(PROLEUKIN™). US patent 4,604,377 teaches how to prepared a pharmaceutical composition of recombinant IL-2 (including aldesleukin) consistent in a sterile, stable lyophilized formulation in which the recombinant IL-2 is admixed with a water soluble carrier such as mannitol that provides bulk, and sufficient amount of sodium dodecyl sulfate to ensure the solubility of the recombinant IL-2 in water. The formulation is suitable for reconstitution in aqueous injections for parenteral administration stable and well tolerated in human patients. On the other hand, a detailed description of the process by which the PROLEUKIN™ product is prepared has been more recently disclosed in the European patent EP 1,688,146 B.

SUMMARY

Accordingly, certain examples of the present disclosure describe methods and compositions concerning the isolation and effective stimulation of cells as well as methods for the use of these cells in the treatment and/or prevention of immune-mediated diseases, cancer, viral infections, or infections mediated by pathogens, neurological disorders and the like.

In certain examples, a method to prepare aldesleukin highly stable in liquid pharmaceutical compositions for parenteral administration to a patient in need of an IL-2 immunotherapy, comprising, in general, the following steps: a) Fermentation of an *E.coli* strain transfected with an expression vector engineered to highly express the aldesleukin gene, b) Bacterial disruption, c) Collection of Inclusion bodies containing aldesleukin aggregates, d) Dissolution of the aldesleukin aggregates using SDS detergent, e) Oxidation with an oxidizing compound e.g. cupric chloride, f) Ceramic hydroxyapatite chromatography, g) Dilution with an organic nitrile, e.g. acetonitrile, h) C4 column HPLC chromatography, i) Diafiltration and j) Filter sterilization.

In certain examples, a method of treating an autoimmune disease or disorder in a subject in need thereof, comprises: administering to a subject in need thereof, a pharmaceutical composition comprising a therapeutically effective amount of aldesleukin wherein the regulatory T cells increase as compared to a control, inducing proliferation of regulatory T cells in vivo. In certain examples, the aldesleukin remains stable in the pharmaceutical liquid composition for at least a year as measured by an in vitro biological activity assay. In certain examples, the aldesleukin comprises a stable aldesleukin/SDS aggregate distribution in the liquid composition at least for a year in a range of about 4 - 18 nm with a peak at about 8 nm as measured by dynamic light scattering. In certain examples, the aldesleukin has a stable therapeutic activity, at least for a year in the liquid composition, as measured in an animal model of human cancer.

In another aspect, the present disclosure describes a use of (i) a pharmaceutical liquid composition comprising IL-2 in a range from 0.001 mg to 3 mg/mL, sodium dodecyl sulfate (SDS) from 0.0001 mg/ml to 10 mg/mL and at least one non-ionic osmolyte; (ii) an expression vector encoding a therapeutic protein; and (iii) one or more mobilization factors selected from the group consisting of chemokines, G-CSF, granulocyte macrophage colony stimulating factor (GM-CSF), AMD3100 and SCF in the manufacture of a medicament for treating an immune deficiency in a subject in need thereof, wherein the subject is to be administered a therapeutically effective amount of the pharmaceutical liquid composition, the expression vector and the one or more mobilization factors to thereby restore T-cell mediated immune responses in the subject in need thereof, wherein the pharmaceutical liquid composition is produced by a method comprising: fermenting a bacterial cell transfected with an expression vector encoding an IL-2 gene; disrupting the bacterial cell; collecting inclusion bodies containing IL-2; subjecting the inclusion bodies to dissolution by a detergent followed by oxidation and then a first chromatography; diluting with an organic nitrile and subjecting to a second chromatography; subjecting the IL-2 to diafiltration and sterilization, thereby producing IL-2 in a pharmaceutical aqueous vehicle, wherein the method does not comprise lyophilization, wherein the immune deficiency is Autoimmune Lymphoproliferative Syndrome (ALPS), APS-1 (APECED), CARD9, Chronic Granulomatous Disease (CGD), Congenital Neutropenia Syndromes, Common Variable Immunodeficiency (CVID), CTLA4 Deficiency, DOCK8 Deficiency, Glycosylation Disorders with Immunodeficiency, Hyper-Immunoglobulin E Syndromes (HIES), PI3 Kinase Disease, PLAID, Severe Combined Immunodeficiency (SCID), STAT3 Dominant-Negative Disease, WHIM Syndrome, X-Linked Agammaglobulinemia (XLA), X-Linked Lymphoproliferative Disease (XLP), Wiscott-Aldrich syndrome, DiGeorge syndrome, Ataxia-telangectasia, Chronic granulomatous disease, Transient hypogammaglobulinemia of infancy, Agammaglobulinemia, Complement deficiencies, or Selective IgA deficiency.

In certain examples, a method of restoring T-cell mediated immune responses in a subject in need thereof comprising administering a therapeutically effective amount of (i) a pharmaceutical composition comprising IL-2 in a range from about 0.001 mg to 3 mg/mL, sodium dodecyl sulfate (SDS) from about 0.0001 mg/ml to about 10 mg/mL, a non-ionic osmolytes(s), or combinations thereof (b) an expression vector encoding a therapeutic protein; (ii) one or more mobilization factors, thereby restoring T-cell mediated immune responses in the subject in need thereof. In certain examples, the IL-2 comprises SEQ ID NO: 1, SEQ ID NO: 2, a combination thereof, or variants thereof. In certain examples, the osmolytes comprise mannitol, sorbitol, xylitol or a pH 7-8 phosphate buffer.

In certain examples, a method of treating a subject suffering from an immune deficiency disease comprising administering to the subject:(i) an expression vector encoding SEQ ID NO: 1; and/or, (ii) a cell comprising an expression vector encoding SEQ ID NO: 1; and/or, (iii) a pharmaceutical composition comprising IL-2 in a range from about 0.001 mg to 3 mg/mL, sodium dodecyl sulfate (SDS) from about 0.0001 mg/ml to about 10 mg/mL, a non-ionic osmolytes(s), or combinations thereof.

In another aspect, the present disclosure describes a use of a cell comprising an expression vector encoding SEQ ID NO: 1, in the manufacture of a medicament for treating cancer, an immune deficiency, immunosuppression, a viral infection, an immune disorder or combinations thereof, wherein the cell is an autologous cell, an allogeneic cell, a haplotype matched cell, a haplotype mismatched cell, a haplo-identical cell, a xenogeneic cell, a cell line; or wherein the cell is a stem cell, a cord blood cell, an adult stem cell, a mesenchymal stem cell, a mesenchymal stromal cell, an induced pluripotent stem cell, an autologous stem cell, a bone marrow cell, a hematopoietic cell, a hematopoietic stem cell, a somatic cell, a germ line cell, a differentiated cell, a somatic stem cell, or an embryonic stem cell, wherein the cancer is carcinoma, lymphoma, blastoma, sarcoma, leukemia, squamous cell cancer, lung cancer, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, head and neck cancer, melanoma B-cell lymphoma, chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), hairy cell leukemia; chronic myeloblastic leukemia; post-transplant lymphoproliferative disorder (PTLD), abnormal vascular proliferation associated with phakomatoses, edema associated with brain tumors, Meigs' syndrome, bone cancer, brain cancer, esophageal cancer, or eye cancer, wherein the immune deficiency is selected from the group consisting of: Autoimmune Lymphoproliferative Syndrome (ALPS), APS-1 (APECED), CARD9, Chronic Granulomatous Disease (CGD), Congenital Neutropenia Syndromes, Common Variable Immunodeficiency (CVID), CTLA4 Deficiency, DOCK8 Deficiency, Glycosylation Disorders with Immunodeficiency, Hyper-Immunoglobulin E Syndromes (HIES), PI3 Kinase Disease, PLAID, Severe Combined Immunodeficiency (SCID), STAT3 Dominant-Negative Disease, WHIM Syndrome, X-Linked Agammaglobulinemia (XLA), X-Linked Lymphoproliferative Disease (XLP), Wiscott-Aldrich syndrome, DiGeorge syndrome, Ataxia-telangectasia, Chronic granulomatous disease, Transient hypogammaglobulinemia of infancy, Agammaglobulinemia, complement deficiencies, or Selective IgA deficiency, and wherein the immune disorder is inflammation, graft versus host disease (GVHD), transplant rejection, an autoimmune disorder, multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, type I diabetes, systemic lupus erythrematosus, contact hypersensitivity, asthma or Sjogren's syndrome.

In certain examples, a method of treating a subject in need of immunotherapy, comprising: administering to the subject: (i) an expression vector encoding SEQ ID NO: 1; and/or, (ii) a cell comprising an expression vector encoding SEQ ID NO: 1, thereby treating the subject. In certain examples, the subject is suffering from cancer, immunodeficiency, immunosuppression, viral infections, an immune disorder or combinations thereof. In certain examples, the immune disorder, inflammation, graft versus host disease (GVHD), transplant rejection, or an autoimmune disorder. In certain examples, the immune disorder is multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, type I diabetes, systemic lupus erythrematosus, contact hypersensitivity, asthma or Sjogren's syndrome. In certain examples, the method further comprises administering IL-2.

In certain examples, the cells comprise: autologous cells, allogeneic cells, haplotype matched cells, haplotype mismatched cells, haplo-identical cells, xenogeneic cells, cell lines or combinations thereof. In certain examples, the cells comprise stem cells, cord blood cells, adult stem cells, mesenchymal stem cells, mesenchymal stromal cells, induced pluripotent stem cells, autologous stem cells, bone marrow cells, hematopoietic cells, hematopoietic stem cells, somatic cells, germ line cells, differentiated cells, somatic stem cells, embryonic stem cells or combinations thereof.

The IL-2 molecules herein include IL-2 variants of the present disclosure comprising an amino acid sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least

What is claimed,

1. Use of:

(i) a pharmaceutical liquid composition comprising IL-2 in a range from 0.001 mg to 3 mg/mL, sodium dodecyl sulfate (SDS) from 0.0001 mg/ml to 10 mg/mL, and at least one non-ionic osmolyte;

(ii) an expression vector encoding a therapeutic protein; and

(iii) one or more mobilization factors selected from the group consisting of chemokines, G-CSF, granulocyte macrophage colony stimulating factor (GM-CSF), AMD3100 and SCF

in the manufacture of a medicament for treating an immune deficiency in a subject in need thereof, wherein the subject is to be administered a therapeutically effective amount of the pharmaceutical liquid composition, the expression vector and the one or more mobilization factors to thereby restore T-cell mediated immune responses in the subject in need thereof

wherein the pharmaceutical liquid composition is produced by a method comprising:

fermenting a bacterial cell transfected with an expression vector encoding an IL-2 gene;

disrupting the bacterial cell;

collecting inclusion bodies containing IL-2;

subjecting the inclusion bodies to dissolution by a detergent followed by oxidation and then a first chromatography;

diluting with an organic nitrile and subjecting to a second chromatography;

subjecting the IL-2 to diafiltration and sterilization, thereby producing IL-2 in a pharmaceutical aqueous vehicle, wherein the method does not comprise lyophilization,

wherein the immune deficiency is Autoimmune Lymphoproliferative Syndrome (ALPS), APS-1 (APECED), CARD9, Chronic Granulomatous Disease (CGD), Congenital Neutropenia Syndromes, Common Variable Immunodeficiency (CVID), CTLA4 Deficiency, DOCK8 Deficiency, Glycosylation Disorders with Immunodeficiency, Hyper-Immunoglobulin E Syndromes (HIES), PI3 Kinase Disease, PLAID, Severe Combined Immunodeficiency (SCID), STAT3 Dominant-Negative Disease, WHIM Syndrome, X-Linked Agammaglobulinemia (XLA), X-Linked Lymphoproliferative Disease (XLP), Wiscott-Aldrich syndrome, DiGeorge syndrome,

Ataxia-telangectasia, Chronic granulomatous disease, Transient hypogammaglobulinemia of infancy, Agammaglobulinemia, Complement deficiencies, or Selective IgA deficiency.

2. The use of claim 1, wherein the IL-2 comprises SEQ ID NO: 1.

3. The use of claim 1, wherein the at least one osmolyte is selected from mannitol, sorbitol or xylitol.

4. Use of a cell comprising an expression vector encoding SEQ ID NO: 1 in the manufacture of a medicament for treating cancer, an immune deficiency, immunosuppression, a viral infection, an immune disorder or combinations thereof,

wherein the cell is an autologous cell, an allogeneic cell, a haplotype matched cell, a haplotype mismatched cell, a haplo-identical cell, a xenogeneic cell, a cell line; or

wherein the cell is a stem cell, a cord blood cell, an adult stem cell, a mesenchymal stem cell, a mesenchymal stromal cell, an induced pluripotent stem cell, an autologous stem cell, a bone marrow cell, a hematopoietic cell, a hematopoietic stem cell, a somatic cell, a germ line cell, a differentiated cell, a somatic stem cell, or an embryonic stem cell,

wherein the cancer is carcinoma, lymphoma, blastoma, sarcoma, leukemia, squamous cell cancer, lung cancer, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, head and neck cancer, melanoma B-cell lymphoma, chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), hairy cell leukemia; chronic myeloblastic leukemia; post-transplant lymphoproliferative disorder (PTLD), abnormal vascular proliferation associated with phakomatoses, edema associated with brain tumors, Meigs' syndrome, bone cancer, brain cancer, esophageal cancer, or eye cancer,

wherein the immune deficiency is selected from the group consisting of: Autoimmune Lymphoproliferative Syndrome (ALPS), APS-1 (APECED), CARD9, Chronic Granulomatous Disease (CGD), Congenital Neutropenia Syndromes, Common Variable Immunodeficiency (CVID), CTLA4 Deficiency, DOCK8 Deficiency, Glycosylation Disorders with

Immunodeficiency, Hyper-Immunoglobulin E Syndromes (HIES), PI3 Kinase Disease, PLAID, Severe Combined Immunodeficiency (SCID), STAT3 Dominant-Negative Disease, WHIM Syndrome, X-Linked Agammaglobulinemia (XLA), X-Linked Lymphoproliferative Disease (XLP), Wiscott-Aldrich syndrome, DiGeorge syndrome, Ataxia-telangectasia, Chronic granulomatous disease, Transient hypogammaglobulinemia of infancy, Agammaglobulinemia, complement deficiencies, or Selective IgA deficiency, and

wherein the immune disorder is inflammation, graft versus host disease (GVHD), transplant rejection, an autoimmune disorder, multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, type I diabetes, systemic lupus erythrematosus, contact hypersensitivity, asthma or Sjogren's syndrome.

5. The use of claim 4, wherein the use further comprises that IL-2 is to be administered.