGVH TFPAVLQSDL	180
	YTLSSSVTVT SSTWPSQSIT CNVAHPASST KVDKKIEPRP KSCDKTHTCP PCPAPELLGG	240
	PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN	300
	STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE	360
	LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW	420
	QQGNVFSCSV MHEALHNHYT QKSLSLSPGK	450
SEQ ID NO:28	QIVLTQSPAI MSASPGEKVT MTCSASSSVS YMNWYQQKSG TSPKRWIYDT SKLASGVPAH	60
Muromonab	FRGSGSGTSY SLTISGMEAE DAATYYCQQW SSNPFTFGSG TKLEINRADT APTVSIFPPS	120
light chain	SEQLTSGGAS VVCFLNNFYP KDINVKWKID GSERQNGVLN SWTDQDSKDS TYSMSSTLTL	180
	TKDEYERHNS YTCEATHKTS TSPIVKSFNR NEC	213

[00102] The term “IL-2” (also referred to herein as “IL2”) refers to the T-cell growth factor known as interleukin-2, and includes all forms of IL-2 including human and mammalian forms, conservative amino acid substitutions, glycoforms, biosimilars, and variants thereof. IL-2 is described, e.g., in Nelson, J. Immunol. 2004, 172, 3983-88 and Malek, Annu. Rev. Immunol. 2008, 26, 453-79, the disclosures of which are incorporated by reference herein. The amino acid sequence of recombinant human IL-2 suitable for use in the invention is given in Table 2 (SEQ ID NO:3). For example, the term IL-2 encompasses human, recombinant forms of IL-2 such as aldesleukin (PROLEUKIN, available commercially from multiple suppliers in 22 million IU per single use vials), as well as the form of recombinant IL-2 commercially supplied by CellGenix, Inc., Portsmouth, NH, USA (CELLGRO GMP) or ProSpec-Tany TechnoGene Ltd., East Brunswick, NJ, USA (Cat. No. CYT-209-b) and other commercial equivalents from other vendors. Aldesleukin (des-alanyl-1, serine-125 human IL-2) is a nonglycosylated human recombinant form of IL-2 with a molecular weight of approximately 15 kDa. The amino acid sequence of aldesleukin suitable for use in the invention is given in Table 2 (SEQ ID NO:30).

The term IL-2 also encompasses pegylated forms of IL-2, as described herein, including the pegylated IL2 prodrug NKTR-214, available from Nektar Therapeutics, South San Francisco, CA, USA. NKTR-214 and pegylated IL-2 suitable for use in the invention is described in U.S. Patent Application Publication No. US 2014/0328791 A1 and International Patent Application Publication No. WO 2012/065086 Al, the disclosures of which are incorporated by reference herein. Alternative forms of conjugated IL-2 suitable for use in the invention are described in U.S. Patent Nos. 4,766,106, 5,206,344, 5,089,261 and 4902,502, the disclosures of which are incorporated by reference herein. Formulations of IL-2 suitable for use in the invention are described in U.S. Patent No. 6,706,289, the disclosure of which is incorporated by reference herein.

[00103] As used herein, interleukins include IL-2, IL-4, IL-7, IL-15, and IL-21. Exemplary sequences for these interleukins are provided in Table 2 below.

TABLE 2. Amino acid sequences of interleukins.

Identifier	Sequence (One-Letter Amino Acid Symbols)	
SEQ ID NO:29 recombinant human IL-2 (rhIL-2)	MAPTSSSTKK TQLQLEHLLL DLQMILNGIN NYKNPKLTRM LTFKFYMPKK ATELKHLQCL EEELKPLEEV LNLAQSKNFH LRPRDLISNI NVIVLELKGS ETTFMCEYAD ETATIVEFLN RWITFCQSII STLT	60 120 134
SEQ ID NO:30 Aldesleukin (Proleukin®)	PTSSSTKKTQ LQLEHLLLDL QMILNGINNY KNPKLTRMLT FKFYMPKKAT ELKHLQCLEE ELKPLEEVLN LAQSKNFHLR PRDLISNINV IVLELKGSET TFMCEYADET ATIVEFLNRW ITFSQSIIST LT	60 120 132
SEQ ID NO:31 recombinant human IL-4 (rhIL-4)	MHKCDITLQE IIKTLNSLTE QKTLCTELTV TDIFAASKNT TEKETFCRAA TVLRQFYSHH EKDTRCLGAT AQQFHRHKQL IRFLKRLDRN LWGLAGLNSC PVKEANQSTL ENFLERLKTI MREKYSKCSS	60 120 130
SEQ ID NO:32 recombinant human IL-7 (rhIL-7)	MDCDIEGKDG KQYESVLMVS IDQLLDSMKE IGSNCLNNEF NFFKRHICDA NKEGMFLFRA ARKLRQFLKM NSTGDFDLHL LKVSEGTTIL LNCTGQVKGR KPAALGEAQP TKSLEENKSL KEQKKLNDLC FLKRLLQEIK TCWNKILMGT KEH	60 120 153
SEQ ID NO:33 recombinant human IL-15 (rhIL-15)	MNWVNVISDL KKIEDLIQSM HIDATLYTES DVHPSCKVTA MKCFLLELQV ISLESGDASI HDTVENLIIL ANNSLSSNGN VTESGCKECE ELEEKNIKEF LQSFVHIVQM FINTS	60 115
SEQ ID NO:34 recombinant human IL-21 (rhIL-21)	MQDRHMIRMR QLIDIVDQLK NYVNDLVPEF LPAPEDVETN CEWSAFSCFQ KAQLKSANTG NNERIINVSI KKLKRKPPST NAGRRQKHRL TCPSCDSYEK KPPKEFLERF KSLLQKMIHQ HLSSRTHGSE DS	60 120 132

[00104] The term "IL-4" (also referred to herein as "IL4") refers to the cytokine known as interleukin 4, which is produced by Th2 T-cells and by eosinophils, basophils, and mast-cells. IL-4 regulates the differentiation of naïve helper T-cells (Th0 cells) to Th2 T-cells. Steinke and Borish, Respir. Res. 2001, 2, 66-70. Upon activation by IL-4, Th2 T-cells subsequently produce

additional IL-4 in a positive feedback loop. IL-4 also stimulates B cell proliferation and class II MHC expression, and induces class switching to IgE and IgG1 expression from B cells. Recombinant human IL-4 suitable for use in the invention is commercially available from multiple suppliers, including ProSpec-Tany TechnoGene Ltd., East Brunswick, NJ, USA (Cat. No. CYT-211) and ThermoFisher Scientific, Inc., Waltham, MA, USA (human IL-15 recombinant protein, Cat. No. Gibco CTP0043). The amino acid sequence of recombinant human IL-4 suitable for use in the invention is given in Table 2 (SEQ ID NO:31).

[00105] The term "IL-7" (also referred to herein as "IL7") refers to a glycosylated tissue-derived cytokine known as interleukin 7, which may be obtained from stromal and epithelial cells, as well as from dendritic cells. Fry and Mackall, Blood 2002, 99, 3892-904. IL-7 can stimulate the development of T-cells. IL-7 binds to the IL-7 receptor, a heterodimer consisting of IL-7 receptor alpha and common gamma chain receptor, which in a series of signals important for T-cell development within the thymus and survival within the periphery. Recombinant human IL-4 suitable for use in the invention is commercially available from multiple suppliers, including ProSpec-Tany TechnoGene Ltd., East Brunswick, NJ, USA (Cat. No. CYT-254) and ThermoFisher Scientific, Inc., Waltham, MA, USA (human IL-15 recombinant protein, Cat. No. Gibco PHC0071). The amino acid sequence of recombinant human IL-7 suitable for use in the invention is given in Table 2 (SEQ ID NO:32).

[00106] The term "IL-15" (also referred to herein as "IL15") refers to the T-cell growth factor known as interleukin-15, and includes all forms of IL-2 including human and mammalian forms, conservative amino acid substitutions, glycoforms, biosimilars, and variants thereof. IL-15 is described, e.g., in Fehniger and Caligiuri, Blood 2001, 97, 14-32, the disclosure of which is incorporated by reference herein. IL-15 shares β and γ signaling receptor subunits with IL-2. Recombinant human IL-15 is a single, non-glycosylated polypeptide chain containing 114 amino acids (and an N-terminal methionine) with a molecular mass of 12.8 kDa. Recombinant human IL-15 is commercially available from multiple suppliers, including ProSpec-Tany TechnoGene Ltd., East Brunswick, NJ, USA (Cat. No. CYT-230-b) and ThermoFisher Scientific, Inc., Waltham, MA, USA (human IL-15 recombinant protein, Cat. No. 34-8159-82). The amino acid sequence of recombinant human IL-15 suitable for use in the invention is given in Table 2 (SEQ ID NO:33).

[00107] The term "IL-21" (also referred to herein as "IL21") refers to the pleiotropic cytokine protein known as interleukin-21, and includes all forms of IL-21 including human and mammalian forms, conservative amino acid substitutions, glycoforms, biosimilars, and variants thereof. IL-21 is described, e.g., in Spolski and Leonard, Nat. Rev. Drug. Disc. 2014, 13, 379-

95, the disclosure of which is incorporated by reference herein. IL-21 is primarily produced by natural killer T-cells and activated human CD4+ T-cells. Recombinant human IL-21 is a single, non-glycosylated polypeptide chain containing 132 amino acids with a molecular mass of 15.4 kDa. Recombinant human IL-21 is commercially available from multiple suppliers, including ProSpec-Tany TechnoGene Ltd., East Brunswick, NJ, USA (Cat. No. CYT-408-b) and ThermoFisher Scientific, Inc., Waltham, MA, USA (human IL-21 recombinant protein, Cat. No. 14-8219-80). The amino acid sequence of recombinant human IL-21 suitable for use in the invention is given in Table 2 (SEQ ID NO:34).

[00108] A "disease" includes a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate.

[00109] In contrast, a "disorder" in an animal includes a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

[00110] A disease or disorder is "alleviated" if the severity of a symptom of the disease or disorder, the frequency with which such a symptom is experienced by a patient, or both, is reduced.

[00111] With respect to the inventive methods, the term "tumor" or "cancer" can be any cancer, including any of acute lymphocytic cancer, acute myeloid leukemia, alveolar rhabdomyosarcoma, bone cancer, brain cancer, breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the vulva, chronic lymphocytic leukemia, chronic myeloid cancer, cervical cancer, glioma, Hodgkin's lymphoma, hypopharynx cancer, kidney cancer, larynx cancer, liver cancer, lung cancer, malignant mesothelioma, melanoma, multiple myeloma, nasopharynx cancer, non-Hodgkin's lymphoma, ovarian cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, renal cancer, skin cancer, soft tissue cancer, testicular cancer, thyroid cancer, ureter cancer, urinary bladder cancer, and digestive tract cancer such as, *e.g.*, esophageal cancer, gastric cancer, pancreatic cancer, stomach cancer, small intestine cancer, gastrointestinal carcinoid tumor, cancer of the oral cavity, colon cancer, and hepatobiliary cancer. In some embodiments, the cancer is melanoma. In some embodiments, the cancer is metastatic melanoma.

[00112] As used herein, the term "mammal" refers to any mammal, including, but not limited to, mammals of the order *Rodentia*, such as mice and hamsters, and mammals of the order *Logomorpha*, such as rabbits. In some embodiments, the mammals are from the order *Carnivora*, including felines (cats) and canines (dogs). In some embodiments, the mammals are from the order *Artiodactyla*, including bovines (cows) and swines (pigs) or of the order *Perssodactyla*, including Equines (horses). It is most preferred that the mammals are of the order *Primates*, *Ceboids*, or *Simoids* (monkeys) or of the order *Anthropoids* (humans and apes). In some embodiments, the mammal is the human.

[00113] The term "regression," as well as words stemming therefrom, as used herein, does not necessarily imply 100% or complete regression. Rather, there are varying degrees of regression of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the inventive methods can provide any amount of any level of regression of cancer in a mammal. Furthermore, the regression provided by the inventive method can include regression of one or more conditions or symptoms of the disease, *e.g.*, cancer. Also, for purposes herein, "regression" can encompass delaying the onset of the disease, or delaying the onset of a symptom and/or delaying the onset of a condition thereof.

[00114] An "effective amount" or "therapeutically effective amount" of a composition includes that amount of the composition which is sufficient to provide a beneficial effect to the subject to which the composition is administered. An "effective amount" of a delivery vehicle includes that amount sufficient to effectively bind or deliver a composition.

[00115] "Encoding" includes the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (*i.e.*, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if, for example, transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

[00116] As used herein "endogenous" includes any material from or produced inside an organism, cell, tissue or system.

[00117] As used herein, the term "exogenous" includes any material introduced from or produced outside an organism, cell, tissue or system.

[00118] An "expression cassette" includes any nucleic acid construct capable of directing the expression of a gene/coding sequence of interest, which is operably linked to a promoter of the expression cassette. Such cassettes can be constructed into a "vector," "vector construct," "expression vector," or "gene transfer vector," in order to transfer the expression cassette into target-cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors.

[00119] As used herein, the term "fragment," as applied to a nucleic acid or polypeptide, includes a subsequence of a larger nucleic acid or polypeptide. A "fragment" of a nucleic acid can be at least about 15 nucleotides in length; for example, at least about 50 nucleotides to about 100 nucleotides; at least about 100 to about 500 nucleotides, at least about 500 to about 1000 nucleotides, at least about 1000 nucleotides to about 1500 nucleotides; or about 1500 nucleotides to about 2500 nucleotides; or about 2500 nucleotides (and any integer value in between). A "fragment" of a polypeptide can be at least about 15 amino acids in length; for example, at least about 50 amino acids to about 100 amino acids; at least about 100 to about 500 amino acids, at least about 500 to about 1000 amino acids, at least about 1000 amino acids to about 1500 amino acids; or about 1500 amino acids to about 2500 amino acids; or about 2500 amino acids (and any integer value in between).

[00120] As used herein, the terms "gene" and "recombinant gene" includes nucleic acid molecules comprising an open reading frame encoding a polypeptide. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of a given gene. Alternative alleles can be identified by sequencing the gene of interest in a number of different individuals. This can be readily carried out by using hybridization probes to identify the same genetic locus in a variety of individuals. Any and all such nucleotide variations and resulting amino acid polymorphisms or variations that are the result of natural allelic variation and that do not alter the functional activity are intended to be within the scope of the invention.

[00121] "Homologous" as used herein, includes the subunit sequence similarity between two polymeric molecules, *e.g.* between two nucleic acid molecules, *e.g.*, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit, *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then they are homologous at that position. The homology between two sequences is a direct function of the number of matching or homologous positions, *e.g.* if half (*e.g.*, five positions in a polymer ten subunits in length) of the positions in two compound sequences are homologous then the two sequences are 50% homologous, if 90% of the positions, *e.g.* 9 of 10, are matched or homologous, the two sequences share 90%

homology. By way of example, the DNA sequences 5'-ATTGCC-3' and 5'-TATGGC-3' share 50% homology.

[00122] "Inducible" expression includes a state in which a gene product is produced in a living cell in response to the presence of a signal in the cell.

[00123] As used herein, an "instructional material" includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness and/or use of a compound, composition, vector, or delivery system of the present disclosure in a kit according to the present disclosure, *e.g.*, a kit for effecting alleviation of the various diseases or disorders recited herein. Optionally, or alternately, the instructional material can describe one or more methods of alleviating the diseases or disorders in a cell or a tissue of a mammal. The instructional material of the kit of the invention can, for example, be affixed to a container which contains the identified compound, composition, vector, or delivery system of the invention or be shipped together with a container which contains the identified compound, composition, vector, or delivery system. Alternatively, the instructional material can be shipped separately from the container with the intention that the instructional material and the compound be used cooperatively by the recipient.

[00124] The term "nucleic acid" includes RNA or DNA molecules having more than one nucleotide in any form including single-stranded, double-stranded, oligonucleotide or polynucleotide. The term "nucleotide sequence" includes the ordering of nucleotides in an oligonucleotide or polynucleotide in a single-stranded form of nucleic acid.

[00125] By "nucleic acid construct" it is meant a nucleic acid sequence that has been constructed to comprise one or more functional units not found together in nature. Examples include circular, linear, double-stranded, extrachromosomal DNA molecules (plasmids), cosmids (plasmids containing COS sequences from lambda phage), viral genomes including non-native nucleic acid sequences, and the like.

[00126] The term "operably linked" as used herein includes a polynucleotide in functional relationship with a second polynucleotide, *e.g.* a single-stranded or double-stranded nucleic acid moiety comprising the two polynucleotides arranged within the nucleic acid moiety in such a manner that at least one of the two polynucleotides is able to exert a physiological effect by which it is characterized, upon the other. By way of example, a promoter operably linked to the coding region of a gene is able to promote transcription of the coding region. The order specified when indicating operably linkage is not important. For example, the phrases: "the promoter is operably linked to the nucleotide sequence" and "the nucleotide sequence is operably linked to the promoter" are used interchangeably herein and are considered equivalent. In some cases,

when the nucleic acid encoding the desired protein further comprises a promoter/regulatory sequence, the promoter/regulatory sequence is positioned at the 5' end of the desired protein coding sequence such that it drives expression of the desired protein in a cell.

[00127] The terms "oligonucleotide," "polynucleotide," and "nucleic acid molecule", used interchangeably herein, refer to a polymeric forms of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. The backbone of the polynucleotide can comprise sugars and phosphate groups (as may typically be found in RNA or DNA), or modified or substituted sugar or phosphate groups. Alternatively, the backbone of the polynucleotide can comprise a polymer of synthetic subunits such as phosphoramidites, and/or phosphorothioates, and thus can be an oligodeoxynucleoside phosphoramidate or a mixed phosphoramidate-phosphodiester oligomer. Peyrottes et al. (1996) Nucl. Acids Res. 24:1841-1848; Chaturvedi et al. (1996) Nucl. Acids Res. 24:2318-2323. The polynucleotide may comprise one or more L-nucleosides. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, and may be interrupted by non-nucleotide components. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, uracyl, other sugars, and linking groups such as fluororibose and thioate, and nucleotide branches. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Other types of modifications included in this definition are caps, substitution of one or more of the naturally occurring nucleotides with an analog, and introduction of means for attaching the polynucleotide to proteins, metal ions, labeling components, other polynucleotides, or a solid support. Whenever a polynucleotide or oligonucleotide is represented by a sequence of letters (upper or lower case), such as "ATGCCTG," it will be understood that the nucleotides are in 5'→3' order from left to right and that "A" denotes deoxyadenosine, "C" denotes deoxycytidine, "G" denotes deoxyguanosine, and "T" denotes thymidine, "I" denotes deoxyinosine, "U" denotes uridine, unless otherwise indicated or obvious from context. Unless otherwise noted the terminology and atom numbering conventions will follow those disclosed in Strachan and Read, Human Molecular Genetics 2 (Wiley-Liss, New York, 1999). Polynucleotides can be single, double, or triplex, linear or

circular, and can be of any length. In discussing polynucleotides, a sequence or structure of a particular polynucleotide may be described herein according to the convention of providing the sequence in the 5' to 3' direction.

[00128] The term "recombinant," as applied to a polynucleotide means the polynucleotide is the product of various combinations of cloning, restriction or ligation steps, and other procedures resulting in a construct distinct and/or different from a polynucleotide found in nature. The terms respectively include replicates of the original polynucleotide construct and progeny of the original virus construct.

[00129] The term "Programmed Death 1 Ligand 1 (PD-L1) binding protein" refers to a polypeptide (*e.g.*, a fusion protein, an scFV, a maxibody, an antibody, and the like), which is capable of specifically binding to Programmed Death 1 Ligand 1 (PD-L1) protein (a.k.a. CD274 or B7-H1) expressed on the surface of a cell.

[00130] The terms "polypeptide," "peptide," and "protein", used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. The term includes polypeptide chains modified or derivatized in any manner, including, but not limited to, glycosylation, formylation, cyclization, acetylation, phosphorylation, and the like. The term includes naturally-occurring peptides, synthetic peptides, and peptides comprising one or more amino acid analogs. The term includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions with heterologous and homologous leader sequences, with or without N-terminal methionine residues; immunologically tagged proteins; and the like.

[00131] The term "tumor-associated antigen" is a term well understood in the art, and refers to molecules that are differentially over-expressed in tumor cells relative to non-cancerous cells of the same cell type. As used herein, "tumor-associated antigen" includes not only complete tumor-associated antigens that can be expressed on the cell surface, but also epitope-comprising portions (fragments) thereof that are recognized by T-cells. A tumor-associated antigen (TAA) may be one found in nature, or may be a synthetic version of a TAA found in nature, or may be a variant of a naturally-occurring TAA, *e.g.*, a variant which has enhanced immunogenic properties. The TAA may be a naturally occurring over-expressed protein or a mutated protein expressed only in tumor cells or other transformed cells in tumors.

[00132] The term "promoter" as used herein includes a DNA sequence operably linked to a nucleic acid sequence to be transcribed such as a nucleic acid sequence encoding a desired molecule. A promoter is generally positioned upstream of a nucleic acid sequence to be

transcribed and provides a site for specific binding by RNA polymerase and other transcription factors.

[00133] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The "polypeptides," "proteins" and "peptides" encoded by the "polynucleotide sequences," include full-length native sequences, as with naturally occurring proteins, as well as functional subsequences, modified forms or sequence variants so long as the subsequence, modified form, or variant retains some degree of functionality of the native full-length protein. In methods and uses of as described herein, such polypeptides, proteins, and peptides encoded by the polynucleotide sequences can be but are not required to be identical to the defective endogenous protein, or whose expression is insufficient, or deficient in the treated mammal. The terms also encompass a modified amino acid polymer; for example, disulfide bond formation, glycosylation, lipidation, phosphorylation, methylation, carboxylation, deamidation, acetylation, or conjugation with a labeling component. Polypeptides such as anti-angiogenic polypeptides, neuroprotective polypeptides, and the like, when discussed in the context of delivering a gene product to a mammalian subject, and compositions therefor, refer to the respective intact polypeptide, or any fragment or genetically engineered derivative thereof, retaining the desired biochemical function of the intact protein.

[00134] A "recombinant polypeptide" includes one which is produced upon expression of a recombinant polynucleotide.

[00135] The term "specifically binds," as used herein, *e.g.*, with respect to an antibody/antigen binding region, includes an antibody/antigen binding region which recognizes a specific antigen, but does not substantially recognize or bind other molecules in a sample. For example, an antibody (*e.g.*, an scFV) that specifically binds to an antigen from one species may also bind to that antigen from one or more other species. But, such cross-species reactivity does not itself alter the classification of an antibody as specific. As another example, an antibody that specifically binds to an antigen may also bind to different allelic forms of the antigen. However, such cross reactivity does not itself alter the classification of an antibody as specific.

[00136] In some instances, the terms "specific binding" or "specifically binding", can be used in reference to the interaction of an antibody, a protein, or a peptide with a second chemical species, to mean that the interaction is dependent upon the presence of a particular structure (*e.g.*, an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free,

unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody.

[00137] The term "synthetic antibody" as used herein includes an antibody which is generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using synthetic DNA or amino acid sequence technology which is available and well known in the art.

[00138] "Variant" as the term is used herein, includes a nucleic acid sequence or a peptide sequence that differs in sequence from a reference nucleic acid sequence or peptide sequence respectively, but retains essential biological properties of the reference molecule. Changes in the sequence of a nucleic acid variant may not alter the amino acid sequence of a peptide encoded by the reference nucleic acid, or may result in amino acid substitutions, additions, deletions, fusions, and truncations. Changes in the sequence of peptide variants are typically limited or conservative, so that the sequences of the reference peptide and the variant are closely similar overall and, in many regions, identical. A variant and reference peptide can differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A variant of a nucleic acid or peptide can be a naturally occurring such as an allelic variant, or can be a variant that is not known to occur naturally. Non-naturally occurring variants of nucleic acids and peptides may be made by mutagenesis techniques or by direct synthesis.

[00139] A "substitution" results from the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively as compared to an amino acid sequence or nucleotide sequence of a polypeptide. If a substitution is conservative, the amino acid that is substituted into a polypeptide has similar structural or chemical properties (*e.g.*, charge, polarity, hydrophobicity, and the like) to the amino acid that it is substituting. Conservative substitutions of naturally occurring amino acids ("conservative amino acid substitutions") usually result in a substitution of a first amino acid with second amino acid from the same group as the first amino acid, where examples of amino acid groups are as follows: (1) acidic (negatively charged) amino acids such as aspartic acid and glutamic acid; (2) basic (positively charged) amino acids such as arginine, histidine, and lysine; (3) neutral polar amino acids such as glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; and (4) neutral non-polar amino acids such as alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine. In some embodiments, polypeptide variants may

have "non-conservative" changes, where the substituted amino acid differs in structural and/or chemical properties.

[00140] A "deletion" is defined as a change in either amino acid or nucleotide sequence in which one or more amino acid or nucleotide residues, respectively, are absent as compared to an amino acid sequence or nucleotide sequence of a naturally occurring polypeptide or polynucleotide. In the context of a polypeptide or polynucleotide sequence, a deletion can involve deletion of 2, 5, 10, up to 20, up to 30, or up to 50 or more amino acids or nucleotide residues, taking into account the length of the polypeptide or polynucleotide sequence being modified.

[00141] An "insertion" or "addition" is that change in an amino acid or nucleotide sequence which has resulted in the addition of one or more amino acid or nucleotide residues, respectively, as compared to an amino acid sequence or nucleotide sequence of a naturally occurring polypeptide or polynucleotide. "Insertion" generally refers to addition of one or more amino acid residues within an amino acid sequence of a polypeptide (or nucleotide residues within a polynucleotide), while "addition" can be an insertion or refer to amino acid residues added at the N- or C-termini of a polypeptide (or nucleotide residues added at the 5′ or 3′ end of a polynucleotide). In the context of a polypeptide or polynucleotide sequence, an insertion or addition may be of up to 10, up to 20, up to 30, or up to 50 or more amino acids (or nucleotide residues).

[00142] An "isolated" plasmid, nucleic acid, vector, or other substance refers to a preparation of the substance devoid of at least some of the other components present where the substance or a similar substance naturally occurs or from which it is initially prepared. Thus, for example, an isolated substance may be prepared by using a purification technique to enrich it from a source mixture. Enrichment can be measured on an absolute basis, such as weight per volume of solution, or it can be measured in relation to a second, potentially interfering substance present in the source mixture. Increasing enrichments of the embodiments of this invention are increasingly more isolated. An isolated plasmid, nucleic acid, vector, or other substance is in some embodiments purified, *e.g.*, from about 80% to about 90% pure, at least about 90% pure, at least about 95% pure, at least about 98% pure, or at least about 99%, or more, pure.

[00143] A "vector" is capable of transferring gene sequences to target-cells. Typically, "vector construct," "expression vector," and "gene transfer vector," mean any nucleic acid construct capable of directing the expression of a gene of interest and which can transfer gene sequences to target-cells, which can be accomplished by genomic integration of all or a portion

of the vector, or transient or inheritable maintenance of the vector as an extrachromosomal element. Thus, the term includes cloning, and expression vehicles, as well as integrating vectors.

[00144] The term "regulatory element" as used herein includes a nucleotide sequence which controls some aspect of the expression of nucleic acid sequences. Examples of regulatory elements illustratively include an enhancer, an internal ribosome entry site (IRES), an intron, an origin of replication, a polyadenylation signal (pA), a promoter, an enhancer, a transcription termination sequence, and an upstream regulatory domain, which contribute to the replication, transcription, and/or post-transcriptional processing of a nucleic acid sequence. In cases, regulatory elements can also include *cis*-regulatory DNA elements as well as transposable elements (TEs). Those of ordinary skill in the art are capable of selecting and using these and other regulatory elements in an expression construct with no more than routine experimentation. Expression constructs can be generated using a genetic recombinant approach or synthetically using well-known methodology.

[00145] A "control element" or "control sequence" is a nucleotide sequence involved in an interaction of molecules contributing to the functional regulation of a polynucleotide, including replication, duplication, transcription, splicing, translation, or degradation of the polynucleotide. The regulation may affect the frequency, speed, or specificity of the process, and may be enhancing or inhibitory in nature. Control elements known in the art include, for example, transcriptional regulatory sequences such as promoters and enhancers. A promoter is a DNA region capable under certain conditions of binding RNA polymerase and initiating transcription of a coding region usually located downstream (in the 3' direction) from the promoter.

[00146] "Operatively linked" or "operably linked" refers to a juxtaposition of genetic elements, wherein the elements are in a relationship permitting them to operate in the expected manner. For instance, a promoter is operatively linked to a coding region if the promoter helps initiate transcription of the coding sequence. There may be intervening residues between the promoter and coding region so long as this functional relationship is maintained.

[00147] The terms "cancer", "neoplasm", "tumor", and "carcinoma", are used interchangeably herein to refer to cells which exhibit relatively autonomous growth, so that they exhibit an aberrant growth phenotype characterized by a significant loss of control of cell proliferation. Cancerous cells can be benign or malignant. Examples of various cancers include but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer and the like.

[00148] The phrase "homologous tumor" is meant any tumor composed of tissue of the same type from which it develops. Additionally, a tumor can be homologous to TILs derived from said tumor. For example, a melanoma tumor can be homologous to TILs derived from the melanoma tumor and/or TILs derived from a melanoma tumor can be used to treat the homologous melanoma tumor from which they were derived. In some embodiments, TILs derived from the homologous tumor can be used to treat the homologous tumor.

[00149] By "individual" or "host" or "subject" or "patient" is meant any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans. Other subjects may include cattle, dogs, cats, guinea pigs, rabbits, rats, mice, horses, and so on.

[00150] As used herein the term "isolated," when used in the context of an isolated compound, refers to a compound of interest that is in an environment different from that in which the compound naturally occurs. "Isolated" is meant to include compounds that are within samples that are substantially enriched for the compound of interest and/or in which the compound of interest is partially or substantially purified.

[00151] As used herein, the term "substantially pure" refers to a compound that is removed from its natural environment and is at least 60% free, 75% free, or 90% free from other components with which it is naturally associated.

[00152] Techniques for determining nucleic acid and amino acid "sequence identity" are known in the art. Typically, such techniques include determining the nucleotide sequence of the mRNA for a gene and/or determining the amino acid sequence encoded thereby, and comparing these sequences to a second nucleotide or amino acid sequence. In general, "identity" refers to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Two or more sequences (polynucleotide or amino acid) can be compared by determining their "percent identity." The percent identity of two sequences, whether nucleic acid or amino acid sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequences and multiplied by 100. An approximate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics*, 2:482-489 (1981). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff, *Atlas of Protein Sequences and Structure*, M.O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA, and normalized by Gribskov, *Nucl. Acids Res.* 14(6):6745-6763 (1986).

[00153] An example of an implementation of this algorithm to determine percent identity of a sequence is provided by the Genetics Computer Group (Madison, WI) in the "BestFit"

utility application. The default parameters for this method are described in the Wisconsin Sequence Analysis Package Program Manual, Version 8 (1995) (available from Genetics Computer Group, Madison, WI). Another method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, CA). From this suite of packages, the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six). From the data generated the "Match" value reflects "sequence identity." Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs can be found at the internet address located by placing http:// in front of blast.ncbi.nlm.nih.gov/Blast.cgi.

[00154] Alternatively, in the context of polynucleotides, homology can be determined by hybridization of polynucleotides under conditions that form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s), and size determination of the digested fragments.

[00155] Two DNA, or two polypeptide sequences are "substantially homologous" to each other when the sequences exhibit at least about 80%-85%, at least about 85%-90%, at least about 90%-95%, or at least about 95%-98% sequence identity over a defined length of the molecules, as determined using the methods above. As used herein, substantially homologous also refers to sequences showing complete identity to the specified DNA or polypeptide sequence. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, *e.g.*, Sambrook and Russel, *Molecular Cloning: A Laboratory Manual Third Edition*, (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

[00156] A first polynucleotide is "derived from" a second polynucleotide if it has the same or substantially the same nucleotide sequence as a region of the second polynucleotide, its cDNA, complements thereof, or if it displays sequence identity as described above. This term is

not meant to require or imply the polynucleotide must be obtained from the origin cited (although such is encompassed), but rather can be made by any suitable method.

[00157] A first polypeptide (or peptide) is "derived from" a second polypeptide (or peptide) if it is (i) encoded by a first polynucleotide derived from a second polynucleotide, or (ii) displays sequence identity to the second polypeptides as described above. This term is not meant to require or imply the polypeptide must be obtained from the origin cited (although such is encompassed), but rather can be made by any suitable method.

[00158] The term "in combination with" as used herein refers to uses where, for example, a first therapy is administered during the entire course of administration of a second therapy; where the first therapy is administered for a period of time that is overlapping with the administration of the second therapy, *e.g.* where administration of the first therapy begins before the administration of the second therapy and the administration of the first therapy ends before the administration of the second therapy ends; where the administration of the second therapy begins before the administration of the first therapy and the administration of the second therapy ends before the administration of the first therapy ends; where the administration of the first therapy begins before administration of the second therapy begins and the administration of the second therapy ends before the administration of the first therapy ends; where the administration of the second therapy begins before administration of the first therapy begins and the administration of the first therapy ends before the administration of the second therapy ends. As such, "in combination" can also refer to regimen involving administration of two or more therapies. "In combination with" as used herein also refers to administration of two or more therapies which may be administered in the same or different formulations, by the same or different routes, and in the same or different dosage form type.

[00159] The terms "treatment", "treating", "treat", and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment", as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, *i.e.*, arresting its development or progression; and (c) relieving the disease, *i.e.*, causing regression of the disease and/or relieving one or more disease symptoms. "Treatment" is also meant to encompass delivery of an agent in order to provide for a pharmacologic effect, even in the absence of a disease or condition. For example, "treatment" encompasses delivery of a

composition that can elicit an immune response or confer immunity in the absence of a disease condition, *e.g.*, in the case of a vaccine.

[00160] Any and all publications (including patents and patent application publications) mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[00161] The publications discussed herein are provided for their disclosure prior to the filing date of the present application. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed. To the extent such publications may set out a definition which conflicts with an explicit or implicit definition or disclosure of the present disclosure, the definition or disclosure of the present disclosure controls.

[00162] As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present invention. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

COMPOSITIONS

Proteins (e.g., antibodies) that specifically bind to PD-L1

[00163] Provided are proteins (*e.g.*, antibodies) that specifically binds to PD-L1, nucleic acids encoding the proteins, and cells that include a subject protein and/or a nucleic acid encoding a subject protein. Throughout this disclosure, a "protein that specifically binds to PD-L1" that includes an antigen binding region as described herein is also referred to as a "PD-L1 binding protein."

[00164] Two new recombinant antibodies are provided (referred to herein as "38A1" and "19H9"), both of which specifically bind to human Programmed Death 1 Ligand (PD-L1). Human PD-L1, NCBI Accession NP_054862 (Version NP_054862.1 GI:7661534), is a 290 aa protein having the amino acid sequence (which includes a putative signal peptide at aa 1-18 and an ectodomain at amino acids 19-238):

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVE
KQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKD
QLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPY
NKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTT
TTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPE

LPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGI
QDTNSKKQSDTHLEET (SEQ ID NO: 21)

PD-L1 is also known as CD274 and B7-H1, and is expressed on the surface of a cell, *e.g.*, a tumor cell.

[00165] Sequence details, including the CDR sequences, and additional information related to the 38A1 and 19H9 antibodies is presented in Figure 7. As such in some cases, a subject protein that specifically binds to PD-L1 has an antigen binding portion that includes a first polypeptide that includes the 3 CDR amino acid sequences set forth in SEQ ID NOs:2-4 (light chain CDRs L1, L2, and L3, respectively of scFV 38A1), and a second polypeptide that includes the 3 CDR amino acid sequences set forth in SEQ ID NOs:6-8 (heavy chain CDRs H1, H2, and H3, respectively of scFV 38A1). In some cases, a subject protein that specifically binds to PD-L1 has an antigen binding portion that includes a first polypeptide that includes the 3 CDR amino acid sequences set forth in SEQ ID NOs:2-4 (light chain CDRs L1, L2, and L3, respectively of scFV 38A1), and a second polypeptide that includes the 3 CDR amino acid sequences set forth in SEQ ID NOs:6-8 (heavy chain CDRs H1, H2, and H3, respectively of scFV 38A1), with the exception that the first and/or the second polypeptide can include one or more conservative amino acid substitutions relative to the specified SEQ ID numbers as long as the protein specifically binds to PD-L1. In some cases, the first and/or the second polypeptide can include two or less (*e.g.*, one or less) conservative amino acid substitutions within each amino acid stretch that corresponds to the specified SEQ ID numbers as long as the protein specifically binds to PD-L1. For example, such a subject protein can in some cases include two or less (*e.g.*, one or less) conservative amino acid substitutions within each CDR.

[00166] In some cases, the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO:1 (38A1 light chain), and the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO:5 (38A1 heavy chain) (*e.g.*, see Figure 7). In some cases, 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, with the exception that the first and/or the second polypeptide can include one or more conservative amino acid substitutions relative to the specified SEQ ID numbers as long as the protein specifically binds to PD-L1. In some cases, the first and/or the second polypeptide can include two or less (*e.g.*, one or less) conservative amino acid substitutions within each amino acid stretch that corresponds to the specified SEQ ID numbers as long as the protein specifically binds to PD-L1. For example, such a subject protein can in some cases include two or less (*e.g.*, one or less) conservative amino acid substitutions within each CDR. In some embodiments, the first

polypeptide comprises the CDRs recited in SEQ ID NOs:2, 3, and 4 and wherein the protein specifically binds to PD-L1. In some embodiments, the second polypeptide comprises the CDRs recited in SEQ ID NOs:6, 7, and 8 and wherein the protein specifically binds to PD-L1. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway.

Light Chain - Antibody 38A1
SYVLTQPPSVSVAPGQTARITCGGNNIGRKIVHWYQQRPGQAPVLVIYYDTDRPAGIPERFSGSN
SGNMATLTISTVGAGDEADYYCQVWDTGSDHVVFGGGTKLTVL
(SEQ ID NO: 1)

CDR1(CDR-L1): NIGRKI (SEQ ID NO:2)
CDR2(CDR-L2): YDT (SEQ ID NO:3)
CDR3(CDR-L3): QVWDTGSDHVV (SEQ ID NO:4)

Heavy Chain- Antibody 38A1
EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSTISGSGGTTYYADSVK
GRFTISRDNSKNTLYLQMNSLRVEDTAVYYCAKDWFRSSSPDAFDIWGQGTTVTVSA
(SEQ ID NO: 5)
CDR1(CDR-H1): GFTFSNYA (SEQ ID NO:6)
CDR2(CDR-H2): ISGSGGTT (SEQ ID NO:7)
CDR3(CDR-H3): AKDWFRSSSPDAFDI (SEQ ID NO:8)

In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding

protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3).

[00167] In some cases, a subject protein that specifically binds to PD-L1 has an antigen binding portion that includes a first polypeptide that includes the 3 CDR amino acid sequences set forth in SEQ ID NOs:10-12 (light chain CDRs L1, L2, and L3, respectively of scFV 19H9), and a second polypeptide that includes the 3 CDR amino acid sequences set forth in SEQ ID NOs:14-16 (heavy chain CDRs H1, H2, and H3, respectively of scFV 19H9). In some cases, a subject protein that specifically binds to PD-L1 has an antigen binding portion that includes a first polypeptide that includes the 3 CDR amino acid sequences set forth in SEQ ID NOs:10-12 (light chain CDRs L1, L2, and L3, respectively of scFV 19H9), and a second polypeptide that includes the 3 CDR amino acid sequences set forth in SEQ ID NOs:14-16 (heavy chain CDRs H1, H2, and H3, respectively of scFV 19H9), with the exception that the first and/or the second polypeptide can include one or more conservative amino acid substitutions relative to the specified SEQ ID numbers as long as the protein specifically binds to PD-L1. In some cases, the first and/or the second polypeptide can include two or less (*e.g.*, one or less) conservative amino acid substitutions within each amino acid stretch that corresponds to the specified SEQ ID numbers as long as the protein specifically binds to PD-L1. For example, such a subject protein can in some cases include two or less (*e.g.*, one or less) conservative amino acid substitutions within each CDR. In some embodiments, the first polypeptide comprises the CDRs recited in SEQ ID NOs:10, 11, and 12 and wherein the protein specifically binds to PD-L1. In some embodiments, the second polypeptide comprises the CDRs recited in SEQ ID NOs:14, 15, and 16 and wherein the protein specifically binds to PD-L1. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity

in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1.

Light Chain - Antibody 19H9

NFMLTQPHSVSESLGKTVTISCTGSSGSIARKFVQWYQQRPGSSPTTVIYENNQRPSGVSDRFSG
SIGSSSNSASLTISGLKTEDEADYYCQSYDSSNVVFGGGTKVTVL

(SEQ ID NO: 9)

CDR1(CDR-L1): SGSIARKF (SEQ ID NO:10)

CDR2(CDR-L2): ENN (SEQ ID NO:11)

CDR3(CDR-L3): QSYDSSNVV (SEQ ID NO:12)

Heavy Chain - Antibody 19H9

QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSGINTAGDTHYPESVKG
RFTISRDNARNSLNLQMNSLRAEDTAVYYCVRERVEREYSGYDAFDIWGQGTTVTVSA

(SEQ ID NO: 13)

CDR1(CDR-H1): GFTFSSYS (SEQ ID NO:14)

CDR2(CDR-H2): INTAGDT (SEQ ID NO:15)

CDR3(CDR-H3): VRERVEREYSGYDAFDI (SEQ ID NO:16)

[00168] In some embodiments, the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO:9 (19H9 light chain), and the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO:13 (19H9 heavy chain) (*e.g.*, see Figure 7). In some embodiments, the first polypeptide comprises the amino acid sequence 90%, 95%, or 98% identical to the amino acid sequence set forth in SEQ ID NO:9 (19H9 light chain), and the second polypeptide comprises the amino acid sequence 90%, 95%, or 98% identical to the amino acid sequence set forth in SEQ ID NO:13 (19H9 heavy chain). In some cases, 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, with the exception that the first and/or the second polypeptide can include one or more conservative amino acid substitutions relative to the specified SEQ ID numbers as long as the protein specifically binds to PD-L1. In some cases, the first and/or the second polypeptide can include two or less (*e.g.*, one or less) conservative amino acid substitutions within each amino acid stretch that corresponds to the specified SEQ ID numbers as long as the protein specifically binds to PD-L1.

For example, such a subject protein can in some cases include two or less (*e.g.*, one or less) conservative amino acid substitutions within each CDR.

[00169] In some embodiments, a subject protein that specifically binds to PD-L1 is a single-chain antibody (scFv) (discussed above). As such, in some cases, a subject protein that specifically binds to PD-L1 is an scFv, and the first and second polypeptides of the antigen binding portion, as described above (*e.g.* in this section), are fused to one another (and are therefore part of the same polypeptide). Examples of suitable scFvs include, but are not limited to those set forth in SEQ ID NOs:17 and 19 (see Figure 7). In some cases, the first and second polypeptides of an scFv are separated from one another via a linker (*e.g.*, a flexible linker). Various linkers will be known to one of ordinary skill in the art and any convenient linker can be used. A flexible linker may include, for example, an amino acid sequence for a hinge region derived from an immunoglobulin heavy chain. A flexible linker can be positioned, for example, between an antigen binding portion (an antigen binding domain) of an antibody (*e.g.*, an scFv) and an immunoglobulin Fc domain. A variety of flexible linkers are known in the art, including, *e.g.*, flexible linkers including one or more Gly, Ser, Asn, and/or Asp amino acids. In some embodiments, the flexible linker is GGGGS. In some embodiments, the l flexible inker is (GGGGS)*n* (SEQ ID NO:35), wherein *n* is an integer between 1 and 10. In some embodiments, the flexible linker is GGGGS. In some embodiments, the flexible linker is GGGGSGGGGS (SEQ ID NO:36). In some embodiments, the flexible linker is GGGGSGGGGSGGGGS (SEQ ID NO:37). In some embodiments, the flexible linker is GGGGSGGGGSGGGGSGGGGS (SEQ ID NO:38). In some embodiments, the flexible linker is GGGGSGGGGSGGGGSGGGGS (SEQ ID NO:39). In some embodiments, the flexible linker comprises a c-terminal Ala and Pro (AP). In some embodiments, the flexible linker is GGGSGG GGSGGGGSGAP (SEQ ID NO:40).

[00170] The flexible linkers of the scFvs of SEQ ID NOs:17 and 19 are bold and underlined in Figure 7. In some cases, a subject protein that specifically binds to PD-L1 includes an amino acid sequence having 80% or more sequence identity (*e.g.*, 85% or more, 90% or more, 95% or more, 98% or more, 99% or more, or 100% sequence identity) with the amino acid sequence set forth in any of SEQ ID NOs:17 and 19 (*e.g.*, see Figure 7).

[00171] In some embodiments, the scFv comprises the following sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSTISGS
GGTTYYADSVKGRFTISRDNSKNTLYLQMNSLRVEDTAVYYCAKDWFRSSSPD
AFDIWGQGTTVTVSAGGGGSGGGGSGGGGSGAPSYVLTQPPSVSVAPGQTARIT
CGGNNIGRKIVHWYQQRPGQAPVLVIYYDTDRPAGIPERFSGSNSGNMATLTIST
VGAGDEADYYCQVWDTGSDHVVFGGGTKLTVL

(SEQ ID NO: 17).

[00172] In some embodiments, the scFv comprises the following sequence:

QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSGINT
AGDTHYPESVKGRFTISRDNARNSLNLQMNSLRAEDTAVYYCVRERVEREYSG
YDAFDIWGQGTTVTVSAGGGGSGGGGSGGGGSGAPNFMLTQPHSVSESLGKTV
TISCTGSSGSIARKFVQWYQQRPGSSPTTVIYENNQRPSGVSDRFSGSIGSSSNSAS
LTISGLKTEDEADYYCQSYDSSNVVFGGGTKVTVL

(SEQ ID NO: 19).

[00173] In some embodiments, a subject protein that specifically binds to PD-L1 (*e.g.*, an scFv) is fused directly or via a linker to an Fc domain (or a fragment thereof) (*e.g.*, an IgG1 Fc, an IgG2 Fc, an IgG3 Fc, an IgG4 Fc, or a fragment thereof) to provide a maxibody. In some cases, the Fc is an IgG1 Fc domain. In some cases, an IgG1 FC domain has the sequence:

CPPCPAPEFEGGPSVFLFPPKPKDTLMetISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP
SSIEKTISKAKGQPREPQVYTLPPSQEEMetTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMetHEALH
NHYTQKSLSLSLGK (SEQ ID NO:22)

[00174] In some embodiments, the Fc is an IgG4 Fc domain. In some cases, an IgG4 FC domain has the sequence:

CPPCPAPEFEGGPSVFLFPPKPKDTLMetISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP
SSIEKTISKAKGQPREPQVYTLPPSQEEMetTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMetHEALH
NHYTQKSLSLSLGK (SEQ ID NO:23)

[00175] In some embodiments, a subject protein that specifically binds to PD-L1 is a humanized antibody. In some cases, a subject protein that specifically binds to PD-L1 is an scFv (*e.g.*, as described above) fused to an Fc domain (or a fragment thereof) (*e.g.*, an IgG1 Fc, an IgG2 Fc, an IgG3 Fc, an IgG4 Fc, or a fragment thereof). In some cases, a subject protein that specifically binds to PD-L1 includes an amino acid sequence having 80% or more sequence identity (*e.g.*, 85% or more, 90% or more, 95% or more, 98% or more, 99% or more, or 100% sequence identity) with the amino acid sequence set forth in any one of SEQ ID NOs:18 and 20 (*e.g.*, see Figure 7).

[00176] In some embodiments, the subject protein that specifically binds to PD-L1 comprises:

MGSTAILALLLAVLQGVSAEVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMS
WVRQAPGKGLEWVSTISGSGGTTYYADSVKGRFTISRDNSKNTLYLQMNSLRV
EDTAVYYCAKDWFRSSSPDAFDIWGQGTTVTVSAGGGGSGGGGSGGGGSGAPS
YVLTQPPSVSVAPGQTARITCGGNNIGRKIVHWYQQRPGQAPVLVIYYDTDRPA
GIPERFSGSNSGNMATLTISTVGAGDEADYYCQVWDTGSDHVVFGGGTKLTVL
GPRANFVYKSGPRPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV
TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK

(SEQ ID NO: 18).

[00177] In some embodiments, the subject protein that specifically binds to PD-L1 comprises:

MGSTAILALLLAVLQGVSAQVQLQESGGGLVKPGGSLRLSCAASGFTFSSYSMN
WVRQAPGKGLEWVSGINTAGDTHYPESVKGRFTISRDNARNSLNLQMNSLRAE
DTAVYYCVRERVEREYSGYDAFDIWGQGTTVTVSAGGGGSGGGGSGGGGSGA
PNFMLTQPHSVSESLGKTVTISCTGSSGSIARKFVQWYQQRPGSSPTTVIYENNQ
RPSGVSDRFSGSIGSSSNSASLTISGLKTEDEADYYCQSYDSSNVVFGGGTKVTV
LGPRANFVYKSGPRPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK

(SEQ ID NO: 20).

[00178] A subject PD-L1 binding protein (*e.g.*, an scFV) can be fused to a fusion partner. Examples of fusion partners for a subject PD-L1 binding protein (*e.g.*, scFV) include but are not limited to: (a) a ligand for an NK activation receptor (*e.g.*, ectodomain ULBP1), (b) the Fc domain of any human immunoglobulin (*e.g.* IgG4 or IgG1) and their variants (*e.g.* monomeric hinge mutant, FcR binding mutant), (c) a self-dimerizing protein (*e.g.*, leucine zipper protein), (d) human CD4 domains 3 and 4 (3/4), and (e) the Carboxy-Terminal Peptide (CTP) of Human Chorionic Gonadotropin (hCG).

[00179] Example sequences of the above fusion partners are as follows, and include sel-dimerizing leucine zipper proteins, human CD4 domains 3 and 4 (3/4), and Carboxy-Terminal Peptide (CTP) of Human Chorionic Gonadotropin (hCG).

[00180] In some embodiments, the self-dimerizing leucine zipper protein comprises:

RSGSSRMetKQIEDKIEEILSKIYHIENEIARIKKLIGERGTSSRG (SEQ ID NO:24)

[00181] In some embodiments, the human CD4 domains 3 and 4 (3/4) comprises

ASSIVYKKEGEQVEFSFPLAFTVEKLTGSGELWWQAERASSSKSWITFDLKNKEVS VKRVTQDPKLQMetGKKLPLHLTLPQALPQYAGSGNLTLALEAKTGKLHQEVNLV VMetRATQLQKNLTCEVWGPTSPKLMetLSLKLENKEAKVSKREKAVWVLNPEAG MetWQCLLSDSGQVLLESNIKVL (SEQ ID NO:25)

[00182] In some embodiments, the Carboxy-Terminal Peptide (CTP) of Human Chorionic Gonadotropin (hCG) comprises:

SSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO:26)

PD-L1 binding protein properties & co-compositions

[00183] In some embodiments, the compositions and methods described include a PD-1 inhibitor that binds human PD-1 with a K_D of about 100 pM or lower, binds human PD-1 with a K_D of about 90 pM or lower, binds human PD-1 with a K_D of about 80 pM or lower, binds human PD-1 with a K_D of about 70 pM or lower, binds human PD-1 with a K_D of about 60 pM or lower, binds human PD-1 with a K_D of about 50 pM or lower, binds human PD-1 with a K_D of about 40 pM or lower, binds human PD-1 with a K_D of about 30 pM or lower, binds human PD-1 with a K_D of about 20 pM or lower, binds human PD-1 with a K_D of about 10 pM or lower, or binds human PD-1 with a K_D of about 1 pM or lower.

[00184] In some embodiments, the compositions and methods described include a PD-1 inhibitor that binds to human PD-1 with a k_{assoc} of about 7.5×10^5 l/M·s or faster, binds to human PD-1 with a k_{assoc} of about 7.5×10^5 1/M·s or faster, binds to human PD-1 with a k_{assoc} of about 8×10^5 1/M·s or faster, binds to human PD-1 with a k_{assoc} of about 8.5×10^5 1/M·s or faster, binds to human PD-1 with a k_{assoc} of about 9×10^5 1/M·s or faster, binds to human PD-1 with a k_{assoc} of about 9.5×10^5 l/M·s or faster, or binds to human PD-1 with a k_{assoc} of about 1×10^6 1/M·s or faster.

[00185] In some embodiments, the compositions and methods described include a PD-1 inhibitor that binds to human PD-1 with a k_{dissoc} of about 2×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.1×10^{-5} 1/s or slower , binds to human PD-1 with a k_{dissoc} of about 2.2×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.3×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.4×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.5×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.6×10^{-5} 1/s

or slower or binds to human PD-1 with a k_{dissoc} of about 2.7×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.8×10^{-5} 1/s or slower, binds to human PD-1 with a k_{dissoc} of about 2.9×10^{-5} 1/s or slower, or binds to human PD-1 with a k_{dissoc} of about 3×10^{-5} 1/s or slower.

[00186] In some embodiments, the compositions and methods described include a PD-1 inhibitor that blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 10 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 9 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 8 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 7 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 6 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 5 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 4 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 3 nM or lower, blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 2 nM or lower, or blocks or inhibits binding of human PD-L1 or human PD-L2 to human PD-1 with an IC_{50} of about 1 nM or lower.

[00187] In some embodiments, the PD-L1 binding protein is a PD-L1 antibody. In some embodiments, the PD-L1 antibody is a high affinity antibody. In some embodiments, the PD-L1 antibody is a human PD-L1 antibody. In some embodiments, the PD-L1 antibody is a murine antibody, a chimeric antibody, or humanized antibody. In some embodiments, the PD-L1 antibody is a monoclonal antibody. In some embodiments, the PD-L1 antibody binds to human PD-1 with a K_D of 5×10^{-8} M or less, binds to human PD-1 with a K_D of 1×10^{-8} M or less, binds to human PD-1 with a K_D of 5×10^{-9} M or less, or binds to human PD-1 with a K_D of between 1×10^{-8} M and 1×10^{-10} M. In some embodiments, the PD-L1 antibody binds to human PD-1 with a KD of 1×10^{-7} M or less.

Nucleic acids encoding a subject protein

Nucleic Acids and Vectors

[00188] Provided are nucleic acids that encode a subject protein (that specifically binds to PD-L1). A subject protein can be encoded by one or more nucleic acids. For example, in cases where the first and second polypeptides of the antigen binding portion are separate polypeptides (*i.e.*, are not fused to one another), the first and second polypeptides can be encoded on the same or on different nucleic acids. In some embodiments, the one or more nucleic acids that encode a

subject PD-L1 binding protein (*e.g.*, antibody, scFv, a maxibody, etc.) are included in an expression vector. In some embodiments, the first and second polypeptides are encoded on the same vector. In some embodiments, the first and second polypeptides are encoded on different vectors. In some embodiments, the first polypeptide is encoded on a first vector. In some embodiments, the second polypeptide is encoded on a second vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the viral vector encodes the PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In

some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the heavy and light chains are encoded on the same vector. In some embodiments, the heavy and light chains are encoded on different vectors. In some embodiments, the heavy chain is encoded on a first vector. In some embodiments, the light chain is encoded on a second vector. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a

sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00189] Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity.

[00190] The expression vector may be provided to a cell (*e.g.*, introduced into a cell) in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al, (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-

associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers.

[00191] A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to the desired cells.

[00192] The present disclosure also provides expression vectors in which a nucleic acid construct of the present disclosure is inserted. In some embodiments, an inducible expression system may be utilized. Where inducible expression is desired, a number of vectors facilitating such inducible expression are available, including, but not limited to, tetracycline- and tetracycline analogue- inducible vectors, tamoxifen-inducible vectors, as well as other inducible transcription system vectors known in the art.

[00193] The nucleic acid construct can be operably linked to control elements directing the transcription or expression thereof in the nucleotide sequence *in vivo*. Such control elements can comprise control sequences normally associated with the selected gene (*e.g.*, endogenous cellular control elements). Alternatively, heterologous control sequences can be employed. Useful heterologous control sequences generally include those derived from sequences encoding mammalian or viral genes. Examples include, but are not limited to, the SV40 early promoter, mouse mammary tumor virus long terminal repeat (LTR) promoter; adenovirus major late promoter (Ad MLP); a herpes simplex virus (HSV) promoter, an endogenous cellular promoter heterologous to the gene of interest, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter region (CMVIE), a rous sarcoma virus (RSV) promoter, synthetic promoters, hybrid promoters, and the like. In addition, sequences derived from nonviral genes, such as the murine metallothionein gene, can also be used. Such promoter sequences are commercially available from, *e.g.*, Stratagene (San Diego, Calif.).

[00194] In some embodiments, a cell type-specific or a tissue-specific promoter can be operably linked to nucleic acid insert encoding the heterologous gene product, and allowing for selectively or preferentially producing a gene product in a particular cell type(s) or tissue(s), for example expression in cytotoxic lymphocytes. In some embodiments, an inducible promoter can be operably linked to the heterologous nucleic acid.

[00195] The vectors disclosed herein can also include conventional control elements operably linked to the nucleic acid insert (also referred to as a heterologous nucleotide sequence)

in a manner permitting transcription, translation and/or expression in a cell transfected with the vector produced according to the present invention. As used herein, "operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act *in trans* or at a distance to control the gene of interest.

[00196] Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (*i.e.*, Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. A great number of expression control sequences, including promoters selected from native, constitutive, inducible and/or tissue-specific, are known in the art and may be utilized.

[00197] Examples of constitutive promoters include, without limitation, the retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), murine cell virus (MSCV) promoter, the cytomegalovirus (CMV) promoter (optionally with the CMV enhancer) (*see*, *e.g.*, Boshart *et al.*, Cell, 41:521-530 (1985)), the SV40 promoter, the dihydrofolate reductase promoter, the beta-actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EF1 promoter (Invitrogen). Inducible promoters allow regulation of gene expression and can be regulated by exogenously supplied compounds, environmental factors such as temperature, or the presence of a specific physiological state, *e.g.*, acute phase, a particular differentiation state of the cell, or in replicating cells only. Inducible promoters and inducible systems are available from a variety of commercial sources, including, without limitation, Invitrogen, Clonetech and Ariad. Many other systems have been described and can be readily selected by one of skill in the art. Examples of inducible promoters regulated by exogenously supplied compounds, include, the zinc-inducible sheep metallothionine (MT) promoter, the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter, the T7 polymerase promoter system (WO 98/10088); the ecdysone insect promoter (No *et al.*, (1996) *Proc. Natl. Acad. Sci. USA*, 93:3346-3351), the tetracycline-repressible system (Gossen *et al.*, (1992) *Proc. Natl. Acad. Sci. USA*, 89:5547-5551), the tetracycline-inducible system (Gossen *et al.*, (1995) *Science*, 268:1766-1769, see also Harvey *et al.*, (1998) *Curr. Opin. Chem. Biol.*, 2:512-518), the RU486-inducible system (Wang *et al.*, (1997) *Nat. Biotech.*, 15:239-243 and Wang *et al.*, (1997) *Gene Ther.*, 4:432-441) and the rapamycin-inducible system (Magari *et al.*, (1997) *J. Clin. Invest.*, 100:2865-2872). Other types of inducible promoters useful in this context are those regulated by a specific physiological state, *e.g.*, temperature, acute phase, a particular differentiation state of the cell, or in replicating cells only. In some embodiments, a lentiviral vector containing a murine cell virus

(MSCV) promoter or a human elongation factor-1 alpha (EF-1 alpha) promoter is utilized to express a nucleic acid encoding a subject PD-L1 binding protein as described herein, *e.g.*, in connection with any embodiment described herein.

[00198] In another embodiment, the native promoter for the nucleic acid insert will be used. The native promoter may be preferred when it is desired that expression of the nucleic acid insert should mimic the native expression. The native promoter may be used when expression of the nucleic acid insert must be regulated temporally or developmentally, or in a tissue-specific manner, or in response to specific transcriptional stimuli. In a further embodiment, other native expression control elements, such as enhancer elements, polyadenylation sites or Kozak consensus sequences may also be used to mimic the native expression.

[00199] Another embodiment of the nucleic acid includes a gene operably linked to a tissue-specific promoter. For instance, if expression in T-cells and/or cytotoxic lymphocytes is desired, a promoter active in T-cells and/or cytotoxic lymphocytes should be employed.

[00200] In various embodiments, the vector carrying one or more nucleic acid inserts also include selectable markers or reporter genes, *e.g.*, sequences encoding geneticin, hygromycin or puromycin resistance, among others. Selectable reporters or marker genes can be used to signal the presence of the plasmids/vectors in bacterial cells, including, for example, examining ampicillin resistance. Other components of the plasmid may include an origin of replication. Selection of these and other promoters and vector elements are conventional and many such sequences are available (*see*, *e.g.*, Sambrook *et al.*, and references cited therein).

[00201] In some cases, a subject nucleic acid includes a promoter that is operably linked to the nucleotide sequence encoding the subject PD-L1 binding protein. Suitable promoters include constitutive promoters (*e.g.*, CMV promoter, EF1alpha promoter, MSCV, and the like) as well as inducible promoters (*e.g.*, Tetracycline- and tetracycline analogue- inducible promoters, tamoxifen-inducible promoters and the like) and conditional promoters such as the NR4A1 promoter. In some embodiments, the vector comprises transposons.

[00202] In some embodiments, the vector is pLEV having a nucleotide sequence as provided in Figure 10. In some embodiments, the pLEV vector containing the PD-L1 binding protein is provided in Figure 11. In some embodiments, the vector is SEQ ID NO:41 (Figure 11A). In some embodiments, the vector is SEQ ID NO:42 (Figure 11B). In some embodiments, the vector is vector shown in Figure 16A. In some embodiments, the vector is shown in Figure 16B.

[00203] In some embodiments, the vector is a retroviral vector, such as a gammaretroviral vector.

[00204] In some cases, a subject nucleic acid encoding a PD-L1 binding protein is included in a plasmid, *e.g.*, for transient expression in a eukaryotic cell, for genomic integration of an expression cassette (which includes the nucleotide sequence encoding the PD-L1 binding protein operably linked to a promoter) in a eukaryotic cell, for propagation in a bacterial cell, and the like.

Cells

[00205] The present disclosure provides cells that include a subject protein that specifically binds to PD-L1, and/or include a nucleic acid (*e.g.*, as discussed above) encoding the protein. For cells that include a nucleic acid encoding a subject protein that specifically binds to PD-L1, the nucleic acid can be maintained episomally (*e.g.*, can be a plasmid), or the nucleic acid can be integrated into the genome. In some embodiments, the first and second polypeptides are encoded on the same nucleic acid. In some embodiments, the first and second polypeptides are encoded on different nucleic acids. In some embodiments, the first polypeptide is encoded on a nucleic acid. In some embodiments, the second polypeptide is encoded on a nucleic acid. In some embodiments, the nucleic acid is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments the nucleic acid encodes a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-

L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the heavy and light chains are encoded on the same vector. In some embodiments, the heavy and light chains are encoded on different vectors. In some embodiments, the heavy chain is encoded on a first vector. In some embodiments, the light chain is encoded on a second vector. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence

having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00206] Cells can be any convenient-cell type. For example, in some cases, a subject-cell is a prokaryotic cell. For example, the can be an *E. coli* cell used for propagating a nucleic acid (*e.g.*, a plasmid) that encodes a subject protein. In some cases, the cell is a eukaryotic cell (*e.g.*, a mammalian cell, a rat-cell, a mouse cell, a human cell). In some cases, the cell is a cytotoxic lymphocyte. In some embodiments, the cell is a cytotoxic lymphocyte and can be referred to as a cytotoxic lymphocyte that is genetically modified to express and secrete a soluble Programmed Death 1 Ligand (PD-L1) binding protein. In such cases, the term "genetically modified" encompasses scenarios in which the nucleic acid is maintained episomally as well as scenarios in which the nucleic acid is integrated into the cell's genome. In some cases, the cell is a cytotoxic lymphocyte that is genetically modified to express and secrete a soluble Programmed Death 1 Ligand (PD-L1) binding protein, where the genetic modification is a genome modification (*i.e.*, a nucleic acid encoding a subject protein is integrated into the genome of the cell.

[00207] Cytotoxic lymphocytes which may be genetically modified to express and secrete a subject PD-L1 binding protein as described herein, *e.g.*, in connection with any embodiment described herein, include cytotoxic T (CTL) cells, natural killer T (NKT) cells and natural killer (NK) cells. Cytotoxic lymphocytes can include, for example, peripheral blood-derived αβ TCR-positive or γδ TCR-positive T-cells activated by tumor associated antigens and/or transduced with tumor specific chimeric antigen receptors or T-cell receptors, and tumor-infiltrating lymphocytes (TILs). In some embodiments, the cytotoxic lymphocyte has been modified to express and secrete a subject PD-L1 binding protein selected from the group consisting of 38A1 and 19H9. In some embodiments, the cytotoxic lymphocyte has been modified to express and secrete 38A1. In some embodiments, the cytotoxic lymphocyte has been modified to express and secrete 19H9.

[00208] In some embodiments, a cytotoxic T-cell according to the present disclosure is a $CD8^+$ T-cell. In some embodiments, a cytotoxic T-cell according to the present disclosure is a $CD4^+$ T-helper cell. In some embodiments, a cytotoxic T-cell according to the present disclosure is a $CD8^+$ T-cell modified to express and secrete a subject PD-L1 binding protein as described herein. In some embodiments, a cytotoxic T-cell according to the present disclosure is a $CD4^+$ T-helper cell modified to express and secrete a subject PD-L1 binding protein as described herein. In some embodiments, a cytotoxic T-cell according to the present disclosure is a $CD8^+$ T-cell modified to express and secrete 38A1. In some embodiments, a cytotoxic T-cell according to the present disclosure is a $CD4^+$ T-helper cell modified to express and secrete 19H9. In some embodiments, a cytotoxic T-cell according to the present disclosure is a $CD8^+$ T-cell modified to

express and secrete a PD-L1 binding protein. In some embodiments, a cytotoxic T-cell according to the present disclosure is a $CD4^+$ T-helper cell modified to express and secrete a PD-L1 binding protein. In some embodiments, the PD-L1 binding protein is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein is 38A1. In some embodiments, the PD-L1 binding protein is 19H9. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody

or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9

and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00209] A cytotoxic lymphocyte which may be genetically modified to express and secrete a subject PD-L1 binding protein as described herein, *e.g.*, in connection with any embodiment described herein, may express one or more activation antigens. For example, a cytotoxic lymphocyte according to the present disclosure may exhibit an increased level of expression of one or more activation antigens relative to a naïve T-cell. The one or more activation antigens may be selected from, *e.g.*, CD25, CD26, CD27, CD28, CD38, CD40L, CD69, CD134, CD137, BTLA, PD-1, HVEM, LIGHT, and HLA-DR. In some embodiments, the PD-L1 binding protein is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein is 38A1. In some embodiments, the PD-L1 binding protein is 19H9. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody

or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises

SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00210] In some embodiments, a cytotoxic lymphocyte which may be genetically modified to express and secrete a subject PD-L1 binding protein as described herein, *e.g.*, in connection with any embodiment described herein, includes or is genetically modified to include a receptor specific for a tumor associated antigen, *e.g.*, a tumor associated antigen from a tumor of a subject to be treated with a genetically modified cytotoxic lymphocyte according to the present disclosure. In some embodiments, the PD-L1 binding protein is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein is 38A1. In some embodiments, the PD-L1 binding protein is 19H9. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the

percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody

or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00211] A cytotoxic lymphocyte which may be genetically modified to express and secrete a subject PD-L1 binding protein as described herein, *e.g.*, in connection with any

embodiment described herein, may be obtained from any suitable tissue or fluid of a subject. For example, in some embodiments a cytotoxic lymphocyte may be obtained from peripheral blood of a subject. In some embodiments, a cytotoxic lymphocyte which may be genetically modified to express and secrete a subject PD-L1 binding protein as described herein, *e.g.*, in connection with any embodiment described herein, is a TIL derived from a tumor of a subject. In some embodiments, the PD-L1 binding protein is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein is 38A1. In some embodiments, the PD-L1 binding protein is 19H9. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy

chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ

ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

Detectable Labels

[00212] In some embodiments, a subject protein that specifically binds to PD-L1 (*i.e.* a subject PD-L1 binding protein) as described herein, *e.g.*, in connection with any embodiment described herein, includes one or more detectable labels. A variety of suitable detectable labels are known in the art including, but not limited to, radioactive isotopes, fluorescers, chemiluminescers, chromophores, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, chromophores, dyes, metal ions, metal sols, ligands (*e.g.*, biotin, avidin, strepavidin or haptens) and the like. The term "fluorescer" refers to a substance or a portion thereof which is capable of exhibiting fluorescence in the detectable range. Examples of detectable labels suitable for use as components of subject PD-L1 binding proteins include affinity tags and fluorescent proteins (*e.g.*, GFP, YFP, RFP, CFP, and the like). In some embodiments, the PD-L1 binding protein is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein is 38A1. In some embodiments, the PD-L1 binding protein is 19H9. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the

percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a

heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00213] The term "affinity tag" is used herein to denote a peptide segment, *e.g.*, a heterologous peptide segment that can be incorporated into a subject PD-L1 binding protein and detected using a molecule that binds the affinity tag and provides a detectable signal (*e.g.*, a fluorescent compound or protein). In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Examples of affinity tags

suitable for use include, but are not limited to, a monocytic adaptor protein (MONA) binding peptide, a T7 binding peptide, a streptavidin binding peptide, a polyhistidine tract, protein A (Nilsson et al., EMBO J. 4:1075 (1985); Nilsson et al., Methods Enzymol. 198:3 (1991)), glutathione S transferase (Smith and Johnson, Gene 67:31 (1988)), Glu-Glu affinity tag (Grussenmeyer et al., Proc. Natl. Acad. Sci. USA 82:7952 (1985)), substance P, FLAG peptide (Hopp et al., Biotechnology 6:1204 (1988)), or other antigenic epitope or binding domain. See, in general, Ford et al., Protein Expression and Purification 2:95 (1991). DNA molecules encoding affinity tags are available from commercial suppliers (*e.g.*, Pharmacia Biotech, Piscataway, N.J.). In some embodiments, the PD-L1 binding protein is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein is 38A1. In some embodiments, the PD-L1 binding protein is 19H9. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased

secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments,

conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00214] Any fluorescent polypeptide (also referred to herein as a fluorescent label) well known in the art is suitable for use as a detectable label directly or in connection with an affinity tag. A suitable fluorescent polypeptide will be one that can be expressed in a desired hosT-cell, such as a bacterial cell or a mammalian cell, and will readily provide a detectable signal that can be assessed qualitatively (positive/negative) and quantitatively (*e.g.*, comparative degree of fluorescence). Examples of fluorescent polypeptides include, but are not limited to, yellow fluorescent protein (YFP), cyan fluorescent protein (CFP), GFP, mRFP, RFP (tdimer2), HCRED, etc., or any mutant (*e.g.*, fluorescent proteins modified to provide for enhanced fluorescence or a shifted emission spectrum), analog, or derivative thereof. Further suitable fluorescent polypeptides, as well as specific examples of those listed herein, are provided in the art and are well known. In some embodiments, the PD-L1 binding protein is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein is 38A1. In some embodiments, the PD-L1 binding protein is 19H9. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the

number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some

embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00215] Biotin-based labels may also find use in the subject PD-L1 binding proteins disclosed herein. Biotinylation of molecules and substrates is well known, for example, a large number of biotinylation agents are known, including amine-reactive and thiol-reactive agents, for the biotinylation of proteins, nucleic acids, carbohydrates, carboxylic acids; see, *e.g.*, chapter

4, Molecular Probes Catalog, Haugland, 6th Ed. 1996, hereby incorporated by reference. A biotinylated substrate can be detected by binding of a detectably labeled biotin binding partner, such as avidin or streptavidin. Similarly, a large number of haptenylation reagents are also known. In some embodiments, the PD-L1 binding protein is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein is 38A1. In some embodiments, the PD-L1 binding protein is 19H9. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or

98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv)

comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

METHODS

Adoptive Cell Transfer

[00216] Adoptive cell transfer (ACT) is a very effective form of immunotherapy and involves the transfer of immune cells with antitumor activity into cancer patients. ACT is a treatment approach that involves the identification, *in vitro*, of lymphocytes with antitumor activity, the in vitro expansion of these cells to large numbers and their infusion into the cancer-bearing host. Lymphocytes used for adoptive transfer can be derived from the stroma of resected tumors (tumor infiltrating lymphocytes or TILs). They can also be derived or from blood if they are genetically engineered to express antitumor T-cell receptors (TCRs) or chimeric antigen receptors (CARs), enriched with mixed lymphocyte tumor cell cultures (MLTCs), or cloned using autologous antigen presenting cells and tumor derived peptides. ACT in which the lymphocytes originate from the cancer-bearing host to be infused is termed autologous ACT. U.S. Publication No. 2011/0052530 relates to a method for performing adoptive cell therapy to promote cancer regression, primarily for treatment of patients suffering from metastatic melanoma, which is incorporated by reference in its entirety for these methods.

[00217] In some embodiments, TILs and/or cytotoxic lymphocytes may be administered in a single dose. Such administration may be by injection, *e.g.*, intravenous injection. In some embodiments, TILs and/or cytotoxic lymphocytes may be administered in multiple doses. Dosing may be once, twice, three times, four times, five times, six times, or more than six times per year. Dosing may be once a month, once every two weeks, once a week, or once every other day. Administration of TILs and/or cytotoxic lymphocytes may continue as long as necessary.

[00218] In some embodiments, an effective dosage of TILs and/or cytotoxic lymphocytes is about 1×10^6, about 2×10^6, about 3×10^6, about 4×10^6, about 5×10^6, about 6×10^6, about 7×10^6, about 8×10^6, about 9×10^6, about 1×10^7, about 2×10^7, about 3×10^7, about 4×10^7, about 5×10^7,

about 6×10^7, about 7×10^7, about 8×10^7, about 9×10^7, about 1×10^8, about 2×10^8, about 3×10^8, about 4×10^8, about 5×10^8, about 6×10^8, about 7×10^8, about 8×10^8, about 9×10^8, about 1×10^9, about 2×10^9, about 3×10^9, about 4×10^9, about 5×10^9, about 6×10^9, about 7×10^9, about 8×10^9, about 9×10^9, about 1×10^{10}, about 2×10^{10}, about 3×10^{10}, about 4×10^{10}, about 5×10^{10}, about 6×10^{10}, about 7×10^{10}, about 8×10^{10}, about 9×10^{10}, about 1×10^{11}, about 2×10^{11}, about 3×10^{11}, about 4×10^{11}, about 5×10^{11}, about 6×10^{11}, about 7×10^{11}, about 8×10^{11}, about 9×10^{11}, about 1×10^{12}, about 2×10^{12}, about 3×10^{12}, about 4×10^{12}, about 5×10^{12}, about 6×10^{12}, about 7×10^{12}, about 8×10^{12}, about 9×10^{12}, about 1×10^{13}, about 2×10^{13}, about 3×10^{13}, about 4×10^{13}, about 5×10^{13}, about 6×10^{13}, about 7×10^{13}, about 8×10^{13}, and about 9×10^{13}. In some embodiments, an effective dosage of TILs and/or cytotoxic lymphocytes is in the range of about 1×10^6 to about 5×10^6, about 5×10^6 to about 1×10^7, about 1×10^7 to about 5×10^7, about 5×10^7 to about 1×10^8, about 1×10^8 to about 5×10^8, about 5×10^8 to about 1×10^9, about 1×10^9 to about 5×10^9, about 5×10^9 to about 1×10^{10}, about 1×10^{10} to about 5×10^{10}, about 5×10^{10} to about 1×10^{11}, about 5×10^{11} to about 1×10^{12}, about 1×10^{12} to about 5×10^{12}, and about 5×10^{12} to about 1×10^{13}. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9 or 38A1. In some embdiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embdiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9 from pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1 from pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embdiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embdiments, the TIL and/or cytotoxic lymphocytes are genetically modified with a lentiviral vector to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to

19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the heavy and light chains are encoded on the same vector or nucleic acid. In some embodiments, the heavy and light chains are encoded on different vectors or nucleic acids. In some embodiments, the heavy chain is encoded on a first vector or nucleic acid. In some embodiments, the light chain is encoded on a second vector or nucleic acid. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID

NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

Contacting cells with a subject protein

[00219] Provided are methods of reducing the interaction between PD-L1 on a first-cell (*e.g.*, a cancer cell such as a tumor cell) and PD-1 on another cell (*e.g.*, an immune cell such as a T-cell). Such methods can include contacting PD-L1 on the first-cell with a subject PD-L1 binding protein (*e.g.*, anti-PD-L1 antibody, anti-PD-L1 scFV, anti-PD-L1 maxibody, etc.). Any amount of reduction can be useful in some cases (depending on context). In some cases, the interaction between PD-L1 and PD-1 is reduced by 10% or more (*e.g.*, 20% or more, 30 % or more, 50% or more, 60% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, or 100%). In some embodiments, the PD-L1 binding protein is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein is 38A1. In some embodiments, the PD-L1 binding protein is 19H9. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling

pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding

protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00220] Any convenient assay can be used to measure the amount of reduction and such assays will be known to one of ordinary skill in the art. Examples of assays for measuring the amount of reduction (*e.g.*, where the assays can be performed in the presence versus absence of a subject PD-L1 binding protein such as an anti-PD-L1 antibody, anti-PD-L1 scFV, anti-PD-L1 maxibody, and the like) can include but are not limited to: measuring the inhibition of cytokine production in mixed lymphocyte reaction assays (MLR assays, *e.g.*, allo MLR assays), measuring interactions between tumor infiltrating lymphocytes (TILs) and a homologous tumor, measuring cytotoxicity (*e.g.*, cytotoxicity assays such as assays to measure cytotoxic T-cell activity toward a PD-L1 expressing cell such as a cancer cell), measuring NFkB translocation to the nucleus, and measuring signaling activity/output of the PI3K/AKT/mTOR signaling pathway (*e.g.*, see Wang *et. al.*, Cancer Immunol Res. 2014 Sep;2(9):846-56).

[00221] The contacting can take place in vitro (*e.g.*, cells in culture). In some cases, the contacting is in vivo. For example, in some cases, the contacting includes administering a subject PD-L1 binding protein to an individual. In such cases, the method can be considered to be a method of treatment (for an individual who has cancer, for an individual who has a solid tumor, for an individual who has a chronic infection (*e.g.*, a chronic viral infection), and the like). In some cases, contacting includes introducing into a cell (*e.g.*, the cell expressing PD-L1, the cell expressing PD-1, or a third cell) a nucleic acid encoding a subject PD-L1 binding protein, and the cell into which the nucleic acid is introduced produces and secretes the PD-L1

binding protein. In cases where the nucleic acid is introduced into a third cell, the third cell can then be used to provide the PD-L1 binding protein via secretion. In some embodiments, where the nucleic acid is introduced into a third cell, the third cell can then be used to provide to the individual or subject in need thereof the PD-L1 binding protein via secretion. In some embodiments, the PD-L1 binding protein is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein is 38A1. In some embodiments, the PD-L1 binding protein is 19H9. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or

98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ

ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00222] Therapeutic formulations comprising one or more PD-L1 binding proteins of the disclosure can be prepared for storage by mixing the PD-L1 binding protein having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. PD-L1 binding protein composition can be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The "therapeutically effective amount" of the PD-L1 binding protein to be administered can be governed by such considerations, and is the minimum amount necessary to treat the patient's disease (*e.g.*, cancer, chronic infection). In some embodiments, the PD-L1 binding protein is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein is 38A1. In some embodiments, the PD-L1 binding protein is 19H9. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry

as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID

NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00223] The therapeutic dose may be at least 0.01 mg/kg body weight, at least 0.05 mg/kg body weight; at least 0.1 mg/kg body weight, at least 0.5 mg/kg body weight, at least 1 mg/kg body weight, at least 2 mg/kg body weight, at least 2.5 mg/kg body weight, at least 5 mg/kg body weight, at least 7.5 mg/kg body weight, at least 10 mg/kg body weight, at least 15 mg/kg body weight, at least 20 mg/kg body weight, at least 25 mg/kg body weight, at least 30 mg/kg body weight, at least 35 mg/kg body weight, at least 40 mg/kg body weight, at least 45 mg/kg

body weight, at least 50 mg/kg body weight, at least 55 mg/kg body weight, at least 60 mg/kg body weight, at least 65 mg/kg body weight, at least 70 mg/kg body weight, at least 75 mg/kg body weight, at least 80 mg/kg body weight, at least 85 mg/kg body weight, at least 90 mg/kg body weight, at least 95 mg/kg body weight, at least 100 mg/kg body weight, at least 110 mg/kg body weight, at least 120 mg/kg body weight, at least 130 mg/kg body weight, at least 140 mg/kg body weight, at least 150 mg/kg body weight, at least 160 mg/kg body weight, at least 170 mg/kg body weight, at least 180 mg/kg body weight, at least 190 mg/kg body weight, at least 200 mg/kg body weight, at least 210 mg/kg body weight, at least 220 mg/kg body weight, at least 230 mg/kg body weight, at least 240 mg/kg body weight, at least 250 mg/kg body weight, at least 260 mg/kg body weight, at least 270 mg/kg body weight, at least 280 mg/kg body weight, at least 290 mg/kg body weight, at least 300 mg/kg body weight, at least 310 mg/kg body weight, at least 320 mg/kg body weight, at least 330 mg/kg body weight, at least 340 mg/kg body weight, at least 350 mg/kg body weight, at least 360 mg/kg body weight, at least 370 mg/kg body weight, at least 380 mg/kg body weight, at least 390 mg/kg body weight, at least 400 mg/kg body weight, at least 410 mg/kg body weight, at least 420 mg/kg body weight, at least 430 mg/kg body weight, at least 440 mg/kg body weight, at least 450 mg/kg body weight, at least 460 mg/kg body weight, at least 470 mg/kg body weight, at least 480 mg/kg body weight, at least 490 mg/kg body weight, and at least 500 mg/kg body weight. In some embodiments, the dosage is not more than 500 mg/kg body weight. It will be understood by one of skill in the art that such guidelines will be adjusted for the molecular weight of the active agent, *e.g.* in the use of antibody fragments, or in the use of antibody conjugates. The dosage may also be varied for localized administration, *e.g.* intranasal, inhalation, *etc.*, or for systemic administration, *e.g.* i.m., i.p., i.v., and the like. In some embodiments, the antibody administered is 19H9 or 38A1, or a combination thereof. In some embodiments, the antibody administered is 19H9. In some embodiments, a PD-L1 binding protein is administered. In some embodiments, the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the antibody administered is 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain

comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2,

IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00224] The PD-L1 binding protein need not be, but is optionally formulated with one or more agents that potentiate activity, or that otherwise increase the therapeutic effect. These are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages. In some embodiments, the PD-L1 binding protein is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein is 38A1. In some embodiments, the PD-L1 binding protein is 19H9. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion

capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments,

conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00225] Acceptable carriers, excipients, or stabilizers are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyidimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Formulations to be used for *in vivo* administration can be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[00226] The PD-L1 binding protein (*e.g.*, anti-PD-L1 antibody, anti-PD-L1 maxibody, anti-PD-L1 scFV, and the like) can be administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In addition, the PD-L1 binding protein (*e.g.*, anti-PD-L1 antibody, anti-PD-L1 maxibody, anti-PD-

L1 scFV, and the like) is suitably administered by pulse infusion, particularly with declining doses of the binding protein. In some cases, a subject PD-L1 binding protein is administered systemically (*e.g.*, i.v.). In some cases, a subject PD-L1 binding protein is administered locally (*e.g.*, intratumorally, *e.g.*, via injection). In some embodiments, the PD-L1 binding protein is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein is 38A1. In some embodiments, the PD-L1 binding protein is 19H9. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or

98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ

ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00227] For the prevention or treatment of disease, the appropriate dosage of PD-L1 binding protein can depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the PD-L1 binding protein is administered for preventive purposes, previous therapy, the patient's clinical history and response to the PD-L1 binding protein, and the discretion of the attending physician. The PD-L1 binding protein can be administered to the patient at one time or over a series of treatments. In some embodiments, the PD-L1 binding protein is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein is 38A1. In some embodiments, the PD-L1 binding protein is 19H9. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell

population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-

chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

Methods of Adoptive Cell Transfer with Cytotoxic Lymphocytes Genetically Modified to Express and Secrete a Soluble Programmed Death 1 Ligand (PD-L1) Binding Protein

[00228] Cytotoxic lymphocytes which express and secrete a subject PD-L1 binding protein as described herein, *e.g.*, in connection with any embodiment described herein, find use in methods of adoptive cell transfer, *e.g.*, in the context of treating cancer or chronic infection.

[00229] In some embodiments, suitable methods according to the present disclosure include, for example, isolating cytotoxic lymphocytes as described herein, *e.g.*, in connection with any embodiment described herein, from a subject, *e.g.*, from a tumor or peripheral blood of a subject.

[00230] In some embodiments, suitable methods according to the present disclosure include, for example, genetically modifying the cytotoxic lymphocytes by introducing into the

cytotoxic lymphocytes a nucleic acid encoding a subject PD-L1 binding protein as described herein, *e.g.*, in connection with any embodiment described herein, wherein the genetically modified cytotoxic lymphocytes express and secrete the subject PD-L1 binding protein as described herein, *e.g.*, in connection with any embodiment described herein. In some embodiments, the PD-L1 binding protein is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein is 38A1. In some embodiments, the PD-L1 binding protein is 19H9. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or

98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ

ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00231] In some embodiments, suitable methods according to the present disclosure include, for example, expanding the genetically modified cytotoxic lymphocyte to provide a population of genetically modified cytotoxic lymphocytes. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9 or 38A1. In some embdiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embdiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9 from pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1 from pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embdiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embdiments, the TIL and/or cytotoxic lymphocytes are genetically modified with a lentiviral vector to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control

antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the heavy and light chains are encoded on the same vector or nucleic acid. In some embodiments, the heavy and light chains are encoded on different vectors or nucleic acids. In some embodiments, the heavy chain is encoded on a first vector or nucleic acid. In some embodiments, the light chain is encoded on a second vector or nucleic acid. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof

comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00232] In some embodiments, suitable methods according to the present disclosure include, for example, administering the population of genetically modified cytotoxic

lymphocytes to the subject to treat a disease or disorder of the subject, *e.g.*, a cancer or chronic infection. In some embdiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9 or 38A1. In some embdiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embdiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9 from pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1 from pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embdiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embdiments, the TIL and/or cytotoxic lymphocytes are genetically modified with a lentiviral vector to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1

and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the heavy and light chains are encoded on the same vector or nucleic acid. In some embodiments, the heavy and light chains are encoded on different vectors or nucleic acids. In some embodiments, the heavy chain is encoded on a first vector or nucleic acid. In some embodiments, the light chain is encoded on a second vector or nucleic acid. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1

binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

Isolating Cytotoxic Lymphocytes from a Subject

[00233] Many suitable methods are known in the art to isolate cytotoxic lymphocytes from a subject, *e.g.*, a human subject, and any convenient method can be used. For example, tumor infiltrating lymphocytes as described herein, *e.g.*, in connection with any embodiment described herein, may be isolated from fresh patient biopsy specimens. Alternatively, cytotoxic lymphocytes may be obtained from peripheral blood of a subject. While the methods described herein are described primarily in the context of autologous cytotoxic lymphocytes used for ACT. It should be noted that in some embodiments cytotoxic lymphocytes may be obtained or generated from a source other than a subject to be treated, as discussed in further detail below.

Genetically Modifying the Cytotoxic Lymphocytes

[00234] Genetically modifying the cytotoxic lymphocytes to express and secrete a subject PD-L1 binding protein as described herein, *e.g.*, in connection with any embodiment described

herein, can include introducing into the cytotoxic lymphocytes a nucleic acid encoding a subject PD-L1 binding protein. In some embodiments, the PD-L1 binding protein comprises a first and second polypeptide. In some embodiments, the first and second polypeptides are encoded on the same vector. In some embodiments, the first and second polypeptides are encoded on different vectors. In some embodiments, the first polypeptide is encoded on a first vector. Many ways to accomplish this are known in the art and any convenient method can be used (*e.g.*, using any convenient vector or vector combination, *e.g.*, a retroviral vector, *e.g.*, a lentiviral vector, an adenoviral vector, or other vectors, *e.g.*, together with suitable packaging and envelope plasmids). Regulatory elements for lentiviral vectors, *e.g.*, promoters, may be selected specifically for stable expression in cytotoxic lymphocytes. See, *e.g.*, Jones et al. *Hum Gene Ther.* 2009 Jun; 20(6): 630–640, describing the use of a lentiviral vector containing a murine cell virus (MSCV) promoter for use with both minimally stimulated and highly activated lymphocytes. In some embodiments, the PD-L1 binding protein encoded by the vector is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein encoded by the vector is 38A1. In some embodiments, the PD-L1 binding protein encoded by the vector is 19H9. In some embodiments the PD-L1 binding protein encoded by the vector comprises an antigen binding portion. In some embodiments the PD-L1 binding protein encoded by the one or more vectors is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein encoded by the one or more vectors is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein encoded by the one or more vectors is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein encoded by the one or more vectors is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein encoded by the one or more vectors is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some

embodiments the PD-L1 binding protein encoded by the one or more vectors is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00235] In some embodiments, the subject PD-L1 binding protein gene can be part of a larger gene expression cassette introduced into the cytotoxic lymphocytes that allows for an inducible expression of the subject PD-L1 binding protein, *e.g.*, using a cell membrane permeant drug that activates the promoter elements driving the subject PD-L1 gene. Withdrawal of the

drug would shut-down the gene expression allowing for a regulatable on/off subject PD-L1 binding protein expression. A number of vectors facilitating this inducible expression are available, including, but not limited to, tetracycline- and tetracycline analogue- inducible vectors, tamoxifen-inducible vectors, as well as other inducible transcription system vectors known in the art. In some embodiments, the PD-L1 binding protein is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein is 38A1. In some embodiments, the PD-L1 binding protein is 19H9. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or

98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ

ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00236] A schematic of an example of an expression vector (*e.g.*, lentivector) encoding an inducible promoter is provided in **Figure 8**. The basic design includes two promoters. One promoter is constitutively active and drives the transcription of a conformational transcription factor (CTF) which changes conformation upon binding to a soluble molecule (SM). The second promoter binds the CTF and drives the gene of interest. There are two possibilities. In both cases, binding of the CTF to its promoter mediates transcription. In case one, addition of the SM releases the CTF from the inducible promoter, stopping transcription. In the second case, addition of SM induces binding of the CTF to the inducible promoter and initiation of transcription.

[00237] A schematic of another example of an expression vector (*e.g.*, lentivector) is provided in **Figure 9**, which depicts schematically an inhibitable expression vector coding for a conformational repressor protein. The basic design includes two promoters. Both promoters are constitutively active. One drives the transcription of a conformational repressor protein (CRP) which changes conformation upon binding to a soluble molecule (SM). The CRP binds to a CRP binding site (CRP-BS) and inhibits transcription of the second constitutive promoter driving transcription of the gene of interest. Addition of the soluble molecule releases the CRP from the CRP-BS "releasing" transcription of the gene of interest.

[00238] Figures 16 provides additional schematics of exemplary viral vectors for use with the present methods, including pLV4301G PDLV scFV 38A1 and pLV4301G PDLV scFV 19H9. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**).

[00239] The soluble molecule utilized as described above with the inducible expression vectors can be any suitable molecule known in the art for use with such inducible expression

vectors, *e.g.*, tetracycline (or analogs thereof) or tamoxifen. Such soluble molecules may be administered to a subject in accordance with one or more of the methods described herein, *e.g.*, prior to, concurrently with, or subsequent to the administration of genetically modified cytotoxic lymphocytes as described herein, *e.g.*, in connection with any embodiment described herein, to a subject. Alternatively, or in addition, expression vectors may be utilized which include elements responsive to conditions and/or molecules present at a desired site of action in a subject, *e.g.*, a tumor microenvironment. For example, expression vectors including a hypoxia response element may be used to selectively induce expression in the hypoxic microenvironment of a solid tumor. See, *e.g.*, Wang et al. *Cancer Gene Ther.* 2005 Mar;12(3):276-83.

[00240] Additional transfection methods, which may include viral and non-viral methods, may be utilized as appropriate to introduce nucleic acids encoding subject PD-L1 binding proteins into cytotoxic lymphocytes. Methods which may be utilized to facilitate delivery into cytotoxic lymphocytes can include, *e.g.*, electroporation, particle bombardment (gene gun), sonoporation, magnetofection, hydrodynamic delivery, nanoparticle delivery, lipofection, etc. In some embodiments, the PD-L1 binding protein encoded is 38A1 or 19H9. In some embodiments, the PD-L1 binding protein encoded is 38A1. In some embodiments, the PD-L1 binding protein encoded is 19H9. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*,

for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein encoded by one or more vectors is an antibody or fragment thereof, wherein the light chain is encoded by a first vector and the heavy chain is encoded by a second vector. In some embodiments the PD-L1 binding protein encoded by one or more vectors is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein encoded by one or more vectors is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein encoded by one or more vectors is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein encoded by one or more vectors is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98%

identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

Viral production

[00241] In some embodiments, the viral vector for transfection is produced using any methods well known in the art. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector

encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some

embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the heavy and light chains are encoded on the same vector. In some embodiments, the heavy and light chains are encoded on different vectors. In some embodiments, the heavy chain is encoded on a first vector. In some embodiments, the light chain is encoded on a second vector. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In

some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00242] In some embodiments, the viral vector is produced for transfection using a method comprising plating $5x10^6$ phoenix cells in 10 mm tissue culture plate containing 5 ml cell culture media. In some embodiments, the virus is produced for transfection using a method further comprising transfecting cells with 10 μg pLEV (lentiviral vector) containing anti-PD-1 scFV and 6 μg pVSV-G using lipofectamine. In some embodiments, the virus is produced for transfection using a method further comprising collecting the supernatant and filter with 70 μM strainer into a 15 ml tube after 60 minutes In some embodiments, the virus is produced for transfection using a method comprising the following steps: 1) plating $5x10^6$ phoenix cells in 10 mm tissue culture plate containing 5 ml cell culture media; 2) transfecting cells with 10 μg pLEV (lentiviral vector) containing anti-PD-1 scFV and 6 μg pVSV-G using lipofectamine; and 3) after 60 hours, collecting the supernatant and filter with 70 μM strainer into a 15 ml tube.

T-cell transduction

[00243] In some embodiments, T-cells, including for example, cytotoxic lymphocytes, are transduced with the viral vector (*i.e.*, genetically modified with the viral vector). In some embodiments, the cytotoxic lymphocytes are genetically modified cytotoxic lymphocytes. In some embodiments, the cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the viral vector is a pMSCIV based vector encoding a PD-L1 binding protein. In some embodiments, the T-cells, TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the T-cells, TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the T-cells, TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry

assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the heavy and light chains are encoded on the same vector. In some embodiments, the heavy and light chains are encoded on different vectors. In some embodiments, the heavy chain is encoded on a first vector. In some embodiments, the light chain is encoded on a second vector. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10

(CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00244] In some embodiments, the T-cells are transduced using a method comprising activating $1x10^6$ TIL with anti-CD3 at concentration of 300 ng/ml in 2 ml TIL culture media in 12-well-plate. In some embodiments, the transduction method further comprises splitting the TIL into two well and adding 1 ml of supernatant (from step 3 in viral production) into each well together with polybrene at a concentration of 8 μg/ml after 48 hours. In some embodiments, the transduction method further comprises centrifuging the cells for 30 minutes at 800 x g at 32 C.

In some embodiments, the transduction method further comprises removing virus containing medium and resuspending cell pellet with 2 ml of fresh complete culture media, and incubating the cells for 24 hours. In some embodiments, the transduction method further comprise propagating the cells using the Expanding the Genetically Modified Cytotoxic Lymphocytes provide below, as well as any other method know in the art for expanding TILs that could be applied to expanding cytotoxic lymphocytes within the TIL population.

Tumor Samples

[00245] In some embodiments, the method comprises obtaining a tumor tissue sample from the mammal, wherein the tumor sample comprises TILs containing the cytotoxic lymphocytes. The tumor tissue sample can be obtained from numerous sources, including but not limited to tumor biopsy or necropsy. The tumor tissue sample may be obtained from any cancer, including but not limited to any of the cancers described herein. In some embodiments, the cancer is melanoma. The tumor tissue sample may be obtained from any mammal. In some embodiments, the tumor tissue sample is obtained from a human. In some embodiments, the tumor tissue sample may be a tumor tissue fragment. The tumor tissue sample may be fragmented, *e.g.*, by dissection, to provide a tumor tissue fragment. In some embodiments, alternatively or additionally, the tumor tissue sample may, optionally, be enzymatically or mechanically digested. Suitable enzymes for fragmenting the tumor tissue sample include, but are not limited to, collagenase. In some embodiments, the tumor tissue sample is fragmented without digestion. The tumor tissue fragment may be any suitable size. Preferably, the tumor tissue fragment has a size of about 1 mm^3 or less to about 8 mm^3 or larger, about 1 mm^3 to about 4 mm^3, about 1 mm^3 to about 2 mm^3, or about 1 mm^3.

[00246] The cancer treated by the disclosed compositions and methods can in some aspects be any solid tumor for which TILs can be produced. The cancer can also be metastatic and/or recurrent. The cancer can be any cancer, including any of acute lymphocytic cancer, acute myeloid leukemia, alveolar rhabdomyosarcoma, bone cancer, brain cancer, breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vulva, chronic lymphocytic leukemia, chronic myeloid cancer, colon cancer, esophageal cancer, cervical cancer, digestive tract cancer, gastric cancer, gastrointestinal carcinoid tumor, glioma, hepatobiliary cancer, Hodgkin's lymphoma, hypopharynx cancer, kidney cancer, larynx cancer, liver cancer, lung cancer, malignant mesothelioma, melanoma, multiple myeloma, nasopharynx cancer, non-

Hodgkin's lymphoma, ovarian cancer, pancreatic cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, renal cancer, skin cancer, small intestine cancer, soft tissue cancer, stomach cancer, testicular cancer, thyroid cancer, ureter cancer, and urinary bladder cancer. In some embodiments, the cancer is melanoma. In some embodiments, the cancer is metastatic melanoma.

[00247] As used herein, the term "mammal" refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. It is preferred that the mammals are from the order Carnivora, including Felines (cats) and Canines (dogs). It is more preferred that the mammals are from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). It is most preferred that the mammals are of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). In some embodiments, the mammal is the human.

TIL Containing Cytotoxic Lymphocyte General Expansion Methods

[00248] Tumor-infiltrating lymphocyte (TIL) containing cytotoxic lymphocyte production is generally a 2-step process: 1) the pre-REP (Rapid Expansion) stage where you the grow the cells in standard lab media such as RPMI and treat the TILs w/reagents such as irradiated feeder cells, and anti-CD3 antibodies to achieve the desired effect; and 2) the REP stage where you expand the TIL containing cytotoxic lymphocyte in a large enough culture amount for treating the patients. The REP stage requires cGMP (current good manufacturing procedures) grade reagents and 30-40 L of culture medium. However, the pre-REP stage can utilize lab grade reagents (under the assumption that the lab grade reagents get diluted out during the REP stage), making it easier to incorporate alternative strategies for improving TIL production. Therefore, in some embodiments, the disclosed TLR agonist and/or peptide or peptidomimetics can be included in the culture medium during the pre-REP stage. The pre-REP culture can in some embodiments, include IL-2.

[00249] In some embodiments, ACT can be performed by (i) obtaining autologous TIL containing cytotoxic lymphocytes from a mammal, (ii) culturing (including, for example, REP) the autologous TIL containing cytotoxic lymphocytes to produce expanded lymphocytes, and (ii) administering the expanded TIL containing cytotoxic lymphocytes to the mammal. In some embodiments, the TIL containing cytotoxic lymphocytes are tumor-derived, *i.e.*, they are TILs containing cytotoxic, and are isolated from the mammal to be treated, *i.e.* autologous transfer. In some embodiments, the autologous TIL containing cytotoxic lymphocytes are genetically

modified cytotoxic lymphocytes. In some embodiments, the autologous cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, the autologous lymphocytes are genetically modified cytotoxic lymphocytes using a viral vector. In some embodiments, the autologous lymphocytes are genetically modified before or after culturing, but prior to administration to the mammal. In some embodiments, the cytotoxic lymphocytes are genetically modified cytotoxic lymphocytes. In some embodiments, the cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity

(MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the heavy and light chains are encoded on the same vector. In some embodiments, the heavy and light chains are encoded on different vectors. In some embodiments, the heavy chain is encoded on a first vector. In some embodiments, the light chain is encoded on a second vector. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID

NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00250] In some embodiments, autologous ACT as described herein can also be performed by (i) culturing autologous TIL containing cytotoxic lymphocytes to produce expanded lymphocytes; (ii) optionally administering nonmyeloablative lymphodepleting chemotherapy to the mammal; and (iii) after optionally administering nonmyeloablative lymphodepleting chemotherapy, administering the expanded TIL containing cytotoxic lymphocytes to the mammal. Autologous TILs may be obtained from the stroma of resected

tumors. For this, tumor samples are obtained from patients and a single cell suspension is obtained. The single cell suspension can be obtained in any suitable manner, *e.g.*, mechanically (disaggregating the tumor using, *e.g.*, a gentleMACS.TM. Dissociator, Miltenyi Biotec, Auburn, Calif.) or enzymatically (*e.g.*, collagenase or DNase). In some embodiments, the autologous cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, the autologous lymphocytes are genetically modified cytotoxic lymphocytes using a viral vector. In some embodiments, the autologous lymphocytes are genetically modified before or after culturing, but prior to administration to the mammal. In some embodiments, the cytotoxic lymphocytes are genetically modified cytotoxic lymphocytes. In some embodiments, the cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive

cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the heavy and light chains are encoded on the same vector. In some embodiments, the heavy and light chains are encoded on different vectors. In some embodiments, the heavy chain is encoded on a first vector. In some embodiments, the light chain is encoded on a second vector. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light

chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00251] Expansion of lymphocytes, including tumor-infiltrating lymphocytes, such as T-cells can be accomplished by any of a number of methods as are known in the art, including those described herein below. For example, T-cells can be rapidly expanded using non-specific

T-cell receptor stimulation in the presence of feeder lymphocytes and interleukin-2 (IL-2), IL-7, IL-15, IL-21, or combinations thereof. The non-specific T-cell receptor stimulus can *e.g.* include around 30 ng/ml of OKT3, a mouse monoclonal anti-CD3 antibody (available from Ortho-McNeil^{RTM}, Raritan, N.J. or Miltenyi Biotec, Bergisch Gladbach, Germany). Alternatively, T-cells can be rapidly expanded by stimulation of peripheral blood mononuclear cells (PBMC) in vitro with one or more antigens (including antigenic portions thereof, such as epitope(s), or a cell of the cancer, which can be optionally expressed from a vector, such as an human leukocyte antigen A2 (HLA-A2) binding peptide, *e.g.*, approximately 0.3 μM MART-1:26-35 (27 L) or gp100:209-217 (210M)), in the presence of a T-cell growth factor, such as around 200-400 μl/ml, such as 300 IU/ml IL-2 or IL-15, with IL-2 being preferred. The in vitro-induced T-cells are rapidly expanded by re-stimulation with the same antigen(s) of the cancer pulsed onto HLA-A2-expressing antigen-presenting cells. Alternatively, the T-cells can be re-stimulated with irradiated, autologous lymphocytes or with irradiated HLA-A2+ allogeneic lymphocytes and IL-2, for example. See, for example, International Patent Publication WO 2015/143328, incorporated by reference herein for all purposes.

[00252] Specific tumor reactivity of the expanded TILs can be tested by any method known in the art, *e.g.*, by measuring cytokine release (*e.g.*, interferon-gamma) following co-culture with tumor cells. In one embodiment, the autologous ACT method comprises enriching cultured TILs for CD8+ T-cells prior to rapid expansion of the cells. Following culture of the TILs in IL-2, the T-cells are depleted of CD4+ cells and enriched for CD8+ cells using, for example, a CD8 microbead separation (*e.g.*, using a CliniMACS<plus>CD8 microbead system (Miltenyi Biotec)). In some embodiments, a T-cell growth factor that promotes the growth and activation of the autologous T-cells is administered to the mammal either concomitantly with the autologous T-cells or subsequently to the autologous T-cells. The T-cell growth factor can be any suitable growth factor that promotes the growth and activation of the autologous T-cells. Examples of suitable T-cell growth factors include interleukin (IL)-2, IL-7, IL-15, IL-12 and IL-21, which can be used alone or in various combinations, such as IL-2 and IL-7, IL-2 and IL-15, IL-7 and IL-15, IL-2, IL-7 and IL-15, IL-12 and IL-7, IL-12 and IL-15, or IL-12 and IL2. In some embodiments, 4-1BB co-stimulation enhancement can be employed to increase active TIL expansion for adoptive cell therapy.

Expanding the Genetically Modified Cytotoxic Lymphocytes

[00253] Expanding the TIL containing cytotoxic lymphocytes and/or expanding (*i.e.*, REP) the genetically modified cytotoxic lymphocytes to provide a population of genetically

modified cytotoxic lymphocytes includes culturing the TIL containing cytotoxic lymphocytes *ex vivo* to provide a population of TIL containing cytotoxic lymphocytes, *e.g.*, for reintroduction to the subject (including, for example, a mammal). In some embodiments, cytotoxic lymphocytes are cultured in suitable media containing irradiated peripheral blood mononuclear cells (PBMCs) (feeder cells), isolated by leukapheresis from the subject or from allogeneic donors, and suitable reagents such as Interleukin 2 (IL-2), *e.g.*, human IL-2, and OKT3 (anti-CD3 antibody). As an alternative to the use of PBMCs, artificial antigen presenting cells could be used in the expansion of cytotoxic lymphocytes, *e.g.*, K562 cells genetically modified to express the human Fc receptors CD32 and CD64, such that the K562 cells can bind and present αCD3 and αCD28 monoclonal antibodies. Such artificial antigen presenting cells, including artificial antigen presenting cells expressing a variety of costimulatory molecules, are described, for example, in Turtle and Riddell *Cancer J.* 2010 Jul–Aug; 16(4): 374–381.

[00254] As part of the expanding step or as an additional step(s) one or more selection steps can be performed to enrich for TIL and/or cytotoxic lymphocytes of interest (*e.g.*, cytotoxic lymphocytes expressing receptors for one or more tumor associated antigens), *e.g.*, via one or more immunological assays.

[00255] In addition to TIL, cytotoxic lymphocytes, macrophages, monocytes, and natural killer (NK) cells may also be obtained from the tumor tissue sample, cultured, and expanded as described herein for TIL. Accordingly, the method may also comprise administering macrophages, monocytes, and natural killer (NK) cells to the mammal. In some embodiments, the methods can be employed for expanding cytotoxic lymphocytes, for example, including REPexpansion step(s).

[00256] Aftter obtaining a tumor sample comprising TIL containing cytotoxic lymphocytes, the TIL and/or cytotoxic lymphocytes are expanded. In some embodiments, the method of propagating the TIL containing cytotoxic lymphocytes and/or genetically modified cytotoxic lymphocytes comprises (i) culturing (*i.e.*, pre-REP) the TIL containing cytotoxic lymphocytes, including using IL-2; (ii) expanding (*i.e.*, REP) the cultured cytotoxic lymphocytes using OKT3 antibody, IL-2, and feeder lymphocytes, wherein the cultured cytotoxic lymphocytes are enriched for CD8+ T-cells prior to expansion of the cytotoxic lymphocytes; (iii) optionally administering to the mammal nonmyeloablative lymphodepleting chemotherapy; and (iv) after optional administration of nonmyeloablative lymphodepleting chemotherapy, administering to the mammal the expanded cytotoxic lymphocytes, wherein the cytotoxic lymphocytes administered to the mammal are about 19 to about 35 days old and have not been screened for specific tumor reactivity, whereupon the regression of the cancer in the mammal is

promoted. In some embodiments, the administered cytotoxic lymphocytes are less than about 35 days old, *e.g.*, about 19 to about 26 days old. *See*, for example those methods described in U.S. Patent No. 8,383,099, as well as the methods provided herein.

[00257] In some embodiments, TIL and/or cytotoxic lymphocytes are genetically modified after obtaining a tumor tissue sample from the mammal. In some embodiments, TIL and/or cytotoxic lymphocytes are genetically modified before or after culturing the TIL containing cytotoxic lymphocytes. In some embodiments, TIL and/or cytotoxic lymphocytes are genetically modified before or after expanding the cultured cytotoxic lymphocytes using OKT3 antibody, IL-2, and feeder lymphocytes, wherein the cultured cytotoxic lymphocytes are enriched for CD8+ T-cells prior to expansion of the TIL and/or cytotoxic lymphocytes. In some embodiments, TIL and/or cytotoxic lymphocytes are genetically modified before administering to the mammal. In some embodiments, the cytotoxic lymphocytes are genetically modified cytotoxic lymphocytes. In some embodiments, the cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, the cytotoxic lymphocytes are genetically modified using a viral vector. In some embodiments, the first and second polypeptides are encoded on the same vector. In some embodiments, the first and second polypeptides are encoded on different vectors. In some embodiments, the first polypeptide is encoded on a first vector. In some embodiments, the second polypeptide is encoded on a second vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embdiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased

binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the heavy and light chains are encoded on the same vector. In some embodiments, the heavy and light chains are encoded on different vectors. In some embodiments, the heavy chain is encoded on a first vector. In some embodiments, the light chain is encoded on a second vector. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or

fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1,

IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00258] In some embodiments, cytotoxic lymphocytes that are about 19 to about 35 days old are believed to provide improved in vivo proliferation, survival, and antitumor activity as compared to cytotoxic lymphocytes that are about 44 days old or older. Moreover, in embodiments that include nonmyeloablative chemotherapy, the inventive methods can advantageously be used to treat patients that would not be eligible for treatments that involve total body irradiation (TBI) such as, for example, patients that have already undergone myeloablative therapy, *e.g.*, radiotherapy, prior to treatment; patients with comorbid conditions; and patients with less than $2x10^6$ $CD34^+$ cells/kg.

[00259] In some embodiments, the period of time required to generate cytotoxic lymphocytes for adoptive cell therapy (ACT) may be shortened from an average of about 44 days to a range of about 19 to about 35 days (or less than about 35 days, *e.g.*, about 19 to about 29 days, or about 19 to about 26 days). Accordingly, more patients may be treated before their disease burden progresses to a stage in which administration of ACT may no longer be safe or possible. In some embodiments, methods do not require *in vitro* testing of specific antigen reactivity prior to administration, the inventive methods reduce the time, expense, and labor associated with the treatment of patients. Additionally, the inventive methods can employ administering cytotoxic lymphocytes that are pooled from bulk cultures instead of those derived from microcultures.

[00260] An embodiment of the method comprises culturing autologous cytotoxic lymphocytes. Tumor samples are obtained from patients and a single cell suspension is obtained. The single cell suspension can be obtained in any suitable manner, *e.g.*, mechanically (disaggregating the tumor using, *e.g.*, a gentleMACS™ Dissociator, Miltenyi Biotec, Auburn, Calif.) or enzymatically (*e.g.*, collagenase or DNase). Single-cell suspensions of tumor enzymatic digests are cultured in interleukin-2 (IL-2), *e.g.*, in multiple wells. The cells are cultured until confluence (*e.g.*, about $2x10^6$ lymphocytes), *e.g.*, from about 5 to about 21 days, preferably from about 10 to about 14 days. For example, the cells may be cultured from 5 days, 5.5 days, or 5.8 days to 21 days, 21.5 days, or 21.8 days, preferably from 10 days, 10.5 days, or 10.8 days to 14 days, 14.5 days, or 14.8 days.

[00261] In some embodiments, the method comprises expanding cultured TILs containing cytotoxic lymphocytes. The cultured TILs containing cytotoxic lymphocytes are pooled and rapidly expanded. Rapid expansion provides an increase in the number of antigen-specific T-cells of at least about 50-fold (*e.g.*, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, or 100-fold, or greater) over a period of about 10 days to about 14 days, and in some embodiments, about 14 days. In some embodiments, rapid expansion provides an increase of at least about 200-fold (*e.g.*, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, or greater) over a period of about 10 to about 14 days, in some embodiments, about 14 days. In some embodiments, rapid expansion provides an increase of at least about 1000-fold over a period of about 10 to about 14 days, in some embodiments, about 14 days. In some embodiments, rapid expansion provides an increase of about 1000-fold over a period of about 14 days. In some embodiments, the cultured TILs containing cytotoxic lymphocytes are genetically modified. In some embodiments, the cytotoxic lymphocytes are genetically modified cytotoxic lymphocytes. In some embodiments, the cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, the cultured TILs containing cytotoxic lymphocytes are genetically modified using a viral vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1

protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or

98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00262] Expansion can be accomplished by any of a number of methods as are known in the art. For example, cytotoxic lymphocytes can be rapidly expanded using non-specific T-cell receptor stimulation in the presence of feeder lymphocytes and either interleukin-2 (IL-2) or interleukin-15 (IL-15), with IL-2 being preferred. The non-specific T-cell receptor stimulus can include around 30 ng/ml of OKT3, a mouse monoclonal anti-CD3 antibody (available from Ortho-McNeil^{RTM}, Raritan, N.J.). Alternatively, cytotoxic lymphocytes can be rapidly expanded by stimulation of peripheral blood mononuclear cells (PBMC) in vitro with one or more antigens (including antigenic portions thereof, such as epitope(s), or a cell) of the cancer, which can be optionally expressed from a vector, such as an human leukocyte antigen A2 (HLA-A2) binding peptide, *e.g.*, 0.3 μM MART-1:26-35 (27 L) or gp100:209-217 (210M), in the presence of a T-cell growth factor, such as 300 IU/ml IL-2 or IL-15. In some embodiments, the T-cell growth factor is IL-2 being preferred. The *in vitro*-induced cytotoxic lymphocytes are rapidly expanded by re-stimulation with the same antigen(s) of the cancer pulsed onto HLA-A2-expressing antigen-presenting cells. In some embodiments, the cytotoxic lymphocytes can be re-stimulated with irradiated, autologous lymphocytes or with irradiated HLA-A2+ allogeneic lymphocytes and IL-2, for example.

[00263] In some embodiments, a T-cell growth factor that promotes the growth and activation of the TIL and/or cytotoxic lymphocytes is administered to the mammal either concomitantly with the TIL or subsequently to the TIL. The T-cell growth factor can be any suitable growth factor that promotes the growth and activation of the TIL. Examples of suitable T-cell growth factors include interleukin (IL)-2, IL-7, IL-15, and IL-12, which can be used alone or in various combinations, such as IL-2 and IL-7, IL-2 and IL-15, IL-7 and IL-15, IL-2, IL-7 and IL-15, IL-12 and IL-7, IL-12 and IL-15, or IL-12 and IL-2. IL-2 is a preferred T-cell growth factor.

[00264] In some embodiments, the TIL and/or cytotoxic lymphocytes are further modified to express a T-cell growth factor that promotes the growth and activation of the TIL and/or cytotoxic lymphocytes. Suitable T-cell growth factors include, for example, any of those described above. Suitable methods of modification are known in the art. See, for instance, Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. 2001; and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, NY, 1994. In some embodiments, modified TIL and/or cytotoxic lymphocytes express the T-cell growth factor at high levels. T-cell growth factor coding sequences, such as that of IL-12, are readily available in the art, as are promoters, the operable linkage of which to a T-cell growth factor coding sequence promote

high-level expression. In some embodiments, the TIL and/or cytotoxic lymphocytes may be modified to express IL-12 as described in World Intellectual Property Organization Patent Application Publication No. WO 2010/126766, which is incorporated herein by reference.

[00265] In some embodiments, two cytokines can be more effective than a single cytokine, and, in some embodiments, three cytokines, *e.g.*, IL-2, IL-7 and IL-15, can be more effective than any two cytokines. It is believed that IL-15 enhances a tumor-specific $CD8^+$ T-cell response. In this regard, the administration of IL-15-cultured cells with IL-2 (such as a bolus injection) can be particularly efficacious. In some embodiments, TIL and/or cytotoxic lymphocytes modified to express IL-15 may be administered with IL-2 as a bolus injection.

[00266] The T-cell growth factor can be administered by any suitable route. In embodiments where more than one T-cell growth factor is administered, they can be administered simultaneously or sequentially, in any order, and by the same route or different routes. In some embodiments, the T-cell growth factor, such as IL-2, is administered intravenously as a bolus injection. In some embodiments, the dosage of the T-cell growth factor, such as IL-2, is what is considered by those of ordinary skill in the art to be high. In some embodiments, a dose of about 720,000 IU/kg of IL-2 is administered three times daily until tolerance, particularly when the cancer is melanoma. In some embodiments, about 5 to about 15 doses of IL-2 are administered, with an average of around 8 doses.

[00267] TIL and/or cytotoxic lymphocytes can recognize any of the unique antigens produced as a result of the estimated 10,000 genetic mutations encoded by each tumor cell genome. The antigen, however, need not be unique. TIL and/or cytotoxic lymphocytes can recognize one or more antigens of a cancer, including an antigenic portion of one or more antigens, such as an epitope, or a cell of the cancer. An “antigen of a cancer” and an “antigen of the cancer” are intended to encompass all of the aforementioned antigens. If the cancer is melanoma, such as metastatic melanoma, the TIL and/or cytotoxic lymphocytes can recognize MART-1 (such as MART-1:26-35 (27L)), gp100 (such as gp100:209-217 (210M)), or a “unique” or patient-specific antigen derived from a tumor-encoded mutation. Other suitable melanoma antigens which may be recognized by TIL can include, but are not limited to, tyrosinase, tyrosinase related protein (TRP)1, TRP2, and MAGE. TIL can also recognize antigens such as, for example, NY-ESO-1, telomerase, p53, HER2/neu, carcinoembryonic antigen, or prostate-specific antigen, for treatment of lung carcinoma, breast cancer, colon cancer, prostate cancer, and the like.

[00268] In some embodiments, the method comprises optionally administering to the mammal nonmyeloablative lymphodepleting chemotherapy. The nonmyeloablative

lymphodepleting chemotherapy can be any suitable such therapy, which can be administered by any suitable route. The nonmyeloablative lymphodepleting chemotherapy can comprise, for example, the administration of cyclophosphamide and fludarabine, particularly if the cancer is melanoma, which can be metastatic. In some embodiments, the route of administering cyclophosphamide and fludarabine is intravenously. Likewise, any suitable dose of cyclophosphamide and fludarabine can be administered. In some embodiments, around 60 mg/kg of cyclophosphamide is administered for two days after which around 25 mg/m^2 fludarabine is administered for five days. In some embodiments, around 60 mg/kg of cyclophosphamide is administered for two days after which around 25 mg/m^2 fludarabine is administered for five days and the cancer/tumor is melanoma.

[00269] In some embodiments, the method comprises, after optionally administering the nonmyeloablative lymphodepleting chemotherapy, administering to the mammal the expanded cytotoxic lymphocytes, wherein the cytotoxic lymphocytes administered to the mammal are about 19 to about 35 days old. For example, the administered cytotoxic lymphocytes may be 19 days, 19.5 days, or 19.8 days to 35 days, 35.5 days, or 35.8 days old. In some embodiments, the cytotoxic lymphocytes administered to the mammal are about 19 days to about 29 days or about 23 days to about 29 days old, or about 26 days old. For example, the administered cytotoxic lymphocytes may be 19 days, 19.5 days, or 19.8 days to 29, 29.5, or 29.8 days old; 23, 23.5, or 23.8 to 29, 29.5, or 29.8 days old; or 26, 26.5, or 26.8 days old. In some embodiments, the cytotoxic lymphocytes that are administered to the mammal are "young" cytotoxic lymphocytes, *i.e.*, minimally cultured cytotoxic lymphocytes or cytotoxic lymphocytes between 19 days to about 29 days or about 23 days to about 29 days old, or about 26 days old.

[00270] Young cytotoxic lymphocytes cultures that are administered to the mammal in accordance with an embodiment of the invention advantageously have features associated with *in vivo* persistence, proliferation, and antitumor activity. For example, young cytotoxic lymphocytes cultures have a higher expression of CD27 and/or CD28 than cytotoxic lymphocytes that are about 44 days old. Without being bound to a particular theory, it is believed that CD27 and CD28 are associated with proliferation, *in vivo* persistence, and a less differentiated state of cytotoxic lymphocytes (while not being bound by theory, the increased differentiation of cytotoxic lymphocytes is believed to negatively affect the capacity of cytotoxic lymphocytes to function in vivo). Cytotoxic lymphocytes expressing higher levels of CD27 are believed to have better antitumor activity than CD27-low cells. Moreover, young cytotoxic lymphocyte cultures (*e.g.*, 19 days to about 29 days or about 23 days to about 29 days old, or

about 26 days old) have a higher frequency of $CD4^+$ cells than cytotoxic lymphocytes that are about 44 days old.

[00271] In addition, young cytotoxic lymphocytes cultures have a mean telomere length that is longer than that of cytotoxic lymphocytes that are about 44 days old. Without being bound to a particular theory, it is believed that cytotoxic lymphocytes lose an estimated telomere length of 0.8 kb per week in culture, and that young cytotoxic lymphocytes cultures have telomeres that are about 1.4 kb longer than cytotoxic lymphocytes that are about 44 days old. Without being bound to a particular theory, it is believed that longer telomere lengths are associated with positive objective clinical responses in subjects and persistence of the cells *in vivo*.

[00272] In some embodiments, the cytotoxic lymphocytes are not tested for specific tumor reactivity to identify tumor reactive cytotoxic lymphocytes prior to administration to the patient. Specific tumor reactivity can be tested by any method known in the art, *e.g.*, by measuring cytokine release (*e.g.*, interferon-γ) following co-culture with tumor cells. The inventive methods advantageously make it possible to promote regression of cancer in a mammal by administering cytotoxic lymphocytes to the mammal without the necessity of prior screening for specific tumor recognition. Embodiments of the methods may, if desired, include testing the cytotoxic lymphocytes for potency in a non-antigen-specific manner prior to administering the cytotoxic lymphocytes to the mammal. In some embodiments, cytotoxic lymphocyte potency may be optionally tested, *e.g.*, by a non-specific potency assay measuring cytokine release following OKT3 stimulation. T-cells may be considered potent if, for example, interferon (IFN) release is greater than about 50 pg/mL, greater than about 100 pg/mL, greater than about 150 pg/mL, or greater than about 200 pg/mL. A less desired embodiment of the method comprises testing the expanded cytotoxic lymphocytes for specific tumor reactivity to identify tumor-reactive cytotoxic lymphocytes.

[00273] In some embodiments, the invention provides a method of promoting regression of a cancer in a mammal comprising (i) culturing (*i.e.*, pre-REP) autologous cytotoxic lymphocytes and/or genetically modified cytotoxic lymphocytes (in some embodiments, autologous genetically modified cytotoxic lymphocytes), including using IL-2; (ii) expanding the cultured cytotoxic lymphocytes using OKT3 antibody, IL-2, and feeder lymphocytes, wherein the cultured cytotoxic lymphocytes are enriched for CD8+ T-cells prior to expansion of the cytotoxic lymphocytes; (iii) optionally administering to the mammal nonmyeloablative lymphodepleting chemotherapy; and (iv) after optionally administering nonmyeloablative lymphodepleting chemotherapy, administering to the mammal the expanded cytotoxic

lymphocytes, wherein the cytotoxic lymphocytes administered to the mammal are about 19 days to about 29 days old, or about 23 days to about 29 days old, or about 26 days old, whereupon the regression of the cancer in the mammal is promoted. For example, the cytotoxic lymphocytes administered to the mammal may be 19 days to about 29 days or about 23 days to about 29 days old. In some embodiments, about 19 days to about 29 days, or about 23 days to about 29 days old, or about 26 days old. In some embodiments, the cultured TILs containing cytotoxic lymphocytes are genetically modified. In some embodiments, the cytotoxic lymphocytes are genetically modified cytotoxic lymphocytes. In some embodiments, the cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, the cultured TILs containing cytotoxic lymphocytes are genetically modified using a viral vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the cultured TILs containing cytotoxic lymphocytes are genetically modified before or after culturing. In some embodiments, the cultured TILs containing cytotoxic lymphocytes are genetically modified before or after expanding the cultured TILs containing cytotoxic lymphocytes using OKT3 antibody, IL-2, and feeder lymphocytes, wherein the cultured cytotoxic lymphocytes are enriched for CD8+ T-cells prior to expansion of the cytotoxic lymphocytes. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or

increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or

98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00274] In some embodiments, the method comprises culturing (for example, pre-REP) autologous T-cells as described herein from about 19 days to about 29 days, or about 23 days to about 29 days, or about 26 days. The method further comprises expanding (for example, REP) the cultured cytotoxic lymphocytes and optionally administering to the mammal nonmyeloablative lymphodepleting chemotherapy as described herein. After optionally administering nonmyeloablative lymphodepleting chemotherapy, the method comprises administering to the mammal the expanded cytotoxic lymphocytes as described herein, whereupon the regression of the cancer in the mammal is promoted. In some embodiments of the method, the administered cytotoxic lymphocytes have not been screened for specific tumor reactivity. In some embodiments, the expanded cytotoxic lymphocytes administered to the mammal are about 19 days to about 29 days or about 23 days to about 29 days old, or about 26 days old. For example, the administered cytotoxic lymphocytes may be 19 days, 19.5 days, or 19.8 days to 29, 29.5, or 29.8 days old; 23, 23.5, or 23.8 to 29, 29.5, or 29.8 days old; or 26, 26.5, or 26.8 days old.

[00275] In some embodiments, the method comprises enriching cultured T-cells containing the cytotoxic lymphocytes for $CD8^+$ T-cells prior to rapid expansion of the cells. Following culture of the TIL containing cytotoxic lymphocytes in IL-2, the TIL containing cytotoxic lymphocytes are depleted of $CD4^+$ cells and enriched for $CD8^+$ cells using, for example, a CD8 microbead separation (*e.g.*, using a CliniMACS® Plus CD8 microbead system (commercially available from Miltenyi Biotec)). In some embodiments, $CD4^+$ and $CD25^+$ regulatory T-cells can impede anti-tumor responses. In some embodiments, enriching cultured cytotoxic lymphocytes for $CD8^+$ cytotoxic lymphocytes and reducing or eliminating $CD4^+$ cells may improve the impact of adoptively transferred anti-tumor $CD8^+$ cells, improve the response rates in patients, and/or reduce the toxicities seen by production of cytokines by $CD4^+$ cells. In some embodiments, $CD8^+$ enrichment of some T-cell cultures reveals *in vitro* tumor recognition that may not be evident in the bulk culture, and improved *in vitro* recognition of tumor in other cultures. In some embodiments, the enriched $CD8^+$ young cytotoxic lymphocytes can function more reliably and predictably in clinical scale rapid expansions than the bulk T-cells.

Expanding the Genetically Modified Cytotoxic Lymphocytes Using Gas Permeable Containers

[00276] An embodiment of the invention provides a method of promoting regression of cancer in a mammal comprising obtaining a tumor tissue sample from the mammal; culturing (*i.e.*, pre-REP) the tumor tissue sample in a first gas permeable container containing cell medium

therein; obtaining cytotoxic lymphocytes from the tumor tissue sample; expanding (*i.e.*, REP) the number of cytotoxic lymphocytes in a second gas permeable container containing cell medium therein using irradiated allogeneic feeder cells and/or irradiated autologous feeder cells; and administering the expanded number of cytotoxic lymphocytes to the mammal. In some embodiments, the cytotoxic lymphocytes are genetically modified after isolation from the tumor tissue sample, but prior to culturing in said first gas permeable container. In some embodiments, the cytotoxic lymphocytes are genetically modified after culturing in said first gas permeable container but prior to culturing in said a second gas permeable container. In some embodiments, the cytotoxic lymphocytes are genetically modified prior to administration of the expanded number of cytotoxic lymphocytes to the mammal. *See*, for example, the methods described in U.S. Patent Publication 2017/0152478, as well as those described herein. In some embodiments, the cytotoxic lymphocytes are genetically modified using a vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-

L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the heavy and light chains are encoded on the same vector. In some embodiments, the heavy and light chains are encoded on different vectors. In some embodiments, the heavy chain is encoded on a first vector. In some embodiments, the light chain is encoded on a second vector. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence

having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00277] In some embodiments, the present methods of promoting regression of cancer using cytotoxic lymphocytes and/or genetically modified cytotoxic lymphocytes which have been expanded using gas permeable containers are simpler, less labor-intensive, use less reagents, and can be performed using simpler equipment than procedures using non-gas permeable containers (*e.g.*, T-flasks or T-175 flasks, bags, and multi-well plates). In addition, gas permeable containers can in some embodiments protect the cells from microbial contamination more effectively than non-gas permeable containers which may be "open" systems. In addition, methods using gas permeable containers can in some embodiments, reduce the number of containers that are used compared to methods using non-gas permeable containers, thereby reducing the amount of labor necessary to carry out the methods and also reducing the risk of microbial contamination. Thus, producing cells in gas permeable containers may be more suitable for compliance with the current good manufacturing practice (cGMP) conditions that are required for, *e.g.*, Phase III clinical trials. In some embodiments, methods using gas-permeable containers reduce the final culture volume to lower than that obtained with non-gas permeable containers, which can lower the incubator capacity required to grow the cells, reduces the amount of reagents (*e.g.*, cell culture medium and additives) necessary to grow the cells, and simplifies the equipment and/or procedures for concentrating and washing the cells. In some embodiments, the cells may be fed less frequently in gas-permeable containers (*e.g.*, about every three to four days) than in non-gas permeable containers (*e.g.*, every other day), particularly when the cells and/or tumor tissue sample are cultured submerged under at least about 1.3 cm of cell culture medium in a gas permeable container. In some embodiments, cells in gas permeable containers can be handled less frequently than cells in non-gas permeable containers (*e.g.*, bags), which may minimize disturbance of the tumor fragment and provide more reproducible cytotoxic lymphocyte growth. In some embodiments, various portions of the method, including, but not limited to, culturing and/or expanding cytotoxic lymphocytes, can be automated. In some embodiments, the use of gas permeable flasks allows for generation of sufficient numbers of cytotoxic lymphocytes allowing for treatment of subjects who previously may not have been successfully treated because sufficient numbers of cytotoxic lymphocytes were not generated due to the technical and logistical complexities of previous methods that do not use gas permeable flasks.

[00278] In some embodiments, the method further comprises culturing the tumor tissue sample in a first gas permeable container containing cell medium therein. In an embodiment, the tumor tissue sample is cultured directly on the gas permeable material in the gas permeable container without digestion. In another embodiment, an enzymatically or mechanically digested

tumor tissue sample may be cultured directly on the gas permeable material. Any suitable cell medium may be used. The cell culture medium may further comprise any suitable T-cell growth factor such as, *e.g.*, interleukin (IL)-2. The cell culture medium may optionally further comprise human AB serum. In some embodiments, the tumor tissue sample may contain TILs that are autologous to the subject. In some embodiments, culturing the tumor tissue sample includes culturing the TILs present in the tumor sample.

[00279] The method also comprises obtaining TIL from the tumor tissue sample. The tumor tissue sample comprises TIL. As the tumor tissue sample is cultured in the gas permeable container, *e.g.*, on gas permeable material in the container, TIL present in the tumor tissue sample also begin to grow in the gas permeable container, *e.g.*, on the gas permeable material. TIL may be obtained from the tumor tissue sample in any suitable manner.

[00280] In some embodiments, the first gas permeable container may be any suitable gas permeable container. In some embodiments, the first gas permeable container comprises a base, sides, and a cap. In some embodiments, the container comprises a gas permeable support and a gas permeable material, *e.g.*, a gas permeable membrane. In some embodiments, the base of the container comprises a gas permeable support and a gas permeable material, *e.g.*, a gas permeable membrane. In some embodiments, the gas permeable material may be positioned inside the container directly on the gas permeable support which comprises openings (*e.g.*, channels) in fluid communication with ambient gas in order to facilitate gas exchange between the interior of the container and the ambient gas. In some embodiments, the cap may comprise a vent and/or a port (*e.g.*, an access port). In some embodiments, the access port may have an opening greater than about 1 mm to about 1 cm (*e.g.*, greater than about 1 mm or greater than about 1 cm). An access port with an opening greater than about 1 mm to about 1 cm may advantageously eliminate or reduce disturbance of the TIL. In some embodiments, the gas permeable container comprises a vent or a vented port, which can be advantageous in the event that the temperature in the container drops during handling. In some embodiments, the first gas permeable container is a gas permeable container as described in U.S. Patent Application Publication No. 2005/0106717, which is incorporated herein by reference, and is commercially available from Wilson Wolf Manufacturing Corporation (*e.g.*, G-Rex10, GP200, G-Rex100, GP2000 containers) (New Brighton, Minn.).

[00281] In some embodiments, the first gas permeable container has any suitable cell medium volume capacity. For example, the first gas permeable container can have a medium volume capacity of about 40 mL or more; about 200 mL or more; about 500 mL or more; about 2,000 mL or more; or about 5,000 mL or more. Although the first gas permeable container can

have any suitable medium volume capacity, the tumor tissue sample and/or TIL containing cytotoxic lymphocytes can be cultured in any suitable volume of medium. In some embodiments, the tumor tissue sample and/or TIL containing cytotoxic lymphocytes are cultured submerged under a height of at least about 1.3 cm of cell culture medium. In some embodiments, the tumor tissue sample and/or TIL containing cytotoxic lymphocytes are cultured submerged under a height of at least about 2.0 cm of cell culture medium. Tumor tissue samples and/or TIL cultured on a gas permeable material submerged under a height of at least about 1.3 cm or a height of at least about 2.0 cm of medium may, advantageously, be handled and fed less frequently.

[00282] In some embodiments, the first gas permeable container can provide any suitable surface area for the growth of the TIL containing cytotoxic lymphocytes. In some embodiments, the gas permeable container can have a surface area for growth of the TIL of about 10 cm^2 or more; about 100 cm^2 or more; or about 650 cm^2 or more.

[00283] In some embodiments, the tumor tissue sample and/or TIL containing cytotoxic lymphocytes are cultured inside the first gas permeable container in contact with the gas permeable material and submerged under a suitable volume of culture medium. Culturing the tumor tissue sample and/or TIL in contact with the gas permeable material facilitates gas exchange between the cells and the ambient air. Facilitating gas exchange between the cells and the ambient air facilitates the respiration, growth, and viability of the cells. In some embodiments, the gas exchange across the gas permeable material can facilitate circulation of the medium (*e.g.*, by convection and diffusion) within the container, which facilitates feeding of the TIL containing cytotoxic lymphocytes. In some embodiments, the cytotoxic lymphocytes are genetically modified before or after culturing in the firs gas permeable container. In some embodiments, the cytotoxic lymphocytes are genetically modified using a vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based

viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the heavy

and light chains are encoded on the same vector. In some embodiments, the heavy and light chains are encoded on different vectors. In some embodiments, the heavy chain is encoded on a first vector. In some embodiments, the light chain is encoded on a second vector. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or

98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00284] In some embodiments, the method further comprises expanding the number of TIL containing cytotoxic lymphocytes in a second gas permeable container containing cell medium therein using irradiated allogeneic feeder cells and/or irradiated autologous feeder cells. In some embodiments, the number of TIL containing cytotoxic lymphocytes is expanded using a ratio of about 1 TIL to at least about 20 feeder cells, about 1 TIL to at least about 25 feeder cells, about 1 TIL to at least about 50 feeder cells, about 1 TIL to at least about 100 feeder cells, about 1 TIL to at least about 200 feeder cells, *e.g.*, a TIL-to-feeder cell ratio of about 1 to about 20, about 1 to about 25, about 1 to about 50, about 1 to about 100, or about 1 to about 200. In some embodiments, the second gas permeable container can be as described for the first container.

[00285] In some embodiments, the cultured TIL containing the cytotoxic lymphocytes are expanded. In some embodiments, the cultured TIL containing the cytotoxic lymphocytes are rapidly expanded. Rapid expansion provides an increase in the number of TIL containing the cytotoxic lymphocytes of at least about 50-fold (or 60-fold, 70-fold, 80-fold, 90-fold, or 100-fold, or greater) over a period of about 10 days to about 14 days, and in some embodiments, over about 14 days. In some embodiments, the rapid expansion provides an increase of at least about 200-fold (or 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, or greater) over a period of about 10 days to about 14 days, and in some embodiments, over about 14 days. In some embodiments, the rapid expansion provides an increase of at least about 1000-fold over a period of about 10 to about 14 days, and in some embodiments, about 14 days. In some embodiments, rapid expansion provides an increase of about 1000-fold to about 2000-fold, *e.g.*, about 1000-fold, about 1500-fold, or about 2,000-fold over a period of about 14 days. In some embodiments, the cytotoxic lymphocytes are genetically modified before or after expanding. In some embodiments, the cytotoxic lymphocytes are genetically modified using a vector. In some

embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some

embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the heavy and light chains are encoded on the same vector. In some embodiments, the heavy and light chains are encoded on different vectors. In some embodiments, the heavy chain is encoded on a first vector. In some embodiments, the light chain is encoded on a second vector. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein

said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00286] In some embodiments, expansion (*i.e.*, REP) can be accomplished in the gas permeable container by any suitable method. For example, TIL containing cytotoxic lymphocytes can be rapidly expanded using non-specific T-cell receptor stimulation in the presence of feeder cells (*e.g.*, irradiated allogeneic feeder cells, irradiated autologous feeder cells, and/or artificial antigen presenting cells (*e.g.*, K562 leukemia cells transduced with nucleic acids encoding CD3 and/or CD8)) and either interleukin-2 (IL-2) or interleukin-15 (IL-15), and in some embodiments, IL-2. In some embodiments, expanding the number of TIL containing cytotoxic lymphocytes uses about $1x10^9$ to about $4x10^9$ allogeneic feeder cells and/or autologous feeder cells, preferably about $2x10^9$ to about $3x10^9$ allogeneic feeder cells and/or autologous feeder cells. The non-specific T-cell receptor stimulus can include, for example, about 30 ng/ml of OKT3, a mouse monoclonal anti-CD3 antibody (available from ORTHO-MCNEIL, Raritan, N.J. or MILTENYI BIOTECH, Auburn, Calif.). Alternatively, TIL containing cytotoxic lymphocytes can be rapidly expanded by, for example, stimulation of the

TIL containing cytotoxic lymphocytes *in vitro* with an antigen (one or more, including antigenic portions thereof, such as epitope(s), or a cell) of the cancer, which can be optionally expressed from a vector, such as an human leukocyte antigen A2 (HLA-A2) binding peptide, *e.g.*, 0.3 μM MART-1:26-35 (27L) or gp100:209-217 (210M), in the presence of a T-cell growth factor, such as 300 IU/ml IL-2 or IL-15, and in some embodiments with IL-2. Other suitable antigens may include, *e.g.*, NY-ESO-1, TRP-1, TRP-2, tyrosinase cancer antigen, MAGE-A3, SSX-2, and VEGFR2, or antigenic portions thereof. The in vitro-induced TIL are rapidly expanded by re-stimulation with the same antigen(s) of the cancer pulsed onto HLA-A2-expressing antigen-presenting cells. Alternatively, the TIL can be re-stimulated with, for example, irradiated, autologous lymphocytes or with irradiated HLA-A2+ allogeneic lymphocytes and IL-2, for example.

[00287] In some embodiments, expanding the number of TIL can comprise using about 5,000 mL to about 10,000 mL of cell medium, preferably about 5,800 mL to about 8,700 mL of cell medium. In an embodiment, expanding the number of TIL uses no more than one type of cell culture medium. Any suitable cell culture medium may be used, *e.g.*, AIM-V cell medium (L-glutamine, 50 μg/ml streptomycin sulfate, and 10 μg/ml gentamicin sulfate) cell culture medium (Invitrogen, Carlsbad Calif.). In this regard, the inventive methods advantageously reduce the amount of medium and the number of types of medium required to expand the number of TIL containing cytotoxic lymphocytes.

[00288] In some embodiments, expanding the number of TIL containing cytotoxic lymphocytes can comprise feeding the cells no more frequently than every third or fourth day. Expanding the number of cells in a gas permeable container advantageously simplifies the procedures necessary to expand the number of cells by reducing the feeding frequency necessary to expand the cells.

[00289] In some embodiments, the cell medium in the first and/or second gas permeable container is unfiltered. Without being bound to a particular theory, it is believed that particulate serum components present in some cell medium supplements (*e.g.*, AB serum) have little or no detrimental effects on TIL growth. The use of unfiltered cell medium may, advantageously, simplify the procedures necessary to expand the number of cells.

[00290] In some embodiments, the cell medium in the first and/or second gas permeable container lacks beta-mercaptoethanol (BME). In some embodiments, the absence of BME from the cell medium can be advantageously more compliant with cGMP (current good manufacturing procedures) and, thus, can advantageously make it easier to gain regulatory approval.

[00291] In some embodiments, the duration of the method comprising obtaining a tumor tissue sample from the mammal; culturing (*i.e.*, pre-REP) the tumor tissue sample in a first gas permeable container containing cell medium therein; obtaining TIL containing cytotoxic lymphocytes from the tumor tissue sample; expanding (*i.e.*, REP) the number of TIL containing cytotoxic lymphocytes in a second gas permeable container containing cell medium therein using irradiated allogeneic feeder cells and/or irradiated autologous feeder cells may be about 28 to about 42 days, *e.g.*, about 28 days. In some embodiments, the cytotoxic lymphocytes are genetically modified before or after culturing, before or after obtaining the TIL-containing cytotoxic lymphocytes, or before or after expanding. In some embodiments, the cytotoxic lymphocytes are genetically modified using a vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry

as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the heavy and light chains are encoded on the same vector. In some embodiments, the heavy and light chains are encoded on different vectors. In some embodiments, the heavy chain is encoded on a first vector. In some embodiments, the light chain is encoded on a second vector. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein

said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00292] In some embodiments, the method comprises administering the expanded TIL containing cytotoxic lymphocytes to the mammal. The TIL containing cytotoxic lymphocytes can be administered by any suitable route as known in the art. In some embodiments the TIL

and/or cytotoxic lymphocytes are administered as an intra-arterial or intravenous infusion, which preferably lasts about 30 to about 60 minutes. Other examples of routes of administration include intraperitoneal, intrathecal and intralymphatic. In some embodiments, the cytotoxic lymphocytes are genetically modified prior to administration. In some embodiments, the cytotoxic lymphocytes are genetically modified using a vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding

protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the heavy and light chains are encoded on the same vector. In some embodiments, the heavy and light chains are encoded on different vectors. In some embodiments, the heavy chain is encoded on a first vector. In some embodiments, the light chain is encoded on a second vector. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ

ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00293] In addition to TIL, cytotoxic lymphocytes, macrophages, monocytes, and natural killer (NK) cells may also be obtained from the tumor tissue sample, cultured, and expanded as described herein for TIL. Accordingly, the method may also comprise administering macrophages, monocytes, and natural killer (NK) cells to the mammal. In some embodiments, the methods can be employed for expanding cytotoxic lymphocytes.

[00294] In some embodiments, a T-cell growth factor that promotes the growth and activation of the TIL and/or cytotoxic lymphocytes is administered to the mammal either concomitantly with the TIL or subsequently to the TIL. The T-cell growth factor can be any

suitable growth factor that promotes the growth and activation of the TIL. Examples of suitable T-cell growth factors include interleukin (IL)-2, IL-7, IL-15, and IL-12, which can be used alone or in various combinations, such as IL-2 and IL-7, IL-2 and IL-15, IL-7 and IL-15, IL-2, IL-7 and IL-15, IL-12 and IL-7, IL-12 and IL-15, or IL-12 and IL-2. IL-2 is a preferred T-cell growth factor.

[00295] In some embodiments, the TIL and/or cytotoxic lymphocytes are modified to express a T-cell growth factor that promotes the growth and activation of the TIL and/or cytotoxic lymphocytes. Suitable T-cell growth factors include, for example, any of those described above. Suitable methods of modification are known in the art. See, for instance, Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. 2001; and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, NY, 1994. In some embodiments, modified TIL and/or cytotoxic lymphocytes express the T-cell growth factor at high levels. T-cell growth factor coding sequences, such as that of IL-12, are readily available in the art, as are promoters, the operable linkage of which to a T-cell growth factor coding sequence promote high-level expression. In some embodiments, the TIL and/or cytotoxic lymphocytes may be modified to express IL-12 as described in World Intellectual Property Organization Patent Application Publication No. WO 2010/126766, which is incorporated herein by reference.

[00296] In some embodiments, two cytokines can be more effective than a single cytokine, and, in some embodiments, three cytokines, *e.g.*, IL-2, IL-7 and IL-15, can be more effective than any two cytokines. It is believed that IL-15 enhances a tumor-specific $CD8^+$ T-cell response. In this regard, the administration of IL-15-cultured cells with IL-2 (such as a bolus injection) can be particularly efficacious. In some embodiments, TIL and/or cytotoxic lymphocytes modified to express IL-15 may be administered with IL-2 as a bolus injection.

[00297] The T-cell growth factor can be administered by any suitable route. In embodiments where more than one T-cell growth factor is administered, they can be administered simultaneously or sequentially, in any order, and by the same route or different routes. In some embodiments, the T-cell growth factor, such as IL-2, is administered intravenously as a bolus injection. In some embodiments, the dosage of the T-cell growth factor, such as IL-2, is what is considered by those of ordinary skill in the art to be high. In some embodiments, a dose of about 720,000 IU/kg of IL-2 is administered three times daily until tolerance, particularly when the cancer is melanoma. In some embodiments, about 5 to about 15 doses of IL-2 are administered, with an average of around 8 doses.

[00298] TIL and/or cytotoxic lymphocytes can recognize any of the unique antigens produced as a result of the estimated 10,000 genetic mutations encoded by each tumor cell genome. The antigen, however, need not be unique. TIL and/or cytotoxic lymphocytes can recognize one or more antigens of a cancer, including an antigenic portion of one or more antigens, such as an epitope, or a cell of the cancer. An "antigen of a cancer" and an "antigen of the cancer" are intended to encompass all of the aforementioned antigens. If the cancer is melanoma, such as metastatic melanoma, the TIL and/or cytotoxic lymphocytes can recognize MART-1 (such as MART-1:26-35 (27L)), gp100 (such as gp100:209-217 (210M)), or a "unique" or patient-specific antigen derived from a tumor-encoded mutation. Other suitable melanoma antigens which may be recognized by TIL can include, but are not limited to, tyrosinase, tyrosinase related protein (TRP)1, TRP2, and MAGE. TIL can also recognize antigens such as, for example, NY-ESO-1, telomerase, p53, HER2/neu, carcinoembryonic antigen, or prostate-specific antigen, for treatment of lung carcinoma, breast cancer, colon cancer, prostate cancer, and the like.

[00299] In some embodiments, the method provided is a method of obtaining an expanded number of TIL and/or cytotoxic lymphocytes from a mammal for adoptive cell immunotherapy comprising obtaining a tumor tissue sample from the mammal; culturing the tumor tissue sample in a first gas permeable container containing cell medium therein; obtaining TIL and/or cytotoxic lymphocytes from the tumor tissue sample; expanding the number of TIL and/or cytotoxic lymphocytes in a second gas permeable container containing cell medium therein using irradiated allogeneic feeder cells and/or irradiated autologous feeder cells. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified. In some embodiments, the cytotoxic lymphocytes are genetically modified cytotoxic lymphocytes. In some embodiments, the cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified with a viral vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral

vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a

sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-

chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00300] In some embodiments, TIL and/or cytotoxic lymphocytes are genetically modified after obtaining a tumor tissue sample from the mammal. In some embodiments, TIL and/or cytotoxic lymphocytes are genetically modified before or after culturing (*i.e.*, pre-REP) the tumor tissue sample in a first gas permeable container containing cell medium therein. In some embodiments, TIL and/or cytotoxic lymphocytes are genetically modified after obtaining the TIL and/or cytotoxic lymphocytes from the tumor tissue sample. In some embodiments, TIL and/or cytotoxic lymphocytes are genetically modified after expanding (*i.e.*, REP) the number of TIL and/or cytotoxic lymphocytes in a second gas permeable container containing cell medium therein using irradiated allogeneic feeder cells and/or irradiated autologous feeder cells. In some embodiments, the cytotoxic lymphocytes are genetically modified cytotoxic lymphocytes. In some embodiments, the cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified with a viral vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In

some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID

NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments,

the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00301] In some embodiments, the method comprises obtaining a tumor tissue sample from the mammal. The tumor tissue sample may be obtained as described herein with respect to any embodiments of the invention.

[00302] In some embodiments, the method comprises culturing the tumor tissue sample in a first gas permeable container containing cell medium therein. The tumor tissue sample may be cultured in a first gas permeable container as described herein with respect to any embodiments of the invention.

[00303] In some embodiments, the method comprises obtaining TIL containing cytotoxic lymphocytes from the tumor tissue sample. The TIL containing cytotoxic lymphocytes may be obtained from the tumor tissue sample as described herein with respect to any embodiments of the invention.

[00304] In some embodiments, the method comprises expanding the number of TIL and/or cytotoxic lymphocytes in a second gas permeable container containing cell medium therein using irradiated allogeneic feeder cells and/or irradiated autologous feeder cells. The number of TIL and/or cytotoxic lymphocytes may be expanded as described herein with respect to any embodiments of the invention.

[00305] In some embodiments, the method involves obtaining an expanded number of TIL and/or cytotoxic lymphocytes from a mammal for adoptive cell immunotherapy, wherein the method comprises obtaining a tumor tissue sample from the mammal; obtaining TIL containing cytotoxic lymphocytes from the tumor tissue sample; expanding the number of TIL and/or cytotoxic lymphocytes in a gas permeable container containing cell medium therein using irradiated allogeneic feeder cells and/or irradiated autologous feeder cells. In some embodiments, the gas permeable container is a first gas permeable container. Obtaining a tumor tissue sample from the mammal, obtaining TIL and/or cytotoxic lymphocytes from the tumor tissue sample, and expanding the number of TIL and/or cytotoxic lymphocytes in a second gas permeable container containing cell medium therein using irradiated allogeneic feeder cells and/or irradiated autologous feeder cells may be carried out as described herein with respect to any embodiments of the invention. In some embodiments, the cytotoxic lymphocytes are genetically modified cytotoxic lymphocytes. In some embodiments, the cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments,

the TIL and/or cytotoxic lymphocytes are genetically modified with a viral vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits

greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID

NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00306] In some embodiments, the method provided is a method of promoting regression of cancer in a mammal comprising obtaining a tumor tissue sample from the mammal; obtaining TIL containing cytotoxic lymphocytes from the tumor tissue sample; expanding the number of TIL and/or cytotoxic lymphocytes in a first gas permeable container and a second gas permeable container containing cell medium therein using irradiated allogeneic feeder cells and/or irradiated autologous feeder cells; and administering the expanded number of TIL and/or cytotoxic lymphocytes to the mammal. In some embodiments, obtaining a tumor tissue sample from the mammal, obtaining TIL from the tumor tissue sample, expanding the number of TIL and/or cytotoxic lymphocytes in a gas permeable container containing cell medium therein using irradiated allogeneic feeder cells and/or irradiated autologous feeder cells, and administering the expanded number of TIL and/or cytotoxic lymphocytes to the mammal can be carried out as described herein with respect to any embodiments described herein. In some embodiments, the method further comprises selecting TIL and/or cytotoxic lymphocytes capable of lysing cancer cells. The TIL and/or cytotoxic lymphocytes may be selected as described herein with respect to any embodiments of the invention. In some embodiments, the TIL and/or cytotoxic lymphocytes

are genetically modified. In some embodiments, the cytotoxic lymphocytes are genetically modified cytotoxic lymphocytes. In some embodiments, the cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified with a viral vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein

an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1

binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00307] In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified after obtaining a tumor tissue sample from the mammal. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified before or after isolating the TIL containing cytotoxic lymphocytes from the tumor tissue sample. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified before or after expanding the number of TIL and/or cytotoxic lymphocytes in a second gas permeable container containing cell medium therein using irradiated allogeneic feeder cells and/or irradiated autologous feeder cells. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified prior to administering the expanded number of TIL and/or cytotoxic lymphocytes to the mammal.

[00308] In some embodiments, the cytotoxic lymphocytes are not tested for specific tumor reactivity to identify tumor reactive cytotoxic lymphocytes prior to administration to the patient. Specific tumor reactivity can be tested by any method known in the art, *e.g.*, by

measuring cytokine release (*e.g.*, interferon-γ) following co-culture with tumor cells. The inventive methods advantageously make it possible to promote regression of cancer in a mammal by administering cytotoxic lymphocytes to the mammal without the necessity of prior screening for specific tumor recognition. Embodiments of the methods may, if desired, include testing the cytotoxic lymphocytes for potency in a non-antigen-specific manner prior to administering the cytotoxic lymphocytes to the mammal. In some embodiments, cytotoxic lymphocyte potency may be optionally tested, *e.g.*, by a non-specific potency assay measuring cytokine release following OKT3 stimulation. T-cells may be considered potent if, for example, interferon (IFN) release is greater than about 50 pg/mL, greater than about 100 pg/mL, greater than about 150 pg/mL, or greater than about 200 pg/mL. A less desired embodiment of the method comprises testing the expanded cytotoxic lymphocytes for specific tumor reactivity to identify tumor-reactive cytotoxic lymphocytes.

Further Methods for Culturing TIL and/or cytotoxic lymphocytes

[00309] In some embodiments, the method can further comprise culturing the tumor tissue by any suitable method that facilitates the obtaining of TIL and/or cytotoxic lymphocytes from the tumor tissue sample. In this regard, culturing the tumor tissue may comprise establishing multiple independent cultures, *e.g.*, microcultures. For example, culturing the tumor tissue may comprise culturing tumor fragments in plates, *e.g.*, 24-well plates. In some embodiments, the tumor tissue is cultured without a gas permeable container.

[00310] In some embodiments, the method further comprises selecting TIL and/or cytotoxic lymphocytes capable of lysing cancer cells while in other embodiments, the method does not include selecting TIL capable of lysing cancer cells. TIL capable of lysing cancer cells may be selected by identifying TILs having any suitable trait associated with the lysis of cancer cells and/or the regression of cancer. Exemplary suitable TIL traits that may serve as the basis for selecting TILs may include any one or more of IFN-γ release upon co-culture with autologous tumor cells; cell surface expression of one or more of CD8, CD27, and CD28; and telomere length. Without being bound to a particular theory, it is believed that cell surface expression of one or more of CD8, CD27, and CD28 and longer telomere lengths are associated with positive objective clinical responses in patients and persistence of the cells in vivo. Preferably the trait is IFN-γ release upon co-culture with autologous tumor cells. In an embodiment of the invention, selected TIL release about 200 pg/ml or more of IFN-γ upon co-culture with tumor cells.

[00311] In some embodiments, selecting TIL capable of lysing cancer cells comprises testing individual cultures for presence of the trait and identifying TIL possessing the trait. Methods of testing cultures for the presence of any one or more of IFN-γ release upon co-culture with autologous tumor cells; cell surface expression of one or more of CD8, CD27, and CD28; and telomere length (longer telomeres being associated with regression of cancer) are known in the art.

[00312] In some embodiments, any number of cultures can be selected. For example, one, two, three, four, five, or more cultures may be selected. In embodiments in which two or more cultures are selected, the selected cultures may be combined and the number of TIL expanded in one (or more) gas permeable containers. In embodiments in which two or more cultures are selected, each selected culture is separately expanded in separate containers, including gas permeable containers when such containers are employed.

[00313] In some embodiments, the method can further comprise expanding the number of TIL and/or cytotoxic lymphocytes in an identified culture in a second gas permeable container containing cell medium therein using irradiated allogeneic feeder cells and/or irradiated autologous feeder cells as described herein with respect to any embodiments of the invention. In some embodiments, the cytotoxic lymphocytes that are expanded in a second gas permeable container are genetically modified cytotoxic lymphocytes. In some embodiments, the cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified with a viral vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments,

the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ

ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

Administering the Population of Genetically Modified Cytotoxic Lymphocytes

[00314] Once expanded to a suitable number, the population of genetically modified cytotoxic lymphocytes may be administered, *e.g.*, infused, to the subject using any suitable method and/or device known in the art. In some embodiments, the cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified with a viral vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector

is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells

as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or

IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00315] The compositions of the present disclosure, *e.g.*, the genetically modified cytotoxic lymphocytes of the present disclosure, may be administered either alone, or as a pharmaceutical composition in combination with diluents and/or with other components such as IL-2 or other cytokines or cell populations. Briefly, pharmaceutical compositions of the present disclosure may include a population of genetically modified cytotoxic lymphocytes as described herein, *e.g.*, in connection with any embodiment described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (*e.g.*, aluminum hydroxide); and preservatives. Compositions of the present invention are preferably formulated for intravenous administration or injection at a tumor site.

[00316] Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type

and severity of the patient's disease, although appropriate dosages may be determined by clinical trials.

[00317] When "an anti-tumor effective amount", "an tumor-inhibiting effective amount", or "therapeutic amount" is indicated, the precise amount of the compositions of the present invention to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject). It can generally be stated that a pharmaceutical composition comprising the genetically modified cytotoxic lymphocytes described herein may be administered at a dosage of 10^4 to 10^{11} cells/kg body weight (*e.g.*, 10^5 to 10^6, 10^5 to 10^{10}, 10^5 to 10^{11}, 10^6 to 10^{10}, 10^6 to 10^{11},10^7 to 10^{11}, 10^7 to 10^{10}, 10^8 to 10^{11}, 10^8 to 10^{10}, 10^9 to 10^{11}, or 10^9 to 10^{10} cells/kg body weight), including all integer values within those ranges. Genetically modified cytotoxic lymphocytes compositions may also be administered multiple times at these dosages. The genetically modified cytotoxic lymphocytes can be administered by using infusion techniques that are commonly known in immunotherapy (see, *e.g.*, Rosenberg et al., *New Eng. J. of Med.* 319: 1676, 1988). The optimal dosage and treatment regime for a particular patient can readily be determined by one skilled in the art of medicine by monitoring the patient for signs of disease and adjusting the treatment accordingly.

[00318] The administration of the subject compositions may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient systemically or locally (*e.g.*, at the site of a tumor). The compositions described herein may be administered to a patient subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous (i.v.) injection, or intraperitoneally. In some embodiments, the compositions of the present disclosure are administered to a patient by intradermal or subcutaneous injection. In some cases, the compositions of the present disclosure are administered by i.v. injection. The compositions of the present disclosure may be injected directly into a tumor, lymph node, or site of infection.

[00319] In some embodiments, the expanded TIL containing cytotoxic lymphocytes, cytotoxic lymphocytes, and/or genetically modified cytotoxic lymphocytes produced by the disclosed methods are administered as an intra-arterial or intravenous infusion. In some embodiments, administration lasts about 30 to about 60 minutes. In some embodiments, routes of administration include intraperitoneal, intrathecal and intralymphatic. In some embodiments, TIL and/or cytotoxic lymphocytes are genetically modified after obtaining a tumor tissue sample from the mammal, anytime during expansion, but prior to administration to a mammal. In some

embodiments, the cytotoxic lymphocytes are genetically modified cytotoxic lymphocytes. In some embodiments, the cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, TIL and/or cytotoxic lymphocytes are genetically modified using a viral vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding

protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein

said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

Lymphodepleting Methods

[00320] In some embodiments, nonmyeloablative lymphodepleting chemotherapy is administered to the mammal prior to administering to the mammal the expanded cytotoxic lymphocytes obtained from the tumor-infiltrating lymphocytes. The purpose of lymphodepletion is to make room for the infused lymphocytes, in particular by eliminating regulatory T-cells and other non-specific T-cells which compete for homeostatic cytokines. Nonmyeloablative lymphodepleting chemotherapy can be any suitable such therapy, which can be administered by any suitable route known to a person of skill. The nonmyeloablative lymphodepleting chemotherapy can comprise, for example, the administration of cyclophosphamide and fludarabine, particularly if the cancer is melanoma, which can be metastatic. In some embodiments, the route of administering cyclophosphamide and fludarabine is intravenously. Likewise, any suitable dose of cyclophosphamide and fludarabine can be administered.

Preferably, around 40-80 mg/kg, such as around 60 mg/kg of cyclophosphamide is administered for approximately two days after which around 15-35 mg/m^2, such as around 25 mg/m^2 fludarabine is administered for around five days, particularly if the cancer is melanoma.

[00321] In some embodiments, nonmyeloablative lymphodepleting chemotherapy is administered to the mammal prior to administering to the mammal the cytotoxic lymphocytes that are genetically modified. In some embodiments, the cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified with a viral vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is

measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-

H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

Combination Therapies

[00322] In some embodiments, a suitable therapy will include exposing a subject to one or more lymphodepletion measures prior to administration of the genetically modified cytotoxic lymphocytes of the present disclosure. Lymphodepletion measures may take the form of chemo- or radiotherapy. Suitable chemotherapy measures may include, *e.g.*, treatment with cyclophosphamide and/or fludarabine.

[00323] In some embodiments of the present disclosure, cells expanded using the methods described herein, *e.g.*, in connection with any embodiment described herein,, or other methods known in the art where lymphocytes are expanded to therapeutic levels, are administered to a patient in conjunction with (*e.g.*, before, simultaneously or following) any number of relevant treatment modalities, including but not limited to treatment with agents such as antiviral therapy, cidofovir and interleukin-2, Cytarabine (also known as ARA-C) or natalizumab treatment for MS patients or efalizumab treatment for psoriasis patients or other treatments for PML patients. In further embodiments, the genetically modified cytotoxic lymphocytes of the invention may be used in combination with chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludaribine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, cytokines, and irradiation, These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin) (Liu et al., *Cell* 66:807-815, 1991; Henderson et al., *Immun.* 73:316-321, 1991; Bierer et al., *Curr. Opin, Immun.* 5:763- 773, 1 993). In a further embodiment, the cell compositions of the present disclosure are administered to a patient in conjunction with (*e.g.*, before, simultaneously or following) bone marrow transplantation, T-cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH. In another embodiment, the cell compositions of the present disclosure are administered following B-cell ablative therapy such as agents that react with CD20, *e.g.*, Rituxan. For example, in one embodiment, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following the transplant, subjects receive an infusion of the expanded genetically modified cytotoxic lymphocytes of the present disclosure. In an additional embodiment, expanded cells are administered before or following surgery.

[00324] In some embodiments, the expanded cells administered before or following surgery are genetically modified. In some embodiments, the cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified with a viral vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B,**

Figure 16B). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition

of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some

embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00325] In some embodiments, genetically modified cytotoxic lymphocytes are administered simultaneously with a standard anti-cancer drug or therapy. Numerous anti-cancer drugs and therapies are available for combination with the present methods and compositions. The following is a non-exhaustive lists of anti-cancer (anti-neoplastic) drugs that can be used in conjunction with irradiation: Acivicin; Aclarubicin; Acodazole Hydrochloride; AcrQnine; Adozelesin; Aldesleukin; Altretamine; Ambomycin; Ametantrone Acetate; Aminoglutethimide; Amsacrine; Anastrozole; Anthramycin; Asparaginase; Asperlin; Azacitidine; Azetepa; Azotomycin; Batimastat; Benzodepa; Bicalutamide; Bisantrene Hydrochloride; Bisnafide Dimesylate; Bizelesin; Bleomycin Sulfate; Brequinar Sodium; Bropirimine; Busulfan; Cactinomycin; Calusterone; Caracemide; Carbetimer; Carboplatin; Carmustine; Carubicin Hydrochloride; Carzelesin; Cedefingol; Chlorambucil; Cirolemycin; Cisplatin; Cladribine; Crisnatol Mesylate; Cyclophosphamide; Cytarabine; Dacarbazine; Dactinomycin; Daunorubicin Hydrochloride; Decitabine; Dexormaplatin; Dezaguanine; Dezaguanine Mesylate; Diaziquone; Docetaxel; Doxorubicin; Doxorubicin Hydrochloride; Droloxifene; Droloxifene Citrate; Dromostanolone Propionate; Duazomycin; Edatrexate; Eflomithine Hydrochloride; Elsamitrucin; Enloplatin; Enpromate; Epipropidine; Epirubicin Hydrochloride; Erbulozole; Esorubicin Hydrochloride; Estramustine; Estramustine Phosphate Sodium; Etanidazole; Ethiodized Oil 1131; Etoposide; Etoposide Phosphate; Etoprine; Fadrozole Hydrochloride; Fazarabine; Fenretinide; Floxuridine; Fludarabine Phosphate; Fluorouracil; Flurocitabine;

Fosquidone; Fostriecin Sodium; Gemcitabine; Gemcitabine Hydrochloride; Gold Au 198; Hydroxyurea; Idarubicin Hydrochloride; Ifosfamide; Ilmofosine; Iproplatin; Irinotecan Hydrochloride; Lanreotide Acetate; Letrozole; Leuprolide Acetate; Liarozole Hydrochloride; Lometrexol Sodium; Lomustine; Losoxantrone Hydrochloride; Masoprocol; Maytansine; Mechlorethamine Hydrochloride; Megestrol Acetate; Melengestrol Acetate; Melphalan; Menogaril; Mercaptopurine; Methotrexate; Methotrexate Sodium; Metoprine; Meturedepa; Mitindomide; Mitocarcin; Mitocromin; Mitogillin; Mitomalcin; Mitomycin; Mitosper; Mitotane; Mitoxantrone Hydrochloride; Mycophenolic Acid; Nocodazole; Nogalamycin; Ormaplatin; Oxisuran; Paclitaxel; Pegaspargase; Peliomycin; Pentamustine; Peplomycin Sulfate; Perfosfamide; Pipobroman; Piposulfan; Piroxantrone Hydrochloride; Plicamycin; Plomestane; Porfimer Sodium; Porfiromycin; Prednimustine; Procarbazine Hydrochloride; Puromycin; Puromycin Hydrochloride; Pyrazofurin; Riboprine; Rogletimide; Safmgol; Safingol Hydrochloride; Semustine; Simtrazene; Sparfosate Sodium; Sparsomycin; Spirogermanium Hydrochloride; Spiromustine; Spiroplatin; Streptonigrin; Streptozocin; Strontium Chloride Sr 89; Sulofenur; Talisomycin; Taxane; Taxoid; Tecogalan Sodium; Tegafur; Teloxantrone Hydrochloride; Temoporfin; Teniposide; Teroxirone; Testolactone; Thiamiprine; Thioguanine; Thiotepa; Tiazofurin; Tirapazamine; Topotecan Hydrochloride; Toremifene Citrate; Trestolone Acetate; Triciribine Phosphate; Trimetrexate; Trimetrexate Glucuronate; Triptorelin; Tubulozole Hydrochloride; Uracil Mustard; Uredepa; Vapreotide; Verteporfin; Vinblastine Sulfate; Vincristine Sulfate; Vindesine; Vindesine Sulfate; Vinepidine Sulfate; Vinglycinate Sulfate; Vinleurosine Sulfate; Vinorelbine Tartrate; Vinrosidine Sulfate; Vinzolidine Sulfate; Vorozole; Zeniplatin; Zinostatin; Zorubicin Hydrochloride.

Subjects Suitable for Treatment

[00326] Subjects suitable for treatment with the disclosed methods and compositions include, but are not limited to, those having (*e.g.*, diagnosed as having) or at risk of having cancer and/or a chronic viral infection. Types of cancer which may be amenable to treatment via the methods and compositions disclosed herein include, but are not limited to, metastatic melanoma, lymphoma, leukemia, and solid tumors of pancreatic or brain origin. Types of chronic viral infection which may be amenable to treatment via the methods and compositions disclosed herein include, but are not limited to, post-transplant lymphoproliferative diseases (PTLD) associated with Epstein-Barr virus (EBV) infection, human papillomavirus (HPV), cytomegalovirus, and adenovirus infection. Subjects infected with hepatitis C and B viruses may also be amendable to treatment via the methods and compositions disclosed herein.

[00327] Cancers that may be treated include tumors that are not vascularized, or not yet substantially vascularized, as well as vascularized tumors. The cancers may comprise non-solid tumors (such as hematological tumors, for example, leukemias and lymphomas) or may comprise solid tumors. Types of cancers to be treated with the methods and compositions of the present disclosure o include, but are not limited to, carcinoma, blastoma, and sarcoma, and certain leukemia or lymphoid malignancies, benign and malignant tumors, and malignancies *e.g.*, sarcomas, carcinomas, and melanomas. Adult tumors/cancers and pediatric tumors/cancers are also included.

[00328] Hematologic cancers are cancers of the blood or bone marrow. Examples of hematological (or hematogenous) cancers include leukemias, including acute leukemias (such as acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia and myeloblastic, promyeiocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (such as chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, and chronic lymphocytic leukemia), polycythemia vera, lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma (indolent and high grade forms), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, myeiodysplastic syndrome, hairy cell leukemia and myelodysplasia.

[00329] Solid tumors are abnormal masses of tissue that usually do not contain cysts or liquid areas. Solid tumors can be benign or malignant. Different types of solid tumors are named for the type of cells that form them (such as sarcomas, carcinomas, and lymphomas). Examples of solid tumors, such as sarcomas and carcinomas, include fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteosarcoma, and other sarcomas, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, lymphoid malignancy, pancreatic cancer, breast cancer, lung cancers, ovarian cancer, prostate cancer, hepatocellular carcinoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, medullary thyroid carcinoma, papillary thyroid carcinoma, pheochromocytomas sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, Wilms' tumor, cervical cancer, testicular tumor, seminoma, bladder carcinoma, melanoma, and CNS tumors (such as a glioma (such as brainstem glioma and mixed gliomas), glioblastoma (also known as glioblastoma multiforme) astrocytoma, CNS lymphoma, germinoma, medulloblastoma, Schwannoma craniopharyogioma, ependymoma, pineaioma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, neuroblastoma, retinoblastoma and brain metastases).

[00330] In some embodiments, the methods promote cancer regression. Promoting regression of cancer in a mammal can comprise treating or preventing cancer in the mammal. The terms "treat," "prevent," and "regression," as well as words stemming therefrom, as used herein, does not necessarily imply 100% or complete regression. Rather, there are varying degrees of treatment, prevention, and regression of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the inventive methods can provide any amount of any level of treatment, prevention, or regression of cancer in a mammal. Furthermore, the treatment, prevention, or regression provided by the inventive method can include treatment, prevention, or regression of one or more conditions or symptoms of the disease, *e.g.*, cancer. Also, for purposes herein, "treatment," "prevention," and "regression" can encompass delaying the onset of the disease, or a symptom or condition thereof.

[00331] In some embodiments, cytotoxic lymphocytes are administered as part of the treatment regimen. In some embodiments, a PD-L1 binding protein is administered as part of the treatment regimen. In some embodiments, cytotoxic lymphocytes in combination with a PD-L1 binding protein are administered as part of the treatment regimen. In some embodiments, genetically modified cytotoxic lymphocytes are administered as part of the treatment regimen. In some embodiments, the cytotoxic lymphocytes are genetically modified cytotoxic lymphocytes. In some embodiments, the cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified with a viral vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express

and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID

NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In

some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

Methods of Making Cytotoxic Lymphocytes Genetically Modified to Express and Secrete a Soluble Programmed Death 1 Ligand 1 (PD-L1) Binding Protein

[00332] Methods of making genetically modified cytotoxic lymphocytes according to the present disclosure may include one or more of the isolating, genetically modifying, and expanding steps described above.

[00333] In some embodiments, the genetically cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments, the genetically modified cytotoxic lymphocytes made according to the present invention have been genetically modified using viral vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embdiments, the genetically modified cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embdiments, the genetically modified cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embdiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells

detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody

or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

KITS

[00334] The present disclosure also provides kits including one or more compositions of the present disclosure, *e.g.*, selected from: (i) a subject PD-L1 binding protein (*e.g.*, scFV, maxibody, and the like), *e.g.*, in connection with any embodiment described herein, (ii) one or more nucleic acids (*e.g.*, vectors) including a nucleotide sequence(s) encoding a subject PD-L1 binding protein according to the present disclosure, *e.g.*, in connection with any embodiment described herein, and (iii) one or more genetically modified cells (*e.g.*, a cytotoxic lymphocyte) (*e.g.*, in a composition including one or more pharmaceutically acceptable excipients) according to the present disclosure, *e.g.*, in connection with any embodiment described herein. In some embodiments, cytotoxic lymphocytes are included in the kit. In some embodiments, a PD-L1 binding protein is included in the kit. In some embodiments, cytotoxic lymphocytes in combination with a PD-L1 binding protein are included in the kit. In some embodiments, genetically modified cytotoxic lymphocytes in the kit. In some embodiments, the cytotoxic lymphocytes are genetically modified cytotoxic lymphocytes. In some embodiments, the cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embdiments, the TIL and/or cytotoxic lymphocytes are genetically modified with a viral vector. In some embodiments, the viral vector is a lentiviral based vector. In some embodiments, the viral vector is pLV4301G PDLV scFV 38A1 (SEQ ID NO:37; **Figure 11A, Figure 16A**). In some embodiments, the viral vector is pLV4301G PDLV scFV 19H9 (SEQ ID NO:38; **Figure 11B, Figure 16B**). In some embodiments, the viral vector is a pLEV based viral vector. In some embodiments, the viral vector is a gammaretroviral based vector. In some embodiments, the viral vector is a pMSCIV based vector. In some embodiments, the viral vector encodes 19H9. In some embodiments, the viral vector is a lentiviral based vector encoding 19H9. In some embodiments, the viral vector is a pLEV based viral vector encoding 19H9. In some embodiments, the viral vector encodes 38A1. In some embodiments, the viral vector is a lentiviral based vector encoding 38A1. In some embodiments, the viral vector is a pLEV based viral vector encoding 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 19H9. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete 38A1. In some embodiments, the TIL and/or cytotoxic lymphocytes are genetically modified to express and secrete a PD-L1 binding protein. In some embodiments the PD-L1 binding protein comprises an antigen binding portion. In some embodiments, the 38A1 protein exhibits greater or increased binding capacity in Jurkat cells as compared to 19H9. In some embodiments, the PD-L1 binding protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In

some embodiments, the 38A1 protein exhibits greater or increased binding capacity in melanoma as compared to 19H9. In some embodiments, the increased binding capacity is measured by (i) determining the percentage of PD-L1 positive cells detected in a flow cytometry assay using a PD-L1 binding protein labeled with or detectable by a fluorescent label, and (ii) comparing the number of PD-L1 positive cells obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the percentage of PD-L1 positive cells detected by flow cytometry as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the increased binding capacity is measured by (i) determining the mean fluorescence intensity (MFI) of PD-L1 positive cells in a flow cytometry assay which have been labeled with a PD-L1 binding protein labeled with or detectable by a fluorescent label and (ii) comparing the MFI obtained with the PD-L1 binding protein to a control antibody or comparing to 19H9, wherein an increase in the MFI of the total cell population by flow cytometry with the PD-L1 binding protein as compared to a control antibody or 19H9 indicates an increase in binding capacity (*see*, for example, the assay in Example 3). In some embodiments, the PD-L1 binding protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, the 38A1 protein exhibits greater or increased biological function in Jurkat cells as compared to 19H9. In some embodiments, biological function includes blockade of the engagement of PD-1 and PD-L1. In some embodiments, biological function includes inhibition of the PD-1 and PD-L1 signaling pathway. In some embodiments, the PD-L1 binding protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments, the 19H9 protein exhibits greater or increased secretion capacity in Jurkat cells as compared to 38A1. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:2 (CDR-L1), SEQ ID NO:3 (CDR-L2), and SEQ ID NO:4 (CDR-L3) and said heavy chain comprises CDRs of SEQ ID NO:6 (CDR-H1), SEQ ID NO:7 (CDR-H2), and SEQ ID NO:8 (CDR-H3). In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises a sequence

having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a heavy chain and a light chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments the PD-L1 binding protein is an antibody or fragment thereof comprising a light chain and a heavy chain, wherein said light chain comprises CDRs of SEQ ID NO:10 (CDR-L1), SEQ ID NO:11 (CDR-L2), and SEQ ID NO:12 (CDR-L3) and said heavy chain comprises CDRs comprising SEQ ID NO:14 (CDR-H1), SEQ ID NO:15 (CDR-H2), and SEQ ID NO:16 (CDR-H3). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:1 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:1 and said heavy chain comprises SEQ ID NO:5. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:17. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:18. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:9 and said heavy chain comprises a sequence having 90%, 95%, or 98% identity to SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising a light chain and a heavy chain, wherein said light chain comprises SEQ ID NO:9 and said heavy chain comprises SEQ ID NO:13. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:19. In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1, IgG2, IgG3, or IgG4 sequence (*i.e.*, conjugated to an IgG1, IgG2, IgG3, or IgG4 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) further comprising an IgG1 sequence (*i.e.*, conjugated to an IgG1 sequence). In some embodiments, the PD-L1 binding protein is a single-chain Fv (scFv) comprising SEQ ID NO:20. In some embodiments, conjugation is direct. In some embodiments, conjugation is via a linker.

[00335] In some cases, a subject kit includes suitable instructional material, *e.g.*, to practice methods as described herein, *e.g.*, in connection with any embodiment described herein.

Examples of Non-Limiting Aspects of the Disclosure

[00336] Aspects, including embodiments, of the present subject matter described above may be beneficial alone or in combination, with one or more other aspects or embodiments. Without limiting the foregoing description, certain non-limiting aspects of the disclosure numbered 1-76 are provided below. As will be apparent to those of skill in the art upon reading this disclosure, each of the individually numbered aspects may be used or combined with any of the preceding or following individually numbered aspects. This is intended to provide support for all such combinations of aspects and is not limited to combinations of aspects explicitly provided below:

1. 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.

2. The protein of 1, 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.

3. The protein of 2, 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.

4. The protein of 1, 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.

5. The protein of 4, 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.

6. The protein of any of 1-5, wherein the first polypeptide is a light chain, and the second polypeptide is a heavy chain.

7. The protein of any of 1-6, 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.

8. The protein of 7, wherein the scFv comprises the amino acid sequence set forth in SEQ ID NO:17 or SEQ ID NO:19.

9. The protein of any of 1-8, wherein the protein is a maxibody comprising an immunoglobulin Fc domain fused directly or via a linker to the antigen binding portion.

10. The protein of 9, wherein the immunoglobulin Fc domain is an IgG1 Fc domain.

11. The protein of 10, wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:18 or SEQ ID NO:20.

12. The protein of 9, wherein the immunoglobulin Fc domain is an IgG4 Fc domain.

13. The protein of any of 1-6, wherein the protein is a humanized antibody.

14. A nucleic acid comprising a nucleotide sequence encoding the protein of any one of 1-13.

15. The nucleic acid of 14, wherein the nucleic acid comprises a promoter that is operably linked to the nucleotide sequence encoding the protein.

16. The nucleic acid of 15, wherein the promoter is a constitutive promoter.

17. The nucleic acid of 15, wherein the promoter is an inducible promoter.

18. A cell comprising the nucleic acid of any one of 14-17.

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

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

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

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

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

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

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

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

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

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

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

32. A method comprising:

genetically modifying a cytotoxic lymphocyte isolated from a tumor of a 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;

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.

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

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

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

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

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

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

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

40. The method 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 method of any one of claims 32-40, comprising isolating the cytotoxic lymphocyte from the subject prior to the genetically modifying.

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

48. The method 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 method 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 method 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 method of claim 50, wherein the immunoglobulin Fc domain is an IgG1 Fc domain.

52. The method 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 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, comprising isolating the cytotoxic lymphocyte from the subject prior to the genetically modifying.

58. The method of claim 57, wherein the isolating comprises isolating the cytotoxic lymphocyte from a tumor of the subject.

59. The method of any one of claims 57, wherein the isolating comprises isolating the cytotoxic lymphocyte 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. 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 according to any of claims 1-13 to the individual.

69. The method of claim 68, wherein the administering comprises introducing into the subject a nucleic acid encoding the protein.

70. The method of claim 68, wherein the administering comprises introducing into the subject a genetically modified cytotoxic lymphocyte that expresses and secretes the protein.

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

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

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

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

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

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

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

78. The method 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 method 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 method 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 first and second cells are in an individual, and the contacting comprises administering the protein of any of claims 1-13 to the individual.

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

84. The method of claim 82, wherein the introducing 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.

EXAMPLES

[00337] As can be appreciated from the disclosure provided above, the present disclosure has a wide variety of applications. Accordingly, the following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Those of skill in the art will readily recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.* amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, *e.g.*, bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); nt, nucleotide(s); and the like.

Example 1: PD-L1 binding proteins with an antigen binding portion

[00338] Recombinant antibodies specific for PD-L1 (anti-PD-L1 scFV fused to the human Fc domain of human IgG) were generated. Using a scFV phage display library (Viva Biotechnologies), several scFVs specific for PD-L1 were isolated. Two scFVs bound to native PD-L1, and were named 38A1 and 19H9. 38A1-scFV, 19H9-scFV and FMC63-scFV (anti-CD19 scFV negative control) were fused to the Fc domain of human IgG1 (38A1-scFV-Fc, 19H9-scFV-Fc and FMC63-scFV-Fc). These were constructed in lentiviral and retroviral plasmids using multisite lambda phage "Gateway" recombination (*e.g.*, see Figure 1). Each scFV sequence was flanked by the recombination sites attL1 and attR5, synthesized by Geneart (Life Technologies) and subcloned into pMK. The sequence for IgG1 Fc was flanked by the recombination sites attL5 and attL2, synthesized by Geneart (Life Technologies) and subcloned into pMK. Each scFV was recombined with IgG1-Fc in either a lentiviral plasmid

(pLVU3pIRESeGFP; Figure 2) or a retroviral plasmid (pMSCVIRESeGFP; Figure 3). The recombined constructs consist of a fusion of the scFV (38A1, 19H9 or FMC63) fused to IgG1-Fc via an attB5 recombination site.

[00339] To determine the affinity of the 38A1-scFV-Fc (SEQ ID NO:17) and 19H9-scFV-Fc (SEQ ID NO:19) maxibodies for PD-L1 (using 12D10-scFV-Fc, a microtubule associated protein specific reagent, as a negative control), a "cell based ELISA" was performed using 293-PD-L1. The affinity of 38A1-scFV-Fc (EC50 = 0.1248) was observed to be 3.2 fold greater than that for 19H9-scFV-Fc (EC50 = 0.4039; Figure 5).

[00340] Jurak T-cells were transduced with lentivirus encoding either 38A1-scFV-Fc, 19H9-scFV-Fc or FMC63-scFV-Fc. 293-PD-L1 were co-cultured at a ratio of 1:1 for 16 hours with Jurkat T-cells stably secreting 38A1-scFV-Fc, 19H9-scFV-Fc or FMC63-scFV-Fc. We found that 38A1-scFV-Fc, 19H9-scFV-Fc but not FMC63-scFV-Fc bound to 293-PD-L1. Compared to 19H9-scFV-Fc, we observed greater than 1.5-fold enhanced binding of 38A1-scFV-Fc to 293-PD-L1 (Figure 5). This result was consistent with a greater affinity of 38A1-scFV-Fc compared to 19H9-scFV-Fc for PD-L1.

[00341] TIL lines M1034 and M1015 were transduced with lentivirus encoding either 38A1-scFV-Fc, 19H9-scFV-Fc or FMC63-scFV-Fc. After sorting for the eGFP co-reporter and expanding in-vitro, supernatants were collected from TIL secreting FMC63-scFV-Fc and 38A1-scFV-Fc, concentrated ten-fold and used to stain 293-PD-L1. Compared to the negative control maxibody (FMC63-scFV-Fc), we observed greater than a 50 fold in increase binding of 38A1-scFV-Fc to 293-PD-L1 (Figure 6).

[00342] The capacity of 38A1-scFV-Fc and 19H9-scFV-Fc to relinquish inhibition of T-cell activity blocked by PD-L1 was next tested. To do so, 12D10-scFV-Fc (microtubule associated protein specific negative control), 19H9-scFV-Fc, and 38A1-scFV-Fc were added to a co-culture of Jurkat T-cells that produce firefly luciferase when activated and CHO cells engineered to activate T-cells but also inhibit such activation via over-expression of PD-L1 as described above. The addition of 38A1-scFV-Fc and 19H9-scFV-Fc but not 12D10-scFV-Fc to the Jurkat/CHO co-culture led to increased bioluminescent activity. In both cases, "de-inhibition" of PD-L1 was dose dependent. The capacity of 38A1-scFV-Fc to induce bioluminescent activity was greater than 3.6 fold that for 19H9-scFV-Fc, consistent with the observed 3.2-fold difference in affinity for PD-L1 (Figure 7).

Example 2: Production of TILs expressing the PD-L1 scFv

Viral production

[00343] Method steps:

1) Plate $5x10^6$ phoenix cells in 10 mm tissue culture plate containing 5 ml cell culture media

2) Transfect cells with 10 ug pLEV (lentiviral vector) containing anti-PD-1 scFV and 6 ug pVSV-G using lipofectamine.

3) After 60 hours, collect the supernatant and filter with 70 uM strainer into a 15 ml tube.

T-cell transduction

[00344] Method steps:

1) Activate $1x10^6$ TIL with anti-CD3 at concentration of 300 ng/ml in 2 ml TIL culture media in 12-well-plate.

2) After 48 hours, split TIL into two well and add 1 ml of supernatant (from step 3 in viral production) into each well together with polybrene at a concentration of 8 ug/ml.

3) Centrifuge cells for 30 minutes at 800 x g at 32 C.

4) Remove virus containing medium and resuspend cell pellet with 2 ml of fresh complete culture media, and incubate the cells for 24 hours.

5) Propagate the cells using Rapid Expansion Protocol (REP).

Example 3: Characterization of 19H9 and 38A1

19H9 Exhibits Greater Secretion Capacity

[00345] Goal: To compare secretion capacity of Jurkat cells overexpressing anti-PD-L1 ScFV clone 19H9 versus 38A1.

[00346] Overall, 19H9 exhibits greater secretion capacity. A comparison of the secretion capacity of Jurkat cells overexpressing anti-PD-L1 ScFV clone 19H9 versus 38A1 is provided in **Figure 12**. Supernatant from Jurkat cells overexpressing anti-PD-L1 ScFV clone 19H9 and 38A1 were harvested and concentrated for IgG ELISA assay to determine the anti-PD-L1 ScFV concentration. It was found that Jurkat cells clone 19H9 had greater secretion capacity as compared with 38A1 (9.82 versus 6.75 µg/cells).

38A1 Exhibits Greater Binding Capacity in Jurkat cells Overexpressing PD-L1

[00347] Goal: To examine the binding affinity of anti-PD-L1 ScFV clone 19H9 and 38A1 using EL4 PD-L1 cells.

[00348] Overall, 38A1 exhibits greater binding capacity in Jurkat cells overexpressing PD-L1. The binding affinity of anti-PD-L1 ScFV clone 19H9 and 38A1 using EL4 PD-L1 cells was examined (**Figure 13**). EL4 PD-L1 were incubated with either anti-PD-L1 ScFV clone 19H9 or 38A1 at concentration of 1, 3, 10, 30, 100, and 300 ng/ml, and stained with Amcyan and goat anti-human FITC. The cells were washed with FACs buffer and analyzed by flow cytometry. Clone 38A1 exhibited greater binding capacity in Jurkat-cells overexpressing PD-L1 in both the percentage of PD-L1 positive cells (**Figure 13A**) and Mean of Fluorescence Intensity (MFI) (**Figure 13B**).

38A1 Exhibits Greater Binding Capacity in Melanoma Tumor Cells

[00349] Goal: To validate the binding of anti-PD-L1 ScFV in tumor cells,

[00350] Overall, 38A1 exhibits greater binding capacity in melanoma tumor cells. A comparison of the binding affinity of anti-PD-L1 ScFV clone 19H9 and 38A1 using EL4 PD-L1 cells is provided in **Figure 14**. EL4 PD-L1 were incubated with either anti-PD-L1 ScFV clone 19H9 or 38A1 at concentration of 1, 3, 10, 30, 100, and 300 ng/ml, and stained with Amcyan and goat anti-human FITC. The cells were washed with FACs buffer and analyzed by flow cytometry. Clone 38A1 exhibited greater binding capacity in Jurkat cells overexpressing PD-L1 in both the percentage of PD-L1 positive cells (**Figure 14A**) and Mean of Fluorescence Intensity (MFI) (**Figure 14B**). To validate the binding of anti-PD-L1 ScFV in tumor cells, three melanoma tumor cells were treated with IFN-gamma (100 ng/ml) to enhance PD-L1 expression. After three days, the cells were harvested with cell dissociation buffer, incubated with either anti-PD-L1 ScFV clone 19H9 or 38A1 at concentration of 100 ng/ml, and stained with goat anti-human FITC. The cells were washed with FACs buffer and analyzed by flow cytometry. We found that clone 38A1 exhibited greater binding affinity in both the percentage of PD-L1 positive cells and MFI. Overall, clone 38A1 has greater binding affinity, but slightly less secretion capacity when compared to Clone 19H9.

38A1 Exhibits Greater Biological Function

[00351] Goal: To further characterize the biological function of two clones of anti-PD-L1 ScFV (19H9 and 38A1).

[00352] Overall, 38A1 exhibits greater biological function than 19H9. To further determine the biological function of two clones of anti-PD-L1 ScFV (19H9 and 38A1), a PD-L1 blockade assay was conducted, which can be used to determine the potency of either anti PD-1 or PD-L1 antibody that block the engagement of PD-1 and PD-L1 interaction (**Figure 15**). The

assay consists of two genetically engineered cell lines: PD-1 effector cell (Jurkat T-cells stably expressing human PD-1 and NFAT-induced luciferase) and PD-L1 aAPC/CHO-K1 Cells stably expressing human PD-L1 with a cell surface protein activating cognate TCRs. When two cell types were co-cultured, the engagement of PD-1 and PD-L1 inhibited TCR signaling and decreased luciferase activity. Addition of either anti-PD-1 or PD-L1 blockade antibody helped release the inhibitory signal, resulting in enhanced TCR signaling and NFAT-mediated luciferase activity. Luciferase signal (RLU) was found to be greater when blocked with anti-PD-L1 as compared to anti-PD-1, suggesting that blocking PD-L1 seemed to be more effective than PD-1 in this context (**Figure 15A**). Expectedly, anti-PD-L1 ScFV clone 38A1, which previously shown to have greater binding affinity, provided greater biological function due to highly increased in luciferase signal in both purified ScFV (P) and non-purified (NP) settings (**Figure 15B**).

[00353] The preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, *i.e.*, any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the example embodiments shown and described herein. Rather, the scope and spirit of present invention is embodied by the appended claims.

[00354] The examples set forth above are provided to give those of ordinary skill in the art a complete disclosure and description of how to make and use the embodiments of the compositions, systems and methods of the invention, and are not intended to limit the scope of

what the inventors regard as their invention. Modifications of the above-described modes for carrying out the invention that are obvious to persons of skill in the art are intended to be within the scope of the following claims. All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

[00355] All headings and section designations are used for clarity and reference purposes only and are not to be considered limiting in any way. For example, those of skill in the art will appreciate the usefulness of combining various aspects from different headings and sections as appropriate according to the spirit and scope of the invention described herein.

[00356] All references cited herein are hereby incorporated by reference herein in their entireties and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[00357] Many modifications and variations of this application can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments and examples described herein are offered by way of example only, and the application is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which the claims are entitled.

CLAIMS

What Is Claimed Is:

1. 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.

2. The protein of claim 1, 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.

3. The protein of claim 2, 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.

4. The protein of claim 1, 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.

5. The protein of claim 4, 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.

6. The protein of any of claims 1-5, wherein the first polypeptide is a light chain, and the second polypeptide is a heavy chain.

7. The protein of any of claims 1-6, 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.

8. The protein of claim 7, wherein the scFv comprises the amino acid sequence set forth in SEQ ID NO:17 or SEQ ID NO:19.

9. The protein of any of claims 1-8, wherein the protein is a maxibody comprising an immunoglobulin Fc domain fused directly or via a linker to the antigen binding portion.

10. The protein of claim 9, wherein the immunoglobulin Fc domain is an IgG1 Fc domain.

11. The protein of claim 10, wherein the protein comprises the amino acid sequence set forth in SEQ ID NO:18 or SEQ ID NO:20.

12. The protein of claim 9, wherein the immunoglobulin Fc domain is an IgG4 Fc domain.

13. The protein of any of claims 1-6, wherein the protein is a humanized antibody.

14. A nucleic acid comprising a nucleotide sequence encoding the protein of any one of claims 1-13.

15. The nucleic acid of claim 14, wherein the nucleic acid comprises a promoter that is operably linked to the nucleotide sequence encoding the protein.

16. The nucleic acid of claim 15, wherein the promoter is a constitutive promoter.

17. The nucleic acid of claim 15, wherein the promoter is an inducible promoter.

18. A cell comprising the nucleic acid of any one of claims 14-17.

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 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. A method comprising:

genetically modifying a cytotoxic lymphocyte isolated from a tumor of a 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;

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.

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

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

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

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

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

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

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

40. The method 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 method of any one of claims 32-40, comprising isolating the cytotoxic lymphocyte from the subject prior to the genetically modifying.

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

48. The method 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 method 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 method 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 method of claim 50, wherein the immunoglobulin Fc domain is an IgG1 Fc domain.

52. The method 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 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, comprising isolating the cytotoxic lymphocyte from the subject prior to the genetically modifying.

58. The method of claim 57, wherein the isolating comprises isolating the cytotoxic lymphocyte from a tumor of the subject.

59. The method of any one of claims 57, wherein the isolating comprises isolating the cytotoxic lymphocyte 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. 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 according to any of claims 1-13 to the individual.

69. The method of claim 68, wherein the administering comprises introducing into the subject a nucleic acid encoding the protein.

70. The method of claim 68, wherein the administering comprises introducing into the subject a genetically modified cytotoxic lymphocyte that expresses and secretes the protein.

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

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

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

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

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

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

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

78. The method 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 method 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 method 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 first and second cells are in an individual, and the contacting comprises administering the protein of any of claims 1-13 to the individual.

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

84. The method of claim 82, wherein the introducing 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.