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(54) Title: COMPOSITIONS AND METHODS FOR MODULATING IMMUNOSUPPRESSION

(57) Abstract: Methods for modulating immunosuppression are disclosed. This invention relates the fields of T cell regulation and therapeutic immunosuppression. More specifically, the invention provides compositions and methods for selective immunosuppressive therapies for the treatment of autoimmune diseases and allograft recipients.



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Compositions and Methods for Modulating Immunosuppression

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Cross-Reference to Related Applications

This application claims priority to US Provisional Patent Application No: 62/731,660 filed
September 14, 2018, the entire contents being incorporated herein by reference as thought set forth in full.

Field of the Invention

This invention relates the fields of T cell regulation and therapeutic immunosuppression.
More specifically, the invention provides compositions and methods for selective
immunosuppressive therapies for the treatment of autoimmune diseases and allograft recipients .

Background of the Invention

15 Several publications and patent documents are cited through the specification in order to
describe the state of the art to which this invention pertains. Each of these citations is
incorporated herein by reference as though set forth in full.

Therapeutic immunosuppression is required for many medical conditions ranging from
autoimmune diseases to transplantation.¹⁻⁶ Unfortunately, current immunosuppressive
20 medications are limited by non-specificity and toxicities,^{7, 8} and more precise and safer drugs are
needed. For example, organ transplantation is potentially curative for patients suffering from
organ failure; however, transplanted allografts require ongoing immunosuppression to prevent
rejection. Current immunosuppressive medications are fairly effective at preventing acute
allograft rejection, but are often associated with substantial toxicity. Prednisone induces weight
25 gain, hypertension, and hyperglycemia, while tacrolimus causes renal toxicity, hypertension, and
can induce diabetes, to name but a few complications associated with current therapies. These

common problems are frequently seen in most transplant programs, and among other patients requiring immunosuppressive therapy, across the United States and worldwide.

There are many examples in mammalian biology of how local environments foster immune regulation through alterations in cell metabolism, such as in the gastrointestinal tract, during pregnancy, and, unfortunately, in the tumor microenvironment (TME). The TME surrounding solid cancers is particularly effective at promoting immune evasion and resistance to anti-tumor therapies. TME metabolism, as well as immune privileged tissues, is characterized by low glucose, glutamine and tryptophan, while lactic acid and kynurenines are enriched, all of which weaken anti-tumor immunity.⁹⁻¹¹ Cytotoxic and effector T cells (CTL, Teff) require glycolysis to proliferate and produce cytokines, and become ineffective in the TME.¹²⁻¹⁵ We observed, that in contrast to Teff and CTL, regulatory T cells (Treg) are very well adapted to low-glucose, high lactate TME metabolism.¹⁶ Hence, the TME suppresses selective effector responses while suppressive and regulatory elements are favored.¹⁷

Current immunosuppressive therapies are limited by toxicities and a lack of specificities. Clearly, a need exists for improved therapies for the selective and effective immunosuppression in patients in need thereof.

Summary of the Invention

The present invention provides a method of modulating immune suppression mediated by T cells (for example, by mediating proliferation or viability or apoptosis or cell death of T cells, or conventional T cells, or mature T cells, or cytotoxic T cells, or effector T cells, or regulatory T cells) in a subject, the method including administering to the subject a metabolic breakdown product of tryptophan, or an analog of a metabolic breakdown product of D-tryptophan, in an amount effective to suppress undesirable immune responses. In preferred embodiments, the product is D-kynureneine. D-kynureneine is preferred over L-kynureneine as D-kynureneine does not exhibit the brain toxicity observed when L-kynureneine is administered.

In one embodiment, the invention provides a method for the treatment of autoimmune disease in a subject in need thereof, the method including administering to the subject a metabolic breakdown product of tryptophan, or an analog of a metabolic breakdown product of tryptophan, and optionally, one or more alloantigens present in the allograft, wherein said product is D-kynureneine.

The present invention also includes a method of preventing allograft rejection in a subject, the method including administering to the subject a metabolic breakdown product of D-tryptophan, or an analog of a metabolic breakdown product of D-tryptophan, and optionally, one or more alloantigens present in the allograft, wherein said product is D-kynurenine.

5 The present invention also includes a method of preventing graft versus host disease in a recipient, the method including administering to the donor a metabolic breakdown product of D-tryptophan, or an analog of a metabolic breakdown product of D-tryptophan, and one or more alloantigens present in the recipient, wherein the metabolic breakdown product of D-tryptophan, or an analog of a metabolic breakdown product of D-tryptophan, and the one or more
10 alloantigens present in the recipient are administered to the donor prior to obtaining donor cells from the donor; obtaining donor cells from the donor; and administering the donor cells to the recipient.

Also provide are compositions comprising at least one metabolic breakdown product of D-tryptophan, and/or an analog of a metabolic breakdown product of tryptophan, for use in the
15 methods and uses described. In one embodiment, the product is D-kynurenine. In one embodiment, the composition further comprises an immunosuppressant which is not a metabolic breakdown product of tryptophan, or an analog of a metabolic breakdown product of tryptophan.

In a preferred embodiment, the methods and compositions disclosed herein may be used along with an immunosuppressant other than the metabolic breakdown product of tryptophan or
20 an analog a metabolic breakdown product of tryptophan. In one embodiment, the metabolic breakdown product is D-kynurenine. In certain embodiments, the metabolic breakdown product of tryptophan or an analog thereof reduces dose of the immunosuppressant and/or administration frequency of the immunosuppressant for the subject compared to a regimen utilizing the immunosuppressant only without the metabolic breakdown product of tryptophan or an analog
25 thereof. In a further embodiment, the metabolic breakdown product of tryptophan or an analog thereof alleviates/mitigates toxicity of the immunosuppressant. In certain embodiments, the metabolic breakdown product of tryptophan or an analog thereof improves specificity of the immunosuppressant while preserving the subject's ability to fight infections and cancer.

Brief Description of the Drawing

Figure 1 provides results of a Kynurenine regulatory T (Treg) cells suppression assay. Purified CD4⁺CD25⁻ T conventional (Tconv) cells from C56BL/6 mice were labeled with carboxyfluorescein succinimidyl ester (CFSE) and co-stimulated with irradiated antigen presenting cells (irradiated CD09.2⁻ APC) plus CD3ε mAb (1 μg /mL, BD PharMingen). Treg (CD4⁺CD25⁺) cells were added at the indicated ratios to suppress Tconv proliferation. After 72 hours, proliferation of Tconv cells was determined by flow cytometric analysis of CFSE or CellTrace dilution. The number in the flow plots shows the percent Tconv cells that underwent one or more cellular division. Decreased effector T (Teff) cells proliferation was observed with higher doses of kynurenine.

Figures 2A and 2B provide analysis on Kynurenine metabolites. CD4⁺CD25⁻ Tconv cells were stimulated with irradiated antigen presenting cells and with CD3/CD28 mAb beads for 16 hours and exposed to 1 mM L- or D-kynurenine for 3 hours. [¹³C₆] glucose at 60 mg/dL was used for a 3-hour labeling. Metabolites were extracted and analyzed by LC-MS. Relative total ion counts of Kynurenine are plotted in Figure 2A while relative total ion counts of Kynurenic acid are shown in Figure 2B.

Figure 3A shows that Foxp3⁺ Induced Treg (iTreg, CD4⁺ CD25⁺ Foxp3⁺) formation is not augmented with kynurenine (decreased with toxicity). Purified CD4⁺CD25⁻ T conventional (Tconv) cells from C56BL/6 mice were labeled with CFSE and co-stimulated with irradiated antigen presenting cells (irradiated CD09.2⁻ APC) plus CD3ε mAb (1 μg /mL, BD PharMingen). CD4⁺ lymphocytes were gated. D-Kynurenine, L-Kynurenine or Ro61-8048 (a Kynurenine 3-monooxygenase (KMO or kynurenine hydroxylase) inhibitor) were incubated with the cells at concentrations indicated in the figure. Figures 3B and 3C show that Kynurenine-induced apoptosis. Purified CD4⁺CD25⁻ T conventional (Tconv) cells from C56BL/6 mice were labeled with CFSE and co-stimulated with irradiated antigen presenting cells (irradiated CD09.2⁻ APC) plus CD3ε mAb (1 μg /mL, BD PharMingen) for 3 days. Staining was performed to distinguish live cells from dead cells, for example using Live/Dead Aqua, an amine reactive dye which binds covalently to amines. Summary of 5 murine Treg suppression assays were plotted as a bar graph shown in Figure 3C. Kynurenine-induced cell death is observed in murine T cells, as has been previously observed.

Figure 4A shows that incubation of human T cells with 1 mM Kynurenine inhibits T cell proliferation and induces apoptosis. As shown in Figure 4A, human CD4⁺ T cells from healthy donor were stimulated with anti-CD3 ϵ /CD28 mAb coated beads for 4 days and labeled with CFSE. Additional staining was performed in order to differentiate live cells from dead cells.

5 CD4⁺ cells were gated. Kynurenine-induced cell death is observed in human T cells, consistent with previous findings in murine T cells.

Figures 5A and 5B show effects of Kynurenines on IFN- γ production. CD8⁺ T cells from C56BL/6 mice were acquired and stimulated via culture on anti-CD3 ϵ /CD28 mAb coated plates overnight and incubated with PMA/ionomycin for five hours. IFN- γ production was evaluated.

10 Representative flow cytometry plots can be found in Figure 5A while Figure 5B provides a bar graph summarizing relative IFN- γ production upon treatment with 250 μ M, 500 μ M, 1 mM, or 2 mM of L- or D-Kynurenine.

Figure 6 shows the effects of Kynurenines on IFN- γ production and cell viability. CD8⁺ T cells from C56BL/6 mice were acquired and stimulated by culture on anti-CD3 ϵ /CD28 mAb coated plates overnight and incubated with PMA/ionomycin for five hours. Staining was performed to distinguish live cells v.s. dead cells.

Figure 7 shows fatty acid depletion by treatment with L- or D-kynurenine. CD4⁺CD25⁻ Tconv cells were stimulated by contact with CD3/CD28 mAb beads for 16 hours and exposed to L- or D-kynurenine for 3 hours. [¹³C₆] glucose at 60 mg/dL was used for a 3-hour labeling.

20 Metabolites were extracted and analyzed. Total ion counts showed a reduction of lipids after 3 hours (10% FBS, RPMI 1640). The results support that Kynurenines induce lipid catabolism.

Figure 8A provides rescue of T cell phenotype with adding Oleate or Palmitate.

CD4⁺CD25⁻ Tconv cells were co-stimulated and exposed to 1 mM L- or D-kynurenine for 3 hours. [¹³C₆] glucose at 60 mg/dL was used for a 3-hour labeling. Consistent with the results

25 shown in Figure 8, Kynurenine ablated T cell proliferation (apoptosis). Addition of Oleate/Palmitate rescued the phenotype and restored T cell proliferation. Figures 8B and 8C show that effects of T cell apoptosis are highly dependent upon experimental conditions, including addition of bovine serum albumin (BSA) or number of cells treated per well. Human T cells were co-stimulated with CD3 ϵ /CD28 monoclonal antibodies (mAb) with 100k cells per well or 200k cells per well, with or without BSA or 0.2 mM Oleate/Palmitate.

Figures 9A to 9E provide *in vivo* results of Kynurenine treatments. B6/Rag1^{-/-} mice (with all the same age and gender, *i.e.*, 16+6 week, female)) were injected with Tconv³⁶. After 1 week, the mice were treated with 30 mg/kg D- or L-kynurenine 5/7 days/week *i.p.* for 3 weeks. Figures 9A to 9B show that Kynurenines suppress T cell function *in vivo*, indicating Kynurenine treated mice showed less homeostatic proliferation than NaCl control (n=5, one-way ANOVA). Figure 9C provides percentages of Foxp3⁺ cells (Treg cells) out of CD4⁺ cells upon treatment with D- or L- kynurenine. No difference in induced Foxp3⁺ cells, suggesting no augmentation of Treg upon treatment. Figures 9D and 9E show Kynurenine slowed weight gain. Weight was normalized to starting weight (day of Tconv injection). Kynurenine treated mice showed less weight gain (L-Kyn, p<0.05) or a trend to less weight gain (D-Kyn) compared to NaCl control (n=5, AUC). Observable effects were found on T cell proliferation *in vivo*, without overt toxicity.

Detailed Description of the Invention

Depletion of tryptophan and an increase of its metabolites from the kynurenine pathway have been recognized as mechanisms of immunosuppression through the induction of T cell apoptosis and the strengthening of regulatory T cells.¹⁸⁻²⁰ The present invention is based on the observation that different isomers of kynurenine drive this pathway in one direction or another. The present inventor has discovered that immunosuppression can be reduced or enhanced depending on which isomer of kynurenine is used to contact T cells. A key difference in D-kynurenine over L-kynurenine is that a key enzyme in L-kynurenine degradation, kynureninase, is specific to the L-kynurenine isomer and cannot metabolize the D-isomer to anthranilic acid.^{44, 45, 21, 22} D-Kynurenine can therefore not contribute to quinolinic acid production, avoiding the excitotoxicity that high dose (>300 mg/kg/d) L-kynurenine metabolism can cause in the brain. Kynurenic acid even has neuroprotective properties⁴⁶. In fact, in Neurobiology, Kynureninase inhibition is a neuroprotective treatment strategy to shunt kynurenine metabolites away from quinolinic acid to kynurenic acid.^{47, 48} In addition, this also avoids mixed or pro-inflammatory metabolites downstream of L-, but not D-kynurenine, and re-route kynurenine metabolites to aryl hydrocarbon receptor (AHR) agonists. AHR agonists have been suggested to be a key mediator of Foxp3⁺ regulatory T cell induction and suppressive function.^{23, 24, 49}

Accordingly, the methods of the present invention may be used in the treatment of an autoimmune disease or other diseases/disorders/conditions where suppression of the immune response is desired.

Autoimmune diseases that may be treated by the methods of the present invention include, but are not limited to, acute disseminated encephalomyelitis (ADEM), Addison's disease, ankylosing spondylitis, antiphospholipid antibody syndrome (APS), aplastic anemia, autoimmune hepatitis, autoimmune uveitis celiac disease, Crohn's disease, Goodpasture's syndrome, Graves' disease, Guillain-Barre syndrome (GBS), Hashimoto's disease, idiopathic thrombocytopenic purpura, insulin dependent diabetes mellitus (IDDM) lupus erythematosus, systemic lupus erythematosus, multiple sclerosis, myasthenia gravis, opsoclonus myoclonus syndrome (OMS), Ord's thyroiditis, pemphigus, pernicious Anaemia, polyarthritis, primary biliary cirrhosis, rheumatoid arthritis, Reiter's syndrome, Sjogren's syndrome, Takayasu's arteritis, temporal arteritis (also known as giant cell arteritis), warm autoimmune hemolytic anemia, and Wegener's granulomatosis.

The methods of the invention can also be used to advantage for the inhibition of allograft rejection and/or in the treatment of graft versus host disorders, for example, after bone marrow transplantation, after a kidney transplantation, after a liver transplantation, or after a cardiac transplantation. In certain embodiments, use of a metabolic breakdown product of tryptophan, or an analog of a metabolic breakdown product of tryptophan as described herein, optionally, in conjunction with an immunosuppressant other than the product or an analog thereof may prevent, delay, slow the progression of, alleviate, or cure an allograft rejection.

Other diseases or disorders or conditions, where suppression of the immune response is desired may include, for example, allergic disease, nephrotic syndrome requiring immunosuppression, and undesirable generation of anti-drug antibody (ADA). Such drug may be a protein, a peptide, an antibody, or a gene therapy vector.

The described methods, uses, and compositions may delay the appearance of a symptom associated with the diseases/disorders/conditions, slow the progression of the diseases/disorders/conditions, alleviate or cure the diseases/disorders/conditions.

In certain embodiments, use of a metabolic breakdown product of D-tryptophan, or an analog of a metabolic breakdown product of D-tryptophan as described herein, in conjunction with an immunosuppressant other than the product or an analog thereof may reduce dose and/or

administration frequency of the immunosuppressant, resulting in an alleviated toxicity effects of the immunosuppressant. In one embodiment, alleviation of a toxicity effect refers to no or delayed appearance of the toxicity effect, and/or a toxicity effect at a less severe level or a lower frequency. Such toxicity effects include, for example, one or more of the following: weight gain: 5 hypertension; hyperglycemia, diabetogenic effects; renal toxicity; diarrhea, constipation, nausea or vomiting; heartburn; stomach pain; loss of appetite; headache; uncontrollable shaking of a part of the body; difficulty falling asleep or staying asleep; dizziness; weakness; joint or back pain; rash or itching; burning, pain, numbness, or tingling in the hands or feet; decreased, painful, and/or burning urination; swelling of the hands, arms, feet, ankles, or lower legs; unusual 10 bruising or bleeding; seizures; coma; blurred vision, eye pain, or seeing halos around lights; swelling, rapid weight gain, feeling short of breath; severe depression, feelings of extreme happiness or sadness, changes in personality or behavior, seizure (convulsions); bloody or tarry stools, coughing up blood; pancreatitis (severe pain in upper stomach spreading to back, nausea and vomiting, fast heart rate); low potassium (confusion, uneven heart rate, extreme thirst, 15 increased urination, leg discomfort, muscle weakness or limp feeling); dangerously high blood pressure (severe headache, blurred vision, buzzing in ears, anxiety, confusion, chest pain, shortness of breath, uneven heartbeats, seizure); sleep problems (insomnia), mood changes; increased appetite, gradual weight gain; acne, increased sweating, dry skin, thinning skin, bruising or discoloration; slow wound healing; headache, dizziness, spinning sensation; nausea, 20 stomach pain, bloating; or changes in the shape or location of body fat (especially in arms, legs, face, neck, breasts, and waist). Other toxicity effects and toxicity effects specific to a certain immunosuppressant can be found on [Drugs.com](https://www.drugs.com) or accessdata.fda.gov/scripts/cder/daf/index.cfm.

In certain embodiments, an “effective amount” of a metabolic breakdown product of 25 tryptophan, or an analog of a metabolic breakdown product of D-tryptophan as provided herein is the amount which achieves desired amelioration of diseases or conditions as described herein and/or alleviation of a toxicity effect of an immunosuppressant. In certain embodiments, an “effective amount” of a metabolic breakdown product of D-tryptophan, or an analog of a metabolic breakdown product of tryptophan as provided herein is the amount which achieves one 30 or more of the following endpoints: a delay in appearance of a symptom associated with a disease/disorder/condition as described herein, a slower progression of a

disease/disorder/condition as described herein, an alleviated symptom or no symptom associated with a disease/disorder/condition as described herein, prevention of a disease/disorder/condition (for example, allograft rejection), and alleviation of a toxicity effect associated with an immunosuppressant which includes no or delayed appearance of a toxicity effect, and/or a toxicity effect at a less severe level or a lower frequency.

The following definitions are provided to facilitate an understanding of the present invention.

The term "indolamine dioxygenase (IDO)" is intended to mean IDO-1 (indoleamine 2,3-dioxygenase, EC 1.13.11.52), IDO-2 (indoleamine-pyrrole 2,3 dioxygenase-like 1, EC 1.13.11.-) or TDO (tryptophan 2,3-dioxygenase, EC 1.13.11.11) and refers to three different proteins that can catabolize tryptophan. IDO-1 can also catabolize serotonin and melatonin although the substrate specificity for IDO-2 and TDO is not so well studied. Metabolites or catabolites from the tryptophan pathway are Tryptophan, N-Formyl-kynurenine, Formylanthranilate, Anthranilate, L-Kynurenine, D-Kynurenine, 4-(2-Aminophenyl)-2,4-dioxybutanoate, Kynurenic acid, 3-Hydroxy-L-kynurenine, 3-Hydroxy-anthranilate, 3-Methoxy-anthranilate, 4-(2-Amino-3-hydroxy-phenyl)-2,4-dioxobutanoate, Xanthurenate, 8-Methoxy-kurenate, 2-Amino-3-carboxy-muconate semialdehyde, 2-Aminomuconate semialdehyde, Quimolinic acid, Cinnavalinate, Tryptamine, N-Methyltryptamine, Indoleacetate, 2-Formamino-benzoylacetate, 5-Hydroxy-L-tryptophan, 5-Hydroxy-N-formylkunerine, 5-Hydroxy-kunerine, 5-Hydroxy-kuneramin, 4,6-Dihydroxy-quinoline, Serotonin, N-Acetyl-serotonin, Melatonin, 6-Hydroxy-melatonin, Formyl-N-acetyl-5-metoxykynurenamine, N-Methylserotonin, Formyl-5-hydroxy-kynurenamine, 5-Metoxytryptamine, 5-Hydroxyindole-acetaldehyde, 5-Hydroxyindoleacetate, 5-Metoxyindoleacetate, or 5-Hydroxyindole-acetylglycine. Agents that potentially enhance the immunosuppressive IDO activity. Examples are D-Kynurenine, 3-hydroxy-kynurenine, anthranilic acid, 3-hydroxy-anthranilic acid, quinolinic acid and picolinic acid. Also enhancements using synthetic variants of tryptophan catabolites, e.g., N-(3,4-Dimethoxycinnamoyl) anthranilic acid.

"Tregs" are potent suppressors of T cell mediated immunity in a range of inflammatory conditions, including infectious disease, autoimmunity, pregnancy and tumors (Sakaguchi, S, Nat Immunol 2005; 6:345-352). Mice lacking Tregs die rapidly of uncontrolled autoimmune disorders (Khattry et al. Nat Immunol 2003; 4:337-342). In vivo, a small percentage of Tregs can

control large numbers of activated effector T cells. Although freshly isolated Tregs exhibit minimal constitutive suppressor functions, ligating the T cell antigen receptor (TCR) in vitro (Thornton et al. Eur J Immunol 2004; 34:366-376), or pre-immunizing mice with high-dose self-antigen in vivo stimulates Treg suppressor functions (Nishikawa et al. J Exp Med 2005; 201:681-5 686).

The term "alloantigen" is defined herein as an antigen present only in an allograft from a allograft donor and capable of inducing the production of an alloantibody response by allograft recipients

10 The term "immunoprotective" is defined herein as an effect which reduces, arrests, or ameliorates immunological insult and is protective, resuscitative or revivative for affected tissue that has suffered cytotoxic insult from immune cells or inflammation.

The phrase "immunoprotective agent" is herein defined as active ingredient or medicament containing an immune insult treatment dose of active ingredient effective in reducing, preventing, arresting, or ameliorating immune insult and provides protection, 15 resuscitation or revival to affected tissue that has suffered immune mediated insult.

The term "buffer" is intended to mean an aqueous solution containing an acid-base mixture with the purpose of stabilising pH. Examples of buffers are Trizma, Bicine, Tricine, MOPS, MOPSO, MOBS, Tris, Hepes, HEPBS, MES, phosphate, carbonate, acetate, citrate, glycolate, lactate, borate, ACES, ADA, tartrate, AMP, AMPD, AMPSO, BES, CABS, 20 cacodylate, CHES, DIPSO, EPPS, ethanolamine, glycine, HEPPSO, imidazole, imidazolelactic acid, PIPES, SSC, SSPE, POPSO, TAPS, TABS, TAPSO and TES.

The term "diluent" is intended to mean an aqueous or non-aqueous solution with the purpose of diluting the medicament. The diluent may be one or more of saline, water, polyethylene glycol, propylene glycol, ethanol or oils (such as safflower oil, corn oil, peanut oil, 25 cottonseed oil or sesame oil).

An excipient may be one or more of carbohydrates, polymers, lipids and minerals. Examples of carbohydrates include lactose, sucrose, mannitol, and cyclodextrines, which are added to the composition, e.g., for facilitating lyophilisation. Examples of polymers are starch, cellulose ethers, cellulose carboxymethylcellulose, hydroxypropylmethyl cellulose, hydroxyethyl 30 cellulose, ethylhydroxyethyl cellulose, alginates, carageenans, hyaluronic acid and derivatives thereof, polyacrylic acid, polysulphonate, polyethyleneglycol/polyethylene oxide,

polyethyleneoxide/polypropylene oxide copolymers, polyvinylalcohol/polyvinylacetate of different degree of hydrolysis, and polyvinylpyrrolidone, all of different molecular weight, which are added to the composition, e.g., for viscosity control, for achieving bioadhesion, or for protecting the lipid from chemical and proteolytic degradation. Examples of lipids are fatty acids, phospholipids, mono-, di-, and triglycerides, ceramides, sphingolipids and glycolipids, all of different acyl chain length and saturation, egg lecithin, soy lecithin, hydrogenated egg and soy lecithin, which are added to the composition for reasons similar to those for polymers. Examples of minerals are talc, magnesium oxide, zinc oxide and titanium oxide, which are added to the composition to obtain benefits such as reduction of liquid accumulation or advantageous pigment properties.

The compositions of the invention may be administrated by any suitable route including oral, sublingual, buccal, nasal, inhalation, parenteral (including intraperitoneal, intraorgan, subcutaneous, intradermal, intramuscular, intra-articular, venous (central, hepatic or peripheral), lymphatic, cardiac, and arterial, with parenteral being the preferred route. The preferred route may vary depending on the condition of the patient.

As described herein, the terms “reduce” “decrease” “alleviate” “ameliorate” “improve” “delay” “earlier” “lower” “mitigate”, any grammatical variation thereof, or any similar terms indication a change, means a variation of about 5 fold, about 2 fold, about 1 fold, about 90%, about 80%, about 70%, about 60%, about 50%, about 40%, about 30%, about 20%, about 10%, about 5 % compared to a reference (*e.g.*, a healthy control, a subject having a disease/disorder/condition as described herein, a subject having transplantation, a subject having an autoimmune disease, a subject having a graft versus host disease, an standard immunosuppressant regimen achieving similar or the same immune suppression effect without using the described metabolic breakdown product of tryptophan or an analog thereof), unless otherwise specified.

In certain embodiments, an immunosuppressant is administered to a subject 0, 1, 2, 3, 4, 5, 6, 7, about 1 week (7 days), about 15 days, about 30 days, about 45 days, about 60 days, or longer, prior to or after the administration of the described metabolic breakdown product of tryptophan or an analog thereof. Such immunosuppressant administration may involve administration of one, two or more immunosuppressants (*e.g.*, glucocorticoids, prednelisone, micophenolate mofetil (MMF) and/or sirolimus (*i.e.*, rapamycin)). Additionally, or alternatively, such immunosuppressant(s) may be administrated to a subject in need once, twice or for more

times at the same dose or an adjusted dose. Immunosuppressants for such co-therapy include, but are not limited to, tacrolimus, prednisone, a glucocorticoid, steroids, antimetabolites, T-cell inhibitors, a macrolide (*e.g.*, a rapamycin or rapalog), and cytostatic agents including an alkylating agent, an anti-metabolite, a cytotoxic antibiotic, an antibody, or an agent active on immunophilin. The immune suppressant may include a nitrogen mustard, nitrosourea, platinum compound, methotrexate, azathioprine, mercaptopurine, fluorouracil, dactinomycin, an anthracycline, mitomycin C, bleomycin, mithramycin, IL-2 receptor- (CD25-) or CD3-directed antibodies, anti-IL-2 antibodies, cyclosporin, tacrolimus, sirolimus, IFN- β , IFN- γ , an opioid, or TNF- α (tumor necrosis factor-alpha) binding agent.

The Example below is provided to illustrate certain embodiments of the invention. It is not intended to limit the invention in anyway.

Example 1

Indoleamine 2,3-dioxygenase (IDO), an enzyme involved in the catabolism of tryptophan, is expressed in certain cells and tissues, particularly in antigen-presenting cells of lymphoid organs and in the placenta. It was shown that IDO prevents rejection of the fetus during pregnancy probably by inhibiting alloreactive T cells, and it was suggested that IDO-expression in antigen-presenting cells may control autoreactive immune responses. Degradation of tryptophan, an essential amino acid required for cell proliferation, was reported to be the mechanism of IDO-induced T cell suppression. As mentioned above, kynurenine is an IDO degradation product.

Kynurenine can be in an L- or D- isomer form. A key difference in D-kynurenine over L-kynurenine is that a key enzyme in L-kynurenine degradation, Kynureninase, is specific to the L-kynurenine isomer and cannot metabolize the D-isomer.^{21, 22} This feature could be exploited for developing agent which avoid mixed or pro-inflammatory metabolites downstream of L-, but not D-kynurenine, thereby re-routing kynurenine metabolites to aryl hydrocarbon receptor (AHR) agonists. AHR agonists have been suggested to be a key mediator of Foxp3⁺ regulatory T cell induction and suppressive function.^{23, 24} AHR receptor knock out mice are also utilized to investigate effects of L- or D- kynurenine.

Our data indicate that exposure to D- and/or L-kynurenine can suppress T cell proliferation. See Figures 1, 3A and 4A. It was also found that 1 mM of Kynurenine induced T cell apoptosis. See, Figures 3B, 3C, 4A, and 6. T cells from C57BL/6 mice were isolated and evaluated for their response to D- or L- kynurenine treatment *in vitro* and *in vivo* as previously reported.²⁵⁻²⁷ Conventional T cells were stimulated under polarizing conditions to induce formation of mature T cells (for example, cytotoxic, effector and regulatory T cells). Stabilization of Foxp3⁺ regulatory T cell phenotype and/ or the effects on immune response suppression was also analyzed. No differential effect was observed on Treg. See, Figure 3A. *In vivo* immunosuppressive effects of D-kynurenine shown in a homeostatic proliferation assay are plotted in Figures 9A to 9C. The results indicate less T cells and CD4⁺ T cells upon treatment of D- or L-kynurenine *in vivo*. The rate of weight gain in the kynurenine homeostatic proliferation experiment was tracked. A slight impairment in weight gain was found with kynurenine treatment (Figures 9D and 9E). Cell death explains most of the T cell phenotypes observed *in vitro*.

Without wishing to be bound by the theory, we hypothesize that exposure to D-kynurenine weakens key cytotoxic and effector T cell functions. In additional experiments, we co-stimulated and exposed murine and human CD4⁺ and CD8⁺ T cells to proliferate (tracked by dye dilution via flow cytometry) to produce cytokines in response to stimulation. see, Figures 5A-5B and 6. Both D- or L-kynurenine were assessed for their ability to impair T cell viability(see, Figures 3B, 3C, 4A, and 6)/proliferation (see, Figures 4A) and effector functions including cytokine production (see, Figures 5A-5B and 6).

Studies on metabolic effects of L- or D-kynurenine were performed, indicating decreasing glycolysis and increasing lipid catabolism, which we found to play a key role in kynurenine-induced T cell apoptosis. In one experiment, Tconv were co-stimulated for 16 hours and exposed to 1 mM L- or D-kynurenine for 3 hours. Metabolites were extracted and analyzed by LC-MS. Results are shown in Figures 2A and 2B. The increase in Kynurenic acid is consistent with the expected D-Kynurenine phenotype of Kynureninase resistance.

Additionally, we observed that kynurenine impairs T cell function by augmenting lipid catabolism. Our studies identified that kynurenine impair T cell glycolysis, which selectively impairs Teff and CTL but spares Treg, creating immunosuppressive conditions.

Example 2

Effects of D-Kynurenine

LC-MS and [$^{13}\text{C}_6$] glucose tracing studies are performed in order to reveal depletion of post-GAPDH glycolytic intermediates with or without L-, D- or mixed isoforms of kynurenine.

5 Briefly, Tconv were co-stimulated and cultured in low glucose medium (30 mg/dL) for 48 hours with or without L-, D- or mixed isoforms of kynurenine, and 1 μM heptelidic acid (GAPDHi) or DMSO vehicle control.

Additionally, Tconv and CTL are co-stimulated and cultured with or without L-, D- or mixed isoforms of kynurenine.

10 Tconv are also stimulated with CD3 ϵ /CD28 for 16 hours and then labeled with 60 mg/dL [$^{13}\text{C}_6$] D-glucose or [$^{13}\text{C}_3$] Pyruvate for 2 hr with or without L-, D- or mixed isoforms of kynurenine.

Metabolites are extracted and analyzed via LC-MS, resulting a heatmap showing increased and decreased glycolytic inter-mediate ion count normalized to control. Glucose derivatives are investigated through [$^{13}\text{C}_6$] D-glucose. The effect of kynurenine on glucose, glutamine and fatty acid metabolism is characterized through heavy carbon [^{13}C] metabolic flux tracer studies. Also assessed are the direction of the Krebs cycle (reductive/oxidative), redox-dependent rerouting of metabolic pathways and effects on the production of immune regulatory metabolites. Glycolytic intermediates can be re-routed to *e.g.* the pentose phosphate pathway⁵⁵,
15 one carbon metabolism⁵⁶, or the methylglyoxal pathway⁵⁷⁻⁶⁰. Such altered pathways can be functionally important to T cell function⁶¹. The amount of heavy carbon isotopes in citrate is tracked, indicating how citrate was formed out of [$^{13}\text{C}_5$] L-glutamine (M+5 reductive vs. M+4 oxidative). Also tracked are other glutamine derived metabolites such as 2-HG.

The effects of kynurenine treatment on lipid metabolism was also determined. Fatty acid
25 metabolism, both β -oxidation and fatty acid synthesis, are crucial metabolic processes that are NAD:NADH regulated, and affect T cell differentiation and function^{35, 61-63}. While [$^{13}\text{C}_6$] D-glucose and [$^{13}\text{C}_5$] L-glutamine inform fatty acid synthesis, [$^{13}\text{C}_{16}$] palmitate tracing allows us to determine the kinetics of β -oxidation under kynurenine exposure.

The data show that a 3-hour exposure to kynurenine depleted fatty acids from cell media,
30 which we found to be causally related to T cell apoptosis (Figures 7 and 8A). Our data showed that kynurenine augments lipid catabolism and palmitate depletion (Figure 7). Restoring

oleate/palmitate rescues apoptosis, indicating fatty acid depletion is a reversible cause of kynurenine induced T cell apoptosis. See, Figure 8A. It is noted that increased number of cells treated per well and addition of albumin (for example, bovine serum albumin (BSA)) also show some effects. See Figure 8B. Albumin partially rescue the apoptosis while cell number affects response.

These data pointed to an important role for lipid catabolism in kynurenine rich environments. We observed, that 3-day co-stimulated Teffs ± 1 mM L- or D-kynurenine had 98.9 ± 0.55 or $97.3 \pm 0.55\%$ cell death (vs. $69.7 \pm 18.84\%$ in untreated Teff, 4/grp). Addition of 0.2-0.6 mM oleate/palmitate rescued the apoptosis phenotype, restoring Teff viability and proliferation. See, Figures 7 and 8A. Our results showed the immune modulatory potential of TME fatty acids, and indicated the possible use of kynurenines to induce lipid catabolism therapeutically.

Example 3

Evaluation of D-Kynurenine as an Immunosuppressive Therapy *in vivo*

– Autoimmune Disease

To assess whether kynurenines alleviate symptoms of autoimmune disease, autoimmune colitis is induced by chronic administration of dextran sulfate sodium to C57BL/6 mice and adoptive transfer of CD4⁺ Tconv isolated from wildtype (WT) C57BL/6 mice into immunodeficient B6/Rag1^{-/-} recipients. Clinical parameters of colitis are determined and inflammation assessed using methods which include, but are not limited to histology and flow cytometry.²⁵ These studies demonstrate the utility of D- and L-kynurenine as immunosuppressive agents.

Example 4

Evaluation of D-Kynurenine as an Immunosuppressive Therapy *in vivo*

– Kidney Transplant Model and Cardiac Transplant Model

In order to access the role of D-/L- kynurenine in supporting and reducing immunosuppressive therapy in transplantation as well as evaluate if D-/L- kynurenine affects allograft function, metabolism, and transplant tolerance, two murine animal models are utilized. In the first kidney transplant model, BALB/c kidneys are transplanted to nephrectomized C57BL/6 recipients who depend on allograft function to survive.²⁹ A second BALB/c to C57BL/6 cardiac allograft model is also used to determine if the dose of tacrolimus (an

immunosuppressant) required to maintain an allograft can be lowered with kynurenine (D- or L- or mixed isoform) treatment. The murine kidney transplant model is not as immunogenic as the cardiac transplant model. In our hands, 40% of the recipients survived for more than 80 days.

This indicates that the kidney transplant model allows investigating long-term differences

5 between the D-/L- kynurenine-treated vs. the control groups than the cardiac transplant model¹⁶.

To minimize effects of microbiota³², home-growing mice are used for these *in vivo* studies. 5-10 or 10-15 mice are used per group of both female and male mice. Statistical analysis (normal distribution testing, comparative statistics) assesses significance.

Na kynurenine in D- or L- or mixed isoform is provided through the drinking water, food,
10 and/or intraperitoneal (*i.p.*) injections. In some experiments, Kynurenine in D- or L- or mixed isoform is administered through *i.p.* injections (30 mg/kg/d), or enteral administration via diet (300 mg/kg/d, more details about this administration can be found in reference 30). NaCl (vs. no additive) is used as a negative control. Animal survival, allograft function and metabolites are monitored, while graft histology and intragraft lymphocytes are evaluated to assess mechanisms
15 linked with graft rejection or survival. For example, median allograft survival is calculated; quantitative data showing BUN, creatinine, calcium, hematocrit and LC-MS assessment of serum kynurenine split product is acquired; and antigen-specific T cell responses is evaluated.

In order to address whether kynurenine prolongs renal allograft dependent survival & allograft function, C57BL/6 mice are transplanted with BALB/c kidneys. The native kidneys are
20 removed after 7 days. As previously reported²⁸, removal of the native kidneys makes the mice dependent on renal allograft function to survive. We track hematocrit, creatinine, and other important parameters on metabolism and renal function with 70 μ L of blood (Chem8, Abbott) which is obtained weekly. This quantity of blood removal is well tolerated and does not affect hematologic parameters^{28,50}.

25 Further, Kynurenine treatment induced donor specific tolerance is evaluated. Mice developing longer than 100 days' allograft survival receive a cervical 3rd party (C3H, H-2k) or a BALB/C (H-2d re-exposure) cardiac allograft to the neck²⁸. Neck cardiac allograft survival are tracked by palpation, histology and immunohistochemistry²⁸.

Further, reduction in using conventional immunosuppressive drugs in MHC-mismatched
30 transplant models is also investigated.

In a second model, it is investigated whether Kynurenine can allow to lower the dose of conventional immunosuppressive therapy (*i.e.* tacrolimus). C57BL/6 mice are transplanted with BALB/c abdominal cardiac allografts^{16, 51, 52}. The recipients were treated with a subtherapeutic dose of tacrolimus (1 mg/kg/d), on which the mice would be expected to reject⁵³. In our previous studies, we have observed, that 1 mg/kg of tacrolimus administered to C57BL/6 mice had barely any effect on T cell function⁵⁴. Kynurenine administration is performed as indicated above and tested if it can prolong cardiac allograft survival. Body NAD:NADH ratios are further investigated via collecting liver, spleen, colon, and allograft tissue in liquid nitrogen, and later measuring NAD:NADH¹⁶ as well as histone acetylation/methylation.

Additionally, transplant-induced weight gain and diabetes are studied in these two models. Detailed methods can be found in the following Example.

Example 5

Kynurenine Treatment Mitigates Prednisone Induced Weight Gain and Hyperglycemia

6-week-old female Rag1^{-/-} mice were injected *i.p.* with 1×10^6 of B6 Tconv cells on Day 0. Starting on Day 7, D-Kynurenine, L-Kynurenine or control was administered *i.p.* at a dose of 30 mg/kg/day on Days 7-11, 14-18 and 21-24. Body weight was measured on days 0, 8, 16, 18, 23 and 25. On Day 25, mice were sacrificed. Serum and other tissues were harvested for further analysis. Immunosuppressive response *in vivo* is also evaluated. We observed that Kynurenine treatment slowed weight gain, suggesting that Kynurenine may affect immunosuppressant associated weight gain. See, Figures 9D and 9E.

Further, an established model of assessing prednisone induced weight gain and hyperglycemia³¹ is used with modifications to control for the effects of gender and microbiota³². Home-bred C57BL/6 are used. Regular chow diet providing 25% kcal from fat is provided to mice after weaning. Four weeks after weaning (*i.e.*, about 9 weeks to 12 weeks of age), all mice (both genders) are exposed to a high fat diet (45% kcal from fat) with high dose methylprednisolone (10 mg/kg/g *i.p.*) injections, with either Na D-Kynurenine *i.p.* injections (30 mg/kg/d) or vehicle control. Body weight is tracked. Serum glucose and insulin are measured weekly. A glucose tolerance test is performed at week 12, after which serum and tissue are collected. Results relating to weight, hyperglycemia, or insulin resistance phenotype are analyzed

to evaluate the alleviation of D-kynurenine treatment. T cell metabolism, caloric intake and energy expenditure are also explored.

Instead of *i.p.* injections for administering D-Kynurenine, enteral formulations which could achieve even higher doses are investigated using the same experimental setting.

5

Example 6

Metabolic Effects of D-Kynurenine

The metabolic effect of kynurenine (L-, D- or mixed isoform) is evaluated on transplant recipient metabolism. The effects of kynurenine on glucose, glutamine and fatty acid metabolism is characterized through heavy carbon [¹³C] metabolic flux tracer studies. Also assessed are the direction of the Krebs cycle (reductive/oxidative), redox-dependent rerouting of metabolic pathways and effects on the production of immune regulatory metabolites.

Metabolic pathways affected by kynurenine are identified, which can include T cell glycolysis (cytokine production,^{13, 33} or intracellular calcium¹⁵), *de novo* lipogenesis of Th17 T cells indicating that targeting acetyl-CoA carboxylase 1 shifts Th17 formation towards Foxp3+ Treg³⁵, the reverse Krebs cycle which is important to highly proliferating cells under hypoxic conditions³⁶ affecting malate dehydrogenase activity and favoring α-ketoglutarate metabolism to L-2-HG and subsequent effects on DNA methylation³⁷, the metabolic sequelae of conventional immunosuppression such as tacrolimus and prednisone (weight gain, diabetogenic)³⁸, and more³⁴. Without wishing to be bound by the theory, kynurenine treatment may alleviate some of these problems by dose reduction of conventional immunosuppressants, and maybe by counteracting certain co-morbid pathologies, such as weight gain.

Additionally, co-stimulated Tconv, CTL, & Tregs, adding L- or D- kynurenine. The T cells are labeled using heavy carbon tracers [¹³C₆] D-glucose, [¹³C₅] L-glutamine, and [¹³C₁₆] palmitate each in separate experiments (all in biological triplicate at minimum). After labeling, metabolites are methanol-extracted and submitted for LC/MS derivative analysis. Methods were detailed^{16, 39, 40-43}.

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While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. It will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the scope of the present invention, as set forth in the following claims.

What is claimed is:

1. A composition comprising a metabolic breakdown product of tryptophan, or an analog of a metabolic breakdown product of tryptophan, and at least one immunosuppressant in a pharmaceutically acceptable carrier, each in an effective amount for use in the treatment of autoimmune disease in a subject, wherein said product is D-kynurenine which is not associated with brain toxicity.

2. A composition comprising a metabolic breakdown product of tryptophan, or an analog of a metabolic breakdown product of tryptophan, and one or more alloantigens present in the allograft in a pharmaceutically acceptable carrier, each in an effective amount for use in preventing allograft rejection in a subject, wherein said product is D-kynurenine which is not associated with brain toxicity.

3. The composition according to claim 2, wherein the composition further comprises at least one immunosuppressant.

4. A composition comprising a metabolic breakdown product of tryptophan, or an analog of a metabolic breakdown product of tryptophan and at least one immunosuppressant in a pharmaceutically acceptable carrier, for use in the treatment of graft versus host disease in a subject, wherein said product is D-kynurenine.

5. The composition according to claim 4, wherein the subject has received a bone marrow transplant, or a kidney transplant, or a liver transplant, or a cardiac transplant.

6. The composition according to any one of claims 1 to 5, wherein the immunosuppressant is not a metabolic breakdown product of D-tryptophan, or an analog of a metabolic breakdown product of tryptophan.

7. The composition according to any one of claims 1 and 3 to 6, whereby the metabolic breakdown product of D-tryptophan or an analog thereof reduces administration frequency of the immunosuppressant.

8. The composition according to any one of claims 1 and 3 to 7, whereby the D-kynureneine also alleviates toxicity of the immunosuppressant absent brain toxicity observed with L- kynurenine is administered.

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9. The composition according to any one of claims 1 and 3 to 8, whereby the metabolic breakdown product of tryptophan or an analog thereof improves specificity of the immunosuppressant while preserving the subject's immune response to infection and cancer.

10. The composition according to any one of claims 1 to 9, wherein the composition further comprises a buffer, a diluent, or an excipient.

11. The composition according to any one of claims 1 to 10, wherein the composition is suitable for an administration via a route selected from the group consisting of parenteral, oral, sublingual, buccal, nasal, inhalation, intraperitoneal, intraorgan, subcutaneous, intradermal, intramuscular, intra-articular, venous, lymphatic, cardiac, and arterial.

12. Use of a metabolic breakdown product of D-tryptophan, or an analog of a metabolic breakdown product of D-tryptophan, as a medicament for modulating immune suppression mediated by T cells in a subject in need thereof, wherein said product is D-kynureneine.

13. Use of a metabolic breakdown product of D-tryptophan, or an analog of a metabolic breakdown product of tryptophan, as a medicament for the treatment of autoimmune disease in a subject, wherein said product is D-kynureneine which does exhibit brain toxicity observed when L- kynurenine is used.

14. Use of a metabolic breakdown product of D-tryptophan, or an analog of a metabolic breakdown product of tryptophan, and optionally, one or more alloantigens present in the allograft, as a medicament for preventing allograft rejection in a subject, wherein said product is D-kynureneine which does exhibit brain toxicity observed when L- kynurenine is used.

15. Use of a metabolic breakdown product of tryptophan, or an analog of a metabolic breakdown product of tryptophan in a pharmaceutically acceptable carrier, as a medicament for the treatment of graft versus host disease in a subject, wherein said product is D-kynurenine, which does exhibit brain toxicity observed when L- kynurenine is used.

16. The use according to claim 15, wherein the subject has received a bone marrow transplant, or a kidney transplant, or a liver transplant, or a cardiac transplant.

17. The use according to any one of claims 12 to 16, wherein the subject further receives at least one immunosuppressant which is not a metabolic breakdown product of tryptophan, or an analog of a metabolic breakdown product of tryptophan.

18. Use of a metabolic breakdown product of D-tryptophan, or an analog of a metabolic breakdown product of D-tryptophan and at least one immunosuppressant, as a medicament for modulating immune suppression mediated by T cells in a subject in need, or for the treatment of autoimmune disease in a subject in need, or for the treatment of autoimmune disease in a subject in need, or for use in preventing allograft rejection in a subject, or for use in the treatment of graft versus host disease in a subject, and wherein the immunosuppressant is not a metabolic breakdown product of tryptophan or an analog a metabolic breakdown product of tryptophan.

19. The use according to claim 18, wherein the product is D-kynurenine which does exhibit brain toxicity observed with L-kynurenine is administered.

20. The use according to any one of claims 12 to 19, wherein the metabolic breakdown product of tryptophan or an analog thereof reduces dose, or administration frequency, of the immunosuppressant.

21. The use according to any one of claims 12 to 20, wherein the metabolic breakdown product of tryptophan or an analog thereof alleviates toxicity of the immunosuppressant, or wherein the metabolic breakdown product of tryptophan or an analog thereof improves specificity of the immunosuppressant and preserves the subject's immune response to infection and cancer.

22. The use according to any one of claims 12 to 21, wherein the metabolic breakdown product of tryptophan or an analog thereof is in a composition which further comprises a buffer or a diluent or an excipient.

23. The use according to any one of claims 12 to 22, wherein the metabolic breakdown product of tryptophan or an analog thereof is suitable for administration via a route selected from the group consisting of parenteral, oral, sublingual, buccal, nasal, inhalation, intraperitoneal, intraorgan, subcutaneous, intradermal, intramuscular, intra-articular, venous, lymphatic, cardiac, and arterial.

24. Use of a metabolic breakdown product of tryptophan or an analog thereof for preparing a medicament.

25. A method of modulating immune suppression mediated by regulatory T cells (Tregs) in a subject, comprising administering to the subject a metabolic breakdown product of D-tryptophan, or an analog of a metabolic breakdown product of D-tryptophan, in an amount effective to suppress undesirable immune responses, wherein said product is D-kynurenine which does exhibit brain toxicity observed with L-kynurenine is administered.

26. A method for the treatment of autoimmune disease in a subject in need thereof, comprising administering to the subject a metabolic breakdown product of tryptophan, or an analog of a metabolic breakdown product of tryptophan, and optionally, one or more alloantigens present in the allograft, wherein said product is D-kynurenine which does exhibit brain toxicity observed with L-kynurenine is administered.

27. A method of preventing allograft rejection in a subject, comprising administering to the subject a metabolic breakdown product of tryptophan, or an analog of a metabolic breakdown product of tryptophan, and optionally, one or more alloantigens present in the allograft, wherein said product is D-kynureneine which does exhibit brain toxicity observed with L-kynureneine is administered.

28. A method of preventing graft versus host disease in a recipient, comprising administering to the donor a metabolic breakdown product of tryptophan, or an analog of a metabolic breakdown product of tryptophan, and one or more alloantigens present in the recipient, wherein the metabolic breakdown product of tryptophan, or an analog of a metabolic breakdown product of tryptophan, and the one or more alloantigens present in the recipient are administered to the donor prior to obtaining donor cells from the donor; obtaining donor cells from the donor; and administering the donor cells to the recipient, wherein said product is D-kynureneine which does exhibit brain toxicity observed with L-kynureneine is administered.

29. The method according to any one of claims 25 to 28, wherein said product is administered via a route selected from the group consisting of parenteral, oral, sublingual, buccal, nasal, inhalation, intraperitoneal, intraorgan, subcutaneous, intradermal, intramuscular, intra-articular, venous, lymphatic, cardiac, and arterial.

30. A method of modulating immune suppression mediated by T cells in a subject, comprising administering to the subject a metabolic breakdown product of D-tryptophan, or an analog of a metabolic breakdown product of D-tryptophan, in an amount effective to suppress undesirable immune responses, wherein said product is D-kynureneine which does exhibit brain toxicity observed with L-kynureneine is administered.

31. The composition, use or method of any of the previous claims, wherein said immunosuppressant is one or more of tacrolimus, prednisone, a glucocorticoid, a steroid, antimetabolites, T-cell inhibitors, a macrolide, rapamycin, rapalog, a cytostatic agent, an alkylating agent, an anti-metabolite, a cytotoxic antibiotic, an antibody, or an agent active on immunophilin.

Kynurenine Treg suppression assay

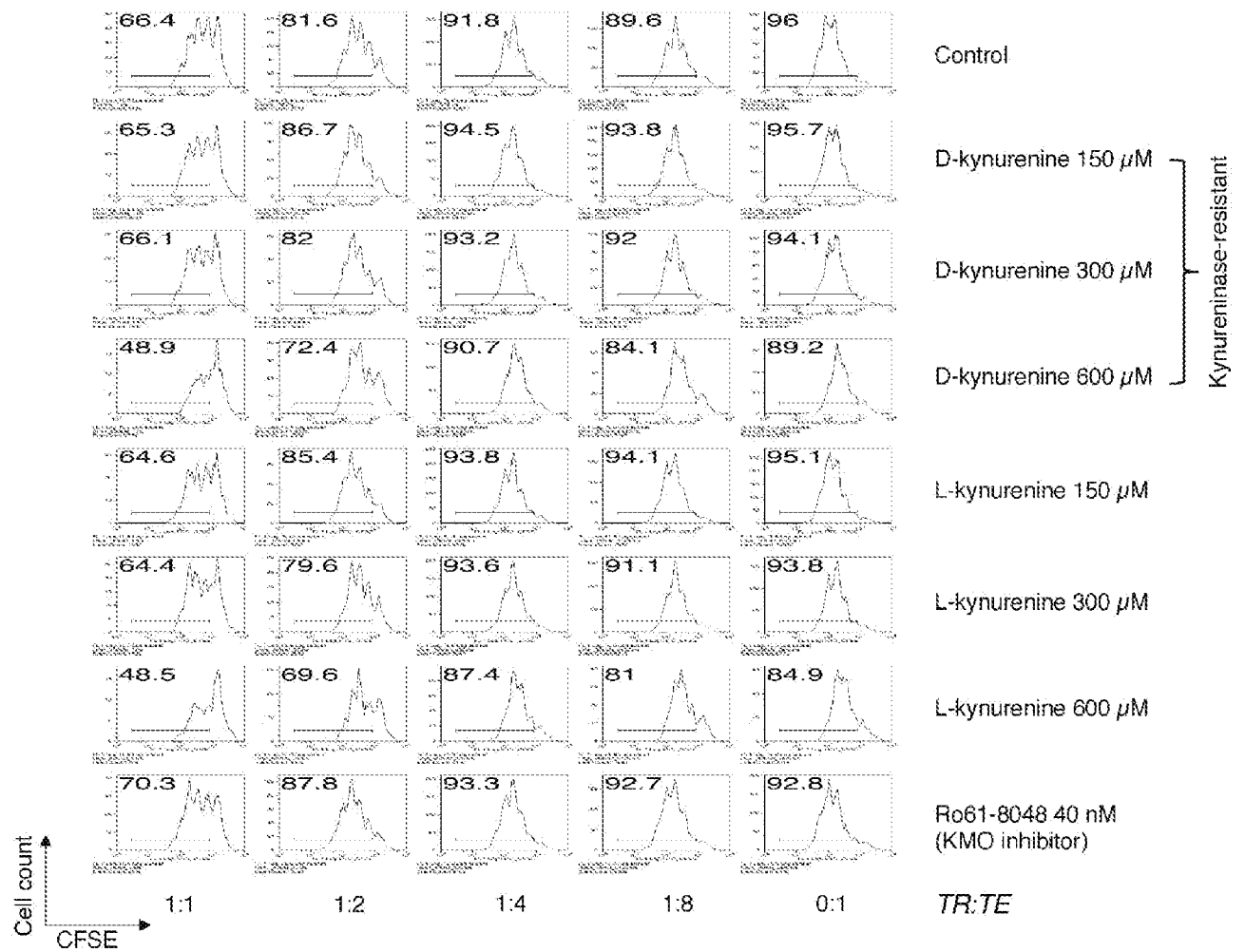


Figure 1

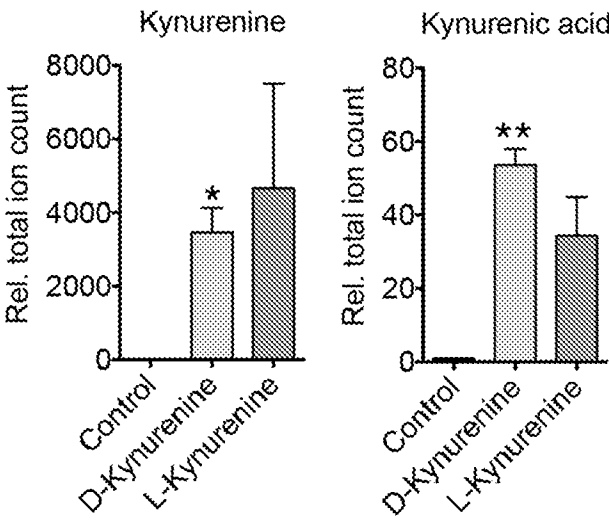


Figure 2A

Figure 2B

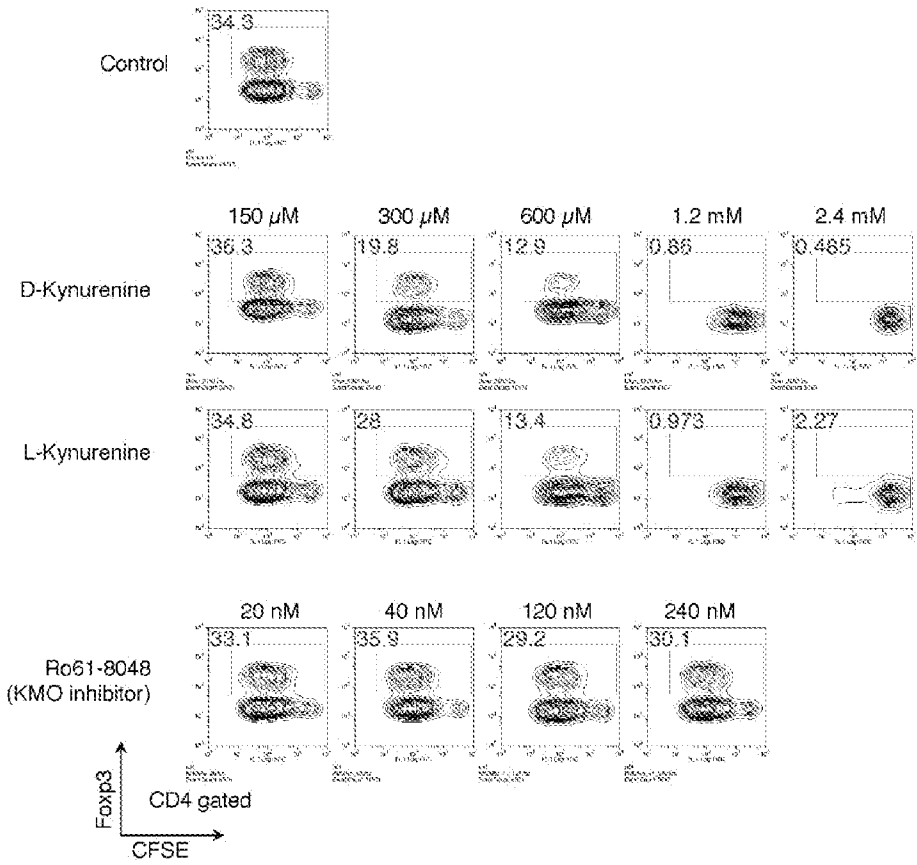


Figure 3A

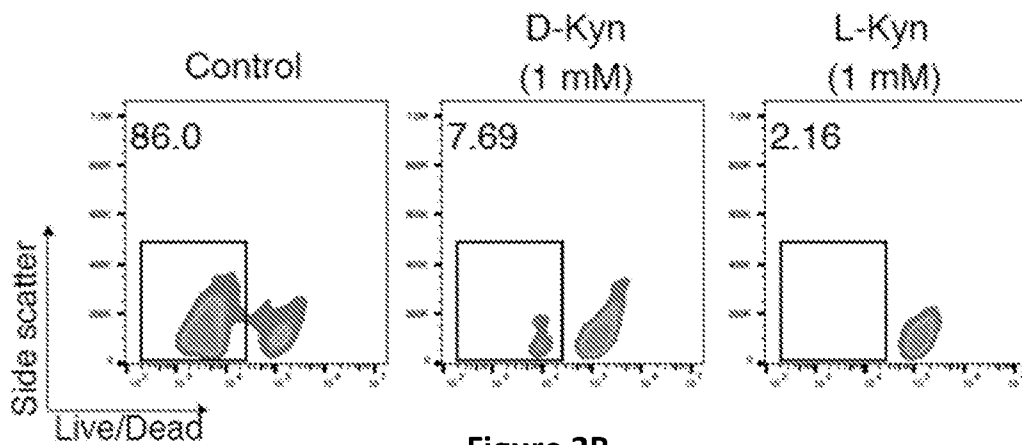


Figure 3B

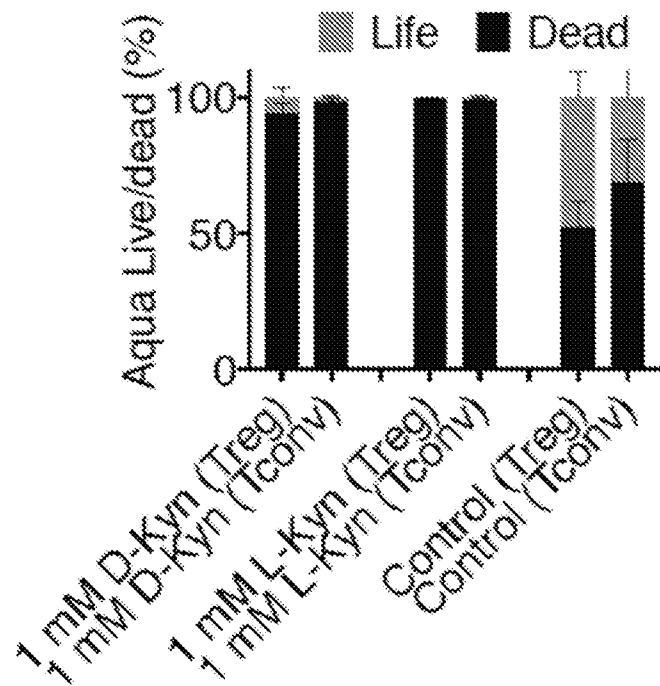


Figure 3C

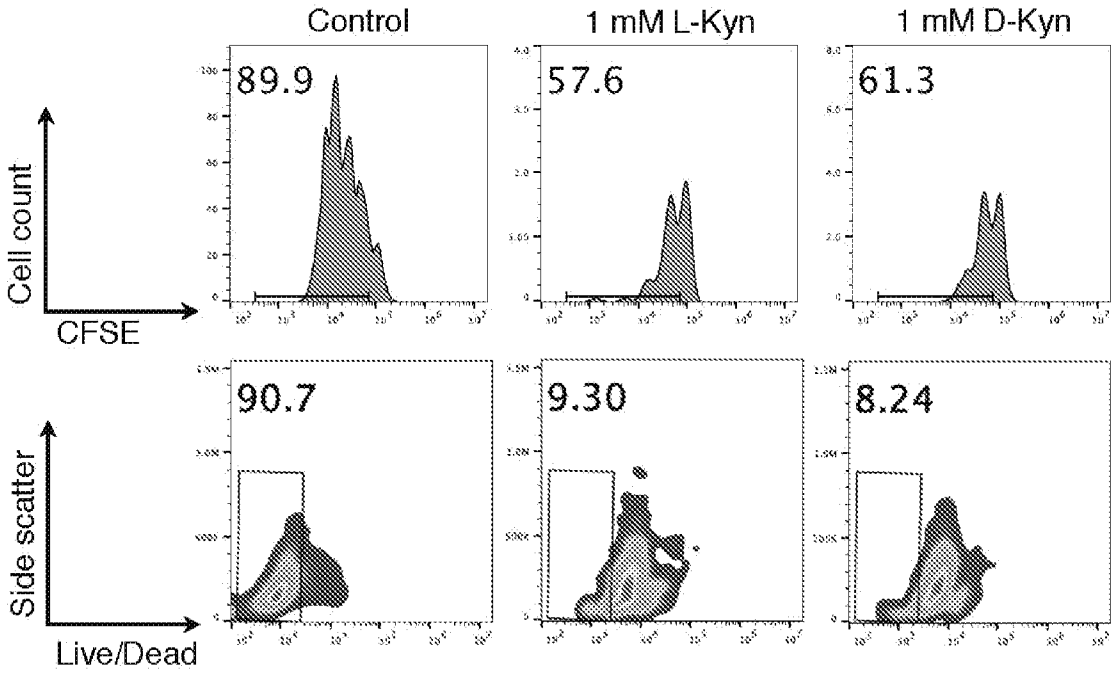


Figure 4A

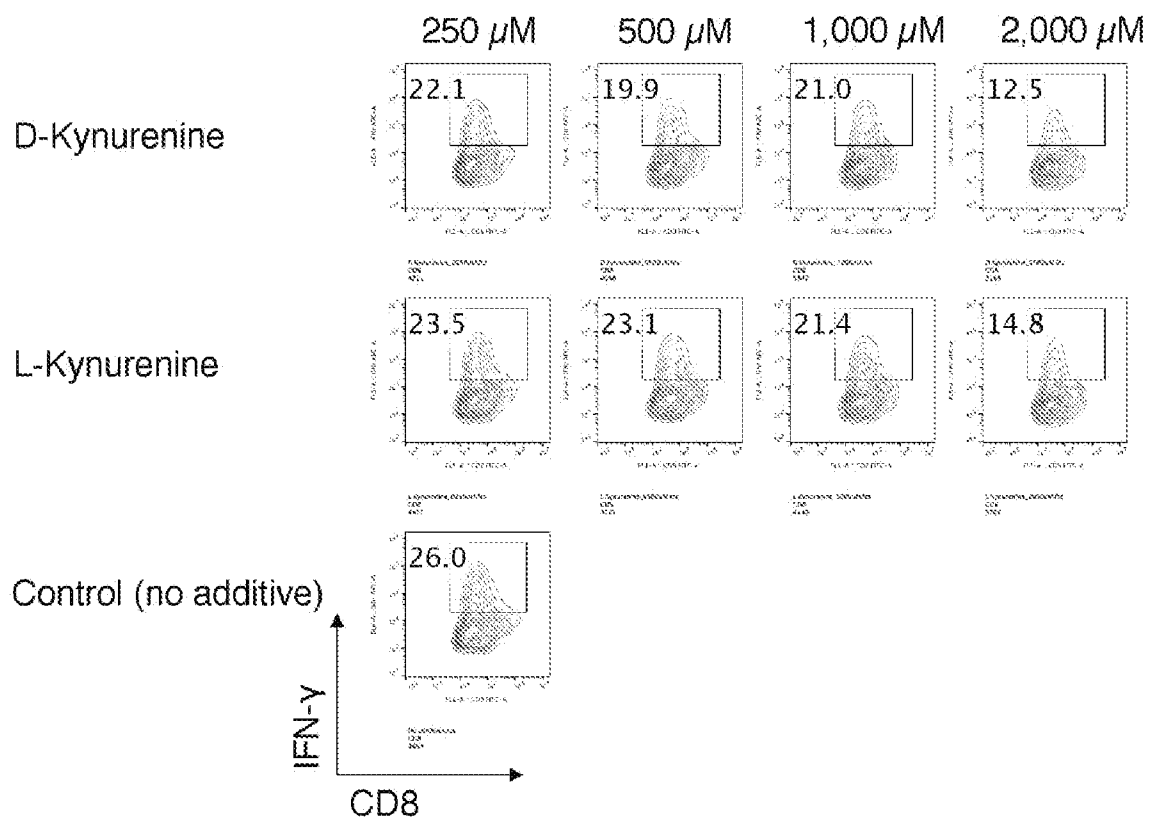


Figure 5A

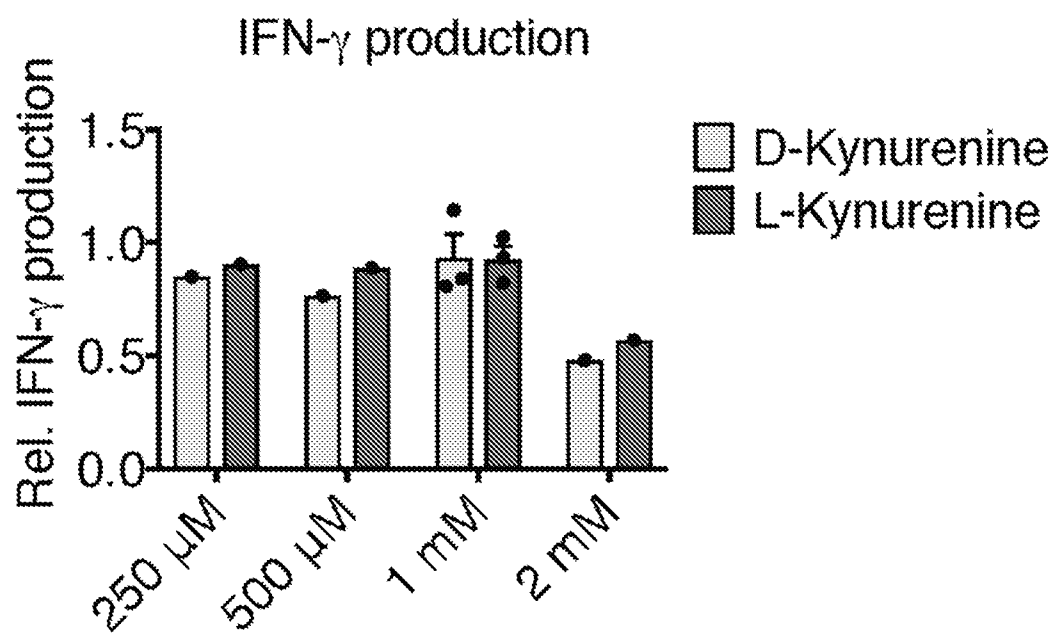


Figure 5B

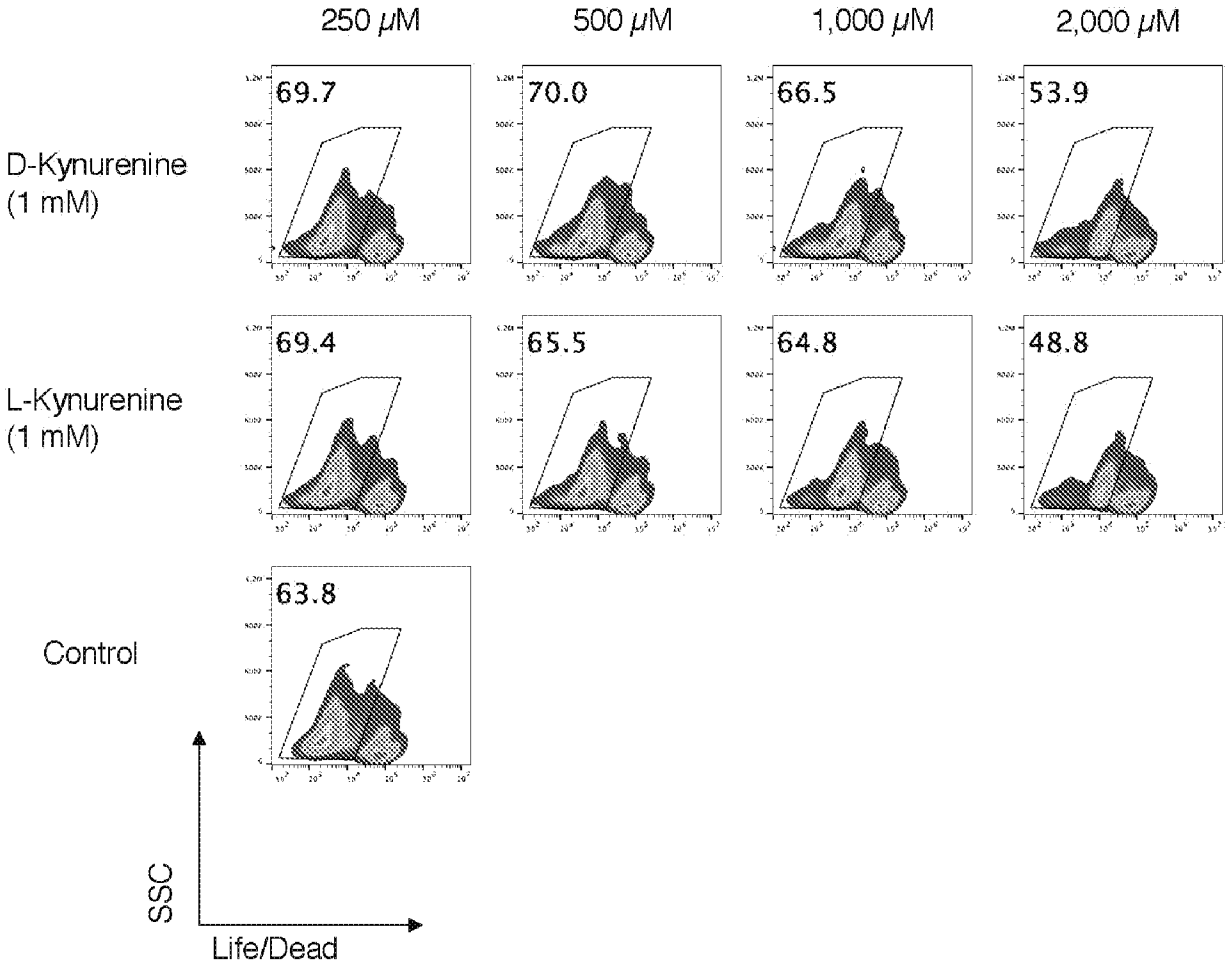


Figure 6

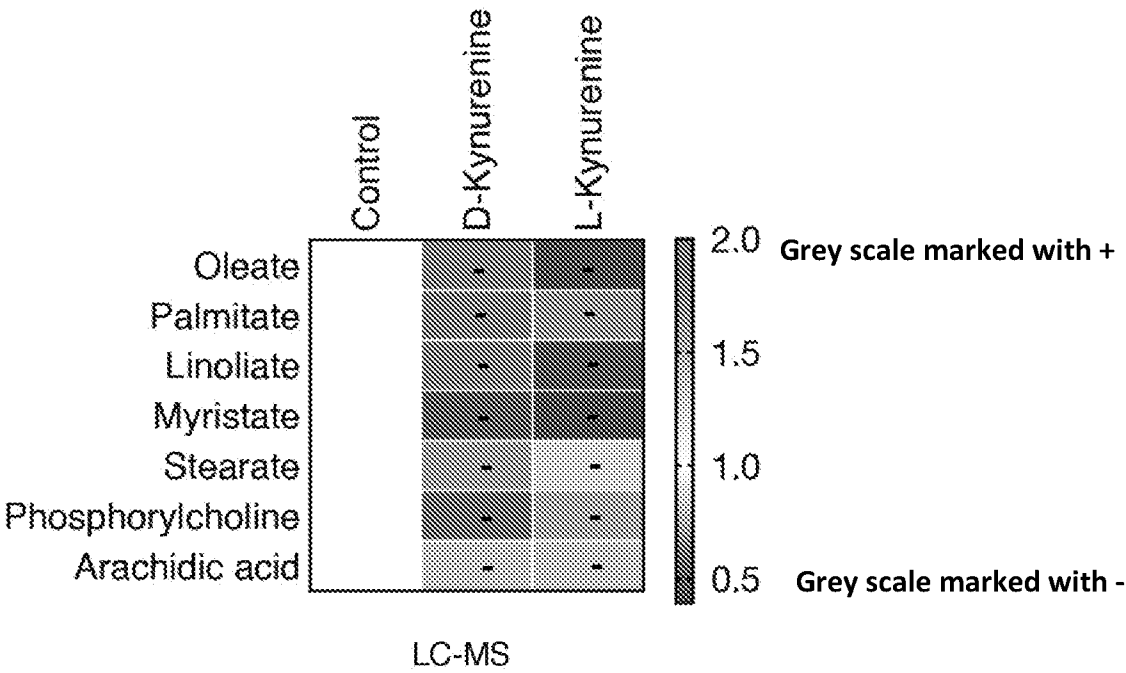


Figure 7

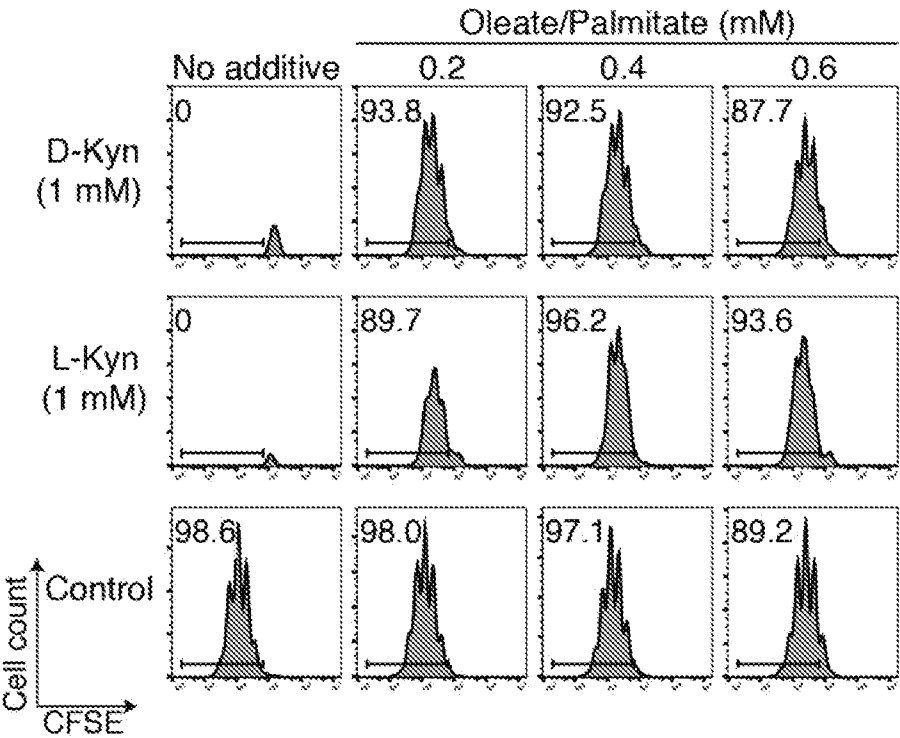


Figure 8A

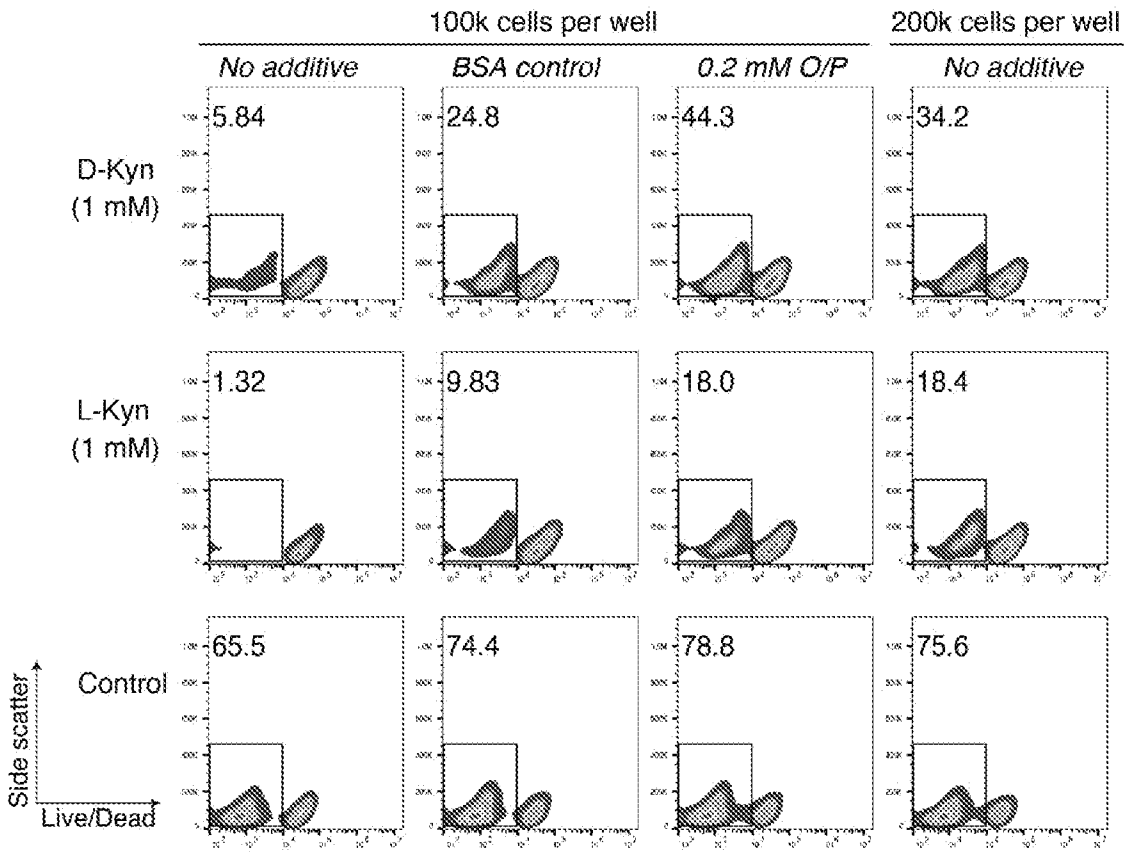


Figure 8B

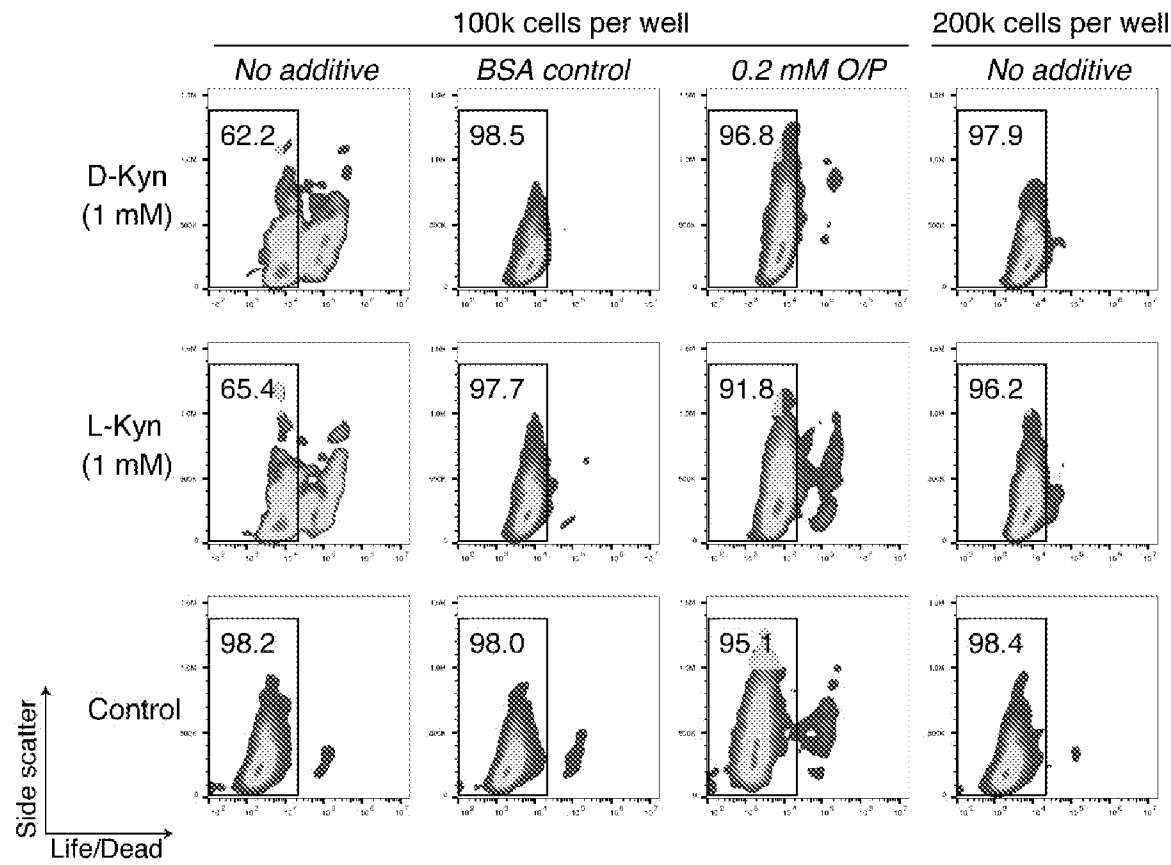


Figure 8C

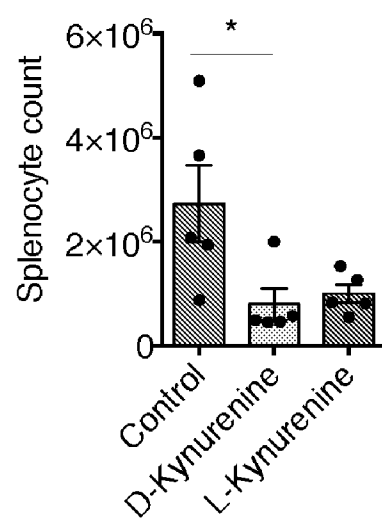


Figure 9A

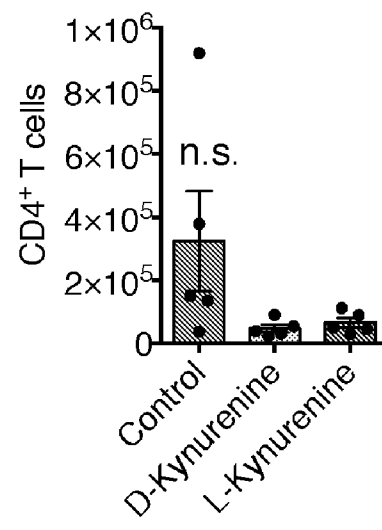


Figure 9B

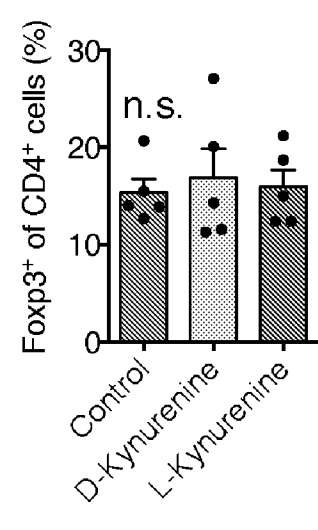


Figure 9C

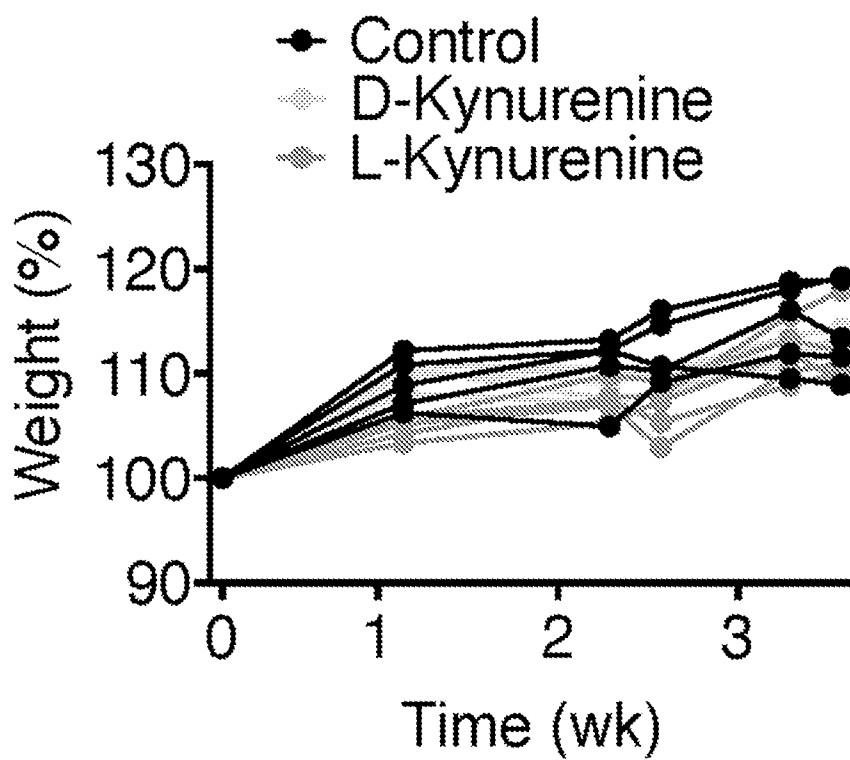


Figure 9D

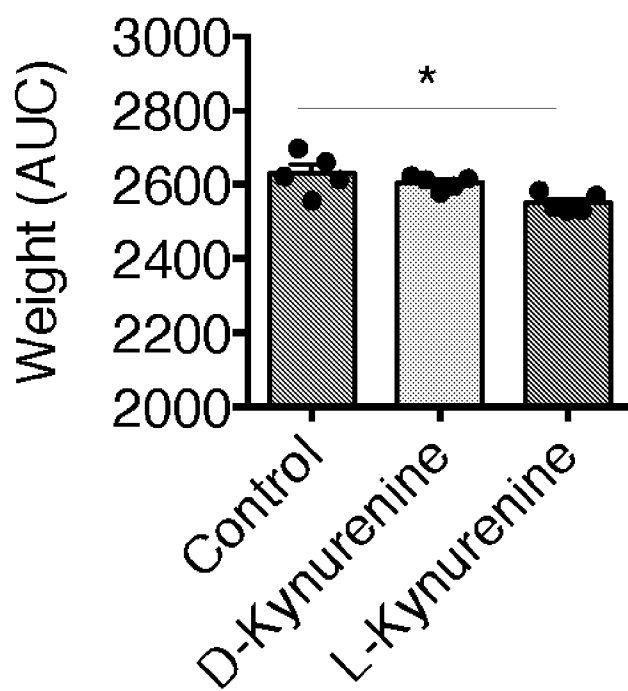


Figure 9E

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/051298

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12P 13/22; A61K 31/405; A61P 37/06; G01N 33/68 (2019.01)

CPC - C12P 13/227; A61K 31/405; A61P 37/06; G01N 33/68 (2019.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 435/106 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	 RADU et al., Immunosuppressive effect of tryptophan metabolites in composite tissue allotransplantation, Plastic and Reconstructive Surgery, Vol. 119, No.7, June 2007 [retrieved on 07 November 2019]. Retrieved from the Internet: <URL: https://www.ncbi.nlm.nih.gov/pubmed/17519695 >. abstract	24
A	 WO 2012/099441 A3 (INJE UNIVERSITY INDUSTRY-ACADEMIC) 18 October 2012 (18.10.2012) see machine translation	1-6, 12-19, 24-30
A	 NOTARANGELO et al., Role of D-amino acid oxidase in the production of kynurenine pathway metabolites from D-tryptophan in mice, Journal of Neurochemistry, Vol. 136, No. 4, 13 January 2016, PMC 10 June 2017 [retrieved on 29 October 2019]. Retrieved from the Internet: <URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4902784/ >. entire document	1-6, 12-19, 24-30

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

31 October 2019

Date of mailing of the international search report

04 DEC 2019

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/051298

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-11, 20-23, 31
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.