(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 one aspect, the present disclosure describes a use of 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 in the manufacture of a medicament for treating an inflammatory disorder, an immune disorder, a viral infection, a bacterial infection, or a neurodegenerative disorder in a subject in need thereof, wherein the subject is to be administered a therapeutically effective amount of the IL-2 such that regulatory T cells increase as compared to a control thereby inducing proliferation of regulatory T cells *in vivo,* 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 disorder is graft versus host disease (GVHD), transplant rejection, multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, type I

diabetes, systemic lupus erythrematosus, contact hypersensitivity, asthma or Sjogren's syndrome, and wherein the inflammatory disorder is atopic disease, paraneoplastic autoimmune disease, cartilage inflammation, arthritis, rheumatoid arthritis, juvenile arthritis, juvenile rheumatoid arthritis, pauciarticular juvenile rheumatoid arthritis, polyarticular juvenile rheumatoid arthritis, systemic onset juvenile rheumatoid arthritis, juvenile ankylosing spondylitis, juvenile enteropathic arthritis, juvenile reactive arthritis, juvenile Reiter's Syndrome, SEA Syndrome (Seronegativity, Enthesopathy, Arthropathy Syndrome), juvenile dermatomyositis, juvenile psoriatic arthritis, juvenile scleroderma, juvenile systemic lupus erythematosus, juvenile vasculitis, pauciarticular rheumatoid arthritis, polyarticular rheumatoid arthritis, systemic onset rheumatoid arthritis, ankylosing spondylitis, enteropathic arthritis, reactive arthritis, Reiter's Syndrome, SEA Syndrome (Seronegativity, Enthesopathy, Arthropathy Syndrome), dermatomyositis, psoriatic arthritis, scleroderma, vasculitis, myolitis, polymyolitis, dermatomyolitis, polyarteritis nodossa, Wegener's granulomatosis, arteritis, ploymyalgia rheumatica, sarcoidosis, sclerosis, primary biliary sclerosis, sclerosing cholangitis, Sjögren's syndrome, psoriasis, plaque psoriasis, guttate psoriasis, inverse psoriasis, pustular psoriasis, erythrodermic psoriasis, dermatitis, atopic dermatitis, dermatitis herpetiformis, Behcet's disease, alopecia, alopecia areata, alopecia totalis, atherosclerosis, lupus, Still's disease, Systemic Lupus Erythematosus, myasthenia gravis, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, celiac disease, multiple sclerosis (MS), asthma, COPD, rhinosinusitis, rhinosinusitis with polyps, eosinophilic esophogitis, eosinophilic bronchitis, Guillain-Barredisease, Type I diabetes mellitus, thyroiditis (e.g., Graves' disease),Addison's disease, Raynaud's phenomenon, autoimmune hepatitis, graft versus host disease, steroid refractory chronic graft versus host disease, transplantation rejection (e.g. kidney, lung, heart, skin, and the like), kidney damage, hepatitis C-induced vasculitis, spontaneous loss of pregnancy, alopecia, vitiligo, focal segmental glomerulosclerosis (FSGS), Minimal Change Disease, Membranous Nephropathy, ANCA Associated Glomerulonephropathy, Membranoproliferative Glomerulonephritis, IgA Nephropathy, or lupus nephritis.

In a further aspect, the present disclosure describes a use of 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 in the manufacture of a medicament for treating cancer in a subject in need thereof, wherein the subject is to be administered a therapeutically effective amount of the IL-2 that stimulates and/or enhances expansion of CD4+ helper cells, CD8+ effector naïve and memory T cells,

NK cells, and/or NKT cells, 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 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.

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 composition comprises aldesleukin in a pharmaceutical suitable aqueous vehicle, for parenteral administration to a patient in need of IL-2 immunotherapy, in which the aldesleukin has been prepared using the production method of the disclosure that ensures that the aldesleukin is stable in a pharmaceutical liquid composition for at least a year. With the composition of aldesleukin of the disclosure it is possible to prepare a pharmaceutical product consistent in one or more pre-filled syringes, each one containing a dose suitable for a given treatment, packaged in a box. This product would not need any manipulation previous to the injection into the patient, avoiding for example microbial or other possible contaminations. On the other hand, the stable liquid aldesleukin preparation of the disclosure could be advantageously introduced and homogeneously distributed in parenteral solutions commonly used in medicine for infusion, transfusion or clinical nutrition. Lately, treatments consisting in a given drug plus aldesleukin has been developed (e.g. CEPLENE™ (histamine dihydrochloride) is administered in conjunction with low dose of proleukin for maintenance of first remission in patients with Acute Myeloid Leukemia). For such treatments, the stable liquid aldesleukin preparation of the disclosure may serve as the base to elaborate a unique combined preparation or a combined kit. Proleukin has been also used to induce *in vitro* proliferation of susceptible cells to be used in cell therapy procedures (5). For these procedure, the stable liquid aldesleukin of the disclosure, formulated in low concentrations, can be very advantageous in order to avoid manipulations that can result in microbial contamination and also in order to obtain a rapid and homogeneous distribution of aldesleukin in the cell culture. These are only few examples, however a person in the art, can easily imagine other applications of the stable liquid aldesleukin preparation of the disclosure. In fact, IL-2 preparations are useful for stimulation of NK, T cells modified T cells, modified NK cells, tumor infiltrating lymphocytes (TIL), and all other cells expressing IL-2 receptors in their membranes and may be find applications as a drug in different medical treatments. In certain examples, the aldesleukin is used as a drug for medical treatments involving cells expressing IL-2 receptors in their membranes, comprising NK cells, T cells, dendritic cells, nonlymphoid cells, cell lines, transformed cells or combinations thereof.

In certain examples, a pharmaceutical composition comprises IL-2, *e.g.* SEQ ID NO: 1, SEQ ID NO: 2, in a range from about 0.001 mg to about 3 mg/mL, SDS 0.0001mg/mL to

about 10 mg/mL, a non-ionic osmolytes, a pH 7-8 phosphate buffer or combinations thereof. In certain examples, a pharmaceutical composition consists of IL-2 in a range from about 0.001 mg to 3 mg/mL, sodium dodecyl sulfate (SDS) in a range of about 0.0001mg/ml to about 10 mg/mL, a non-ionic osmolytes and a pH 7-8 phosphate buffer. In certain examples, an osmolyte comprises mannitol, sorbitol, xylitol or a pH 7-8 phosphate buffer. In certain examples composition may comprise an IL-2 protein, *e.g*. SEQ ID NO: 1, plus a buffer, an antioxidant such as ascorbic acid, a low molecular weight polypeptide(such as those having less than 10 amino acids), a protein, amino acids, carbohydrates such as glucose, sucrose, or dextrins, chelating agent such as EDTA, glutathione, and/or other stabilizers, excipients, and/or preservatives. The composition may be formulated as a liquid or a lyophilizate. Further examples of components that may be employed in pharmaceutical formulations are presented in Remington's Pharmaceutical Sciences, 23rd Ed., Mack Publishing Company, Easton, Pa., (2020) and others as described herein.

In certain examples, the methods comprise administering a pharmaceutical composition comprising the IL-2, *e.g*. SEQ ID NO: 1, SEQ ID NO: 2 or the combination thereof, to the subject. In some examples, the subject is a subject in need thereof.

In certain examples, the subject in need thereof is suffering from an inflammatory disorder. In some examples, the inflammatory disorder comprises inflammation, autoimmune disease, atopic diseases, paraneoplastic autoimmune diseases, cartilage inflammation, arthritis, rheumatoid arthritis (e.g. active), juvenile arthritis, juvenile rheumatoid arthritis, pauciarticular juvenile rheumatoid arthritis, polyarticular juvenile rheumatoid arthritis, systemic onset juvenile rheumatoid arthritis, juvenile ankylosing spondylitis, juvenile enteropathic arthritis, juvenile reactive arthritis, juvenile Reiter's Syndrome, SEA Syndrome (Seronegativity, Enthesopathy, Arthropathy Syndrome), juvenile dermatomyositis, juvenile psoriatic arthritis, juvenile scleroderma, juvenile systemic lupus erythematosus, juvenile vasculitis, pauciarticular rheumatoid arthritis, polyarticular rheumatoid arthritis, systemic onset rheumatoid arthritis, ankylosing spondylitis, enteropathic arthritis, reactive arthritis, Reiter's Syndrome, SEA Syndrome (Seronegativity, Enthesopathy, Arthropathy Syndrome), dermatomyositis, psoriatic arthritis, scleroderma, vasculitis, myolitis, polymyolitis, dermatomyolitis, polyarteritis nodossa, Wegener's granulomatosis, arteritis, ploymyalgia rheumatica, sarcoidosis, sclerosis, primary biliary sclerosis, sclerosing cholangitis, Sjögren's syndrome, psoriasis, plaque psoriasis, guttate psoriasis, inverse psoriasis, pustular psoriasis, erythrodermic psoriasis, dermatitis, atopic dermatitis, dermatitis herpetiformis, Behcet's

disease, including but not limited to the effects on the skin, alopecia, alopecia areata, alopecia totalis, atherosclerosis, lupus, Still's disease, Systemic Lupus Erythematosus(SLE) (e.g. active), myasthenia gravis, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, celiac disease, multiple sclerosis(MS), asthma, COPD, rhinosinusitis, rhinosinusitis with polyps, eosinophilic esophogitis, eosinophilic bronchitis, Guillain-Barredisease, Type I diabetes mellitus, thyroiditis (e.g., Graves' disease),Addison's disease, Raynaud's phenomenon, autoimmune hepatitis, graft versus host disease, steroid refractory chronic graft versus host disease, transplantation rejection (e.g. kidney, lung, heart, skin, and the like), kidney damage, hepatitis C-induced vasculitis, spontaneous loss of pregnancy, alopecia, vitiligo, focal segmental glomerulosclerosis (FSGS), Minimal Change Disease, Membranous Nephropathy, ANCA Associated Glomerulonephropathy, Membranoproliferative Glomerulonephritis, IgA Nephropathy, lupus nephritis, and the like.

In certain examples, the immune disorder is inflammation, graft versus host disease, transplant rejection, or an autoimmune disorder. In certain examples, the immune disorder is graft versus host disease (GVHD). In specific examples, the GVHD is chronic GVHD. In certain examples, the immune disorder is transplant rejection. In certain examples, the transplant is an organ transplant, bone marrow or other cell transplant, composite tissue transplant, or a skin graft. 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 immune disorder is cancer.

In certain examples, a method of treating a subject at risk of or is suffering from an immune disorder comprises obtaining an isolated population of T cells, and culturing the isolated population of T cells in the presence of a pharmaceutical composition comprising IL-2, *e.g*. SEQ ID NO: 1, for a period of time sufficient to induce proliferation of a population of T cells; and wherein, the T cells are adoptively transferred to the subject. In certain examples, the T cells are regulatory T (Treg) cells. Regulatory T (Tregs) cells are T lymphocytes having immunosuppressive activity. Natural Tregs are characterized as $CD4^+CD25^+Foxp3^+$ cells. Humans and mice presenting a genetic deficit in Tregs develop multiple T-cell mediated organ-specific autoimmune diseases. A Treg quantitative or qualitative defect has been described in many human autoimmune diseases, including systemic lupus erythematosus (SLE), Type 1 Diabetes, Multiple Sclerosis, uveitis and myositis. Conversely,

addition/restoration of Treg induces clinical improvements in most animal models of these diseases.

Tregs also play a major role in the control of inflammatory diseases, although their mode of action in such disease is not well understood. In fact, in most inflammatory diseases, Treg, depletion exacerbates disease while Treg addition decreases it. This is for example shown in the context of atherosclerosis. Although this disease is not primarily an inflammatory disease, its development involves an inflammatory component/loop. In apolipoprotein E (ApoE) deficient mice that spontaneously develop atherosclerosis, Treg depletion significantly aggravated the plaque formation, while injection of polyclonal Tregs significantly improved the disease. Most Tregs are $CD4^+$ cells, although there also a rare population of $CD8^+$ $Foxp3^+$ T lymphocytes with a suppressive activity.

In certain examples, a method of inducing proliferation of regulatory T cells in vivo, comprises administering to a subject in need thereof, 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 is comprised in a pharmaceutical composition. In certain examples, the aldesleukin comprises SEQ ID NO: 1, SEQ ID NO: 2, or variants thereof. 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 certain examples, a method of regulating an immune response associated with a disease or disorder in a subject in need thereof, comprising: 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 certain examples, the disease or disorder comprises: an autoimmune disease, cancer, inflammation, a viral infection, a bacterial infection, a neurodegenerative disorder or combinations thereof.

In certain examples, a method of inducing proliferation of a population of T cells, comprises obtaining an isolated population of T cells, and culturing the isolated population of T cells in the presence of a pharmaceutical composition comprising IL-2, *e.g.* SEQ ID NO: 1, for a period of time sufficient to induce proliferation of a population of T cells. In certain examples, the T cells are regulatory T (Treg) cells.

In certain examples, an *in vitro* method of producing a stimulated population of T cells, comprises obtaining an isolated population of T cells, and culturing the isolated population of T cells in the presence a pharmaceutical composition comprising IL-2, *e.g.* SEQ ID NO: 1, SEQ ID NO: 2 or the combination thereof, for a period of time sufficient to produce a stimulated population of T cells.

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 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, immunodeficiency, immunosuppression, viral infections, 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.

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

71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the IL-2 amino acid sequence (SEQ ID NO: 1). These include IL-2 variants that comprise an amino acid sequence having an N88R mutation 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 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the wild-type IL-2 amino acid sequence (i.e. SEQ ID NO: 1). Examples also include IL-2 variants that preferentially stimulate Treg cells and comprise an amino acid sequence having N88R and C125S mutations 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 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, or at least 98% sequence identity to the IL-2 amino acid sequence (SEQ ID NO: 1). Examples also include IL-2 variants that preferentially stimulate Treg cells and comprise an amino acid sequence having 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 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the IL-2 amino acid sequence (SEQ ID NO: 1).

Any compositions or methods described herein can be combined with one or more of any of the other compositions and methods described herein.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1: Amino acid sequence of the recombinant human interleukin 2 (Aldesleukin) (SEQ ID NO: 1).

FIG. 2: Comparison of the aldesleukin/SDS particle size range, as measured by dynamic light scattering, between PROLEUKIN™ and the aldesleukin prepared by the method of this disclosure.

FIG. 3: Aldesleukin/SDS particle size range of the aldesleukin prepared by the method of this disclosure after 0, 6 or 12 month storage at 0-8°C.

DETAILED DESCRIPTION

Embodiments of the disclosure are directed to a highly stable IL-2 in a liquid formulation and methods of producing the stable IL-2.

Definitions

What is claimed,

1. Use of 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 in the manufacture of a medicament for treating an inflammatory disorder, an immune disorder, a viral infection, a bacterial infection, or a neurodegenerative disorder in a subject in need thereof, wherein the subject is to be administered a therapeutically effective amount of the IL-2 such that regulatory T cells increase as compared to a control thereby inducing proliferation of regulatory T cells *in vivo,*

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

wherein the inflammatory disorder is atopic disease, paraneoplastic autoimmune disease, cartilage inflammation, arthritis, rheumatoid arthritis, juvenile arthritis, juvenile rheumatoid arthritis, pauciarticular juvenile rheumatoid arthritis, polyarticular juvenile rheumatoid arthritis, systemic onset juvenile rheumatoid arthritis, juvenile ankylosing spondylitis, juvenile enteropathic arthritis, juvenile reactive arthritis, juvenile Reiter's Syndrome, SEA Syndrome (Seronegativity, Enthesopathy, Arthropathy Syndrome), juvenile dermatomyositis, juvenile psoriatic arthritis, juvenile scleroderma, juvenile systemic lupus erythematosus, juvenile vasculitis, pauciarticular rheumatoid arthritis, polyarticular rheumatoid arthritis, systemic onset rheumatoid arthritis, ankylosing spondylitis, enteropathic arthritis, reactive arthritis, Reiter's Syndrome, SEA Syndrome

(Seronegativity, Enthesopathy, Arthropathy Syndrome), dermatomyositis, psoriatic arthritis, scleroderma, vasculitis, myolitis, polymyolitis, dermatomyolitis, polyarteritis nodossa, Wegener's granulomatosis, arteritis, ploymyalgia rheumatica, sarcoidosis, sclerosis, primary biliary sclerosis, sclerosing cholangitis, Sjögren's syndrome, psoriasis, plaque psoriasis, guttate psoriasis, inverse psoriasis, pustular psoriasis, erythrodermic psoriasis, dermatitis, atopic dermatitis, dermatitis herpetiformis, Behcet's disease, alopecia, alopecia areata, alopecia totalis, atherosclerosis, lupus, Still's disease, Systemic Lupus Erythematosus, myasthenia gravis, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, celiac disease, multiple sclerosis (MS), asthma, COPD, rhinosinusitis, rhinosinusitis with polyps, eosinophilic esophogitis, eosinophilic bronchitis, Guillain-Barredisease, Type I diabetes mellitus, thyroiditis (e.g., Graves' disease),Addison's disease, Raynaud's phenomenon, autoimmune hepatitis, graft versus host disease, steroid refractory chronic graft versus host disease, transplantation rejection (e.g. kidney, lung, heart, skin, and the like), kidney damage, hepatitis C-induced vasculitis, spontaneous loss of pregnancy, alopecia, vitiligo, focal segmental glomerulosclerosis (FSGS), Minimal Change Disease, Membranous Nephropathy, ANCA Associated Glomerulonephropathy, Membranoproliferative Glomerulonephritis, IgA Nephropathy, or lupus nephritis.

2. Use of 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 in the manufacture of a medicament for treating cancer in a subject in need thereof, wherein the subject is to be administered a therapeutically effective amount of the IL-2 that stimulates and/or enhances expansion of CD4+ helper cells, CD8+ effector naïve and memory T cells, NK cells, and/or NKT cells,

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 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.

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

4. The use of claim 1 or 2, wherein the at least one osmolyte is selected from the group consisting of: mannitol, sorbitol or xylitol.

5. The use of claim 1 or 2, wherein the IL-2 comprises a stable IL-2/SDS aggregate distribution in the medicament at least for a year in a range of 4 - 18 nm with a peak at about 8 nm as measured by dynamic light scattering, wherein the medicament is in form of a pharmaceutical liquid.

6. 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

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.

7. The use of claim 6, wherein the IL-2 comprises SEQ ID NO: 1.

8. The use of claim 6, wherein the at least one osmolyte is selected from mannitol, sorbitol or xylitol.

9. Use of a cell comprising an expression vector encoding SEQ ID NO: 1 in the manufacture of a medicament for treating cancer, immunodeficiency, immunosuppression, viral infections, 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.

10. The use of claim 9, wherein the immune disorder is inflammation, graft versus host disease (GVHD), transplant rejection, or an autoimmune disorder.

11. The use of claim 9, wherein the immune disorder is multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, type I diabetes, systemic lupus erythrematosus, contact hypersensitivity, asthma or Sjogren's syndrome.

12. The use of claim 9, wherein the use further comprises that IL-2 is to be administered.