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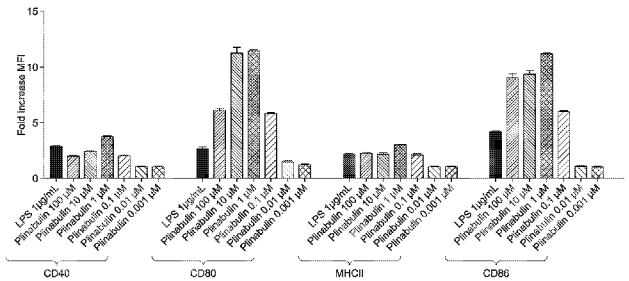

FIG. 1A

(57) Abstract: Disclosed herein are compositions comprising Plinabulin and one or more immune checkpoint inhibitor for treating cancer. Some embodiments relate to methods of treating cancer by co-administering Plinabulin and one or more immune checkpoint inhibitor to a subject in need thereof.

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USE OF PLINABULIN IN COMBINATION WITH IMMUNE CHECKPOINT INHIBITORS

INCORPORATION BY REFERENCE TO ANY PRIORITY APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/115,468, filed February 12, 2015, and U.S. Provisional Application No. 62/255,259, filed November 13, 2015, the disclosures of which are incorporated herein by reference in their entireties.

BACKGROUND

Field

[0002] The present invention relates to the field of chemistry and medicine. More particularly, the present invention relates to Plinabulin, compositions containing Plinabulin, and its use in treatment.

Description of the Related Art

[0003] Human cancers harbor numerous genetic and epigenetic alterations, generating neoantigens potentially recognizable by the immune system (Sjoblom et al, 2006). The adaptive immune system, comprised of T and B lymphocytes, has powerful anti-cancer potential, with a broad capacity and exquisite specificity to respond to diverse tumor antigens.

[0004] Recent cancer immunotherapy research has focused substantial effort on approaches that enhance anti-tumor immunity by adoptive-transfer of activated effector cells, immunization against relevant antigens, providing non-specific immune- stimulatory agents such as cytokines, or removing inhibitors to anti-cancer effector cells. Efforts to develop specific immune checkpoint inhibitors have begun to provide new immunotherapeutic approaches for treating cancer, including the development of an antibody, ipilimumab, that binds to and inhibits Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) for the treatment of patients with advanced melanoma (Hodi et al., 2010). While cancer remains as an incurable disease for the great majority of patients, there exists a particular need for developing effective therapeutic agents that can be used in cancer immunotherapy.

SUMMARY OF THE INVENTION

[0005] Some embodiments relate to a pharmaceutical composition including Plinabulin and one or more immune checkpoint inhibitor.

[0006] Some embodiments relate to a method for treating cancer, the method including co-administering Plinabulin and one or more immune checkpoint inhibitor to a subject in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIG. 1A shows the expression of DC maturation markers CD40, CD80, CD86, and MHCII in dendritic cells treated with Plinabulin at various concentrations and with LPS control; FIG. 1B shows the viability of dendritic cells treated with Plinabulin and LPS.

[0008] FIG. 2A shows the expression of the CD40 marker in dendritic cells treated with Plinabulin, Paclitaxel, Etoposide, or control; FIG. 2B shows the expression of the CD80 marker in dendritic cells treated with Plinabulin, Paclitaxel, Etoposide, or control; FIG. 2C shows the expression of the CD86 marker in dendritic cells treated with Plinabulin, Paclitaxel, Etoposide, or control; FIG. 2D shows the expression of the MHCII marker in dendritic cells treated with Plinabulin, Paclitaxel, Etoposide, or control.

[0009] FIG. 3A shows the production of IL-1β in dendritic cells treated with Plinabulin, Paclitaxel. Etoposide, and control; FIG. 3B shows the production of IL-6 marker in dendritic cells treated with Plinabulin, Paclitaxel. Etoposide, and control; FIG. 3C shows the production of IL-12p40 in dendritic cells treated with Plinabulin, Paclitaxel. Etoposide, and control.

[0010] FIGS. 4A-4C show the plinabulin-induced enhancement of the anti-tumor effect of the PD-1 antibody plus CTLA-4 antibody in the MC-38 tumor model in immune competent mice. FIG 4A shows the effect on tumor growth; FIG 4B shows the effect on the mean tumor weight at necropsy; Fig 4C shows the time for tumors to reach 10 fold of their starting volume.

[0011] FIGS. 5A-5C show the results of Fluorescence-activated cell sorting (FACS) analysis of the tumors at necropsy from the study described in Example 6. FIG. 5A

shows the effect on Treg cells; FIG 5B shows the ratio of CD8+ cells to Treg cells; FIG 5C shows the effect on macrophages.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0012] Plinabulin, (3*Z*,6*Z*)-3-Benzylidene-6-{[5-(2-methyl-2-propanyl)-1*H*-imidazol-4-yl]methylene}-2,5-piperazinedione, is a synthetic analog of the natural compound phenylahistin. Plinabulin can be readily prepared according to methods and procedures detailed in U.S. Patent Nos. 7,064,201 and 7,919,497, which are incorporated herein by reference in their entireties. In some embodiments, Plinabulin can efficiently promote antigen uptake and migration of dendritic cells to lymph nodes where tumor-specific antigens are presented by dendritic cells to prime immune effector cells. Exposure of dendritic cells to Plinabulin can induce maturation of dendritic cells and significantly increase their capacity to prime T cells. In some embodiments, Plinabulin can mediate tumor size reduction through immune modulation of the tumor microenvironment to promote anti-tumor immune enhancing effects. In some embodiments, substantial therapeutic synergies can be achieved when combining Plinabulin with immune checkpoint inhibitors.

[0013] Some embodiments relate to the use of Plinabulin in combination with one or more immune checkpoint inhibitors, such as inhibitors of CTLA4 (cytotoxic T lymphocyte antigen-4), PD-1 (programmed cell death protein 1), PD-L1 (programmed cell death ligand 1), PD-L2(programmed cell death ligand 2), PD-L3(programmed cell death ligand 3), PD-L4(programmed cell death ligand 4), LAG-3 (lymphocyte activation gene-3), and TIM-3 (T cell immunoglobulin and mucin protein-3). In some embodiments, the immune checkpoint inhibitor is a binding ligand of PD-1. In some embodiments, the immune checkpoing inhibitor is a binding ligand of CTLA-4.

[0014] PD-1 is a key immune checkpoint receptor expressed by activated T and B cells and mediates immunosuppression. PD-1 is a member of the CD28 family of receptors, which includes CD28, CTLA-4, ICOS, PD-1, and BTLA. The term "PD-1" as used herein includes human PD-1 (hPD-1), variants, isoforms, and species homologs of hPD-1, and analogs having at least one common epitope with hPD-1.

[0015] Various cell surface glycoprotein ligands for PD-1 have been identified, including PD-L1, PD-L2, PD-L3, and PD-L4, that are expressed on antigen- presenting cells as well as many human cancers and have been shown to downregulate T cell activation and cytokine secretion upon binding to PD-1. The term "PD-L1" as used herein includes human PD-L1 (hPD-Ll), variants, isoforms, and species homologs of hPD-Ll, and analogs having at least one common epitope with hPD-Ll. The term "PD-L2" as used herein includes human PD-L2 (hPD-L2), variants, isoforms, and species homologs of hPD-L2, and analogs having at least one common epitope with hPD-L2. The term "PD-L3" as used herein includes human PD-L3 (hPD-L3), variants, isoforms, and species homologs of hPD-L3, and analogs having at least one common epitope with hPD-L3. The term "PD-L4" as used herein includes human PD-L4 (hPD-L4), variants, isoforms, and species homologs of hPD-L4, and analogs having at least one common epitope with hPD-L4.

[0016] CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) is a protein receptor that, functioning as an immune checkpoint, downregulates the immune system. CTLA4 is found on the surface of T cells, is also a member of the immunoglobulin (Ig) superfamily; CTLA-4 comprises a single extracellular Ig domain. CTLA-4 transcripts have been found in T cell populations having cytotoxic activity, suggesting that CTLA-4 might function in the cytolytic response.

Definitions

[0017] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this disclosure belongs. All patents, applications, published applications, and other publications are incorporated by reference in their entirety. In the event that there is a plurality of definitions for a term herein, those in this section prevail unless stated otherwise.

[0018] The term “pharmaceutically acceptable carrier” or “pharmaceutically acceptable excipient” includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. In addition, various adjuvants such as are

commonly used in the art may be included. Considerations for the inclusion of various components in pharmaceutical compositions are described, e.g., in Gilman et al. (Eds.) (1990); Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th Ed., Pergamon Press, which is incorporated herein by reference in its entirety. The pharmaceutically acceptable excipient can be a monosaccharide or monosaccharide derivative.

[0019] "Subject" as used herein, means a human or a non-human mammal, e.g., a dog, a cat, a mouse, a rat, a cow, a sheep, a pig, a goat, a non-human primate or a bird, e.g., a chicken, as well as any other vertebrate or invertebrate.

[0020] The term "mammal" is used in its usual biological sense. Thus, it specifically includes, but is not limited to, primates, including simians (chimpanzees, apes, monkeys) and humans, cattle, horses, sheep, goats, swine, rabbits, dogs, cats, rodents, rats, mice, guinea pigs, or the like.

[0021] An "effective amount" or a "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent that is effective to relieve, to some extent, or to reduce the likelihood of onset of, one or more of the symptoms of a disease or condition, and can include curing a disease or condition.

[0022] "Treat," "treatment," or "treating," as used herein refers to administering a compound or pharmaceutical composition to a subject for prophylactic and/or therapeutic purposes. The term "prophylactic treatment" refers to treating a subject who does not yet exhibit symptoms of a disease or condition, but who is susceptible to, or otherwise at risk of, a particular disease or condition, whereby the treatment reduces the likelihood that the patient will develop the disease or condition. The term "therapeutic treatment" refers to administering treatment to a subject already suffering from a disease or condition.

[0023] As used herein, the term "chemotherapeutic agent" refers to an agent that reduces, prevents, mitigates, limits, and/or delays the growth of metastases or neoplasms, or kills neoplastic cells directly by necrosis or apoptosis of neoplasms or any other mechanism, or that can be otherwise used, in a pharmaceutically-effective amount, to reduce, prevent, mitigate, limit, and/or delay the growth of metastases or neoplasms in a subject with neoplastic disease. Chemotherapeutic agents include but are not limited to, for example,

fluoropyrimidines; pyrimidine nucleosides; purine nucleosides; anti-folates, platinum-based agents; anthracyclines/anthracenediones; epipodophyllotoxins; camptothecins; hormones; hormonal complexes; antihormonals; enzymes, proteins, peptides and polyclonal and/or monoclonal antibodies; vinca alkaloids; taxanes; epothilones; antimicrotubule agents; alkylating agents; antimetabolites; topoisomerase inhibitors; antivirals; and various other cytotoxic and cytostatic agents.

Administration and Pharmaceutical Compositions

[0024] Some embodiments relate to a pharmaceutical composition, comprising Plinabulin and one or more immune checkpoint inhibitor.

[0025] In some embodiments, the immune checkpoint inhibitor is an inhibitor of PD-1, PD-L1, PD-L2, PD-L3, PD-L4, CTLA-4, LAG3, B7-H3, B7-H4, KIR or TIM3. In some embodiments, the immune checkpoint inhibitor is a PD-1 inhibitor. In some embodiments, the immune checkpoint inhibitor is a binding ligand of PD-L1. In some embodiments, the immune checkpoint inhibitor is a PD-L1 inhibitor. In some embodiments, the immune checkpoint inhibitor is a PD-L2 inhibitor or a combined PD-L1/PD-L2 inhibitor. In some embodiments, the immune checkpoint inhibitor is a CTLA-4 inhibitor.

[0026] In some embodiments, the composition described herein includes a first immune checkpoint inhibitor and a second immune checkpoint inhibitor, wherein the first immune checkpoint inhibitor is different from the second immune checkpoint inhibitor. In some embodiments, the first and the second immune checkpoint inhibitor is independently an inhibitor of PD-1, PD-L1, PD-L2, PD-L3, PD-L4, CTLA-4, LAG3, B7-H3, B7-H4, KIR or TIM3. In some embodiments, the first immune checkpoint inhibitor is a PD-1 inhibitor, and the second immune checkpoint inhibitor is a CTLA-4 inhibitor. In some embodiments, the first immune checkpoint inhibitor is a PD-L1 inhibitor, and the second immune checkpoint inhibitor is a CTLA-4 inhibitor. In some embodiments, the first immune checkpoint inhibitor is a PD-L2 inhibitor, and the second immune checkpoint inhibitor is a CTLA-4 inhibitor.

[0027] In some embodiments, the immune checkpoint inhibitor can be a small peptide agent that can inhibit T cell regulation function. In some embodiments, the immune checkpoint inhibitor can be a small molecule (*e.g.* less than 500 Daltons) that can inhibit T cell regulation function. In some embodiments, the immune checkpoint inhibitor can be a

molecule providing co-stimulation of T-cell activation. In some embodiments, the immune checkpoint inhibitor can be a molecule providing co-stimulation of natural killer cell activation. In some embodiments, the immune checkpoint inhibitor can be an antibody. In some embodiments, the immune checkpoint inhibitor is a PD-1 antibody. In some embodiments, the immune checkpoint inhibitor is a PD-L1 antibody. In some embodiments, the immune checkpoint inhibitor is a PD-L2 antibody. In some embodiments, the immune checkpoint inhibitor is a PD-L3 antibody. In some embodiments, the immune checkpoint inhibitor is a PD-L4 antibody. In some embodiments, the immune checkpoint inhibitor is a CTLA-4 antibody. In some embodiments, the immune checkpoint inhibitor is an antibody of CTLA-4, LAG3, B7-H3, B7-H4, KIR, or TIM3.

[0028] The antibody can be selected from α-CD3-APC, α-CD3-APC-H7, α-CD4-ECD, α-CD4-PB, α-CD8-PE-Cy7, α-CD-8-PerCP-Cy5.5, α-CD11c-APC, α-CD11b-PE-Cy7, α-CD11b-AF700, α-CD14-FITC, α-CD16-PB, α-CD19-AF780, α-CD19-AF700, α-CD20-PO, α-CD25-PE-Cy7, α-CD40-APC, α-CD45-Biotin, Streptavidin-BV605, α-CD62L-ECD, α-CD69-APC-Cy7, α-CD80-FITC, α-CD83-Biotin, Streptavidin-PE-Cy7, α-CD86-PE-Cy7, α-CD86-PE, α-CD123-PE, α-CD154-PE, α-CD161-PE, α-CTLA4-PE-Cy7, α-FoxP3-AF488 (clone 259D), IgG1-isotype-AF488, α-ICOS (CD278)-PE, α-HLA-A2-PE, α-HLA-DR-PB, α-HLA-DR-PerCPCy5.5, α-PD1-APC, VISTA, co-stimulatory molecule OX40, and CD137.

[0029] A variety of antibodies (Abs) can be used in the composition described herein, including antibodies having high-affinity binding to PD-1 PD-Ll, PD-L2, PD-L3, or PD-L4. Human mAbs (HuMAbs) that bind specifically to PD-1 (e.g., bind to human PD-1 and may cross-react with PD-1 from other species, such as cynomolgus monkey) with high affinity have been disclosed in U.S. Patent No. 8,008,449, which is incorporated herein by reference in its entirety. HuMAbs that bind specifically to PD-Ll with high affinity have been disclosed in U.S. Patent No. 7,943,743, which is incorporated herein by reference in its entirety. Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 6,808,710, 7,488,802 and 8,168,757, and PCT Publication No. WO 2012/145493, all of which are incorporated herein by reference in their entireties. Anti-PD-Ll mAbs have been described in, for example, U.S. Patent Nos. 7,635,757 and 8,217,149, U.S. Publication No.

2009/0317368, and PCT Publication Nos. WO 2011/066389 and WO 2012/14549, all of which are incorporated herein by reference in their entireties.

[0030] In some embodiments, the anti-PD-1 HuMAbs can be selected from 17D8, 2D3, 4H1, 5C4 (also referred to herein as nivolumab), 4A1 1, 7D3 and 5F4, all of which are described in U.S. Patent No. 8,008,449. In some embodiments, the anti-PD-1 HuMAbs can be selected from 3G10, 12A4 (also referred to herein as BMS-936559), 10A5, 5F8, 10H10, 1B12, 7H1, 1 1E6, 12B7, and 13G4, all of which are described in U.S. Patent No. 7,943,743.

[0031] In some embodiments, the composition can further include one or more pharmaceutically acceptable diluents. In some embodiments, the pharmaceutically acceptable diluent can include Kolliphor HS15® (Polyoxyl (15)-hydroxystearate). In some embodiments, the pharmaceutically acceptable diluent can include propylene glycol. In some embodiments, the pharmaceutically acceptable diluents can include kolliphor and propylene glycol. In some embodiments, the pharmaceutically acceptable diluents can include kolliphor and propylene glycol, wherein the kolliphor is about 40% by weight and propylene glycol is about 60% by weight based on the total weight of the diluents. In some embodiments, the composition can further include one or more other pharmaceutically acceptable excipients.

[0032] Standard pharmaceutical formulation techniques can be used to make the pharmaceutical compositions described herein, such as those disclosed in Remington's The Science and Practice of Pharmacy, 21st Ed., Lippincott Williams & Wilkins (2005), incorporated herein by reference in its entirety. Accordingly, some embodiments include pharmaceutical compositions comprising: (a) a safe and therapeutically effective amount of Plinabulin or pharmaceutically acceptable salts thereof; (b) an immune checkpoint inhibitor and (c) a pharmaceutically acceptable carrier, diluent, excipient or combination thereof.

[0033] Other embodiments include co-administering Plinabulin and one or more immune checkpoint inhibitor in separate compositions. Thus, some embodiments include a first pharmaceutical compositions comprising: (a) a safe and therapeutically effective amount of Plinabulin or pharmaceutically acceptable salts thereof and (b) a pharmaceutically acceptable carrier, diluent, excipient or combination thereof; and a second pharmaceutical composition comprising: (a) one or more immune checkpoint inhibitor and (b) a pharmaceutically acceptable carrier, diluent, excipient or combination thereof.

[0034] Administration of the pharmaceutical compositions described herein can be via any of the accepted modes of administration for agents that serve similar utilities including, but not limited to, orally, sublingually, buccally, subcutaneously, intravenously, intranasally, topically, transdermally, intradermally, intraperitoneally, intramuscularly, intrapulmonarilly, vaginally, rectally, or intraocularly. Oral and parenteral administrations are customary in treating the indications that are the subject of the preferred embodiments.

[0035] The term "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. In addition, various adjuvants such as are commonly used in the art may be included. Considerations for the inclusion of various components in pharmaceutical compositions are described, e.g., in Gilman et al. (Eds.) (1990); Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th Ed., Pergamon Press, which is incorporated herein by reference in its entirety.

[0036] Some examples of substances, which can serve as pharmaceutically-acceptable carriers or components thereof, are sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose, and methyl cellulose; powdered tragacanth; malt; gelatin; talc; solid lubricants, such as stearic acid and magnesium stearate; calcium sulfate; vegetable oils, such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil of theobroma; polyols such as propylene glycol, glycerine, sorbitol, mannitol, and polyethylene glycol; alginic acid; emulsifiers, such as the TWEENS; wetting agents, such sodium lauryl sulfate; coloring agents; flavoring agents; tableting agents, stabilizers; antioxidants; preservatives; pyrogen-free water; isotonic saline; and phosphate buffer solutions.

[0037] The compositions described herein are preferably provided in unit dosage form. As used herein, a "unit dosage form" is a composition containing an amount of a compound or composition that is suitable for administration to an animal, preferably mammal subject, in a single dose, according to good medical practice. The preparation of a single or

unit dosage form however, does not imply that the dosage form is administered once per day or once per course of therapy. Such dosage forms are contemplated to be administered once, twice, thrice or more per day and may be administered as infusion over a period of time (e.g., from about 30 minutes to about 2-6 hours), or administered as a continuous infusion, and may be given more than once during a course of therapy, although a single administration is not specifically excluded. The skilled artisan will recognize that the formulation does not specifically contemplate the entire course of therapy and such decisions are left for those skilled in the art of treatment rather than formulation.

[0038] The compositions useful as described above may be in any of a variety of suitable forms for a variety of routes for administration, for example, for oral, sublingual, buccal, nasal, rectal, topical (including transdermal and intradermal), ocular, intracerebral, intracranial, intrathecal, intra-arterial, intravenous, intramuscular, or other parental routes of administration. The skilled artisan will appreciate that oral and nasal compositions include compositions that are administered by inhalation, and made using available methodologies. Depending upon the particular route of administration desired, a variety of pharmaceutically-acceptable carriers well-known in the art may be used. Pharmaceutically-acceptable carriers include, for example, solid or liquid fillers, diluents, hydrotropies, surface-active agents, and encapsulating substances. Optional pharmaceutically-active materials may be included, which do not substantially interfere with the inhibitory activity of the compound or composition. The amount of carrier employed in conjunction with the compound or composition is sufficient to provide a practical quantity of material for administration per unit dose of the compound. Techniques and compositions for making dosage forms useful in the methods described herein are described in the following references, all incorporated by reference herein: Modern Pharmaceutics, 4th Ed., Chapters 9 and 10 (Banker & Rhodes, editors, 2002); Lieberman *et al.*, Pharmaceutical Dosage Forms: Tablets (1989); and Ansel, Introduction to Pharmaceutical Dosage Forms 8th Edition (2004).

[0039] Various oral dosage forms can be used, including such solid forms as tablets, capsules (*e.g.* solid gel capsules and liquid gel capsules), granules and bulk powders. Tablets can be compressed, tablet triturates, enteric-coated, sugar-coated, film-coated, or multiple-compressed, containing suitable binders, lubricants, diluents, disintegrating agents,

coloring agents, flavoring agents, flow-inducing agents, and melting agents. Liquid oral dosage forms include aqueous solutions, emulsions, suspensions, solutions and/or suspensions reconstituted from non-effervescent granules, and effervescent preparations reconstituted from effervescent granules, containing suitable solvents, preservatives, emulsifying agents, suspending agents, diluents, sweeteners, melting agents, coloring agents and flavoring agents.

[0040] The pharmaceutically-acceptable carriers suitable for the preparation of unit dosage forms for peroral administration is well-known in the art. Tablets typically comprise conventional pharmaceutically-compatible adjuvants as inert diluents, such as calcium carbonate, sodium carbonate, mannitol, lactose and cellulose; binders such as starch, gelatin and sucrose; disintegrants such as starch, alginic acid and croscarmelose; lubricants such as magnesium stearate, stearic acid and talc. Glidants such as silicon dioxide can be used to improve flow characteristics of the powder mixture. Coloring agents, such as the FD&C dyes, can be added for appearance. Sweeteners and flavoring agents, such as aspartame, saccharin, menthol, peppermint, and fruit flavors, are useful adjuvants for chewable tablets. Capsules typically comprise one or more solid diluents disclosed above. The selection of carrier components depends on secondary considerations like taste, cost, and shelf stability, which are not critical, and can be readily made by a person skilled in the art.

[0041] Peroral compositions also include liquid solutions, emulsions, suspensions, and the like. The pharmaceutically-acceptable carriers suitable for preparation of such compositions are well known in the art. Typical components of carriers for syrups, elixirs, emulsions and suspensions include ethanol, glycerol, propylene glycol, polyethylene glycol, liquid sucrose, sorbitol and water. For a suspension, typical suspending agents include methyl cellulose, sodium carboxymethyl cellulose, AVICEL RC-591, tragacanth and sodium alginate; typical wetting agents include lecithin and polysorbate 80; and typical preservatives include methyl paraben and sodium benzoate. Peroral liquid compositions may also contain one or more components such as sweeteners, flavoring agents and colorants disclosed above.

[0042] Such compositions may also be coated by conventional methods, typically with pH or time-dependent coatings, such that the subject composition is released in the

gastrointestinal tract in the vicinity of the desired topical application, or at various times to extend the desired action. Such dosage forms typically include, but are not limited to, one or more of cellulose acetate phthalate, polyvinylacetate phthalate, hydroxypropyl methyl cellulose phthalate, ethyl cellulose, Eudragit coatings, waxes and shellac.

[0043] Compositions described herein may optionally include other drug actives.

[0044] Other compositions useful for attaining systemic delivery of the subject compounds include sublingual, buccal and nasal dosage forms. Such compositions typically comprise one or more of soluble filler substances such as sucrose, sorbitol and mannitol; and binders such as acacia, microcrystalline cellulose, carboxymethyl cellulose and hydroxypropyl methyl cellulose. Glidants, lubricants, sweeteners, colorants, antioxidants and flavoring agents disclosed above may also be included.

[0045] A liquid composition, which is formulated for topical ophthalmic use, is formulated such that it can be administered topically to the eye. The comfort may be maximized as much as possible, although sometimes formulation considerations (e.g. drug stability) may necessitate less than optimal comfort. In the case that comfort cannot be maximized, the liquid may be formulated such that the liquid is tolerable to the patient for topical ophthalmic use. Additionally, an ophthalmically acceptable liquid may either be packaged for single use, or contain a preservative to prevent contamination over multiple uses.

[0046] For ophthalmic application, solutions or medicaments are often prepared using a physiological saline solution as a major vehicle. Ophthalmic solutions may preferably be maintained at a comfortable pH with an appropriate buffer system. The formulations may also contain conventional, pharmaceutically acceptable preservatives, stabilizers and surfactants.

[0047] Preservatives that may be used in the pharmaceutical compositions disclosed herein include, but are not limited to, benzalkonium chloride, PHMB, chlorobutanol, thimerosal, phenylmercuric, acetate and phenylmercuric nitrate. A useful surfactant is, for example, Tween 80. Likewise, various useful vehicles may be used in the ophthalmic preparations disclosed herein. These vehicles include, but are not limited to,

polyvinyl alcohol, povidone, hydroxypropyl methyl cellulose, poloxamers, carboxymethyl cellulose, hydroxyethyl cellulose and purified water.

[0048] Tonicity adjustors may be added as needed or convenient. They include, but are not limited to, salts, particularly sodium chloride, potassium chloride, mannitol and glycerin, or any other suitable ophthalmically acceptable tonicity adjustor.

[0049] Various buffers and means for adjusting pH may be used so long as the resulting preparation is ophthalmically acceptable. For many compositions, the pH will be between 4 and 9. Accordingly, buffers include acetate buffers, citrate buffers, phosphate buffers and borate buffers. Acids or bases may be used to adjust the pH of these formulations as needed.

[0050] Ophthalmically acceptable antioxidants include, but are not limited to, sodium metabisulfite, sodium thiosulfate, acetylcysteine, butylated hydroxyanisole and butylated hydroxytoluene.

[0051] Other excipient components, which may be included in the ophthalmic preparations, are chelating agents. A useful chelating agent is edetate disodium, although other chelating agents may also be used in place or in conjunction with it.

[0052] For topical use, creams, ointments, gels, solutions or suspensions, etc., containing the composition disclosed herein are employed. Topical formulations may generally be comprised of a pharmaceutical carrier, co-solvent, emulsifier, penetration enhancer, preservative system, and emollient.

[0053] For intravenous administration, the compositions described herein may be dissolved or dispersed in a pharmaceutically acceptable diluent, such as a saline or dextrose solution. Suitable excipients may be included to achieve the desired pH, including but not limited to NaOH, sodium carbonate, sodium acetate, HCl, and citric acid. In various embodiments, the pH of the final composition ranges from 2 to 8, or preferably from 4 to 7. Antioxidant excipients may include sodium bisulfite, acetone sodium bisulfite, sodium formaldehyde, sulfoxylate, thiourea, and EDTA. Other non-limiting examples of suitable excipients found in the final intravenous composition may include sodium or potassium phosphates, citric acid, tartaric acid, gelatin, and carbohydrates such as dextrose, mannitol, and dextran. Further acceptable excipients are described in Powell, et al., Compendium of

Excipients for Parenteral Formulations, *PDA J Pharm Sci and Tech* **1998**, *52* 238-311 and Nema et al., Excipients and Their Role in Approved Injectable Products: Current Usage and Future Directions, *PDA J Pharm Sci and Tech* **2011**, *65* 287-332, both of which are incorporated herein by reference in their entirety. Antimicrobial agents may also be included to achieve a bacteriostatic or fungistatic solution, including but not limited to phenylmercuric nitrate, thimerosal, benzethonium chloride, benzalkonium chloride, phenol, cresol, and chlorobutanol.

[0054] The compositions for intravenous administration may be provided to caregivers in the form of one more solids that are reconstituted with a suitable diluent such as sterile water, saline or dextrose in water shortly prior to administration. In other embodiments, the compositions are provided in solution ready to administer parenterally. In still other embodiments, the compositions are provided in a solution that is further diluted prior to administration. In embodiments that include administering a combination of a compound described herein and another agent, the combination may be provided to caregivers as a mixture, or the caregivers may mix the two agents prior to administration, or the two agents may be administered separately.

[0055] The actual dose of the active compounds described herein depends on the specific compound, and on the condition to be treated; the selection of the appropriate dose is well within the knowledge of the skilled artisan. In some embodiments, a daily dose of Plinabulin may be from about 0.25 mg/kg to about 120 mg/kg or more of body weight, from about 0.5 mg/kg or less to about 70 mg/kg, from about 1.0 mg/kg to about 50 mg/kg of body weight, or from about 1.5 mg/kg to about 10 mg/kg of body weight. Thus, for administration to a 70 kg person, the dosage range would be from about 17 mg per day to about 8000 mg per day, from about 35 mg per day or less to about 7000 mg per day or more, from about 70 mg per day to about 6000 mg per day, from about 100 mg per day to about 5000 mg per day, or from about 200 mg to about 3000 mg per day.

[0056] In some embodiments, the compositions described herein can be used in combination with other therapeutic agents. In some embodiments, the compositions described herein can be administered or used in combination with treatments such as chemotherapy, radiation, and biologic therapies.

Method of Treatment

[0057] Some embodiments relate to a method for treating cancer using the pharmaceutical composition described herein to a subject in need thereof. Some embodiments relate to a method for treating cancer, comprising co-administering Plinabulin and one or more immune checkpoint inhibitor to a subject in need thereof. In some embodiments, the subject can be an animal, e.g., a mammal, a human. In some embodiments, the subject is a human.

[0058] Some embodiments relate to methods of providing co-stimulation of T-cell activation against cancer by co-administering plinabulin and one or more immune checkpoint inhibitor. Some embodiments relate to methods of providing co-stimulation of natural killer cells against cancer by co-administering plinabulin and one or more immune checkpoint inhibitor.

[0059] In some embodiments, the cancer comprises cancer cells expressing a binding ligand of PD-1. In some embodiments, the binding ligand of PD-1 is PD-L1. In some embodiments, the binding ligand of PD-1 is PD-L2.

[0060] In some embodiments, the method of treating cancer described herein further includes identifying cancer cells expressing a binding ligand of PD-1. In some embodiments, the method of treating cancer described herein further includes identifying cancer cells expressing PD-L1. In some embodiments, the method of treating cancer described herein further includes identifying cancer cells expressing PD-L2. In some embodiments, the method of treating cancer described herein further includes identifying cancer cells expressing PD-L3 or PD-L4.

[0061] In some embodiments, identifying cancer cells expressing a binding ligand of PD-1 includes using an assay to detect the presence of the binding ligand. Examples of applicable assay include but are not limited to PD-L1 IHC 22C3 pharmDx kit and PD-L1 IHC 28-8 pharmDx available from Dako.

[0062] In some embodiments, the cancer comprises cancer cells expressing a binding ligand of CTLA-4. In some embodiments, the binding ligand of CTLA-4 is B7.1 or B7.2.

[0063] In some embodiments, the method of treating cancer described herein further includes identifying cancer cells expressing a binding ligand of CTLA-4. In some

embodiments, the method of treating cancer described herein further includes identifying cancer cells expressing B7.1 or B7.2.

[0064] In some embodiments, the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, ipilimumab, dacarbazine, BMS 936559, atezolizumab, durvalimumab, or any combinations thereof.

[0065] In some embodiments, cancer is head and neck cancer, lung cancer, stomach cancer, colon cancer, pancreatic cancer, prostate cancer, breast cancer, kidney cancer, bladder cancer, ovary cancer, cervical cancer, melanoma, glioblastoma, myeloma, lymphoma, or leukemia. In some embodiments, the cancer is renal cell carcinoma, malignant melanoma, non-small cell lung cancer (NSCLC), ovarian cancer, Hodgkin's lymphoma or squamous cell carcinoma. In some embodiments, the cancer is selected from breast cancer, colon cancer, rectal cancer, lung cancer, prostate cancer, melanoma, leukemia, ovarian cancer, gastric cancer, renal cell carcinoma, liver cancer, pancreatic cancer, lymphomas and myeloma. In some embodiments, the cancer is a solid tumor or hematological cancer.

[0066] In some embodiments, the cancer does not have any cells expressing PD-1, PD-L1, or PD-L2 at detectable levels.

[0067] In some embodiments, the cancer is selected from breast cancer, colon cancer, rectal cancer, lung cancer, prostate cancer, melanoma, leukemia, ovarian cancer, gastric cancer, renal cell carcinoma, liver cancer, pancreatic cancer, lymphomas and myeloma. In some embodiments, the cancer is a solid tumor or hematological cancer.

[0068] Some embodiments relate to a method of inducing dendritic cell maturation in a cancer patient, comprising administering to a composition comprising Plinabulin to a cancer patient.

[0069] Some embodiments relate to a method of disrupting cancer associated tumor vasculature in a subject comprising co-administering to the subject a compound of plinabulin and one or more immune checkpoint inhibitor.

[0070] Various cancers are associated the formation of tumor vasculature. In some embodiments, the cancer is selected from the group consisting of a melanoma, a pancreatic cancer, a colorectal adenocarcinoma, a brain tumor, acute lymphoblastic leukemia, chronic lymphocytic leukemia, hormone refractory metastatic prostate cancer, metastatic

breast cancer, non-small cell lung cancer, renal cell carcinoma, head and neck cancer, prostate cancer, colon cancer, anaplastic thyroid cancer.

[0071] Some embodiments include co-administering a composition, and/or pharmaceutical composition described herein, with an additional medicament. For example, as described above, some embodiments include co-administering Plinabulin with one or more immune checkpoint inhibitor. By "co-administration," it is meant that the two or more agents are administered in such a manner that administration of one or more agent has an effect on the efficacy and/or safety of the one or more other agent, regardless of when or how they are actually administered. In one embodiment, the agents are administered simultaneously. In one such embodiment, administration in combination is accomplished by combining the agents in a single dosage form. In another embodiment, the agents are administered sequentially. In one embodiment the agents are administered through the same route, such as orally or intravenously. In another embodiment, the agents are administered through different routes, such as one being administered orally and another being administered i.v. In some embodiments, the time period between administration of one or more agent and administration of the co-administered one or more agent can be about 1 hour, 2 hours, 3 hours, 5 hours, 8 hours, 10 hours, 12 hours, 15 hours, 18 hours, 20 hours, 24 hours, 36 hours, 48 hours, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 14 days, 21 days, 28 days, or 30 days.

[0072] In some embodiments, the treatment cycle can include co-administering Plinabulin and one or more immune checkpoint inhibitors in combination with administering Plinabulin alone or administering one or more checkpoint inhibitor alone. In some embodiments, plinabulin and one or more immune checkpoint inhibitor are co-administered on day 1, followed by administration of plinabulin alone after 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, or 3 weeks, and then followed by co-administration of plinabulin and one or more immune checkpoint inhibitor after 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, or 3 weeks. In some embodiments, plinabulin and one or more immune checkpoint inhibitor are administered simultaneously on day 1, followed by administration of plinabulin or one or more immune checkpoint inhibitor alone on a day selected between day 2 and day 31, and then followed by co-administration of plinabulin and

one or more immune checkpoint inhibitor on a day selected between day 3 and day 31. In some embodiments, plinabulin and one or more immune checkpoint inhibitor are co-administered on day 1, followed by administration of plinabulin alone on day 8, and then followed by co-administration of plinabulin and one or more immune checkpoint inhibitor on day 15. In some embodiments, the treatment cycle can be repeated two or more times.

[0073] Examples of additional medicaments include other chemotherapeutic agents.

[0074] In some embodiments, the chemotherapeutic agent can be selected from the group consisting of Abiraterone Acetate, Abitrexate (Methotrexate), Abraxane (Paclitaxel Albumin-stabilized Nanoparticle Formulation), ABVD, ABVE, ABVE-PC , AC, AC-T, Adcetris (Brentuximab Vedotin), ADE, Ado-Trastuzumab Emtansine ,Adriamycin (Doxorubicin Hydrochloride) , Afatinib Dimaleate, Afinitor (Everolimus), Akynzeo (Netupitant and Palonosetron Hydrochloride), Aldara (Imiquimod), Aldesleukin, Alecensa (Alectinib), Alectinib, Alemtuzumab, Alimta (Pemetrexed Disodium), Aloxi (Palonosetron Hydrochloride), Ambochlorin (Chlorambucil), Amboclorin (Chlorambucil), Aminolevulinic Acid, \ Anastrozole, Aprepitant, Aredia (Pamidronate Disodium), Arimidex (Anastrozole), Aromasin (Exemestane), Arranon (Nelarabine), Arsenic Trioxide, Arzerra (Ofatumumab), Asparaginase Erwinia chrysanthemi , Avastin (Bevacizumab), Axitinib, Azacitidine, BEACOPP, Becenum (Carmustine), Beleodaq (Belinostat), Belinostat, Bendamustine Hydrochloride, BEP, Bevacizumab, Bexarotene, Bexxar (Tositumomab and Iodine I 131 Tositumomab), Bicalutamide, BiCNU (Carmustine), Bleomycin, Blinatumomab, Blincyto (Blinatumomab), Bortezomib, Bosulif (Bosutinib), Bosutinib, Brentuximab Vedotin, Busulfan, Cabazitaxel, Cabozantinib-S-Malate, CAF, Campath (Alemtuzumab), Camptosar (Irinotecan Hydrochloride), Capecitabine, CAPOX, Carac (Fluorouracil--Topical), Carboplatin, CARBOPLATIN-TAXOL, Carfilzomib, Carmubris (Carmustine), Carmustine, Carmustine Implant, Casodex (Bicalutamide), CeeNU (Lomustine), Ceritinib, Cerubidine (Daunorubicin Hydrochloride), Cervarix (Recombinant HPV Bivalent Vaccine) , Cetuximab, Chlorambucil, CHLORAMBUCIL-PREDNISONE, CHOP, Cisplatin, Clafen (Cyclophosphamide), Clofarabine, Clofarex (Clofarabine), Clolar (Clofarabine), CMF, Cobimetinib, Cometriq (Cabozantinib-S-Malate), COPDAC, COPP, COPP-ABV, Cosmegen

(Dactinomycin), Cotellic (Cobimetinib), Crizotinib, CVP, Cyclophosphamide, Cyfos (Ifosfamide), Cyramza (Ramucirumab), Cytarabine, Cytarabine Liposome, Cytosar-U (Cytarabine), Cytoxan (Cyclophosphamide), Dabrafenib, Dacarbazine, Dacogen (Decitabine), Dactinomycin, Daratumumab, Darzalex (Daratumumab), Dasatinib, Daunorubicin Hydrochloride, Decitabine, Degarelix, Denileukin Diftitox, Denosumab, DepoCyt (Cytarabine Liposome), Dexamethasone, Dexrazoxane Hydrochloride, Dinutuximab, Docetaxel, Doxil (Doxorubicin Hydrochloride Liposome), Doxorubicin Hydrochloride, Doxorubicin Hydrochloride Liposome, Dox-SL (Doxorubicin Hydrochloride Liposome), DTIC-Dome (Dacarbazine), Efudex (Fluorouracil--Topical), Elitek (Rasburicase), Ellence (Epirubicin Hydrochloride), Elotuzumab, Eloxatin (Oxaliplatin), Eltrombopag Olamine, Emend (Aprepitant), Empliciti (Elotuzumab), Enzalutamide, Epirubicin Hydrochloride, EPOCH, Erbitux (Cetuximab), Eribulin Mesylate, Erivedge (Vismodegib), Erlotinib Hydrochloride, Erwinaze (Asparaginase Erwinia chrysanthemi), Etopophos (Etoposide Phosphate), Etoposide, Etoposide Phosphate, Evacet (Doxorubicin Hydrochloride Liposome), Everolimus, Evista (Raloxifene Hydrochloride), Exemestane, 5-FU (Fluorouracil Injection), 5-FU (Fluorouracil--Topical), Fareston (Toremifene), Farydak (Panobinostat), Faslodex (Fulvestrant), FEC, Femara (Letrozole), Filgrastim, Fludara (Fludarabine Phosphate), Fludarabine Phosphate, Fluoroplex (Fluorouracil--Topical), Fluorouracil Injection, Fluorouracil—Topical, Flutamide, Folex (Methotrexate), Folex PFS (Methotrexate), FOLFIRI, FOLFIRI-BEVACIZUMAB, FOLFIRI-CETUXIMAB, FOLFIRINOX, FOLFOX, Folotyn (Pralatrexate), FU-LV, Fulvestrant, Gardasil (Recombinant HPV Quadrivalent Vaccine), Gardasil 9 (Recombinant HPV Nonavalent Vaccine), Gazyva (Obinutuzumab), Gefitinib, Gemcitabine Hydrochloride, GEMCITABINE-CISPLATIN, GEMCITABINE-OXALIPLATIN ,Gemtuzumab Ozogamicin, Gemzar (Gemcitabine Hydrochloride), Gilotrif (Afatinib Dimaleate), Gleevec (Imatinib Mesylate), Gliadel (Carmustine Implant), Gliadel wafer (Carmustine Implant), Glucarpidase, Goserelin Acetate, Halaven (Eribulin Mesylate),Herceptin (Trastuzumab), HPV Bivalent Vaccine, Recombinant, HPV Nonavalent Vaccine, Recombinant, HPV Quadrivalent Vaccine, Recombinant, Hycamtin (Topotecan Hydrochloride), Hyper-CVAD, Ibrance (Palbociclib), Ibritumomab Tiuxetan, Ibrutinib, ICE, Iclusig (Ponatinib Hydrochloride), Idamycin (Idarubicin Hydrochloride), Idelalisib, Ifex

(Ifosfamide), Ifosfamide, IL-2 (Aldesleukin), Imatinib Mesylate, Imbruvica (Ibrutinib), Imiquimod, Imlygic (Talimogene Laherparepvec), Inlyta (Axitinib), Interferon Alfa-2b, Recombinant, Interleukin-2 (Aldesleukin), Intron A (Recombinant Interferon Alfa-2b), Iodine I 131 Tositumomab and Tositumomab, Ipilimumab, Iressa (Gefitinib), Irinotecan Hydrochloride, Irinotecan Hydrochloride Liposome, Istodax (Romidepsin), Ixabepilone, Ixazomib Citrate, Ixempra (Ixabepilone), Jakafi (Ruxolitinib Phosphate), Jevtana (Cabazitaxel), Kadcyla (Ado-Trastuzumab Emtansine), Keoxifene (Raloxifene Hydrochloride), Kepivance (Palifermin), Keytruda (Pembrolizumab), Kyprolis (Carfilzomib), Lanreotide Acetate, Lapatinib Ditosylate, Lenalidomide, Lenvatinib Mesylate, Lenvima (Lenvatinib Mesylate), Letrozole, Leucovorin Calcium, Leukeran (Chlorambucil), Leuprolide Acetate, Levulan (Aminolevulinic Acid), Linfolizin (Chlorambucil), LipoDox (Doxorubicin Hydrochloride Liposome), Lomustine, Lonsurf (Trifluridine and Tipiracil Hydrochloride), Lupron (Leuprolide Acetate) ,Lupron Depot (Leuprolide Acetate), Lupron Depot-Ped (Leuprolide Acetate), Lupron Depot-3 Month (Leuprolide Acetate), Lupron Depot-4 Month (Leuprolide Acetate), Lynparza (Olaparib), Marqibo (Vincristine Sulfate Liposome), Matulane (Procarbazine Hydrochloride), Mechlorethamine Hydrochloride, Megace (Megestrol Acetate), Megestrol Acetate, Mekinist (Trametinib), Mercaptopurine, Mesna, Mesnex (Mesna), Methazolastone (Temozolomide), Methotrexate, Methotrexate LPF (Methotrexate), Mexate (Methotrexate), Mexate-AQ (Methotrexate), Mitomycin C, Mitoxantrone Hydrochloride, Mitozytrex (Mitomycin C), MOPP,Mozobil (Plerixafor), Mustargen (Mechlorethamine Hydrochloride),,Mutamycin (Mitomycin C), Myleran (Busulfan), Mylosar (Azacitidine), Mylotarg (Gemtuzumab Ozogamicin), Nanoparticle Paclitaxel (Paclitaxel Albumin-stabilized Nanoparticle Formulation), Navelbine (Vinorelbine Tartrate), Necitumumab, Nelarabine, Neosar (Cyclophosphamide), Netupitant and Palonosetron Hydrochloride, Neupogen (Filgrastim), Nexavar (Sorafenib Tosylate) , Nilotinib, Ninlaro (Ixazomib Citrate), Nivolumab, Nolvadex (Tamoxifen Citrate), Nplate (Romiplostim), Obinutuzumab, Odomzo (Sonidegib), OEPA, Ofatumumab, OFF, Olaparib, Omacetaxine Mepesuccinate, Oncaspar (Pegaspargase), Ondansetron Hydrochloride, Onivyde (Irinotecan Hydrochloride Liposome), Ontak (Denileukin Diftitox), Opdivo (Nivolumab), OPPA , Osimertinib, Oxaliplatin, Paclitaxel, Paclitaxel Albumin-stabilized

Nanoparticle Formulation, PAD, Palbociclib, Palifermin, Palonosetron Hydrochloride, Palonosetron Hydrochloride and Netupitant, Pamidronate Disodium, Panitumumab, Panobinostat, Paraplat (Carboplatin), Paraplatin (Carboplatin), Pazopanib Hydrochloride, PCV , Pegaspargase, Peginterferon Alfa-2b, PEG-Intron (Peginterferon Alfa-2b), Pembrolizumab, Pemetrexed Disodium Perjeta (Pertuzumab), Pertuzumab, Platinol (Cisplatin), Platinol-AQ (Cisplatin), Plerixafor, Pomalidomide, Pomalyst (Pomalidomide), Ponatinib Hydrochloride, Portrazza (Necitumumab), Pralatrexate, Prednisone, Procarbazine Hydrochloride, Proleukin (Aldesleukin), Prolia (Denosumab), Promacta (Eltrombopag Olamine), Provenge (Sipuleucel-T), Purinethol (Mercaptopurine), Purixan (Mercaptopurine), Radium 223 Dichloride, Raloxifene Hydrochloride, Ramucirumab, Rasburicase, R-CHOP, R-CVP, Recombinant Human Papillomavirus (HPV) Bivalent Vaccine, Recombinant Human Papillomavirus (HPV) Nonavalent Vaccine, Recombinant Human Papillomavirus (HPV) Quadrivalent Vaccine, Recombinant Interferon Alfa-2b, Regorafenib, R-EPOCH, Revlimid (Lenalidomide) , Rheumatrex (Methotrexate), Rituximab, Rolapitant Hydrochloride, Romidepsin , Romiplostim, Rubidomycin (Daunorubicin Hydrochloride), Ruxolitinib Phosphate, Sclerosol Intrapleural Aerosol (Talc),Siltuximab, Sipuleucel-T, Somatuline Depot (Lanreotide Acetate), Sonidegib, Sorafenib Tosylate, Sprycel (Dasatinib), STANFORD V, Sterile Talc Powder (Talc), Steritalc (Talc), Stivarga (Regorafenib), Sunitinib Malate, Sutent (Sunitinib Malate), Sylatron (Peginterferon Alfa-2b), Sylvant (Siltuximab), Synovir (Thalidomide), Synribo (Omacetaxine Mepesuccinate), Tabloid (Thioguanine), TAC, Tafinlar (Dabrafenib), Tagrisso (Osimertinib), Talc, Talimogene Laherparepvec, Tamoxifen Citrate, Tarabine PFS (Cytarabine), Tarceva (Erlotinib Hydrochloride), Targretin (Bexarotene), Tasigna (Nilotinib), Taxol (Paclitaxel), Taxotere (Docetaxel), Temodar (Temozolomide), Temozolomide, Temsirolimus, Thalidomide, Thioguanine, Thiotepa, Tolak (Fluorouracil--Topical), Toposar (Etoposide), Topotecan Hydrochloride, Toremifene, Torisel (Temsirolimus), Tositumomab and Iodine I 131, Tositumomab, Totect (Dexrazoxane Hydrochloride), TPF, Trabectedin, Trametinib, Trastuzumab, Treanda (Bendamustine Hydrochloride) , Trifluridine and Tipiracil Hydrochloride, Trisenox (Arsenic Trioxide), Tykerb (Lapatinib Ditosylate), Unituxin (Dinutuximab), Uridine Triacetate, VAC, Vandetanib, VAMP, Varubi (Rolapitant

Hydrochloride), Vectibix (Panitumumab), VelP, Velban (Vinblastine Sulfate), Velcade (Bortezomib), Velsar (Vinblastine Sulfate), Vemurafenib, VePesid (Etoposide), Viadur (Leuprolide Acetate), Vidaza (Azacitidine), Vinblastine Sulfate, Vincasar PFS (Vincristine Sulfate), Vincristine Sulfate, Vincristine Sulfate Liposome, Vinorelbine Tartrate, VIP, Vismodegib, Vistogard (Uridine Triacetate), Voraxaze (Glucarpidase), Vorinostat, Votrient (Pazopanib Hydrochloride), Wellcovorin (Leucovorin Calcium), Xalkori (Crizotinib), Xeloda (Capecitabine), XELIRI,XELOX, Xgeva (Denosumab), Xofigo (Radium 223 Dichloride), Xtandi (Enzalutamide), Yervoy (Ipilimumab),Yondelis (Trabectedin), Zaltrap (Ziv-Aflibercept), Zarxio (Filgrastim), Zelboraf (Vemurafenib), Zevalin (Ibritumomab Tiuxetan), Zinecard (Dexrazoxane Hydrochloride), Ziv-Aflibercept, Zofran (Ondansetron Hydrochloride), Zoladex (Goserelin Acetate), Zoledronic Acid, Zolinza (Vorinostat), Zometa (Zoledronic Acid), Zydelig (Idelalisib), Zykadia (Ceritinib), and Zytiga (Abiraterone Acetate).

[0075] To further illustrate this invention, the following examples are included. The examples should not, of course, be construed as specifically limiting the invention. Variations of these examples within the scope of the claims are within the purview of one skilled in the art and are considered to fall within the scope of the invention as described, and claimed herein. The reader will recognize that the skilled artisan, armed with the present disclosure, and skill in the art is able to prepare and use the invention without exhaustive examples.

EXAMPLES

Example 1. Plinabulin Effect on Dendritic Cell Maturation

[0076] Cell lines: The immature mouse DC cell line SP37A3 (provided by Merck KGaA) was cultured in Iscove's Modified Dulbecco's Medium (IMDM; Sigma) supplemented with 10% heat-inactivated and endotoxin-tested FBS (PAA), sodium pyruvate (Gibco), penicillin/streptomycin L-glutamine mix (Gibco), Eagle's Minimum Essential Medium (MEM) nonessential amino acids (Sigma), Ciproxin (Bayer), and 0.05 mmol/L 2-mercaptoethanol (Gibco). IMDM complete medium was supplemented with 20 ng/mL recombinant mouse GM-CSF and 20 ng/mL recombinant mouse M-CSF (both Peprotech). The murine tumor cell lines EG7 and 3LL-OVA were obtained from ATCC or provided by

Douglas T. Fearon (Cancer Research UK Cambridge Institute, Li Ka Shing Centre, University of Cambridge, Cambridge, UK), respectively. All cell lines were tested and validated to be Mycoplasma-free. Expression of OVA in EG7 and 3LL-OVA, and of Thy1.1 in RMAThy1.1, respectively, was confirmed; no genomic authentication was performed.

[0077] SP37A3 DCs (murine DC line, Merck) were plated ($8x10^4$ cells/well, 96-well flat bottom, tissue-culture treated) in 180 uL IMDM complete medium [IMDM medium (Sigma) supplemented with 10% heat-inactivated and endotoxin-tested FBS (PAA), sodium pyruvate (Gibco), penicillin/streptomycin L-glutamine mix (Gibco), MEM nonessential amino acids (Sigma) and 0.05 mM 2-mercaptoethanol (Gibco)]. IMDM complete medium was supplemented with 20 ng/mL recombinant mouse GM-CSF. DCs were allowed to adhere for two hours before Plinabulin, medium, or LPS as controls were added 10x concentrated in 20 uL. DCs were incubated with Plinabulin in various concentrations (0.001 μM, 0.01 μM, 0.1 μM, 1 μM, 10 μM), medium, and LPS respectively for 20 h. Supernatants of these cultures were collected and used for detection of cytokine production by ELISA (kits available from BD) and the cells were stained with the LD-IR viability dye (Invitrogen) as well as with fluorochrom-labeled monoclonal antibodies against CD80, CD86, CD40 and MHCII for flow cytometric analysis. Cells were analyzed using a BD Fortessa Cytometer equipped with DIVA software. Mean fluorescence intensity (MFI) of the DC maturation markers CD40, CD80, CD86 and MHCII in live cells was normalized to the MFI of those markers detected in untreated (medium) DCs. As shown in FIG. 1A, Plinabulin significantly increased expression of all four DC maturation markers: CD40, CD80, CD86 and MHCII. DC viability did not change significantly at any of the drug concentrations tested, as determined using SytoxGreen staining, as shown in FIG. 1B.

Example 2. Plinabulin in comparison with Paclitaxel and Etoposide on Dendritic Cell Maturation

[0078] Two other cancer drugs, Paclitaxel and Etoposide, were also tested to compare their effects on DC maturation with Plinabulin. SP37A3 DCs (murine DC line, Merck) were plated ($8x10^4$ cells/well, 96-well flat bottom, tissue-culture treated) in 180 uL IMDM complete medium [IMDM medium (Sigma) supplemented with 10% heat-inactivated and endotoxin-tested FBS (PAA), sodium pyruvate (Gibco), penicillin/streptomycin L-

glutamine mix (Gibco), MEM nonessential amino acids (Sigma) and 0.05 mM 2-mercaptoethanol (Gibco)]. IMDM complete medium was supplemented with 20 ng/mL recombinant mouse GM-CSF. DCs were allowed to adhere for two hours before Plinabulin, Paclitaxel, Etoposide, medium, or LPS (positive control) were added 10x concentrated in 20 uL. DCs were incubated with Plinabulin (0.001 μM, 0.01 μM, 0.1 μM, 1 μM, 10 μM), Paclitaxel (0.001 μM, 0.01 μM, 0.1 μM, 1 μM, 10 μM), Etoposide (0.001 μM, 0.01 μM, 0.1 μM, 1 μM, 10 μM), medium, and LPS (positive control) respectively for 20h. Supernatants of these cultures were collected and used for detection of cytokine production by ELISA (kits available from BD) and the cells were stained with the LD-IR viability dye (Invitrogen) as well as with fluorochrom-labeled monoclonal antibodies against CD80, CD86, CD40 and MHCII for flow cytometric analysis. Cells were analyzed using a BD Fortessa Cytometer equipped with DIVA software. Mean fluorescence intensity (MFI) of the DC maturation markers CD40 (FIG. 2A), CD80(FIG. 2B), CD86 (FIG. 2C) and MHCII(FIG. 2D) in live cells was normalized to the MFI of those markers detected in untreated (medium) DCs. The production of the pro-inflammatory cytokines IL-1β(Fig. 3A), IL-6(Fig. 3B), and IL-12p40(Fig. 3C) were also determined by ELISA. Supernatants from the DC cultures were analyzed for these proinflammatory cytokines that have been demonstrated to play critical roles in regulating T-cell function and antitumor immune responses.

[0079] It was noted that Plinabulin was the most potent inducer of DC maturation among all three drugs. Plinabulin showed much greater expression of all four DC maturation markers, CD 40, CD 80, MHCII, and CD 86 than Paclitaxel and Etoposide. Plinabulin also showed significantly increased expression of all four markers when compared with the positive control LPS. Plinabulin triggered increased production of IL1b, IL6, and IL12, compared to in contrast to Paclitaxel, Etoposide, and LPS. Therefore, Plinabulin increased up-regulation of maturation markers and production of pro-inflammatory cytokines, resulting in an enhanced T cell stimulatory capacity.

Example 3. Synergy of Plinabulin and immune checkpoint inhibitors (PD-1 antibody)

[0080] The combined treatment with Plinabulin and a PD-1 checkpoint inhibitor is tested in comparison with the treatment with Plinabulin alone and the treatment with PD-1 antibody alone. The tests are performed using seven to ten-week old mice that are injected

subcutaneously with MC-38 tumor cells. Five testing groups are prepared, and each group includes 9 mice.

[0081] Group 1 is administered with saline; Group 2 is administered with the Plinabulin diluent (in the absence of Plinabulin); Group 3 is administered with Plinabulin dissolved in diluent at a concentration of 7.5 mg/kg; Group 4 is administered with PD-1 antibody; and Group 5 is administered with a Plinabulin/PD-1 antibody combined treatment. For the Plinabulin/PD-1 antibody combined treatment (Group 5), the mice are administered twice per week (Day 1 and Day 4 of each week) with Plinabulin (7.5 mg/kg) that is dissolved in diluent, followed by administering PD-1 antibody one hour after each Plinabulin administration. For the Plinabulin only treatment (Group 3) or the antibody only treatment (Group 4), mice are administered Plinabulin (7.5 mg/kg dissolved in diluent) or antibody alone twice per week (Day 1 and Day 4 of each week). For Groups 1 and 2, the mice are administered with saline or the Plinabulin diluent alone twice per week.

[0082] Each treatment starts at tumor size of around 125 mm^3 and continues until tumor size of 1500 mm^3 is reached. If the mean tumor size in any group has not reached 1500 mm^3 by Experimental Day 45, treatment will be stopped and tumor size continued to be assessed. To determine the efficacy of each treatment, the following data are collected: mortality rate prior to tumor size reaching 1500 mm^3; the body weight of the mice assessed twice weekly both prior to treatments; the rate of tumor growth as determined by the tumor size measurement (twice every week); the tumor growth index; overall survival rate; and the time required to double tumor size. The test results of the combined treatment with Plinabulin and PD-1 antibody show that Plinabulin acts in synergy with PD-1 antibody in inhibiting tumor growth.

Example 4. *In vivo* stimulation of OVA specific OT-I and OT-II T cells

[0083] SP37A3 cells or day 7 BMDCs are pulsed for 1 hour with OVA full-length protein (0.1 mg/mL) before activation with Plinabulin or with OVA257–264 peptide (T4)/OVA323–339 peptide (500 ng/mL; after activation) and added at the indicated ratios to $CD8^+/CD4^+$T cells purified from OT-I/OT-II transgenic mice ($2x10^5$ total cells/well, 96-well round bottomed plate). $CD4^+$ T cells are loaded with the proliferation dye eFluor670 before co-culture. Proliferation is assessed after 3 days using flow cytometry.

Example 5. *In vivo* stimulation of antigen specific CD4 and CD8 T cells

[0084] Langerhans cells (LC) and spleen cells from naive OT-I and OT-II transgenic mice (Ly5.2) are labeled with eFluor670 and adoptively transferred into C57BL/6-Ly5.1 mice. After 24 hours, mice are immunized via tail-base injection with OVA257–264 peptide (T4: SIINFEKL; low-affinity variant of SIINFEKL) or OVA323–339 peptide together with Plinabulin or LPS. Proliferation of OT-I $CD8^+$ and OT-II $CD4^+$ T cells is assessed 4 days after adoptive transfer by flow cytometry.

Example 6. Analysis of DC homing to tumor draining LNs

[0085] For detection of DC homing upon injection of Plinabulin, mice bearing subcutaneous EG7 tumors are injected intratumorally with FITC-conjugated dextran (100 mg/mouse; Sigma) together with Plinabulin or PBS/carrier (mock control). Single-cell suspensions from tumor draining and nondraining LNs are prepared 48 hours after injection of Plinabulin and analyzed by flow cytometry.

Example 7. Synergy of Plinabulin and immune checkpoint inhibitors (PD-1 antibody and CTLA-4 antibody)

[0086] The combined treatment with Plinabulin and a PD-1 checkpoint inhibitor in combination with a CTLA-4 checkpoint inhibitor was tested in comparison with the treatment with Plinabulin alone, the treatment with PD-1 antibody alone, or the treatment with PD-1 antibody in combination with CTLA-4 antibody. The tests were performed using seven to ten-week old mice that were injected subcutaneously with MC-38 tumor cells. Six testing groups were prepared, and each group included 10 mice.

[0087] Group 1 was administered with IgG2a and plinabulin vehicle; Group 2 was administered with Plinabulin dissolved in diluent at a concentration of 7.5 mg/kg; Group 3 was administered with PD-1 antibody; Group 4 was administered with a Plinabulin/PD-1 antibody combined treatment; Group 5 was administered combined PD-1/CTLA-4 antibodies; and Group 6 was administered combined PD-1 antibody/CTLA-4 antibody/Plinabulin treatment. For the Plinabulin/PD-1 antibody combined treatment (Group 4) and the Plinabulin/PD-1/CTLA-4 antibody treatment (Group 6), the mice were administered twice per week (Day 1 and Day 4 of each week) with Plinabulin (7.5 mg/kg)

that was dissolved in diluent, followed by administering antibody (ies) one hour after each Plinabulin administration. For the Plinabulin only treatment (Group 2) or the antibody (ies) only treatment (Groups 3 and 5), mice were administered Plinabulin (7.5 mg/kg dissolved in diluent) or antibody (ies) alone twice per week (Day 1 and Day 4 of each week).

[0088] Each treatment started at tumor size of around 125 mm^3 and continued until tumor size of 3000 mm^3 was reached. When the mean tumor size for Group 1 reached 3000 mm^3 , the experiment ended. To determine the efficacy of each treatment, the following data were collected: mortality rate prior to tumor size reaching 3000 mm^3; the body weight of the mice assessed twice weekly both prior to treatments; the rate of tumor growth as determined by the tumor size measurement (twice every week); the tumor growth index; overall survival rate; the tumor weight at necropsy; and the time required to increase tumor size 10 fold. At necropsy the tissues were weighed and subjected to FACS analysis.

[0089] The test results of the combined treatment with Plinabulin and PD-1antibody and CTLA-4-antibody showed that Plinabulin acted in synergy with the antibodies in inhibiting tumor growth and had the longest time to reach 10-fold increased tumor weight among the six test groups. FIG. 4A shows the effects of Groups 1, 5, and 6 on tumor growth. As shown in FIG. 4A, Group 6, the combined treatment with Plinabulin, PD-1 antibody and CTLA-4-antibody, had better inhibition of tumor growth than Group 5, the combination of PD-1 antibody and CTLA-4 antibody treatment group, and both groups 5 and 6 showed inhibition of tumor growth when compared with the control group 1. FIG. 4B shows the effects of the six treatment groups on the mean tumor weight at necropsy. As shown in FIG. 4B, the combined treatment with Plinabulin, PD-1 antibody and CTLA-4-antibody produced the lowest mean tumor weight at necropsy, followed by the treatment group with Plinabulin and PD-1 antibody. Fig 4C shows the time for tumors to reach 10 fold of their starting volume in the six treatment groups. As shown in FIG. 4C, the treatment group with Plinabulin, PD-1 antibody and CTLA-4-antibody combined had the longest time for the tumors to reach 10 fold of their starting volume. Therefore, Plinabulin treatment either alone or in combination with PD-1 antibody or PD-1 plus CTLA-4 antibodies, resulted in a decreased tumor weight at necropsy. The combined treatment of Plinabulin, PD-1 antibody and CTLA-4-antibody had better tumor inhibitor effect than the treatment of Plinabulin and

PD-1 antibody, which showed had better tumor inhibitor effect than the treatment of Plinabulin alone.

[0090] FIG.5 shows the results of FACS analysis of the tumors at necropsy, including the percentage change of Treg cells, the ration of CD8+/Treg, and the percentage of macrophages in CD45+ lymphocytes, in the MC-38 CRC tumor model described above. FIG. 5A shows the effects of the six treatment groups on the percentage of Treg cells. As shown in FIG. 5A, the treatment of Plinabulin, PD-1 antibody and CTLA-4-antibody , the treatment of Plinabulin and PD-1 antibody and the treatment of Plinabulin alone all showed a reduction in % Treg cells as compared to the comparator group without plinabulin.. FIG 5B shows the ratio of CD8+ cells to Treg cells. As shown in FIG. 5B, the treatment of Plinabulin, PD-1 antibody and CTLA-4-antibody showed the highest ratio of CD8+/Treg cells. FIG 5C shows the effects of the six treatment groups on macrophages. As shown in FIG. 5C, the treatment group of Plinabulin, PD-1 antibody and CTLA-4-antibody, the treatment group of Plinabulin, and the treatment group of PD-1 antibody and CTLA-4-antibody all showed decreased percentage of macrophage when compared with the respective comparator groups.

[0091] Therefore, the FACS analysis of the tumor tissue demonstrated that treatments of Plinabulin alone, Plinabulin and the immune checkpoint inhibitors (*e.g.*, plinabulin with PD-1 antibody, Plinabulin with PD-1 antibody and CTLA-4-antibody) were associated with a decreased percentage of Regulatory T cells (Treg cells), a decreased percentage of macrophage stained cells, and a concomitant increase in the ratio of CD8+/Treg cells. The decrease of the Treg cells percentage and macrophage stained cells and the increase in the ratio of CD8+/Treg cells were more significant in the treatment groups with plinabulin and immune checkpoint inhibitors than the group with plinabulin alone or antibody(antibodies) alone. These data has demonstrated the synergistic immuno-oncology properties of the combined treatment using Plinabulin and the immune checkpoint inhibitors (*e.g.*, PD-1 antibody and CTLA-4-antibody).

WHAT IS CLAIMED IS:

1. A pharmaceutical composition, comprising Plinabulin and one or more immune checkpoint inhibitor.

2. The composition of claim 1, wherein the immune checkpoint inhibitor is an inhibitor of PD-1, PD-L1, PD-L2, PD-L3, PD-L4, CTLA-4, LAG3, B7-H3, B7-H4, KIR or TIM3.

3. The composition of claim 2, wherein the immune checkpoint inhibitor is a PD-1 inhibitor.

4. The composition of claim 2, wherein the immune checkpoint inhibitor is a PD-L1 inhibitor.

5. The composition of claim 2, wherein the immune checkpoint inhibitor is a PD-L2 inhibitor.

6. The composition of claim 2, wherein the immune checkpoint inhibitor is a CTLA-4 inhibitor.

7. The composition of claim 1, comprising a first immune checkpoint inhibitor and a second immune checkpoint inhibitor, wherein the first immune checkpoint inhibitor is different from the second immune checkpoint inhibitor.

8. The composition of claim 7, wherein the first and the second immune checkpoint inhibitor is independently an inhibitor of PD-1, PD-L1, PD-L2, PD-L3, PD-L4, CTLA-4, LAG3, B7-H3, B7-H4, KIR or TIM3.

9. The composition of claim 8, wherein the first immune checkpoint inhibitor is a PD-1 inhibitor, and the second immune checkpoint inhibitor is a CTLA-4 inhibitor.

10. The composition of claim 8, wherein the first immune checkpoint inhibitor is a PD-L1 inhibitor, and the second immune checkpoint inhibitor is a CTLA-4 inhibitor.

11. The composition of claim 8, wherein the first immune checkpoint inhibitor is a PD-L2 inhibitor, and the second immune checkpoint inhibitor is a CTLA-4 inhibitor.

12. The composition of any one of claims 1 to 11, wherein the immune checkpoint inhibitor is an antibody.

13. The composition of claim 12, wherein the immune checkpoint inhibitor is a PD-1 antibody.

14. The composition of claim 12, wherein the immune checkpoint inhibitor is a PD-L1 antibody.

15. The composition of claim 12, wherein the immune checkpoint inhibitor is a PD-L2 antibody.

16. The composition of claim 12, wherein the immune checkpoint inhibitor is a CTLA-4 antibody.

17. The composition of claim 12, wherein the antibody is selected from α-CD3-APC, α-CD3-APC-H7, α-CD4-ECD, α-CD4-PB, α-CD8-PE-Cy7, α-CD-8-PerCP-Cy5.5, α-CD11c-APC, α-CD11b-PE-Cy7, α-CD11b-AF700, α-CD14-FITC, α-CD16-PB, α-CD19-AF780, α-CD19-AF700, α-CD20-PO, α-CD25-PE-Cy7, α-CD40-APC, α-CD45-Biotin, Streptavidin-BV605, α-CD62L-ECD, α-CD69-APC-Cy7, α-CD80-FITC, α-CD83-Biotin, Streptavidin-PE-Cy7, α-CD86-PE-Cy7, α-CD86-PE, α-CD123-PE, α-CD154-PE, α-CD161-PE, α-CTLA4-PE-Cy7, α-FoxP3-AF488 (clone 259D), IgG1-isotype-AF488, α-ICOS (CD278)-PE, α-HLA-A2-PE, α-HLA-DR-PB, α-HLA-DR-PerCPCy5.5, α-PD1-APC, VISTA, co-stimulatory molecule OX40, and CD137.

18. The composition of anyone of claims 1 to 17, further comprising one or more pharmaceutically acceptable excipients.

19. The composition of anyone of claims 1 to 18, further comprising one or more additional chemotherapeutic agent.

20. The composition of anyone of claims 1 to 19, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, ipilimumab, dacarbazine, BMS 936559, atezolizumab, durvalimumab, or any combinations thereof.

21. A method for treating cancer, comprising administering the pharmaceutical composition of any one of claims 1 to 20 to a subject in need thereof.

22. A method for treating cancer, comprising co-administering Plinabulin and one or more immune checkpoint inhibitor to a subject in need thereof.

23. The method of claim 22, further comprising co-administering one or more additional chemotherapeutic agent.

24. The method of any one of claims 21 to 23, wherein the cancer comprises cancer cells expressing a binding ligand of PD-1.

25. The method of claim 24, wherein the binding ligand of PD-1 is PD-L1 or PD-L2.

26. The method of claim 24, wherein the cancer is head and neck cancer, lung cancer, stomach cancer, colon cancer, pancreatic cancer, prostate cancer, breast cancer, kidney cancer, bladder cancer, ovary cancer, cervical cancer, melanoma, glioblastoma, myeloma, lymphoma, or leukemia.

27. The method of claim 24, wherein the cancer is renal cell carcinoma, malignant melanoma, non-small cell lung cancer (NSCLC), ovarian cancer, Hodgkin's lymphoma or squamous cell carcinoma.

28. The method of any one of claims 21 to 27, wherein the cancer comprises cancer cells expressing a binding ligand of CTLA-4.

29. The method of claim 28, wherein the binding ligand of CTLA-4 is B7.1 or B7.2.

30. The method of any one of claims 22 to 29, wherein the immune checkpoint inhibitor is an inhibitor of PD-1, PD-L1, PD-L2, PD-L3, PD-L4, CTLA-4, LAG3, B7-H3, B7-H4, KIR or TIM3.

31. The method of claim 30, wherein the immune checkpoint inhibitor is a PD-1 inhibitor.

32. The method of claim 30, wherein the immune checkpoint inhibitor is a PD-L1 inhibitor.

33. The method of claim 30, wherein the immune checkpoint inhibitor is a PD-L2 inhibitor.

34. The method of claim 30, wherein the immune checkpoint inhibitor is a CTLA inhibitor.

35. The method of claim any one of claims 22 to 29, comprising a first immune checkpoint inhibitor and a second immune checkpoint inhibitor, wherein the first immune checkpoint inhibitor is different from the second immune checkpoint inhibitor.

36. The method of claim 35, wherein the first and the second immune checkpoint inhibitor is independently an inhibitor of PD-1, PD-L1, PD-L2, PD-L3, PD-L4, CTLA-4, LAG3, B7-H3, B7-H4, KIR or TIM3.

37. The method of claim 36, wherein the first immune checkpoint inhibitor is a PD-1 inhibitor, and the second immune checkpoint inhibitor is a CTLA-4 inhibitor.

38. The method of any one of claims 22 to 29, wherein the immune checkpoint inhibitor is an antibody.

39. The method of claim 38, wherein the immune checkpoint inhibitor is a PD-1 antibody.

40. The method of claim 38, wherein the immune checkpoint inhibitor is a PD-L1 antibody.

41. The method of claim 38, wherein the immune checkpoint inhibitor is a PD-L2 antibody.

42. The method of claim 38, wherein the immune checkpoint inhibitor is a CTLA-4 antibody.

43. The method of claim 38, wherein the antibody is selected from α-CD3-APC, α-CD3-APC-H7, α-CD4-ECD, α-CD4-PB, α-CD8-PE-Cy7, α-CD-8-PerCP-Cy5.5, α-CD11c-APC, α-CD11b-PE-Cy7, α-CD11b-AF700, α-CD14-FITC, α-CD16-PB, α-CD19-AF780, α-CD19-AF700, α-CD20-PO, α-CD25-PE-Cy7, α-CD40-APC, α-CD45-Biotin, Streptavidin-BV605, α-CD62L-ECD, α-CD69-APC-Cy7, α-CD80-FITC, α-CD83-Biotin, Streptavidin-PE-Cy7, α-CD86-PE-Cy7, α-CD86-PE, α-CD123-PE, α-CD154-PE, α-CD161-PE, α-CTLA4-PE-Cy7, α-FoxP3-AF488 (clone 259D), IgG1-isotype-AF488, α-ICOS (CD278)-PE, α-HLA-A2-PE, α-HLA-DR-PB, α-HLA-DR-PerCPCy5.5, α-PD1-APC, VISTA, co-stimulatory molecule OX40, and CD137.

44. The method of any one of claims 22 to 43, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, ipilimumab, dacarbazine, BMS 936559, atezolizumab, durvalimumab, or any combinations thereof.

45. The method of 21 or 22, wherein the cancer is selected from breast cancer, colon cancer, rectal cancer, lung cancer, prostate cancer, melanoma, leukemia, ovarian cancer, gastric cancer, renal cell carcinoma, liver cancer, pancreatic cancer, lymphomas and myeloma.

46. The method of 21 or 22, wherein the cancer is a solid tumor or hematological cancer.

47. The method of claim 21 or 22, wherein the cancer does not have any cells expressing PD-1, PD-L1, or PD-L2.

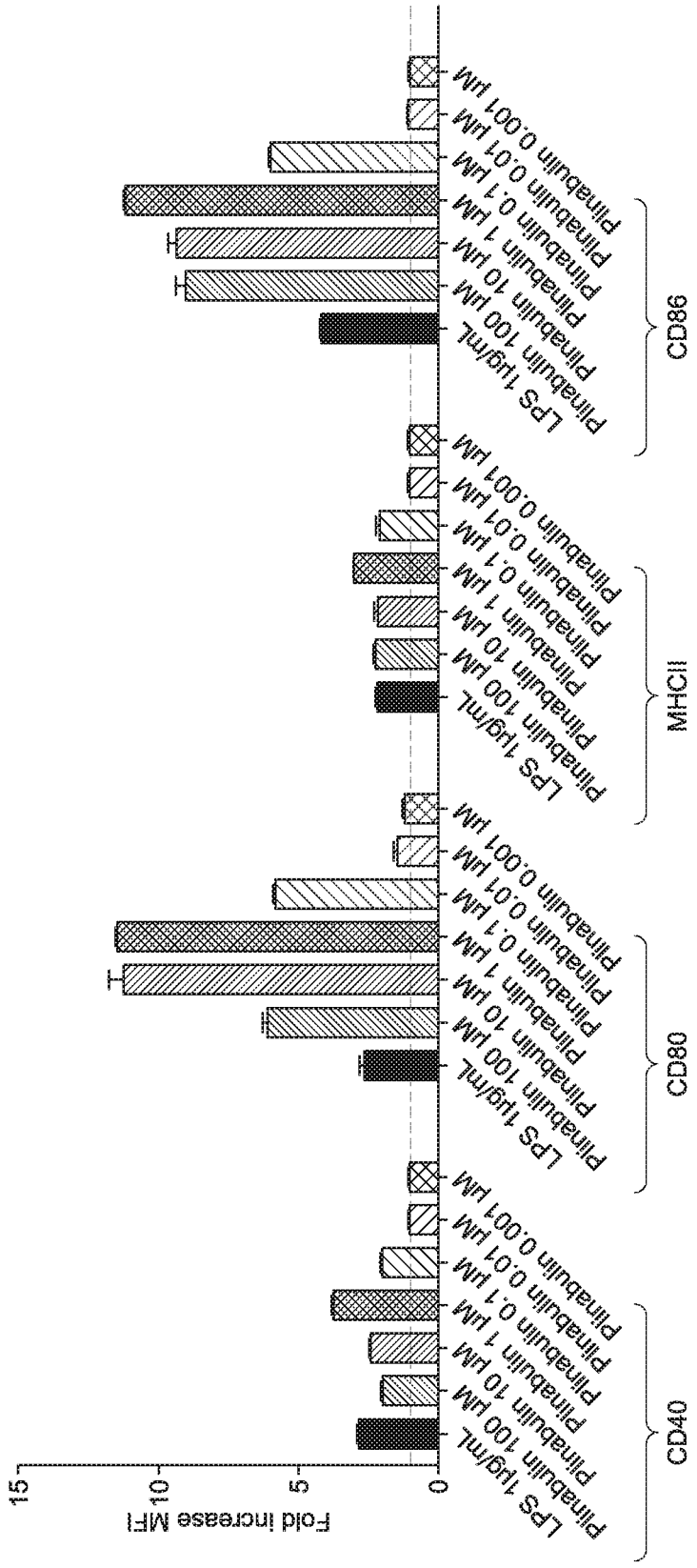

Fold increase MFI
0
5
10
15
CD40
CD80
MHCII
CD86
LPS 1ug/mL
Pilnabulin 100 uM
Pilnabulin 10 uM
Pilnabulin 1 uM
Pilnabulin 0.1 uM
Pilnabulin 0.01 uM
Pilnabulin 0.001 uM

FIG. 1A

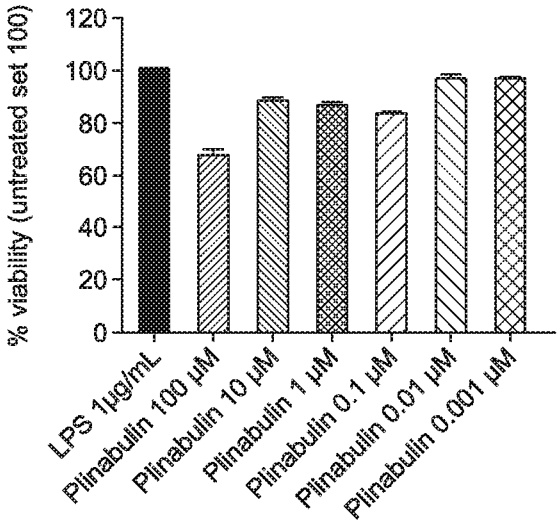

% viability (untreated set 100)
120
100
80
60
40
20
0
LPS 1µg/mL
Plinabulin 100 µM
Plinabulin 10 µM
Plinabulin 1 µM
Plinabulin 0.1 µM
Plinabulin 0.01 µM
Plinabulin 0.001 µM

FIG. 1B

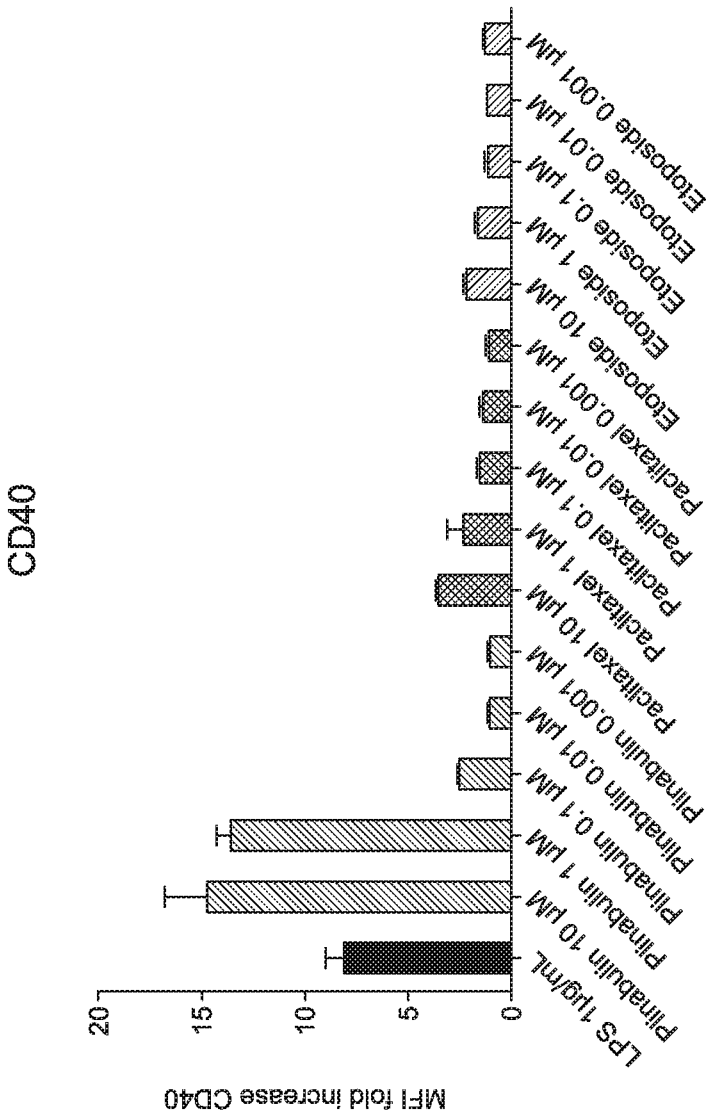

CD40
MFI fold increase CD40
20
15
10
5
0
LPS 1ug/mL
Pilnabulin 10 uM
Pilnabulin 1 uM
Pilnabulin 0.1 uM
Pilnabulin 0.01 uM
Pilnabulin 0.001 uM
Paclitaxel 10 uM
Paclitaxel 1 uM
Paclitaxel 0.1 uM
Paclitaxel 0.01 uM
Paclitaxel 0.001 uM
Etoposide 10 uM
Etoposide 1 uM
Etoposide 0.1 uM
Etoposide 0.01 uM
Etoposide 0.001 uM

FIG. 2A

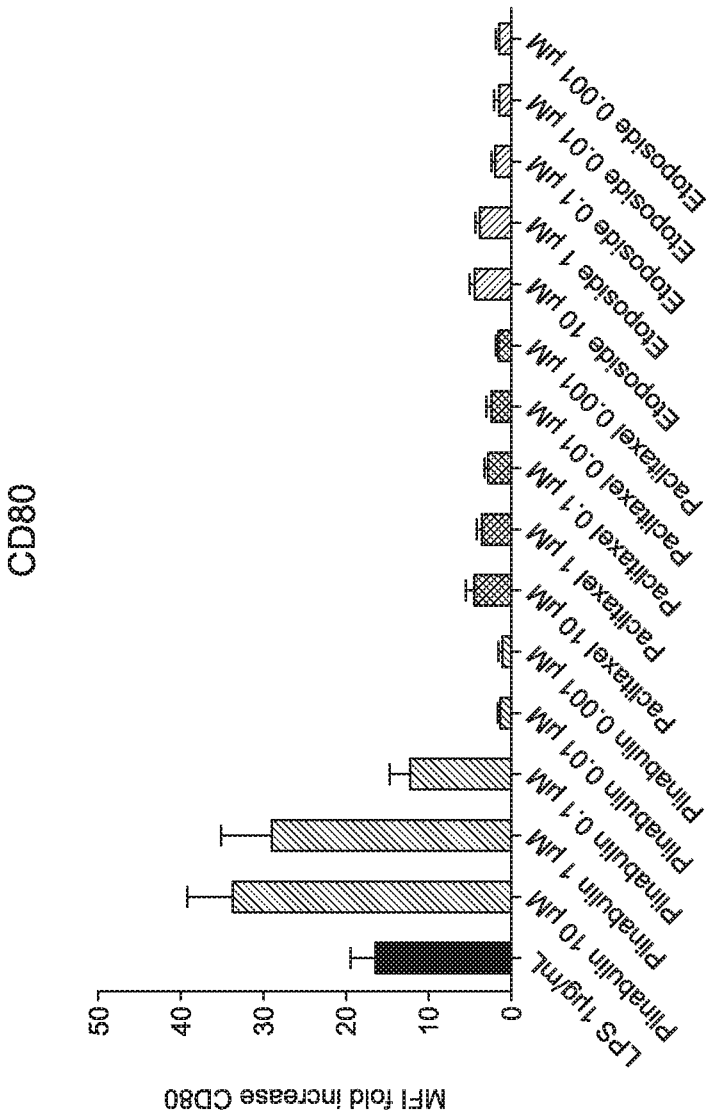

CD80
MFI fold increase CD80
50
40
30
20
10
0
LPS 1ug/mL
Pilnabulin 10 uM
Pilnabulin 1 uM
Pilnabulin 0.1 uM
Pilnabulin 0.01 uM
Pilnabulin 0.001 uM
Paclitaxel 10 uM
Paclitaxel 1 uM
Paclitaxel 0.1 uM
Paclitaxel 0.01 uM
Paclitaxel 0.001 uM
Etoposide 10 uM
Etoposide 1 uM
Etoposide 0.1 uM
Etoposide 0.01 uM
Etoposide 0.001 uM

FIG. 2B


CD86
MFI fold increase CD86
20
15
10
5
0
LPS 1ug/mL
Pilnabulin 10 uM
Pilnabulin 1 uM
Pilnabulin 0.1 uM
Pilnabulin 0.01 uM
Pilnabulin 0.001 uM
Paclitaxel 10 uM
Paclitaxel 1 uM
Paclitaxel 0.1 uM
Paclitaxel 0.01 uM
Paclitaxel 0.001 uM
Etoposide 10 uM
Etoposide 1 uM
Etoposide 0.1 uM
Etoposide 0.01 uM
Etoposide 0.001 uM

FIG. 2C

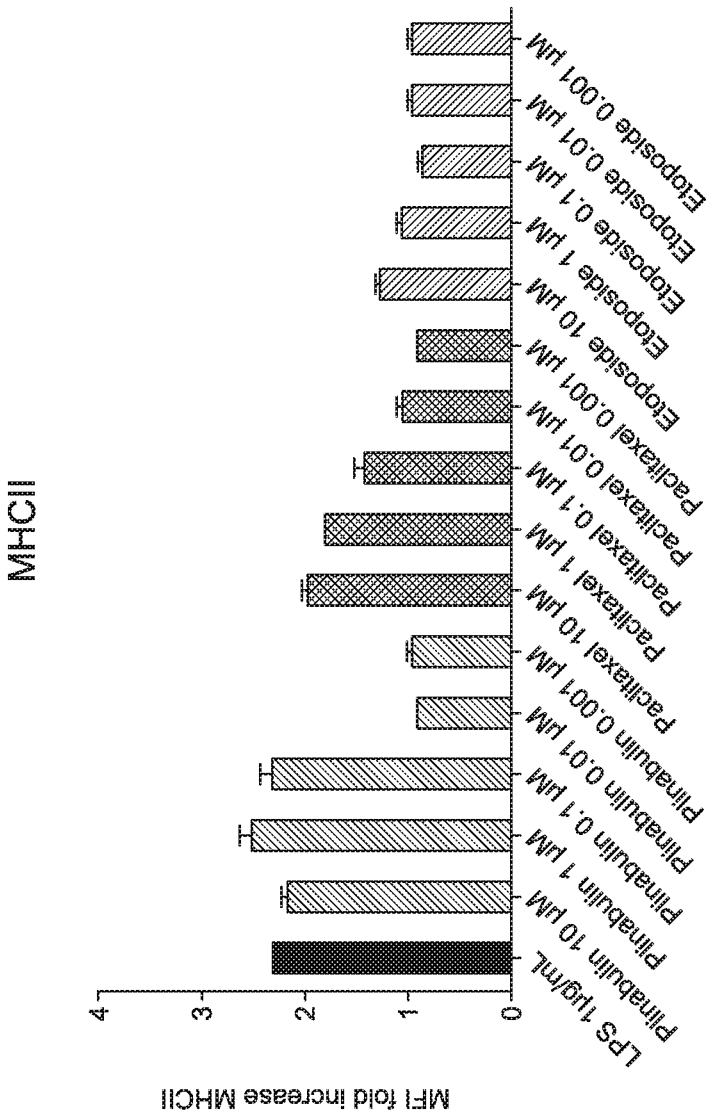

MHCII
MFI fold increase MHCII
0
1
2
3
4
LPS 1ug/mL
Pilnabulin 10 uM
Pilnabulin 1 uM
Pilnabulin 0.1 uM
Pilnabulin 0.01 uM
Pilnabulin 0.001 uM
Paclitaxel 10 uM
Paclitaxel 1 uM
Paclitaxel 0.1 uM
Paclitaxel 0.01 uM
Paclitaxel 0.001 uM
Etoposide 10 uM
Etoposide 1 uM
Etoposide 0.1 uM
Etoposide 0.01 uM
Etoposide 0.001 uM

FIG. 2D

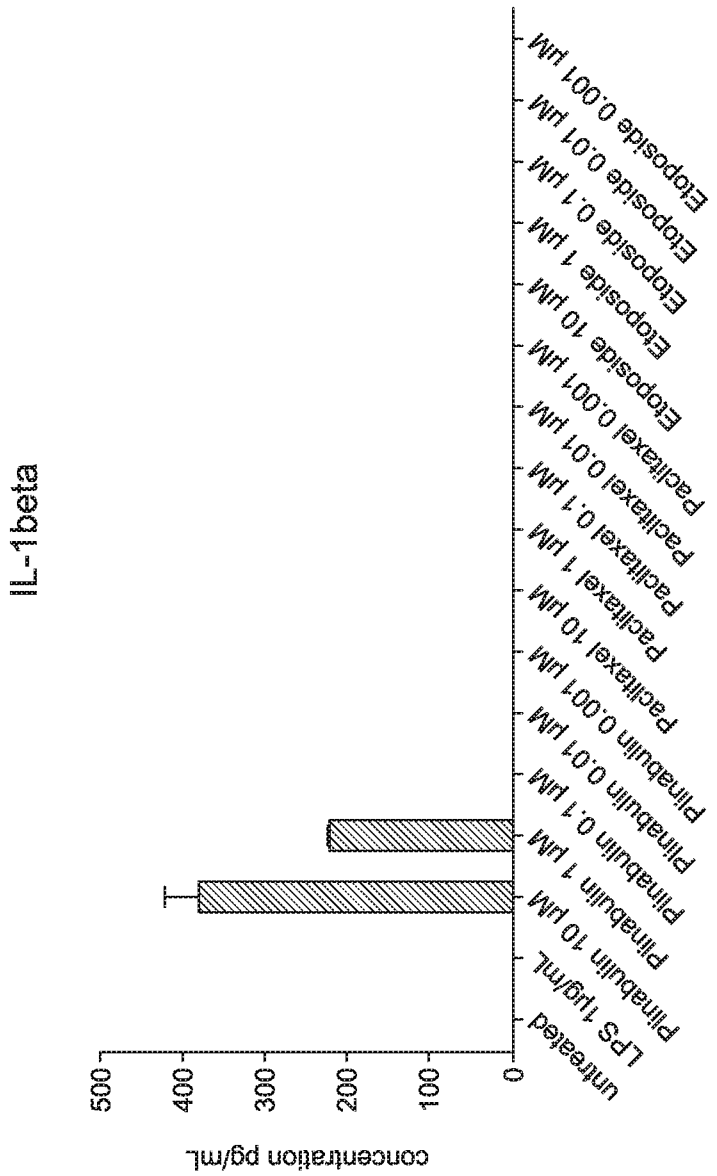

IL-1beta
concentration pg/mL
500
400
300
200
100
0
untreated
LPS 1ug/mL
Pilnabulin 10 uM
Pilnabulin 1 uM
Pilnabulin 0.1 uM
Pilnabulin 0.01 uM
Pilnabulin 0.001 uM
Paclitaxel 10 uM
Paclitaxel 1 uM
Paclitaxel 0.1 uM
Paclitaxel 0.01 uM
Paclitaxel 0.001 uM
Etoposide 10 uM
Etoposide 1 uM
Etoposide 0.1 uM
Etoposide 0.01 uM
Etoposide 0.001 uM

FIG. 3A

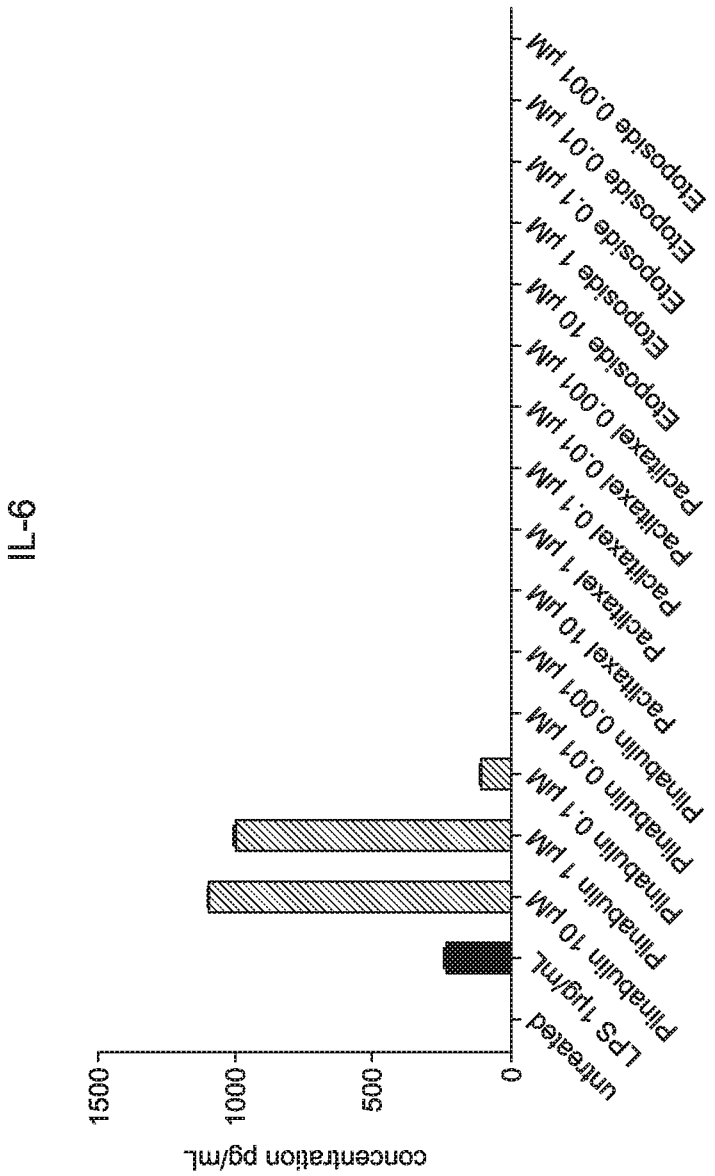

IL-6
concentration pg/mL
1500
1000
500
0
untreated
LPS 1ug/mL
Pilnabulin 10 uM
Pilnabulin 1 uM
Pilnabulin 0.1 uM
Pilnabulin 0.01 uM
Pilnabulin 0.001 uM
Paclitaxel 10 uM
Paclitaxel 1 uM
Paclitaxel 0.1 uM
Paclitaxel 0.01 uM
Paclitaxel 0.001 uM
Etoposide 10 uM
Etoposide 1 uM
Etoposide 0.1 uM
Etoposide 0.01 uM
Etoposide 0.001 uM

FIG. 3B

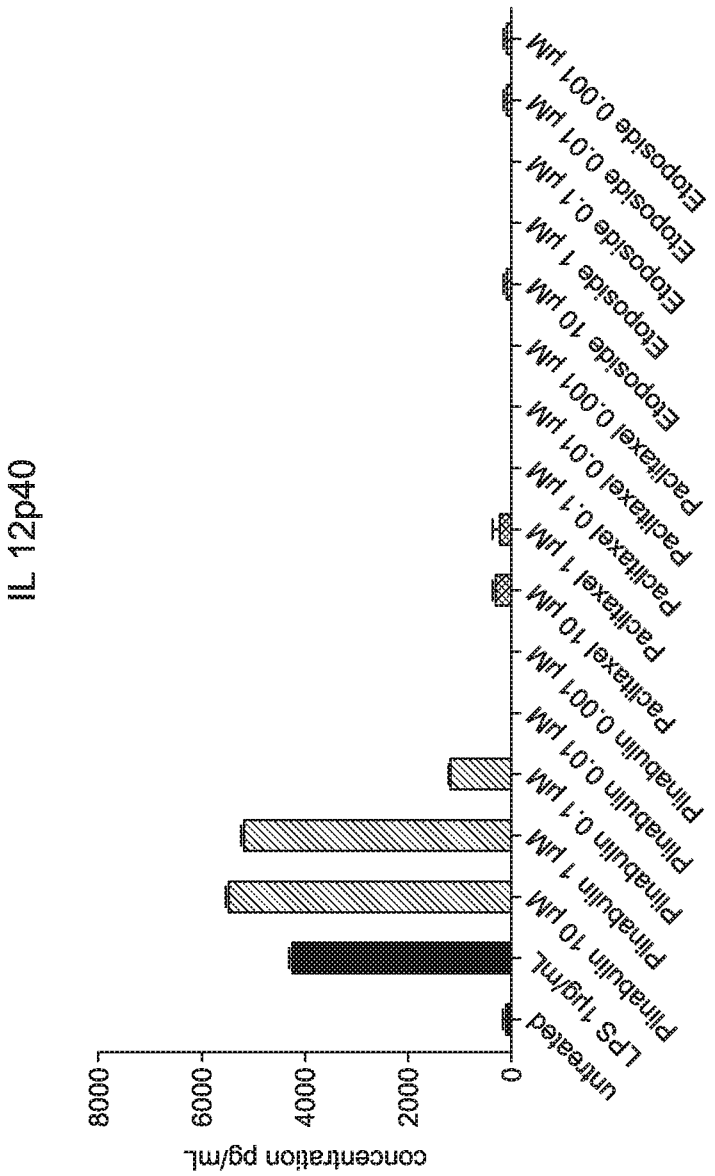

IL 12p40
concentration pg/mL
8000
6000
4000
2000
0
untreated
LPS 1ug/mL
Pilnabulin 10 uM
Pilnabulin 1 uM
Pilnabulin 0.1 uM
Pilnabulin 0.01 uM
Pilnabulin 0.001 uM
Paclitaxel 10 uM
Paclitaxel 1 uM
Paclitaxel 0.1 uM
Paclitaxel 0.01 uM
Paclitaxel 0.001 uM
Etoposide 10 uM
Etoposide 1 uM
Etoposide 0.1 uM
Etoposide 0.01 uM
Etoposide 0.001 uM

FIG. 3C

FIGURE 4A

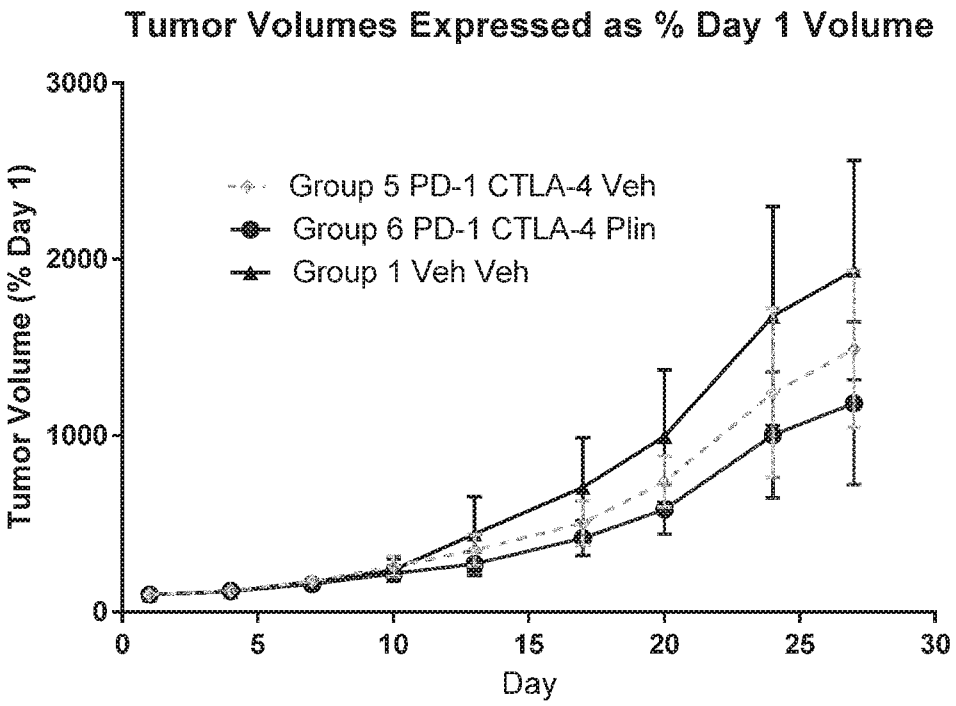


FIGURE 4B

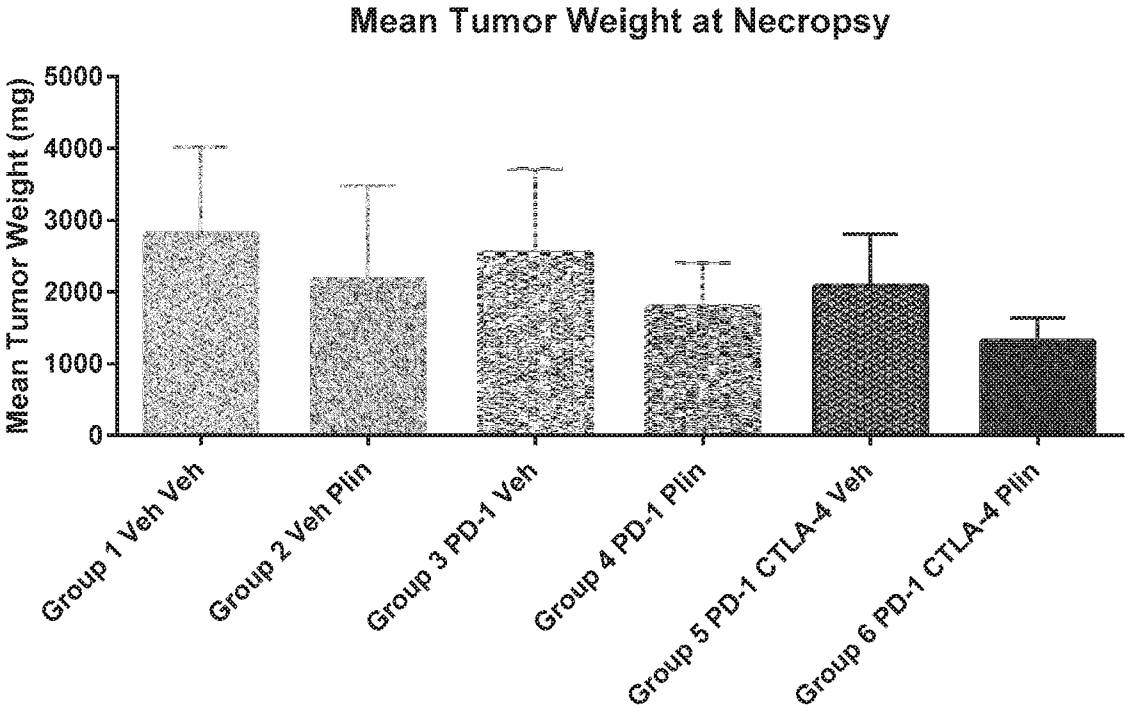


FIGURE 4C

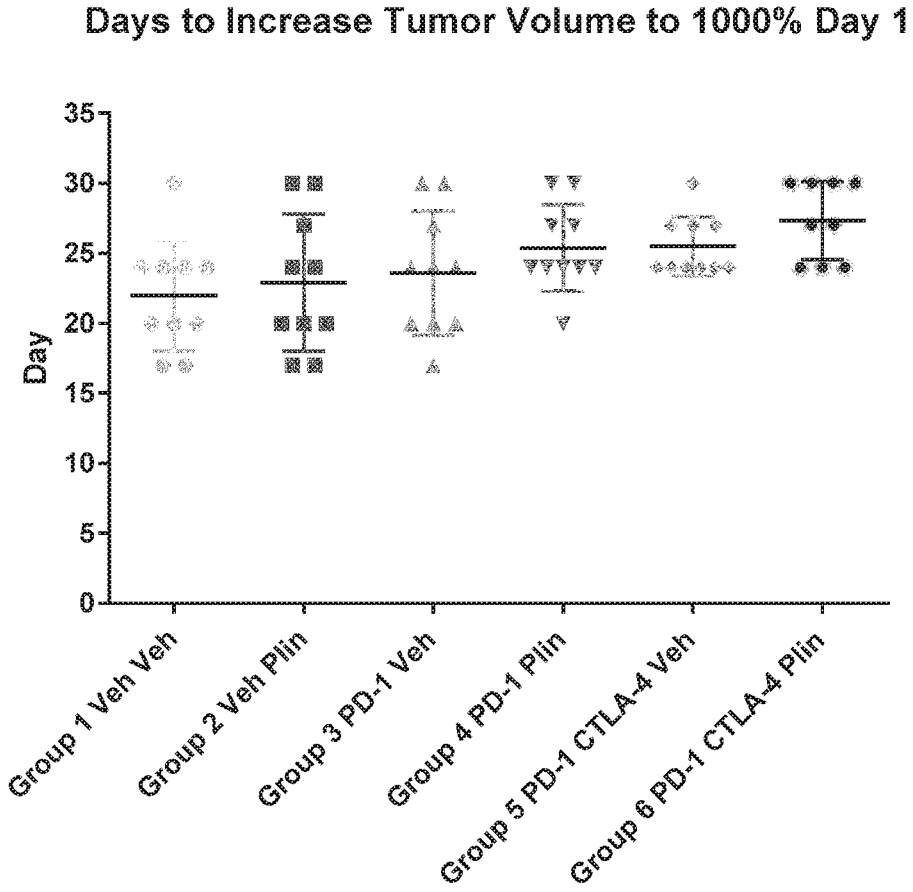

Days to Increase Tumor Volume to 1000% Day 1
Day
35
30
25
20
15
10
5
0
Group 1 Veh Veh
Group 2 Veh Plin
Group 3 PD-1 Veh
Group 4 PD-1 Plin
Group 5 PD-1 CTLA-4 Veh
Group 6 PD-1 CTLA-4 Plin

FIGURE 5A

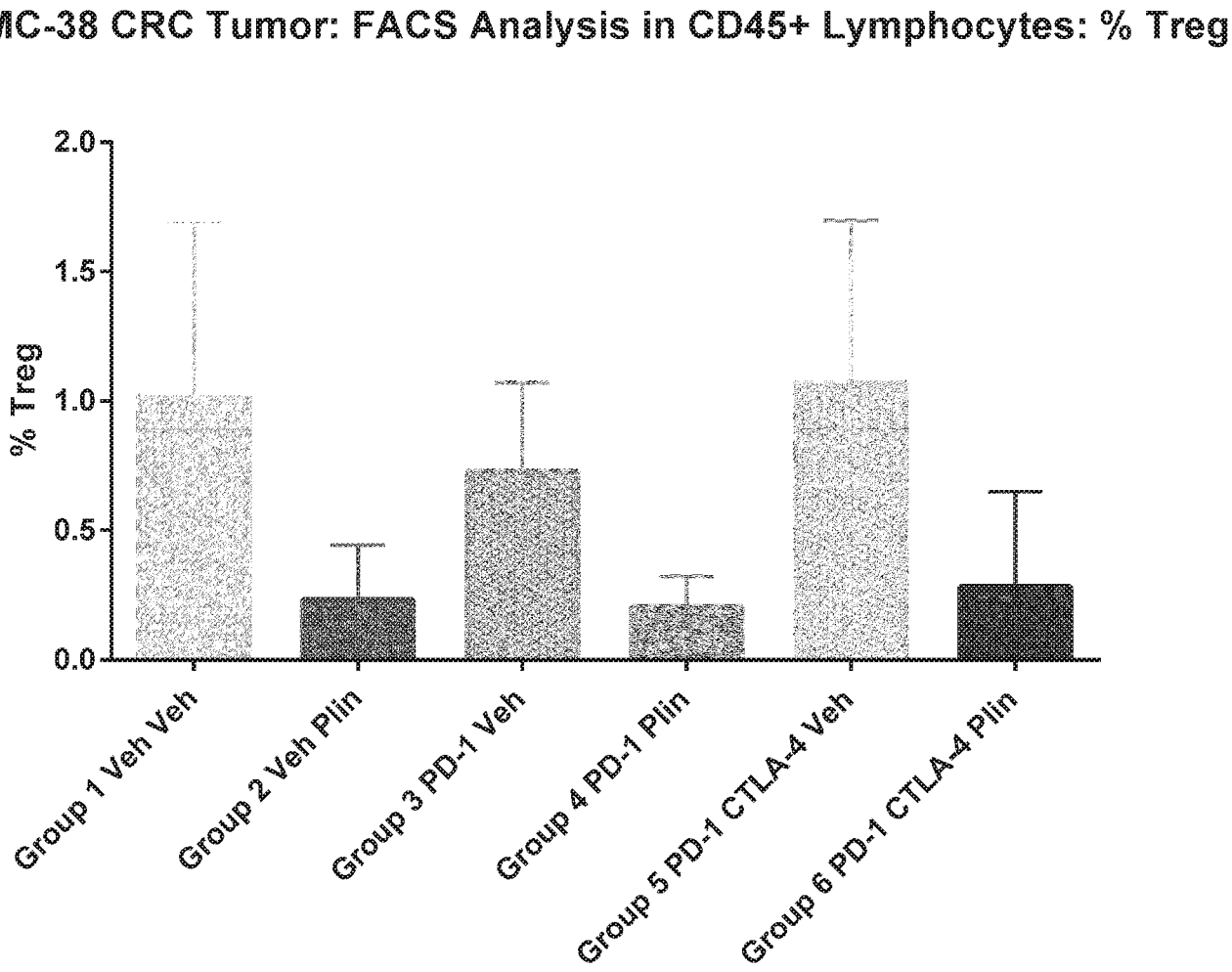

MC-38 CRC Tumor: FACS Analysis in CD45+ Lymphocytes: % Treg
% Treg
2.0
1.5
1.0
0.5
0.0
Group 1 Veh Veh
Group 2 Veh Plin
Group 3 PD-1 Veh
Group 4 PD-1 Plin
Group 5 PD-1 CTLA-4 Veh
Group 6 PD-1 CTLA-4 Plin

FIGURE 5B

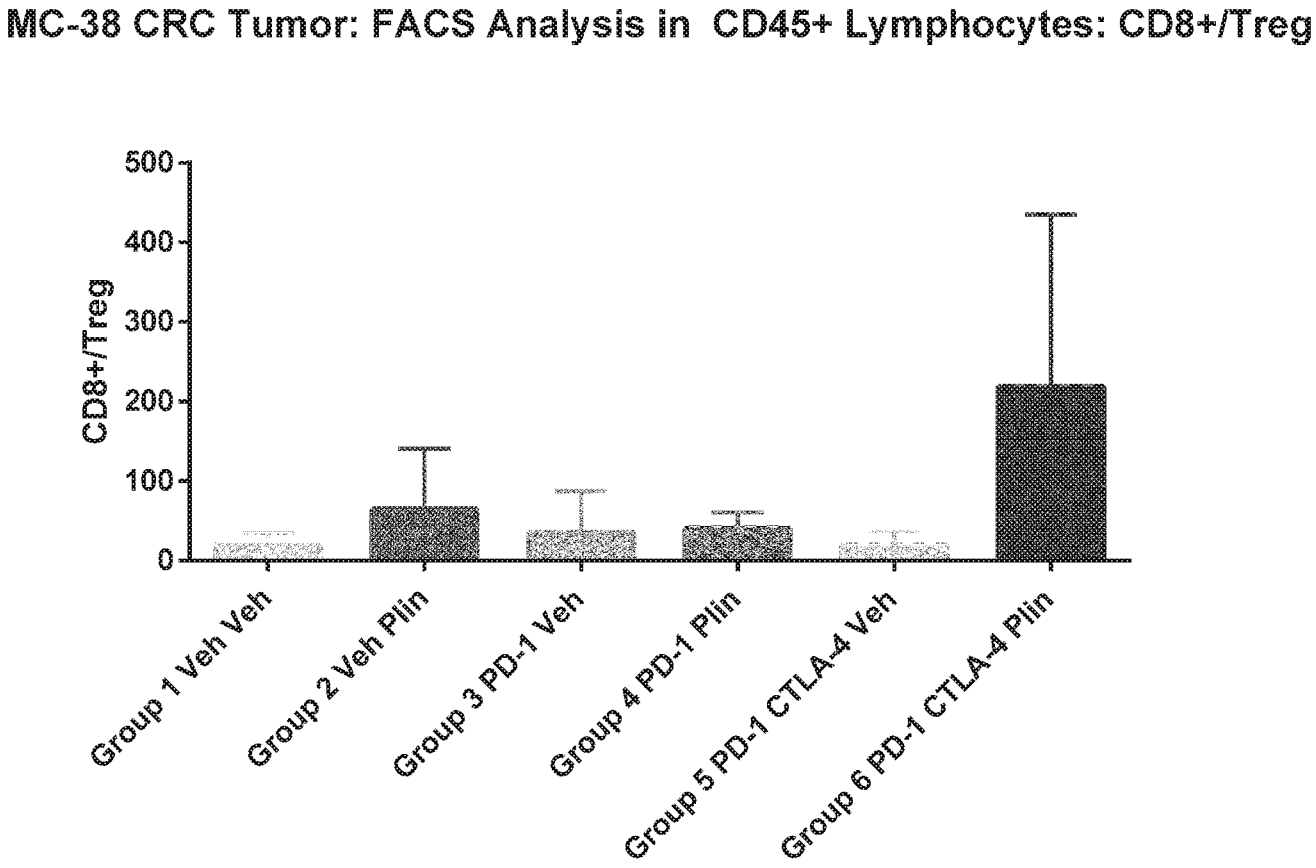
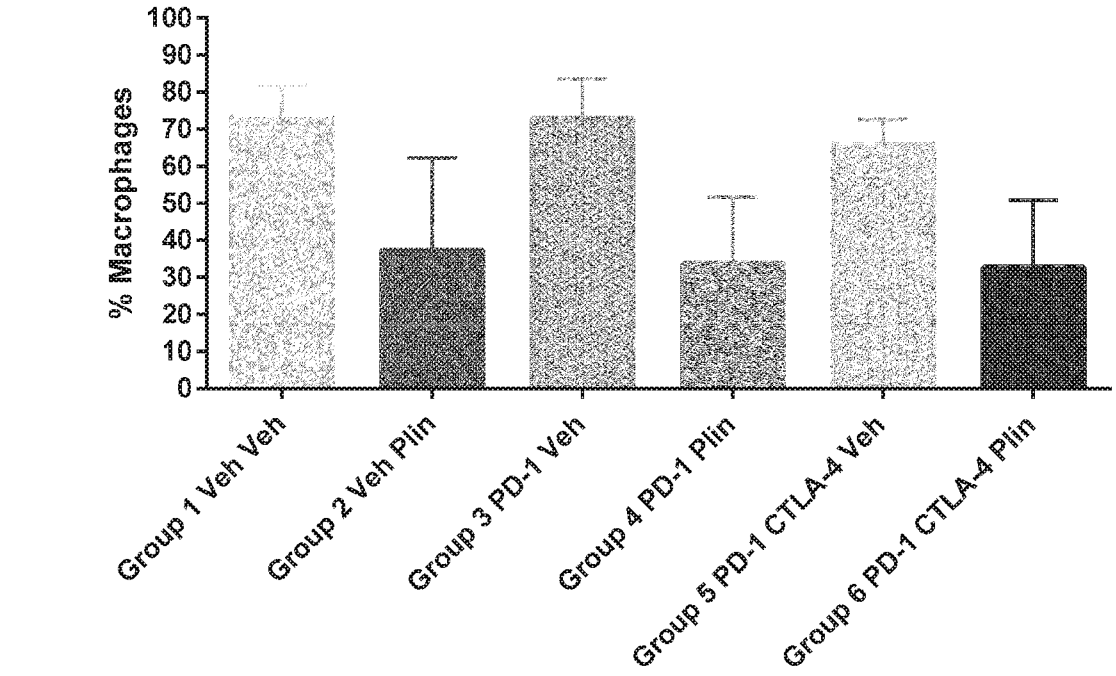

MC-38 CRC Tumor: FACS Analysis in CD45+ Lymphocytes: CD8+/Treg
CD8+/Treg
500
400
300
200
100
0
Group 1 Veh Veh
Group 2 Veh Plin
Group 3 PD-1 Veh
Group 4 PD-1 Plin
Group 5 PD-1 CTLA-4 Veh
Group 6 PD-1 CTLA-4 Plin

FIGURE 5C


MC-38 CRC Tumor: FACS Analysis in CD45+ Lymphocytes: % Macrophages
% Macrophages
100
90
80
70
60
50
40
30
20
10
0
Group 1 Veh Veh
Group 2 Veh Plin
Group 3 PD-1 Veh
Group 4 PD-1 Plin
Group 5 PD-1 CTLA-4 Veh
Group 6 PD-1 CTLA-4 Plin

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/017602

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/496 (2006.01) A61K 39/395 (2006.01) A61P 35/00 (2006.01)

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)

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

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

EPODOC, WIPA, Medline, Registry, CAplus, Biosis, Embase & keywords: immune checkpoint inhibitor, PD-1, PD-L1, PD-L2, PD-L3, PD-L4, CTLA4, cytotoxic t lymphocyte antigen 4, programmed cell death ligand, nivolumab, pembrolizumab, pidilizumab, ipilimumab, BMS936559, atezolizumab, durvalimumab, plinabulin, antiangiogen, diketopeperazine, phenylahistin, vascular disrupting, tubulin, mitosis inhibit and similar terms

PubMed, AUSPAT, Patentscope, internal database: Applicant/inventors

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] 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	Date of mailing of the international search report
1 April 2016	01 April 2016
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Nathalie Tochon-Danguy AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262833101

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/US2016/017602**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2011/146382 A1 (BRISTOL-MYERS SQUIBB COMPANY) 24 November 2011 Abstract, pages 6, 23, 27-29, 33-36, figures 2, 5, Examples 1	1-47
A	BERTELSEN L.B. et al., "Vascular effects of plinabulin (NPI-2358) and the influence on tumour response when given alone or combined with radiation", Int. J. Radiat. Biol., 2011 November, vol. 87. no. 11, pages 1126–1134 Abstract, Figure 2	1-47
A	WO 2009/089260 A2 (BRISTOL-MYERS SQUIBB COMPANY) 16 July 2009 Abstract, pages 3, 10-12, 14-26, 38, 39, 41, Tables 2-5, Example 3-5	1-47
A	WO 2012/035436 A1 (TOKYO UNIVERSITY OF PHARMACY AND LIFE SCIENCES) 22 March 2012 whole document	1-47
A	JURE-KUNKEL M. et al.,"Synergy between chemotherapeutic agents and CTLA-4 blockade in preclinical tumor models", Cancer Immunol. Immunother. 2013, vol. 62, pages 1533-1545 Abstract	1-47
A	KREAMER K., "Immune checkpoint blockade: a new paradigm in treating advanced cancer", J. Adv. Pract. Oncol., 2014, vol. 5, pages 418-431 Abstract, pages 419, 421, Table	1-47
P,A	WO 2015/051543 A1 (DALIAN WANCHUN BIOTECHNOLOGY CO. LTD. & BEYONDSPRING PHARMACEUTICALS, INC.) 16 April 2015 Abstract	1-47

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2016/017602**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2011/146382 A1	24 November 2011	WO 2011146382 A1	24 Nov 2011
		EP 2571577 A1	27 Mar 2013
		US 2013064831 A1	14 Mar 2013
WO 2009/089260 A2	16 July 2009	WO 2009089260 A2	16 Jul 2009
		AU 2009204194 A1	16 Jul 2009
		CN 101909693 A	08 Dec 2010
		EP 2227296 A2	15 Sep 2010
		EP 2227296 B1	25 Nov 2015
		EP 2962731 A1	06 Jan 2016
		JP 2011509299 A	24 Mar 2011
		KR 20100100949 A	15 Sep 2010
		US 2010278828 A1	04 Nov 2010
		US 8449886 B2	28 May 2013
WO 2012/035436 A1	22 March 2012	WO 2012035436 A1	22 Mar 2012
WO 2015/051543 A1	16 April 2015	WO 2015051543 A1	16 Apr 2015

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

(19)中华人民共和国国家知识产权局

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(51)Int.Cl.
A61K 31/496(2006.01)
A61K 39/395(2006.01)
A61P 35/00(2006.01)

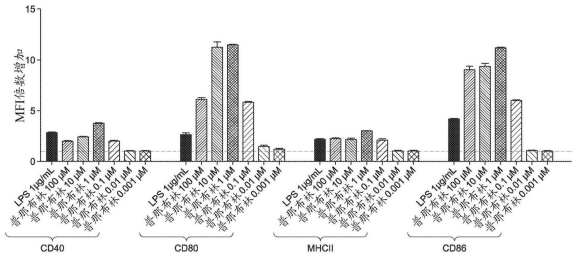
权利要求书3页 说明书16页 附图14页

(54)发明名称

普那布林联合免疫检查点抑制剂的用途

(57)摘要

本申请公开了用于治疗癌症的组合物,其包含普那布林以及一种或多种免疫检查点抑制剂。一些实施方案涉及通过将普那布林以及一种或多种免疫检查点抑制剂共同施用于有需要的对象来治疗癌症的方法。



CN 107427510 A

1.药物组合物,其包含普那布林以及一种或多种免疫检查点抑制剂。

2.如权利要求1所述的组合物,其中所述免疫检查点抑制剂是PD-1、PD-L1、PD-L2、PD-L3、PD-L4、CTLA-4、LAG3、B7-H3、B7-H4、KIR或TIM3的抑制剂。

3.如权利要求2所述的组合物,其中所述免疫检查点抑制剂是PD-1抑制剂。

4.如权利要求2所述的组合物,其中所述免疫检查点抑制剂是PD-L1抑制剂。

5.如权利要求2所述的组合物,其中所述免疫检查点抑制剂是PD-L2抑制剂。

6.如权利要求2所述的组合物,其中所述免疫检查点抑制剂是CTLA-4抑制剂。

7.如权利要求1所述的组合物,其包含第一免疫检查点抑制剂和第二免疫检查点抑制剂,其中所述第一免疫检查点抑制剂不同于所述第二免疫检查点抑制剂。

8.如权利要求7所述的组合物,其中所述第一免疫检查点抑制剂和所述第二免疫检查点抑制剂独立地为PD-1、PD-L1、PD-L2、PD-L3、PD-L4、CTLA-4、LAG3、B7-H3、B7-H4、KIR或TIM3的抑制剂。

9.如权利要求8所述的组合物,其中所述第一免疫检查点抑制剂是PD-1抑制剂,且所述第二免疫检查点抑制剂是CTLA-4抑制剂。

10.如权利要求8所述的组合物,其中所述第一免疫检查点抑制剂是PD-L1抑制剂,且所述第二免疫检查点抑制剂是CTLA-4抑制剂。

11.如权利要求8所述的组合物,其中所述第一免疫检查点抑制剂是PD-L2抑制剂,且所述第二免疫检查点抑制剂是CTLA-4抑制剂。

12.如权利要求1-11中任一项所述的组合物,其中所述免疫检查点抑制剂是抗体。

13.如权利要求12所述的组合物,其中所述免疫检查点抑制剂是PD-1抗体。

14.如权利要求12所述的组合物,其中所述免疫检查点抑制剂是PD-L1抗体。

15.如权利要求12所述的组合物,其中所述免疫检查点抑制剂是PD-L2抗体。

16.如权利要求12所述的组合物,其中所述免疫检查点抑制剂是CTLA-4抗体。

17.如权利要求12所述的组合物,其中所述抗体选自:α-CD3-APC、α-CD3-APC-H7、α-CD4-ECD、α-CD4-PB、α-CD8-PE-Cy7、α-CD-8-PerCP-Cy5.5、α-CD11c-APC、α-CD11b-PE-Cy7、α-CD11b-AF700、α-CD14-FITC、α-CD16-PB、α-CD19-AF780、α-CD19-AF700、α-CD20-PO、α-CD25-PE-Cy7、α-CD40-APC、α-CD45-生物素、链霉亲和素-BV605、α-CD62L-ECD、α-CD69-APC-Cy7、α-CD80-FITC、α-CD83-生物素、链霉亲和素-PE-Cy7、α-CD86-PE-Cy7、α-CD86-PE、α-CD123-PE、α-CD154-PE、α-CD161-PE、α-CTLA4-PE-Cy7、α-FoxP3-AF488(克隆259D)、IgG1-同种型-AF488、α-ICOS(CD278)-PE、α-HLA-A2-PE、α-HLA-DR-PB、α-HLA-DR-PerCPCy5.5、α-PD1-APC、VISTA、共刺激分子OX40和CD137。

18.如权利要求1-17中任一项所述的组合物,其还包含一种或多种药学可接受的赋形剂。

19.如权利要求1-18中任一项所述的组合物,其还包含一种或多种另外的化疗剂。

20.如权利要求1-19中任一项所述的组合物,其中所述免疫检查点抑制剂是纳武单抗、派姆单抗、哌利珠单抗(pidilizumab)、伊匹单抗(ipilimumab)、达卡巴嗪、BMS 936559、阿特珠单抗(atezolizumab)、杜瓦单抗(durvalimumab)、或以上抑制剂的任何组合。

21.治疗癌症的方法,其包括将权利要求1-20中任一项所述的药物组合物施用于有需要的对象。

22.治疗癌症的方法，其包括将普那布林以及一种或多种免疫检查点抑制剂共同施用于有需要的对象。

23.如权利要求22所述的方法，其还包括共同施用一种或多种另外的化疗剂。

24.如权利要求21-23中任一项所述的方法，其中所述癌症包含表达结合PD-1的配体的癌症细胞。

25.如权利要求24所述的方法，其中所述结合PD-1的配体是PD-L1或PD-L2。

26.如权利要求24所述的方法，其中所述癌症是头颈癌、肺癌、胃癌、结肠癌、胰腺癌、前列腺癌、乳腺癌、肾癌、膀胱癌、卵巢癌、子宫颈癌、黑素瘤、胶质母细胞瘤、骨髓瘤、淋巴瘤或白血病。

27.如权利要求24所述的方法，其中所述癌症是肾细胞癌、恶性黑素瘤、非小细胞肺癌(NSCLC)、卵巢癌、霍奇金淋巴瘤或鳞状细胞癌。

28.如权利要求21-27中任一项所述的方法，其中所述癌症包含表达结合CTLA-4的配体的癌症细胞。

29.如权利要求28所述的方法，其中所述结合CTLA-4的配体是B7.1或B7.2。

30.如权利要求22-29中任一项所述的方法，其中所述免疫检查点抑制剂是PD-1、PD-L1、PD-L2、PD-L3、PD-L4、CTLA-4、LAG3、B7-H3、B7-H4、KIR或TIM3的抑制剂。

31.如权利要求30所述的方法，其中所述免疫检查点抑制剂是PD-1抑制剂。

32.如权利要求30所述的方法，其中所述免疫检查点抑制剂是PD-L1抑制剂。

33.如权利要求30所述的方法，其中所述免疫检查点抑制剂是PD-L2抑制剂。

34.如权利要求30所述的方法，其中所述免疫检查点抑制剂是CTLA抑制剂。

35.如权利要求22-29中任一项所述的方法，其包括第一免疫检查点抑制剂和第二免疫检查点抑制剂，其中所述第一免疫检查点抑制剂不同于所述第二免疫检查点抑制剂。

36.如权利要求35所述的方法，其中所述第一免疫检查点抑制剂和所述第二免疫检查点抑制剂独立地为PD-1、PD-L1、PD-L2、PD-L3、PD-L4、CTLA-4、LAG3、B7-H3、B7-H4、KIR或TIM3的抑制剂。

37.如权利要求36所述的方法，其中所述第一免疫检查点抑制剂是PD-1抑制剂，且所述第二免疫检查点抑制剂是CTLA-4抑制剂。

38.如权利要求22-29中任一项所述的方法，其中所述免疫检查点抑制剂是抗体。

39.如权利要求38所述的方法，其中所述免疫检查点抑制剂是PD-1抗体。

40.如权利要求38所述的方法，其中所述免疫检查点抑制剂是PD-L1抗体。

41.如权利要求38所述的方法，其中所述免疫检查点抑制剂是PD-L2抗体。

42.如权利要求38所述的方法，其中所述免疫检查点抑制剂是CTLA-4抗体。

43.如权利要求38所述的方法，其中所述抗体选自：α-CD3-APC、α-CD3-APC-H7、α-CD4-ECD、α-CD4-PB、α-CD8-PE-Cy7、α-CD-8-PerCP-Cy5.5、α-CD11c-APC、α-CD11b-PE-Cy7、α-CD11b-AF700、α-CD14-FITC、α-CD16-PB、α-CD19-AF780、α-CD19-AF700、α-CD20-PO、α-CD25-PE-Cy7、α-CD40-APC、α-CD45-生物素、链霉亲和素-BV605、α-CD62L-ECD、α-CD69-APC-Cy7、α-CD80-FITC、α-CD83-生物素、链霉亲和素-PE-Cy7、α-CD86-PE-Cy7、α-CD86-PE、α-CD123-PE、α-CD154-PE、α-CD161-PE、α-CTLA4-PE-Cy7、α-FoxP3-AF488(克隆259D)、IgG1-同种型-AF488、α-ICOS(CD278)-PE、α-HLA-A2-PE、α-HLA-DR-PB、α-HLA-DR-PerCPCy5.5、α-PD1-APC、

VISTA、共刺激分子OX40和CD137。

44.如权利要求22-43中任一项所述的方法，其中所述免疫检查点抑制剂是纳武单抗、派姆单抗、哌利珠单抗、伊匹单抗、达卡巴嗪、BMS936559、阿特珠单抗、杜瓦单抗、或以上抑制剂的任何组合。

45.如权利要求21或22所述的方法，其中所述癌症选自：乳腺癌、结肠癌、直肠癌、肺癌、前列腺癌、黑素瘤、白血病、卵巢癌、胃癌、肾细胞癌、肝癌、胰腺癌、淋巴瘤和骨髓瘤。

46.如权利要求21或22所述的方法，其中所述癌症是实体瘤或血液癌症。

47.如权利要求21或22所述的方法，其中所述癌症不具有任何表达PD-1、PD-L1或PD-L2的细胞。

普那布林联合免疫检查点抑制剂的用途

[0001] 通过引用并入任何优先权申请

[0002] 本申请要求2015年2月12日提交的美国临时申请第62/115,468号和2015年11月13日提交的美国临时申请第62/255,259号的权益,将其公开内容通过引用整体并入本文。

背景技术

[0003] 领域

[0004] 本发明涉及化学和医药领域。更具体地,本发明涉及普那布林(Plinabulin)、含有普那布林的组合物及其治疗用途。

[0005] 相关技术的描述

[0006] 人类癌症具有许多遗传和表观遗传改变,产生潜在可被免疫系统识别的新抗原(Sjoblom等,2006)。由T淋巴细胞和B淋巴细胞组成的适应性免疫系统具有强大的抗癌潜力,具有应对多种肿瘤抗原的广泛能力和精确的特异性。

[0007] 最近的癌症免疫治疗研究已经将大量努力集中在通过过继转移活化的效应细胞来增强抗肿瘤免疫力、针对相关抗原的免疫的方法,提供非特异性免疫刺激剂如细胞因子,或除去抗-癌症效应细胞的抑制剂。开发特异性免疫检查点抑制剂(immune checkpoint inhibitor)的努力已经开始为治疗癌症提供新的免疫治疗方法,包括开发结合和抑制细胞毒性T淋巴细胞抗原-4(CTLA-1)的抗体伊匹单抗(ipilimumab),用于治疗晚期黑素瘤患者(Hodi等,2010)。虽然对于大多数患者,癌症仍然是不治之症,但是特别需要开发可用于癌症免疫治疗的有效治疗剂。

[0008] 发明概述

[0009] 一些实施方案涉及药物组合物,其包含普那布林以及一种或多种免疫检查点抑制剂。

[0010] 一些实施方案涉及治疗癌症的方法,所述方法包括将普那布林以及一种或多种免疫检查点抑制剂共同施用于有需要的对象。

[0011] 附图简述

[0012] 图1A显示在用不同浓度的普那布林和LPS对照处理的树突细胞中,DC成熟标志物CD40、CD80、CD86和MHCII的表达;图1B显示用普那布林和LPS处理的树突细胞的活力。

[0013] 图2A显示在用普那布林、紫杉醇、依托泊苷或对照处理的树突细胞中CD40标志物的表达;图2B显示在用普那布林、紫杉醇、依托泊苷或对照处理的树突细胞中CD80标志物的表达;图2C显示在用普那布林、紫杉醇、依托泊苷或对照处理的树突细胞中CD86标志物的表达;图2D显示在用普那布林、紫杉醇、依托泊苷或对照处理的树突细胞中MHCII标志物的表达。

[0014] 图3A显示在用普那布林、紫杉醇、依托泊苷和对照处理的树突细胞中IL-1β的产生;图3B显示在用普那布林、紫杉醇、依托泊苷和对照处理的树突细胞中IL-6标志物的产生;图3C显示在用普那布林、紫杉醇、依托泊苷和对照处理的树突细胞中IL-12p40的产生。

[0015] 图4A-4C显示在免疫活性小鼠的MC-38肿瘤模型中,普那布林(Plin)诱导的PD-1抗

体加CTLA-4抗体的抗肿瘤作用增加。图4A显示对肿瘤生长的影响;图4B显示对尸体剖检时的平均肿瘤重量的影响;图4C显示肿瘤达到其10倍初始体积的时间。

[0016] 图5A-5C显示在实施例6所述的研究中尸体剖检时的肿瘤的荧光激活细胞分选(FACS)分析结果。图5A显示对Treg细胞的影响;图5B显示CD8+细胞与Treg细胞的比例;图5C显示对巨噬细胞的影响。

[0017] 优选实施方案详述

[0018] 普那布林,即(3Z,6Z)-3-苯亚甲基-6-{[5-(2-甲基-2-丙基)-1H-咪唑-4-基]亚甲基}-2,5-哌嗪二酮,是天然化合物Phenylahistin的合成类似物。可根据美国专利第7,064,201号和第7,919,497号(其通过引用将其整体并入本文)中详细描述的方法和步骤容易地制备普那布林。在一些实施方案中,普那布林可有效促进抗原摄取以及树突细胞迁移至淋巴结,在这里肿瘤特异性抗原由树突细胞呈递给初始免疫效应细胞。将树突细胞暴露于普那布林可诱导树突细胞的成熟,并显著增加其刺激T细胞的能力。在一些实施方案中,普那布林可以通过免疫调节肿瘤微环境来介导肿瘤大小的减小,以促进抗肿瘤免疫增强作用。在一些实施方案中,当普那布林与免疫检查点抑制剂结合时,可以实现显著的治疗协同作用。

[0019] 一些实施方案涉及普那布林与一种或多种免疫检查点抑制剂联用,所述免疫检查点抑制剂如CTLA4(细胞毒性T淋巴细胞抗原-4)、PD-1(程序性细胞死亡蛋白1)、PD-L1(程序性细胞死亡配体1)、PD-L2(程序性细胞死亡配体2)、PD-L3(程序性细胞死亡配体3)、PD-L4(程序性细胞死亡配体4)、LAG-3(淋巴细胞活化基因-3)和TIM-3(T细胞免疫球蛋白和粘蛋白-3)的抑制剂。在一些实施方案中,所述免疫检查点抑制剂是结合PD-1的配体。在一些实施方案中,所述免疫检查点抑制剂是结合CTLA-4的配体。

[0020] PD-1是由激活的T细胞和B细胞表达的重要免疫检查点受体,并介导免疫抑制。PD-1是受体CD28家族的成员,该家族包括CD28、CTLA-4、ICOS、PD-1和BTLA。如本文所用,术语“PD-1”包括人PD-1(hPD-1)、hPD-1的变体、同种型和物种同系物,以及与hPD-1具有至少一个共同表位的类似物。

[0021] 已经鉴定了针对PD-1的各种细胞表面糖蛋白配体,包括在抗原呈递细胞以及许多人类癌症上表达的PD-L1、PD-L2、PD-L3和PD-L4,所述细胞表面糖蛋白配体已显示在结合PD-1后下调T细胞活化和细胞因子分泌。如本文所用,术语“PD-L1”包括人PD-L1(hPD-L1)、hPD-L1的变体、同种型和物种同系物,以及与hPD-L1具有至少一个共同表位的类似物。如本文所用,术语“PD-L2”包括人PD-L2(hPD-L2)、hPD-L2的变体、同种型和物种同系物,以及与hPD-L2具有至少一个共同表位的类似物。如本文所用,术语“PD-L3”包括人PD-L3(hPD-L3)、hPD-L3的变体、同种型和物种同系物,以及与hPD-L3具有至少一个共同表位的类似物。如本文所用,术语“PD-L4”包括人PD-L4(hPD-L4)、hPD-L4的变体、同种型和物种同系物,以及与hPD-L4具有至少一个共同表位的类似物。

[0022] CTLA-4(细胞毒性T淋巴细胞相关蛋白4)是这样的蛋白受体,其作为免疫检查点起作用,下调免疫系统。CTLA-4存在于T细胞表面,也是免疫球蛋白(Ig)超家族的成员;CTLA-4包含单个细胞外Ig结构域。已经在具有细胞毒性活性的T细胞群中发现CTLA-4转录物,这表明CTLA-4可能在细胞溶解反应中起作用。

[0023] 定义

[0024] 除非另有定义，本文使用的所有技术和科学术语具有与本公开所属领域的普通技术人员通常理解的相同的含义。所有专利、申请、公开的申请和其他出版物均通过引用整体并入本文。如果本文中有一个术语的多个定义，除非另有说明，否则以本节中的定义为准。

[0025] 术语“药学可接受的载体”或“药学可接受的赋形剂”包括任何和所有溶剂、分散介质、包衣、抗菌剂和抗真菌剂、等渗剂和吸收延迟剂等。将这种介质和试剂用于药物活性物质是本领域公知的。除了任何常规介质或试剂与活性成分不相容的情形之外，本文预期其在治疗组合物中的用途。此外，可以包括例如本领域常用的各种佐剂。以下描述了将各种组分包含于药物组合物的考虑，例如，Gilman等(Eds.)(1990)；Goodman and Gilman's:The Pharmacological Basis of Therapeutics,8th Ed.,Pergamon Press，通过引用将其整体并入本文。所述药学可接受的赋形剂可为单糖或单糖衍生物。

[0026] 如本文所用，“对象”意指人或非人哺乳动物，例如狗、猫、小鼠、大鼠、牛、绵羊、猪、山羊、非人灵长类动物或鸟如鸡，以及任何其他脊椎动物或无脊椎动物。

[0027] 术语“哺乳动物”以其通常的生物学意义使用。因此，其具体包括但不限于：灵长类动物(包括类人猿(黑猩猩、猿、猴)和人)、牛、马、绵羊、山羊、猪、兔、狗、猫、啮齿动物、大鼠、小鼠、豚鼠等。

[0028] 如本文所用，“有效量”或“治疗有效量”是指治疗剂的量，其在一定程度上有效地缓解或降低发生疾病或病况的一种或多种症状的可能性，并且可以包括治愈疾病或病况。

[0029] 如本文所用，“治疗”(Treat/treatment/treating)是指向对象施用化合物或药物组合物以用于预防和/或治疗目的。术语“预防性治疗”是指治疗尚未表现出疾病或病况的症状但是易患或以其他方式具有特定疾病或病况风险的对象，从而该治疗降低患者将来发展疾病或病况的可能性。术语“治疗性治疗”是指对已患有疾病或病况的对象进行治疗。

[0030] 如本文所用，术语“化疗剂”是指这样的试剂，其可以降低、预防、减轻、限制和/或延迟转移瘤或肿瘤的生长，或通过肿瘤坏死或细胞凋亡或任何其他机制直接杀死肿瘤细胞，或可以在其他方面按药学有效量使用以减少、预防、减轻、限制和/或延缓肿瘤疾病对象中的转移瘤或肿瘤生长。化学剂包括但不限于，例如，氟嘧啶；嘧啶核苷；嘌呤核苷；抗叶酸制剂、铂类药剂；蒽环类/蒽二酮类；表鬼臼毒素；喜树碱；激素；激素复合物；抗激素类；酶、蛋白质、肽和多克隆和/或单克隆抗体；长春花生物碱；紫杉烷类；埃博霉素；抗微管剂；烷化剂；抗代谢物；拓扑异构酶抑制剂；抗病毒剂；和各种其他细胞毒性剂和细胞生长抑制剂。

[0031] 给药和药物组合物

[0032] 一些实施方案涉及药物组合物，其包含普那布林以及一种或多种免疫检查点抑制剂。

[0033] 在一些实施方案中，所述免疫检查点抑制剂是PD-1、PD-L1、PD-L2、PD-L3、PD-L4、CTLA-4、LAG3、B7-H3、B7-H4、KIR或TIM3的抑制剂。在一些实施方案中，所述免疫检查点抑制剂是PD-1抑制剂。在一些实施方案中，所述免疫检查点抑制剂是结合PD-L1的配体。在一些实施方案中，所述免疫检查点抑制剂是PD-L1抑制剂。在一些实施方案中，所述免疫检查点抑制剂是PD-L2抑制剂或组合的PD-L1/PD-L2抑制剂。在一些实施方案中，所述免疫检查点抑制剂是CTLA-4抑制剂。

[0034] 在一些实施方案中，本文所述的组合物包含第一免疫检查点抑制剂和第二免疫检查点抑制剂，其中所述第一免疫检查点抑制剂不同于所述第二免疫检查点抑制剂。在一些

实施方案中，所述第一免疫检查点抑制剂和所述第二免疫检查点抑制剂独立地为PD-1、PD-L1、PD-L2、PD-L3、PD-L4、CTLA-4、LAG3、B7-H3、B7-H4、KIR或TIM3的抑制剂。在一些实施方案中，所述第一免疫检查点抑制剂是PD-1抑制剂，且所述第二免疫检查点抑制剂是CTLA-4抑制剂。在一些实施方案中，所述第一免疫检查点抑制剂是PD-L1抑制剂，且所述第二免疫检查点抑制剂是CTLA-4抑制剂。在一些实施方案中，所述第一免疫检查点抑制剂是PD-L2抑制剂，且所述第二免疫检查点抑制剂是CTLA-4抑制剂。

[0035] 在一些实施方案中，所述免疫检查点抑制剂可以为能抑制T细胞调控作用的小肽剂。在一些实施方案中，所述免疫检查点抑制剂可以为能抑制T细胞调控作用的小分子(例如小于500道尔顿)。在一些实施方案中，所述免疫检查点抑制剂可以为提供T细胞激活的共刺激的分子。在一些实施方案中，所述免疫检查点抑制剂可以为提供自然杀伤细胞激活的共刺激的分子。在一些实施方案中，所述免疫检查点抑制剂可以为抗体。在一些实施方案中，所述免疫检查点抑制剂是PD-1抗体。在一些实施方案中，所述免疫检查点抑制剂是PD-L1抗体。在一些实施方案中，所述免疫检查点抑制剂是PD-L2抗体。在一些实施方案中，所述免疫检查点抑制剂是PD-L3抗体。在一些实施方案中，所述免疫检查点抑制剂是PD-L4抗体。在一些实施方案中，所述免疫检查点抑制剂是CTLA-4抗体。在一些实施方案中，所述免疫检查点抑制剂是CTLA-4、LAG3、B7-H3、B7-H4、KIR或TIM3的抗体。

[0036] 所述抗体可选自：α-CD3-APC、α-CD3-APC-H7、α-CD4-ECD、α-CD4-PB、α-CD8-PE-Cy7、α-CD-8-PerCP-Cy5.5、α-CD11c-APC、α-CD11b-PE-Cy7、α-CD11b-AF700、α-CD14-FITC、α-CD16-PB、α-CD19-AF780、α-CD19-AF700、α-CD20-PO、α-CD25-PE-Cy7、α-CD40-APC、α-CD45-生物素、链霉亲和素-BV605、α-CD62L-ECD、α-CD69-APC-Cy7、α-CD80-FITC、α-CD83-生物素、链霉亲和素-PE-Cy7、α-CD86-PE-Cy7、α-CD86-PE、α-CD123-PE、α-CD154-PE、α-CD161-PE、α-CTLA4-PE-Cy7、α-FoxP3-AF488(克隆259D)、IgG1-同种型-AF488、α-ICOS(CD278)-PE、α-HLA-A2-PE、α-HLA-DR-PB、α-HLA-DR-PerCPCy5.5、α-PD1-APC、VISTA、共刺激分子OX40和CD137。

[0037] 多种抗体(Ab)可用于本文所述的组合物，所述抗体包括具有高亲和力结合PD-1、PD-L1、PD-L2、PD-L3或PD-L4的抗体。以高亲和力与PD-1特异性结合的人mAb(HuMAb)(例如，结合人PD-1并可能与来自其他物种如食蟹猴的PD-1交叉反应)已公开于美国专利第8,008,449号，通过引用将其整体并入本文。以高亲和力与PD-L1特异性结合的HuMAb已公开于美国专利第7,943,743号，通过引用将其整体并入本文。其他抗-PD-1mAb已描述于例如，美国专利第6,808,710号、第7,488,802号和第8,168,757号，以及PCT公开号WO 2012/145493，以上所有文献均通过引用将其整体并入本文。抗-PD-L1 mAb已描述于例如，美国专利第7,635,757号和第8,217,149号，美国公开第2009/0317368号，以及PCT公开号WO 2011/066389和WO 2012/14549，以上所有文献均通过引用将其整体并入本文。

[0038] 在一些实施方案中，抗-PD-1HuMAb可选自17D8、2D3、4H1、5C4(本文中也称为纳武单抗)、4A1 1、7D3和5F4，它们均描述于美国专利第8,008,449号。在一些实施方案中，抗-PD-1HuMAb可选自3G10、12A4(本文中也称为BMS-936559)、10A5、5F8、10H10、1B12、7H1、11E6、12B7和13G4，它们均描述于美国专利第7,943,743号。

[0039] 在一些实施方案中，所述组合物还可包含一种或多种药学可接受的稀释剂。在一些实施方案中，所述药学可接受的稀释剂可包括KolliphorHS15®(聚乙二醇(15)-羟基硬

脂酸)。在一些实施方案中,所述药学可接受的稀释剂可包括丙二醇。在一些实施方案中,所述药学可接受的稀释剂可包括Kolliphor和丙二醇。在一些实施方案中,所述药学可接受的稀释剂可包括Kolliphor和丙二醇,其中基于稀释剂的总重量,所述Kolliphor为约40重量%,丙二醇为约60重量%。在一些实施方案中,所述组合物还可包含一种或多种其他药学可接受的赋形剂。

[0040] 标准的药物制剂技术可用于制备本文所述的药物组合物,如Remington's The Science and Practice of Pharmacy,21st Ed.,Lippincott Williams&Wilkins(2005)中公开的那些,通过引用将其整体并入本文。因此,一些实施方案包括药物组合物,其包含:(a)安全和治疗有效量的普那布林或其药学可接受的盐,(b)免疫检查点抑制剂,和(c)药学可接受的载体、稀释剂、赋形剂或它们的组合。

[0041] 其他实施方案包括在分别的组合物中共同施用普那布林以及一种或多种免疫检查点抑制剂。因此,一些实施方案包括第一药物组合物,其包含:(a)安全和治疗有效量的普那布林或其药学可接受的盐,和(b)药学可接受的载体、稀释剂、赋形剂或它们的组合;以及第二药物组合物,其包含:(a)一种或多种免疫检查点抑制剂,和(b)药学可接受的载体、稀释剂、赋形剂或它们的组合。

[0042] 可以通过任何一种可以接受的适用于类似用途的试剂的施用模式施用本文所述的药物组合物,所述施用模式包括但不限于:口服、舌下、口腔、皮下、静脉内、鼻内、局部、经皮、皮内、腹膜内、肌肉内、肺内、阴道、直肠或眼内。在治疗适应症(其为优选实施方案的对象)中,口服和肠胃外施用是常用的。

[0043] 术语"药学可接受的载体"或"药学可接受的赋形剂"包括任何和所有溶剂、分散介质、包衣、抗菌剂和抗真菌剂、等渗剂和吸收延迟剂等。将这种介质和试剂用于药物活性物质是本领域公知的。除了任何常规介质或试剂与活性成分不相容的情况之外,本文预期其在用于治疗组合物中的用途。此外,可以包括例如本领域常用的各种佐剂。以下描述了在药物组合物中包含各种成分的考虑,例如,Gilman等(Eds.)(1990);Goodman and Gilman's:The Pharmacological Basis of Therapeutics,8th Ed.,Pergamon Press,通过引用将其整体并入本文。

[0044] 作为药学可接受的载体或其组分的物质的一些实例是:糖,如乳糖、葡萄糖和蔗糖;淀粉,如玉米淀粉和马铃薯淀粉;纤维素及其衍生物,如羧甲纤维素钠、乙基纤维素和甲基纤维素;粉状黄蓍胶;麦芽;明胶;滑石;固体润滑剂,如硬脂酸和硬脂酸镁;硫酸钙;植物油,如花生油、棉籽油、芝麻油、橄榄油、玉米油和可可油;多元醇,如丙二醇、甘油、山梨糖醇、甘露糖醇和聚乙二醇;海藻酸;乳化剂,如TWEENS;润湿剂,如十二烷基硫酸钠;着色剂;调味剂;压片剂;稳定剂;抗氧化剂;防腐剂;无热原水;等渗盐水和磷酸盐缓冲溶液。

[0045] 本文所述的组合物优选以单位剂型提供。如本文所用,"单位剂型"是根据良好医疗实践(good medical practice)的以下组合物:其包含的化合物或组合物的量适于以单一剂量向动物、优选哺乳动物对象施用。然而,单一或单位剂型的制剂并不意味着该剂型每天施用一次或每个疗程施用一次。预期这种剂型以每天一次、两次、三次或更多次施用,和以输注一段时间施用(例如,约30分钟至约2-6小时),或作为连续输注施用,以及可以在治疗过程中给药不止一次,尽管没有特别排除单一施用。本领域技术人员将认识到,该制剂没有具体预期整个治疗过程,并且将这些决定留给治疗领域而不是制剂领域的技术人员。

[0046] 如上所述有用的组合物可以是用于多种施用途径的多种合适形式中的任何一种，例如用于口服、舌下、口腔、鼻、直肠、局部(包括透皮和皮内)、眼、脑内、颅内、鞘内、动脉内、静脉内、肌肉内施用，或其他肠胃外施用途径。本领域技术人员将理解，口服和鼻用组合物包括通过吸入给药并以可用的方法制备的组合物。根据所需的特定给药途径，可以使用本领域众所周知的多种药学可接受的载体。药学可接受的载体包括例如，固体或液体填充剂、稀释剂、水溶助剂、表面活性剂和封装物质。可以包括任选的药物活性物质，其不会实质上干扰化合物或组合物的抑制活性。与化合物或组合物结合使用的载体的量，足以提供用于每单位剂量的化合物施用的实际量的材料。制备可用于本文所述方法的剂型的技术和组合物描述于以下文献(其全体通过引用并入本文)：Modern Pharmaceutics,4th Ed., Chapters 9and 10(Banker&Rhodes,editors,2002)；Lieberman等,Pharmaceutical Dosage Forms:Tablets(1989)；和Ansel,Introduction to Pharmaceutical Dosage Forms 8th Edition(2004)。

[0047] 可以使用各种口服剂型，包括片剂、胶囊(例如固体凝胶胶囊和液体凝胶胶囊)、颗粒剂和散装粉末等固体形式。片剂可以为压缩的、研磨片剂、包有肠溶衣、糖衣、薄膜包衣或多次压缩的，其含有合适的粘合剂、润滑剂、稀释剂、崩解剂、着色剂、调味剂、流动诱导剂和熔化剂。液体口服剂型包括水溶液、乳液、悬浮液、由非泡腾颗粒重新构成的溶液和/或悬浮液，以及由泡腾颗粒重新构成的泡腾制剂，该剂型中含有合适的溶剂、防腐剂、乳化剂、悬浮剂、稀释剂、甜味剂、熔化剂、着色剂和调味剂。

[0048] 适于制备经口施用的单位剂型的药学可接受的载体是本领域熟知的。片剂通常包含作为惰性稀释剂的常规药学上相容的佐剂如碳酸钙、碳酸钠、甘露糖醇、乳糖和纤维素；粘合剂如淀粉、明胶和蔗糖；崩解剂如淀粉、海藻酸和交联羧甲纤维素；润滑剂如硬脂酸镁、硬脂酸和滑石。助流剂如二氧化硅可用于改善粉末混合物的流动特性。为了外观，可以添加着色剂，如FD&C染料。甜味剂和调味剂，例如阿斯巴甜、糖精、薄荷醇、薄荷和水果香料是咀嚼片的有用的佐剂。胶囊通常包含一种或多种上述公开的固体稀释剂。载体组分的选择取决于次要考虑因素如味道、成本和贮存稳定性，这不是关键的并且可以由本领域技术人员容易地进行。

[0049] 口服组合物还可以包含液体溶液、乳剂、悬浮液等。适于制备这种组合物的药学可接受的载体是本领域公知的。用于糖浆、酏剂、乳剂和悬浮液的载体的典型组分，包括乙醇、甘油、丙二醇、聚乙二醇、液体蔗糖、山梨糖醇和水。对于悬浮液，典型的悬浮剂包括：甲基纤维素、羧甲基纤维素钠、AVICEL RC-591、黄蓍胶和海藻酸钠；典型的润湿剂包括卵磷脂和聚山梨醇酯80；典型的防腐剂包括对羟基苯甲酸甲酯和苯甲酸钠。口服液体组合物还可以含有诸如上文公开的甜味剂、调味剂和着色剂中的一种或多种组分。

[0050] 这种组合物也可以通过常规方法进行包衣，通常使用pH或时间依赖性包衣，使得主题组合物在期望的局部应用附近的胃肠道中释放，或者在不同时间释放以延长期望的作用。这种剂型通常包括但不限于以下的一种或多种：乙酸邻苯二甲酸纤维素、聚乙烯乙酸邻苯二甲酸酯、羟丙基甲基纤维素邻苯二甲酸酯、乙基纤维素、Eudragit包衣、蜡和虫胶。

[0051] 本文描述的组合物可任选地包括其他药物活性物质。

[0052] 用于实现全身递送主题化合物的其他组合物，包括舌下、口腔和鼻用剂型。这样的组合物通常包含一种或多种可溶性填料物质如蔗糖、山梨糖醇和甘露醇；和粘合剂如阿拉

伯树胶、微晶纤维素、羧甲基纤维素和羟丙基甲基纤维素。也可以包括上述公开的助流剂、润滑剂、甜味剂、着色剂、抗氧化剂和调味剂。

[0053] 配制液体组合物(将其配制为用于局部眼科应用)使得其可以向眼睛局部施用。应当尽可能使舒适感最大化,但有时出于配制考虑(例如药物稳定性)可能无法达到最佳舒适感。在不能将舒适感最大化的情况下,应当将其配制成液体,这样液体对局部眼科应用的患者而言是可容忍的。另外,眼科可接受的液体应当被包装成单次使用,或包含防腐剂以预防经多次使用污染。

[0054] 对于眼科应用,通常用生理盐水溶液作为主要介质制备溶液或药物。可以优选用适当的缓冲系统使眼科溶液保持舒适的pH。制剂还可以包含常规的药学可接受的防腐剂、稳定剂和表面活性剂。

[0055] 可用于本文所公开的药物组合物的防腐剂包括但不限于,苯扎氯铵、PHMB、氯丁醇、硫柳汞、醋酸苯汞和硝酸苯汞。有用的表面活性剂为例如Tween 80。同样地,多种有用的介质可用于本文所公开的眼科制剂中。这些介质包括但不限于,聚乙烯醇、聚维酮、羟丙基甲基纤维素、泊洛沙姆、羧甲基纤维素、羟乙基纤维素和纯化水。

[0056] 可根据需要或便利添加张力调节剂。它们包括但不限于,盐(尤其是氯化钠、氯化钾)、甘露糖醇和甘油,或任何其他合适的眼科可接受的张力调节剂。

[0057] 可以使用多种用于调节pH的缓冲液和方法,只要所得的制剂是眼科可接受的。对于许多组合物,pH为4至9。因此,缓冲液包括醋酸盐缓冲液、柠檬酸盐缓冲液、磷酸盐缓冲液和硼酸盐缓冲液。根据需要,可用酸或碱调节这些制剂的pH。

[0058] 眼科可接受的抗氧化剂包括但不限于,焦亚硫酸钠、硫代硫酸钠、乙酰半胱氨酸、丁基羟基茴香醚和丁羟甲苯。

[0059] 可包含在眼科制剂中的其他赋形剂组分为螯合剂。有用的螯合剂为依地酸二钠,但是还可用其他螯合试剂代替它或与其结合。

[0060] 对于局部应用,可使用包含本文所公开的组合物的乳膏剂、软膏剂、凝胶、溶液或悬浮液等。局部制剂可以通常由药物载体、共溶剂、乳化剂、渗透促进剂、防腐剂系统和软化剂组成。

[0061] 对于静脉内施用,可将本文所述的组合物溶解或分散在药学可接受的稀释剂(例如盐水或葡萄糖溶液)中。还可包含合适的赋形剂以达到期望的pH,其包括但不限于NaOH、碳酸钠、醋酸钠、HCl和柠檬酸。在多种实施方案中,最终组合物的pH为2至8,或优选4至7。抗氧化剂赋形剂可包含亚硫酸氢钠、丙酮合亚硫酸氢钠、甲醛次硫酸氢钠、硫脲和EDTA。可见于最终静脉内组合物中的合适的赋形剂的其他非限制性实例可包括磷酸钠或磷酸钾、柠檬酸、酒石酸、明胶和诸如葡萄糖、甘露糖醇和葡聚糖的碳水化合物。其他可接受的赋形剂在以下中有所描述:Powell等,Compendium of Excipients for Parenteral Formulations,PDA J Pharm Sci and Tech 1998,52 238-311和Nema等,Excipients and Their Role in Approved Injectable Products:Current Usage and Future Directions,PDA J Pharm Sci and Tech 2011,65 287-332,两者通过引用整体并入本文中。还可包含抗微生物试剂以获得抑制细菌的或抑制真菌的溶液,其包括但不限于硝酸苯汞、硫柳汞、苄索氯铵、苯扎氯铵、苯酚、甲酚和氯丁醇。

[0062] 可以以一种或多种固体形式向护理人员提供用于静脉内施用的组合物,在施用之

前可以立即用合适的稀释剂如无菌水、盐水或葡萄糖水溶液复水。在其他的实施方案中，以准备好的溶液形式提供组合物以进行肠胃外施用。在其他实施方案中，以在施用之前需进一步稀释的溶液形式提供组合物。在包括施用本文所述的化合物与其他试剂的组合的实施方案中，作为混合物向护理人员提供该组合，或护理人员在施用之前混合两种试剂，或可单独施用两种试剂。

[0063] 本文所述的活性化合物的实际剂量取决于具体化合物和待治疗的病况；适当剂量的选择是技术人员所熟知的。在一些实施方案中，普那布林的每日剂量可为约0.25mg/kg体重至约120mg/kg体重或更多，约0.5mg/kg体重或更少至约70mg/kg体重，约1.0mg/kg体重至约50mg/kg体重，或约1.5mg/kg体重至约10mg/kg体重。因此，对于施用于70kg的人，剂量范围可为：约17mg/天至约8000mg/天，约35mg/天或更少至约7000mg/天或更多，约70mg/天至约6000mg/天，约100mg/天至约5000mg/天，或约200mg至约3000mg/天。

[0064] 在一些实施方案中，本文所述的组合物可以与其他治疗剂组合使用。在一些实施方案中，本文所述的组合物可以与诸如化学疗法、放射疗法和生物疗法的治疗组合施用或应用。

[0065] 治疗方法

[0066] 一些实施方案涉及治疗癌症的方法，其将本文所述的药物组合物应用于有需要的对象。一些实施方案涉及治疗癌症的方法，其包括将普那布林以及一种或多种免疫检查点抑制剂共同施用于有需要的对象。在一些实施方案中，所述对象可以为动物，例如哺乳动物、人。在一些实施方案中，所述对象为人。

[0067] 一些实施方案涉及通过共同施用普那布林以及一种或多种免疫检查点抑制剂来提供针对癌症的T细胞激活的共刺激的方法。一些实施方案涉及通过共同施用普那布林以及一种或多种免疫检查点抑制剂来提供针对癌症的自然杀伤细胞的共刺激的方法。

[0068] 在一些实施方案中，所述癌症包含表达结合PD-1的配体的癌症细胞。在一些实施方案中，所述结合PD-1的配体是PD-L1。在一些实施方案中，所述结合PD-1的配体是PD-L2。

[0069] 在一些实施方案中，本文所述的治疗癌症的方法还包括鉴别表达结合PD-1的配体的癌症细胞。在一些实施方案中，本文所述的治疗癌症的方法还包括鉴别表达PD-L1的癌症细胞。在一些实施方案中，本文所述的治疗癌症的方法还包括鉴别表达PD-L2的癌症细胞。在一些实施方案中，本文所述的治疗癌症的方法还包括鉴别表达PD-L3或PD-L4的癌症细胞。

[0070] 在一些实施方案中，鉴别表达结合PD-1的配体的癌症细胞，包括使用分析测定来检测结合配体的存在。适用的分析测定的实例包括但不限于：Dako公司提供的PD-L1IHC 22C3pharmDx试剂盒和PD-L1IHC 28-8pharmDx。

[0071] 在一些实施方案中，所述癌症包含表达结合CTLA-4的配体的癌症细胞。在一些实施方案中，所述结合CTLA-4的配体是B7.1或B7.2。

[0072] 在一些实施方案中，本文所述的治疗癌症的方法还包括鉴别表达结合CTLA-4的配体的癌症细胞。在一些实施方案中，本文所述的治疗癌症的方法还包括鉴别表达B7.1或B7.2的癌症细胞。

[0073] 在一些实施方案中，所述免疫检查点抑制剂是纳武单抗、派姆单抗、哌利珠单抗(pidilizumab)、伊匹单抗、达卡巴嗪、BMS 936559、阿特珠单抗(atezolizumab)、杜瓦单抗

(durvalimumab)或以上抑制剂的任何组合。

[0074] 在一些实施方案中，癌症是头颈癌、肺癌、胃癌、结肠癌、胰腺癌、前列腺癌、乳腺癌、肾癌、膀胱癌、卵巢癌、子宫颈癌、黑素瘤、胶质母细胞瘤、骨髓瘤、淋巴瘤或白血病。在一些实施方案中，所述癌症是肾细胞癌、恶性黑素瘤、非小细胞肺癌(NSCLC)、卵巢癌、霍奇金淋巴瘤或鳞状细胞癌。在一些实施方案中，所述癌症选自：乳腺癌、结肠癌、直肠癌、肺癌、前列腺癌、黑素瘤、白血病、卵巢癌、胃癌、肾细胞癌、肝癌、胰腺癌、淋巴瘤和骨髓瘤。在一些实施方案中，所述癌症是实体瘤或血液癌症。

[0075] 在一些实施方案中，所述癌症不具有在可检测水平的任何表达PD-1、PD-L1或PD-L2的细胞。

[0076] 在一些实施方案中，所述癌症选自：乳腺癌、结肠癌、直肠癌、肺癌、前列腺癌、黑素瘤、白血病、卵巢癌、胃癌、肾细胞癌、肝癌、胰腺癌、淋巴瘤和骨髓瘤。在一些实施方案中，所述癌症是实体瘤或血液癌症。

[0077] 一些实施方案涉及在癌症患者中诱导树突状细胞成熟的方法，其包括将含有普那布林的组合物施用于癌症患者。

[0078] 一些实施方案涉及破坏对象的癌症相关肿瘤血管系统的方法，其包括将普那布林以及一种或多种免疫检查点抑制剂的混合物共同施用于对象。

[0079] 多种癌症与肿瘤血管系统的形成有关。在一些实施方案中，所述癌症选自：黑素瘤、胰腺癌、结肠直肠腺癌、脑肿瘤、急性淋巴细胞性白血病、慢性淋巴细胞性白血病、激素难治性转移性前列腺癌、转移性乳腺癌、非小细胞肺癌、肾细胞癌、头颈癌、前列腺癌、结肠癌、间变性甲状腺癌。

[0080] 一些实施方案包括将本文所述的组合物和/或药物组合物与另外的药物共同施用。例如，如上所述，一些实施方案包括将普那布林与一种或多种免疫检查点抑制剂共同施用。“共同施用”意指两种或更多种试剂以这样的方式施用，即一种或多种试剂的施用对一种或多种其他试剂的功效和/或安全性有影响，不管它们实际上是何时或怎样被施用。在一个实施方案中，所述试剂是同时施用的。在一个这样的实施方案中，通过将所述试剂组合在一个剂型中来实现组合施用。在另一实施方案中，所述试剂是依次施用的。在一个实施方案中，通过相同的途径如口服或静脉内来施用所述试剂。在另一实施方案中，通过不同的途径施用所述试剂，如一种试剂口服施用，另一种试剂静脉内施用。在一些实施方案中，施用一种或多种试剂与施用共同施用的一种或多种试剂之间的时间段可以为：约1小时、2小时、3小时、5小时、8小时、10小时、12小时、15小时、18小时、20小时、24小时、36小时、48小时、3天、4天、5天、6天、7天、10天、14天、21天、28天或30天。

[0081] 在一些实施方案中，治疗周期可包括将共同施用普那布林以及一种或多种免疫检查点抑制剂，与单独施用普那布林或单独施用一种或多种免疫检查点抑制剂组合。在一些实施方案中，第一天共同施用普那布林以及一种或多种免疫检查点抑制剂，然后在1天、2天、3天、4天、5天、6天、7天、2周或3周后单独施用普那布林，接着在1天、2天、3天、4天、5天、6天、7天、2周或3周后共同施用普那布林以及一种或多种免疫检查点抑制剂。在一些实施方案中，在第一天同时施用普那布林以及一种或多种免疫检查点抑制剂，然后在第2天至第31天选择一天单独施用普那布林或一种或多种免疫检查点抑制剂，接着在第3天至第31天选择一天共同施用普那布林以及一种或多种免疫检查点抑制剂。在一些实施方案中，在第一

天共同施用普那布林以及一种或多种免疫检查点抑制剂，然后在第8天单独施用普那布林，接着在第15天共同施用普那布林以及一种或多种免疫检查点抑制剂。在一些实施方案中，所述治疗周期可重复两次或更多次。

[0082] 其他药物的实例包括其他化疗剂。

[0083] 在一些实施方案中，所述化疗剂可选自：醋酸阿比特龙、氨甲喋呤(甲氨喋呤)、Abraxane(紫杉醇白蛋白-稳定的纳米微粒制剂)、ABVD、ABVE、ABVE-PC、AC、AC-T、Adcetris(贝伦妥单抗-维多汀)、ADE、Ado-曲妥珠单抗-艾美坦辛、阿霉素(盐酸多柔比星)、双马来酸盐阿法替尼、Afinitor(依维莫司)、Akynzeo(奈妥吡坦和盐酸帕洛诺司琼)、Aldara(咪喹莫特)、阿地白介素、Alecensa(艾乐替尼)、艾乐替尼、阿伦单抗、爱宁达(培美曲塞二钠)、阿乐喜(盐酸帕洛诺司琼)、瘤可宁(苯丁酸氮芥)、瘤可宁(苯丁酸氮芥)、氨基乙酰丙酸、阿那曲唑、阿瑞匹坦、阿可达(Aredia)(帕米磷酸二钠)、瑞宁得(阿那曲唑)、阿诺新(依西美坦)、Arranon(奈拉滨)、三氧化二砷、Arzerra(奥法木单抗)、菊欧文氏菌天冬酰胺酶、阿瓦斯汀(贝伐单抗)、阿西替尼、阿扎胞苷、BEACOPP、Becenum(卡莫司汀)、Beleodaq(贝利司他)、贝利司他、盐酸苯达莫司汀、BEP、贝伐单抗、蓓萨罗丁、百克沙(Bexxar)(托西莫单抗和碘131托西莫单抗)、比卡鲁胺、BiCNU(卡莫司汀)、博来霉素、博纳吐单抗、Blincyto(博纳吐单抗)、硼替佐米、Bosulif(博舒替尼)、博舒替尼、贝伦妥单抗-维多汀、白消安、卡巴他赛、卡博替尼-S-苹果酸盐、CAF、Campath(阿伦单抗)、Camptosar(盐酸伊立替康)、卡培他滨、CAPOX、Carac(氟尿嘧啶-局部用)、卡铂、卡铂-紫杉酚、卡非佐米、Carmubris(卡莫司汀)、卡莫司汀、卡莫司汀植入物、康士得(Casodex)(比卡鲁胺)、CeeNU(洛莫司汀)、色瑞替尼、Cerubidine(盐酸柔红霉素)、希瑞适(Cervarix)(重组HPV双价疫苗)、西妥昔单抗、瘤可宁、瘤可宁-强的松、CHOP、顺铂、Clafen(环磷酰胺)、氯法拉滨、Clofarex(氯法拉滨)、克罗拉(Clolar)(氯法拉滨)、CMF、考比替尼、Cometriq(卡博替尼-S-苹果酸盐)、COPDAC、COPP、COPP-ABV、Cosmegen(更生霉素)、Cotellic(考比替尼)、克唑替尼、CVP、环磷酰胺、Cyfos(异环磷酰胺)、Cyramza(雷莫芦单抗)、阿糖胞苷、阿糖胞苷脂质体、Cytosar-U(阿糖胞苷)、癌得星(Cytoxan)(环磷酰胺)、达拉菲尼、达卡巴嗪、达珂(Dacogen)(地西他滨)、更生霉素、达雷木单抗、Darzalex(达雷木单抗)、达沙替尼、盐酸道诺霉素、地西他滨、地加瑞克(Degarelix)、地尼白介素-白喉毒素连接物、地诺单抗、DepoCyt(阿糖胞苷脂质体)、地塞米松、盐酸右雷佐生、地妥昔单抗(Dinutuximab)、多西他赛、Doxil(盐酸多柔比星脂质体)、盐酸多柔比星、盐酸多柔比星脂质体、Dox-SL(盐酸多柔比星脂质体)、DTIC-Dome(达卡巴嗪)、Efudex(氟尿嘧啶-局部用)、Elitek(拉布立酶)、Ellence(盐酸表柔比星)、埃罗妥珠单抗、乐沙定(Eloxatin)(奥沙利铂)、艾曲波帕乙醇胺、Emend(阿瑞匹坦)、Empliciti(埃罗妥珠单抗)、恩杂鲁胺、盐酸表柔比星、EPOCH、爱必妥(Erbitux)(西妥昔单抗)、甲磺酸艾日布林、Erivedge(维莫德吉)、盐酸埃罗替尼、Erwinaze(菊欧文氏菌天冬酰胺酶)、凡毕复(Etopophos)(磷酸依托泊苷)、依托泊苷、磷酸依托泊苷、Evacet(盐酸多柔比星脂质体)、依维莫司、易维特(Evista)(盐酸雷洛昔芬)、依西美坦、5-FU(氟尿嘧啶注射液)、5-FU(氟尿嘧啶-局部用)、法乐通(Fareston)(托瑞米芬)、Farydak(帕比司他)、Faslodex(氟维司群)、FEC、弗隆(Femara)(来曲唑)、非格司亭、福达华(Fludara)(磷酸氟达拉滨)、磷酸氟达拉滨、Fluoroplex(氟尿嘧啶-局部用)、氟尿嘧啶注射液、氟尿嘧啶-局部用、氟他米特、Folex(甲氨蝶呤)、Folex PFS(甲氨蝶呤)、FOLFIRI、FOLFIRI-贝伐单抗、FOLFIRI-西妥昔单抗、

FOLFIRINOX、FOLFOX、Folotyn(普拉曲沙)、FU-LV、氟维司群、加卫苗(Gardasil)(重组HPV四价疫苗)、加卫苗(Gardasil)9(重组HPV九价疫苗)、Gazyva(阿妥珠单抗)、吉非替尼、盐酸吉西他滨、吉西他滨-顺铂、吉西他滨-奥沙利铂、吉妥珠单抗-奥佐米星、健择(Gemzar)(盐酸吉西他滨)、Gilotrif(双马来酸盐阿法替尼)、格列卫(Gleevec)(甲磺酸伊马替尼)、Gliadel(卡莫司汀植入物)、Gliadel圆片(卡莫司汀植入物)、羧肽酶、乙酸戈舍瑞林、Halaven(甲磺酸艾日布林)、赫赛汀(Herceptin)(曲妥珠单抗)、HPV二价疫苗、重组HPV九价疫苗、重组HPV四价疫苗、重组和美新(Hycamtin)(盐酸拓扑替康)、Hyper-CVAD、Ibrance(帕博西尼)、替伊莫单抗-Tiuxetan、依鲁替尼、ICE、Iclusig(盐酸帕纳替尼)、善唯达(Idamycin)(盐酸伊达比星)、艾代拉利司、Ifex(异环磷酰胺)、异环磷酰胺、IL-2(阿地白介素)、甲磺酸伊马替尼、Imbruvica(依鲁替尼)、咪喹莫特、Imlygic(Talimogene Laherparepvec)、Inlyta(阿西替尼)、干扰素α-2b、重组白介素-2(阿地白介素)、Intron A(重组干扰素α-2b)、碘131托西莫单抗和托西莫单抗、伊匹单抗、易瑞沙(Iressa)(吉非替尼)、盐酸伊立替康、盐酸伊立替康脂质体、Istodax(罗米地辛)、伊沙匹隆、柠檬酸伊沙佐米、Ixempra(伊沙匹隆)、Jakafi(磷酸卢索替尼)、Jevtana(卡巴他赛)、Kadcyla(Ado-曲妥珠单抗Emtansine)、Keoxifene(盐酸雷洛昔芬)、Kepivance(帕利夫明)、Keytruda(派姆单抗)、Kyprolis(卡非佐米)、乙酸兰瑞肽、二甲苯磺酸拉帕替尼、来那度胺、甲磺酸乐伐替尼、Lenvima(甲磺酸乐伐替尼)、来曲唑、亚叶酸钙、Leukeran(瘤可宁)、乙酸亮丙瑞林、Levulan(氨基乙酰丙酸)、Linfolizin(瘤可宁)、LipoDox(盐酸多柔比星脂质体)、洛莫司汀、Lonsurf(三氟尿苷和盐酸替比嘧啶)、利普安(Lupron)(乙酸亮丙瑞林)、储库型利普安(Lupron Depot)(乙酸亮丙瑞林)、储库型利普安-Ped(乙酸亮丙瑞林)、储库型利普安-3个月(乙酸亮丙瑞林)、储库型利普安-4个月(乙酸亮丙瑞林)、Lynparza(奥拉帕尼)、Marqibo(硫酸长春新碱脂质体)、Matulane(盐酸甲基苄肼)、盐酸氮芥、Megace(乙酸甲地孕酮)、乙酸甲地孕酮、Mekinist(曲美替尼)、巯嘌呤、美司钠、Mesnex(美司钠)、Methazolastone(替莫唑胺)、甲氨蝶呤、甲氨蝶呤LPF(甲氨蝶呤)、Mexate(甲氨蝶呤)、Mexate-AQ(甲氨蝶呤)、丝裂霉素C、盐酸米托蒽醌、Mitozytrex(丝裂霉素C)、MOPP、Mozobil(普乐沙福)、Mustargen(盐酸氮芥)、Mutamycin(丝裂霉素C)、马勒兰(Myleran)(白消安)、Mylosar(阿扎胞苷)、米罗他(Mylotarg)(吉妥珠单抗-奥佐米星)、纳米颗粒紫杉醇(紫杉醇白蛋白-稳定的纳米颗粒制剂)、Navelbine(酒石酸长春瑞滨)、耐昔妥单抗、奈拉滨、Neosar(环磷酰胺)、奈妥吡坦和盐酸帕洛诺司琼、优保津(Neupogen)(非格司亭)、多吉美(Nexavar)(甲苯磺酸索拉非尼)、尼罗替尼、Ninlaro(柠檬酸伊沙佐米)、纳武单抗、Nolvadex(柠檬酸泰莫西芬)、Nplate(罗米司亭)、阿妥珠单抗、Odomzo(索尼吉布)、OEPA、奥法木单抗(Ofatumumab)、OFF、奥拉帕尼、高三尖杉酯碱(Omacetaxine Mepesuccinate)、Oncaspar(培门冬酶)、盐酸昂丹司琼、Onivyde(盐酸伊立替康脂质体)、Ontak(地尼白介素-白喉毒素连接物)、Opdivo(纳武单抗)、OPPA、奥斯替尼、奥沙利铂、紫杉醇、紫杉醇白蛋白-稳定的纳米颗粒制剂、PAD、帕博西尼、帕利夫明、盐酸帕洛诺司琼、盐酸帕洛诺司琼和奈妥吡坦、帕米磷酸二钠、帕尼单抗、帕比司他、Paraplat(卡铂)、Paraplatin(卡铂)、盐酸帕唑帕尼、PCV、培门冬酶、聚乙二醇干扰素α-2b、PEG-Intron(聚乙二醇干扰素α-2b)、派姆单抗、培美曲塞二钠Perjeta(帕妥珠单抗)、帕妥珠单抗、Platinol(顺铂)、Platinol-AQ(顺铂)、普乐沙福、泊马度胺、Pomalyst(泊马度胺)、盐酸帕纳替尼、Portrazza(耐昔妥单抗)、普拉曲沙、强的松、盐酸甲基苄肼、

Proleukin(阿地白介素)、Prolia(地诺单抗)、Promacta(艾曲波帕乙醇胺)、Provenge(Sipuleucel-T)、Purinethol(巯嘌呤)、Purixan(巯嘌呤)、镭223二氯化物、盐酸雷洛昔芬、雷莫芦单抗、拉布立酶、R-CHOP、R-CVP、重组人乳头瘤病毒(HPV)二价疫苗、重组人乳头瘤病毒(HPV)九价疫苗、重组人乳头瘤病毒(HPV)四价疫苗、重组干扰素α-2b、瑞戈非尼、R-EPOCH、Revlimid(来那度胺)、Rheumatrex(甲氨蝶呤)、利妥昔单抗、盐酸罗拉吡坦、罗米地辛、罗米司亭、红比霉素(盐酸道诺霉素)、磷酸卢索替尼、司兰索胸膜内气溶胶(Talc)、司妥昔单抗、Sipuleucel-T、索马杜林储库型(乙酸兰瑞肽)、索尼吉布、甲苯磺酸索拉非尼、施达赛(Sprycel)(达沙替尼)、STANFORD V、无菌滑石粉(Talc)、Steritalc(Talc)、Stivarga(瑞戈非尼)、苹果酸舒尼替尼、索坦(Sutent)(苹果酸舒尼替尼)、Sylatron(聚乙二醇干扰素α-2b)、Sylvant(司妥昔单抗)、Synovir(萨力多胺)、Synribo(高三尖杉酯碱)、Tabloid(硫鸟嘌呤)、TAC、Tafinlar(达拉菲尼)、Tagrisso(奥斯替尼)、Talc、Talimogene Laherparepvec、柠檬酸泰莫西芬、Tarabine PFS(阿糖胞苷)、特罗凯(Tarceva)(盐酸埃罗替尼)、Targretin(蓓萨罗丁)、泰息安(Tasigna)(尼罗替尼)、Taxol(紫杉醇)、泰索帝(Taxotere)(多西他赛)、Temodar(替莫唑胺)、替莫唑胺、坦罗莫司、萨力多胺、硫鸟嘌呤、塞替派、Tolak(氟尿嘧啶-局部用)、Toposar(依托泊苷)、盐酸拓扑替康、托瑞米芬、Torisel(坦罗莫司)、托西莫单抗和碘131托西莫单抗、Totect(盐酸右雷佐生)、TPF、曲贝替定、曲美替尼、曲妥珠单抗、Treanda(盐酸苯达莫司汀)、三氟尿苷和盐酸替比嘧啶、Trisenox(三氧化砷)、泰立沙(Tykerb)(二甲苯磺酸拉帕替尼)、Unituxin(地妥昔单抗)、三乙酸尿苷、VAC、凡得他尼、VAMP、Varubi(盐酸罗拉吡坦)、Vectibix(帕尼单抗)、VeIP、Velban(硫酸长春花碱)、万珂(Velcade)(硼替佐米)、Velsar(硫酸长春花碱)、维罗非尼、VePesid(依托泊苷)、Viadur(乙酸亮丙瑞林)、Vidaza(阿扎胞苷)、硫酸长春花碱、Vincasar PFS(硫酸长春新碱)、硫酸长春新碱、硫酸长春新碱脂质体、酒石酸长春瑞滨、VIP、维莫德吉、Vistogard(三乙酸尿苷)、Voraxaze(羧肽酶)、伏立诺他、福退癌(Votrient)(盐酸帕唑帕尼)、Wellcovorin(亚叶酸钙)、Xalkori(克唑替尼)、希罗达(Xeloda)(卡培他滨)、XELIRI、XELOX、Xgeva(地诺单抗)、Xofigo(镭223二氯化物)、Xtandi(恩杂鲁胺)、Yervoy(伊匹单抗)、Yondelis(曲贝替定)、Zaltrap(Ziv-阿柏西普)、Zarxio(非格司亭)、Zelboraf(维罗非尼)、泽娃灵(Zevalin)(替伊莫单抗-Tiuxetan)、Zinecard(盐酸右雷佐生)、Ziv-阿柏西普、枢复宁(Zofran)(盐酸昂丹司琼)、诺雷得(Zoladex)(乙酸戈舍瑞林)、唑来膦酸、Zolinza(伏立诺他)、择泰(Zometa)(唑来膦酸)、Zydelig(艾代拉利司)、Zykadia(色瑞替尼)和Zytiga(醋酸阿比特龙)。

[0084] 为了进一步说明本发明,包括以下实施例。这些实施例当然不应被解释为具体限制本发明。在权利要求范围内的这些实施例的变化在本领域技术人员的范围内,并且被视为落入本文所述和所要求保护的本发明范围内。读者将认识到借助本公开后,技术人员和本领域技术人员能够准备和使用本发明而无需详尽的实例。

实施例

[0085] 实施例1.普那布林对树突细胞成熟的影响

[0086] 细胞系:将未成熟小鼠DC细胞系SP37A3(由Merck KGaA提供)培养于补充有以下成分的Iscove改良杜氏培养基(IMDM;Sigma):10%热灭活和内毒素测试的FBS(PAA)、丙酮酸

钠(Gibco)、青霉素/链霉素L-谷氨酰胺混合物(Gibco)、Eagle最低必需培养基(MEM)非必需氨基酸(Sigma)、Ciproxin(Bayer)和0.05mmol/L 2-巯基乙醇(Gibco)。IMDM完全培养基补充有20ng/mL重组小鼠GM-CSF和20ng/mL重组小鼠M-CSF(两者均来自Peprotech)。鼠肿瘤细胞系EG7和3LL-OVA分别获自ATCC或由Douglas T.Fearon(Cancer Research UK Cambridge Institute,Li Ka Shing Center,University of Cambridge,Cambridge,UK)提供。检测所有细胞系并验证为无支原体。分别证实了OVA在EG7和3LL-OVA中的表达及Thy1.1在RMAThy1.1中的表达;没有进行基因组鉴定。

[0087] 将SP37A3DC(鼠DC系,Merck)在补充有以下成分的180uL IMDM完全培养基[IMDM培养基(Sigma)中铺板($8x10^4$个细胞/孔,96-孔平底,经组织培养处理的):10%热灭活和内毒素测试的FBS(PAA)、丙酮酸钠(Gibco)、青霉素/链霉素L-谷氨酰胺混合物(Gibco)、MEM非必需氨基酸(Sigma)和0.05mM 2-巯基乙醇(Gibco)]。IMDM完全培养基补充有20ng/mL重组小鼠GM-CSF。允许DC贴附2小时,然后添加普那布林、培养基或LPS作为对照,10x浓缩于20uL。将DC与不同浓度的普那布林(0.001μM,0.01μM,0.1μM,1μM,10μM)、培养基和LPS分别孵育20小时。收集这些培养物的上清液,将其用于通过ELISA(来自BD的试剂盒)检测细胞因子产生,并用LD-IR细胞活性鉴定染料(Invitrogen)以及针对CD80、CD86、CD40和MHCII的荧光染料标记的单克隆抗体对细胞进行染色,用于进行流式细胞术分析。使用配有DIVA软件的BD Fortessa细胞计数器分析细胞。将活细胞中DC成熟标志物CD40、CD80、CD86和MHCII的平均荧光强度(MFI)归一化为在未处理的(培养基)DC中检测到的那些标志物的MFI。如图1A所示,普那布林显著增加了所有四种DC成熟标志物CD40、CD80、CD86和MHCII的表达。如图1B所示,如使用SytoxGreen染色所测定,在所测试的任何药物浓度下,DC活力没有显著变化。

[0088] 实施例2.相比紫杉醇和依托泊苷,普那布林对树突细胞成熟的影响

[0089] 还测定了其他两种癌症药物紫杉醇和依托泊苷,以和普那布林比较它们对DC成熟的影响。将SP37A3DC(鼠DC系,Merck)在180uL IMDM完全培养基[IMDM培养基(Sigma),其补充有:10%热灭活和内毒素测试的FBS(PAA)、丙酮酸钠(Gibco)、青霉素/链霉素L-谷氨酰胺混合物(Gibco)、MEM非必需氨基酸(Sigma)和0.05mM 2-巯基乙醇(Gibco)]中接种($8x10^4$个细胞/孔,96-孔平底,经组织培养处理的)。IMDM完全培养基补充有20ng/mL重组小鼠GM-CSF。允许DC贴附2小时,然后添加普那布林、紫杉醇、依托泊苷、培养基或LPS(阳性对照),10x浓缩于20uL。将DC与普那布林(0.001μM,0.01μM,0.1μM,1μM,10μM)、紫杉醇(0.001μM,0.01μM,0.1μM,1μM,10μM)、依托泊苷(0.001μM,0.01μM,0.1μM,1μM,10μM)、培养基和LPS(阳性对照)分别孵育20h。收集这些培养物的上清液,将其用于通过ELISA(来自BD的试剂盒)检测细胞因子产生,并用LD-IR细胞活性鉴定染料(Invitrogen)以及针对CD80、CD86、CD40和MHCII的荧光染料标记的单克隆抗体对细胞进行染色,用于进行流式细胞术分析。使用配有DIVA软件的BD Fortessa细胞计数器分析细胞。将活细胞中DC成熟标志物CD40(图2A)、CD80(图2B)、CD86(图2C)和MHCII(图2D)的平均荧光强度(MFI)归一化为在未处理的(培养基)DC中检测到的那些标志物的MFI。还通过ELISA测定促炎细胞因子IL-1β(图3A)、IL-6(图3B)和IL-12p40(图3C)的产生。分析了来自DC培养物上清液的这些促炎细胞因子,其已经被证明在调节T细胞功能和抗肿瘤免疫应答中发挥关键作用。

[0090] 注意到,普那布林是所有三种药物中对DC成熟最有效的诱导剂。相比紫杉醇和依托泊苷,普那布林显示出所有四种DC成熟标志物(CD40、CD 80、MHCII和CD 86)的更高表达。

与阳性对照LPS相比，普那布林也显示出所有四种标志物的显著增加的表达。与紫杉醇、依托泊苷和LPS相比，普那布林引发IL1b、IL6和IL12的产生增加。因此，普那布林增加了成熟标志物的上调和促炎细胞因子的产生，从而产生了增强的T细胞刺激能力。

[0091] 实施例3.普那布林和免疫检查点抑制剂(PD-1抗体)的协同作用

[0092] 将普那布林和PD-1检查点抑制剂的联合治疗与用单独的普那布林和用单独的PD-1抗体的治疗进行比较。使用皮下注射MC-38肿瘤细胞的7-10周龄小鼠进行试验。准备了五个试验组，每组包括9只小鼠。

[0093] 第1组施用盐水；第2组施用普那布林稀释剂(无普那布林)；第3组施用浓度为7.5mg/kg的溶于稀释剂的普那布林；第4组施用PD-1抗体；以及第5组施用普那布林/PD-1抗体联合治疗。对于普那布林/PD-1抗体联合治疗(第5组)，给小鼠每周2次(每周的第1天和第4天)施用溶于稀释剂的普那布林(7.5mg/kg)，然后在每次施用普那布林后1小时施用PD-1抗体。对于仅有普那布林治疗(第3组)或仅有抗体治疗(第4组)，给小鼠每周2次(每周的第1天和第4天)单独施用普那布林(7.5mg/kg，溶解于稀释剂中)或抗体。对于第1组和第2组，给小鼠每周2次单独施用盐水或普那布林稀释剂。

[0094] 每次治疗开始于大约125mm^3的肿瘤大小，并持续到肿瘤大小达1500mm^3。如果实验45天时，任何组中的平均肿瘤大小未达到1500mm^3，则将停止治疗并继续评估肿瘤大小。为了确定每次治疗的疗效，收集以下数据：肿瘤大小达到1500mm^3之前的死亡率；在治疗前每周两次评估的小鼠体重；由肿瘤大小测量(每周两次)确定的肿瘤生长率；肿瘤生长指数；总体生存率；和使肿瘤大小加倍所需的时间。普那布林和PD-1抗体联合治疗的检测结果表明，普那布林与PD-1抗体在抑制肿瘤生长中起着协同作用。

[0095] 实施例4.OVA特异性OT-I和OT-II T细胞的体内刺激

[0096] 在用普那布林活化前用OVA全长蛋白(0.1mg/mL)或用OVA257-264肽(T4)/OVA323-339肽(500ng/mL；活化后)，将SP37A3细胞或第7天的BMDC脉冲1小时，并以指定的比例加入从OT-I/OT-II转基因小鼠(2x10^5个总细胞/孔，96孔圆底板)纯化的$CD8^+$/$CD4^+$T细胞。在共培养前，使$CD4^+$T细胞装载有增殖染料eFluor670。3天后使用流式细胞术评估增殖。

[0097] 实施例5.抗原特异性CD4和CD8T细胞的体内刺激

[0098] 将来自初始OT-I和OT-II转基因小鼠(Ly5.2)的朗格汉斯细胞(LC)和脾细胞用eFluor670标记，并过继转移到C57BL/6-Ly5.1小鼠中。24小时后，通过尾巴注射用OVA257-264肽(T4:SIINFEKL;SIINFEKL的低亲和力变体)或OVA323-339肽与普那布林或LPS一起对小鼠进行免疫。在用流式细胞术过继转移后4天，评估OT-1$CD8^+$和OT-II $CD4^+$T细胞的增殖。

[0099] 实施例6.DC归巢到肿瘤引流LN的分析

[0100] 为了在注射普那布林时检测DC归巢，用FITC-缀合的葡聚糖(100mg/小鼠；Sigma)和普那布林或PBS/载体(模拟对照)瘤内注射具有皮下EG7肿瘤的小鼠。在注射普那布林后48小时，制备来自肿瘤引流和非引流LN的单细胞悬浮液，并通过流式细胞术进行分析。

[0101] 实施例7.普那布林和免疫检查点抑制剂(PD-1抗体和CTLA-4抗体)的协同作用

[0102] 将普那布林和PD-1检查点抑制剂与CTLA-4检查点抑制剂组合的联合治疗与用单独的普那布林治疗、用单独的PD-1抗体治疗、或用PD-1抗体和CTLA-4抗体的组合治疗进行比较测试。使用皮下注射MC-38肿瘤细胞的7-10周龄小鼠进行试验。准备了六个试验组，每组包括10只小鼠。

[0103] 第1组施用IgG2a和普那布林载体;第2组施用浓度为7.5mg/kg的溶于稀释剂的普那布林;第3组施用PD-1抗体;第4组施用普那布林/PD-1抗体联合治疗;第5组施用组合的PD-1/CTLA-4抗体;以及第6组施用组合的PD-1抗体/CTLA-4抗体/普那布林治疗。对于普那布林/PD-1抗体联合治疗(第4组),以及普那布林/PD-1/CTLA-4抗体治疗(第6组),给小鼠每周2次(每周的第1天和第4天)施用溶于稀释剂的普那布林(7.5mg/kg),然后在每次施用普那布林后1小时施用所述抗体。对于仅有普那布林治疗(第2组)或仅有抗体治疗(第3和5组),给小鼠每周2次(每周的第1天和第4天)单独施用普那布林(7.5mg/kg,溶解于稀释剂中)或抗体。

[0104] 每次治疗开始于大约125mm^{3}的肿瘤大小,并持续到肿瘤大小达3000mm^3。当第1组中的平均肿瘤大小达到3000mm^{3}时,结束实验。为了确定每次治疗的疗效,收集以下数据:肿瘤大小达到3000mm^{3}之前的死亡率;在治疗前每周两次评估的小鼠体重;由肿瘤大小测量(每周两次)确定的肿瘤生长率;肿瘤生长指数;总体生存率;尸体剖检时的肿瘤重量;和肿瘤大小增大10倍所需的时间。尸体剖检时对组织称重并进行FACS分析。

[0105] 普那布林和PD-1抗体与CTLA-4-抗体联合治疗的检测结果表明,普那布林与抗体在抑制肿瘤生长中起协同作用,并且在这6个试验组中,具有达到10倍增加的肿瘤重量的最长时间。图4A显示第1组、第5组和第6组对肿瘤生长的影响。如图4A所示,第6组的普那布林、PD-1抗体和CTLA-4抗体的联合治疗,具有比第5组的PD-1抗体和CTLA-4抗体治疗组的组合更好的肿瘤生长抑制作用,并且与对照第1组相比,第5组和第6组均显示对肿瘤生长的抑制作用。图4B显示六个治疗组对尸体剖检时平均肿瘤重量的影响。如图4B所示,用普那布林、PD-1抗体和CTLA-4-抗体的联合治疗产生了尸体剖检时最低的平均肿瘤重量,其次为普那布林和PD-1抗体的治疗组。图4C显示在六个治疗组中肿瘤达到其起始体积10倍的时间。如图4C所示,用普那布林、PD-1抗体和CTLA-4-抗体联合的治疗组,肿瘤达到其起始体积10倍的时间最长。因此,单独的普那布林治疗或普那布林与PD-1抗体或PD-1加CTLA-4抗体的联合治疗,导致尸体剖检时肿瘤重量减少。普那布林、PD-1抗体和CTLA-4-抗体的联合治疗具有比普那布林和PD-1抗体的治疗更好的肿瘤抑制剂作用,普那布林和PD-1抗体的治疗显示出具有比单独的普那布林治疗更好的肿瘤抑制剂作用。

[0106] 图5显示在上文所述的MC-38CRC肿瘤模型中尸体剖检时肿瘤的FACS分析结果,包括Treg细胞的百分比变化,CD8$^+$/Treg的比例,以及CD45+淋巴细胞中巨噬细胞的百分比。图5A显示六个治疗组对Treg细胞百分比的影响。如图5A所示,与没有普那布林的比较组相比,普那布林、PD-1抗体和CTLA-4-抗体的治疗,普那布林和PD-1抗体的治疗,以及单独普那布林的治疗均显示出%Treg细胞的减少。图5B显示CD8+细胞与Treg细胞的比例。如图5B所示,普那布林、PD-1抗体和CTLA-4-抗体的治疗显示CD8+/Treg细胞的最高比例。图5C显示六个治疗组对巨噬细胞的影响。如图5C所示,与各比较组相比,普那布林、PD-1抗体和CTLA-4-抗体的治疗组,普那布林的治疗组,以及PD-1抗体和CTLA-4-抗体的治疗组均显示降低的巨噬细胞百分比。

[0107] 因此,肿瘤组织的FACS分析表明,单独普那布林治疗,普那布林和免疫检查点抑制剂(例如,普那布林与PD-1抗体,普那布林与PD-1抗体和CTLA-4-抗体)治疗均与调节性T细胞(Treg细胞)的百分比降低、巨噬细胞染色细胞的百分比降低和CD8+/Treg细胞比例的伴随增加相关。与单独的普那布林或单独的抗体的组相比,Treg细胞百分比和巨噬细胞染色

细胞的减少,以及CD8+/Treg细胞比例的增加,在普那布林和免疫检查点抑制剂的治疗组中更显著。这些数据已经证明,使用普那布林和免疫检查点抑制剂(例如PD-1抗体和CTLA-4-抗体)联合治疗的协同免疫肿瘤学特性。

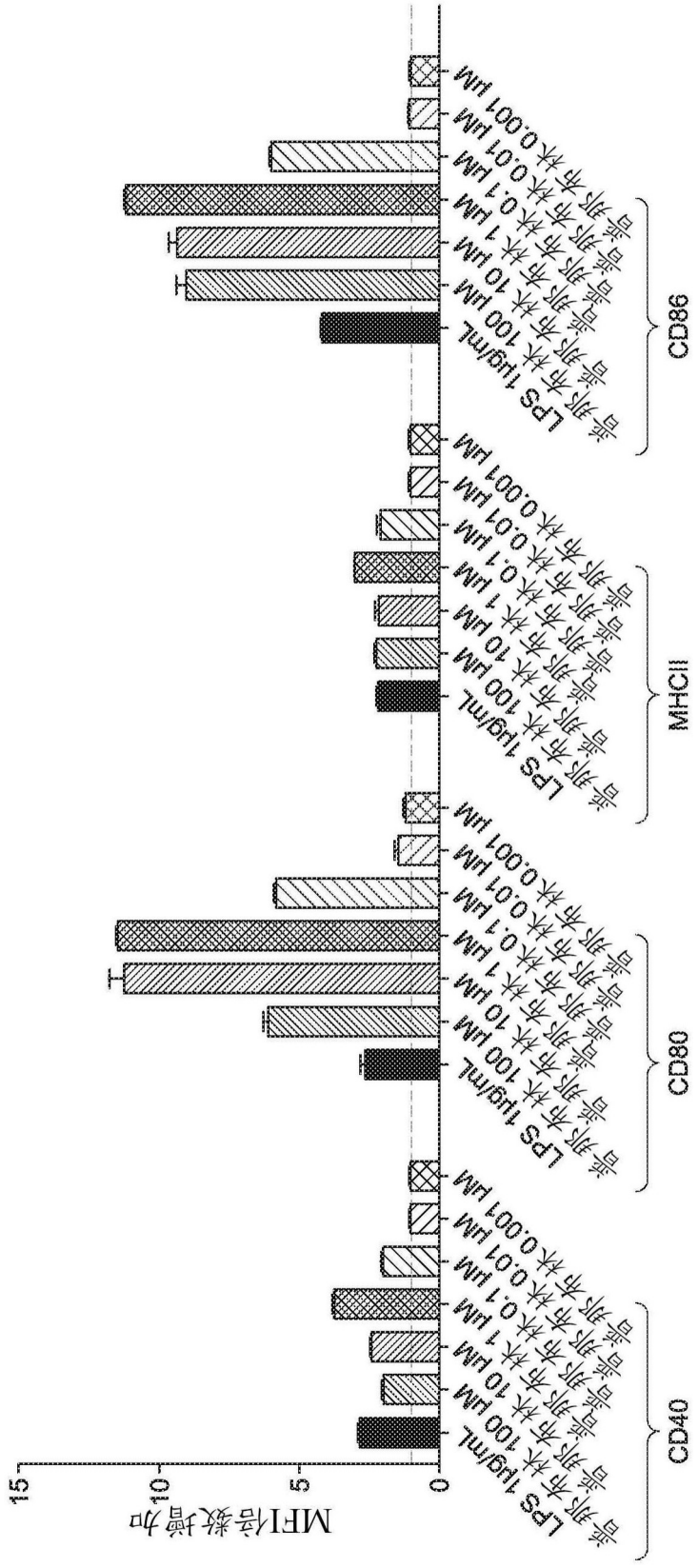

MFI倍数增加
15
10
5
0
CD40
CD80
MHCII
CD86
LPS 1μg/mL
奈那韦钠 100 μM
奈那韦钠 10 μM
奈那韦钠 1 μM
奈那韦钠 0.1 μM
奈那韦钠 0.01 μM
奈那韦钠 0.001 μM

图1A

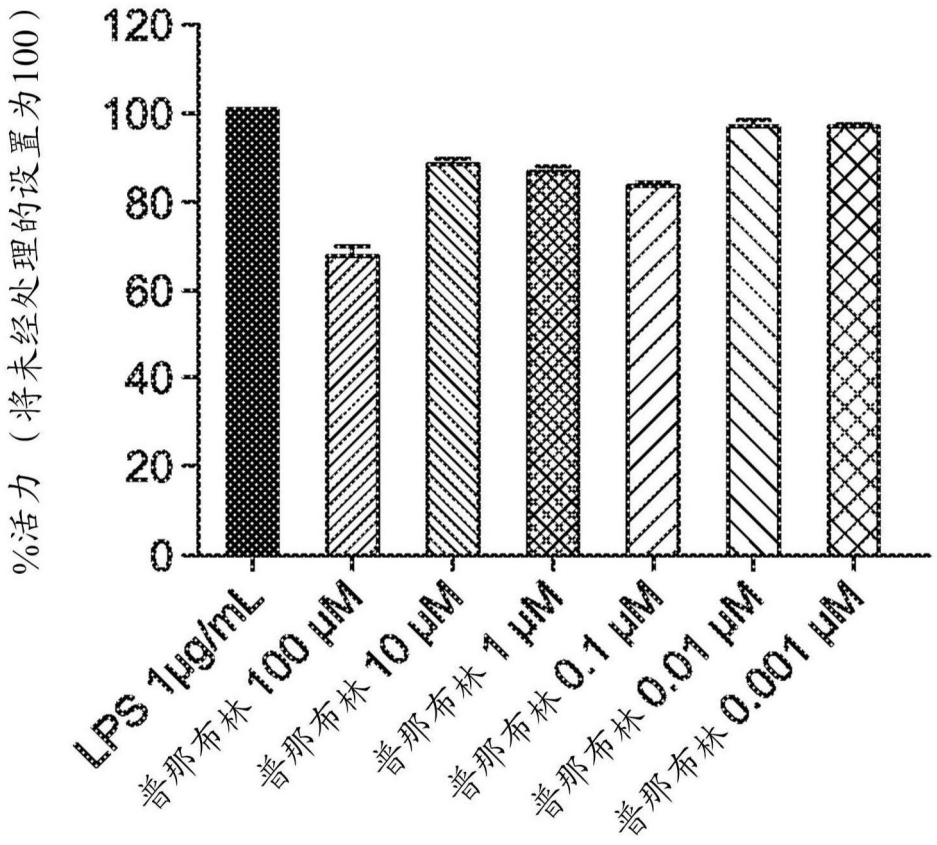

%活力（将未经处理的设置为100）
120
100
80
60
40
20
0
LPS 1μg/mL
普那布林 100 μM
普那布林 10 μM
普那布林 1 μM
普那布林 0.1 μM
普那布林 0.01 μM
普那布林 0.001 μM

图1B

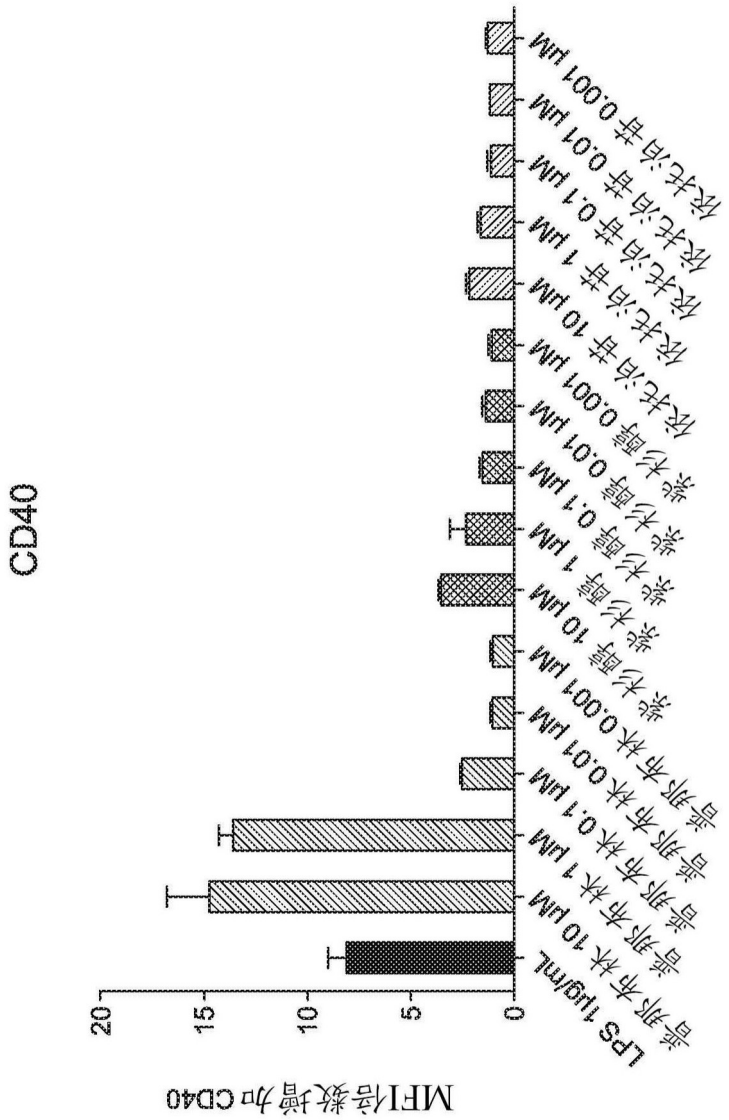

CD40
MFI倍数增加 CD40
0
5
10
15
20
LPS 1μg/mL
索那布林 10 μM
索那布林 1 μM
索那布林 0.1 μM
索那布林 0.01 μM
索那布林 0.001 μM
紫杉醇 10 μM
紫杉醇 1 μM
紫杉醇 0.1 μM
紫杉醇 0.01 μM
紫杉醇 0.001 μM
尿无治林 10 μM
尿无治林 1 μM
尿无治林 0.1 μM
尿无治林 0.01 μM
尿无治林 0.001 μM

图2A

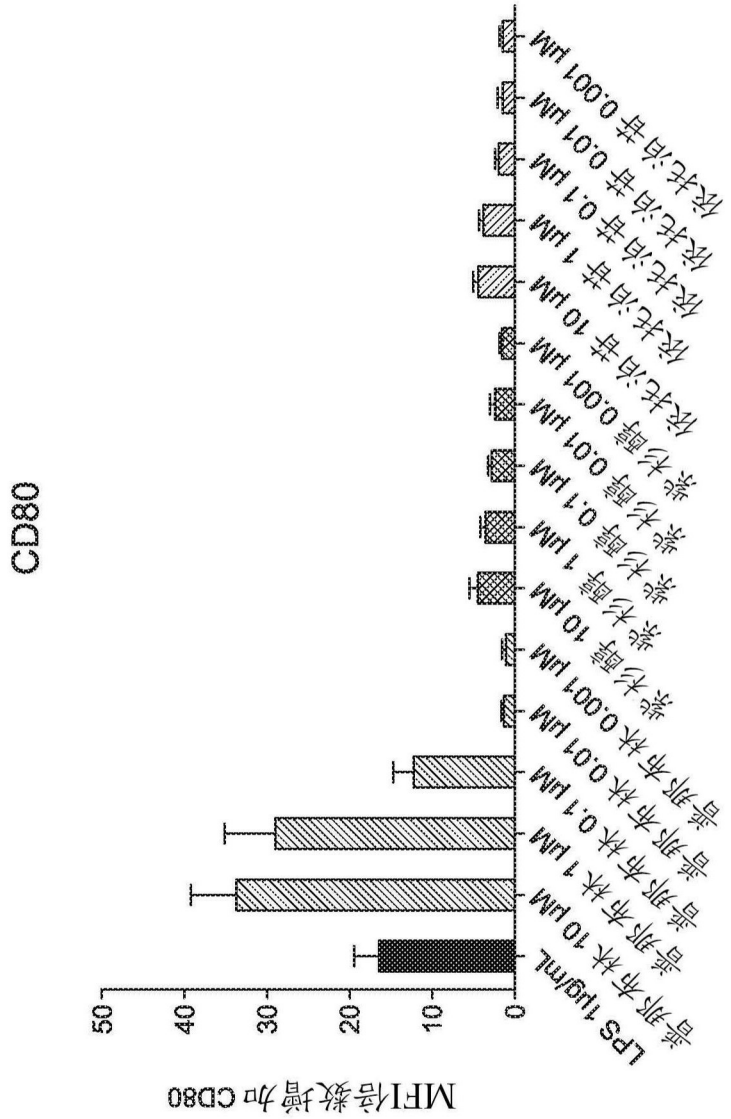

CD80
MFI倍数增加 CD80
0
10
20
30
40
50
LPS 1ug/mL
索那布林 10 μM
索那布林 1 μM
索那布林 0.1 μM
索那布林 0.01 μM
索那布林 0.001 μM
紫杉醇 10 μM
紫杉醇 1 μM
紫杉醇 0.1 μM
紫杉醇 0.01 μM
紫杉醇 0.001 μM
依托泊苷 10 μM
依托泊苷 1 μM
依托泊苷 0.1 μM
依托泊苷 0.01 μM
依托泊苷 0.001 μM

图2B

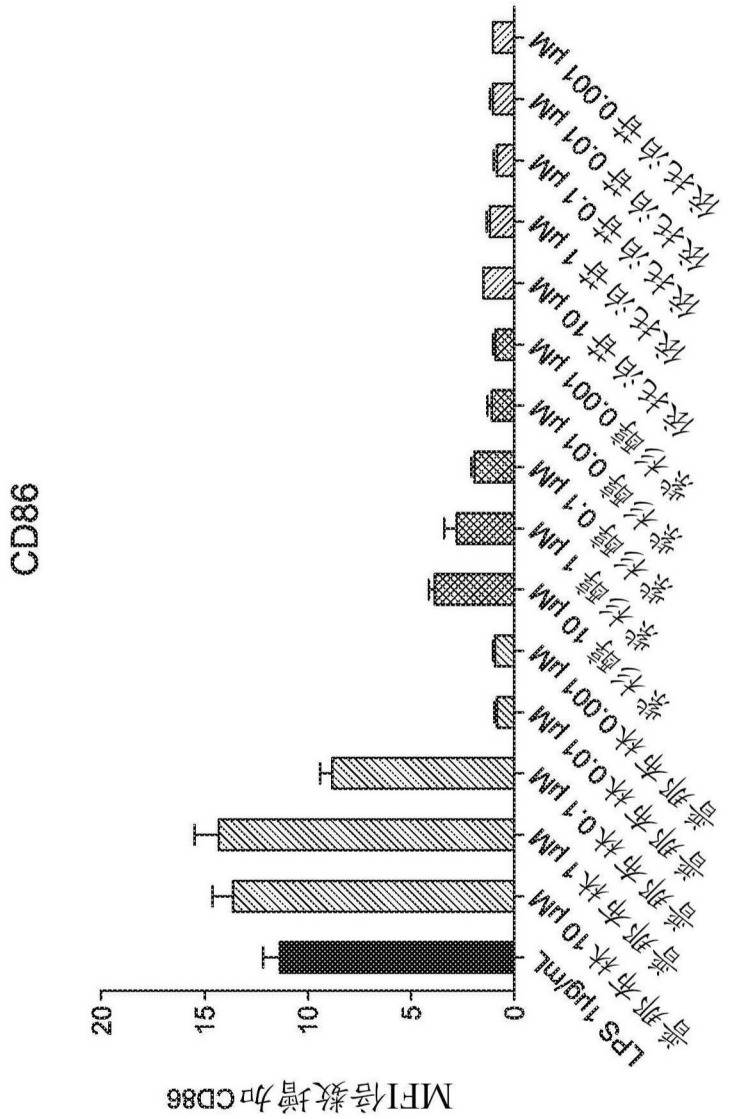

CD86
MFI倍数增加 CD86
0
5
10
15
20
LPS 1μg/mL
奇那布林 10 μM
奇那布林 1 μM
奇那布林 0.1 μM
奇那布林 0.01 μM
奇那布林 0.001 μM
紫杉醇 10 μM
紫杉醇 1 μM
紫杉醇 0.1 μM
紫杉醇 0.01 μM
紫杉醇 0.001 μM
依托泊苷 10 μM
依托泊苷 1 μM
依托泊苷 0.1 μM
依托泊苷 0.01 μM
依托泊苷 0.001 μM

图2C


MHCII
MFI/各数增加 MHCII
0
1
2
3
4
LPS 1ug/mL
奇那布林 10 μM
奇那布林 1 μM
奇那布林 0.1 μM
奇那布林 0.01 μM
奇那布林 0.001 μM
紫杉醇 10 μM
紫杉醇 1 μM
紫杉醇 0.1 μM
紫杉醇 0.01 μM
紫杉醇 0.001 μM
依托泊苷 10 μM
依托泊苷 1 μM
依托泊苷 0.1 μM
依托泊苷 0.01 μM
依托泊苷 0.001 μM

图2D

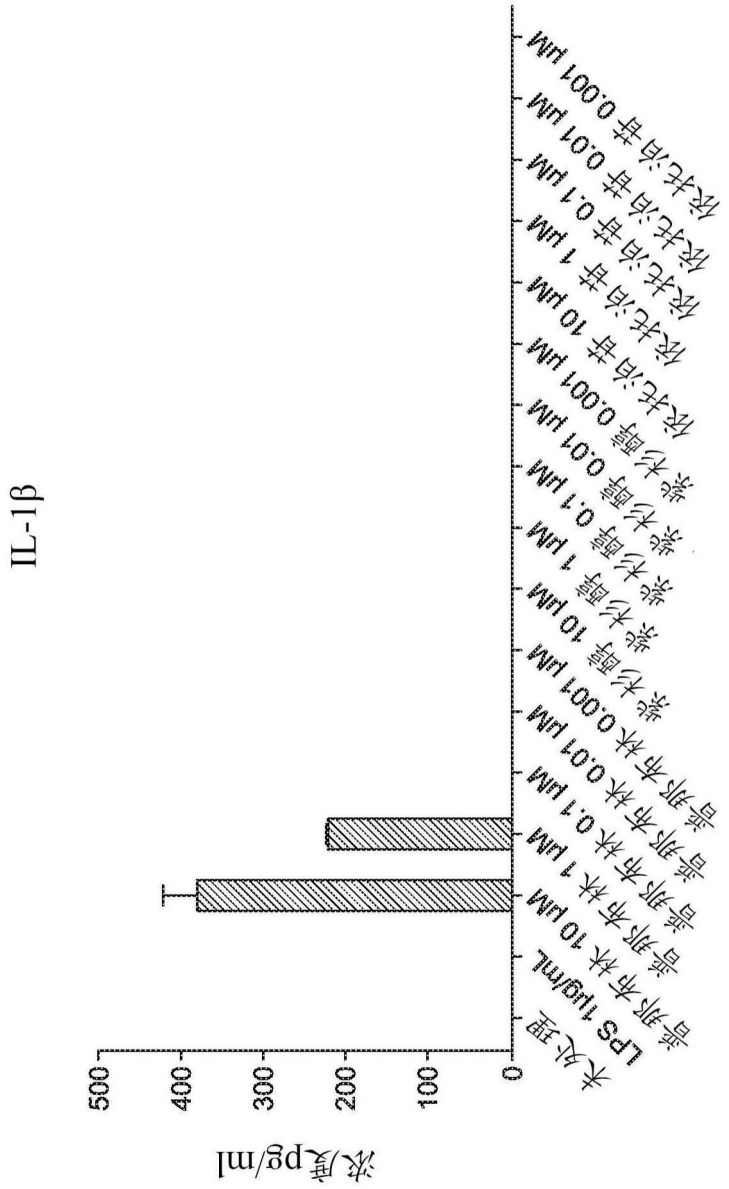

IL-1β
浓度pg/ml
500
400
300
200
100
0
未处理
LPS 1ug/mL
肾那布林 10 μM
肾那布林 1 μM
肾那布林 0.1 μM
肾那布林 0.01 μM
肾那布林 0.001 μM
紫杉醇 10 μM
紫杉醇 1 μM
紫杉醇 0.1 μM
紫杉醇 0.01 μM
紫杉醇 0.001 μM
依托泊苷 10 μM
依托泊苷 1 μM
依托泊苷 0.1 μM
依托泊苷 0.01 μM
依托泊苷 0.001 μM

图3A

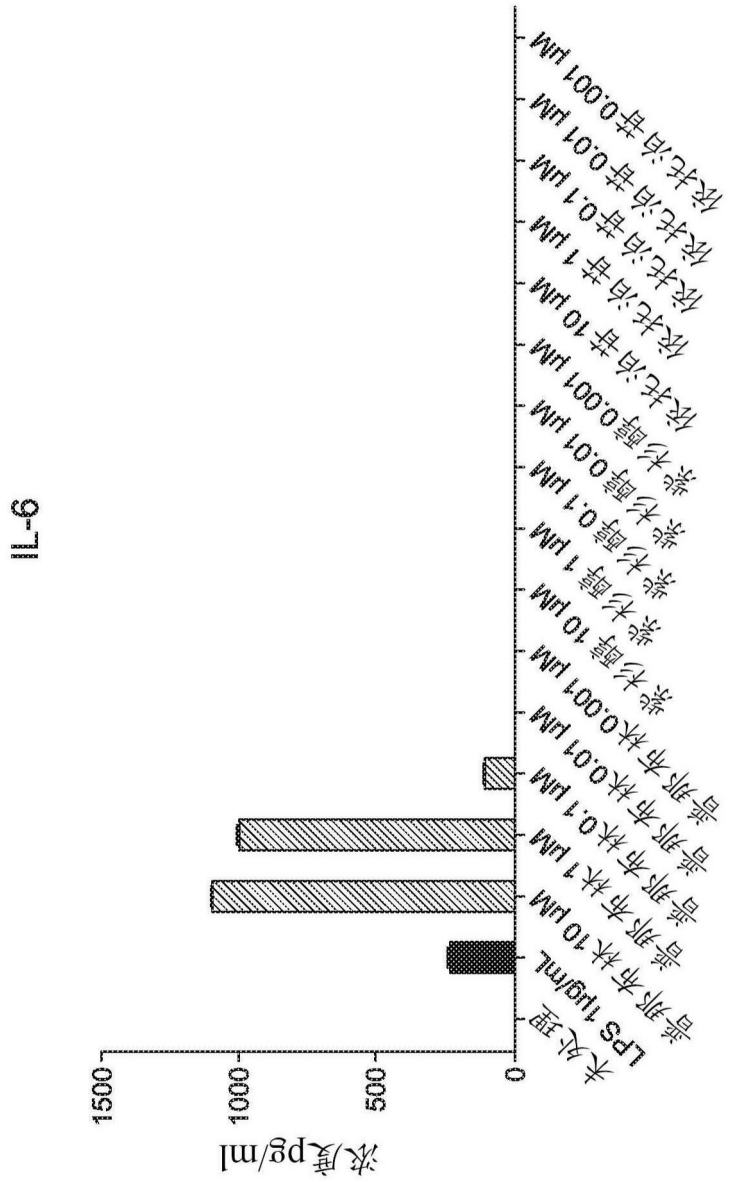

IL-6
浓度pg/ml
1500
1000
500
0
未处理
LPS 1ug/mL
普那布林 10 μM
普那布林 1 μM
普那布林 0.1 μM
普那布林 0.01 μM
普那布林 0.001 μM
紫杉醇 10 μM
紫杉醇 1 μM
紫杉醇 0.1 μM
紫杉醇 0.01 μM
紫杉醇 0.001 μM
依托泊苷 10 μM
依托泊苷 1 μM
依托泊苷 0.1 μM
依托泊苷 0.01 μM
依托泊苷 0.001 μM

图3B

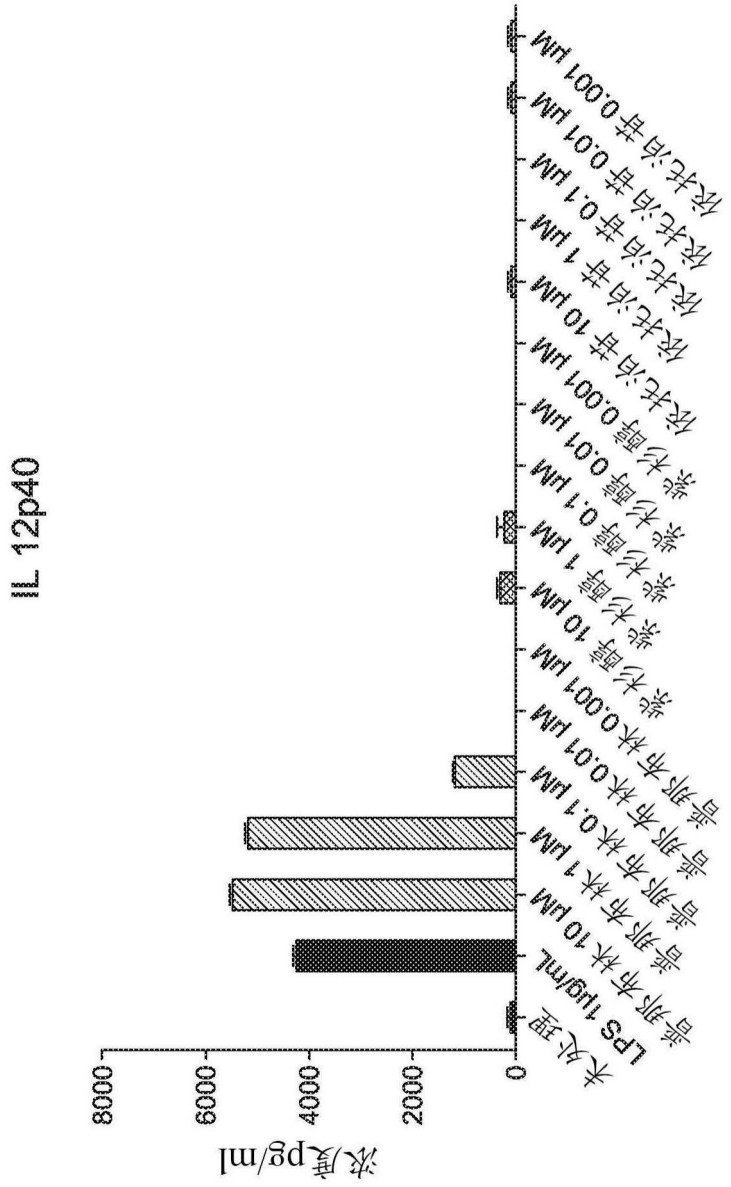

IL 12p40
浓度pg/ml
8000
6000
4000
2000
0
未处理
LPS 1ug/mL
奇那布林 10 μM
奇那布林 1 μM
奇那布林 0.1 μM
奇那布林 0.01 μM
奇那布林 0.001 μM
紫杉醇 10 μM
紫杉醇 1 μM
紫杉醇 0.1 μM
紫杉醇 0.01 μM
紫杉醇 0.001 μM
依托泊苷 10 μM
依托泊苷 1 μM
依托泊苷 0.1 μM
依托泊苷 0.01 μM
依托泊苷 0.001 μM

图3C

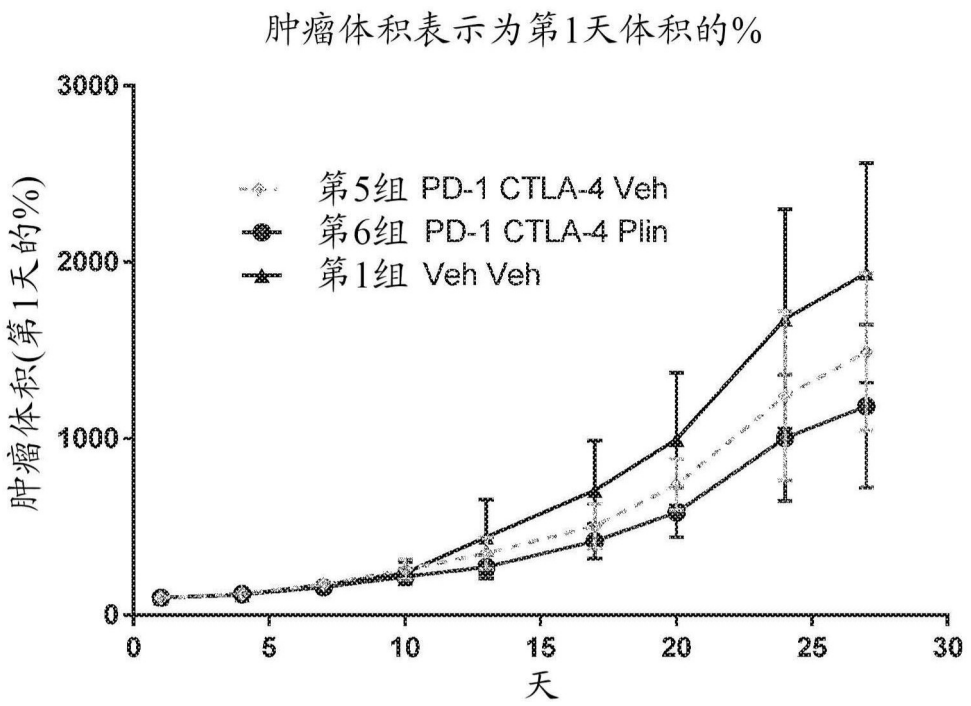

肿瘤体积表示为第1天体积的%
肿瘤体积(第1天的%)
3000
2000
1000
0
0
5
10
15
20
25
30
天
第5组 PD-1 CTLA-4 Veh
第6组 PD-1 CTLA-4 Plin
第1组 Veh Veh

图4A

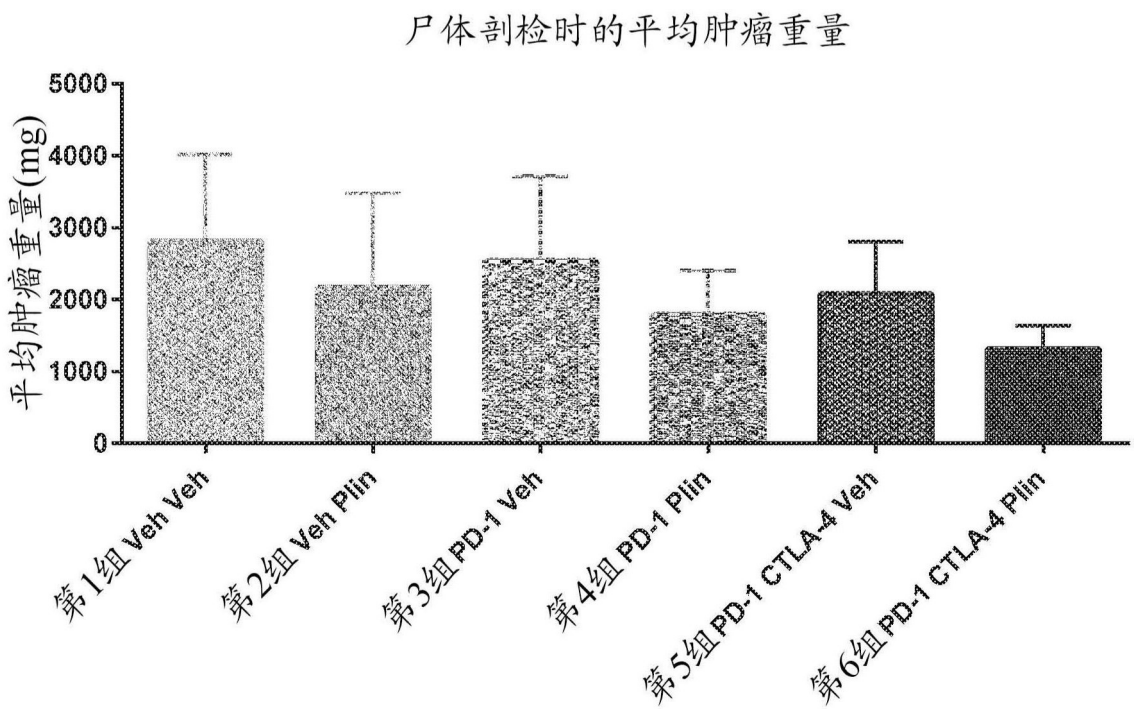

尸体剖检时的平均肿瘤重量
平均肿瘤重量(mg)
5000
4000
3000
2000
1000
0
第1组 Veh Veh
第2组 Veh Plin
第3组 PD-1 Veh
第4组 PD-1 Plin
第5组 PD-1 CTLA-4 Veh
第6组 PD-1 CTLA-4 Plin

图4B

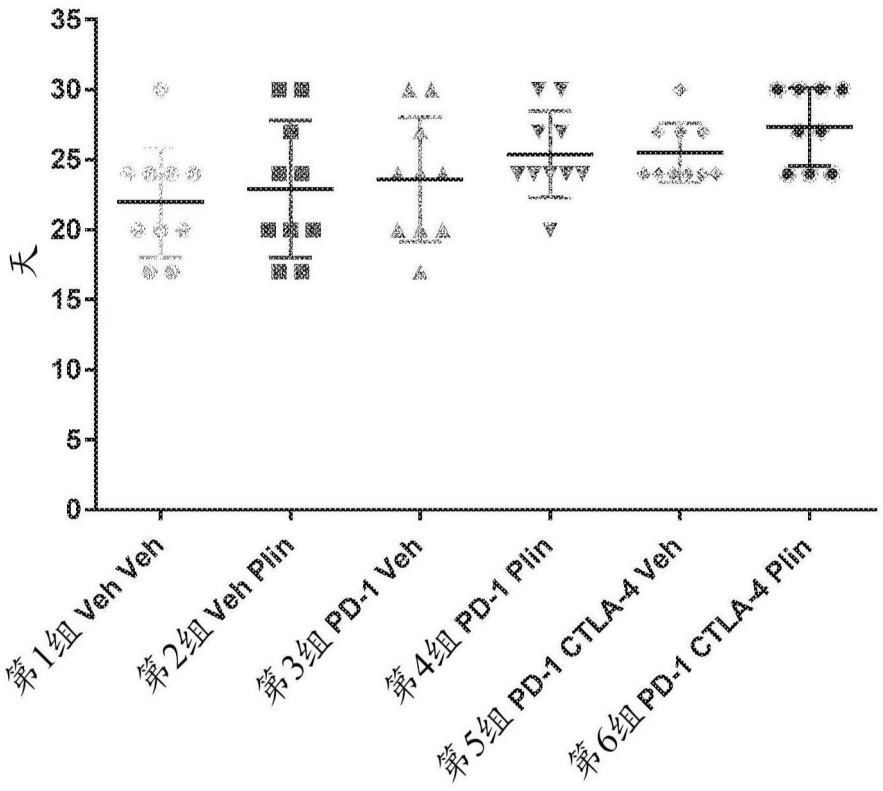

肿瘤体积增长为第1天的1000%的天数
天
35
30
25
20
15
10
5
0
第1组 Veh Veh
第2组 Veh Plin
第3组 PD-1 Veh
第4组 PD-1 Plin
第5组 PD-1 CTLA-4 Veh
第6组 PD-1 CTLA-4 Plin

图4C

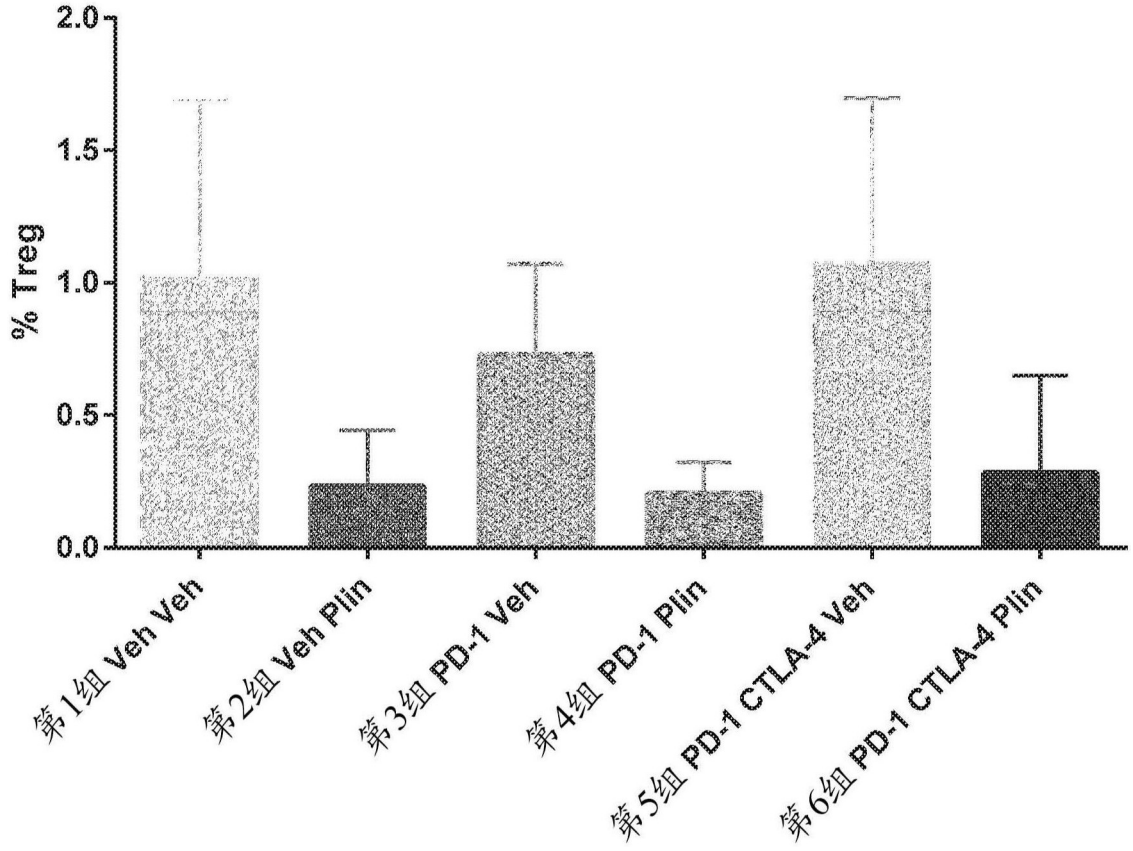

MC-38 CRC肿瘤: 在CD45+淋巴细胞中的FACS分析: % Treg
% Treg
2.0
1.5
1.0
0.5
0.0
第1组 Veh Veh
第2组 Veh Plin
第3组 PD-1 Veh
第4组 PD-1 Plin
第5组 PD-1 CTLA-4 Veh
第6组 PD-1 CTLA-4 Plin

图5A

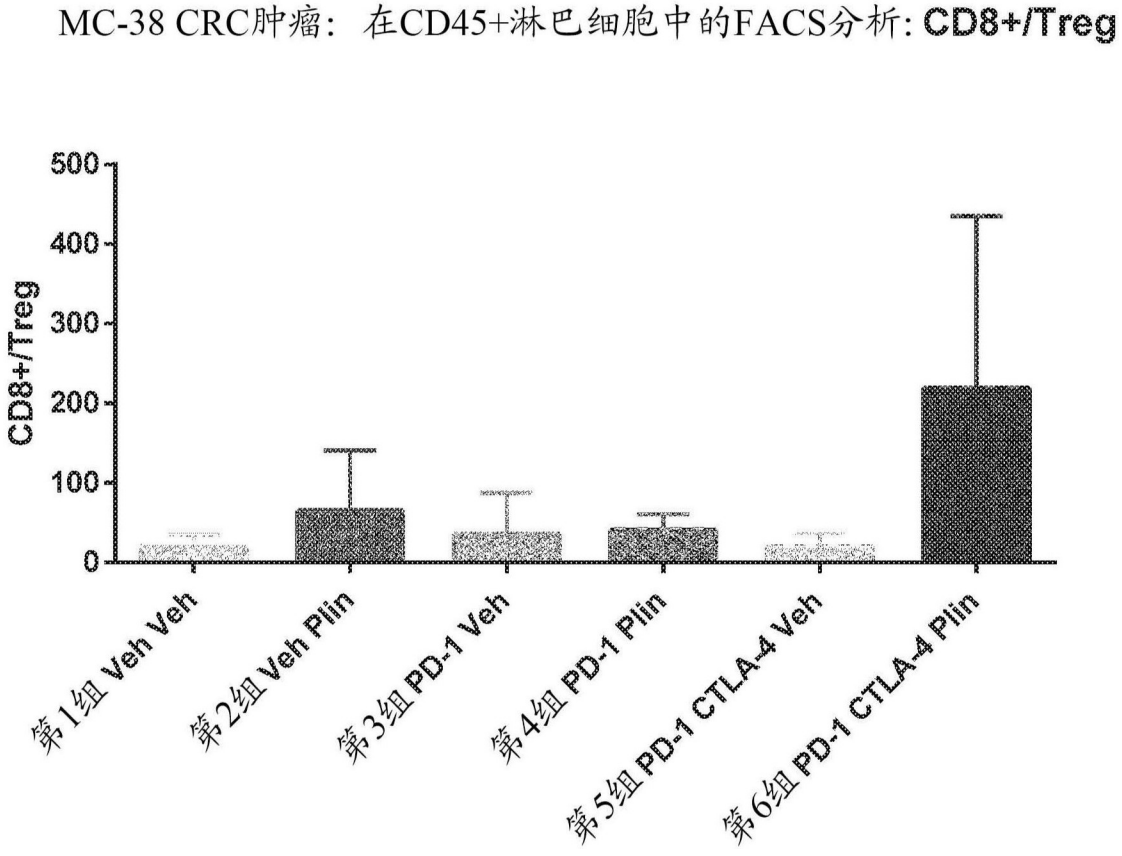

MC-38 CRC肿瘤: 在CD45+淋巴细胞中的FACS分析: CD8+/Treg
CD8+/Treg
500
400
300
200
100
0
第1组 Veh Veh
第2组 Veh Plin
第3组 PD-1 Veh
第4组 PD-1 Plin
第5组 PD-1 CTLA-4 Veh
第6组 PD-1 CTLA-4 Plin

图5B

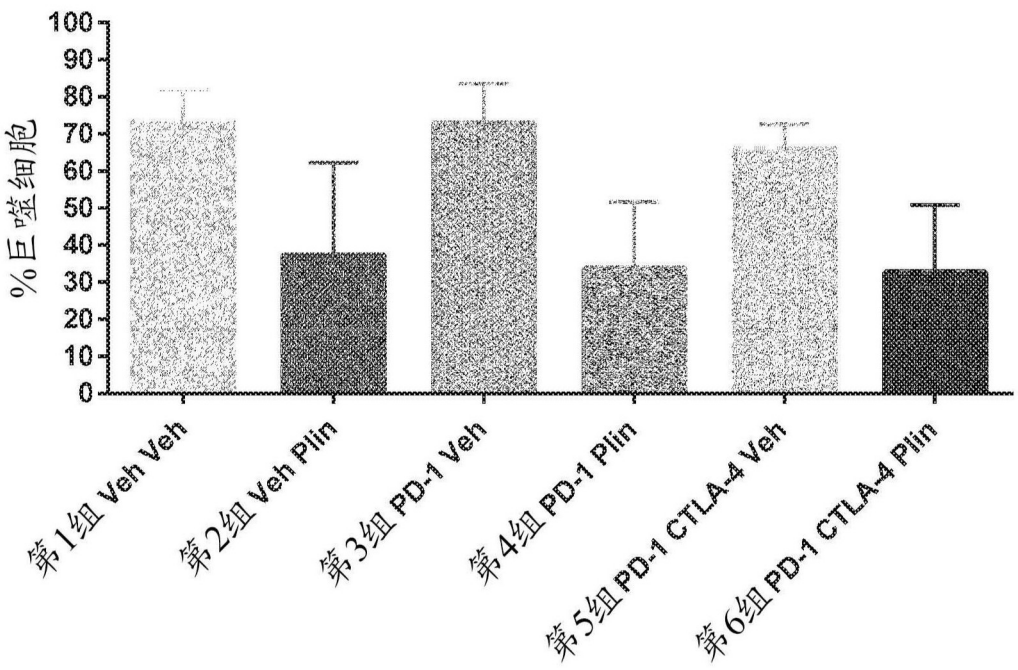

MC-38 CRC肿瘤：在CD45+淋巴细胞中的FACS分析:%巨噬细胞
%巨噬细胞
100
90
80
70
60
50
40
30
20
10
0
第1组 Veh Veh
第2组 Veh Plin
第3组 PD-1 Veh
第4组 PD-1 Plin
第5组 PD-1 CTLA-4 Veh
第6组 PD-1 CTLA-4 Plin

图5C