



(51) International Patent Classification:

A61K 39/395 (2006.01) A61P 35/02 (2006.01)

(21) International Application Number:

PCT/US2024/051589

(22) International Filing Date:

16 October 2024 (16.10.2024)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/591,060 17 October 2023 (17.10.2023) US

(71) Applicants: **MEMORIAL SLOAN-KETTERING CANCER CENTER** [US/US]; 1275 York Avenue, New York, New York 10065 (US). **MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES** [US/US]; 1275 York Avenue, New York, New York 10065 (US). **SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH** [US/US]; 1275 York Avenue, New York, New York 10065 (US).

(72) Inventors: **HSU, Katharine**; c/o MEMORIAL SLOAN KETTERING CANCER CENTER, 1275 York Avenue, New York, New York 10065 (US). **SHAFFER, Brian**; c/o MEMORIAL SLOAN KETTERING CANCER CENTER, 1275 York Avenue, New York, New York 10065 (US).

(74) Agent: **FERNANDES, Jolene S.** et al.; c/o FOLEY & LARDNER LLP, 3000 K Street NW, Suite 600, Washington, District of Columbia 20007 (US).

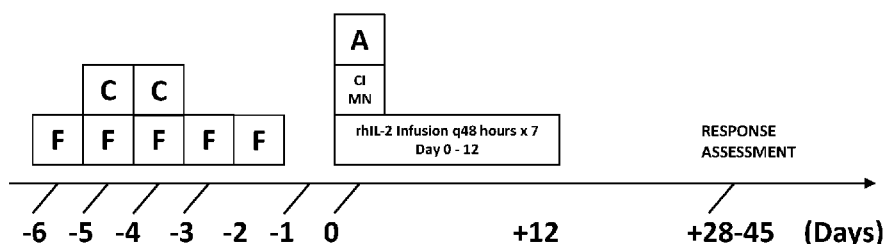
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report (Rule 48.2(g))

(54) Title: COMBINATION THERAPY WITH CYTOKINE INDUCED MEMORY- LIKE NATURAL KILLER (CIMN) CELLS AND PD-L1 INHIBITORS TO TREAT RELAPSED OR REFRACTORY ACUTE MYELOGENOUS LEUKEMIA



C = Cyclophosphamide 50 mg/kg
F = Fludarabine 25 mg/m²
A = Atezolizumab 1,200 mg flat dose
CIMN = Cytokine induced memory NK cells
rhIL-2 = recombinant human IL-2, 1 million IU SQ

Optional dose level 0 = 0.5 x 10⁶ CIMN cells/recipient kg
Dose level 1 = 1 x 10⁶ CIMN cells/recipient kg
Dose level 2 = Up to 1 x 10⁷ CIMN cells/recipient kg

FIG. 1

(57) Abstract: The present disclosure provides methods for treating acute myelogenous leukemia (AML) in a subject in need thereof comprising administering to the subject an effective amount of an anti-PD-L1 antibody or antigen binding fragment thereof and cytokine induced memory-like natural killer (CIMN) cells.



COMBINATION THERAPY WITH CYTOKINE INDUCED MEMORY-LIKE NATURAL KILLER (CIMN) CELLS AND PD-L1 INHIBITORS TO TREAT RELAPSED OR REFRACTORY ACUTE MYELOGENOUS LEUKEMIA

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Appl. No. 63/591,060, filed October 17, 2023, the contents of which are incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] The present disclosure relates to methods for treating acute myelogenous leukemia (AML) in a subject in need thereof comprising administering to the subject an effective amount of an anti-PD-L1 antibody or antigen binding fragment thereof and cytokine induced memory-like natural killer (CIMN) cells.

BACKGROUND

[0003] The following description of the background of the present technology is provided simply as an aid in understanding the present technology and is not admitted to describe or constitute prior art to the present technology.

[0004] Acute myeloid leukemia (AML) is a heterogenous myeloproliferative cancer arising from hematopoietic progenitor cells.¹ Proliferation of neoplastic cells within the bone marrow leads to loss of normal hematopoiesis, resulting in pancytopenia and subsequent complications from bone marrow failure. AML is common: There are roughly 18,000-20,000 new cases in the United States per year.² Long term survival in AML patients is extremely poor. Younger patients have <50% probability of survival without leukemia at 10 years, whereas older patients have <5% long-term disease free survival. A recent analysis of patients treated for AML without allogeneic hematopoietic cell transplantation (allo HCT) in the cooperative group setting a 10-year disease free survival of 3% in older patients and 16% in younger patients.³

[0005] The standard approach to newly diagnosed, fit patients with AML is administration of systemic chemotherapy, such as daunorubicin and cytarabine (“7+3”).²

Hypomethylating agents with or without venetoclax are active and are an appropriate therapy for less fit patients.⁴ Approximately 20-30% of younger adults and 50% of older adults fail to achieve a remission after initial treatment.⁵ Among patients who do obtain an initial remission, relapse within the first year is common. The typical strategy to manage patients with relapsed or refractory AML (r/r-AML) is to administer salvage chemotherapy and proceed to allogeneic hematopoietic cell transplant (allo HCT). This strategy is only successful in approximately 10% of patients, due to lack of response or excessive toxicity from the salvage treatment.⁵ Thus, current therapies for r/r-AML are insufficient to control leukemia, particularly in older patients or those with high-risk prognostic features.

[0006] Accordingly, there is an urgent need for methods and compositions that effectively treat AML.

SUMMARY OF THE PRESENT TECHNOLOGY

[0007] In one aspect, the present disclosure provides a method for treating acute myelogenous leukemia (AML) in a subject in need thereof comprising administering to the subject an effective amount of an anti-PD-L1 antibody or antigen binding fragment thereof, and an effective amount of cytokine induced memory-like natural killer (CIMN) cells. The AML may be relapsed AML or refractory AML. Additionally or alternatively, in some embodiments, the CIMN cells are autologous or obtained from a haploidentical donor.

[0008] In some embodiments of the methods disclosed herein, the anti-PD-L1 antibody or antigen binding fragment comprises a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein the V_H comprises a V_H -CDR1 sequence of SEQ ID NO: 4, a V_H -CDR2 sequence of SEQ ID NO: 6, and a V_H -CDR3 sequence of SEQ ID NO: 7 and the V_L comprises a V_L -CDR1 sequence of SEQ ID NO: 9, a V_L -CDR2 sequence of SEQ ID NO: 11, and a V_L -CDR3 sequence of SEQ ID NO: 13. Additionally or alternatively, in certain embodiments, the V_H comprises the amino acid sequence of SEQ ID NO: 1, and/or the V_L comprises the amino acid sequence of SEQ ID NO: 2.

[0009] In some embodiments of the methods disclosed herein, the anti-PD-L1 antibody or antigen binding fragment comprises a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein the V_H comprises a V_H -CDR1 sequence of SEQ ID NO: 17, a V_H -CDR2 sequence of SEQ ID NO: 19, and a V_H -

CDR3 sequence of SEQ ID NO: 21 and the V_L comprises a V_L -CDR1 sequence of SEQ ID NO: 23, a V_L -CDR2 sequence of SEQ ID NO: 25, and a V_L -CDR3 sequence of SEQ ID NO: 27. Additionally or alternatively, in certain embodiments, the V_H comprises the amino acid sequence of SEQ ID NO: 14, and/or the V_L comprises the amino acid sequence of SEQ ID NO: 15.

[0010] In some embodiments of the methods disclosed herein, the anti-PD-L1 antibody or antigen binding fragment comprises a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein the V_H comprises a V_H -CDR1 sequence of SEQ ID NO: 30, a V_H -CDR2 sequence of SEQ ID NO: 31, and a V_H -CDR3 sequence of SEQ ID NO: 32 and the V_L comprises a V_L -CDR1 sequence of SEQ ID NO: 33, a V_L -CDR2 sequence of SEQ ID NO: 34, and a V_L -CDR3 sequence of SEQ ID NO: 35. Additionally or alternatively, in certain embodiments, the V_H comprises the amino acid sequence of SEQ ID NO: 28; and/or the V_L comprises the amino acid sequence of SEQ ID NO: 29.

[0011] Additionally or alternatively, in some embodiments of the methods disclosed herein, the CIMN cells are derived from CD3⁺CD56⁺ NK cells. In certain embodiments, the CIMN cells are generated by stimulating donor NK cells with at least one cytokine for about 12-18 hours. In some embodiments, the CIMN cells are generated by stimulating donor NK cells with at least one cytokine for about 12 hours, about 12.5 hours, about 13 hours, about 13.5 hours, about 14 hours, about 14.5 hours, about 15 hours, about 15.5 hours, about 16 hours, about 16.5 hours, about 17 hours, about 17.5 hours, or about 18 hours. The at least one cytokine may comprise one or more of IL-12, IL-15, and/or IL-18.

[0012] Additionally or alternatively, in certain embodiments of the methods disclosed herein, the CIMN cells further comprises a chimeric antigen receptor (CAR) that specifically binds an AML-specific tumor antigen. Examples of AML-specific tumor antigens include, but are not limited to, CD33 (Siglec-3), CD123 (IL3R α), Siglec-6, TIM3 (HAVCR2), CD7, CD70, ILT3 (LILRB4), NKG2D ligand (NKG2DL), CD276 (B7-H3), CD117 (c-kit), FLT3 (CD135), CD19, CD174 (Lewis-Y, LeY), CLL-1 (CLEC12A), CD38, CD44v6, FR β , GM-CSF (CD116/CD131), CD25 (IL-2R α), CD32, CD47, CD56, CD90 (Thy1), CD96, IL1RAP, MUC1, WT1, PR1/HLA-A2 (h8F4), CD93, PD1, PRAME, mLPA, IDH1 (R132), IDH2 (R140), NPM1mut, NOTCH2, PRL3, IL12RB1, CD244/2B4, RHAMM, Survivin, hTERT or CD4.

[0013] Additionally or alternatively, in some embodiments, the CAR comprises (i) an extracellular antigen binding domain; (ii) a transmembrane domain; and (iii) an intracellular domain comprising one or more co-stimulatory domains, wherein the extracellular antigen binding domain binds to the AML-specific tumor antigen. The transmembrane domain of the CAR may comprise a CD8 transmembrane domain, a CD28 transmembrane domain, a NKG2D transmembrane domain, a CD3 ζ transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, or a BTLA transmembrane domain. In any of the preceding embodiments, the one or more co-stimulatory domains of the CAR are selected from the group consisting of a CD28 co-stimulatory domain, a 4-1BB co-stimulatory domain, an OX40 co-stimulatory domain, an ICOS co-stimulatory domain, a DAP-10 co-stimulatory domain, a PD-1 co-stimulatory domain, a CTLA-4 co-stimulatory domain, a LAG-3 co-stimulatory domain, a 2B4 co-stimulatory domain, a BTLA co-stimulatory domain, a NKG2C co-stimulatory domain, a NKG2D co-stimulatory domain, and any combination thereof.

[0014] Additionally or alternatively, in some embodiments of the methods disclosed herein, the CIMN cells are administered pleurally, intravenously, subcutaneously, intranodally, intratumorally, intrathecally, intrapleurally or intraperitoneally. In certain embodiments, the subject receives an infusion of the CIMN cells in two escalating dose levels.

[0015] Additionally or alternatively, in certain embodiments of the methods disclosed herein, the anti-PD-L1 antibody or antigen binding fragment is administered pleurally, intravenously, subcutaneously, intranodally, intratumorally, intrathecally, intrapleurally or intraperitoneally.

[0016] In any and all embodiments of the methods disclosed herein, administration of the CIMN cells and the anti-PD-L1 antibody or antigen binding fragment prevents Graft versus host disease (GvHD) in the subject.

BRIEF DESCRIPTION OF DRAWINGS

[0017] **FIG. 1** shows study schema of phase I trial.

[0018] **FIG. 2A:** Donor cell dose and KIR gene profile of treated patients. **FIG. 2B:** Kaplan-Meier estimate of overall survival. **FIG. 2C:** Incidence of neutrophil recovery in treated patients.

[0019] **FIGs. 3A-3C: Results of cytotoxicity assays using cytokine-induced memory-like NK cells with and without PD-L1 inhibition with atezolizumab.** **FIG. 3A:** *In vitro* cytotoxicity of purified NK cells treated for 18 hours with IL12 (10ng/ml), IL15 (50ng/ml) and IL18 (50ng/ml). NK cells were incubated with K562, THP1 and SET2 target cells for 6 hours without or with 20µg/ml of anti-PDL1 antibody Atezolizumab (AZ). Target cell killing was assessed using a 6 hour bioluminescence assay. One representative experiment is shown. **FIG. 3B:** Cumulative analysis of three independent experiments performed with the same setting described in **FIG. 3A**. For each target two Effector:Target (E:T) ratio were analyzed using a Wilcoxon matched-pairs signed rank test. **FIG. 3C:** PD-L1 surface expression on NK cells not treated or treated overnight with IL12 (10ng/ml), IL15 (50ng/ml) and IL18 (50ng/ml). ** P-value < 0.01, * P-value ≤ 0.05.

[0020] **FIG. 4A:** Volcano plot illustrating key differentially regulated genes in CIMN cells cultured with atezolizumab compared to those without. **FIG. 4B:** Differentially regulated immune checkpoint mediators in control, CIMN, and CIMN + atezolizumab.

DETAILED DESCRIPTION

[0021] It is to be appreciated that certain aspects, modes, embodiments, variations and features of the present methods are described below in various levels of detail in order to provide a substantial understanding of the present technology.

[0022] In practicing the present methods, many conventional techniques in molecular biology, protein biochemistry, cell biology, immunology, microbiology and recombinant DNA are used. *See, e.g.,* Sambrook and Russell eds. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd edition; the series Ausubel *et al.* eds. (2007) *Current Protocols in Molecular Biology*; the series *Methods in Enzymology* (Academic Press, Inc., N.Y.); MacPherson *et al.* (1991) *PCR 1: A Practical Approach* (IRL Press at Oxford University Press); MacPherson *et al.* (1995) *PCR 2: A Practical Approach*; Harlow and Lane eds. (1999) *Antibodies, A Laboratory Manual*; Freshney (2005) *Culture of Animal Cells: A Manual of Basic Technique*, 5th edition; Gait ed. (1984) *Oligonucleotide Synthesis*; U.S. Patent No. 4,683,195; Hames and Higgins eds. (1984) *Nucleic Acid Hybridization*;

Anderson (1999) *Nucleic Acid Hybridization*; Hames and Higgins eds. (1984) *Transcription and Translation; Immobilized Cells and Enzymes* (IRL Press (1986)); Perbal (1984) *A Practical Guide to Molecular Cloning*; Miller and Calos eds. (1987) *Gene Transfer Vectors for Mammalian Cells* (Cold Spring Harbor Laboratory); Makrides ed. (2003) *Gene Transfer and Expression in Mammalian Cells*; Mayer and Walker eds. (1987) *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); and Herzenberg *et al.* eds (1996) *Weir's Handbook of Experimental Immunology*. Methods to detect and measure levels of polypeptide gene expression products (*i.e.*, gene translation level) are well-known in the art and include the use of polypeptide detection methods such as antibody detection and quantification techniques. (*See also*, Strachan & Read, *Human Molecular Genetics*, Second Edition. (John Wiley and Sons, Inc., NY, 1999)).

Definitions

[0023] Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this technology belongs. As used in this specification and the appended claims, the singular forms “a”, “an” and “the” include plural referents unless the content clearly dictates otherwise. For example, reference to “a cell” includes a combination of two or more cells, and the like. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry, analytical chemistry and nucleic acid chemistry and hybridization described below are those well-known and commonly employed in the art.

[0024] As used herein, the term “about” in reference to a number is generally taken to include numbers that fall within a range of 1%, 5%, or 10% in either direction (greater than or less than) of the number unless otherwise stated or otherwise evident from the context (except where such number would be less than 0% or exceed 100% of a possible value).

[0025] As used herein, the “administration” of an agent or drug to a subject includes any route of introducing or delivering to a subject a compound to perform its intended function. Administration can be carried out by any suitable route, including but not limited to, orally, intranasally, intrathecally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, intrathecally, intraocularly, intradermally, transmucosally, iontophoretically, or topically. Administration includes self-administration and the

administration by another. "Administration" of a cell or vector or other agent and compositions containing same can be performed in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are known to those of skill in the art and will vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician or in the case of animals, by the treating veterinarian. In some embodiments, administering or a grammatical variation thereof also refers to more than one doses with certain interval. In some embodiments, the interval is 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 10 days, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 1 year or longer. In some embodiments, one dose is repeated for once, twice, three times, four times, five times, six times, seven times, eight times, nine times, ten times or more. Suitable dosage formulations and methods of administering the agents are known in the art. Route of administration can also be determined and method of determining the most effective route of administration are known to those of skill in the art and will vary with the composition used for treatment, the purpose of the treatment, the health condition or disease stage of the subject being treated, and target cell or tissue. Non-limiting examples of route of administration include oral administration, intraperitoneal, infusion, nasal administration, inhalation, injection, and topical application. In some embodiments, the administration is an infusion (for example to peripheral blood of a subject) over a certain period of time, such as about 30 minutes, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 24 hours or longer.

[0026] As used herein "adoptive cell therapeutic composition" refers to any composition comprising cells suitable for adoptive cell transfer. In exemplary embodiments, the adoptive cell therapeutic composition comprises a cell type selected from a group consisting of T-cells, CD8⁺ cells, CD4⁺ cells, NK-cells, delta-gamma T-cells, regulatory T-cells and peripheral blood mononuclear cells. In another embodiment, TILs, T-cells, CD8⁺ cells, CD4⁺ cells, NK-cells, delta-gamma T-cells, regulatory T-cells or peripheral blood mononuclear cells form the adoptive cell therapeutic composition. In one

embodiment, the adoptive cell therapeutic composition comprises cytokine-induced memory-like NK cells (CIMN).

[0027] The term “amino acid” refers to naturally occurring and non-naturally occurring amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally encoded amino acids are the 20 common amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine) and pyrrolysine and selenocysteine. Amino acid analogs refer to agents that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, such as, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (such as, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. In some embodiments, amino acids forming a polypeptide are in the D form. In some embodiments, the amino acids forming a polypeptide are in the L form. In some embodiments, a first plurality of amino acids forming a polypeptide are in the D form, and a second plurality of amino acids are in the L form.

[0028] Amino acids are referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, are referred to by their commonly accepted single-letter code.

[0029] As used herein, “cytokine-induced memory-like NK cells” or “CIMN” refer to memory-like NK cell populations having enhanced anti-malignancy potential that are generated by stimulation of donor NK cells with IL-12, IL-15, and IL-18. In some embodiments, a single 12-18 hour cytokine stimulation of donor NK cells results in sufficient expansion for $10^6 - 10^7$ CIMN cells/kg in adult patients.

[0030] As used herein, the term “antibody” collectively refers to immunoglobulins or immunoglobulin-like molecules including by way of example and without limitation, IgA, IgD, IgE, IgG and IgM, combinations thereof, and similar molecules produced during an immune response in any vertebrate, for example, in mammals such as humans, goats,

rabbits and mice, as well as non-mammalian species, such as shark immunoglobulins. As used herein, “antibodies” (includes intact immunoglobulins) and “antigen binding fragments” specifically bind to a molecule of interest (or a group of highly similar molecules of interest) to the substantial exclusion of binding to other molecules (for example, antibodies and antibody fragments that have a binding constant for the molecule of interest that is at least 10^3 M^{-1} greater, at least 10^4 M^{-1} greater or at least 10^5 M^{-1} greater than a binding constant for other molecules in a biological sample). The term “antibody” also includes genetically engineered forms such as chimeric antibodies (for example, humanized murine antibodies), heteroconjugate antibodies (such as, bispecific antibodies). See also, Pierce Catalog and Handbook, 1994-1995 (Pierce Chemical Co., Rockford, Ill.); Kuby, J., *Immunology*, 3rd Ed., W.H. Freeman & Co., New York, 1997.

[0031] More particularly, antibody refers to a polypeptide ligand comprising at least a light chain immunoglobulin variable region or heavy chain immunoglobulin variable region which specifically recognizes and binds an epitope of an antigen. Antibodies are composed of a heavy and a light chain, each of which has a variable region, termed the variable heavy (V_H) region and the variable light (V_L) region. Together, the V_H region and the V_L region are responsible for binding the antigen recognized by the antibody. Typically, an immunoglobulin has heavy (H) chains and light (L) chains interconnected by disulfide bonds. There are two types of light chain, lambda (λ) and kappa (κ). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each heavy and light chain contains a constant region and a variable region, (the regions are also known as “domains”). In combination, the heavy and the light chain variable regions specifically bind the antigen. Light and heavy chain variable regions contain a “framework” region interrupted by three hypervariable regions, also called “complementarity-determining regions” or “CDRs”. The extent of the framework region and CDRs have been defined (see, Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, 1991, which is hereby incorporated by reference). The Kabat database is now maintained online. The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, largely adopt a β -sheet conformation and the CDRs form loops which connect, and in some cases form part of, the

β -sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions.

[0032] The CDRs are primarily responsible for binding to an epitope of an antigen. The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3, numbered sequentially starting from the N-terminus, and are also typically identified by the chain in which the particular CDR is located. Thus, a V_H CDR3 is located in the variable domain of the heavy chain of the antibody in which it is found, whereas a V_L CDR1 is the CDR1 from the variable domain of the light chain of the antibody in which it is found. An antibody that binds PD-L1 protein will have a specific V_H region and the V_L region sequence, and thus specific CDR sequences. Antibodies with different specificities (*i.e.* different combining sites for different antigens) have different CDRs. Although it is the CDRs that vary from antibody to antibody, only a limited number of amino acid positions within the CDRs are directly involved in antigen binding. These positions within the CDRs are called specificity determining residues (SDRs). “Immunoglobulin-related compositions” as used herein, refers to antibodies (including monoclonal antibodies, polyclonal antibodies, humanized antibodies, chimeric antibodies, recombinant antibodies, multi-specific antibodies, bispecific antibodies, *etc.*) as well as antibody fragments. An antibody or antigen binding fragment thereof specifically binds to an antigen.

[0033] As used herein, the term “antibody-related polypeptide” means antigen-binding antibody fragments, including single-chain antibodies, that can comprise the variable region(s) alone, or in combination, with all or part of the following polypeptide elements: hinge region, CH_1 , CH_2 , and CH_3 domains of an antibody molecule. Also included in the technology are any combinations of variable region(s) and hinge region, CH_1 , CH_2 , and CH_3 domains. Antibody-related molecules useful in the present methods, *e.g.*, but are not limited to, Fab, Fab' and $F(ab')_2$, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain. Examples include: (i) a Fab fragment, a monovalent fragment consisting of the V_L , V_H , CL and CH_1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and CH_1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, *Nature* 341: 544-546, 1989), which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR). As such

“antibody fragments” or “antigen binding fragments” can comprise a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments or antigen binding fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multi-specific antibodies formed from antibody fragments.

[0034] As used herein, the term “diabodies” refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen binding sites. Diabodies are described more fully in, *e.g.*, EP 404,097; WO 93/11161; and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90: 6444-6448 (1993).

[0035] As used herein, the term “single-chain variable fragment” or “scFv” is a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of an immunoglobulin (*e.g.*, mouse or human) covalently linked to form a V_H:V_L heterodimer. The heavy (V_H) and light chains (V_L) are either joined directly or joined by a peptide-encoding linker (*e.g.*, about 10, 15, 20, 25 amino acids), which connects the N-terminus of the V_H with the C-terminus of the V_L, or the C-terminus of the V_H with the N-terminus of the V_L. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility. The linker can link the heavy chain variable region and the light chain variable region of the extracellular antigen binding domain.

[0036] Despite removal of the constant regions and the introduction of a linker, scFv proteins retain the specificity of the original immunoglobulin. Single chain Fv polypeptide antibodies can be expressed from a nucleic acid comprising V_H- and V_L-encoding sequences as described by Huston, *et al.* (*Proc. Nat. Acad. Sci. USA*, 85:5879-5883 (1988)). See, also, U.S. Patent Nos. 5,091,513, 5,132,405 and 4,956,778; and U.S. Patent Publication Nos. 20050196754 and 20050196754. Antagonistic scFvs having inhibitory activity have been described (see, *e.g.*, Zhao *et al.*, *Hybridoma* (Larchmt) 27(6):455-51 (2008); Peter *et al.*, *J Cachexia Sarcopenia Muscle* (2012); Shieh *et al.*, *J Immunol* 183(4):2277-85 (2009); Giomarelli *et al.*, *Thromb Haemost* 97(6):955-63 (2007); Fife *et al.*, *J Clin Invest* 116(8):2252-61 (2006); Brocks *et al.*, *Immunotechnology* 3(3): 173-84 (1997); Moosmayer *et al.*, *Ther Immunol* 2(10):31-40 (1995). Agonistic scFvs having stimulatory activity have been

described (see, *e.g.*, Peter *et al.*, *J Biol Chem* 25278(38):36740-7 (2003); Xie *et al.*, *Nat Biotech* 15(8):768-71 (1997); Ledbetter *et al.*, *Crit Rev Immunol* 17(5-6):427-55 (1997); Ho *et al.*, *Bio Chim Biophys Acta* 1638(3):257-66 (2003)).

[0037] The term “antigen binding fragment” refers to a fragment of the whole immunoglobulin structure which possesses a part of a polypeptide responsible for binding to antigen. Examples of the antigen binding fragment useful in the present technology include scFv, (scFv)₂, scFvFc, Fab, Fab' and F(ab')₂, but are not limited thereto. Any of the above-noted antibody fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for binding specificity and neutralization activity in the same manner as are intact antibodies.

[0038] As used herein, an “antigen” refers to a molecule to which an antibody (or antigen binding fragment thereof) can selectively bind. The target antigen may be a protein, carbohydrate, nucleic acid, lipid, hapten, or other naturally occurring or synthetic compound. In some embodiments, the target antigen may be a polypeptide (*e.g.*, a PD-L1 polypeptide). An antigen may also be administered to an animal to generate an immune response in the animal.

[0039] As used herein, the term “cell population” refers to a group of at least two cells expressing similar or different phenotypes. In non-limiting examples, a cell population can include at least about 10, at least about 100, at least about 200, at least about 300, at least about 400, at least about 500, at least about 600, at least about 700, at least about 800, at least about 900, at least about 1000 cells, at least about 10,000 cells, at least about 100,000 cells, at least about 1×10^6 cells, at least about 1×10^7 cells, at least about 1×10^8 cells, at least about 1×10^9 cells, at least about 1×10^{10} cells, at least about 1×10^{11} cells, at least about 1×10^{12} cells, or more cells expressing similar or different phenotypes.

[0040] As used herein, the term “chimeric antibody” means an antibody in which the Fc constant region of a monoclonal antibody from one species (*e.g.*, a mouse Fc constant region) is replaced, using recombinant DNA techniques, with an Fc constant region from an antibody of another species (*e.g.*, a human Fc constant region). *See generally*, Robinson *et al.*, PCT/US86/02269; Akira *et al.*, European Patent Application 184,187; Taniguchi, European Patent Application 171,496; Morrison *et al.*, European Patent Application 173,494; Neuberger *et al.*, WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly

et al., European Patent Application 0125,023; Better *et al.*, *Science* 240: 1041-1043, 1988; Liu *et al.*, *Proc. Natl. Acad. Sci. USA* 84: 3439-3443, 1987; Liu *et al.*, *J. Immunol* 139: 3521-3526, 1987; Sun *et al.*, *Proc. Natl. Acad. Sci. USA* 84: 214-218, 1987; Nishimura *et al.*, *Cancer Res* 47: 999-1005, 1987; Wood *et al.*, *Nature* 314: 446-449, 1885; and Shaw *et al.*, *J. Natl. Cancer Inst.* 80: 1553-1559, 1988.

[0041] As used herein, the term “conjugated” refers to the association of two molecules by any method known to those in the art. Suitable types of associations include chemical bonds and physical bonds. Chemical bonds include, for example, covalent bonds and coordinate bonds. Physical bonds include, for instance, hydrogen bonds, dipolar interactions, van der Waal forces, electrostatic interactions, hydrophobic interactions and aromatic stacking.

[0042] As used herein, a "control" is an alternative sample used in an experiment for comparison purpose. A control can be "positive" or "negative." For example, where the purpose of the experiment is to determine a correlation of the efficacy of a therapeutic agent for the treatment for a particular type of disease, a positive control (a compound or composition known to exhibit the desired therapeutic effect) and a negative control (a subject or a sample that does not receive the therapy or receives a placebo) are typically employed.

[0043] As used herein, the term “effective amount” refers to a quantity sufficient to achieve a desired therapeutic and/or prophylactic effect, *e.g.*, an amount which results in the prevention of, or a decrease in a disease or condition described herein or one or more signs or symptoms associated with a disease or condition described herein. In the context of therapeutic or prophylactic applications, the amount of a composition administered to the subject will vary depending on the composition, the degree, type, and severity of the disease and on the characteristics of the individual, such as general health, age, sex, body weight and tolerance to drugs. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. The compositions can also be administered in combination with one or more additional therapeutic compounds. In the methods described herein, the therapeutic compositions may be administered to a subject having one or more signs or symptoms of a disease or condition described herein. As used herein, a "therapeutically effective amount" of a composition refers to composition levels in which

the physiological effects of a disease or condition are ameliorated or eliminated. A therapeutically effective amount can be given in one or more administrations.

[0044] As used herein, the term “epitope” means a protein determinant capable of specific binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents. In some embodiments, an “epitope” of the PD-L1 protein is a region of the protein to which the anti-PD-L1 antibodies of the present technology specifically bind. In some embodiments, the epitope is a conformational epitope or a non-conformational epitope. To screen for anti-PD-L1 antibodies which bind to an epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. This assay can be used to determine if an anti-PD-L1 antibody binds the same site or epitope as an anti-PD-L1 antibody of the present technology. Alternatively, or additionally, epitope mapping can be performed by methods known in the art. For example, the antibody sequence can be mutagenized such as by alanine scanning, to identify contact residues. In a different method, peptides corresponding to different regions of PD-L1 protein can be used in competition assays with the test antibodies or with a test antibody and an antibody with a characterized or known epitope.

[0045] As used herein, “expression” includes one or more of the following: transcription of the gene into precursor mRNA; splicing and other processing of the precursor mRNA to produce mature mRNA; mRNA stability; translation of the mature mRNA into protein (including codon usage and tRNA availability); and glycosylation and/or other modifications of the translation product, if required for proper expression and function.

[0046] As used herein, an “expression vector” includes vectors capable of expressing DNA that is operably linked with regulatory sequences, such as promoter regions, that are capable of effecting expression of such DNA fragments. Such additional segments can include promoter and terminator sequences, and optionally can include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, and the like. Expression vectors are generally derived from plasmid or viral DNA, or can

contain elements of both. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the cloned DNA.

Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome.

[0047] As used herein, the term “gene” means a segment of DNA that contains all the information for the regulated biosynthesis of an RNA product, including promoters, exons, introns, and other untranslated regions that control expression.

[0048] As used herein, “haploidentical donor” refers to a tissue donor whose HLA tissue type partially matches (usually 50%) the HLA tissue type of a person receiving a stem cell or organ transplant. HLAs are a set of cell surface markers found on a person’s cells and tissues that play an important role in the body’s immune response to foreign substances. Prior to a transplant, the HLA tissue type of a recipient will be matched with that of a potential donor. A haploidentical donor, such as a parent, child, or sibling, may be used in a stem cell or organ transplant when a fully or closely matched donor is not available.

[0049] “Homology” or “identity” or “similarity” refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%) of “sequence identity” to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art. In some embodiments, default parameters are used for alignment. One alignment program is BLAST, using default parameters. In particular, programs are BLASTN and BLASTP, 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+SwissProtein+SPupdate+PIR. Details of these programs can be found at the National Center for Biotechnology Information. Biologically equivalent polynucleotides are those having the specified percent homology and encoding a polypeptide having the same or similar biological activity. Two sequences are deemed “unrelated” or “non-homologous” if they share less than 40% identity, or less than 25% identity, with each other.

[0050] As used herein, “humanized” forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some embodiments, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance such as binding affinity. Generally, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains (*e.g.*, Fab, Fab', F(ab')₂, or Fv), in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus FR sequence although the FR regions may include one or more amino acid substitutions that improve binding affinity. The number of these amino acid substitutions in the FR are typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally may also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, *Nature* 321:522-525 (1986); Reichmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See *e.g.*, Ahmed & Cheung, *FEBS Letters* 588(2):288-297 (2014).

[0051] As used herein, the term “hypervariable region” refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a “complementarity determining region” or “CDR” (*e.g.*, around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the V_L, and

around about 31-35B (H1), 50-65 (H2) and 95-102 (H3) in the V_H (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a “hypervariable loop” (*e.g.*, residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the V_L, and 26-32 (H1), 52A-55 (H2) and 96-101 (H3) in the V_H (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)).

[0052] As used herein, the terms “identical” or percent “identity”, when used in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.*, about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region (*e.g.*, nucleotide sequence encoding an antibody described herein or amino acid sequence of an antibody described herein)), when compared and aligned for maximum correspondence over a comparison window or designated region as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (*e.g.*, NCBI web site). Such sequences are then said to be “substantially identical.” This term also refers to, or can be applied to, the complement of a test sequence. The term also includes sequences that have deletions and/or additions, as well as those that have substitutions. In some embodiments, identity exists over a region that is at least about 25 amino acids or nucleotides in length, or 50-100 amino acids or nucleotides in length.

[0053] As used herein, the term “intact antibody” or “intact immunoglobulin” means an antibody that has at least two heavy (H) chain polypeptides and two light (L) chain polypeptides interconnected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH₁, CH₂ and CH₃. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxyl-terminus in the following order: FR₁, CDR₁, FR₂, CDR₂, FR₃, CDR₃, FR₄. The variable regions of

the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies can mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system.

[0054] The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. For example, a monoclonal antibody can be an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including, *e.g.*, but not limited to, hybridoma, recombinant, and phage display technologies. For example, the monoclonal antibodies to be used in accordance with the present methods may be made by the hybridoma method first described by Kohler *et al.*, *Nature* 256:495 (1975), or may be made by recombinant DNA methods (*See, e.g.*, U.S. Patent No. 4,816,567). The “monoclonal antibodies” may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature* 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), for example.

[0055] As used herein, the term “pharmaceutically-acceptable carrier” is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal compounds, isotonic and absorption delaying compounds, and the like, compatible with pharmaceutical administration. Pharmaceutically-acceptable carriers and their formulations are known to one skilled in the art and are described, for example, in Remington's Pharmaceutical Sciences (20th edition, ed. A. Gennaro, 2000, Lippincott, Williams & Wilkins, Philadelphia, Pa.).

[0056] As used herein, the term “polynucleotide” or “nucleic acid” means any RNA or DNA, which may be unmodified or modified RNA or DNA. Polynucleotides include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, RNA that is mixture of single- and double-stranded regions, and hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, polynucleotide refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons.

[0057] As used herein, the terms “polypeptide,” “peptide” and “protein” are used interchangeably herein to mean a polymer comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, *i.e.*, peptide isosteres. Polypeptide refers to both short chains, commonly referred to as peptides, glycopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. Polypeptides include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques that are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature.

[0058] As used herein, “prevention” or “preventing” of a disorder or condition refers to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset of one or more symptoms of the disorder or condition relative to the untreated control sample.

[0059] As used herein, the term “recombinant” when used with reference, *e.g.*, to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the material is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

[0060] As used herein, a “sample” or “biological sample” refers to a body fluid or a tissue sample isolated from a subject. In some cases, a biological sample may consist of or comprise whole blood, platelets, red blood cells, white blood cells, plasma, sera, urine, feces, epidermal sample, vaginal sample, skin sample, cheek swab, sperm, amniotic fluid, cultured cells, bone marrow sample, tumor biopsies, aspirate and/or chorionic villi, cultured cells, endothelial cells, synovial fluid, lymphatic fluid, ascites fluid, interstitial or extracellular fluid and the like. The term “sample” may also encompass the fluid in spaces between cells, including gingival crevicular fluid, bone marrow, cerebrospinal fluid (CSF), saliva, mucus, sputum, semen, sweat, urine, or any other bodily fluids. Samples can be obtained from a subject by any means including, but not limited to, venipuncture, excretion, ejaculation, massage, biopsy, needle aspirate, lavage, scraping, surgical incision, or intervention or other means known in the art. A blood sample can be whole blood or any fraction thereof, including blood cells (red blood cells, white blood cells or leukocytes, and platelets), serum and plasma.

[0061] As used herein, the term “separate” therapeutic use refers to an administration of at least two active ingredients at the same time or at substantially the same time by different routes.

[0062] As used herein, the term “sequential” therapeutic use refers to administration of at least two active ingredients at different times. More particularly, sequential use refers to the whole administration of one of the active ingredients before administration of the other or others commences. It is thus possible to administer one of the active ingredients over several minutes, hours, or days before administering the other active ingredient or ingredients. There is no simultaneous treatment in this case.

[0063] As used herein, “specifically binds” refers to a molecule (*e.g.*, an antibody or antigen binding fragment thereof) which recognizes and binds another molecule (*e.g.*, an antigen), but that does not substantially recognize and bind other molecules. The terms “specific binding,” “specifically binds to,” or is “specific for” a particular molecule (*e.g.*, a polypeptide, or an epitope on a polypeptide), as used herein, can be exhibited, for example, by a molecule having a K_D for the molecule to which it binds to of about 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, or 10^{-12} M. The term “specifically binds” may also refer to binding where a molecule (*e.g.*, an antibody or antigen binding fragment thereof) binds to a particular polypeptide (*e.g.*, a PD-L1 polypeptide), or an epitope on a

particular polypeptide, without substantially binding to any other polypeptide, or polypeptide epitope.

[0064] As used herein, the term “simultaneous” therapeutic use refers to the administration of at least two active ingredients by the same route and at the same time or at substantially the same time.

[0065] As used herein, the terms “subject”, “patient”, or “individual” can be an individual organism, a vertebrate, a mammal, or a human. In some embodiments, the subject, patient or individual is a human.

[0066] “Treating” or “treatment” as used herein covers the treatment of a disease or disorder described herein, in a subject, such as a human, and includes: (i) inhibiting a disease or disorder, *i.e.*, arresting its development; (ii) relieving a disease or disorder, *i.e.*, causing regression of the disorder; (iii) slowing progression of the disorder; and/or (iv) inhibiting, relieving, or slowing progression of one or more symptoms of the disease or disorder. In some embodiments, treatment means that the symptoms associated with the disease are, *e.g.*, alleviated, reduced, cured, or placed in a state of remission.

[0067] It is also to be appreciated that the various modes of treatment of disorders as described herein are intended to mean “substantial,” which includes total but also less than total treatment, and wherein some biologically or medically relevant result is achieved. The treatment may be a continuous prolonged treatment for a chronic disease or a single, or few time administrations for the treatment of an acute condition.

[0068] Amino acid sequence modification(s) of the anti-PD-L1 antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an anti-PD-L1 antibody are prepared by introducing appropriate nucleotide changes into the antibody nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution is made to obtain the antibody of interest, as long as the obtained antibody possesses the desired properties. The modification also includes the change of the pattern of glycosylation of the protein. The sites of greatest interest for substitutional mutagenesis include the

hypervariable regions, but FR alterations are also contemplated. “Conservative substitutions” are shown in the Table below.

Table 1. Amino Acid Substitutions		
Original Residue	Exemplary Substitutions	Conservative Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; asp, lys; arg	gln
Asp (D)	glu; asn	glu
Cys (C)	ser; ala	ser
Gln (Q)	asn; glu	asn
Glu (E)	asp; gln	asp
Gly (G)	ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	tyr
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr

Table 1. Amino Acid Substitutions		
Original Residue	Exemplary Substitutions	Conservative Substitutions
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

[0069] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Specifically, several hypervariable region sites (*e.g.*, 6-7 sites) are mutated to generate all possible amino acid substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e.g.*, binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and the antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with similar or superior properties in one or more relevant assays may be selected for further development.

Immune Checkpoint Inhibition in Cancer Therapy

[0070] Inhibitors of the programmed death-1 (PD-1)/programmed death ligand-1 (PD-L1) pathway via blockade with monoclonal antibody therapeutics have emerged as effective tools in cancer immunotherapy. PD-1/PD-L1 antagonists have limited efficacy against AML as a stand-alone therapy, however, can result in meaningful responses when used after allo HCT.^{26,27} Inhibition of PD-1/PD-L1 results in a broad activation of autologous lymphocytes. In-class adverse events are typically related to the manifestations of autoimmunity and can include thyroiditis, colitis, pneumonitis, and others.²⁸ Despite this,

PD-1/PD-L1 inhibitors are generally well tolerated and have gained approval from the Food and Drug Administration for several indications against solid-organ malignancies.

[0071] The anti-PD-L1 immunoglobulin-related compositions of the present disclosure may be useful in the treatment of AML. Anti-PD-L1 immunoglobulin-related compositions within the scope of the present technology include, *e.g.*, but are not limited to, monoclonal, chimeric, humanized, bispecific antibodies and diabodies that specifically bind the target polypeptide, a homolog, derivative or a fragment thereof. The present disclosure also provides antigen binding fragments of any of the anti-PD-L1 antibodies disclosed herein, wherein the antigen binding fragment is selected from the group consisting of Fab, F(ab)'₂, Fab', scF_v, and F_v.

Atezolizumab

[0072] Atezolizumab is a glyco-engineered monoclonal IgG1 antibody targeting PD-L1. The directed N297A mutation in the Fc region leads to loss of glycosylation and abrogation of antibody-dependent cellular cytotoxicity. Importantly, this eliminates fratricide of PD-L1⁺ effector lymphocytes when deployed as an immune checkpoint inhibitor in cancer therapy. Several late-phase clinical trials support the safety and efficacy of atezolizumab in patients with non-small cell lung adenocarcinoma.²⁹⁻³¹ For example, the randomized phase III OAK study demonstrated a favorable rate of CTCAE grade 3-4 adverse events associated with atezolizumab monotherapy compared to chemotherapy in this population.³¹ Importantly, rates of grade 3-4 immune related adverse events (irAE) were less than 20% in this study and others.

[0073] Information regarding atezolizumab (or antigen binding fragments thereof) for use in the methods provided herein can be found in U.S. Patent No. US 8,217,149, the disclosure of which is incorporated herein by reference in its entirety. Atezolizumab and antigen-binding fragments thereof for use in the methods provided herein comprises a heavy chain and a light chain or a heavy chain variable region and a light chain variable region.

[0074] Those of ordinary skill in the art would easily be able to identify Chothia-defined, Abm-defined or other CDRs.

SEQ ID NO: 1	Atezolizumab V _H domain
--------------	------------------------------------

	EVQLVESGGGLVQPGGSLRLSCAAS <u>GFTFS</u> DSWIHWVRQAPGK GLEWV <u>AWISPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSL</u> RAEDTAVYYCARRHWP <u>GGFDY</u> WGQGTLVTVSA
SEQ ID NO: 2	Atezolizumab V _L domain DIQMTQSPSSLSASVGDRVTITC <u>RASQDVSTAVAWYQQKPGKAP</u> KWYS <u>SASFLYSGVPSRFS</u> SGSGSGTDFTLTISLQPEDFATYYC <u>QQY</u> <u>LYHPATFGQG</u> TKVEIKR
SEQ ID NO: 3	V _H CDR1 consensus GFTFSX ₁ SWIH, wherein: X ₁ is D or G
SEQ ID NO: 4	Atezolizumab V _H CDR1 GFTFSDSWIH
SEQ ID NO: 5	V _H CDR2 consensus AWIX ₂ PYGGSX ₃ YYADSVKG, wherein X ₂ is S or L; X ₃ is T or S.
SEQ ID NO: 6	Atezolizumab V _H CDR2 AWISPYGGSTYYADSVKG
SEQ ID NO: 7	Atezolizumab V _H CDR3 RHWPGGFDY
SEQ ID NO: 8	V _L CDR1 consensus RASQX ₄ X ₅ X ₆ TX ₇ X ₈ A, wherein X ₄ is D or V; X ₅ is V or I; X ₆ is S or N; X ₇ is A or F; X ₈ is V or L
SEQ ID NO: 9	Atezolizumab V _L CDR1 RASQDVSTAVA
SEQ ID NO: 10	V _L CDR2 consensus SASX ₉ LX ₁₀ S, wherein X ₉ is F or T; X ₁₀ is Y or A
SEQ ID NO: 11	Atezolizumab V _L CDR2 SASFLYS

SEQ ID NO: 12	Atezolizumab V _L CDR3 QQX ₁₁ X ₁₂ X ₁₃ X ₁₄ PX ₁₅ T, wherein X ₁₁ is Y, G, F, or S; X ₁₂ , is L, Y, F or W; X ₁₃ is Y, N, A, T, G, F or I; X ₁₄ is H, V, P, T or I; X ₁₅ is A, W, R, P or T.
SEQ ID NO: 13	QQYLYHPAT

Avelumab

[0075] Avelumab is a humanized recombinant IgG1 antibody that targets PD-L1 and is utilized in cancer immunotherapy. Avelumab could induce objective responses in patients with advanced, metastatic, or unresectable malignant neoplasms in various multicenter studies, with a proportion of patients experiencing long-term remission

[0076] Information regarding avelumab (or antigen binding fragments thereof) for use in the methods provided herein can be found in U.S. Patent No. US 11,884,724, the disclosure of which is incorporated herein by reference in its entirety. Avelumab and antigen-binding fragments thereof for use in the methods provided herein comprises a heavy chain and a light chain or a heavy chain variable region and a light chain variable region.

[0077] Those of ordinary skill in the art would easily be able to identify Chothia-defined, Abm-defined or other CDRs.

SEQ ID NO: 14	Avelumab V _H domain EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYIMMW VRQAPGKGLEWVSSIYPSSGGITFYADTVKGRFTISR NSKNTLYLQMNSLRAEDTAVYYCARIKLGTVTTV D YWGQGTLLVTVSS
SEQ ID NO: 15	Avelumab V _L domain QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSW YQQHPGKAPKLMIDVSNRPSGVSNRFSGSKSGNTA SLTISGLQAEDEADYYC SSYTSSSTRVFGTGTKVTVL
SEQ ID NO: 16	V _H CDR1 consensus

	$X_1YX_2MX_3$, wherein X_1 is K, R, T, Q, G, A, W, M, I or S; X_2 is V, R, K, L, M or I; X_3 is H, T, N, Q, A, V, Y, W, F or M
SEQ ID NO: 17	Avelumab V_H CDR1 SYIMM
SEQ ID NO: 18	V_H CDR2 consensus $SIYPSGGX_4TFYADX_5VKG$, wherein X_4 is F or I; X_5 is S or T
SEQ ID NO: 19	Avelumab V_H CDR2 SIYPSGGITFYADTVKG
SEQ ID NO: 20	V_H CDR3 consensus $IKLGTVTTVX_6Y$, wherein X_6 is E or D.
SEQ ID NO: 21	Avelumab V_H CDR3 IKLGTVTTVDY
SEQ ID NO: 22	V_L CDR1 consensus $TGTX_7X_8DVGX_9YNYVS$, wherein X_7 is N or S; X_8 is T, R or S; X_9 is A or G;
SEQ ID NO: 23	Avelumab V_L CDR1 TGTSSDVGGYNYVS
SEQ ID NO: 24	V_L CDR2 consensus $X_{10}VX_{11}X_{12}RPS$, wherein X_{10} is E or D; X_{11} is I, N or S; X_{12} is D, H or N
SEQ ID NO: 25	Avelumab V_L CDR2 DVSNRPS
SEQ ID NO: 26	V_L CDR3 consensus

	SSX ₁₃ TX ₁₄ X ₁₅ X ₁₆ X ₁₇ RV, wherein X ₁₃ is F or Y; X ₁₄ is N or S; X ₁₅ is R, T or S; X ₁₆ is G or S; and X ₁₇ is I or T.
SEQ ID NO: 27	Avelumab V _L CDR3 SSYTSSSTRV

Durvalumab

[0078] Durvalumab binds to the protein PD-L1 to help immune cells kill cancer cells better and is used to treat different types of cancer. Durvalumab is used alone or with other drugs to treat adults with certain types of biliary tract cancer (including bile duct cancer and gallbladder cancer), endometrial cancer, hepatocellular carcinoma (a type of liver cancer), non-small cell lung cancer, and small cell lung cancer.

[0079] Information regarding durvalumab (or antigen binding fragments thereof) for use in the methods provided herein can be found in U.S. Patent No. US9,493,565, the disclosure of which is incorporated herein by reference in its entirety. Durvalumab and antigen-binding fragments thereof for use in the methods provided herein comprises a heavy chain and a light chain or a heavy chain variable region and a light chain variable region.

[0080] Those of ordinary skill in the art would easily be able to identify Chothia-defined, Abm-defined or other CDRs.

SEQ ID NO: 28	Durvalumab V _H domain EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMS WVRQAPGKGLEWVANIKQDGSEKYYVDSVKGRFTI SRDNAKNSLYLQMNSLRAEDTAVYYCAREGGWFG ELAFDYWGQGTLVTVSS
SEQ ID NO: 29	Durvalumab V _L domain EIVLTQSPGTLSPGERATLSCRASQRVSSSYLAWY QQKPGQAPRLLIYDASSRATGIPDRFSGSGSGTDFTL TISRLEPEDFAVYYCQQYGSLPWTFGQGTKVEIK
SEQ ID NO: 30	Durvalumab V _H CDR1

	RYWMS
SEQ ID NO: 31	Durvalumab V _H CDR2 NIKQDGSEKYYVDSVK
SEQ ID NO: 32	Durvalumab V _H CDR3 EGGWFGELAFDY
SEQ ID NO: 33	Durvalumab V _L CDR1 RASQRVSSSYLA
SEQ ID NO: 34	Durvalumab V _L CDR2 DASSRAT
SEQ ID NO: 35	Durvalumab V _L CDR3 QQYGSLPWT

[0081] In one aspect, the present disclosure provides an anti-PD-L1 antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 4, a V_H-CDR2 sequence of SEQ ID NO: 6, and a V_H-CDR3 sequence of SEQ ID NO: 7 and the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 9, a V_L-CDR2 sequence of SEQ ID NO: 11, and a V_L-CDR3 sequence of SEQ ID NO: 13. In one aspect, the present disclosure provides an anti-PD-L1 antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein: (a) the V_H comprises an amino acid sequence of SEQ ID NO: 1; and/or (b) the V_L comprises an amino acid sequence of SEQ ID NO: 2.

[0082] In another aspect, the present disclosure provides an anti-PD-L1 antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein the V_H comprises a V_H-CDR1 sequence of GFTFSX₁SWIH (SEQ ID NO: 3), wherein: X₁ is D or G, a V_H-CDR2 sequence of AWIX₂PYGGSX₃YYADSVKG (SEQ ID NO: 5), wherein X₂ is S or L; X₃ is T or S, and a V_H-CDR3 sequence of SEQ ID NO: 7 and the V_L comprises a V_L-CDR1

sequence of RASQX₄X₅X₆TX₇X₈A (SEQ ID NO: 8), wherein X₄ is D or V; X₅ is V or I; X₆ is S or N; X₇ is A or F; X₈ is V or L, a V_L-CDR2 sequence of SASX₉LX₁₀S (SEQ ID NO: 10), wherein X₉ is F or T; X₁₀ is Y or A, and a V_L-CDR3 sequence of QQX₁₁X₁₂X₁₃X₁₄PX₁₅T (SEQ ID NO: 12), wherein X₁₁ is Y, G, F, or S; X₁₂ is L, Y, F or W; X₁₃ is Y, N, A, T, G, F or I; X₁₄ is H, V, P, T or I; X₁₅ is A, W, R, P or T.

[0083] In one aspect, the present disclosure provides an anti-PD-L1 antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 17, a V_H-CDR2 sequence of SEQ ID NO: 19, and a V_H-CDR3 sequence of SEQ ID NO: 21 and the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 23, a V_L-CDR2 sequence of SEQ ID NO: 25, and a V_L-CDR3 sequence of SEQ ID NO: 27. In one aspect, the present disclosure provides an anti-PD-L1 antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein: (a) the V_H comprises an amino acid sequence of SEQ ID NO: 14; and/or (b) the V_L comprises an amino acid sequence of SEQ ID NO: 15.

[0084] In another aspect, the present disclosure provides an anti-PD-L1 antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein the V_H comprises a V_H-CDR1 sequence of X₁YX₂MX₃ (SEQ ID NO: 16), wherein X₁ is K, R, T, Q, G, A, W, M, I or S; X₂ is V, R, K, L, M or I; X₃ is H, T, N, Q, A, V, Y, W, F or M, a V_H-CDR2 sequence of SIYPSGGX₄TFYADX₅VKG (SEQ ID NO: 18), wherein X₄ is F or I; X₅ is S or T and a V_H-CDR3 sequence of IKLGTVTTVX₆Y (SEQ ID NO: 20), wherein X₆ is E or D, and the V_L comprises a V_L-CDR1 sequence of TGTX₇X₈DVGX₉YNYVS (SEQ ID NO: 22), wherein X₇ is N or S; X₈ is T, R or S; X₉ is A or G, a V_L-CDR2 sequence of X₁₀VX₁₁X₁₂RPS (SEQ ID NO: 24), wherein X₁₀ is E or D; X₁₁ is I, N or S; X₁₂ is D, H or N, and a V_L-CDR3 sequence of SSX₁₃TX₁₄X₁₅X₁₆X₁₇RV (SEQ ID NO: 26), wherein X₁₃ is F or Y; X₁₄ is N or S; X₁₅ is R, T or S; X₁₆ is G or S; and X₁₇ is I or T.

[0085] In one aspect, the present disclosure provides an anti-PD-L1 antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 30, a V_H-CDR2 sequence of SEQ ID NO: 31, and a V_H-

CDR3 sequence of SEQ ID NO: 32 and the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 33, a V_L-CDR2 sequence of SEQ ID NO: 34, and a V_L-CDR3 sequence of SEQ ID NO: 35. In one aspect, the present disclosure provides an anti-PD-L1 antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein: (a) the V_H comprises an amino acid sequence of SEQ ID NO: 28; and/or (b) the V_L comprises an amino acid sequence of SEQ ID NO: 29.

[0086] In any of the above embodiments, the antibody further comprises a Fc domain of any isotype, *e.g.*, but are not limited to, IgG (including IgG1, IgG2, IgG3, and IgG4), IgA (including IgA₁ and IgA₂), IgD, IgE, or IgM, and IgY. Non-limiting examples of constant region sequences include:

[0087] Human IgD constant region, Uniprot: P01880 (SEQ ID NO: 36)

APTKAPDVFPIISGCRHPKDNSPVVLACLITGYHPTSVTVTWYMGTSQSPQRTFPEI
QRRDSYYMTSSQLSTPLQQWRQGEYKCVVQHTASKSKKEIFRWPESPKAQASSVP
TAQPQAEGLAKATTAPATTRNTGRGGEEKKKEKEKEEQEERETKTPECPSHTQPL
GVYLLTPAVQDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGVEEGLLER
HSNGSQSQHSRLTLPRSLWNAGTSVTCTLNHPSLPPQRLMALREPAAQAPVKLSLN
LLASSDPPEAASWLLCEVSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFW
AWSVLRVPAPPSPQPATYTCVVSHEDSRTLLNASRSLEVSYVTDHGPMK

[0088] Human IgG1 constant region, Uniprot: P01857 (SEQ ID NO: 37)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLSPGK

[0089] Human IgG2 constant region, Uniprot: P01859 (SEQ ID NO: 38)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPP
VAGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVQFNWYVDGVEVHNAKT
KPREEQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPRE

PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0090] Human IgG3 constant region, Uniprot: P01860 (SEQ ID NO: 39)

ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYTCNVNHKPSNTKVDKRVELKTPLGDTTHTCPR
CPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPAPELLGGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNS
TFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK

[0091] Human IgM constant region, Uniprot: P01871 (SEQ ID NO: 40)

GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITLSWKYKNNSDISSTRGFPS
VLRGGKYAATSQVLLPSKDVMQGTDEHVCKVQHPNGNKEKNVPLPVIAELPPKV
SVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSVTTDQVQAEAK
ESGPTTYKVTSTLTIKESDWLGQSMFTCRVDHRGLTFQQNASSMCVPDQDPAIRVF
AIPPSFASIFLTKSTKLTVCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSA
VGEASICEDDWNNGERFTCTVTHTDLPSPKQKTISRPKGVALHRPDVYLLPPAREQL
NLRESATITCLVTGFSPADVVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSIL
TVSEEEWNTGETYTCVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY

[0092] Human IgG4 constant region, Uniprot: P01861 (SEQ ID NO: 41)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYTCNVNHDHKPSNTKVDKRVESKYGPPCSPCAPEF
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT
KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE
PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0093] Human IgA1 constant region, Uniprot: P01876 (SEQ ID NO: 42)

ASPTSPKVFPLSLCSTQPDGNVVIACLVQGFFPQEPLSVTWSESGQGVARNFPSPQ
DASGDLYTTSSQLTLPATQCLAGKSVTCHVKHYTNPSQDVTVPVPCVPSTPPTPSPST
PPTPSPSCCHPRLSLHRPALEDLLLGSEANLTCTLTGLRDASGVTFTWTPSSGKSAV
QGPPERDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRP
EVHLLPPPSEELALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYL TWASRQE

PSQGTTFFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVN
VSVVMAEVDGTCY

[0094] Human IgA2 constant region, Uniprot: P01877 (SEQ ID NO: 43)

ASPTSPKVFPLSLDSTPQDGNVVVACLVQGFFPQEPLSVTWSESGQNVTARNFPPSQ
DASGDLYTTSSQLTLPATQCPDGKSVTCHVKHYTNPSQDVTVPVPPPPPCCHPRL
SLHRPALEDLLLSEANLTCTLTGLRDASGATFTWTPSSGKSAVQGPPERDLCGCY
SVSSVLPGCAQPWNHGETFTCTAAHPELKTPLTANITKSGNTFRPEVHLLPPPSEEL
ALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQGTTFFAVTS
ILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRMAGKPTHVNVSVVMAEVDG
TCY

[0095] Human Ig kappa constant region, Uniprot: P01834 (SEQ ID NO: 44)

TVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYPREAKVQWKVDNALQSGNSQESVT
EQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0096] In some embodiments, the immunoglobulin-related compositions of the present technology comprise a heavy chain constant region that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or is 100% identical to SEQ ID NOS: 36-43. Additionally or alternatively, in some embodiments, the immunoglobulin-related compositions of the present technology comprise a light chain constant region that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or is 100% identical to SEQ ID NO: 44.

[0097] In some embodiments, the PD-L1 immunoglobulin-related compositions of the present technology bind to an epitope within the extracellular region of human PD-L1. In certain embodiments, the epitope is a conformational epitope or non-conformational epitope. In some embodiments, the PD-L1 polypeptide has the amino acid sequence of SEQ ID NO: 45.

MRIFAVFIFMTYWHLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVY
WEMEDKNIIQFVHGEECLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGV
YRCMISYGGADYKRITVKVNAPYNKINQRILVVDPTSEHELTCQAEGYPKAEVIW
TSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELV
IPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQ
SDTHLEET (SEQ ID NO: 45)

Underline: Extracellular domain of PD-L1

[0098] Additionally or alternatively, in some embodiments, the antibody or antigen binding fragment binds to the extracellular domain of a PD-L1 polypeptide. In some embodiments, the PD-L1 immunoglobulin-related compositions of the present technology bind to an epitope comprising residues 19-238 of SEQ ID NO: 45.

[0099] In some embodiments, the V_H and V_L immunoglobulin variable domain sequences are components of the same polypeptide chain. In other embodiments, the V_H and V_L immunoglobulin variable domain sequences are components of different polypeptide chains. In certain embodiments, the antibody is a full-length antibody.

[00100] In some embodiments, the immunoglobulin-related compositions of the present technology bind specifically to at least one PD-L1 polypeptide. In some embodiments, the immunoglobulin-related compositions of the present technology bind at least one PD-L1 polypeptide with a dissociation constant (K_D) of about 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, or 10^{-12} M. In certain embodiments, the immunoglobulin-related compositions are monoclonal antibodies, chimeric antibodies, humanized antibodies, bispecific antibodies, or multi-specific antibodies. In some embodiments, the antibodies comprise a human antibody framework region.

[00101] In certain embodiments, the immunoglobulin-related composition includes one or more of the following characteristics: (a) a light chain immunoglobulin variable domain sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the light chain immunoglobulin variable domain sequence of any one of SEQ ID NOs: 2, 15 or 29 ; and/or (b) a heavy chain immunoglobulin variable domain sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the heavy chain immunoglobulin variable domain sequence of any one of SEQ ID NOs: 1, 14 or 28. In another aspect, one or more amino acid residues in the immunoglobulin-related compositions provided herein are substituted with another amino acid. The substitution may be a “conservative substitution” as defined herein.

[00102] In certain embodiments, the immunoglobulin-related compositions contain an IgG1 constant region comprising one or more amino acid substitutions selected from the group consisting of N297A and K322A. Additionally or alternatively, in some embodiments, the immunoglobulin-related compositions contain an IgG4 constant region comprising a S228P mutation.

[00103] In some aspects, the anti-PD-L1 immunoglobulin-related compositions described herein contain structural modifications to facilitate rapid binding and cell uptake and/or slow release. In some aspects, the anti-PD-L1 immunoglobulin-related composition of the present technology (*e.g.*, an antibody) may contain a deletion in the CH2 constant heavy chain region to facilitate rapid binding and cell uptake and/or slow release. In some aspects, a Fab fragment is used to facilitate rapid binding and cell uptake and/or slow release. In some aspects, a F(ab)₂ fragment is used to facilitate rapid binding and cell uptake and/or slow release.

[00104] In one aspect, the present technology provides a nucleic acid sequence encoding any of the immunoglobulin-related compositions described herein. Also disclosed herein are recombinant nucleic acid sequences encoding any of the antibodies described herein.

[00105] In another aspect, the present technology provides a host cell expressing any nucleic acid sequence encoding any of the immunoglobulin-related compositions described herein.

[00106] The immunoglobulin-related compositions of the present technology (*e.g.*, an anti-PD-L1 antibody) can be monospecific, bispecific, trispecific or of greater multi-specificity. Multi-specific antibodies can be specific for different epitopes of one or more PD-L1 polypeptides or can be specific for the PD-L1 polypeptide(s) as well as for heterologous compositions, such as a heterologous polypeptide or solid support material. *See, e.g.*, WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt *et al.*, *J. Immunol.* 147: 60-69 (1991); U.S. Pat. Nos. 5,573,920, 4,474,893, 5,601,819, 4,714,681, 4,925,648; 6,106,835; Kostelny *et al.*, *J. Immunol.* 148: 1547-1553 (1992). In some embodiments, the immunoglobulin-related compositions are chimeric. In certain embodiments, the immunoglobulin-related compositions are humanized.

[00107] The immunoglobulin-related compositions of the present technology can further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, the immunoglobulin-related compositions of the present technology can be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, or toxins.

See, e.g., WO 92/08495; WO 91/14438; WO 89/12624; U.S. Pat. No. 5,314,995; and EP 0 396 387.

[00108] In any of the above embodiments of the immunoglobulin-related compositions of the present technology, the antibody or antigen binding fragment may be optionally conjugated to an agent selected from the group consisting of isotopes, dyes, chromagens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, metals, liposomes, nanoparticles, RNA, DNA or any combination thereof. For a chemical bond or physical bond, a functional group on the immunoglobulin-related composition typically associates with a functional group on the agent. Alternatively, a functional group on the agent associates with a functional group on the immunoglobulin-related composition.

[00109] The functional groups on the agent and immunoglobulin-related composition can associate directly. For example, a functional group (*e.g.*, a sulfhydryl group) on an agent can associate with a functional group (*e.g.*, sulfhydryl group) on an immunoglobulin-related composition to form a disulfide. Alternatively, the functional groups can associate through a cross-linking agent (*i.e.*, linker). Some examples of cross-linking agents are described below. The cross-linker can be attached to either the agent or the immunoglobulin-related composition. The number of agents or immunoglobulin-related compositions in a conjugate is also limited by the number of functional groups present on the other. For example, the maximum number of agents associated with a conjugate depends on the number of functional groups present on the immunoglobulin-related composition. Alternatively, the maximum number of immunoglobulin-related compositions associated with an agent depends on the number of functional groups present on the agent.

[00110] In yet another embodiment, the conjugate comprises one immunoglobulin-related composition associated to one agent. In one embodiment, a conjugate comprises at least one agent chemically bonded (*e.g.*, conjugated) to at least one immunoglobulin-related composition. The agent can be chemically bonded to an immunoglobulin-related composition by any method known to those in the art. For example, a functional group on the agent may be directly attached to a functional group on the immunoglobulin-related composition. Some examples of suitable functional groups include, for example, amino, carboxyl, sulfhydryl, maleimide, isocyanate, isothiocyanate and hydroxyl.

[00111] The agent may also be chemically bonded to the immunoglobulin-related composition by means of cross-linking agents, such as dialdehydes, carbodiimides, dimaleimides, and the like. Cross-linking agents can, for example, be obtained from Pierce Biotechnology, Inc., Rockford, Ill. The Pierce Biotechnology, Inc. web-site can provide assistance. Additional cross-linking agents include the platinum cross-linking agents described in U.S. Pat. Nos. 5,580,990; 5,985,566; and 6,133,038 of Kreatech Biotechnology, B.V., Amsterdam, The Netherlands.

[00112] Alternatively, the functional group on the agent and immunoglobulin-related composition can be the same. Homobifunctional cross-linkers are typically used to cross-link identical functional groups. Examples of homobifunctional cross-linkers include EGS (*i.e.*, ethylene glycol bis[succinimidylsuccinate]), DSS (*i.e.*, disuccinimidyl suberate), DMA (*i.e.*, dimethyl adipimidate.2HCl), DTSSP (*i.e.*, 3,3'-dithiobis[sulfosuccinimidylpropionate])), DPDPB (*i.e.*, 1,4-di-[3'-(2'-pyridyldithio)-propionamido]butane), and BMH (*i.e.*, bis-maleimido-hexane). Such homobifunctional cross-linkers are also available from Pierce Biotechnology, Inc.

[00113] In other instances, it may be beneficial to cleave the agent from the immunoglobulin-related composition. The web-site of Pierce Biotechnology, Inc. described above can also provide assistance to one skilled in the art in choosing suitable cross-linkers which can be cleaved by, for example, enzymes in the cell. Thus the agent can be separated from the immunoglobulin-related composition. Examples of cleavable linkers include SMPT (*i.e.*, 4-succinimidylloxycarbonyl-methyl-a-[2-pyridyldithio]toluene), Sulfo-LC-SPDP (*i.e.*, sulfosuccinimidyl 6-(3-[2-pyridyldithio]-propionamido)hexanoate), LC-SPDP (*i.e.*, succinimidyl 6-(3-[2-pyridyldithio]-propionamido)hexanoate), Sulfo-LC-SPDP (*i.e.*, sulfosuccinimidyl 6-(3-[2-pyridyldithio]-propionamido)hexanoate), SPDP (*i.e.*, N-succinimidyl 3-[2-pyridyldithio]-propionamido-hexanoate), and AEDP (*i.e.*, 3-[(2-aminoethyl)dithio]propionic acid HCl).

[00114] In another embodiment, a conjugate comprises at least one agent physically bonded with at least one immunoglobulin-related composition. Any method known to those in the art can be employed to physically bond the agents with the immunoglobulin-related compositions. For example, the immunoglobulin-related compositions and agents can be mixed together by any method known to those in the art. The order of mixing is not important. For instance, agents can be physically mixed with immunoglobulin-related

compositions by any method known to those in the art. For example, the immunoglobulin-related compositions and agents can be placed in a container and agitated, by for example, shaking the container, to mix the immunoglobulin-related compositions and agents.

[00115] The immunoglobulin-related compositions can be modified by any method known to those in the art. For instance, the immunoglobulin-related composition may be modified by means of cross-linking agents or functional groups, as described above.

[00116] In some embodiments, the antibodies of the present technology comprise pharmaceutical formulations which may be administered to subjects in need thereof in one or more doses. Dosage regimens can be adjusted to provide the desired response (*e.g.*, a therapeutic response).

[00117] Typically, an effective amount of the antibody compositions of the present technology, sufficient for achieving a therapeutic effect, range from about 0.000001 mg per kilogram body weight per day to about 10,000 mg per kilogram body weight per day. Typically, the dosage ranges are from about 0.0001 mg per kilogram body weight per day to about 100 mg per kilogram body weight per day. For administration of anti-PD-L1 antibodies, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg every week, every two weeks or every three weeks, of the subject body weight. For example, dosages can be 1 mg/kg body weight or 10 mg/kg body weight every week, every two weeks or every three weeks or within the range of 1-10 mg/kg every week, every two weeks or every three weeks. In one embodiment, a single dosage of antibody ranges from 0.1-10,000 micrograms per kg body weight. In one embodiment, antibody concentrations in a carrier range from 0.2 to 2000 micrograms per delivered milliliter. An exemplary treatment regime entails administration once per every two weeks or once a month or once every 3 to 6 months. Anti-PD-L1 antibodies may be administered on multiple occasions. Intervals between single dosages can be hourly, daily, weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of the antibody in the subject. In some methods, dosage is adjusted to achieve a serum antibody concentration in the subject of from about 75 µg/mL to about 125 µg/mL, 100 µg/mL to about 150 µg/mL, from about 125 µg/mL to about 175 µg/mL, or from about 150 µg/mL to about 200 µg/mL. Alternatively, anti-PD-L1 antibodies can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the subject. The dosage and frequency of

administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, or until the subject shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

[00118] *Toxicity.* Optimally, an effective amount (*e.g.*, dose) of an anti-PD-L1 antibody described herein will provide therapeutic benefit without causing substantial toxicity to the subject. Toxicity of the anti-PD-L1 antibody described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, by determining the LD₅₀ (the dose lethal to 50% of the population) or the LD₁₀₀ (the dose lethal to 100% of the population). The dose ratio between toxic and therapeutic effect is the therapeutic index. The data obtained from these cell culture assays and animal studies can be used in formulating a dosage range that is not toxic for use in human. The dosage of the anti-PD-L1 antibody described herein lies within a range of circulating concentrations that include the effective dose with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the subject's condition. *See, e.g.*, Fingl *et al.*, In: *The Pharmacological Basis of Therapeutics*, Ch. 1 (1975).

[00119] *Formulations of Pharmaceutical Compositions.* According to the methods of the present technology, the anti-PD-L1 antibody can be incorporated into pharmaceutical compositions suitable for administration. The pharmaceutical compositions generally comprise recombinant or substantially purified antibody and a pharmaceutically-acceptable carrier in a form suitable for administration to a subject. Pharmaceutically-acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions for administering the antibody compositions (*See, e.g.*, *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA 18th ed., 1990). The pharmaceutical compositions are generally formulated as sterile, substantially isotonic and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration.

[00120] The terms “pharmaceutically-acceptable,” “physiologically-tolerable,” and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a subject without the production of undesirable physiological effects to a degree that would prohibit administration of the composition. For example, “pharmaceutically-acceptable excipient” means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for veterinary use as well as for human pharmaceutical use. Such excipients can be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous. “Pharmaceutically-acceptable salts and esters” means salts and esters that are pharmaceutically-acceptable and have the desired pharmacological properties. Such salts include salts that can be formed where acidic protons present in the composition are capable of reacting with inorganic or organic bases. Suitable inorganic salts include those formed with the alkali metals, *e.g.*, sodium and potassium, magnesium, calcium, and aluminum. Suitable organic salts include those formed with organic bases such as the amine bases, *e.g.*, ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like. Such salts also include acid addition salts formed with inorganic acids (*e.g.*, hydrochloric and hydrobromic acids) and organic acids (*e.g.*, acetic acid, citric acid, maleic acid, and the alkane- and arene-sulfonic acids such as methanesulfonic acid and benzenesulfonic acid). Pharmaceutically-acceptable esters include esters formed from carboxy, sulfonyloxy, and phosphonoxy groups present in the anti-PD-L1 antibody, *e.g.*, C₁₋₆ alkyl esters. When there are two acidic groups present, a pharmaceutically-acceptable salt or ester can be a mono-acid-mono-salt or ester or a di-salt or ester; and similarly where there are more than two acidic groups present, some or all of such groups can be salified or esterified. An anti-PD-L1 antibody named in this technology can be present in unsalified or unesterified form, or in salified and/or esterified form, and the naming of such anti-PD-L1 antibody is intended to include both the original (unsalified and unesterified) compound and its pharmaceutically-acceptable salts and esters. Also, certain embodiments of the present technology can be present in more than one stereoisomeric form, and the naming of such anti-PD-L1 antibody is intended to include all single stereoisomers and all mixtures (whether racemic or otherwise) of such stereoisomers. A person of ordinary skill in the art,

would have no difficulty determining the appropriate timing, sequence and dosages of administration for particular drugs and compositions of the present technology.

[00121] Examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and compounds for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or compound is incompatible with the anti-PD-L1 antibody, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[00122] A pharmaceutical composition of the present technology is formulated to be compatible with its intended route of administration. The anti-PD-L1 antibody compositions of the present technology can be administered by parenteral, topical, intravenous, oral, subcutaneous, intraarterial, intradermal, transdermal, rectal, intracranial, intrathecal, intraperitoneal, intranasal; or intramuscular routes, or as inhalants. The anti-PD-L1 antibody can optionally be administered in combination with other agents that are at least partly effective in treating AML.

[00123] Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial compounds such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating compounds such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and compounds for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00124] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy

syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, *e.g.*, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, *e.g.*, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal compounds, *e.g.*, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be desirable to include isotonic compounds, *e.g.*, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition a compound which delays absorption, *e.g.*, aluminum monostearate and gelatin.

[00125] Sterile injectable solutions can be prepared by incorporating an anti-PD-L1 antibody of the present technology in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the anti-PD-L1 antibody into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The antibodies of the present technology can be administered in the form of a depot injection or implant preparation which can be formulated in such a manner as to permit a sustained or pulsatile release of the active ingredient.

[00126] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the anti-PD-L1 antibody can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding compounds, and/or adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating compound such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening compound such as sucrose or saccharin; or a flavoring compound such as peppermint, methyl salicylate, or orange flavoring.

[00127] For administration by inhalation, the anti-PD-L1 antibody is delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[00128] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, *e.g.*, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the anti-PD-L1 antibody is formulated into ointments, salves, gels, or creams as generally known in the art.

[00129] The anti-PD-L1 antibody can also be prepared as pharmaceutical compositions in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[00130] In one embodiment, the anti-PD-L1 antibody is prepared with carriers that will protect the anti-PD-L1 antibody against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically-acceptable carriers. These can be prepared according to methods known to those skilled in the art, *e.g.*, as described in U.S. Pat. No. 4,522,811.

Methods of Preparing Anti-PD-L1 Antibodies of the Present Technology

[00131] *General Overview.* Initially, a target polypeptide is chosen to which an antibody of the present technology can be raised. For example, an antibody may be raised against the full-length PD-L1 protein, or to a portion of the extracellular domain of the PD-L1 protein. Techniques for generating antibodies directed to such target polypeptides are well known to those skilled in the art. Examples of such techniques include, for example, but are not limited to, those involving display libraries, xeno or human mice, hybridomas, and the like. Target polypeptides within the scope of the present technology include any polypeptide derived from PD-L1 protein containing the extracellular domain which is capable of eliciting an immune response.

[00132] It should be understood that recombinantly engineered antibodies and antibody fragments, *e.g.*, antibody-related polypeptides, which are directed to PD-L1 protein and fragments thereof are suitable for use in accordance with the present disclosure.

[00133] Anti-PD-L1 antibodies that can be subjected to the techniques set forth herein include monoclonal and polyclonal antibodies, and antibody fragments such as Fab, Fab', F(ab')₂, Fd, scFv, diabodies, antibody light chains, antibody heavy chains and/or antibody fragments. Methods useful for the high yield production of antibody Fv-containing polypeptides, *e.g.*, Fab' and F(ab')₂ antibody fragments have been described. *See* U.S. Pat. No. 5,648,237.

[00134] Generally, an antibody is obtained from an originating species. More particularly, the nucleic acid or amino acid sequence of the variable portion of the light chain, heavy chain or both, of an originating species antibody having specificity for a target polypeptide antigen is obtained. An originating species is any species which was useful to generate the antibody of the present technology or library of antibodies, *e.g.*, rat, mouse, rabbit, chicken, monkey, human, and the like.

[00135] Phage or phagemid display technologies are useful techniques to derive the antibodies of the present technology. Techniques for generating and cloning monoclonal antibodies are well known to those skilled in the art. Expression of sequences encoding antibodies of the present technology, can be carried out in *E. coli*.

[00136] Due to the degeneracy of nucleic acid coding sequences, other sequences which encode substantially the same amino acid sequences as those of the naturally occurring

proteins may be used in the practice of the present technology. These include, but are not limited to, nucleic acid sequences including all or portions of the nucleic acid sequences encoding the above polypeptides, which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. It is appreciated that the nucleotide sequence of an immunoglobulin according to the present technology tolerates sequence homology variations of up to 25% as calculated by standard methods ("Current Methods in Sequence Comparison and Analysis," *Macromolecule Sequencing and Synthesis, Selected Methods and Applications*, pp. 127-149, 1998, Alan R. Liss, Inc.) so long as such a variant forms an operative antibody which recognizes PD-L1 proteins. For example, one or more amino acid residues within a polypeptide sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Also included within the scope of the present technology are proteins or fragments or derivatives thereof which are differentially modified during or after translation, *e.g.*, by glycosylation, proteolytic cleavage, linkage to an antibody molecule or other cellular ligands, *etc.* Additionally, an immunoglobulin encoding nucleic acid sequence can be mutated *in vitro* or *in vivo* to create and/or destroy translation, initiation, and/or termination sequences or to create variations in coding regions and/or form new restriction endonuclease sites or destroy pre-existing ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used, including but not limited to *in vitro* site directed mutagenesis, *J. Biol. Chem.* 253:6551, use of Tab linkers (Pharmacia), and the like.

[00137] *Monoclonal Antibody.* In one embodiment of the present technology, the antibody is an anti-PD-L1 monoclonal antibody. For example, in some embodiments, the anti-PD-L1 monoclonal antibody may be a human or a mouse anti-PD-L1 monoclonal antibody. For preparation of monoclonal antibodies directed towards the PD-L1 protein, or

derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture can be utilized. Such techniques include, but are not limited to, the hybridoma technique (*See, e.g., Kohler & Milstein, 1975. Nature 256: 495-497*); the trioma technique; the human B-cell hybridoma technique (*See, e.g., Kozbor, et al., 1983. Immunol. Today 4: 72*) and the EBV hybridoma technique to produce human monoclonal antibodies (*See, e.g., Cole, et al., 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96*). Human monoclonal antibodies can be utilized in the practice of the present technology and can be produced by using human hybridomas (*See, e.g., Cote, et al., 1983. Proc. Natl. Acad. Sci. USA 80: 2026-2030*) or by transforming human B-cells with Epstein Barr Virus *in vitro* (*See, e.g., Cole, et al., 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96*). For example, a population of nucleic acids that encode regions of antibodies can be isolated. PCR utilizing primers derived from sequences encoding conserved regions of antibodies is used to amplify sequences encoding portions of antibodies from the population and then DNAs encoding antibodies or fragments thereof, such as variable domains, are reconstructed from the amplified sequences. Such amplified sequences also can be fused to DNAs encoding other proteins – *e.g., a bacteriophage coat, or a bacterial cell surface protein* – for expression and display of the fusion polypeptides on phage or bacteria. Amplified sequences can then be expressed and further selected or isolated based, *e.g., on the affinity of the expressed antibody or fragment thereof for an antigen or epitope present on the PD-L1 protein*. Alternatively, hybridomas expressing anti-PD-L1 monoclonal antibodies can be prepared by immunizing a subject and then isolating hybridomas from the subject's spleen using routine methods. *See, e.g., Milstein et al., (Galfre and Milstein, Methods Enzymol (1981) 73: 3-46)*. Screening the hybridomas using standard methods will produce monoclonal antibodies of varying specificity (*i.e., for different epitopes*) and affinity. A selected monoclonal antibody with the desired properties, *e.g., PD-L1 binding*, can be used as expressed by the hybridoma, it can be bound to a molecule such as polyethylene glycol (PEG) to alter its properties, or a cDNA encoding it can be isolated, sequenced and manipulated in various ways. Synthetic dendromeric trees can be added to reactive amino acid side chains, *e.g., lysine*, to enhance the immunogenic properties of PD-L1 protein. Also, CPG-dinucleotide techniques can be used to enhance the immunogenic properties of the PD-L1 protein. Other manipulations include substituting or

deleting particular amino acyl residues that contribute to instability of the antibody during storage or after administration to a subject, and affinity maturation techniques to improve affinity of the antibody of the PD-L1 protein.

[00138] *Hybridoma Technique.* In some embodiments, the antibody of the present technology is an anti-PD-L1 monoclonal antibody produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell. Hybridoma techniques include those known in the art and taught in Harlow *et al.*, *Antibodies: A Laboratory Manual* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 349 (1988); Hammerling *et al.*, *Monoclonal Antibodies And T-Cell Hybridomas*, 563-681 (1981). Other methods for producing hybridomas and monoclonal antibodies are well known to those of skill in the art.

[00139] *Phage Display Technique.* As noted above, the antibodies of the present technology can be produced through the application of recombinant DNA and phage display technology. For example, anti-PD-L1 antibodies, can be prepared using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of a phage particle which carries polynucleotide sequences encoding them. Phages with a desired binding property are selected from a repertoire or combinatorial antibody library (*e.g.*, human or murine) by selecting directly with an antigen, typically an antigen bound or captured to a solid surface or bead. Phages used in these methods are typically filamentous phage including fd and M13 with Fab, Fv or disulfide stabilized Fv antibody domains that are recombinantly fused to either the phage gene III or gene VIII protein. In addition, methods can be adapted for the construction of Fab expression libraries (*See, e.g.*, Huse, *et al.*, *Science* 246: 1275-1281, 1989) to allow rapid and effective identification of monoclonal Fab fragments with the desired specificity for a PD-L1 polypeptide, *e.g.*, a polypeptide or derivatives, fragments, analogs or homologs thereof. Other examples of phage display methods that can be used to make the antibodies of the present technology include those disclosed in Huston *et al.*, *Proc. Natl. Acad. Sci U.S.A.*, 85: 5879-5883, 1988; Chaudhary *et al.*, *Proc. Natl. Acad. Sci U.S.A.*, 87: 1066-1070, 1990; Brinkman *et al.*, *J. Immunol. Methods* 182: 41-50, 1995; Ames *et al.*, *J. Immunol. Methods* 184: 177-186, 1995; Kettleborough *et al.*, *Eur. J. Immunol.* 24: 952-958, 1994; Persic *et al.*, *Gene* 187: 9-18, 1997; Burton *et al.*, *Advances in Immunology* 57: 191-280,

1994; PCT/GB91/01134; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; WO 96/06213; WO 92/01047 (Medical Research Council *et al.*); WO 97/08320 (Morphosys); WO 92/01047 (CAT/MRC); WO 91/17271 (Affymax); and U.S. Pat. Nos. 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727 and 5,733,743. Methods useful for displaying polypeptides on the surface of bacteriophage particles by attaching the polypeptides *via* disulfide bonds have been described by Lohning, U.S. Pat. No. 6,753,136. As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host including mammalian cells, insect cells, plant cells, yeast, and bacteria. For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in WO 92/22324; Mullinax *et al.*, *BioTechniques* 12: 864-869, 1992; and Sawai *et al.*, *AJRI* 34: 26-34, 1995; and Better *et al.*, *Science* 240: 1041-1043, 1988.

[00140] Generally, hybrid antibodies or hybrid antibody fragments that are cloned into a display vector can be selected against the appropriate antigen in order to identify variants that maintain good binding activity, because the antibody or antibody fragment will be present on the surface of the phage or phagemid particle. *See, e.g.*, Barbas III *et al.*, *Phage Display, A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001). However, other vector formats could be used for this process, such as cloning the antibody fragment library into a lytic phage vector (modified T7 or Lambda Zap systems) for selection and/or screening.

[00141] *Expression of Recombinant Anti-PD-L1 Antibodies.* As noted above, the antibodies of the present technology can be produced through the application of recombinant DNA technology. Recombinant polynucleotide constructs encoding an anti-PD-L1 antibody of the present technology typically include an expression control sequence operably-linked to the coding sequences of anti-PD-L1 antibody chains, including naturally-associated or heterologous promoter regions. As such, another aspect of the technology includes vectors containing one or more nucleic acid sequences encoding an anti-PD-L1 antibody of the present technology. For recombinant expression of one or more of the polypeptides of the present technology, the nucleic acid containing all or a portion of the

nucleotide sequence encoding the anti-PD-L1 antibody is inserted into an appropriate cloning vector, or an expression vector (*i.e.*, a vector that contains the necessary elements for the transcription and translation of the inserted polypeptide coding sequence) by recombinant DNA techniques well known in the art and as detailed below. Methods for producing diverse populations of vectors have been described by Lerner *et al.*, U.S. Pat. Nos. 6,291,160 and 6,680,192.

[00142] In general, expression vectors useful in recombinant DNA techniques are often in the form of plasmids. In the present disclosure, “plasmid” and “vector” can be used interchangeably as the plasmid is the most commonly used form of vector. However, the present technology is intended to include such other forms of expression vectors that are not technically plasmids, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions. Such viral vectors permit infection of a subject and expression of a construct in that subject. In some embodiments, the expression control sequences are eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences encoding the anti-PD-L1 antibody, and the collection and purification of the anti-PD-L1 antibody, *e.g.*, cross-reacting anti-PD-L1 antibodies. *See generally*, U.S. 2002/0199213. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors contain selection markers, *e.g.*, ampicillin-resistance or hygromycin-resistance, to permit detection of those cells transformed with the desired DNA sequences. Vectors can also encode signal peptide, *e.g.*, pectate lyase, useful to direct the secretion of extracellular antibody fragments. *See* U.S. Pat. No. 5,576,195.

[00143] The recombinant expression vectors of the present technology comprise a nucleic acid encoding a protein with PD-L1 binding properties in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression that is operably-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, “operably-linked” is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that

allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term “regulatory sequence” is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, *e.g.*, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of polypeptide desired, *etc.* Typical regulatory sequences useful as promoters of recombinant polypeptide expression (*e.g.*, anti-PD-L1 antibody), include, *e.g.*, but are not limited to, promoters of 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilization. In one embodiment, a polynucleotide encoding an anti-PD-L1 antibody of the present technology is operably-linked to an *ara B* promoter and expressible in a host cell. *See* U.S. Pat. 5,028,530. The expression vectors of the present technology can be introduced into host cells to thereby produce polypeptides or peptides, including fusion polypeptides, encoded by nucleic acids as described herein (*e.g.*, anti-PD-L1 antibody, *etc.*).

[00144] Another aspect of the present technology pertains to anti-PD-L1 antibody-expressing host cells, which contain a nucleic acid encoding one or more anti-PD-L1 antibodies. The recombinant expression vectors of the present technology can be designed for expression of an anti-PD-L1 antibody in prokaryotic or eukaryotic cells. For example, an anti-PD-L1 antibody can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors), fungal cells, *e.g.*, yeast, yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, *e.g.*, using T7 promoter regulatory sequences and T7 polymerase. Methods useful for the preparation and screening of polypeptides having a

predetermined property, *e.g.*, anti-PD-L1 antibody, *via* expression of stochastically generated polynucleotide sequences has been previously described. *See* U.S. Pat. Nos. 5,763,192; 5,723,323; 5,814,476; 5,817,483; 5,824,514; 5,976,862; 6,492,107; 6,569,641.

[00145] Expression of polypeptides in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion polypeptides. Fusion vectors add a number of amino acids to a polypeptide encoded therein, usually to the amino terminus of the recombinant polypeptide. Such fusion vectors typically serve three purposes: (i) to increase expression of recombinant polypeptide; (ii) to increase the solubility of the recombinant polypeptide; and (iii) to aid in the purification of the recombinant polypeptide by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant polypeptide to enable separation of the recombinant polypeptide from the fusion moiety subsequent to purification of the fusion polypeptide. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding polypeptide, or polypeptide A, respectively, to the target recombinant polypeptide.

[00146] Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69: 301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89). Methods for targeted assembly of distinct active peptide or protein domains to yield multifunctional polypeptides *via* polypeptide fusion has been described by Pack *et al.*, U.S. Pat. Nos. 6,294,353; 6,692,935. One strategy to maximize recombinant polypeptide expression, *e.g.*, an anti-PD-L1 antibody, in *E. coli* is to express the polypeptide in host bacteria with an impaired capacity to proteolytically cleave the recombinant polypeptide. *See, e.g.*, Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in the expression host, *e.g.*, *E. coli* (*See, e.g.*, Wada, *et al.*, 1992. *Nucl.*

Acids Res. 20: 2111-2118). Such alteration of nucleic acid sequences of the present technology can be carried out by standard DNA synthesis techniques.

[00147] In another embodiment, the anti-PD-L1 antibody expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerevisiae* include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, *Cell* 30: 933-943, 1982), pJRY88 (Schultz *et al.*, *Gene* 54: 113-123, 1987), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (Invitrogen Corp, San Diego, Calif.). Alternatively, an anti-PD-L1 antibody can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of polypeptides, *e.g.*, anti-PD-L1 antibody, in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith, *et al.*, *Mol. Cell. Biol.* 3: 2156-2165, 1983) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

[00148] In yet another embodiment, a nucleic acid encoding an anti-PD-L1 antibody of the present technology is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include, *e.g.*, but are not limited to, pCDM8 (Seed, *Nature* 329: 840, 1987) and pMT2PC (Kaufman, *et al.*, *EMBO J.* 6: 187-195, 1987). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells that are useful for expression of the anti-PD-L1 antibody of the present technology, see, *e.g.*, Chapters 16 and 17 of Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

[00149] In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid in a particular cell type (*e.g.*, tissue-specific regulatory elements). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert, *et al.*, *Genes Dev.* 1: 268-277, 1987), lymphoid-specific promoters (Calame and Eaton, *Adv. Immunol.* 43: 235-275, 1988), promoters of T cell receptors (Winoto and Baltimore, *EMBO J.* 8: 729-733, 1989) and immunoglobulins (Banerji, *et al.*, 1983. *Cell* 33: 729-740; Queen and Baltimore, *Cell* 33: 741-748, 1983.), neuron-specific

promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle, *Proc. Natl. Acad. Sci. USA* 86: 5473-5477, 1989), pancreas-specific promoters (Edlund, *et al.*, 1985. *Science* 230: 912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss, *Science* 249: 374-379, 1990) and the α -fetoprotein promoter (Campes and Tilghman, *Genes Dev.* 3: 537-546, 1989).

[00150] Another aspect of the present methods pertains to host cells into which a recombinant expression vector of the present technology has been introduced. The terms “host cell” and “recombinant host cell” are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[00151] A host cell can be any prokaryotic or eukaryotic cell. For example, an anti-PD-L1 antibody can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells. Mammalian cells are a suitable host for expressing nucleotide segments encoding immunoglobulins or fragments thereof. See Winnacker, *From Genes To Clones*, (VCH Publishers, NY, 1987). A number of suitable host cell lines capable of secreting intact heterologous proteins have been developed in the art, and include Chinese hamster ovary (CHO) cell lines, various COS cell lines, HeLa cells, L cells and myeloma cell lines. In some embodiments, the cells are non-human. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer, and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Queen *et al.*, *Immunol. Rev.* 89: 49, 1986. Illustrative expression control sequences are promoters derived from endogenous genes, cytomegalovirus, SV40, adenovirus, bovine papillomavirus, and the like. Co *et al.*, *J Immunol.* 148: 1149, 1992. Other suitable host cells are known to those skilled in the art.

[00152] Vector DNA can be introduced into prokaryotic or eukaryotic cells *via* conventional transformation or transfection techniques. As used herein, the terms

“transformation” and “transfection” are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, electroporation, biolistics or viral-based transfection. Other methods used to transform mammalian cells include the use of polybrene, protoplast fusion, liposomes, electroporation, and microinjection (*See generally*, Sambrook *et al.*, *Molecular Cloning*). Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals. The vectors containing the DNA segments of interest can be transferred into the host cell by well-known methods, depending on the type of cellular host.

[00153] For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding the anti-PD-L1 antibody or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

[00154] A host cell that includes an anti-PD-L1 antibody of the present technology, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) recombinant anti-PD-L1 antibody. In one embodiment, the method comprises culturing the host cell (into which a recombinant expression vector encoding the anti-PD-L1 antibody has been introduced) in a suitable medium such that the anti-PD-L1 antibody is produced. In another embodiment, the method further comprises the step of isolating the anti-PD-L1 antibody from the medium or the host cell. Once expressed, collections of the anti-PD-L1 antibody, *e.g.*, the anti-PD-L1 antibodies or the anti-PD-L1 antibody-related polypeptides are purified from culture media and host cells. The anti-PD-L1 antibody can be purified

according to standard procedures of the art, including HPLC purification, column chromatography, gel electrophoresis and the like. In one embodiment, the anti-PD-L1 antibody is produced in a host organism by the method of Boss *et al.*, U.S. Pat. No. 4,816,397. Usually, anti-PD-L1 antibody chains are expressed with signal sequences and are thus released to the culture media. However, if the anti-PD-L1 antibody chains are not naturally secreted by host cells, the anti-PD-L1 antibody chains can be released by treatment with mild detergent. Purification of recombinant polypeptides is well known in the art and includes ammonium sulfate precipitation, affinity chromatography purification technique, column chromatography, ion exchange purification technique, gel electrophoresis and the like (*See generally* Scopes, Protein Purification (Springer-Verlag, N.Y., 1982).

[00155] Polynucleotides encoding anti-PD-L1 antibodies, *e.g.*, the anti-PD-L1 antibody coding sequences, can be incorporated in transgenes for introduction into the genome of a transgenic animal and subsequent expression in the milk of the transgenic animal. *See, e.g.*, U.S. Pat. Nos. 5,741,957, 5,304,489, and 5,849,992. Suitable transgenes include coding sequences for light and/or heavy chains in operable linkage with a promoter and enhancer from a mammary gland specific gene, such as casein or β -lactoglobulin. For production of transgenic animals, transgenes can be microinjected into fertilized oocytes, or can be incorporated into the genome of embryonic stem cells, and the nuclei of such cells transferred into enucleated oocytes.

[00156] *Single-Chain Antibodies.* In one embodiment, the anti-PD-L1 antibody of the present technology is a single-chain anti-PD-L1 antibody. According to the present technology, techniques can be adapted for the production of single-chain antibodies specific to a PD-L1 protein (*See, e.g.*, U.S. Pat. No. 4,946,778). Examples of techniques which can be used to produce single-chain Fvs and antibodies of the present technology include those described in U.S. Pat. Nos. 4,946,778 and 5,258,498; Huston *et al.*, *Methods in Enzymology*, 203: 46-88, 1991; Shu, L. *et al.*, *Proc. Natl. Acad. Sci. USA*, 90: 7995-7999, 1993; and Skerra *et al.*, *Science* 240: 1038-1040, 1988.

[00157] *Chimeric and Humanized Antibodies.* In one embodiment, the anti-PD-L1 antibody of the present technology is a chimeric anti-PD-L1 antibody. In one embodiment, the anti-PD-L1 antibody of the present technology is a humanized anti-PD-L1 antibody. In one embodiment of the present technology, the donor and acceptor antibodies are

monoclonal antibodies from different species. For example, the acceptor antibody is a human antibody (to minimize its antigenicity in a human), in which case the resulting CDR-grafted antibody is termed a “humanized” antibody.

[00158] Recombinant anti-PD-L1 antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, can be made using standard recombinant DNA techniques, and are within the scope of the present technology. For some uses, including *in vivo* use of the anti-PD-L1 antibody of the present technology in humans as well as use of these agents in *in vitro* detection assays, it is possible to use chimeric or humanized anti-PD-L1 antibodies. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art. Such useful methods include, *e.g.*, but are not limited to, methods described in International Application No. PCT/US86/02269; U.S. Pat. No. 5,225,539; European Patent No. 184187; European Patent No. 171496; European Patent No. 173494; PCT International Publication No. WO 86/01533; U.S. Pat. Nos. 4,816,567; 5,225,539; European Patent No. 125023; Better, *et al.*, 1988. *Science* 240: 1041-1043; Liu, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 3439-3443; Liu, *et al.*, 1987. *J. Immunol.* 139: 3521-3526; Sun, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 214-218; Nishimura, *et al.*, 1987. *Cancer Res.* 47: 999-1005; Wood, *et al.*, 1985. *Nature* 314: 446-449; Shaw, *et al.*, 1988. *J. Natl. Cancer Inst.* 80: 1553-1559; Morrison (1985) *Science* 229: 1202-1207; Oi, *et al.* (1986) *BioTechniques* 4: 214; Jones, *et al.*, 1986. *Nature* 321: 552-525; Verhoeyan, *et al.*, 1988. *Science* 239: 1534; Morrison, *Science* 229: 1202, 1985; Oi *et al.*, *BioTechniques* 4: 214, 1986; Gillies *et al.*, *J. Immunol. Methods*, 125: 191-202, 1989; U.S. Pat. No. 5,807,715; and Beidler, *et al.*, 1988. *J. Immunol.* 141: 4053-4060. For example, antibodies can be humanized using a variety of techniques including CDR-grafting (EP 0 239 400; WO 91/09967; U.S. Pat. No. 5,530,101; 5,585,089; 5,859,205; 6,248,516; EP460167), veneering or resurfacing (EP 0 592 106; EP 0 519 596; Padlan E. A., *Molecular Immunology*, 28: 489-498, 1991; Studnicka *et al.*, *Protein Engineering* 7: 805-814, 1994; Roguska *et al.*, *PNAS* 91: 969-973, 1994), and chain shuffling (U.S. Pat. No. 5,565,332). In one embodiment, a cDNA encoding a murine anti-PD-L1 monoclonal antibody is digested with a restriction enzyme selected specifically to remove the sequence encoding the Fc constant region, and the equivalent portion of a cDNA encoding a human Fc constant region is substituted (*See Robinson et al.*, PCT/US86/02269; Akira *et al.*, European Patent Application 184,187; Taniguchi, European Patent Application

171,496; Morrison *et al.*, European Patent Application 173,494; Neuberger *et al.*, WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent Application 125,023; Better *et al.* (1988) *Science* 240: 1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84: 3439-3443; Liu *et al.* (1987) *J Immunol* 139: 3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84: 214-218; Nishimura *et al.* (1987) *Cancer Res* 47: 999-1005; Wood *et al.* (1985) *Nature* 314: 446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80: 1553-1559; U.S. Pat. No. 6,180,370; U.S. Pat. Nos. 6,300,064; 6,696,248; 6,706,484; 6,828,422.

[00159] In one embodiment, the present technology provides the construction of humanized anti-PD-L1 antibodies that are unlikely to induce a human anti-mouse antibody (hereinafter referred to as “HAMA”) response, while still having an effective antibody effector function. As used herein, the terms “human” and “humanized”, in relation to antibodies, relate to any antibody which is expected to elicit a therapeutically tolerable weak immunogenic response in a human subject. In one embodiment, the present technology provides for a humanized anti-PD-L1 antibodies, heavy and light chain immunoglobulins.

[00160] *CDR Antibodies.* In some embodiments, the anti-PD-L1 antibody of the present technology is an anti-PD-L1 CDR antibody. Generally the donor and acceptor antibodies used to generate the anti-PD-L1 CDR antibody are monoclonal antibodies from different species; typically the acceptor antibody is a human antibody (to minimize its antigenicity in a human), in which case the resulting CDR-grafted antibody is termed a “humanized” antibody. The graft may be of a single CDR (or even a portion of a single CDR) within a single V_H or V_L of the acceptor antibody, or can be of multiple CDRs (or portions thereof) within one or both of the V_H and V_L. Frequently, all three CDRs in all variable domains of the acceptor antibody will be replaced with the corresponding donor CDRs, though one needs to replace only as many as necessary to permit adequate binding of the resulting CDR-grafted antibody to PD-L1 protein. Methods for generating CDR-grafted and humanized antibodies are taught by Queen *et al.* U.S. Pat. No. 5,585,089; U.S. Pat. No. 5,693,761; U.S. Pat. No. 5,693,762; and Winter U.S. 5,225,539; and EP 0682040. Methods useful to prepare V_H and V_L polypeptides are taught by Winter *et al.*, U.S. Pat. Nos. 4,816,397; 6,291,158; 6,291,159; 6,291,161; 6,545,142; EP 0368684; EP0451216; and EP0120694.

[00161] After selecting suitable framework region candidates from the same family and/or the same family member, either or both the heavy and light chain variable regions are produced by grafting the CDRs from the originating species into the hybrid framework regions. Assembly of hybrid antibodies or hybrid antibody fragments having hybrid variable chain regions with regard to either of the above aspects can be accomplished using conventional methods known to those skilled in the art. For example, DNA sequences encoding the hybrid variable domains described herein (*i.e.*, frameworks based on the target species and CDRs from the originating species) can be produced by oligonucleotide synthesis and/or PCR. The nucleic acid encoding CDR regions can also be isolated from the originating species antibodies using suitable restriction enzymes and ligated into the target species framework by ligating with suitable ligation enzymes. Alternatively, the framework regions of the variable chains of the originating species antibody can be changed by site-directed mutagenesis.

[00162] Since the hybrids are constructed from choices among multiple candidates corresponding to each framework region, there exist many combinations of sequences which are amenable to construction in accordance with the principles described herein. Accordingly, libraries of hybrids can be assembled having members with different combinations of individual framework regions. Such libraries can be electronic database collections of sequences or physical collections of hybrids.

[00163] This process typically does not alter the acceptor antibody's FRs flanking the grafted CDRs. However, one skilled in the art can sometimes improve antigen binding affinity of the resulting anti-PD-L1 CDR-grafted antibody by replacing certain residues of a given FR to make the FR more similar to the corresponding FR of the donor antibody. Suitable locations of the substitutions include amino acid residues adjacent to the CDR, or which are capable of interacting with a CDR (*See, e.g.*, US 5,585,089, especially columns 12-16). Or one skilled in the art can start with the donor FR and modify it to be more similar to the acceptor FR or a human consensus FR. Techniques for making these modifications are known in the art. Particularly if the resulting FR fits a human consensus FR for that position, or is at least 90% or more identical to such a consensus FR, doing so may not increase the antigenicity of the resulting modified anti-PD-L1 CDR-grafted antibody significantly compared to the same antibody with a fully human FR.

[00164] *Fc Modifications.* In some embodiments, the anti-PD-L1 antibodies of the present technology comprise a variant Fc region, wherein said variant Fc region comprises at least one amino acid modification relative to a wild-type Fc region (or the parental Fc region), such that said molecule has an altered affinity for an Fc receptor (*e.g.*, an FcγR), provided that said variant Fc region does not have a substitution at positions that make a direct contact with Fc receptor based on crystallographic and structural analysis of Fc-Fc receptor interactions such as those disclosed by Sondermann *et al.*, *Nature*, 406:267-273 (2000). Examples of positions within the Fc region that make a direct contact with an Fc receptor such as an FcγR, include amino acids 234-239 (hinge region), amino acids 265-269 (B/C loop), amino acids 297-299 (C7E loop), and amino acids 327-332 (F/G) loop.

[00165] In some embodiments, an anti-PD-L1 antibody of the present technology has an altered affinity for activating and/or inhibitory receptors, having a variant Fc region with one or more amino acid modifications, wherein said one or more amino acid modification is a N297 substitution with alanine, or a K322 substitution with alanine.

[00166] *Glycosylation Modifications.* In some embodiments, anti-PD-L1 antibodies of the present technology have an Fc region with variant glycosylation as compared to a parent Fc region. In some embodiments, variant glycosylation includes the absence of fucose; in some embodiments, variant glycosylation results from expression in GnT1-deficient CHO cells.

[00167] In some embodiments, the antibodies of the present technology, may have a modified glycosylation site relative to an appropriate reference antibody that binds to an antigen of interest (*e.g.*, PD-L1), without altering the functionality of the antibody, *e.g.*, binding activity to the antigen. As used herein, "glycosylation sites" include any specific amino acid sequence in an antibody to which an oligosaccharide (*i.e.*, carbohydrates containing two or more simple sugars linked together) will specifically and covalently attach.

[00168] Oligosaccharide side chains are typically linked to the backbone of an antibody *via* either N-or O-linkages. N-linked glycosylation refers to the attachment of an oligosaccharide moiety to the side chain of an asparagine residue. O-linked glycosylation refers to the attachment of an oligosaccharide moiety to a hydroxyamino acid, *e.g.*, serine, threonine. For example, an Fc-glycoform (hPD-L1-IgGln) that lacks certain

oligosaccharides including fucose and terminal N- acetylglucosamine may be produced in special CHO cells and exhibit enhanced ADCC effector function.

[00169] In some embodiments, the carbohydrate content of an immunoglobulin-related composition disclosed herein is modified by adding or deleting a glycosylation site. Methods for modifying the carbohydrate content of antibodies are well known in the art and are included within the present technology, see, *e.g.*, U.S. Patent No. 6,218,149; EP 0359096B1; U.S. Patent Publication No. US 2002/0028486; International Patent Application Publication WO 03/035835; U.S. Patent Publication No. 2003/0115614; U.S. Patent No. 6,218,149; U.S. Patent No. 6,472,511; all of which are incorporated herein by reference in their entirety. In some embodiments, the carbohydrate content of an antibody (or relevant portion or component thereof) is modified by deleting one or more endogenous carbohydrate moieties of the antibody. In some certain embodiments, the present technology includes deleting the glycosylation site of the Fc region of an antibody, by modifying position 297 from asparagine to alanine.

[00170] Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing effector function. Engineered glycoforms may be generated by any method known to one skilled in the art, for example by using engineered or variant expression strains, by co-expression with one or more enzymes, for example N-acetylglucosaminyltransferase III (GnTIII), by expressing a molecule comprising an Fc region in various organisms or cell lines from various organisms, or by modifying carbohydrate(s) after the molecule comprising Fc region has been expressed. Methods for generating engineered glycoforms are known in the art, and include but are not limited to those described in Umana *et al.*, 1999, *Nat. Biotechnol.* 17: 176-180; Davies *et al.*, 2001, *Biotechnol. Bioeng.* 74:288-294; Shields *et al.*, 2002, *J. Biol. Chem.* 277:26733-26740; Shinkawa *et al.*, 2003, *J. Biol. Chem.* 278:3466-3473; U.S. Patent No. 6,602,684; U.S. Patent Application Serial No. 10/277,370; U.S. Patent Application Serial No. 10/113,929; International Patent Application Publications WO 00/61739A1 ; WO 01/292246A1; WO 02/311140A1; WO 02/30954A1; POTILLEGENT™ technology (Biowa, Inc. Princeton, N.J.); GLYCOMAB™ glycosylation engineering technology (GLYCART biotechnology AG, Zurich, Switzerland); each of which is incorporated herein by reference in its entirety. See, *e.g.*, International Patent Application Publication WO 00/061739; U.S. Patent Application Publication No. 2003/0115614; Okazaki *et al.*, 2004, *JMB*, 336: 1239-49.

[00171] *Fusion Proteins.* In one embodiment, the anti-PD-L1 antibody of the present technology is a fusion protein. The anti-PD-L1 antibodies of the present technology, when fused to a second protein, can be used as an antigenic tag. Examples of domains that can be fused to polypeptides include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but can occur through linker sequences. Moreover, fusion proteins of the present technology can also be engineered to improve characteristics of the anti-PD-L1 antibodies. For instance, a region of additional amino acids, particularly charged amino acids, can be added to the N-terminus of the anti-PD-L1 antibody to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties can be added to an anti-PD-L1 antibody to facilitate purification. Such regions can be removed prior to final preparation of the anti-PD-L1 antibody. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art. The anti-PD-L1 antibody of the present technology can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In select embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., Chatsworth, Calif), among others, many of which are commercially available. As described in Gentz *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 821-824, 1989, for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the “HA” tag, corresponds to an epitope derived from the influenza hemagglutinin protein. Wilson *et al.*, *Cell* 37: 767, 1984.

[00172] Thus, any of these above fusion proteins can be engineered using the polynucleotides or the polypeptides of the present technology. Also, in some embodiments, the fusion proteins described herein show an increased half-life *in vivo*.

[00173] Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can be more efficient in binding and neutralizing other molecules compared to the monomeric secreted protein or protein fragment alone. Fountoulakis *et al.*, *J. Biochem.* 270: 3958-3964, 1995.

[00174] Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or a fragment thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, *e.g.*, improved

pharmacokinetic properties. *See* EP-A 0232 262. Alternatively, deleting or modifying the Fc part after the fusion protein has been expressed, detected, and purified, may be desired. For example, the Fc portion can hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, *e.g.*, human proteins, such as hPD-L1, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hPD-L1. Bennett *et al.*, *J. Molecular Recognition* 8: 52-58, 1995; Johanson *et al.*, *J. Biol. Chem.*, 270: 9459-9471, 1995.

[00175] *Labeled Anti-PD-L1 antibodies.* In one embodiment, the anti-PD-L1 antibody of the present technology is coupled with a label moiety, *i.e.*, detectable group. The particular label or detectable group conjugated to the anti-PD-L1 antibody is not a critical aspect of the technology, so long as it does not significantly interfere with the specific binding of the anti-PD-L1 antibody of the present technology to the PD-L1 protein. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well-developed in the field of immunoassays and imaging. In general, almost any label useful in such methods can be applied to the present technology. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Labels useful in the practice of the present technology include magnetic beads (*e.g.*, Dynabeads™), fluorescent dyes (*e.g.*, fluorescein isothiocyanate, Texas red, rhodamine, and the like), radiolabels (*e.g.*, ³H, ¹⁴C, ³⁵S, ¹²⁵I, ¹²¹I, ¹³¹I, ¹¹²In, ^{99m}Tc), other imaging agents such as microbubbles (for ultrasound imaging), ¹⁸F, ¹¹C, ¹⁵O, ⁸⁹Zr (for Positron emission tomography), ^{99m}Tc, ¹¹¹In (for Single photon emission tomography), enzymes (*e.g.*, horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and calorimetric labels such as colloidal gold or colored glass or plastic (*e.g.*, polystyrene, polypropylene, latex, and the like) beads. Patents that describe the use of such labels include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241, each incorporated herein by reference in their entirety and for all purposes. *See also* Handbook of Fluorescent Probes and Research Chemicals (6th Ed., Molecular Probes, Inc., Eugene OR.).

[00176] The label can be coupled directly or indirectly to the desired component of an assay according to methods well known in the art. As indicated above, a wide variety of labels can be used, with the choice of label depending on factors such as required

sensitivity, ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions.

[00177] Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule (*e.g.*, biotin) is covalently bound to the molecule. The ligand then binds to an anti-ligand (*e.g.*, streptavidin) molecule which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. A number of ligands and anti-ligands can be used. Where a ligand has a natural anti-ligand, *e.g.*, biotin, thyroxine, and cortisol, it can be used in conjunction with the labeled, naturally-occurring anti-ligands. Alternatively, any haptenic or antigenic compound can be used in combination with an antibody, *e.g.*, an anti-PD-L1 antibody.

[00178] The molecules can also be conjugated directly to signal generating compounds, *e.g.*, by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidoreductases, particularly peroxidases. Fluorescent compounds useful as labeling moieties, include, but are not limited to, *e.g.*, fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, and the like. Chemiluminescent compounds useful as labeling moieties, include, but are not limited to, *e.g.*, luciferin, and 2,3-dihydrophthalazinediones, *e.g.*, luminol. For a review of various labeling or signal-producing systems which can be used, see U.S. Pat. No. 4,391,904.

[00179] Means of detecting labels are well known to those of skill in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it can be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence. The fluorescence can be detected visually, by means of photographic film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels can be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product. Finally, simple colorimetric labels can be detected simply by observing the color associated with the label. Thus, in various dipstick assays, conjugated gold often appears pink, while various conjugated beads appear the color of the bead.

[00180] Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies, *e.g.*, the anti-PD-L1 antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need be labeled and the presence of the target antibody is detected by simple visual inspection.

Natural Killer Cells as Adoptive Cellular Therapy for AML

[00181] Natural killer cells are lymphocytes defined by their innate capacity to recognize and exert cytotoxicity against malignant or virally infected cells without prior sensitization.⁶⁻⁸ This attribute distinguishes NK cells from T and B cells, which rely on receptor gene rearrangement for antigen specificity and expansion of antigen-experienced clones. Central to NK cell immunity is the detection of cellular stress in target cells, whereby loss of HLA class I combined with increased expression of NK activating receptor ligands on the target cell tilts the NK cells towards cytotoxicity.⁹⁻¹¹ NK cell target recognition is largely dependent on expression of germline encoded receptors, such as the killer Ig-like receptor family, other activating and inhibitory receptors, and cytokine receptors.

[00182] The relevance of NK cells as immunotherapy for AML first became apparent in the context of HLA-haploidentical transplantation using CD34⁺-selected allograft products.^{12,13} Here, Ruggeri and colleagues reported that absence of inhibitory killer Ig-like receptor signaling to educated donor NK cells associated with decreased relapse of myeloid neoplasia after allogeneic hematopoietic transplantation (allo HCT). Isolated donor NK cells expressing cognate KIR for the missing donor KIR ligand in the patient demonstrated reactivity to recipient leukemia cells, revealing the previously unrecognized contribution of NK cells to the graft *versus* leukemia (GVL) phenomenon.

[00183] Following these data, a number of clinical trials were conducted to evaluate whether adoptive transfer of haploidentical NK cells could exert a GVL phenomenon independent from allo HCT.^{14,15} These studies demonstrated that unstimulated or minimally stimulated NK cells induced a limited overall response rate in subjects with myeloid neoplasia. For example, Bachanova and colleagues demonstrated an overall response rate of 27% in 42 patients treated with haploidentical NK cell infusion after fludarabine and cyclophosphamide pre-conditioning in patients with refractory AML.¹⁶ Other studies

demonstrate a similar overall response rate.^{17,18} We performed an initial phase II study of haploidentical NK cells in 7 patients with myeloid neoplasia (**FIGs. 2A-2C**).¹⁹ Peripheral blood NK cells were isolated using a two-step procedure including a CD3⁺ cell depletion step followed by CD56⁺ cell enrichment step using the CliniMACS System (Miltenyi Biotech, Glabach, Germany). Donor NK cells did not receive cytokine stimulation. All subjects received fludarabine and cyclophosphamide pre-conditioning followed by recombinant human IL-2 infusion post-NK cell infusion. The median number of infused CD3⁺CD56⁺ cells 1.1 x 10⁶ cells/recipient kg (range 4.3 -22.4 x 10⁶/kg). There was minimal infusion related toxicities and no patient developed GVHD; however, only 2/7 patients had an objective response. Poor *in vivo* persistence, expansion, and activation of donor NK cells are contributors to the lack of response seen in this study and others.

Cytokine Induction of a Memory-like Phenotype in NK cells for Adoptive Transfer

[00184] As innate lymphocytes, NK cells were canonically thought to not form long-lasting populations with antigen specificity after a pathogenic stimulus. Recent discoveries demonstrated that subpopulations of NK cells with the capacity for enhanced reactivity and pathogen specificity do indeed form after cytomegalovirus infection.^{20,21} These so-called “memory NK cells” also demonstrate enhanced anti-malignancy potential.²² Cooper and colleagues generated a “memory-like” NK cell population with similar phenotype and anti-malignancy potential via stimulation of donor NK cells with IL-12, -15, and -18.^{21,23,24} These so-called “cytokine-induced memory-like” NK cells (CIMN) were examined by Romee and colleagues in a phase I study.²³ The authors noted that a single 12-18 hour stimulation resulted in sufficient expansion for 10⁶ – 10⁷ cells/kg in adult patients. Thirteen patients were treated at increasing dose levels with four complete responses and one morphological leukemia free state (MLFS) response. Importantly, no patient developed evidence of GVHD after CIMN infusion. An additional 9 pediatric patients were treated with CIMN cells following allo HCT, where 4 of 8 evaluable patients achieved a complete remission and no significant GVHD was observed.²⁵

[00185] Collectively these data demonstrate that infusion of CIMN cells are feasible, do not exert significant clinical graft *versus* host disease, and demonstrate responsiveness in patients with r/r-AML.

[00186] The CIMN cells can be generated from peripheral donor lymphocytes. The CIMN cells can be autologous, non-autologous (*e.g.*, allogeneic), or derived *in vitro* from progenitor or stem cells.

[00187] In some embodiments, the CIMN cells may further comprise an engineered receptor, such as a chimeric antigen receptor (*e.g.*, a CAR) or other ligand that comprises an extracellular antigen-binding domain, a transmembrane domain and an intracellular domain, where the extracellular antigen-binding domain specifically binds an AML-specific tumor antigen, including a tumor receptor or ligand. Examples of AML-specific tumor antigens include, but are not limited to, CD33 (Siglec-3), CD123 (IL3R α), Siglec-6, TIM3 (HAVCR2), CD7, CD70, ILT3 (LILRB4), NKG2D ligand (NKG2DL), CD276 (B7-H3), CD117 (c-kit), FLT3 (CD135), CD19, CD174 (Lewis-Y, LeY), CLL-1 (CLEC12A), CD38, CD44v6, FR β , GM-CSF (CD116/CD131), CD25 (IL-2R α), CD32, CD47, CD56, CD90 (Thy1), CD96, IL1RAP, MUC1, WT1, PR1/HLA-A2 (h8F4), CD93, PD1, PRAME, mLPA, IDH1 (R132), IDH2 (R140), NPM1mut, NOTCH2, PRL3, IL12RB1, CD244/2B4, RHAMM, Survivin, hTERT or CD4. In some embodiments of the CIMN cells described herein, the CAR comprises (i) an extracellular antigen binding domain; (ii) a transmembrane domain; and (iii) an intracellular domain comprising one or more co-stimulatory domains, wherein the extracellular antigen binding domain binds to the AML-specific tumor antigen. Additionally or alternatively, in certain embodiments of the CIMN cells described herein, the transmembrane domain of the CAR comprises a CD8 transmembrane domain, a CD28 transmembrane domain, a NKG2D transmembrane domain, a CD3 ζ transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, or a BTLA transmembrane domain. Additionally or alternatively, in some embodiments of the CIMN cells described herein, the one or more co-stimulatory domains of the CAR are selected from the group consisting of a CD28 co-stimulatory domain, a 4-1BB co-stimulatory domain, an OX40 co-stimulatory domain, an ICOS co-stimulatory domain, a DAP-10 co-stimulatory domain, a PD-1 co-stimulatory domain, a CTLA-4 co-stimulatory domain, a LAG-3 co-stimulatory domain, a 2B4 co-stimulatory domain, a BTLA co-stimulatory domain, a NKG2C co-stimulatory domain, a NKG2D co-stimulatory domain, and any combination thereof.

[00188] The presently disclosed CIMN cells of the present technology may further include at least one recombinant or exogenous co-stimulatory ligand. For example, the presently disclosed CIMN cells can be further transduced with at least one co-stimulatory ligand, such that the CIMN cells co-expresses or is induced to co-express any CAR disclosed herein and the at least one co-stimulatory ligand. Co-stimulatory ligands include, but are not limited to, members of the tumor necrosis factor (TNF) superfamily, and immunoglobulin (Ig) superfamily ligands. TNF is a cytokine involved in systemic inflammation and stimulates the acute phase reaction. Its primary role is in the regulation of immune cells. Members of TNF superfamily share a number of common features. The majority of TNF superfamily members are synthesized as type II transmembrane proteins (extracellular C-terminus) containing a short cytoplasmic segment and a relatively long extracellular region. TNF superfamily members include, without limitation, nerve growth factor (NGF), CD40L (CD40L)/CD 154, CD137L/4-1BBL, TNF- α , CD134L/OX40L/CD252, CD27L/CD70, Fas ligand (FasL), CD30L/CD153, tumor necrosis factor beta (TNFP)/lymphotoxin- α (LT α), lymphotoxin-beta O-T β), CD257/B cell-activating factor (B AFF)/Bly s/THANK/Tall- 1, glucocorticoid-induced TNF Receptor ligand (GITRL), and TF-related apoptosis-inducing ligand (TRAIL), LIGHT (TNFSF14). In certain embodiments, the at least one co-stimulatory ligand is selected from the group consisting of 4-1BBL, CD80, CD86, CD70, OX40L, CD48, TNFRSF14, PD-L1, and combinations thereof. In certain embodiments, the CIMN cells comprises one recombinant co-stimulatory ligand (*e.g.*, 4-1BBL). In certain embodiments, the CIMN cells comprises two recombinant co-stimulatory ligands (*e.g.*, 4-1BBL and CD80). CARs comprising at least one co-stimulatory ligand are described in U.S. Patent No. 8,389,282, which is incorporated by reference in its entirety.

[00189] The unpurified source of CIMN cells can be any known in the art, such as the bone marrow, fetal, neonate or adult or other hematopoietic cell source, *e.g.*, fetal liver, peripheral blood or umbilical cord blood. Various techniques can be employed to separate the cells. For instance, negative selection methods can remove non-immune cell initially. Monoclonal antibodies are particularly useful for identifying markers associated with particular cell lineages and/or stages of differentiation for both positive and negative selections.

[00190] A large proportion of terminally differentiated cells can be initially removed by a relatively crude separation. For example, magnetic bead separations can be used initially to remove large numbers of irrelevant cells. In some embodiments, at least about 80%, usually at least 70% of the total hematopoietic cells will be removed prior to cell isolation.

[00191] Procedures for separation include, but are not limited to, density gradient centrifugation; resetting; coupling to particles that modify cell density; magnetic separation with antibody-coated magnetic beads; affinity chromatography; cytotoxic agents joined to or used in conjunction with a mAb, including, but not limited to, complement and cytotoxins; and panning with antibody attached to a solid matrix, *e.g.*, plate, chip, elutriation or any other convenient technique.

[00192] Techniques for separation and analysis include, but are not limited to, flow cytometry, which can have varying degrees of sophistication, *e.g.*, a plurality of color channels, low angle and obtuse light scattering detecting channels, impedance channels.

[00193] The cells can be selected against dead cells, by employing dyes associated with dead cells such as propidium iodide (PI). In some embodiments, the cells are collected in a medium comprising 2% fetal calf serum (FCS) or 0.2% bovine serum albumin (BSA) or any other suitable, preferably sterile, isotonic medium.

[00194] In some embodiments, the CIMN cells comprise one or more additional modifications. For example, in some embodiments, the CIMN cells comprise and express (is transduced to express) a chimeric co-stimulatory receptor (CCR). CCR is described in Krause *et al.* (1998) *J. Exp. Med.* 188(4):619-626, and US20020018783, the contents of which are incorporated by reference in their entireties. CCRs mimic co-stimulatory signals, but do not provide a T-cell activation signal, *e.g.*, CCRs lack a CD3 ζ polypeptide. CCRs provide co-stimulation, *e.g.*, a CD28-like signal, in the absence of the natural co-stimulatory ligand on the antigen-presenting cell.

[00195] In some embodiments, the CIMN cells are further modified to suppress expression of one or more genes. In some embodiments, the CIMN cells are further modified via genome editing. Various methods and compositions for targeted cleavage of genomic DNA have been described. Such targeted cleavage events can be used, for example, to induce targeted mutagenesis, induce targeted deletions of cellular DNA sequences, and facilitate targeted recombination at a predetermined chromosomal locus.

See, for example, U.S. Patent Nos. 7,888,121; 7,972,854; 7,914,796; 7,951,925; 8,110,379; 8,409,861; 8,586,526; U.S. Patent Publications 20030232410; 20050208489; 20050026157; 20050064474; 20060063231; 201000218264; 20120017290; 20110265198; 20130137104; 20130122591; 20130177983 and 20130177960, the disclosures of which are incorporated by reference in their entireties. These methods often involve the use of engineered cleavage systems to induce a double strand break (DSB) or a nick in a target DNA sequence such that repair of the break by an error born process such as non-homologous end joining (NHEJ) or repair using a repair template (homology directed repair or HDR) can result in the knock out of a gene or the insertion of a sequence of interest (targeted integration). Cleavage can occur through the use of specific nucleases such as engineered zinc finger nucleases (ZFN), transcription-activator like effector nucleases (TALENs), or using the CRISPR/Cas system with an engineered crRNA/tracr RNA ('single guide RNA') to guide specific cleavage.

Administration

[00196] The CIMN cells of the presently disclosed subject matter can be provided systemically or directly to a subject for treating cancer. In certain embodiments, the CIMN cells of the present technology are provided to the organ of interest, for example, by administration into the circulatory system (*e.g.*, the tumor vasculature). Expansion and differentiation agents can be provided prior to, during or after administration of cells and compositions to increase production of the CIMN cells either *in vitro* or *in vivo*.

[00197] CIMN cells of the presently disclosed subject matter can be administered in any physiologically acceptable vehicle, systemically or regionally, normally intravascularly, intraperitoneally, intrathecally, or intrapleurally, although they may also be introduced into bone or other convenient site where the cells may find an appropriate site for regeneration and differentiation (*e.g.*, thymus). In certain embodiments, at least 1×10^5 cells can be administered, eventually reaching 1×10^{10} or more. In certain embodiments, at least 1×10^6 cells can be administered. A cell population comprising CIMN cells can comprise a purified population of CD3⁻CD56⁺ cells. Those skilled in the art can readily determine the percentage of CIMN cells in a cell population using various well-known methods, such as fluorescence activated cell sorting (FACS). The ranges of purity in cell populations comprising CIMN cells can be from about 50% to about 55%, from about 55% to about 60%, about 60% to about 65%, from about 65% to about 70%, from about 70% to about 75%, from about 75% to about 80%, from about 80% to about 85%; from about 85% to

about 90%, from about 90% to about 95%, or from about 95 to about 100%. Dosages can be readily adjusted by those skilled in the art (*e.g.*, a decrease in purity may require an increase in dosage). The CIMN cells can be introduced by injection, catheter, or the like. If desired, factors can also be included, including, but not limited to, interleukins, *e.g.*, IL-2, IL-4, IL-7, IL-12, IL-15, IL-18, IL-21, IL-23, as well as the other interleukins, the colony stimulating factors, such as G-, M- and GM-CSF, interferons, *e.g.*, γ -interferon.

[00198] In certain embodiments, compositions of the presently disclosed subject matter comprise any and all embodiments of the CIMN cells of the present technology with a pharmaceutically acceptable carrier. Administration can be autologous or non-autologous. For example, the CIMN cells of the present technology and compositions comprising the same can be obtained from one subject, and administered to the same subject or a different, compatible subject. Peripheral blood derived CIMN cells of the presently disclosed subject matter or their progeny (*e.g.*, *in vivo*, *ex vivo* or *in vitro* derived) can be administered via localized injection, including catheter administration, systemic injection, localized injection, intravenous injection, or parenteral administration. When administering a pharmaceutical composition of the presently disclosed subject matter (*e.g.*, a pharmaceutical composition comprising any and all embodiments of the CIMN cells disclosed herein), it can be formulated in a unit dosage injectable form (solution, suspension, emulsion).

[00199] The compositions used in accordance with the disclosure can be packaged in dosage unit form for ease of administration and uniformity of dosage. The term "unit dose" or "dosage" refers to physically discrete units suitable for use in a subject, each unit containing a predetermined quantity of the composition calculated to produce the desired responses in association with its administration, *i.e.*, the appropriate route and regimen. The quantity to be administered, both according to number of treatments and unit dose, depends on the result and/or protection desired. Precise amounts of the composition also depend on the judgment of the practitioner and are peculiar to each individual. Factors affecting dose include physical and clinical state of the subject, route of administration, intended goal of treatment (alleviation of symptoms versus cure), and potency, stability, and toxicity of the particular composition. Upon formulation, solutions are administered in a manner compatible with the dosage formulation and in such amount as is therapeutically or prophylactically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described herein.

Formulations

[00200] The CIMN cells of the present technology and compositions comprising the same can be conveniently provided as sterile liquid preparations, *e.g.*, isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions, which may be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like) and suitable mixtures thereof.

[00201] Sterile injectable solutions can be prepared by incorporating the compositions of the presently disclosed subject matter, *e.g.*, a composition comprising the CIMN cells of the present technology, in the required amount of the appropriate solvent with various amounts of the other ingredients, as desired. Such compositions may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting, dispersing, or emulsifying agents (*e.g.*, methylcellulose), pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts, such as “REMINGTON'S PHARMACEUTICAL SCIENCE”, 17th edition, 1985, incorporated herein by reference, may be consulted to prepare suitable preparations, without undue experimentation.

[00202] Various additives which enhance the stability and sterility of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. According to the presently disclosed subject matter, however, any vehicle, diluent, or

additive used would have to be compatible with the CIMN cells of the presently disclosed subject matter.

[00203] The compositions can be isotonic, *i.e.*, they can have the same osmotic pressure as blood and lacrimal fluid. The desired isotonicity of the compositions of the presently disclosed subject matter may be accomplished using sodium chloride, or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol or other inorganic or organic solutes. Sodium chloride is suitable particularly for buffers containing sodium ions.

[00204] Viscosity of the compositions, if desired, can be maintained at the selected level using a pharmaceutically acceptable thickening agent. Methylcellulose can be used because it is readily and economically available and is easy to work with. Other suitable thickening agents include, for example, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, carbomer, and the like. The concentration of the thickener can depend upon the agent selected. The important point is to use an amount that will achieve the selected viscosity. Obviously, the choice of suitable carriers and other additives will depend on the exact route of administration and the nature of the particular dosage form, *e.g.*, liquid dosage form (*e.g.*, whether the composition is to be formulated into a solution, a suspension, gel or another liquid form, such as a time release form or liquid-filled form).

[00205] Those skilled in the art will recognize that the components of the compositions should be selected to be chemically inert and will not affect the viability or efficacy of the CIMN cells as described in the presently disclosed subject matter. This will present no problem to those skilled in chemical and pharmaceutical principles, or problems can be readily avoided by reference to standard texts or by simple experiments (not involving undue experimentation), from this disclosure and the documents cited herein.

[00206] One consideration concerning the therapeutic use of the CIMN cells of the presently disclosed subject matter is the quantity of cells necessary to achieve an optimal effect. The quantity of cells to be administered will vary for the subject being treated. In certain embodiments, from about 10^2 to about 10^{12} , from about 10^3 to about 10^{11} , from about 10^4 to about 10^{10} , from about 10^5 to about 10^9 , or from about 10^6 to about 10^8 CIMN cells of the presently disclosed subject matter are administered to a subject. More effective cells may be administered in even smaller numbers. In some embodiments, at least about 1

$\times 10^8$, about 2×10^8 , about 3×10^8 , about 4×10^8 , about 5×10^8 , about 1×10^9 , about 5×10^9 , about 1×10^{10} , about 5×10^{10} , about 1×10^{11} , about 5×10^{11} , about 1×10^{12} or more CIMN cells of the presently disclosed subject matter are administered to a human subject. The precise determination of what would be considered an effective dose may be based on factors individual to each subject, including their size, age, sex, weight, and condition of the particular subject. Dosages can be readily ascertained by those skilled in the art from this disclosure and the knowledge in the art. Generally, CIMN cells are administered at doses that are nontoxic or tolerable to the patient.

[00207] The skilled artisan can readily determine the amount of cells and optional additives, vehicles, and/or carrier in compositions to be administered in methods of the presently disclosed subject matter. Typically, any additives (in addition to the active cell(s) and/or agent(s)) are present in an amount of from about 0.001% to about 50% by weight) solution in phosphate buffered saline, and the active ingredient is present in the order of micrograms to milligrams, such as from about 0.0001 wt % to about 5 wt %, from about 0.0001 wt% to about 1 wt %, from about 0.0001 wt% to about 0.05 wt%, from about 0.001 wt% to about 20 wt %, from about 0.01 wt% to about 10 wt %, or from about 0.05 wt% to about 5 wt %. For any composition to be administered to an animal or human, and for any particular method of administration, toxicity should be determined, such as by determining the lethal dose (LD) and LD50 in a suitable animal model *e.g.*, rodent such as mouse; and, the dosage of the composition(s), concentration of components therein and timing of administering the composition(s), which elicit a suitable response. Such determinations do not require undue experimentation from the knowledge of the skilled artisan, this disclosure and the documents cited herein. And, the time for sequential administrations can be ascertained without undue experimentation.

Methods for Treating AML Comprising CIMN cells and Anti-PD-L1 Antibodies or Antigen Binding Fragments Thereof

[00208] The presently disclosed subject matter provides methods of using any and all embodiments of the CIMN cells described herein and any and all embodiments of the anti-PD-L1 antibodies or antigen binding fragments thereof for the treatment of AML. In some embodiments, the anti-PD-L1 antibodies or antigen binding fragments may comprise atezolizumab, avelumab or durvalumab.

[00209] In one aspect, the present disclosure provides a method for treating acute myelogenous leukemia (AML) in a subject in need thereof comprising administering to the subject an effective amount of an anti-PD-L1 antibody or antigen binding fragment thereof, and an effective amount of cytokine induced memory-like natural killer (CIMN) cells. The AML may be relapsed AML or refractory AML. Additionally or alternatively, in some embodiments, the CIMN cells are autologous or obtained from a haploidentical donor.

[00210] In some embodiments of the methods disclosed herein, the anti-PD-L1 antibody or antigen binding fragment comprises a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein the V_H comprises a V_H -CDR1 sequence of SEQ ID NO: 4, a V_H -CDR2 sequence of SEQ ID NO: 6, and a V_H -CDR3 sequence of SEQ ID NO: 7 and the V_L comprises a V_L -CDR1 sequence of SEQ ID NO: 9, a V_L -CDR2 sequence of SEQ ID NO: 11, and a V_L -CDR3 sequence of SEQ ID NO: 13. Additionally or alternatively, in certain embodiments, the V_H comprises the amino acid sequence of SEQ ID NO: 1, and/or the V_L comprises the amino acid sequence of SEQ ID NO: 2.

[00211] In some embodiments of the methods disclosed herein, the anti-PD-L1 antibody or antigen binding fragment comprises a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein the V_H comprises a V_H -CDR1 sequence of SEQ ID NO: 17, a V_H -CDR2 sequence of SEQ ID NO: 19, and a V_H -CDR3 sequence of SEQ ID NO: 21 and the V_L comprises a V_L -CDR1 sequence of SEQ ID NO: 23, a V_L -CDR2 sequence of SEQ ID NO: 25, and a V_L -CDR3 sequence of SEQ ID NO: 27. Additionally or alternatively, in certain embodiments, the V_H comprises the amino acid sequence of SEQ ID NO: 14, and/or and the V_L comprises the amino acid sequence of SEQ ID NO: 15.

[00212] In some embodiments of the methods disclosed herein, the anti-PD-L1 antibody or antigen binding fragment comprises a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein the V_H comprises a V_H -CDR1 sequence of SEQ ID NO: 30, a V_H -CDR2 sequence of SEQ ID NO: 31, and a V_H -CDR3 sequence of SEQ ID NO: 32 and the V_L comprises a V_L -CDR1 sequence of SEQ ID NO: 33, a V_L -CDR2 sequence of SEQ ID NO: 34, and a V_L -CDR3 sequence of SEQ ID NO: 35. Additionally or alternatively, in certain embodiments, the V_H comprises the amino

acid sequence of SEQ ID NO: 28; and/or the V_L comprises the amino acid sequence of SEQ ID NO: 29.

[00213] Additionally or alternatively, in some embodiments of the methods disclosed herein, the CIMN cells are derived from CD3⁺CD56⁺ NK cells. In certain embodiments, the CIMN cells are generated by stimulating donor NK cells with at least one cytokine for about 12-18 hours. In some embodiments, the CIMN cells are generated by stimulating donor NK cells with at least one cytokine for about 12 hours, about 12.5 hours, about 13 hours, about 13.5 hours, about 14 hours, about 14.5 hours, about 15 hours, about 15.5 hours, about 16 hours, about 16.5 hours, about 17 hours, about 17.5 hours, or about 18 hours. The at least one cytokine may comprise one or more of IL-12, IL-15, and/or IL-18.

[00214] Additionally or alternatively, in certain embodiments of the methods disclosed herein, the CIMN cells further comprises a chimeric antigen receptor (CAR) that specifically binds an AML-specific tumor antigen. Examples of AML-specific tumor antigens include, but are not limited to, CD33 (Siglec-3), CD123 (IL3R α), Siglec-6, TIM3 (HAVCR2), CD7, CD70, ILT3 (LILRB4), NKG2D ligand (NKG2DL), CD276 (B7-H3), CD117 (c-kit), FLT3 (CD135), CD19, CD174 (Lewis-Y, LeY), CLL-1 (CLEC12A), CD38, CD44v6, FR β , GM-CSF (CD116/CD131), CD25 (IL-2R α), CD32, CD47, CD56, CD90 (Thy1), CD96, IL1RAP, MUC1, WT1, PR1/HLA-A2 (h8F4), CD93, PD1, PRAME, mLPA, IDH1 (R132), IDH2 (R140), NPM1mut, NOTCH2, PRL3, IL12RB1, CD244/2B4, RHAMM, Survivin, hTERT or CD4.

[00215] Additionally or alternatively, in some embodiments, the CAR comprises (i) an extracellular antigen binding domain; (ii) a transmembrane domain; and (iii) an intracellular domain comprising one or more co-stimulatory domains, wherein the extracellular antigen binding domain binds to the AML-specific tumor antigen. The transmembrane domain of the CAR may comprise a CD8 transmembrane domain, a CD28 transmembrane domain, a NKG2D transmembrane domain, a CD3 ζ transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, or a BTLA transmembrane domain. In any of the preceding embodiments, the one or more co-stimulatory domains of the CAR are selected from the group consisting of a CD28 co-stimulatory domain, a 4-1BB co-stimulatory domain, an OX40 co-stimulatory domain, an

ICOS co-stimulatory domain, a DAP-10 co-stimulatory domain, a PD-1 co-stimulatory domain, a CTLA-4 co-stimulatory domain, a LAG-3 co-stimulatory domain, a 2B4 co-stimulatory domain, a BTLA co-stimulatory domain, a NKG2C co-stimulatory domain, a NKG2D co-stimulatory domain, and any combination thereof.

[00216] Additionally or alternatively, in some embodiments of the methods disclosed herein, the CIMN cells can be administered by any methods known in the art, including, but not limited to, pleural administration, intravenous administration, subcutaneous administration, intranodal administration, intratumoral administration, intrathecal administration, intrapleural administration, intraperitoneal administration, and direct administration to the thymus. In certain embodiments, the CIMN cells and the compositions comprising thereof are intravenously administered to the subject in need. Methods for administering cells for adoptive cell therapies, including, for example, donor lymphocyte infusion and immune cell therapies, and regimens for administration are known in the art and can be employed for administration of the CIMN cells provided herein. In certain embodiments, the subject receives an infusion of the CIMN cells in two escalating dose levels. For treatment, the amount of the CIMN cells provided herein administered is an amount effective in producing the desired effect, for example, treatment of AML or one or more symptoms of AML. An effective amount can be provided in one or a series of administrations of the CIMN cells provided herein. An effective amount can be provided in a bolus or by continuous perfusion. For adoptive immunotherapy using CIMN cells, cell doses in the range of about 10^6 to about 10^{10} may be infused. Lower doses of the CIMN cells may be administered, *e.g.*, about 10^4 to about 10^8 .

[00217] Additionally or alternatively, in certain embodiments of the methods disclosed herein, the anti-PD-L1 antibody or antigen binding fragment is administered pleurally, intravenously, subcutaneously, intranodally, intratumorally, intrathecally, intrapleurally or intraperitoneally.

[00218] In any and all embodiments of the methods disclosed herein, administration of the CIMN cells and the anti-PD-L1 antibody or antigen binding fragment prevents Graft versus host disease (GvHD) in the subject.

[00219] The presently disclosed combination therapy methods can reduce the number of tumor cells, reduce tumor size, and/or eradicate the tumor in the subject. The presently

disclosed combination therapy methods may increase or lengthen survival of a subject with AML. In one non-limiting example, the method of increasing or lengthening survival of a subject with AML comprises administering an effective amount of the CIMN cells and the anti-PD-L1 antibody or antigen binding fragment to the subject.

[00220] The presently disclosed subject matter further provides methods for treating or preventing AML in a subject, comprising administering an effective amount of the CIMN cells and the anti-PD-L1 antibody or antigen binding fragment to the subject. Also provided herein are methods for treating or inhibiting tumor growth or metastasis in a subject comprising contacting a tumor cell with an effective amount of any and all embodiments of the CIMN cells and the anti-PD-L1 antibody or antigen binding fragment described herein.

EXAMPLES

[00221] The present technology is further illustrated by the following Examples, which should not be construed as limiting in any way.

Example 1: Combinatorial Approach of CIMN cells with PD-L1 Blockade

[00222] Responses in PD-L1⁺ tumors to PD-L1 antagonism prompted Dong and colleagues to examine whether PD-L1 inhibition could enhance NK cell reactivity against such tumors.³² These investigators found that co-incubation of cytokine activated NK cells with PD-L1 inhibitors resulted in a p38 pathway dependent activation of PD-L1⁺ NK cells, suggesting that PD-L1 inhibitors act directly on NK cells to enhance their cytotoxicity. We examined NK cells for PD-L1 expression before and after overnight stimulation with IL-12/15/18. CIMN cells demonstrated broad expression of PD-L1 (**FIG. 3C**) suggesting they may be sensitive to PD-L1 inhibitor co-incubation. Given these findings, we tested CIMN cells against three PD-L1⁺ negative AML cell lines with and without co-incubation with the PD-L1 antagonist atezolizumab (**FIGs. 3A-3B**). Here, co-culture resulted in a mean 4.2 fold (3.0 – 5.5 fold) upregulation in target killing across different effector:target ratios.

[00223] We further examined the transcriptomic signature in CIMN cells with and without atezolizumab. Bulk RNA sequencing was performed on healthy donor NK cells stimulated overnight with IL-12/15/18 with or without atezolizumab. Gene set enrichment analysis demonstrated upregulation of IFN- γ and related pathways. Importantly, we found down-regulation of several immune checkpoint mediators downstream from IFN- γ ,

suggesting that atezolizumab both directly activates PD-L1⁺ NK cells and prevents checkpoint mediated dampening of the activation cascade.

[00224] Collectively these data indicate that PD-L1 inhibition acts directly on activated NK cells to increase their persistence and cytotoxicity *in vivo*. Here, we propose to “boost” CIMN cells with co-infusion of the PD-L1 specific monoclonal antibody atezolizumab. Immune related adverse events associated with atezolizumab monotherapy are emergent after 4-10 weeks of therapy. In the context of a single dose of atezolizumab we anticipate limited side effects related to administration of this medication. In order to confirm safety of the CIMN cells when co-administered with immune checkpoint inhibition we will perform a limited dose escalation study of CIMN cells in a standard 3+3 fashion.

Example 2: Phase I Study

[00225] This is a phase I dose escalation study designed to determine the safety of CIMN cells when combined with atezolizumab in patients with relapsed or refractory acute myelogenous leukemia (AML). Study treatment consists of a standard pre-cellular therapy conditioning program of fludarabine 25 mg/m² on days -6 to -2, cyclophosphamide 50 mg/kg on days -5 and -4, and the selective anti-programmed death ligand-1 monoclonal antibody atezolizumab 1,200 mg IV as a flat dose on day 0. Subjects will then receive an infusion of CIMN cells on day 0 in two escalating dose levels. Post infusion, patients will receive rhIL-2 (1 x 10⁶ IU/m² IV) every other day x 7 doses beginning on day +0. The dose escalation portion of the study will employ a standard 3+3 design with two dose levels of CIMN cells: The initial cohort will consist of 1 x 10⁶ CIMN cells per recipient kg and the second cohort will consist of all of the CIMN cells generated up to a maximum of 1 x 10⁷ cells/recipient kg. If excess toxicity is seen in dose level 1 there will be an optional dose level zero of 0.5 x 10⁶ CIMN cells per recipient kg. Dose limiting toxicity (DLT) is defined as any of the following: A grade 4-5 adverse event (AE) according to the CTCAE v5.0, severe ICANS, severe seizure disorder, or grade 3-4 AE that does not resolve after 7 days as defined in section 13. The total number of anticipated patients treated in this study is 9-12.

[00226] *Therapeutic Agents*

[00227] Cytokine Induced Memory NK cells (CIMN): Haploidentical donors undergo a standard 6-12 L leukapheresis to isolate peripheral blood mononuclear cells (PBMC). Following this, the PBMC fraction undergoes a two-step isolation procedure (CD3⁺ cell depletion; CD56⁺ cell enrichment) using the Miltenyi Prodigy system. The isolated CD3⁻

CD56⁺ fraction undergoes a 12-18 hour cytokine stimulation with IL-12 (10 ng/mL), IL-15 (50 ng/mL), and IL-18 (50 ng/mL). The cells are then washed to remove the cytokines prior to their infusion into the patients. Quality control (QC) testing will be performed on the final product and standard lot release criteria will be followed before releasing the product for patient infusion. The final CIMN product will undergo labelling and tracking according to standard protocols. CIMN cells are infused fresh without cryopreservation.

[00228] *Atezolizumab:* Atezolizumab is a commercially available IgG1 monoclonal antibody directed at programmed death ligand-1 (PD-L1). Atezolizumab has undergone glycol-engineering in the Fc region to limit antibody-dependent cellular cytotoxicity. Atezolizumab is FDA approved for use in patients with non-small cell lung cancer but not in patients with AML.

[00229] *Objectives*

[00230] To determine the maximum tolerated dose (MTD) of the combination of CIMN cells with atezolizumab in subjects with r/r-AML

[00231] To further characterize adverse events including non-DLT events following CIMN cells with atezolizumab at the MTD.

[00232] To estimate the duration of CIMN *in vivo* persistence.

[00233] To determine transcriptomic changes in CIMN cells after infusion with PD-L1 blockade.

[00234] To characterize the incidence of formation of donor-HLA specific anti-HLA antibodies in recipients.

[00235] *Design*

[00236] This is a single-arm, phase I study examining two escalating dose levels of CIMN cells infused following a standard pre-conditioning chemotherapy program. In addition, all subjects will receive a single dose of atezolizumab prior to CIMN infusion. The study will be designed in a standard 3+3 format whereby if no dose limiting toxicity occurs in the first three patients the next dose level will begin. If 1/3 patients experiences a DLT the dose level will enroll an additional 3 patients. If 2/6 patients in a dose level have a DLT the previous dose level will be examined as the maximum tolerated dose (MTD). If 2/6

DLTs occur in the initial dose cohort, we will dose-deescalate to a dose level 0 cohort of 0.5×10^6 CIMN cells/recipient kg.

[00237] The primary endpoint of the study will be to determine the MTD as outlined above. The secondary endpoints of the study will be to estimate the response rate (CR/CRi), estimate the overall rate of AEs, estimate the persistence of CIMN cells *in vivo*, characterize transcriptomic changes in CIMN cells co-infused with atezolizumab, and estimate the likelihood of donor-HLA specific anti-HLA antibody formation in recipients treated in both the dose escalation and expansion phases.

[00238] *Intervention*

[00239] All study subjects will receive the same therapy as outlined below in the study schema. Pre-conditioning chemotherapy will consist of a standard lymphoreductive regimen including fludarabine 25 mg/m² IV daily x 5 on days -6 to -2 and cyclophosphamide 50 mg/kg IV x 2 on days -5 and -4 prior to the infusion of the CIMN cells. Standard MSKCC supportive care guidelines for infusion of chemotherapy including cyclophosphamide will be employed.

[00240] On the day of infusion subjects will receive a single dose of atezolizumab IV over 60 minutes IV. Subjects will receive subcutaneous or IV recombinant human IL-2 (rh-IL2) 1 million IU every other day x 6 doses beginning on day 0. Response assessment will occur between days 28-45 depending on hematopoietic cell recovery. If the patient has not recovered by day 38, or if disease restaging is required earlier in the opinion of the treating physician, restaging will occur without hematopoietic recovery.

[00241] Supportive care will include opportunistic infection prophylaxis for Herpesviridae and pneumocystis *jirovecii* per standard Adult BMT service guidelines for 3 months following therapy, standard anti-emesis support per MSKCC guidelines, and other standard supportive care peri-administration of high-dose cyclophosphamide.

[00242] *Dose escalation and expansion design:*

[00243] A dose limiting toxicity is defined as any of the following:

[00244] Any non-hematologic AE grade 4-5 as per CTCAE v5.0 with the exception of the following:

[00245] Individual signs and symptoms that occur in the context of grade <3 CRS will not be considered a DLT.

[00246] Any grade III-IV acute GvHD not responsive to glucocorticoid therapy. Non-response to glucocorticoid therapy is defined as either progression to a higher grade after 3 days of therapy or lack of a partial response after 7 days of therapy with prednisone 1 mg/kg/d or equivalent.

[00247] Grade 3 immune effector cell-associated neurotoxicity syndrome of any duration.

[00248] The study will proceed in a standard 3+3 design with the below dose levels:

Dose level	CIMN Cells	Number of subjects
DL 0	$0.5 \times 10^5/\text{kg}$	Optional 3-6
DL 1	$1 \times 10^6/\text{kg}$	3-6
DL 2	Up to $1 \times 10^7/\text{kg}$	3-6

[00249] If 1/3 subjects has a DLT the dose level will accrue 6 subjects. If no further subject experiences a DLT the next dose level may be employed. If a second DLT is observed the next lowest dose level will be declared the MTD. In the instance that two DLTs occur in DL1 an optional DL0 may be opened to evaluate this lower cell dose. DL2 will accrue 6 patients regardless of whether a DLT occurs in the first three.

[00250] In the dose expansion portion of the study an additional 6 patients will be accrued at the MTD. If two subjects experience a DLT in this expansion cohort the recommended phase II dose level will be one dose level below the identified dose level in the escalation portion of the study. Subject enrollment spacing will be performed as outlined below.

[00251] *Participant Inclusion Criteria*

[00252] Subjects must have histologically confirmed acute myeloid leukemia with $\geq 5\%$ marrow blasts that meets any of the following criteria:

[00253] Refractory to at least two attempts at prior induction therapy. An attempt is defined as either a single cycle of intensive chemotherapy (e.g. cytarabine/anthracycline, high-dose cytarabine, FLAG, or other) OR a single monthly cycle of a hypomethylating

agent with venetoclax OR if a hypomethylating agent alone is used subjects must have either progression after 2 monthly cycles or persistent disease after 4 monthly cycles.

[00254] Patients with mutations in TP53 are eligible if they do not respond to a single initial cytoreduction attempt with a hypomethylating agent alone or in combination with venetoclax as defined above

[00255] First relapse of AML when relapse occurred within 6 months of achieving an initial complete remission.

[00256] Second or greater relapse following previous therapy.

[00257] Patients aged 18 through 70 years old are eligible.

[00258] Must have an available, haplotype mismatched related individual that meets criteria for cell donation according to the FACT guidelines.

[00259] Patients must have Karnofsky performance status $\geq 70\%$.

[00260] Adequate cardiac function as defined as a systolic LV ejection fraction $\geq 50\%$ at rest and absence of New York Heart Association stage III or IV congestive heart failure.

[00261] Adequate pulmonary function as defined as a resting SpO₂ $\geq 92\%$ on room air at rest.

[00262] Serum bilirubin ≤ 2 mg/dL

[00263] AST and ALT $\leq 2.5\times$ ULN unless thought to be disease related.

[00264] Estimated or measured creatinine clearance > 50 mL/min.

[00265] Subjects must be free from all systemic immune suppression for at least 4 weeks prior to the start of intended therapy.

[00266] *Participant Exclusion Criteria*

[00267] Prior allogeneic hematopoietic cell transplantation.

[00268] Subjects with active/uncontrolled CNS leukemia as defined by routine histopathologic evaluation of the CSF specimen.

[00269] Subjects requiring systemic immunosuppression for any indication are excluded. Glucocorticoid supplementation for hypoadrenalism is acceptable.

- [00270] Myocardial infarction or cerebrovascular accident within 6 months of planned therapy.
- [00271] Subjects with isolated extramedullary disease without evidence of bone marrow involvement by immunohistochemistry.
- [00272] Female patients who are pregnant or breast-feeding.
- [00273] Persons with an infection that is not responding to antimicrobial therapy.
- [00274] Persons who have detected HIV, Hepatitis B, or Hepatitis C blood PCR prior to study therapy.
- [00275] Persons who do not meet the age and organ function criteria specified above.
- [00276] Presence of psychiatric or neurologic disease, or lack of social support that limits the patient's ability to comply with the treatment protocol including supportive care, follow-up, and research tests.
- [00277] Concurrent active malignancy with the exception of non-melanomatous skin cancer.
- [00278] *Donor Inclusion Criteria*
- [00279] Donors must be eligible for apheresis according to standard FACT guidelines.
- [00280] Donors must not have an HLA genotype reactive against anti-HLA antibodies in the recipient.
- [00281] The following tests will be performed within 60 days prior to therapy:
- [00282] Evaluation for anti-HLA antibodies
- [00283] Electrocardiogram
- [00284] Echocardiogram
- [00285] The following tests will be performed within 30 days of the start of study therapy:
- [00286] CBC
- [00287] Complete metabolic panel
- [00288] TSH and free T4
- [00289] Lipase

- [00290] PT/PTT
- [00291] Urinalysis
- [00292] CT scan or PET/CT scan for individuals with a history of extramedullary AML
- [00293] Bone marrow biopsy with immunohistochemical enumeration of blast percentage.
- [00294] CT scan based restaging study if extramedullary disease is present.
- [00295] Complete history and physical examination.
- [00296] Pulse oximetry at rest
- [00297] Urine or blood pregnancy test for women of childbearing potential.
- [00298] Serum evaluation for seroconversion against cytomegalovirus, hepatitis B, hepatitis C, Epstein-Barr virus, toxoplasmosis, and HIV.
- [00299] *Regimen for Cytoreduction*
- [00300] All drugs are administered relative to the date of stem cell infusion (day 0).
- [00301] *Fludarabine administration*: Fludarabine is administered intravenously over 30 minutes.
- [00302] *Cyclophosphamide administration*: Subjects will receive standard supportive care for high-dose cyclophosphamide administration including adjuvant fluid hydration and MESNA administration according to MSKCC standard chemotherapy administration guidelines
- [00303] *Modification of chemotherapy dose based on adjusted body weight*: Patients who are >125% of ideal body weight will receive cyclophosphamide based on adjusted body weight. Fludarabine will be dosed based on actual body weight. Ideal and adjusted body weights are calculated as below:
- [00304] Ideal body weight (IBW) formula:
- [00305] Male IBW = 50 kg + 2.3 kg/inch over 60 inches
- [00306] Female IBW = 45.5 kg + 2.3 kg/inches over 60 inches
- [00307] Adjusted body weight (ABW) formula:

[00308] $ABW = IBW + (0.4 \times (\text{actual weight} - IBW))$

[00309] *Donor lymphapheresis:* Collection of peripheral blood mononuclear cells will be performed according to standard collection SOP outlined in the Adult BMT Service Guidelines.

[00310] *Infusion of atezolizumab:* Infusion of atezolizumab will be according to the FDA approved package insert as follows: Infusion time will be over 60 minutes through an intravenous line.

[00311] *Infusion of cytokine-induced memory-like NK cells:* CIMN infusion pretreatment: Prior to each T cell infusion, patients will be given acetaminophen and diphenhydramine (po or iv) to prevent infusion-related reactions. Substitutions/modifications to these pre-medications are permitted at the discretion of the treating physician.

[00312] *Tracking/labeling:* A standard operating procedure for product labeling and Verification of Patient Identity are established. Labels with appropriate identifiers (unique identification number) will be provided to the collection staff. In-processing labels and final product labels will have the same identifiers to assure product identity throughout production and administration. Confirmation of identity of product and intended patient by two parties will take place prior to administration by review of product labeling and patient identification. Product labeling includes unique patient identifiers, product name, date of manufacture, storage conditions, and expiration date/time, we well as collection date/time, product volume, identification and address of the processing lab.

[00313] *CIMN infusion:* CIMN cells will be infused based on institutional guidelines (approximately over 15-120 minutes based off final product formulation).

[00314] *Dose escalation and expansion plan and patient enrollment spacing:* Dose levels: The following dose levels will be employed. Infusion of CIMN cells is based on actual body weight of the recipient. The starting dose level is DL 1.

Dose level	CIMN Cells	Number of subjects
DL 0	$0.5 \times 10^5/\text{kg}$	3-6
DL 1	$1 \times 10^6/\text{kg}$	3-6
DL 2	Up to $1 \times 10^7/\text{kg}$	3-6

[00315] Dose escalation plan: The study will enroll subjects in a standard 3+3 format. If 1/3 subjects has a DLT the dose level will accrue 6 subjects. If no further subject experiences a DLT the next dose level may be employed. If a second DLT is observed the next lowest dose level will be declared the MTD. In the instance that two DLTs occur in DL1 an optional DL0 may be opened to evaluate this lower cell dose. DL2 will accrue 6 patients regardless of whether a DLT occurs in the first three.

[00316] Dose expansion: The study will accrue an additional 6 patients at the MTD. If two subjects experience a DLT in this expansion cohort the recommended phase II dose level will be one dose level below the identified dose level in the escalation portion of the study.

[00317] Enrollment spacing: The first three patients in a dose level may be enrolled without spacing between patients. If no patients develop a DLT the remaining patients may be enrolled (if required) again without spacing. If any patient develops a DLT a minimum of 28 days will pass between enrollment of the next patient until the dose level is complete.

[00318] A minimum of 30 days will pass between enrollment of the last patient on a lower dose cohort and the first patient in the next dose level.

[00319] The DLT monitoring period for each patient will be up to day 30 post CIMN cell infusion.

[00320] *Administration of recombinant human IL-2:* Recombinant human IL-2 (rh-IL2) will be administered according to the manufacturer guidelines as a subcutaneous injection on days 0, 2, 4, 6, 8, 10, 12. If a subject develops treatment emergent complications of rh-IL2 administration dose modification should proceed according to the manufacturer's guidelines.

[00321] *Management of immune related adverse events (irAE):* Immune related adverse events may arise from either administration of atezolizumab or GVHD related to CIMN. Management of irAE will follow standard guidelines or MSKCC Adult BMT Service guidelines for management of GVHD syndromes.³⁵ Grade 2 irAEs may be managed according to the prerogative of the treating clinician. Therapies may include topical glucocorticoid, budesonide, or low dose systemic glucocorticoid. Grade 3-4 irAEs will be treated with glucocorticoid at a starting dose of prednisone 1 mg/kg/d or equivalent.

[00322] *Management of Cytokine Release Syndrome (CRS):* Cytokine release syndrome is not described after administration of CIMN cells; however, is theoretically possible. CRS will be graded according to standard clinical criteria (Appendix 1).³⁶ If CRS occurs following infusion of CIMN cells intuitional guidelines will be used for management.

[00323] *Management of Infusion-Related Reactions:*

[00324] If a *grade 1* infusion-related reaction occurs, cell infusion may be continued at the same dose and rate of administration. Fever, chills, and rigors may be treated with acetaminophen, diphenhydramine, and meperidine as clinically indicated. Nausea and vomiting may be treated using previously established MSKCC guidelines.

[00325] If a *grade 2* infusion-related reaction occurs, treatment may be continued with a 50% reduction in the infusion rate of CIMN cells. Symptoms may be treated as above, as clinically indicated.

[00326] If a *grade 3* infusion-related reaction occurs, the infusion of CIMN cells will be interrupted. Symptomatic treatment, as outlined above with the addition of hydrocortisone or methylprednisolone as clinically indicated and with approval of the treating physician. The infusion can be resumed at a 50% reduction in the rate after resolution of symptoms. If grade 3 reactions recur after resumption of the infusion, CIMN cell infusion will be discontinued.

[00327] If a *grade 4* infusion-related toxicity recurs, CIMN cell infusion will be discontinued. Symptoms will be treated using the guidelines above, and no further CIMN cells will be administered.

[00328] *Routine clinical assessments on day 0:* The subject will undergo a history and physical examination prior to infusion of CIMN cells on day 0 in addition to routine blood work.

[00329] *Monitoring during day 1-14:* The patient will undergo a clinical examination and basic clinical laboratories (CBC, CMP) daily between days 0-7. The patient will undergo a clinical examination and basic clinical laboratories every other day between days 8-14. Follow up between days 15-28 will be twice weekly.

[00330] *Late monitoring and disease response assessment:* The study will require a single response assessment between days 28-45. Response assessment should occur after

the absolute neutrophil count increases to 1.0 K/mcL or greater AND the platelet count is > 100,000/mcL, or at day 38-45 if hematopoietic recovery has not occurred by day 38.

Assessment may occur earlier without hematopoietic recovery if the treating physician feels there is sufficient evidence of ongoing myeloid leukemia that requires subsequent therapy.

[00331] *Monitoring for persistence of donor CIMN cells:* CIMN cells may be identified readily using HLA-specific monoclonal antibodies in *most* donor recipient pairs (depending on the specific HLA genotypes). We will enumerate the fraction of allogeneic cells in the CD3⁺CD56⁺ PBMC fraction in surviving subjects at day 7, 14, 28, and 45 in the sub-cohort of individuals amenable to HLA-based multicolor flow cytometry. The peak fraction of CIMN cells will be used to determine the timing for the studies outlined below.

[00332] *Genomic interrogation of donor derived CIMN cells following infusion.*

[00333] Peripheral blood will be collected from all subjects at days 7 and 14 post infusion of CIMN cells. Each participant will give 2 x 10 mL green-top tubes at these collection points. Following collection, PBMC will be extracted and cryopreserved. Pre-infusion CIMN cells from the donor will be obtained by washing of the infusion bag/tubing and cryopreserved. Based on donor/recipient HLA genotypes, we will perform CITE-Seq based transcriptomic analysis of lymphocytes subsets on 3-6 subjects comparing donor lymphocytes at the single time point following infusion that correlates with peak CIMN blood concentration.

[00334] *Assessment for formation of donor-HLA specific antibodies following CIMN infusion.* Subjects will undergo assessment for anti-HLA antibodies at day 28-45 post CIMN infusion. New anti-HLA antibodies associated with donor HLA genotype with a mean fluorescence intensity > 1,000 will be considered an induced HLA antibody by the study therapeutic and recorded as an endpoint.

[00335] *Donor assessment prior to apheresis.* Donors will be required to complete a donor screen comparable to current FACT and Adult BMT Service standard guidelines. Standard tests for infectious disease will be performed, including assays for the detection of HIV and HCV (by nucleic acid testing), anti-HIV I/II, anti-HBc Ab, HBsAG, anti-HCV, anti-CMV and Treponema pallidum (by serology). If a test is positive, donor will be notified of the result, and the need for further testing will be determined through

consultation with the donor's physician. The above donor screening will be done prior to starting chemotherapy for the patient.

[00336] *Criteria for determination of a dose limiting toxicity*

[00337] A dose limiting toxicity is defined as any of the following, occurring within 30 days of infusion of CIMN cells:

[00338] Any non-hematologic AE grade 4-5 as per CTCAE v5.0 Any grade 3 non-hematologic AE as per CTCAE v5.0 that does not resolve to grade ≤ 2 within 7 days.

[00339] Any grade 3-4 seizure.

[00340] Any grade III-IV acute GvHD syndrome not responsive to glucocorticoid therapy. Non-response to glucocorticoid therapy is defined as either progression to a higher grade after 3 days of therapy or lack of a partial response after 7 days of therapy with prednisone 1 mg/kg/d or equivalent.

[00341] Grade 4 immune effector cell-associated neurotoxicity not resolving to grade ≤ 2 within 72 hours of institution of corticosteroid therapy.

[00342] Grade 3-4 CRS not improving to grade ≤ 2 within 48 hours of initiation of systemic corticosteroids as defined in Appendix 1.

[00343] *Criteria for Study Endpoint Evaluability*

[00344] Subjects that receive infusion of CIMN cells will be evaluated for the study endpoints. In dose level 2: If a subject receives $<10^6$ CIMN cells/kg recipient body weight the subject will continue to be followed for outcomes; however, we will replace this subject in the dosing cohort.

[00345] Subjects that do not receive the CIMN infusion will not be considered for study endpoints but will be monitored for survival and ongoing toxicity.

[00346] *Criteria for determination of secondary endpoints*

[00347] Determination of formation of donor-HLA specific anti-HLA antibodies is described herein.

[00348] Transcriptomic changes are a qualitative appraisal without a specific endpoint. We will use descriptive statistics as appropriate to study changes in the overall transcriptomic landscape of the infused cellular product.

[00349] Disease response assessments will be according to the ELN2017 criteria.²

Specific response criteria are given below:

[00350] Complete remission: Bone marrow blasts <5%; absence of circulating blasts and blasts with Auer rods; absence of extramedullary disease; ANC $\geq 1.0 \times 10^9/\text{L}$ (1000/ μL); platelet count $\geq 100 \times 10^9/\text{L}$ (100 000/ μL).

[00351] CR with incomplete hematologic recovery (CRi): All CR criteria except for residual neutropenia ($< 1.0 \times 10^9/\text{L}$ [1000/ μL]) or thrombocytopenia ($< 100 \times 10^9/\text{L}$ [100 000/ μL]).

[00352] Morphologic leukemia-free state (MLFS): Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required. Marrow should not merely be “aplastic”; at least 200 cells should be enumerated, or cellularity should be at least 10%.

[00353] Partial response: All hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%.

[00354] Stable disease: Absence of CR_{MRD}-, CR, CRi, PR, MLFS; and criteria for PD not met. Period of stable disease should last at least 3 mo.

[00355] Progressive disease: Evidence for an increase in bone marrow blast percentage and/or increase of absolute blast counts in the blood:

[00356] >50% increase in marrow blasts over baseline (a minimum 15% point increase is required in cases with <30% blasts at baseline; or persistent marrow blast percentage of >70% over at least 3 months; without at least a 100% improvement in ANC to an absolute level ($> 0.5 \times 10^9/\text{L}$ [500/ μL], and/or platelet count to $> 50 \times 10^9/\text{L}$ [50 000/ μL] non-transfused).

[00357] >50% increase in peripheral blasts (WBC $\times$ % blasts) to $> 25 \times 10^9/\text{L}$ (> 25 000/ μL) (in the absence of differentiation syndrome).

[00358] New extramedullary disease.

[00359] Definition for survival, event-free survival, and cumulative incidence of relapse

[00360] Overall survival: measured from the date of cell infusion to the date of death from any cause; patients not known to have died at last follow-up are censored on the date they were last known to be alive.

[00361] Event-free survival: measured from the date of cell infusion to the date of primary refractory disease, or relapse from CR, or CR_i, or death from any cause; patients not known to have any of these events are censored on the date they were last examined.

[00362] Defined for all patients achieving CR, CR_i; measured from the date of achievement of a remission until the date of relapse; patients not known to have relapsed are censored on the date they were last examined; patients who died without relapse are counted as a competing cause of failure.

[00363] *Grading of acute GVHD and Cytokine Release Syndrome*

[00364] Grading of CRS will be according to Lee et al.³⁶ The specific grading system is outlined in Appendix 1.

Appendix 1: ASTCT Cytokine Release Syndrome Consensus Grading

CRS Parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever*	Temperature $\geq 38^{\circ}\text{C}$	Temperature $\geq 38^{\circ}\text{C}$	Temperature $\geq 38^{\circ}\text{C}$	Temperature $\geq 38^{\circ}\text{C}$
With				
Hypotension	None	Not requiring vasopressors	Requiring a vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
And/or				
Hypoxia	None	Requiring low-flow nasal	Requiring high-flow nasal cannula, facemask,	Requiring positive pressure (e.g., CPAP,

CRS Parameter	Grade 1	Grade 2	Grade 3	Grade 4
		cannula or blow-by	nonrebreather mask, or Venturi mask	BiPAP, intubation and mechanical ventilation)

[00365] Grading for acute GVHD will be according to the Glucksberg criteria.³⁷ The specific grading system is outlined in the Appendix 2.

Appendix 2: Acute GVHD Grading Criteria

Stage	Skin	Liver	Intestinal tract ¹
1	Maculopapular rash < 25% of body surface	Bilirubin 34-50 mmol/L	> 500 mL diarrhea/day
2	Maculopapular rash 25 -50% of body surface	Bilirubin 51-102 mmol/L	> 1000 mL diarrhea/day
3	Maculopapular rash >50% of body surface	Bilirubin 103-225 mmol/L	> 1500 mL diarrhea/day
4	Maculopapular rash with bullous formation and desquamation	Bilirubin > 255 mmol/L	Severe abdominal pain ± ileus

[00366] *Criteria for stopping of the study*

[00367] If 2 or more DLTs occur in dose level 1 the study will de-escalate to dose level 0. If 2 or more DLTs occur in dose level zero, the intervention will be declared not promising and the study will be terminated.

[00368] *DLT-Evaluable Population:*

[00369] DLT-evaluable participants are defined as those participants who were infused with CIMN cells and who were monitored for toxicities during the first 30 days of post infusion. Participant removed from the study due to a DLT within first 30 days will be included to DLT-Evaluable Population as the primary endpoint has been met. A participant removed from the study treatment due to toxicity at least possibly related to the cell product that does not meet the DLT definition will be considered as a DLT. Although we anticipate achieving the desired CIMN dose through our proposed manufacturing process for most patient samples, in cases where less than 50% of the planned CIMN dose is generated, the

patient will still receive the infusion. However, for the escalation phase of the study, the following scenarios will be considered for DLT evaluability and replacement of these patients:

[00370] 1. If the patient does not have a DLT during the DLT evaluation window, this patient will be considered as inevaluable and replaced.

[00371] 2. If the patient does have a DLT during the DLT evaluation window, this patient will be considered as having a DLT at the current dose level studied (i.e. DLT-Evaluable).

[00372] Any patient that is not included in the DLT-evaluable population will be replaced in the dose escalation part of the study.

[00373] *Primary Objective:*

[00374] This is a phase 1 dose-escalation study designed to determine the recommended phase II dose (RP2D) of CIMN cells in patients with relapsed or refractory AML. The RP2D will be identified at or below the established maximum tolerated dose (MTD). Dose escalation will use a 3+3 design.

[00375] The MTD is defined as the highest dose with an observed incidence of DLT in no more than one out of six patients treated at a particular dose level. The definition of DLT is defined in section 13.1.

[00376] All patients treated in a cohort will be observed a minimum of 30 days before the CIMN dose can be escalated. The anticipated trial size for the escalation component of this study is 9 to 12 patients, with 1 patient every month to be entered onto the study. The 3+3 dose-escalation algorithm is as follows:

[00377] If none of the initial 3 patients in a cohort experienced a dose-limiting toxicity (DLT), then the next dose level will be studied in another cohort of 3 patients.

[00378] If 1 of the initial 3 patients at a given dose level experience a DLT, up to 3 additional patients will be treated at that same dose level. Escalation will continue if not more than 1 of the 6 patients experience a DLT.

[00379] If 2 or more patients experience a DLT at a given dose level, the MTD will have been exceeded.

[00380] If 3 or fewer patients are treated at a dose under consideration as the MTD, additional patients to a total of 6 will be treated at that level to confirm the MTD.

[00381] Escalation to the next dose level is probable if the risk of DLT is low, and the likelihood of escalation decreases as the risk of DLT increases.

[00382]	True Risk of Toxicity	.10	.20	.30	.40	.50
		.60				

[00383]	Probability of Escalation	.91	.71	.49	.31	.17
		.08				

[00384] *Secondary Objectives:*

[00385] To further characterize adverse events including non-DLT events following CIMN cells with atezolizumab at the MTD.

[00386] To estimate the duration of CIMN *in vivo* persistence.

[00387] To determine transcriptomic changes in CIMN cells after infusion with PD-L1 blockade.

[00388] To characterize the incidence of formation of donor-HLA specific anti-HLA antibodies in recipients.

EQUIVALENTS

[00389] The present technology is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the present technology. Many modifications and variations of this present technology can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatuses within the scope of the present technology, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing descriptions. Such modifications and variations are intended to fall within the scope of the present technology. It is to be understood that this present technology is not limited to particular methods, reagents, compounds compositions or biological systems, which can, 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.

[00390] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[00391] As will be understood by one skilled in the art, for any and all purposes, particularly in terms of providing a written description, all ranges disclosed herein also encompass any and all possible subranges and combinations of subranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, *etc.* As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, *etc.* As will also be understood by one skilled in the art all language such as “up to,” “at least,” “greater than,” “less than,” and the like, include the number recited and refer to ranges which can be subsequently broken down into subranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 cells refers to groups having 1, 2, or 3 cells. Similarly, a group having 1-5 cells refers to groups having 1, 2, 3, 4, or 5 cells, and so forth.

[00392] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

REFERENCES

1. Döhner H, Weisdorf DJ, Bloomfield CD: Acute Myeloid Leukemia. New England Journal of Medicine 373:1136-1152, 2015
2. Döhner H, Estey E, Grimwade D, et al: Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 129:424-447, 2017
3. Vasu S, Kohlschmidt J, Mrózek K, et al: Ten-year outcome of patients with acute myeloid leukemia not treated with allogeneic transplantation in first complete remission. Blood Advances 2:1645-1650, 2018

4. DiNardo CD, Jonas BA, Pullarkat V, et al: Azacitidine and Venetoclax in Previously Untreated Acute Myeloid Leukemia. *New England Journal of Medicine* 383:617-629, 2020
5. Forman SJ, Rowe JM: The myth of the second remission of acute leukemia in the adult. *Blood* 121:1077-1082, 2013
6. Herberman RB, Nunn ME, Lavrin DH: Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic acid allogeneic tumors. I. Distribution of reactivity and specificity. *Int J Cancer* 16:216-29, 1975
7. Herberman RB, Nunn ME, Holden HT, et al: Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. *Int J Cancer* 16:230-9, 1975
8. Kiessling R, Klein E, Pross H, et al: "Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. *Eur J Immunol* 5:117-21, 1975
9. Gleimer M, Parham P: Stress Management: MHC Class I and Class I-like Molecules as Reporters of Cellular Stress. *Immunity* 19:469-477, 2003
10. Bauer S, Groh V, Wu J, et al: Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* 285:727-9, 1999
11. Cerwenka A, Baron JL, Lanier LL: Ectopic expression of retinoic acid early inducible-1 gene (RAE-1) permits natural killer cell-mediated rejection of a MHC class I-bearing tumor in vivo. *Proc Natl Acad Sci U S A* 98:11521-6, 2001
12. Ruggeri L, Mancusi A, Capanni M, et al: Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value. *Blood* 110:433-40, 2007
13. Ruggeri L, Capanni M, Urbani E, et al: Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* 295:2097-100, 2002
14. Ruggeri L, Capanni M, Casucci M, et al: Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. *Blood* 94:333-9, 1999

15. Mancusi A, Ruggeri L, Urbani E, et al: Haploidentical hematopoietic transplantation from KIR ligand–mismatched donors with activating KIRs reduces nonrelapse mortality. *Blood* 125:3173-3182, 2015
16. Bachanova V, Cooley S, Defor TE, et al: Clearance of acute myeloid leukemia by haploidentical natural killer cells is improved using IL-2 diphtheria toxin fusion protein, 2014
17. Lee DA, Denman CJ, Rondon G, et al: Haploidentical Natural Killer Cells Infused before Allogeneic Stem Cell Transplantation for Myeloid Malignancies: A Phase I Trial. *Biol Blood Marrow Transplant* 22:1290-1298, 2016
18. Miller JS, Soignier Y, Panoskaltsis-Mortari A, et al: Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. *Blood* 105:3051-7, 2005
19. Shaffer BC, Le Luque J-B, Forlenza C, et al: Phase II Study of Haploidentical Natural Killer Cell Infusion for Treatment of Relapsed or Persistent Myeloid Malignancies Following Allogeneic Hematopoietic Cell Transplantation. *Biology of Blood and Marrow Transplantation* 22:705-709, 2016
20. Hammer Q, Rückert T, Borst EM, et al: Peptide-specific recognition of human cytomegalovirus strains controls adaptive natural killer cells. *Nature Immunology* 19:453-463, 2018
21. Cooper MA, Elliott JM, Keyel PA, et al: Cytokine-induced memory-like natural killer cells. *Proc Natl Acad Sci U S A* 106:1915-9, 2009
22. Merino A, Dougherty P, Zhang B, et al: Adaptive NK Cells Are Expanded By NKG2C-Agonist Antibodies and Retain Their Cytotoxic and Secretory Properties. *Blood* 132:3695-3695, 2018
23. Romee R, Rosario M, Berrien-Elliott MM, et al: Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. *Science translational medicine* 8:357ra123-357ra123, 2016
24. Keppel MP, Yang L, Cooper MA: Murine NK cell intrinsic cytokine-induced memory-like responses are maintained following homeostatic proliferation. *J Immunol* 190:4754-62, 2013

25. Bednarski JJ, Zimmerman C, Berrien-Elliott MM, et al: Donor Memory-like NK cells Persist and Induce Remissions in Pediatric Patients with Relapsed AML after Transplant. *Blood*, 2021
26. Shallis RM, Bewersdorf JP, Gowda L, et al: Immune Checkpoint Inhibitor Therapy for Acute Myeloid Leukemia and Higher-Risk Myelodysplastic Syndromes: A Single-Center Experience. *Blood* 134:1330-1330, 2019
27. Davids MS, Kim HT, Bachireddy P, et al: Ipilimumab for Patients with Relapse after Allogeneic Transplantation. *New England Journal of Medicine* 375:143-153, 2016
28. Martins F, Sofiya L, Sykietis GP, et al: Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance. *Nature Reviews Clinical Oncology* 16:563-580, 2019
29. Herbst RS, Giaccone G, de Marinis F, et al: Atezolizumab for First-Line Treatment of PD-L1–Selected Patients with NSCLC. *New England Journal of Medicine* 383:1328-1339, 2020
30. Herbst RS, Soria JC, Kowanetz M, et al: Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 515:563-7, 2014
31. Rittmeyer A, Barlesi F, Waterkamp D, et al: Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet* 389:255-265, 2017
32. Dong W, Wu X, Ma S, et al: The Mechanism of Anti–PD-L1 Antibody Efficacy against PD-L1–Negative Tumors Identifies NK Cells Expressing PD-L1 as a Cytolytic Effector. *Cancer Discovery* 9:1422, 2019
33. Adusumilli PS, Zauderer MG, Rusch VW, et al: Abstract CT036: A phase I clinical trial of malignant pleural disease treated with regionally delivered autologous mesothelin-targeted CAR T cells: Safety and efficacy. *Cancer Research* 79:CT036-CT036, 2019
34. Chong EA, Melenhorst JJ, Lacey SF, et al: PD-1 blockade modulates chimeric antigen receptor (CAR)–modified T cells: refueling the CAR. *Blood* 129:1039-1041, 2017

35. Brahmer JR, Abu-Sbeih H, Ascierto PA, et al: Society for Immunotherapy of Cancer (SITC) clinical practice guideline on immune checkpoint inhibitor-related adverse events. *Journal for ImmunoTherapy of Cancer* 9:e002435, 2021

36. Lee DW, Santomasso BD, Locke FL, et al: ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. *Biology of Blood and Marrow Transplantation* 25:625-638, 2019

37. Rowlings PA, Przepiorka D, Klein JP, et al: IBMTR Severity Index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade. *Br J Haematol* 97:855-64, 1997

WHAT IS CLAIMED IS

1. A method for treating acute myelogenous leukemia (AML) in a subject in need thereof comprising administering to the subject an effective amount of an anti-PD-L1 antibody or antigen binding fragment thereof, and an effective amount of cytokine induced memory-like natural killer (CIMN) cells.
2. The method of claim 1, wherein the AML is relapsed or refractory AML.
3. The method of claim 1 or 2, wherein the anti-PD-L1 antibody or antigen binding fragment comprises a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 4, a V_H-CDR2 sequence of SEQ ID NO: 6, and a V_H-CDR3 sequence of SEQ ID NO: 7 and the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 9, a V_L-CDR2 sequence of SEQ ID NO: 11, and a V_L-CDR3 sequence of SEQ ID NO: 13.
4. The method of claim 3, wherein the V_H comprises the amino acid sequence of SEQ ID NO: 1, and/or the V_L comprises the amino acid sequence of SEQ ID NO: 2.
5. The method of claim 1 or 2, wherein the anti-PD-L1 antibody or antigen binding fragment comprises a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 17, a V_H-CDR2 sequence of SEQ ID NO: 19, and a V_H-CDR3 sequence of SEQ ID NO: 21 and the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 23, a V_L-CDR2 sequence of SEQ ID NO: 25, and a V_L-CDR3 sequence of SEQ ID NO: 27.
6. The method of claim 5, wherein the V_H comprises the amino acid sequence of SEQ ID NO: 14, and/or and the V_L comprises the amino acid sequence of SEQ ID NO: 15.
7. The method of claim 1 or 2, wherein the anti-PD-L1 antibody or antigen binding fragment comprises a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 30, a V_H-CDR2 sequence of SEQ ID NO: 31, and a V_H-CDR3 sequence of SEQ ID NO: 32 and the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 33, a V_L-CDR2 sequence of SEQ ID NO: 34, and a V_L-CDR3 sequence of SEQ ID NO: 35.

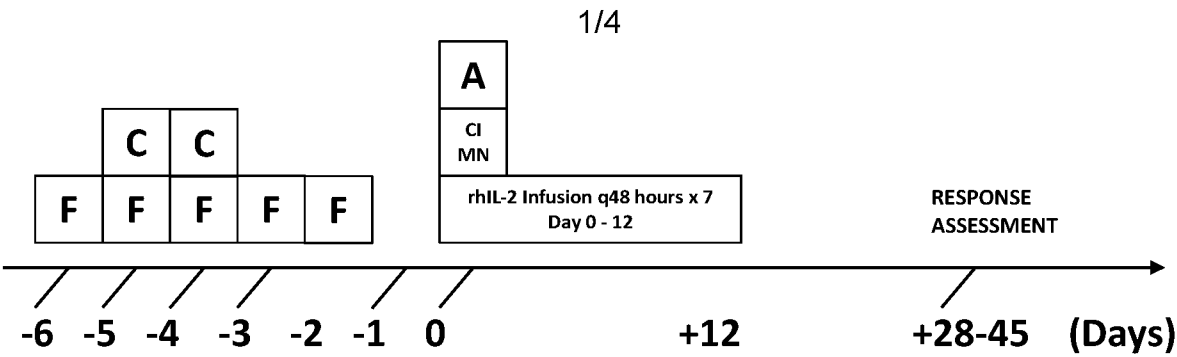
8. The method of claim 7, wherein the V_H comprises the amino acid sequence of SEQ ID NO: 28; and/or the V_L comprises the amino acid sequence of SEQ ID NO: 29.
9. The method of any one of claims 1-8, wherein the CIMN cells are derived from CD3⁺CD56⁺ NK cells
10. The method of any one of claims 1-9, wherein the CIMN cells are generated by stimulating donor NK cells with at least one cytokine for about 12-18 hours.
11. The method of claim 10, wherein the at least one cytokine comprises IL-12, IL-15, and/or IL-18.
12. The method of any one of claims 1-11, wherein the CIMN cells are autologous or obtained from a haploidentical donor.
13. The method of any one of claims 1-12, wherein the CIMN cells further comprises a chimeric antigen receptor (CAR) that specifically binds an AML-specific tumor antigen.
14. The method of claim 13, wherein the CAR comprises (i) an extracellular antigen binding domain; (ii) a transmembrane domain; and (iii) an intracellular domain comprising one or more co-stimulatory domains, wherein the extracellular antigen binding domain binds to the AML-specific tumor antigen.
15. The method of claim 14, wherein the transmembrane domain of the CAR comprises a CD8 transmembrane domain, a CD28 transmembrane domain, a NKG2D transmembrane domain, a CD3 ζ transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, or a BTLA transmembrane domain.
16. The method of any one of claims 14-15, wherein the one or more co-stimulatory domains of the CAR are selected from the group consisting of a CD28 co-stimulatory domain, a 4-1BB co-stimulatory domain, an OX40 co-stimulatory domain, an ICOS co-stimulatory domain, a DAP-10 co-stimulatory domain, a PD-1 co-stimulatory domain, a CTLA-4 co-stimulatory domain, a LAG-3 co-stimulatory domain, a 2B4 co-stimulatory domain, a BTLA co-stimulatory domain, a NKG2C co-stimulatory domain, a NKG2D co-stimulatory domain, and any combination thereof.

17. The method of any one of claims 1-16, wherein the CIMN cells are administered pleurally, intravenously, subcutaneously, intranodally, intratumorally, intrathecally, intrapleurally or intraperitoneally.

18. The method of any one of claims 1-17, wherein the subject receives an infusion of the CIMN cells in two escalating dose levels.

19. The method of any one of claims 1-18, wherein the anti-PD-L1 antibody or antigen binding fragment is administered pleurally, intravenously, subcutaneously, intranodally, intratumorally, intrathecally, intrapleurally or intraperitoneally.

20. The method of any one of claims 1-19, wherein administration of the CIMN cells and the anti-PD-L1 antibody or antigen binding fragment prevents Graft versus host disease (GvHD) in the subject.



C = Cyclophosphamide 50 mg/kg
F = Fludarabine 25 mg/m²
A = Atezolizumab 1,200 mg flat dose
CIMN = Cytokine induced memory NK cells
rhIL-2 = recombinant human IL-2, 1 million IU SQ

Optional dose level 0 = 0.5 x 10⁶ CIMN cells/recipient kg
Dose level 1 = 1 x 10⁶ CIMN cells/recipient kg
Dose level 2 = Up to 1 x 10⁷ CIMN cells/recipient kg

FIG. 1

Donor Cell Dose and KIR Gene Profile

Patient	CD3 ⁺ CD56 ⁺ /kg × 10 ⁵	CD3 ⁺ /kg × 10 ⁵	Viability, %	CD3 ⁺ CD56 ⁺ , %	CD3 ⁺ CD56 ⁺ , Yield %	Donor KIR Haplotype	Recipient Missing Ligand
1	8.2	1.9	93	98.5	64.4	B,B	None
2	4.3	0.9	82	97.8	59.9	A,B	Bw4
3	5.77	0.58	84	98.9	40.2	B,B	C2
4	1.2	7.2	92	99.7	88.8	A,B	Bw4, C2 [*]
5	30	3.3	89	99.8	73.0	A,B	None
6	19.9	40	88	99.9	41.3	A,A	Bw4, C2 [*]
7	22.4	2.9	100	96.7	50.9	A,B	None
8	5.36	2.2	88	98.5	41.7	A,B	Bw4

* Ligand(s) expressed in donor.

FIG. 2A

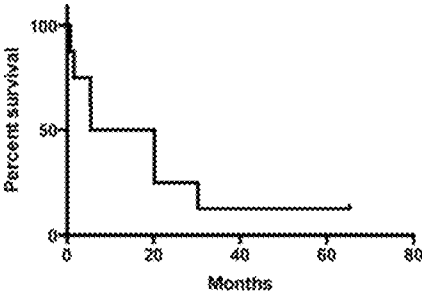


FIG. 2B

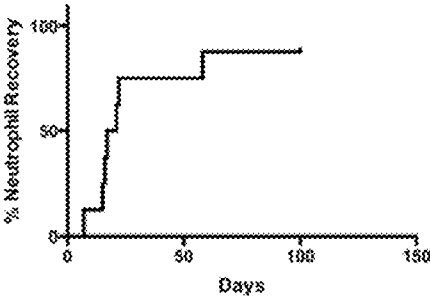


FIG. 2C

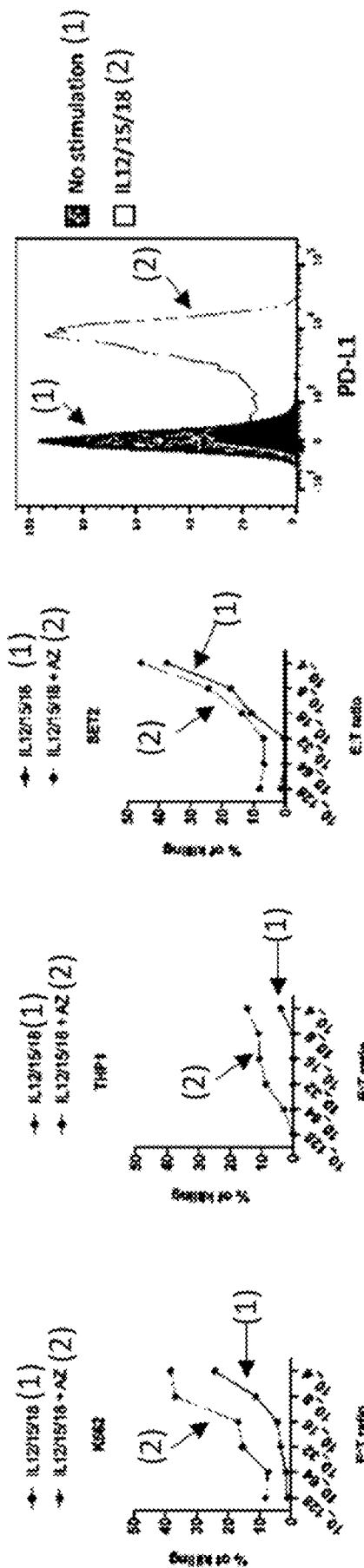


FIG. 3A

FIG. 3C

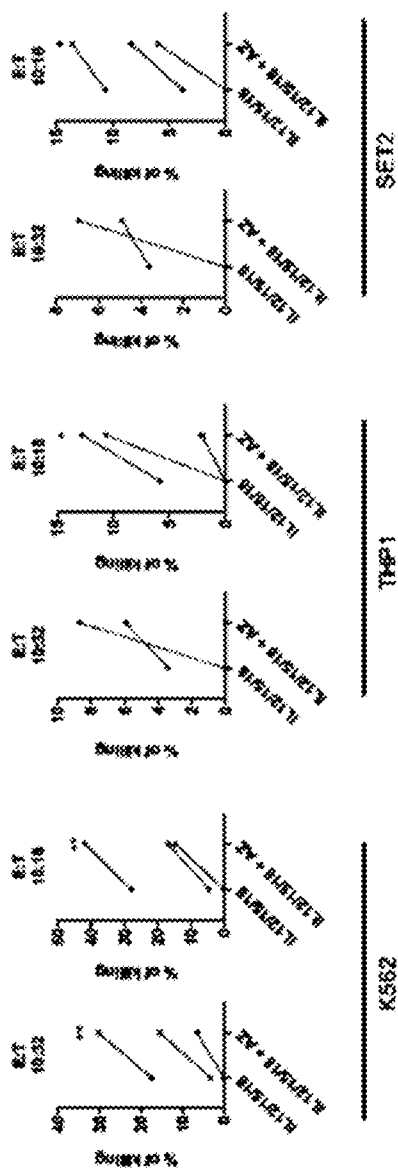


FIG. 3B

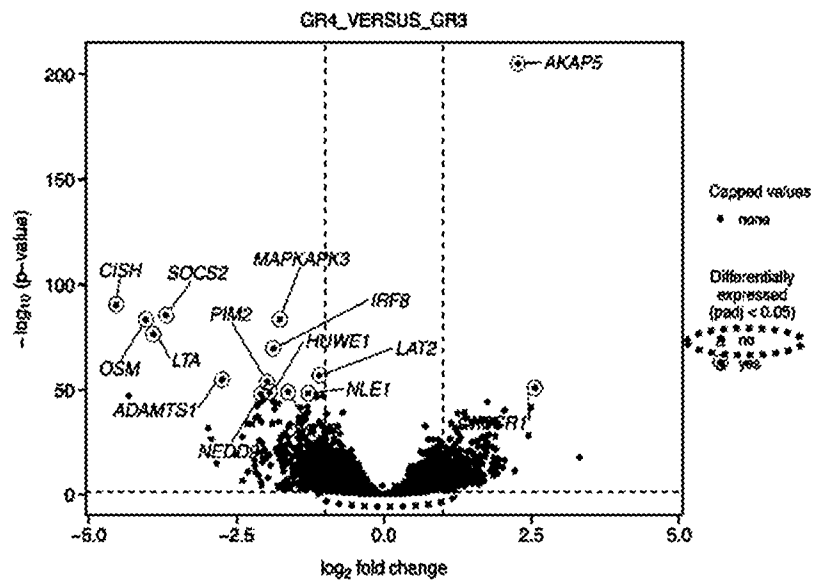


FIG. 4A

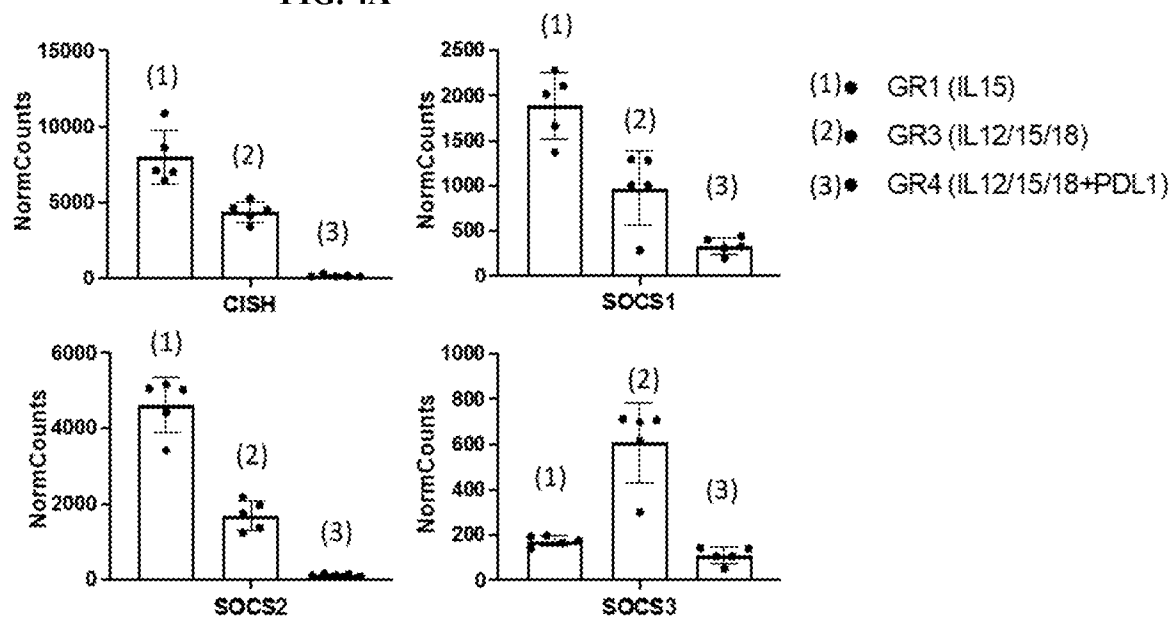


FIG. 4B