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(71) Applicant: THE REGENTS OF THE UNIVERSITY OF COLORADO, A BODY CORPORATE [US/US]: 1800 Grant Street, 8th Floor, Denver, Colorado 80203 (US).

(72) Inventors: CAMBIER, John; 2559 S. Sherman Street, Denver, Colorado 80210 (US). FRANKS, Elizabeth; 2820 Syracuse Court, Denver, Colorado 80238 (US).

(74) Agent: TRAVER, Robert D.; Sheridan Ross P.C., 1560 Broadway, Suite 1200, Denver, Colorado 80202 (US).

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(54) Title: P110-DELTA INHIBITORS TREAT AND PREVENT AUTOIMMUNITY WHILE SPARING THE ABILITY TO MOUNT AN IMMUNE RESPONSE TO EXOGENOUS IMMUNOGENS

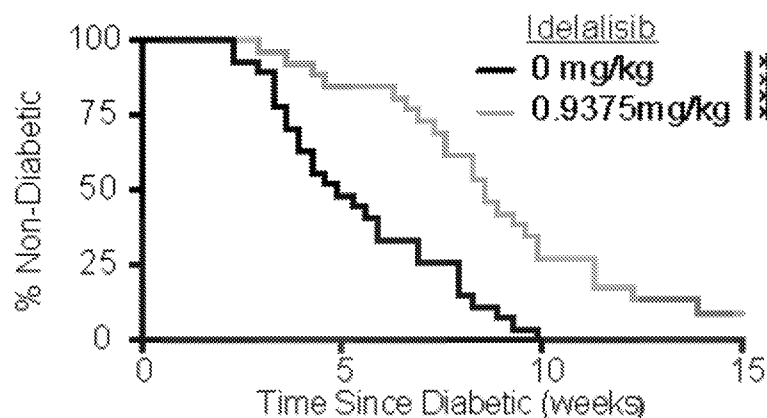


FIG. 1B

(57) Abstract: Methods of using a phosphoinositide 3-kinase p110-delta inhibitor to treat, delay the onset, or slow the progression of an autoimmune disease or disorder in a subject, without suppressing the subject's B cell responses to exogenous antigens or rendering the subject immunocompromised, as well as pharmaceutical compositions containing phosphoinositide 3-kinase p110-delta inhibitors in amounts suitable for convenient and accurate administration within these therapeutic methods.



CROSS REFERENCE TO RELATED APPLICATION

STATEMENT OF FEDERALLY SPONSORED RESEARCH

TECHNICAL FIELD

BACKGROUND

The p110 δ isoform has been implicated in biological functions related to immune-inflammatory diseases, including signaling from the B cell receptor, T cell receptor, FcR signaling of mast cells and monocyte/macrophage, and osteoclast function/RANKL signaling. Deletion of the PI3K δ gene or selective introduction of a catalytically inactive mutant of PI3K δ causes a nearly complete ablation of B cell proliferation and signaling, and impairment of signaling through T cell receptors as well.

Multiple mechanisms are involved in the maintenance of B cell tolerance to autoantigens. In the bone marrow, receptor editing and clonal deletion ensure that B cells undergoing high avidity interactions with self-antigens are removed from the repertoire. However, B cells recognizing lower avidity self-antigens do not undergo receptor editing, but instead are released
5 into the periphery where they are maintained transiently in an unresponsive state called anergy. Anergy is rapidly reversible, requiring chronic receptor stimulation by self-antigen, suggesting maintenance by nondurable biochemical mechanisms.

Maintenance of the anergic state of peripheral autoreactive B cells involves multiple regulatory mechanisms that operate proximally in BCR signaling. Among these are inositol lipid
10 phosphatases, PTEN and SHIP-1 that, in anergic cells prevent the BCR-mediated accumulation of PI(3,4,5)P3. Acute deletion of SHIP-1 or PTEN and expression of a constitutively-active catalytic subunit of PI3K in anergic B cells leads to immediate loss of anergy followed by cell proliferation, differentiation, and production of autoantibodies, thus demonstrating the importance of these proteins and their regulation of the PI3K pathway in maintaining B cell anergy. Importantly, B
15 cells from SLE, Type 1 Diabetes (T1D), and Autoimmune Thyroiditis (AITD) patients express reduced levels of PTEN, consistent with a role in autoimmunity.

There remains an unmet need for effective treatments of autoimmune diseases and disorders.

SUMMARY

20 The inventors have unexpectedly discovered that administration of low doses of PI3K-p110 δ inhibitors compensate for failed PI3K pathway regulation, and thereby delay development of autoimmunity in a murine model of type 1 diabetes. Surprisingly, the chronically treated animals remained immunocompetent as indicated by production of class switched high affinity antibodies in response to immunization. Low dose p110 δ inhibition selectively inhibited
25 participation in autoimmunity of autoreactive B cells that have lost anergy due to defective PI3K pathway regulation and did not affect in vitro or in vivo T cell responses.

Thus, this disclosure provides methods of treating autoimmune disorders by modifying B cell antigen receptor signaling to prevent or significantly reduce responses to self or endogenous antigens while ensuring that protective immunological responses are mounted against exogenous
30 pathogens. These methods comprise administering compounds with inhibitory activity and selective binding to the p110 delta isoform of the PI3 kinase ("PI3K-p110 δ inhibitors"). An exemplary PI3K-p110 δ inhibitor for use in these methods is idelalisib. In these methods, the PI3K-p110 δ inhibitor may be administered in a dosage or administration schedule that treats, prevents, and/or delays the onset of an autoimmune disorder while sparing B cell responses to exogenous
35 antigens.

In these methods, the PI3K-p110 δ inhibitors may be used for in vitro, in situ, and in vivo diagnosis or treatment of mammalian cells, organisms, or associated pathological autoimmune conditions, such as autoimmune diabetes mellitus (type 1 diabetes mellitus; T1D), systemic lupus erythematosus, autoimmune thyroiditis, rheumatoid arthritis, psoriasis, and multiple sclerosis.

5 These methods may therefore include treating or delaying the onset or slowing the progression of an autoimmune disease or disorder in a subject by administering a therapeutically effective amount of a phosphoinositide 3-kinase p110-delta (PI3K-p110 δ) inhibitor to the subject at a dosage that does not reduce immune response to exogenous immunogens in the subject. In these methods, the PI3K-p110 δ inhibitor may be idelalisib. The idelalisib may be administered at a
10 dosage of between about 0.23 mg/kg/day and about 3.6 mg/kg/day, or between about 0.45 mg/kg/day and about 1.8 mg/kg/day, or between about 0.9 mg/kg/day and about 1.8 mg/kg/day, or between about 1.8 mg/kg/day and about 3.6 mg/kg/day. The idelalisib may be administered at a dosage of about 0.9 mg/kg/day, or about 1.8 mg/kg/day.

15 In these methods, the autoimmune disease or disorder may be selected from autoimmune diabetes mellitus (type 1 diabetes mellitus; T1D), systemic lupus erythematosus, autoimmune thyroiditis, rheumatoid arthritis, psoriasis, and multiple sclerosis. In exemplary methods, the autoimmune disease is type 1 diabetes mellitus (T1D).

In these methods, the PI3K-p110 δ inhibitor may be administered to a subject displaying reduced activity of phosphatase and tensin homolog (PTEN) in B cells.

20 In these methods, the PI3K-p110 δ inhibitor may be administered to a subject displaying reduced activity of the SH-2-containing inositol phosphatase SHIP-1 in B cells.

Another aspect of the invention provides the use of a PI3K-p110 δ inhibitor in the manufacture of a medicament for treating an autoimmune disease or disorder, in particular an autoimmune disorder that may be mediated by PI3 kinase including by selective inhibition of the
25 p110 delta isoform.

These methods and uses may further comprise administering an additional therapeutic agent, such as an anti-inflammatory agent, an immunomodulatory agent, an antidiabetic agent, and/or an agent for treating immunodeficiency disorders.

Another aspect of the invention provides a pharmaceutical composition comprising a
30 PI3K-p110 δ inhibitor and a pharmaceutically acceptable carrier, glidant, diluent, and/or excipient. In these pharmaceutical compositions, the PI3K-p110 δ inhibitor is preferably present in a dose that can be easily and accurately administered to a subject to treat, prevent, and/or delay the onset of an autoimmune disorder while sparing B cell responses to exogenous antigens. An exemplary PI3K-p110 δ inhibitor that may be present in these pharmaceutical compositions is idelalisib. An
35 exemplary oral dosage formulation of idelalisib for use in the therapeutic methods of this disclosure includes idelalisib in an amount between 5mg and 25mg

Another aspect of the invention provides a kit for treating an autoimmune disease or disorder mediated by the p110 delta isoform of PI3 kinase, comprising a pharmaceutical composition comprising a PI3K-p110 δ inhibitor, such as idelalisib, and instructions for use.

This Summary is neither intended nor should it be construed as being representative of the full extent and scope of the present disclosure. Moreover, references made herein to “the present disclosure,” or aspects thereof, should be understood to mean certain embodiments of the present disclosure and should not necessarily be construed as limiting all embodiments to a particular description. The present disclosure is set forth in various levels of detail in this Summary as well as in the attached drawings and the Detailed Description and no limitation as to the scope of the present disclosure is intended by either the inclusion or non-inclusion of elements, components, etc. in this Summary. Additional aspects of the present disclosure will become more readily apparent from the Detailed Description, particularly when taken together with the drawings.

BRIEF DESCRIPTION OF FIGURES

FIGS. 1A-1E show low dose idelalisib delays disease progression in VH125.NOD mice without compromising response to immunization. FIG. 1A is a schematic representation of the survival experimental protocol. FIG. 1B shows disease incidence as measured by % non-diabetic (blood glucose <600mg/dl) mice receiving vehicle control chow (black line) or 0.9375mg/kg Idelalisib containing chow (grey line) (n=25/group). FIG. 1C is a schematic representation of the immunization experimental protocol. FIG. 1D shows the total NP-specific (NP27 binding) and high affinity NP-specific (NP2 binding) IgM (left) and IgG (right) antibody secreting cell response (ASCs/Spleen) 14 days post immunization of mice receiving vehicle control chow (white bar) or 0.9375mg/kg Idelalisib chow (grey bar) (n=7/group. Error bars represent mean $\pm$ SEM). FIG. 1E shows the total circulating NP-specific (NP27 binding) IgM (left) and IgG (center) and high affinity NP-specific (NP2 binding) IgG (right) at day 14 post-immunization of mice receiving vehicle control chow (white bars) or 0.9375mg/kg Idelalisib containing chow (grey bars). (Idel= idelalisib. BG= blood glucose. Bars in FIGS. 1D & 1E represent mean $\pm$ SEM. Log-rank (Mantel-Cox) test was used to calculate statistics in FIG. 1B. One-Way ANOVA was used to calculate statistics in FIGS. 1D & 1E. *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.005$, ****= $p < 0.0001$).

FIGS. 2A-2H show autoreactive PTEN^{fl/+} x SHIP-1^{fl/+} B cells re-establish anergy when treated with idelalisib. FIG. 2A is a schematic representation of the experimental protocol. FIG. 2B shows WT Ars/A1 or PTEN^{fl/wt} x SHIP-1^{fl/wt} Ars/A1-derived IgM^a anti-Ars antibody detected in serum 14 days post tamoxifen treatment of mice receiving vehicle control chow (open circles), 0.9375mg/kg (closed squares), 3.75mg/kg (closed triangles), 30mg/kg (closed circles) and WT Ars/A1 receiving 0mg/kg (open triangles) idelalisib-containing chow. FIG. 2C shows quantification of relative response of PTEN^{fl/wt} x SHIP-1^{fl/wt} Ars/A1-derived IgM^a anti-Ars antibody detected in the serum 14 days post tamoxifen treatment. FIG. 2D shows quantification of relative

response of PTEN^{fl/wt}xSHIP-1^{fl/wt} Ars/A1-derived IgM^a anti-Ars ASCs/spleen 14 days post tamoxifen treatment. FIG. 2E shows proliferation (top row) and plasmablast differentiation (bottom row) of splenic Ars/A1 Idiotypic+ YFP+ B cells of mice on vehicle control chow (open black line, top row) or indicated doses of idelalisib (shaded line, top row) 14 days post tamoxifen treatment. Enumeration of (FIG. 2F) total recovered transferred PTEN^{fl/wt}xSHIP-1^{fl/wt} cells, (FIG. 2G) the unproliferated Ars/A1 Idiotypic+ YFP+ population and (FIG. 2H) plasmablasts in the spleens of recipient mice 14 days post tamoxifen treatment (For FIGS. 2E, 2F, 2G, & 2H gated on: B220+ Ars/A1 Id+ YFP+). (Idel:= idelalisib. $n = 8/\text{group}$. Data shown are representative of at least three replicated experiments. Bars in FIGS. 2C, 2D, 2F, 2G, & 2H represent mean $\pm$ SEM. One-Way ANOVA was used to calculate statistics in FIGS. 2C, 2D, 2F, 2G, & 2H. *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.005$, ****= $p < 0.0001$, ND=undetectable).

FIGS. 3A-3H show autoreactive SHP-1^{-/-} B cells do not maintain anergy when treated with low dose idelalisib. FIG. 3A schematic representation of the experimental protocol. FIG. 3B shows WT Ars/A1 or SHP-1^{fl/fl} Ars/A1-derived IgM^a anti-Ars antibody detected in serum 14 days post tamoxifen treatment of mice receiving vehicle control chow (open circles), 0.9375mg/kg (closed squares), 3.75mg/kg (closed triangles), 30mg/kg (closed circles) or WT Ars/A1 receiving 0mg/kg (open triangles) idelalisib-containing chow. FIG. 3C shows quantification of relative response of SHP-1^{fl/fl} Ars/A1-derived IgM^a anti-Ars antibody detected in the serum 14 days post tamoxifen treatment. FIG. 3D shows quantification of relative response of SHP-1^{fl/fl} Ars/A1-derived IgM^a anti-Ars ASCs/spleen 14 days post tamoxifen treatment. FIG. 3E shows proliferation (top row) and plasmablast differentiation (bottom row) of splenic Ars/A1 Idiotypic+ CD45.1+ B cells of mice on vehicle control chow (open black line, top row) or indicated doses of idelalisib (shaded line, top row). Enumeration of (FIG. 3F) total recovered transferred SHP-1^{fl/fl} cells, (FIG. 3G) the unproliferated Ars/A1 Idiotypic+ CD45.1+ population and (FIG. 3H) plasmablasts in the spleens of recipient mice 14 days post tamoxifen treatment (For FIGS. 3E, 3F, 3G, & 3H gated on: B220+ Ars/A1 Id+ CD45.1+). (Idel:= idelalisib. $n = 8/\text{group}$. Data shown are representative of at least three replicated experiments. Bars in FIGS. 3C, 3D, 3F, 3G, & 3H represent mean $\pm$ SEM. One-Way ANOVA was used to calculate statistics in FIGS. 3C, 3D, 3F, 3G, & 3H. *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.005$, ****= $p < 0.0001$, ND=undetectable).

FIGS. 4A-4C show p110 δ inhibition suppresses B cell calcium flux and reduces phosphorylation of downstream signaling intermediaries. FIG. 4A shows calcium flux of B220+ cells stimulated with anti-H&L with simultaneous addition of 0nM (black line), 15nM (solid grey line), 60nM (grey long dashed line) or 490nM (grey dashed line) idelalisib. FIG. 4B shows quantification of area under the curve (AUC) seen in FIG. 4A. FIG. 4C shows quantification of phosphorylated signaling intermediaries after preincubation with indicated doses of idelalisib and BCR stimulation. (idel:= idelalisib. $n=5/\text{group}$. Data shown are representative of at least three

replicated experiments. Bars in FIGS. 4B & 4C represent mean $\pm$ SEM. Student T test was used to calculate statistics in FIG. 4B. One-Way ANOVA was used to calculate statistics in FIG. 4C.

*= $p<0.05$, **= $p<0.01$, ***= $p<0.005$, ****= $p<0.0001$).

FIGS. 5A-5G show dose-dependent idelalisib inhibition of antibody responses. FIG. 5A is a schematic representation of the experimental protocol. FIG. 5B shows enumeration of total recovered transferred MD4 B cells and (FIG. 5C) quantification of the unproliferated population of recovered MD4+ B cells in the spleen of recipient mice 5 days post-immunization. FIG. 5D shows MD4-derived IgM^a anti-HEL antibody detected in serum 5 days post immunization of mice receiving vehicle control chow (open circles), 0.9375mg/kg (closed squares), 3.75mg/kg (closed triangles), 30mg/kg (closed circles), and unimmunized mice receiving 0mg/kg (open triangles) idelalisib-containing chow. FIG. 5E shows quantification of relative response of MD4-derived IgM^a anti-HEL antibody detected in serum and (FIG. 5F) MD4-derived IgM^a anti-HEL ASCs/spleen 5 days post immunization. FIG. 5G shows proliferation of splenic MD4+ B cells 5 days post-immunization of mice receiving vehicle control chow (unfilled black line) or indicated doses of idelalisib (shaded grey line). (Idel:= idelalisib. -imm= unimmunized. $n = 8$ /group. Data shown are representative of at least three replicated experiments. FIGS. 5B, 5C, & 5G gated on: B220+ HEL binding+. Bars in FIGS. 5B, 5C, 5E, & 5F represent mean $\pm$ SEM. One-Way ANOVA was used to calculate statistics. *= $p<0.05$, **= $p<0.01$, ***= $p<0.005$, ****= $p<0.0001$).

FIGS. 6A-6F show low dose p110 δ inhibition does not inhibit CD4+ T cell responses *in vitro* or *in vivo*. FIG. 6A shows calcium flux of CD4+ T cells stained with anti-CD3 biotin with simultaneous addition of 0nM (black line) or 15nM (grey line) Idelalisib, and crosslinked with avidin. FIG. 6B shows quantification of area under the curve of A. FIG. 6C is a schematic representation of the experimental protocol. FIG. 6D shows representative flow cytometric plots of proliferation (CFSE) and upregulation of CD44 following immunization. FIG. 6E shows representative histogram (left) and quantification (right) of proliferation (CFSE) of recovered OT-II T cells from the spleens of recipient mice. FIG. 6F shows representative histogram (left) and quantification (right) of upregulation of CD44 of recovered OT-II T cells from the spleens of recipient mice. (Idel:= idelalisib. imm+/- = immunized/unimmunized. $n = 5$ /group. Bars in FIGS. 6B, 6E, & 6F represent mean $\pm$ SEM. Student's T test was used to calculate statistics in B. One-Way ANOVA was used to calculate statistics in FIGS. 6E & 6F. *= $p<0.05$, **= $p<0.01$, ***= $p<0.005$, ****= $p<0.0001$).

DETAILED DESCRIPTION

In the description that follows, a number of terms are used and the following definitions are provided to facilitate understanding of the claimed subject matter. Terms that are not expressly defined herein are used in accordance with their plain and ordinary meanings. Abbreviations used herein have their conventional meaning within the chemical and biological arts.

Unless otherwise specified, “a” or “an” means “one or more.”

The term “about” is used herein to mean plus or minus ten percent (10%) of a value. For example, “about 100” refers to any number between 90 and 110.

When ranges are used herein for physical properties, such as molecular weight, or chemical properties, or dosage amounts, all combinations and subcombinations of ranges and specific embodiments therein are intended to be included.

The term “comprising” (and related terms such as “comprise” or “comprises” or “having” or “including”) includes those embodiments, for example, an embodiment of any composition of matter, composition, method, or process, or the like, that “consist of” or “consist essentially of” the described features.

The terms “coadministration,” “administered in combination with,” and their grammatical equivalents, as used herein, encompass administration of two or more agents to an animal so that both agents and/or their metabolites are present in the animal at the same time. Co-administration includes simultaneous administration in separate compositions, administration at different times in separate compositions, or administration in a composition in which both agents are present.

The term “effective amount” or “therapeutically effective amount” refers to that amount of a PI3K-p110 δ inhibitor that is sufficient to affect the intended application, including but not limited to disease treatment, prevention, or delay, as defined below. The therapeutically effective amount may vary depending upon the intended application (in vitro or in vivo), or the subject and disease condition being treated, e.g., the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can be determined by one of ordinary skill in the art. The term also applies to a dose that will induce a particular response in target cells, e.g. reduction of B cell proliferation or activation, and/or stabilization of B cell anergy. The specific dose will vary depending on the particular compounds chosen, the dosing regimen to be followed, whether it is administered in combination with other compounds, timing of administration, the tissue to which it is administered, and the physical delivery system in which it is carried.

As used herein, “treatment,” “treating,” or “ameliorating” are used interchangeably. These terms refer to an approach for obtaining beneficial or desired results including but not limited to, therapeutic benefit and/or a prophylactic benefit. By therapeutic benefit is meant eradication or amelioration of the underlying autoimmune disorder being treated. Also, a therapeutic benefit is achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the underlying autoimmune disorder such that an improvement is observed in the patient, notwithstanding that the patient may still be afflicted with the underlying autoimmune disorder. For prophylactic benefit, the pharmaceutical compositions may be administered to a patient at risk of developing a particular disease, or to a patient reporting one or more of the

physiological symptoms of an autoimmune disease, even though a diagnosis of this disease may not have been made.

“Inflammatory disorder” as used herein can refer to any disease, disorder, or syndrome in which an excessive or unregulated inflammatory response leads to excessive inflammatory
5 symptoms, host tissue damage, or loss of tissue function. “Inflammatory disorder” also refers to a pathological state mediated by influx of leukocytes and/or neutrophil chemotaxis.

“Inflammation” as used herein refers to a localized, protective response elicited by injury or destruction of tissues, which serves to destroy, dilute, or wall off (sequester) both the injurious agent and the injured tissue. Inflammation is notably associated with influx of leukocytes and/or
10 neutrophil chemotaxis. Inflammation can result from infection with pathogenic organisms and viruses and from noninfectious means such as trauma or reperfusion following myocardial infarction or stroke, immune response to foreign antigen, and autoimmune responses. Accordingly, inflammatory disorders amenable to treatment with Formula I compounds encompass disorders associated with reactions of the specific defense system as well as with reactions of the nonspecific
15 defense system.

“Specific defense system” refers to the component of the immune system that reacts to the presence of specific antigens. Examples of inflammation resulting from a response of the specific defense system include the classical response to foreign antigens, autoimmune diseases, and delayed type hypersensitivity response mediated by T cells. Chronic inflammatory diseases, the
20 rejection of solid transplanted tissue and organs, e.g., kidney and bone marrow transplants, and graft versus host disease (GVHD), are further examples of inflammatory reactions of the specific defense system.

“Inflammatory cell activation” refers to the induction by a stimulus (including, but not limited to, cytokines, antigens or autoantibodies) of a proliferative cellular response, the
25 production of soluble mediators (including but not limited to cytokines, oxygen radicals, enzymes, prostanoids, or vasoactive amines), or cell surface expression of new or increased numbers of mediators (including, but not limited to, major histocompatibility antigens or cell adhesion molecules) in inflammatory cells (including but not limited to monocytes, macrophages, T lymphocytes, B lymphocytes, granulocytes (i.e., polymorphonuclear leukocytes such as
30 neutrophils, basophils, and eosinophils), mast cells, dendritic cells, Langerhans cells, and endothelial cells). It will be appreciated by persons skilled in the art that the activation of one or a combination of these phenotypes in these cells can contribute to the initiation, perpetuation, or exacerbation of an inflammatory disorder.

The term “nonspecific defense system” as used herein refers to inflammatory disorders
35 that are mediated by leukocytes that are incapable of immunological memory (e.g., granulocytes, and macrophages). Examples of inflammation that result, at least in part, from a reaction of the

nonspecific defense system include inflammation associated with conditions such as adult (acute) respiratory distress syndrome (ARDS) or multiple organ injury syndromes; reperfusion injury; acute glomerulonephritis; reactive arthritis; dermatoses with acute inflammatory components; acute purulent meningitis or other central nervous system inflammatory disorders such as stroke; thermal injury; inflammatory bowel disease; granulocyte transfusion associated syndromes; and cytokine-induced toxicity.

A “therapeutic effect” encompasses a therapeutic benefit and/or a prophylactic benefit as described above. A prophylactic effect includes delaying or eliminating the appearance (i.e., slowing the onset) of an autoimmune disease or condition, delaying or eliminating the onset of symptoms of an autoimmune disease or condition, slowing, halting, or reversing the progression of an autoimmune disease or condition, or any combination thereof.

The term “subject” or “patient” refers to a mammal, for example a human, mouse, rat, dog, cat, horse, or pig. The methods described herein can be useful in both human therapeutics and veterinary applications. It is not intended that the term be limited to a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are encompassed by the term. Doses given herein are for humans, but can be adjusted to the size of other mammals, as well as children, in accordance with weight or square meter size.

The term “pharmaceutically acceptable carrier” or “pharmaceutically acceptable excipient” includes, but is not limited to, any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, one or more suitable diluents, fillers, salts, disintegrants, binders, lubricants, glidants, wetting agents, controlled release matrices, colorants/flavoring, carriers, excipients, buffers, stabilizers, solubilizers, and combinations thereof. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions of the invention is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The phrase “pharmaceutically acceptable salt” as used herein, refers to pharmaceutically acceptable organic or inorganic salts of a compound of the invention. Exemplary salts include, but are not limited, to sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate “mesylate”, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, and pamoate (i.e., 1,1'-methylene-bis(2-hydroxy-3-naphthoate)) salts. A pharmaceutically acceptable salt may involve the inclusion of another molecule such as an acetate ion, a succinate ion or other counter ion. The counter ion may be any organic or inorganic moiety that stabilizes the charge on the parent compound. Furthermore, a pharmaceutically acceptable salt may have more than one charged atom in its

structure. Instances where multiple charged atoms are part of the pharmaceutically acceptable salt can have multiple counter ions. Hence, a pharmaceutically acceptable salt can have one or more charged atoms and/or one or more counter ion.

“Autoimmune disease” as used herein refers to any group of disorders in which tissue injury is associated with humoral or cell-mediated responses to the body's own constituents. Exemplary autoimmune disorders that are amenable to treatment in the methods of this disclosure include autoimmune diabetes mellitus (type 1 diabetes mellitus; T1D), systemic lupus erythematosus, autoimmune thyroiditis, rheumatoid arthritis, psoriasis, and multiple sclerosis.

The methods of the invention may be applied to cell populations in vivo or ex vivo. “In vivo” means within a living individual, as within an animal or human or in a subject's body. In this context, the methods of the invention may be used therapeutically or prophylactically in an individual. “Ex vivo” or “in vitro” means outside of a living individual. Examples of ex vivo cell populations include in vitro cell cultures and biological samples including but not limited to fluid or tissue samples obtained from individuals. Such samples may be obtained by methods known in the art. Exemplary biological fluid samples include blood, cerebrospinal fluid, urine, and saliva. Exemplary tissue samples include tumors and biopsies thereof. In this context, the invention may be used for a variety of purposes, including therapeutic and experimental purposes. For example, the invention may be used ex vivo or in vitro to determine the optimal schedule and/or dosing of administration of a PI3-kinase p110 δ selective inhibitor for a given indication, cell type, individual, and other parameters. Information gleaned from such use may be used for experimental or diagnostic purposes or in the clinic to set protocols for in vivo treatment or to select patients for treatment using the methods of this disclosure.

PI3K-p110 δ inhibitor compounds

The PI3K-p110 δ inhibitor compounds useful in the methods of this disclosure may exhibit one or more functional characteristics disclosed herein. For example, the PI3K-p110 δ inhibitor compounds may bind specifically to a PI3 kinase with an IC₅₀ for the 110 δ isoform of less than about 1 μ M, less than about 100 nM, less than about 50 nM, less than about 10 nM, less than about 1 nM, less than about 0.5 nM, less than about 100 pM, or less than about 50 pM. As an additional example, these PI3K-p110 δ inhibitor compounds may selectively inhibit one or more members of type I or class I phosphatidylinositol 3-kinases with an IC₅₀ value of about 100 nM or less, about 50 nM or less, about 10 nM or less, about 5 nM or less, about 100 pM or less, about 10 pM or less, or about 1 pM or less as measured in an in vitro kinase assay. As an additional example, the PI3K-p110 δ inhibitor compounds may selectively inhibit one or more members of type I PI3-kinases or more type I PI3-kinase mediated signaling pathways, with an IC₅₀ with respect to a p110 δ isoform, that is at least 10-fold lower, at least 20-fold lower, at least 50-fold lower, at least 100-

fold lower, at least 1000-fold lower than the inhibitor's IC₅₀ with respect to the other isoforms of type I PI3-kinases.

The PI3K-p110 δ inhibitor compounds useful in the methods of this disclosure may include any PI3K-p110 delta-specific inhibitor compound, including those disclosed in U.S. Patent Pub.

5 Nos. 2011/0021497; 2012/0245144; 2013/0344061; 2014/0011819; 2015/0011569; 2015/0272936; 2016/0207929; 2016/0222012; or U.S. Patent Nos. 6,518,277; 6,074,426; 6,074,427, each of which are incorporated herein by reference.

Idelalisib is a reversible p110 δ inhibitor that noncovalently binds the ATP binding pocket of the catalytic subunit and targets the p110 δ isoform with 110-453 fold more selectivity than
10 other class I isoforms. Thus, idelalisib is an exemplary PI3K-p110 δ inhibitor compound useful in the methods of this disclosure. Idelalisib (5-fluoro-3-phenyl-2-[(1S)-1-(7H-purin-6-ylamino)propyl]-4(3H)-quinazolinone; or CAL-101), a PI3K δ inhibitor marketed under the tradename ZYDELIGTM by Gilead Sciences, Inc. ZYDELIGTM, is indicated for the treatment of patients with chronic lymphocytic leukemia, in combination with rituximab, in patients for whom
15 rituximab alone would be considered appropriate therapy due to other co-morbidities. It is also indicated for treating patients with relapsed follicular B-cell non-Hodgkin lymphoma in patients who have received at least two prior systemic therapies and patients with relapsed small lymphocytic lymphoma in patients who have received at least two prior systemic therapies. The doses of idelalisib (30mg/kg) used in these approved applications significantly depletes B cells,
20 and a black box warning has been issued for fatal and/or severe colitis, pneumonitis, and infection following the administration of idelalisib at these doses. Idelalisib is disclosed in U.S. Pat. No. RE44638, International Publication Nos. WO2013/134288, WO2015/014315, WO2015/092810, and WO2005/113556, and US Patent Pub. Nos. 2018/0093987, 2018/0064714, 2018/0037584, and 2017/0260186, each of which is incorporated herein by reference.

25 **Therapeutic Treatment**

This disclosure provides methods of treating a subject by administering a therapeutically effective amount of a PI3K-p110 δ inhibitor, as described herein, to a subject. Diseases that may be treated with the PI3K-p110 δ inhibitor include, but are not limited to, autoimmune disease or disorder such as autoimmune diabetes mellitus (type 1 diabetes mellitus; T1D), systemic lupus
30 erythematosus, autoimmune thyroiditis, rheumatoid arthritis, psoriasis, and multiple sclerosis. In exemplary embodiments, the autoimmune disease is type 1 diabetes mellitus (T1D). In other embodiments PI3K-p110 δ inhibitors can be used to treat immune system dysfunction (e.g., graft-versus-host disease, organ transplant rejection).

Autoimmune diseases that may be treated with PI3K-p110 δ inhibitors, such as idelalisib,
35 in the methods of this disclosure may include acute type 1 diabetes mellitus, systemic lupus erythematosus, multiple sclerosis, idiopathic thrombocytopenic purpura, chronic idiopathic

thrombocytopenic purpura, dermatomyositis, Sydenham's chorea, myasthenia gravis, lupus nephritis, rheumatic fever, polyglandular syndromes, bullous pemphigoid, Henoch-Schonlein purpura, post-streptococcal nephritis, erythema nodosum, Takayasu's arteritis, ANCA-associated vasculitides, Addison's disease, rheumatoid arthritis, sarcoidosis, ulcerative colitis, erythema
5 multiforme, IgA nephropathy, polyarteritis nodosa, ankylosing spondylitis, Goodpasture's syndrome, thromboangitis obliterans, Sjogren's syndrome, primary biliary cirrhosis, Hashimoto's thyroiditis, thyrotoxicosis, scleroderma, chronic active hepatitis, polymyositis/dermatomyositis, polychondritis, bullous pemphigoid, pemphigus vulgaris, Wegener's granulomatosis, membranous nephropathy, amyotrophic lateral sclerosis, tabes dorsalis, giant cell arteritis/polymyalgia,
10 pernicious anemia, rapidly progressive glomerulonephritis, psoriasis, or fibrosing alveolitis.

Optimal dosing of PI3K-p110 δ inhibitors, such as idelalisib, in the methods of this disclosure may include a dosage of between 0.01 mg/kg and 4 mg/kg, preferably given either daily, twice daily, or every other day. The dosage may be up to 10 mg/kg. Exemplary dosages may include between 0.23 mg/kg/day and about 3.6 mg/kg/day, including dosages of idelalisib
15 administered at a dosage of between about 0.45 mg/kg/day and about 1.8 mg/kg/day, between about 0.9 mg/kg/day and about 1.8 mg/kg/day, between about 1.8 mg/kg/day and about 3.6 mg/kg/day, or a dosage of idelalisib of about 0.9 mg/kg/day or about 1.8 mg/kg/day. Thus, the individual dose of PI3K-p110 δ inhibitors, such as idelalisib, in the methods of this disclosure may include a dosage of 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg,
20 0.8 mg/kg, 0.9 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg. The person of ordinary skill will realize that a variety of factors, such as age, general health, specific organ function or weight, as well as effects of prior therapy on specific organ systems (e.g., bone marrow) may be considered in selecting an optimal dosage of the PI3K-p110 δ inhibitor, and that the dosage and/or frequency of administration may be increased or
25 decreased during the course of therapy. The dosage may be repeated as needed, with evidence of anergy or autoimmune treatment or abatement observed after as few as 2 to 10 doses. The optimized dosages and schedules of administration disclosed herein show unexpected efficacy and reduced toxicity in treated subjects, while sparing B cell responses to exogenous antigen in the subject.

30 The dosage of the PI3K-p110 δ inhibitor is preferably administered multiple times, once or twice a day, or a week. The schedule of administration may comprise administration once or twice a day, on a cycle selected from the group consisting of: (i) daily; (ii) every other day; (iii) one day of therapy followed by two, three, or four days off; (iv) two days of therapy followed by one, two, three or four days off; (v) three days of therapy followed by one, two, three, four or five days off;
35 (vi) four days of therapy followed by one, two, three, four, or five days off; (vii) five days of

therapy followed by one, two, three, four, or five days off; and (viii) weekly or monthly. Such administration cycles may be repeated 4, 6, 8, 10, 12, 16, or 20 times, or more.

Suitable routes of administration of the PI3K-p110 δ inhibitor, such as idelalisib, within the methods of this disclosure include, without limitation, oral, parenteral, subcutaneous, rectal, transmucosal, intestinal administration, intramuscular, intramedullary, intrathecal, direct intraventricular, intravenous, intravitreal, intraperitoneal, intranasal, or intraocular injections. The preferred routes of administration are parenteral. Alternatively, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into an organ or joint.

Diagnostic assays may be employed in the therapeutic methods of this disclosure to identify subjects with activated PI3K or reduced SH2-containing inositol lipid phosphatase (SHIP-1) and/or reduced phosphatase and tensin homolog (PTEN) expression or activity, particularly in B cells from the subject, defining a subject most likely to respond to PI3K-p110 δ inhibitor therapy. Therefore, the inhibition of PI3K-p110 δ in cells with low or absent PTEN and/or SHIP-1 expression and activity may be an effective autoimmune disease treatment strategy. Such selection of patients based on PTEN and SHIP-1 activity may also be useful in the clinical evaluation of efficacy of existing or future PI3K inhibitors, as well as in the future prescription of such drugs to patients diagnosed or suspected of having an autoimmune disease or disorder. Accordingly, this disclosure also provides methods for selecting a patient suitable for PI3K-p110 δ inhibitor therapy by obtaining at least one biological sample from a subject that is a candidate for therapy with a PI3K-p110 δ inhibitor, such as idelalisib, and detecting the activity of PTEN and/or SHIP-1 in the sample, in particular the expression of these proteins in B cells in the sample, thereby identifying the subject with reduced or absent phosphatase activity as particularly suitable for PI3K-p110 δ inhibitor therapy.

Methods of determining the presence or absence of PTEN and/or SHIP-1 expression, mutations, and activity in a patient and/or a tumor cell are known in art, for example as described in U.S. Patent Pub. No. 2018/0338977, which is incorporated herein by reference for this purpose.

Thus, one aspect of this disclosure is a method of treating an autoimmune disease or disorder in a subject expressing low or absent PTEN and/or SHIP-1 protein (e.g., B cells displaying low or absent enzymatic activity or gene expression) by administering a PI3K-p110 δ inhibitor, or pharmaceutical composition comprising a PI3K-p110 δ inhibitor, to the subject. Said another way, these methods include testing a subject and/or a biological sample from a subject for PTEN and SHIP-1 activity, expression and/or mutations, and treating an autoimmune disease or disorder in the subject in which PTEN and/or SHIP-1 protein activity and/or expression is low (i.e., below wild-type expression or protein activity levels) or absent by administering a PI3K-p110 δ inhibitor, or pharmaceutical composition comprising a PI3K-p110 δ inhibitor, to the subject.

Conversely, administration of a PI3K-p110 δ inhibitor, or pharmaceutical composition comprising a PI3K-p110 δ inhibitor, may be withheld from a subject in which wild-type or elevated PTEN protein activity and/or expression is detected.

According to this aspect, this disclosure provides for the use of a PI3K-p110 δ inhibitor, or pharmaceutical composition comprising a PI3K-p110 δ inhibitor, in the manufacture of a medicament for the treatment of an autoimmune disease or disorder in a subject expressing reduced or absent PTEN and/or SHIP-1. This aspect also provides a PI3K-p110 δ inhibitor, or pharmaceutical composition comprising a PI3K-p110 δ inhibitor, for use in the treatment of an autoimmune disease or disorder in a subject expressing reduced or absent PTEN and/or SHIP-1.

10 **Combination Therapy**

In the therapeutic methods of this disclosure, the PI3K-p110 δ inhibitor compounds may be employed alone or in combination with other therapeutic agents for the treatment of an autoimmune disease or disorder, such as Type 1 Diabetes, SLE, or autoimmune thyroiditis. In these methods, a PI3K-p110 δ inhibitor compound is combined in a pharmaceutical combination formulation, or dosing regimen as combination therapy, with a second therapeutic compound that has anti-inflammatory or immune suppressing properties or is otherwise useful for treating an autoimmune disease or symptoms thereof. For example, the second therapeutic agent may be a non-steroidal anti-inflammatory agent, or an antidiabetic agent. The second compound of the pharmaceutical combination formulation or dosing regimen preferably has complementary activities to the PI3K-p110 δ inhibitor such that they do not adversely affect each other. Such compounds are suitably present in combination in amounts that are effective for the purpose intended.

The combination therapy may be administered as a simultaneous or sequential regimen. When administered sequentially, the combination may be administered in two or more administrations. The combined administration includes coadministration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein there is a time period while both (or all) active agents simultaneously exert their biological activities.

Suitable dosages for any of the above coadministered agents are those described herein for the PI3K-p110 δ inhibitor, such as idelalisib, and may be lowered due to the combined action (synergy) of the second identified agent and other therapeutic agents or treatments.

The combination therapy may provide “synergy” and prove “synergistic”, i.e., the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect may be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined, unit dosage formulation; (2) delivered by alternation or in parallel as separate formulations; or (3)

by some other regimen. When delivered in alternating therapy, a synergistic effect may be attained when the compounds are administered or delivered sequentially, e.g., by different injections in separate syringes, separate tablets or capsules, or separate infusions. In general, during alternating therapy, an effective dosage of each active ingredient is administered sequentially, i.e., serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.

Pharmaceutical Formulations

The PI3K-p110 δ inhibitor can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the PI3K-p110 δ inhibitor is combined in a mixture with a pharmaceutically suitable excipient. Suitable excipients are well-known to those in the art. See, for example, Ansel et al., PHARMACEUTICAL DOSAGE FORMS AND DRUG DELIVERY SYSTEMS, 5th Edition (Lea & Febiger 1990), and Gennaro (ed.), REMINGTON'S PHARMACEUTICAL SCIENCES, 18th Edition (Mack Publishing Company 1990), and revised editions thereof.

Additional pharmaceutical methods may be employed to control the duration of action of the PI3K-p110 δ inhibitor. Controlled release preparations can be prepared through the use of polymers to complex or adsorb the PI3K-p110 δ inhibitor.

In order to use a PI3K-p110 δ inhibitor compound for the therapeutic treatment (including prophylactic treatment) of mammals, it is normally formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition. Thus, this disclosure provides a pharmaceutical composition comprising a PI3K-p110 δ inhibitor in association with a pharmaceutically acceptable diluent or carrier.

A typical formulation is prepared by mixing a PI3K-p110 δ inhibitor compound and a carrier, diluent or excipient. Suitable carriers, diluents and excipients are well known to those skilled in the art and include materials such as carbohydrates, waxes, water soluble and/or swellable polymers, hydrophilic or hydrophobic materials, gelatin, oils, solvents, water and the like. The particular carrier, diluent or excipient used will depend upon the means and purpose for which the PI3K-p110 δ inhibitor compound is being applied. Solvents are generally selected based on solvents recognized by persons skilled in the art as safe (GRAS) to be administered to a mammal. In general, safe solvents are non-toxic aqueous solvents such as water and other non-toxic solvents that are soluble or miscible in water. Suitable aqueous solvents include water, ethanol, propylene glycol, polyethylene glycols (e.g., PEG 400, PEG 300), etc. and mixtures thereof. The formulations may also include one or more buffers, stabilizing agents, surfactants, wetting agents, lubricating agents, emulsifiers, suspending agents, preservatives, antioxidants, opaquing agents, glidants, processing aids, colorants, sweeteners, perfuming agents, flavoring agents and other known additives to provide an elegant presentation of the drug (i.e., a compound

of the present invention or pharmaceutical composition thereof) or aid in the manufacturing of the pharmaceutical product (i.e., medicament).

The formulations may be prepared using conventional dissolution and mixing procedures. For example, the bulk drug substance (i.e., compound of the present invention or stabilized form of the compound (e.g., complex with a cyclodextrin derivative or other known complexation agent) is dissolved in a suitable solvent in the presence of one or more of the excipients described above. The PI3K-p110 δ inhibitor compound is typically formulated into pharmaceutical dosage forms to provide an easily controllable dosage of the drug and to enable patient compliance with the prescribed regimen.

The pharmaceutical composition (or formulation) for application may be packaged in a variety of ways depending upon the method used for administering the drug. Generally, an article for distribution includes a container having deposited therein the pharmaceutical formulation in an appropriate form. Suitable containers are well known to those skilled in the art and include materials such as bottles (plastic and glass), sachets, ampoules, plastic bags, metal cylinders, and the like. The container may also include a tamper-proof assemblage to prevent indiscreet access to the contents of the package. In addition, the container has deposited thereon a label that describes the contents of the container. The label may also include appropriate warnings.

Exemplary oral dosage formulations of PI3K-p110 δ inhibitors, useful in practicing the therapeutic methods of this disclosure, include at least one PI3K-p110 δ inhibitor, such as idelalisib, in an amount that is convenient and accurate for administration of the surprisingly low doses that the inventors have discovered to be efficacious in treating an autoimmune disease or disorder. Thus, exemplary oral dosage formulations of this disclosure may include tablets or capsules containing idelalisib ((S)-5-fluoro-3-phenyl-2-[1-(9H-purin-6-ylamino)-propyl]-3H-quinazolin-4-one) or a pharmaceutically acceptable salt thereof, in an amount between 5mg and 25mg, and at least one pharmaceutically acceptable excipient. Such tablet or capsule may contain 5mg, 6mg, 7mg, 8mg, 9mg, 10mg, 11mg, 12mg, 13mg, 14mg, 15mg, 16mg, 17mg, 18mg, 19mg, 20mg, 21mg, 22mg, 23mg, 24mg, or 25mg of idelalisib.

Articles of Manufacture

In another aspect of this disclosure, an article of manufacture, or “kit”, containing materials useful for the treatment of autoimmune diseases and disorders in the methods described above is provided. The kit may include a container comprising a compound of PI3K-p110 δ inhibitor. The kit may further comprise a label or package insert, on or associated with the container. The term “package insert” is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. Suitable containers include, for example, bottles, vials, syringes, blister pack,

etc. The container may be formed from a variety of materials such as glass or plastic. The container may hold a PI3K-p110 δ inhibitor or a formulation thereof which is effective for treating the autoimmune disease or disorder. At least one active agent in the composition is a PI3K-p110 δ inhibitor compound. The kit may further comprise directions for the administration of the PI3K-p110 δ inhibitor compound and, if present, a second pharmaceutical formulation. For example, if the kit comprises a first composition comprising a PI3K-p110 δ inhibitor compound and a second pharmaceutical formulation, the kit may further comprise directions for the simultaneous, sequential or separate administration of the first and second pharmaceutical compositions to a patient in need thereof.

These kits may be suitable for the delivery of solid oral forms of a PI3K-p110 δ inhibitor compound, such as tablets or capsules. Such a kit preferably includes a number of unit dosages and may further include a card having the dosages oriented in the order of their intended use. An example of such a kit is a “blister pack”. Blister packs are well known in the packaging industry and are widely used for packaging pharmaceutical unit dosage forms. If desired, a memory aid can be provided, for example in the form of numbers, letters, or other markings or with a calendar insert, designating the days in the treatment schedule in which the dosages can be administered.

The following examples are intended to be purely exemplary and should not be considered to be limiting in any way.

EXAMPLES

The following methods were used to conduct the experiments described in Examples 1-7, below:

Mice: Except where otherwise indicated, 6-16 week old mice were used in all experiments. Both male and female mice were used, but experiments were sex matched and both sexes gave identical results, with the exception of only female mice being used in the VH125.NOD experiments as female mice develop accelerated disease. hCD20-TamCre animals (Khalil, et al. Science 336:

1178-81) were intercrossed with mice carrying the rosa26-flox-STOP-YFP allele (Srinivas, et al., BMC Dev. Biol. 1:4), generating mice in which YFP is expressed in B cells upon Cre activation.

These mice were crossed with Ars/A1 (Benschop, et al., Immunity 14: 33-43) transgenic mice to generate hCD20-TamCre x rosa26-flox-STOP-YFP x Ars/A1 mice. B cells from these mice will be referred to as WT Ars/A1. These mice were also crossed with SHIP-1^{flox/flox} mice (Karlsson, et al. J Exp Med 198: 333-40). hCD20-TamCre x rosa26-flox-STOP-YFP x Ars/A1 mice were also crossed to PTEN^{flox/flox} mice (Anzelon, et al., Nat Immunol 4: 287-94) and SHP-1^{flox/flox} mice to generate mice in which SHIP-1, PTEN and SHP-1 deletion can be induced in anergic B cells.

hCD20-TamCre x rosa26-flox-STOP-YFP x PTEN^{flox/flox} x Ars/A1 were crossed to hCD20-TamCre x rosa26-flox-STOP-YFP x SHIP-1^{flox/flox} x Ars/A1 to generate hCD20-TamCre x rosa26-flox-STOP-YFP x PTEN^{flox/wt} x SHIP-1^{flox/wt} x Ars/A1 to allow for the double haploinsufficiency of both PTEN and SHIP-1 within B cells.

Adoptive transfers and tamoxifen induction: 2 hours before adoptive transfer, C57BL/6 recipient mice received 200 rads irradiation. For MD4 transfers, recipients did not receive prior irradiation. B cells from donor mice were isolated via depletion of CD43⁺ cells with anti-CD43-conjugated magnetic beads (MACS anti-mouse CD43; Miltenyi Biotec). Alternatively, CD4⁺ T cells were isolated via CD4 positive selection (MACS anti-mouse CD4 (L3T4) Miltenyi Biotec). Resultant populations were >97% pure based on flow cytometric analyses. Donor B cells were labeled with either CellTrace Violet (Molecular Probes) or CFSE (Molecular Probes) at 5 μ M for 5 minutes at room temperature prior to transfer. Donor CD4 T cells were labeled with CFSE (Molecular Probes) at 5 μ M for 5 minutes at room temperature prior to transfer. 1-2x10⁶ donor cells in 200 μ l PBS were adoptively transferred via IV injection. 24 hours post transfer, tamoxifen was administered to activate Cre. Tamoxifen (Sigma-Aldrich) was dissolved in 100% corn oil (Sigma-Aldrich) at 20mg/ml. Recipient mice were injected IP with 100 μ l (2mg) on two consecutive days.

Manufacturing and administration of idelalisib containing rodent chow: Idelalisib (LC Laboratories) was shipped to ResearchDiets Inc. for blending the compound homogenously into modified OSD with 24 kcal% protein, 16 kcal% fat, 60% kcal% carbohydrate, 100g of cellulose and 25g inulin. Diet dose (DD) is calculated by multiplying the single daily dose (SD) by the body weight of a mouse (BW) and dividing that by the daily food intake (FI) [DD= (SD x BW)/FI]. Chow used includes base chow as described above with: + 0mg idelalisib/kg diet (Vehicle Control), + 600mg idelalisib/kg diet (30mg/kg ingested idelalisib dose), + 75mg idelalisib/kg diet (3.75mg/kg ingested idelalisib dose), and + 18.75mg idelalisib/kg diet (0.9375mg/kg ingested idelalisib dose).

For PTEN^{fl/wt} x SHIP-1^{fl/wt} and SHP-1^{fl/fl} adoptive transfers, control chow and idelalisib containing chow was given to mice on day 7 post-tamoxifen administration. For MD4 and OT-II adoptive transfers, control chow and idelalisib containing chow was given to mice 2 days post transfer. For VH125.NOD experiments animals are placed on control chow immediately after weaning. Following 2 consecutive diabetic blood glucose readings (between 150-200mg/dl) animals either remain on vehicle control chow or are placed on 0.9375mg/kg idelalisib containing chow and disease progression is monitored. For NP₄Ova-alum experiments, animals were placed on either vehicle control chow or 0.9375mg/kg idelalisib chow for 28 days and subsequently immunized.

Antigens and immunization: HEL conjugated to SRBCs was used to produce antigen for experiments with MD4 B cells. SRBCs (Colorado Serum Company) were stored in Alsever's solution at 4°C. SRBCs are washed 3 times in PBS prior to use. 1ml of 50mg/ml of the chemical crosslinker N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (Sigma-Aldrich) was added to 1ml packed SRBCs and 15ml of 5mg/ml HEL (Sigma-Aldrich), mixed and rotated at room temperature for 45 minutes. Mice were immunized IP with 200 μ l of a 5% HEL-SRBC in

PBS. For NP₄Ova-Alum immunizations, 10mg/ml alum and 5mg/ml NP₄Ova were mixed to a final concentration of 250µg/ml NP₄Ova and 2.5mg/ml alum and rotated for 3 hours at room temperature. NOD mice were placed either on vehicle control chow or 0.9375mg/kg idelalisib chow for 28 days. Mice were then immunized with NP₄Ova-alum IP in 200µl/mouse, and responses were measured on day 7 and day 14 post-immunization.

Phenotypic analysis by FACS: Spleens were mechanically disrupted, single-cell suspensions were generated, and red blood cells were lysed with ammonium chloride TRIS. Cells were resuspended in PBS containing 1% FBS and incubated with indicated antibodies. For analysis of cell surface markers, antibodies against the following molecules were used: B220-PE (BioLegend), B220-BV786 (BD Biosciences), B220-BV510 (BioLegend), CD4-BV711 (BioLegend), CD8-BV421 (BioLegend), CD138-PECy7 (BioLegend), CD69-BV786 (BD Biosciences), CD86-PerCPCy5.5 (BioLegend). After cell surface staining, the cells were fixed and permeabilized with Cytofix/Cytoperm (BD) per manufacturer's instructions and stained with Dylight650-E4 anti-Ars/A1 idiotype (produced and conjugated in our laboratory) OR HEL-650 (produced and conjugated in our laboratory) and Alexa Fluor 488-anti-GFP (rabbit polyclonal; Life Technologies). Events were collected on a CyAn ADP (Dako) and subsequent analysis using FlowJo software (Tree Star).

Analysis of calcium mobilization: For measurements of intracellular free calcium concentration ($[Ca^{2+}]_i$), RBC-depleted single-cell suspended splenocytes were simultaneously stained with CD8-PE (BD Biosciences), CD4-APC (BioLegend) or B220-PE and loaded with Indo-1 acetoxymethyl (Molecular Probes), as described previously (Gauld, et al., Nat Immunol 6: 1160-1167). For analysis of ($[Ca^{2+}]_i$), cells were suspended at 10×10^6 cells/ml in warm IMDM + 2% FBS in a 500ul volume. Cells were acquired for 30 seconds to establish a baseline, and then stimulated with 5µg/ml of F(ab')₂ rabbit anti-mouse anti-IgG (H&L; Invitrogen) +/- indicated doses of idelalisib and acquired for 3 minutes. For CD4 and CD8 T cells, cells were acquired for 30 seconds to establish a baseline, and then 10µg/ml of anti-CD3-biotin (BD Biosciences) +/- indicated doses of idelalisib was added, 60 seconds later 20µg/ml streptavidin (Sigma-Aldrich) was added and acquired for 3 minutes. Mean relative ($[Ca^{2+}]_i$) was monitored over time using an LSR Fortessa X-20 (BD) with analysis using FlowJo software.

Analysis of phosphorylated signaling intermediaries: RBC-depleted single-cell suspended splenocytes were suspended at 10×10^6 cells/ml in serum free IMDM, +/- indicated doses of idelalisib (as indicated in FIG. 4) and rested for 1 hour at 37°C. Cells are then washed 2X in serum free IMDM, and stimulated with 5µg/ml of F(ab')₂ rabbit anti-mouse anti-IgG (H&L; Invitrogen) or 10µg/ml anti-CD3-biotin (BD Biosciences) + 20µg/ml streptavidin (Sigma-Aldrich) for 2 minutes. Signaling was stopped by addition of 20% PFA to a final concentration of 2%, incubated at 37°C for 15 minutes and resuspended in 100% ice-cold MeOH (directly from -80°C). Cells were

then placed on ice for 30 minutes and placed at -20°C for storage. For analysis, cells were stained with B220-BV786, CD4-BV711, CD8-BV421, and/or pAKT-Alexa Fluor 647 (BD Biosciences), pPLC γ -PE (BD Biosciences), pBTK-BV421 (BD Biosciences) pSyk/Zap70-PE (BD Biosciences) at room temperature for 1 hour. Cells were washed 3 times and samples were acquired in triplicate on an LSR Fortessa X-20 (BD) with analysis using FlowJo software.

Enzyme linked immunosorbent assay: For detection of IgM^a anti-Ars antibodies, microtiter plates were coated with 10 μ g/ml Ars-BSA16 in PBS and blocked with 2mg/ml BSA in PBS 0.05% Tween-20. For detection of IgM^a anti-HEL antibodies, microtiter plates were coated with 10 μ g/ml HEL in PBS and blocked with 2mg/ml BSA in PBS 0.05% Tween-20. For detection of total NP-specific IgM and IgG, microtiter plates were coated with 20 μ g/ml NP₂₇BSA and blocked with 2mg/ml BSA in PBS 0.05% Tween-20. For detection of high affinity NP-specific IgM and IgG, microtiter plates were coated with 20 μ g/ml NP₂BSA and blocked with 2mg/ml BSA in PBS 0.05% Tween-20. Serial dilutions of mouse serum in PBS were added and incubated overnight at 4°C. Ars/A1-derived IgM^a antibodies and MD4-derived HEL IgM^a antibodies were detected with biotinylated DS.1 anti-IgM^a (BD Pharmingen) in PBS, followed by Streptavidin-HRP (Thermo Fisher Scientific). For NP-specific IgM antibodies were detected using goat-anti-mouse IgM-HRP (SouthernBiotech). For NP-specific IgG antibodies were detected using goat-anti-mouse IgG-HRP (SouthernBiotech). Between all steps, plates were washed 3 times with PBS 0.05% Tween-20. The ELISA was developed with TMB single solution (Invitrogen) and the reaction was stopped with 1M HCl. OD was measured at 450nm using a VERSAMax plate reader (Molecular Devices) and data analyzed with SoftMax Pro6 software.

ELISPOT: For detection of IgM^a anti-Ars antibodies, microtiter plates were coated with 10 μ g/ml Ars-BSA16 in PBS and blocked with 2mg/ml BSA in PBS 0.05% Tween-20. For detection of IgM^a anti-HEL antibodies, microtiter plates were coated with 10 μ g/ml HEL in PBS and blocked with 2mg/ml BSA in PBS 0.05% Tween-20. For detection of total NP-specific IgM and IgG, microtiter plates were coated with 20 μ g/ml NP₂₇BSA and blocked with 2mg/ml BSA in PBS 0.05% Tween-20. For detection of high affinity NP-specific IgM and IgG, microtiter plates were coated with 20 μ g/ml NP₂BSA and blocked with 2mg/ml BSA in PBS 0.05% Tween-20. Plates were washed 3 times prior to use with PBS 0.05% Tween-20. RBC-depleted single-cell suspension of splenocytes in complete medium were added in two-fold serial dilutions starting at 1/100th of a spleen in the first well. Plates were incubated overnight at 37°C. Ars/A1-derived IgM^a antibodies and MD4-derived HEL IgM^a antibodies were detected with biotinylated DS.1 anti-IgM^a (BD Pharmingen) in PBS, followed by Streptavidin-AP (SouthernBiotech). For NP-specific IgM antibodies were detected using goat-anti-mouse IgM-AP (SouthernBiotech). For NP-specific IgG antibodies were detected using goat-anti-mouse IgG-AP (SouthernBiotech). Between all steps,

plates were washed 3 times with PBS 0.05% Tween-20. The plates were developed by incubated with ELISPOT developing buffer (25 μ m 5-bromochloro-3-indolyl phosphate p-toluidine, 100mM NaCl, 100mM Tris, and 10mM MgCl₂, pH 9.5) for 1 hour. The reaction was stopped by washing the plates 3 times with PBS 0.05% Tween-20. The number of spots at a cell dilution in the linear range was determined, and the number of ASCs was calculated.

Statistics: Data were analyzed using Prism GraphPad Software. Statistical analyses were performed using the indicated statistical tests in the brief description of the figures. P values ≤ 0.05 were considered statistically significant. Throughout the figures, asterisks are used to denote *p*-values of: * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.005$ and **** = $p \leq 0.0001$.

Example 1

Idelalisib prevents T1D progression in VH125.NOD mice

The inventors' began exploring the ability of low doses of p110 δ inhibitor to arrest progression of T1D in a genetically complex model of autoimmunity. The most commonly used mouse model of T1D, the non-obese diabetic mouse, reflects disease progression in the human. Female NOD mice develop overt diabetes at ~20 weeks of age, with lymphocytic infiltration of the islets and autoantibody production preceding hyperglycemia and diabetes. NOD mice are protected from disease development upon deletion of the B cell compartment (NOD.uMT^{-/-}), or upon skewing of the BCR repertoire away from insulin reactivity (VH281.NOD), but not upon removal of autoantibody (mIgM NOD). In these studies the inventors utilized the VH125.NOD mouse model of disease in which mice carry an immunoglobulin heavy chain transgene specific for insulin, the dominant autoantigen in T1D. Importantly, the transgenic heavy chain can pair with any endogenous light chain, resulting in a frequency of peripheral B cells reactive with insulin of 1-3%. In WT female NOD mice, disease penetrance only reaches ~70%, but skewing of the B cell repertoire towards insulin reactivity leads to 100% penetrance of disease in female mice and earlier disease onset.

While multiple insulin-dependent (Type 1) diabetes (Idd) loci contribute to disease development in NODs, B cells in these mice exhibit a marked reduction in PTEN levels in both insulin-reactive B cells and total B cells compared to closely related, autoimmunity resistant VH125.C57BL/6-H2g7 mice. On this background, but not VH125.NOD, high affinity insulin-reactive B cells are anergic. NOD mice have increased susceptibility to additional autoimmune diseases, such as Rheumatoid Arthritis (RA), SLE and the Experimental Autoimmune Encephalomyelitis (EAE) mouse model of MS. Reduced B cell expression of PTEN has been reported in lupus patients, and the inventors have observed reduced PTEN levels in the B cells of both T1D and AITD patients. Thus, loss of B cell tolerance in both man and mouse may be driven in part by PI3K pathway dysregulation. We therefore postulated VH125.NOD mice and T1D

patients, both of which have PI3K pathway regulation defects, may benefit from low dose idelalisib, a p110 δ inhibitor, to reinstate anergy of autoreactive B cells.

To test this possibility, immediately post-weaning, female VH125.NOD mice were placed on vehicle control chow to allow habituation to the diet. Upon two consecutive blood glucose readings in the pre-diabetic range, some mice were maintained on the vehicle control chow, while others were fed 0.9375mg/kg idelalisib containing chow (FIG. 1A). Disease progression was monitored based on blood glucose levels and visible signs of disease (i.e., hunching, scruffy fur, excessive urination). In mice receiving 0.9375mg/kg idelalisib, disease progression was significantly delayed, and survival was extended (FIG. 1B).

Example 2

NOD mice on low-dose idelalisib treatment remain immunocompetent

B cell depletion therapies are somewhat efficacious in T1D, but are not without safety concerns. Removal of an arm of the adaptive immune system can leave patients susceptible to infection and prevent proper response to immunization. The inventors therefore sought to determine if low dose idelalisib treatment would spare responsiveness to immunization. Non-diabetic female NOD mice were placed on vehicle control or 0.9375mg/kg idelalisib containing chow for 4 weeks, immunized with NP4Ova + Alum, and their antibody response assessed (FIG. 1C). 14 days post-immunization, the anti-NP IgM response in the spleen was not different between the vehicle control and 0.9375mg/kg idelalisib-treated cohorts. Further, the number of total anti-NP IgM antibody secreting cells (ASCs) per spleen was equivalent, as were the number of high-affinity IgM anti-NP ASCs/spleen (FIG. 1D, left panel). There was no observed difference between the vehicle control and 0.9375mg/kg idelalisib treated cohorts in either total IgG anti-NP or high affinity IgG anti-NP ASCs/spleen (FIG. 1D, right panel). The levels of IgM anti-NP and IgG anti-NP found in the periphery was also equivalent (FIG. 1E). There was no difference in B cell numbers in the spleens of animals receiving vehicle control and 0.9375mg/kg idelalisib containing chow. These data show low dose idelalisib treatment does not affect the ability to respond to immunization, as evidenced by equivalent levels of class switching and affinity maturation between the treated and untreated groups. Thus, animals receiving doses of idelalisib sufficient to slow progression of disease and prolong survival in VH125.NOD mice remain immunocompetent, alleviating potential adverse outcomes inherently associated with B cell-depleting therapies.

Example 3

Autoreactive PTEN^{+/-} x SHIP-1^{+/-} B cells re-establish anergy when treated with idelalisib

The inventors next sought to confirm the specificity of the idelalisib effect for autoimmunity driven by dysregulation of the PI3K pathway. Regulation of the PI3K pathway by the inositol phosphatases PTEN and SHIP-1 is required for maintenance of B cell anergy. B cells

in T1D, AITD, and SLE patients express reduced PTEN and SHIP-1, presumably due to an increase in the miRNAs that regulate them, e.g. mir-7 and mir-155. Studies in animal models utilizing B cell targeted conditional deletion of either of these molecules is sufficient to drive autoreactive B cells out of anergy leading to rapid proliferation and differentiation into antibody secreting cells. Removal of a single allele of both PTEN and SHIP-1 is also sufficient to allow for loss of anergy because both degrade PI(3,4,5)P3. To best approximate physiologic conditions, the inventors utilized B cell-targeted conditional deletion (huCD20cre^{tam}) of one allele of PTEN (PTEN^{flox/+}) and one allele of SHIP-1 (SHIP-1^{flox/+}), coupled with a YFP-reporter to determine cre-activity, and crossed onto an anti-DNA (Ars/A1) transgenic background that renders B cells anergic (as described above).

Anergic B cells were adoptively transferred into C57BL/6 recipients as shown diagrammatically in FIG. 2A. Seven days post tamoxifen treatment, anergy was lost and idelalisib treatment was begun. Fourteen days post adoptive transfer, mice on 0.9375mg/kg, 3.75mg/kg, and the clinically-prescribed dose of 30mg/kg of idelalisib-containing chow had significantly decreased serum autoantibody relative to untreated controls (FIG. 2B, quantified FIG. 2C). Furthermore, the number of autoreactive ASCs/spleen was significantly reduced in cohorts receiving 0.9375mg/kg and 3.75mg/kg idelalisib containing chow, with undetected ASCs in cohorts receiving 30mg/kg idelalisib containing chow (FIG. 2D). The reduction in peripheral autoantibody, as well as ASCs/spleen was not due to a differential recovery of transferred cells among treatment groups, as no significant difference in total Ars/A1 idiotype+ YFP+ B cells in the spleens of animals at day 14 post transfer was found, regardless of treatment (FIG. 2F). Adoptively transferred PTEN^{+/-} x SHIP-1^{+/-} B cells from cohorts receiving tamoxifen and 0.9375mg/kg, 3.75mg/kg, and 30mg/kg idelalisib-containing chow underwent significantly decreased proliferation (FIG. 2E top panel, quantified FIG. 2G), and plasmablast differentiation as measured by CD138 positivity (FIG. 2E bottom panel, quantified FIG. 2H) relative to mice receiving vehicle control chow. These data demonstrate that 0.9375mg/kg idelalisib, as well as higher doses, is sufficient to constrain an autoreactive B cell response driven by haploinsufficiency of the inositol phosphatases that regulate the PI3K pathway.

Example 4

Autoreactive SHP-1^{-/-} B cells do not maintain anergy when treated with low dose idelalisib

SHP-1 is a regulatory SH2-domain containing tyrosine phosphatase which mediates the function of inhibitory receptors such as CD22, PD1, and FCγRIIB, and is necessary for maintenance of B cell tolerance. Allelic variants of SHP-1 have been shown to increase risk of developing SLE, with studies indicating a subset of SLE patients having reduced SHP-1 protein in their B cells. Additionally, reductions in SHP-1 mRNA and protein have been observed in peripheral blood B cells of MS patients. Studies in viable motheaten mice have revealed that a

mutation in a splice site in *Ptpn6*, the gene that encodes SHP-1, resulting in an 80-90% reduction in enzymatic activity, leads to severe B cell immunodeficiency and autoantibody production. Our laboratory has shown B cell-targeted conditional deletion of SHP-1 from anergic B cells *in vivo* leads to proliferation and autoantibody production. SHP-1 is required to maintain B cell anergy, acting through a pathway distinct from SHIP-1 and PTEN.

To test the specificity of idelalisib effects on non-PI3K pathway dysregulation-mediated autoimmunity we used the adoptive transfer system described in FIG. 3A in conjunction with SHP-1^{flox/flox} B cells. Idelalisib-containing chow was administered at day 7 post transfer, upon removal of SHP-1 protein from transferred cells. Unlike its enforcement of anergy caused by PI3K pathway dysregulation, low and intermediate dose idelalisib treatment had no effect on loss of anergy caused by SHP-1 induced deficiency. However, autoimmunity was blocked by high dose idelalisib (FIG. 3B, quantified in FIGS. 3C and 3D). Although a trend of reduced recovery of adoptively transferred SHP-1^{-/-} B cells from the spleens of animals receiving idelalisib-containing chow was observed, this was not significant (FIG. 3F). In animals receiving vehicle, 0.9375mg/kg or 3.75mg/kg idelalisib-containing chow, SHP-1^{-/-} B cells proliferated (FIG. 3E top panel, quantified FIG. 3G) and differentiated (FIG. 3E bottom panel, quantified FIG. 3H) equivalent amounts. Only in animals receiving 30mg/kg idelalisib-containing chow did SHP-1^{-/-} B cells undergo decreased proliferation and differentiation (FIGS. 3E, 3G, and 3H). These findings demonstrate that the particular risk allele-mimetic conditions at play determine the ability of partial p110δ inhibition to enforce tolerance.

Example 5

p110δ inhibition suppresses BCR-mediated calcium flux and reduces phosphorylation of its downstream signaling intermediaries

In naïve B cells, antigen receptor stimulation leads to phosphorylation of the two conserved tyrosines in the ITAMs of CD79a/b leading to recruitment of Lyn and Syk to the receptor complex. Lyn phosphorylates CD19, allowing its interaction with Lyn and PI3K and subsequent activation of p110δ. p110δ converts PI(4,5)P2 to PI(3,4,5)P3, generating docking sites for PH-domain containing downstream BCR effectors such as PLCγ, AKT, and BTK. Multiple parallel pathways emanate from this signalosome, ultimately leading to cell activation, differentiation, proliferation and migration.

To determine whether p110δ inhibition blocks proximal BCR signaling events, the inventors stimulated splenic B cells from C57BL/6 mice with polyclonal F(ab')₂ anti-Ig heavy and light chain antibodies with simultaneous addition of 0nM, 15nM, 60nM and 490nM idelalisib. It is noteworthy that these are equivalent to doses of 0mg/kg, 0.9375mg/kg, 3.75mg/kg and 30mg/kg of idelalisib, respectively, used *in vivo*. All doses of idelalisib tested suppressed calcium mobilization (FIG. 4A, quantified FIG. 4B). Similarly, phosphorylation of AKT, BTK, and PLCγ are

significantly reduced following exposure to all doses of inhibitor (FIG. 4C). These findings demonstrate that low doses of p110 δ inhibitor that enforce anergy while sparing the antibody response have an inhibitory effect on early events in BCR signaling that are predicted to be dependent on PI3K activation.

5

Example 6

Dose-dependent idelalisib inhibition of antibody responses

As shown in FIG. 1, low dose idelalisib does not inhibit antibody responses in NOD mice. To investigate this further the effect of a range of idelalisib doses on *in vivo* B cell responses to immunization was investigated. MD4 B cells loaded with dilution dye were adoptively transferred
10 into C57BL/6 recipients, and, after allowing the cells to rest for 48 hours, recipient mice were placed on various doses of idelalisib-containing chow. Twenty-four hours later, the inventors immunized with HEL conjugated to SRBC, and analyzed the B cell response 5 days later (FIG. 5A). MD4 B cells from mice receiving vehicle control chow and 0.9375mg/kg idelalisib-containing chow mounted equivalent IgM anti-HEL antibody responses and generated equivalent
15 HEL-specific ASCs/spleen (FIGS. 5D, 5E, and 5F). MD4 B cells in mice receiving 3.75mg/kg and 30mg/kg idelalisib-containing chow prior to immunization mounted significantly reduced IgM anti-HEL antibody responses and generated a reduced number of ASCs/spleen in comparison to MD4 B cells from vehicle control cohorts (FIGS. 5D, 5E, and 5F). This dose-dependent reduction in HEL-specific antibody is further reflected in the recovery of MD4 B cells in the spleens of mice
20 from the various cohorts. Mice receiving 3.75mg/kg and 30mg/kg idelalisib containing chow had significant decreases in recoverable transferred cells, while recovered cells proliferated less than in controls (FIGS. 5B, 5G, quantified FIG. 5C). These data clearly define doses that enforce anergy of autoreactive B cells while sparing B cell responses to exogenous antigen.

Example 7

Low dose p110 δ inhibition does not inhibit T cell responses in vitro or in vivo

T cells are essential components of antibody responses to most proteinaceous antigens, including autoantigens. T cells utilize the p110 δ isoform, but the role of low dose inhibition of this isoform on T cell function has not been studied. Studies of p110 δ knockout mice or functionally inactive p110 δ mice have yielded conflicting results with respect to the requirement of this isoform
30 for T cell responses. Autoantibody responses caused here by compromise of Ars/A1 anti-chromatin B cells is T cell dependent. Upon transfer into TCR $\alpha^{-/-}$ recipients these B cells fail to proliferate, differentiate and secrete autoantibody. This raises the possibility that idelalisib is mediating its effect by inhibiting T cell function. It is noteworthy, however, that if this were the case, the inhibitor should have been equally effective in inhibiting autoimmunity caused by B cell

targeted PTEN^{-/+} x SHIP-1^{-/+} and SHP-1^{-/-} conditions. Nonetheless, the inventors set out to determine the consequence of low dose p110δ inhibition on T cell responses *in vitro* and *in vivo*.

Because a therapeutic effect of the inhibitor on VH125.NOD disease progression and survival was observed, reduction in autoantibody responses in PTEN^{-/+} x SHIP-1^{-/+} cells, yet
5 equivalent autoantibody responses by SHP-1^{-/-} and MD4 B cells in mice receiving 0.9375mg/kg idelalisib-containing chow, we chose to focus our analysis on effects of this dose on T cell function. 15nM idelalisib (the *in vitro* equivalent to 0.9375mg/kg), failed to inhibit calcium mobilization of CD4 T cells stimulated by TCR aggregation with biotin-anti-CD3 and avidin (FIG. 6A, quantified in FIG. 6B). To analyze *in vivo* CD4 T cell responses, OT-II CD4 T cells were
10 adoptively transferred into congenically mismatched recipients and immunized with OVA+P:IC as represented diagrammatically in FIG. 6C. Five days post-immunization with OVA+P:IC, mice that received vehicle control chow and mice that received 0.9375mg/kg idelalisib containing chow proliferated (FIG. 6E left panel, quantified right panel), and upregulated the activation marker CD44 to equivalent levels (FIG. 6F left panel, quantified right panel). The transferred OT-II cells
15 that proliferated most underwent the greatest upregulation of CD44 (FIG. 6D). Doses of idelalisib that enforce anergy of autoreactive B cells, delay disease progression and prolong survival in VH125.NOD mice do not affect CD4 T cell responses *in vitro* or *in vivo*.

The various features and processes described above may be used independently of one another or may be combined in various ways. All possible combinations and subcombinations are
20 intended to fall within the scope of this disclosure. In addition, certain method or process blocks may be omitted in some implementations. The methods and processes described herein are also not limited to any particular sequence, and the blocks or states relating thereto can be performed in other sequences that are appropriate. For example, described blocks or states may be performed in an order other than that specifically disclosed, or multiple blocks or states may be combined in a
25 single block or state. The example blocks or states may be performed in serial, in parallel, or in some other manner. Blocks or states may be added to or removed from the disclosed example embodiments. The example systems and components described herein may be configured differently than described. For example, elements may be added to, removed from, or rearranged compared to the disclosed example embodiments.

30 Conditional language used herein, such as, among others, “can,” “could,” “might,” “may,” “e.g.,” and the like, unless specifically stated otherwise, or otherwise understood within the context as used, is generally intended to convey that certain embodiments include, while other embodiments do not include, certain features, elements, and/or steps. Thus, such conditional language is not generally intended to imply that features, elements and/or steps are in any way
35 required for one or more embodiments or that one or more embodiments necessarily include logic for deciding, with or without author input or prompting, whether these features, elements and/or

steps are included or are to be performed in any particular embodiment. The terms “comprising,” “including,” “having,” and the like are synonymous and are used inclusively, in an open-ended fashion, and do not exclude additional elements, features, acts, operations, and so forth. Also, the term “or” is used in its inclusive sense (and not in its exclusive sense) so that when used, for
5 example, to connect a list of elements, the term “or” means one, some, or all of the elements in the list.

While certain example embodiments have been described, these embodiments have been presented by way of example only and are not intended to limit the scope of the inventions disclosed herein. Thus, nothing in the foregoing description is intended to imply that any particular
10 feature, characteristic, step, module, or block is necessary or indispensable. Indeed, the novel methods and systems described herein may be embodied in a variety of other forms; furthermore, various omissions, substitutions and changes in the form of the methods and systems described herein may be made without departing from the spirit of the inventions disclosed herein. The accompanying claims and their equivalents are intended to cover such forms or modifications as
15 would fall within the scope and spirit of certain of the inventions disclosed herein.

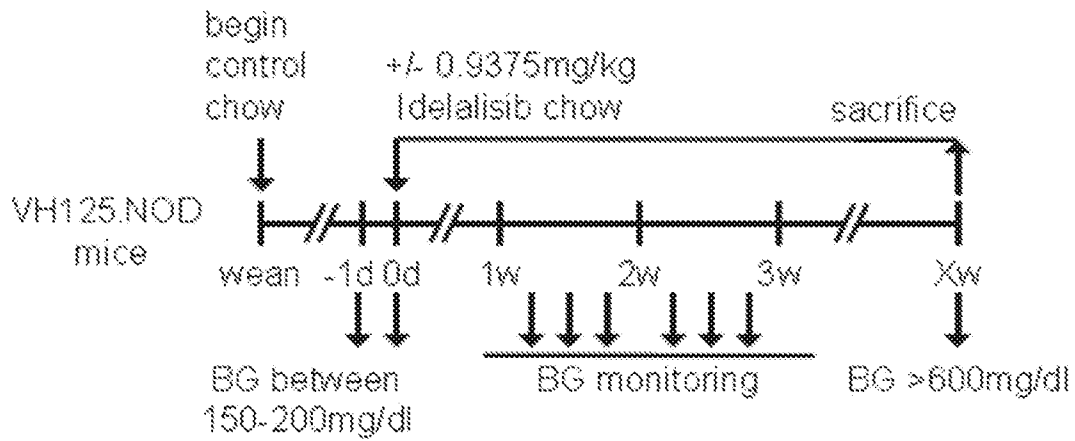
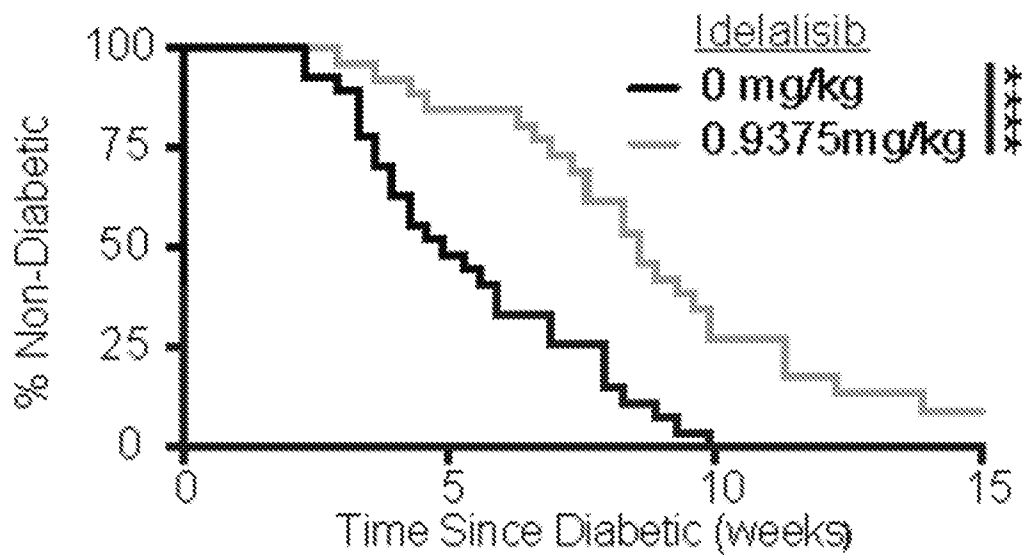
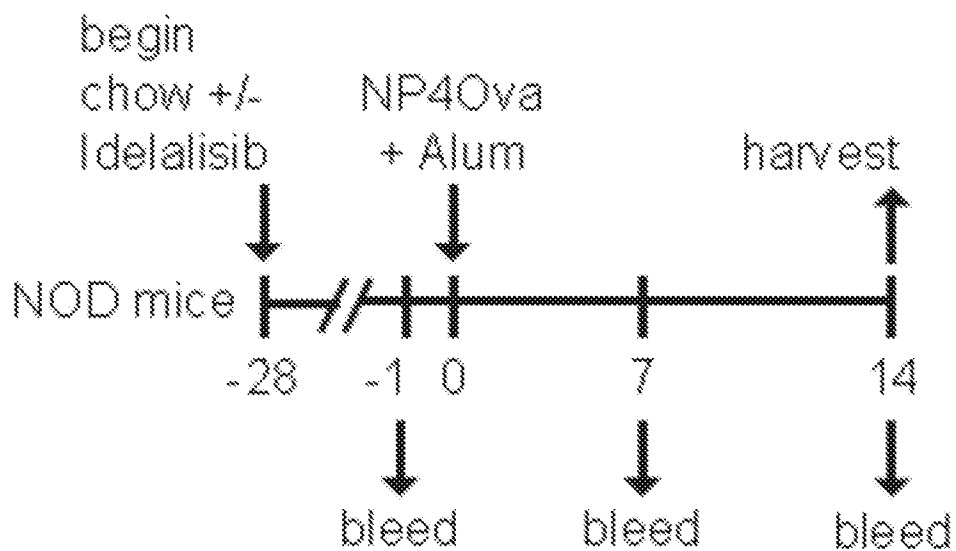
What is claimed is:

1. A method of treating or delaying the onset or slowing the progression of an autoimmune disease or disorder in a subject comprising administering a therapeutically effective amount of a phosphoinositide 3-kinase p110 δ (PI3K-p110 δ) inhibitor to the subject at a dosage that does not reduce immune response to exogenous immunogens in the subject.
2. The method of claim 1, wherein the PI3K-p110 δ inhibitor is idelalisib.
3. The method of claim 2, wherein the idelalisib is administered at a dosage of between about 0.23 mg/kg/day and about 3.6 mg/kg/day.
4. The method of claim 2, wherein the idelalisib is administered at a dosage of between about 0.45 mg/kg/day and about 1.8 mg/kg/day.
5. The method of claim 2, wherein the idelalisib is administered at a dosage of between about 0.9 mg/kg/day and about 1.8 mg/kg/day.
6. The method of claim 2, wherein the idelalisib is administered at a dosage of between about 1.8 mg/kg/day and about 3.6 mg/kg/day.
7. The method of claim 2, wherein the idelalisib is administered at a dosage of about 0.9 mg/kg/day.
8. The method of claim 2, wherein the idelalisib is administered at a dosage of about 1.8 mg/kg/day.
9. The method of any one of claims 1-8, wherein the autoimmune disease or disorder is selected from autoimmune diabetes mellitus (type 1 diabetes mellitus; T1D), systemic lupus erythematosus, autoimmune thyroiditis, rheumatoid arthritis, psoriasis, and multiple sclerosis.
10. The method of any one of claims 1-8, wherein the autoimmune disease is type 1 diabetes mellitus (T1D).
11. A method of treating or delaying the onset or slowing the progression of an autoimmune disease or disorder in a subject, comprising administering a therapeutically effective amount of a PI3K-p110 δ inhibitor to a subject displaying reduced activity of phosphatase and tensin homolog (PTEN) and/or the SH2-containing inositol lipid phosphatase (SHIP-1) in B cells.
12. The method of claim 11, wherein the administration of the PI3K-p110 δ inhibitor does not significantly reduce immune response to exogenous immunogens in the subject.
13. The method of claim 11, wherein the administration of the PI3K-p110 δ inhibitor comprises first determining at least one of:
 - a) presence of a mutation in PTEN in a tumor cell from the subject;
 - b) presence of PTEN expression in B cells from the subject;
 - c) presence of PTEN protein activity in B cells from the subject;

- d) presence of a mutation in SHIP-1 in a tumor cell from the subject;
- e) presence of SHIP-1 expression in B cells from the subject; and
- f) presence of SHIP-1 protein activity in B cells from the subject.

- 5 14. The method of claim 11, wherein the PI3K-p110 δ inhibitor is idelalisib.
15. The method of claim 14, wherein the idelalisib is administered at a dosage of between about 0.23 mg/kg/day and about 3.6 mg/kg/day.
16. The method of claim 14, wherein the idelalisib is administered at a dosage of between about 0.45 mg/kg/day and about 1.8 mg/kg/day.
- 10 17. The method of claim 14, wherein the idelalisib is administered at a dosage of between about 0.9 mg/kg/day and about 1.8 mg/kg/day.
18. The method of claim 14, wherein the idelalisib is administered at a dosage of between about 1.8 mg/kg/day and about 3.6 mg/kg/day.
19. The method of claim 14, wherein the idelalisib is administered at a dosage of about 0.9
- 15 mg/kg/day.
20. The method of claim 14, wherein the idelalisib is administered at a dosage of about 1.8 mg/kg/day.
21. The method of any one of claims 11-20, wherein the autoimmune disease or disorder is selected from autoimmune diabetes mellitus (type 1 diabetes mellitus; T1D), systemic
- 20 lupus erythematosus, autoimmune thyroiditis, rheumatoid arthritis, psoriasis, and multiple sclerosis.
22. The method of any one of claims 11-20, wherein the autoimmune disease is type 1 diabetes mellitus (T1D).
23. Use of a PI3K-p110 δ inhibitor in the manufacture of a medicament for treating or delaying
- 25 the onset or slowing the progression of an autoimmune disease or disorder in a subject.
24. Use of a PI3K-p110 δ inhibitor in the manufacture of a medicament for treating or delaying the onset or slowing the progression of an autoimmune disease or disorder in a subject with reduced activity of PTEN in B cells.
25. A PI3K-p110 δ inhibitor for use in treating or delaying the onset or slowing the progression
- 30 of an autoimmune disease or disorder in a subject.
26. A PI3K-p110 δ inhibitor for use in treating or delaying the onset or slowing the progression of an autoimmune disease or disorder in a subject with reduced activity of PTEN in B cells.
27. The use of any one of claims 23-26, wherein the PI3K-p110 δ inhibitor does not reduce
- 35 immune response to exogenous immunogens in the subject.
28. The use of any one of claims 23-26, wherein the PI3K-p110 δ inhibitor is idelalisib.

29. The use of claim 28, wherein the idelalisib dosage is between about 0.23 mg/kg/day and about 3.6 mg/kg/day.
30. The use of claim 28, wherein the idelalisib dosage is between about 0.45 mg/kg/day and about 1.8 mg/kg/day.
- 5 31. The use of claim 28, wherein the idelalisib dosage is between about 0.9 mg/kg/day and about 1.8 mg/kg/day.
32. The use of claim 28, wherein the idelalisib dosage is between about 1.8 mg/kg/day and about 3.6 mg/kg/day.
33. The use of claim 28, wherein the idelalisib dosage is about 0.9 mg/kg/day.
- 10 34. The use of claim 28, wherein the idelalisib dosage is about 1.8 mg/kg/day.
35. The method of any one of claims 23-34, wherein the autoimmune disease or disorder is selected from autoimmune diabetes mellitus (type 1 diabetes mellitus; T1D), systemic lupus erythematosus, autoimmune thyroiditis, rheumatoid arthritis, psoriasis, and multiple sclerosis.
- 15 36. The method of any one of claims 23-34, wherein the autoimmune disease is type 1 diabetes mellitus (T1D).
37. An oral dosage formulation formulated as a tablet or capsule comprising idelalisib ((S)-5-fluoro-3-phenyl-2-[1-(9H-purin-6-ylamino)-propyl]-3H-quinazolin-4-one) or a pharmaceutically acceptable salt thereof, in an amount between 5mg and 25mg, and at
- 20 least one pharmaceutically acceptable excipient.
38. The oral dosage formulation of claim 37 comprising 5mg, 6mg, 7mg, 8mg, 9mg, 10mg, 11mg, 12mg, 13mg, 14mg, 15mg, 16mg, 17mg, 18mg, 19mg, 20mg, 21mg, 22mg, 23mg, 24mg, or 25mg of idelalisib.

**FIG. 1A****FIG. 1B****FIG. 1C**

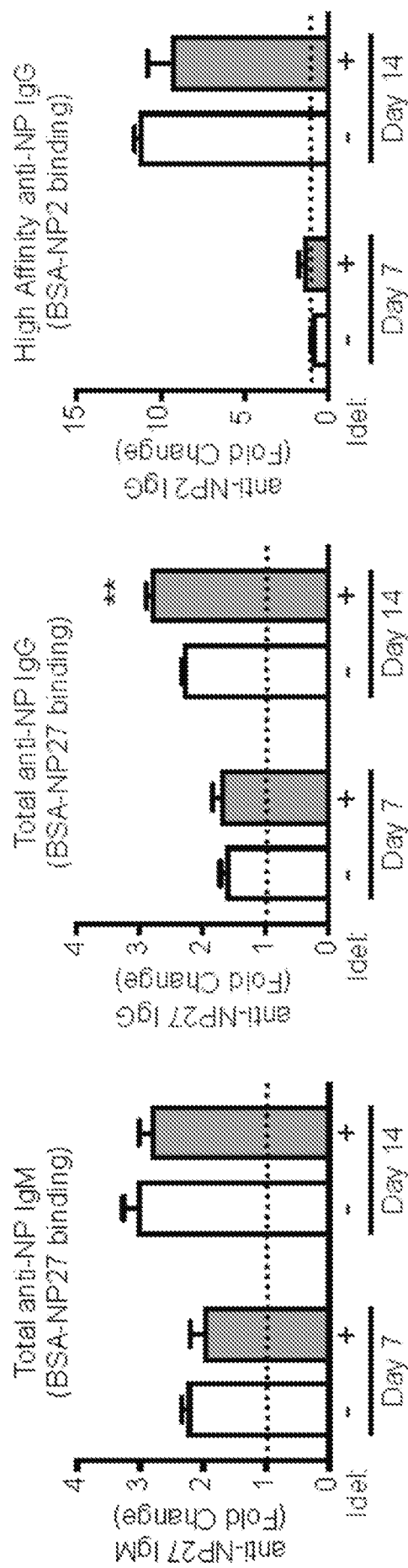
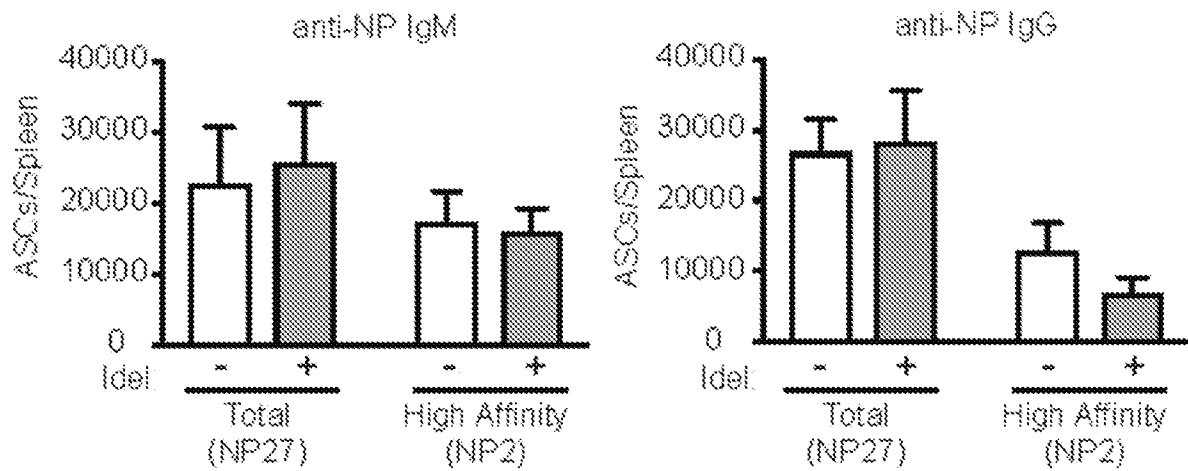
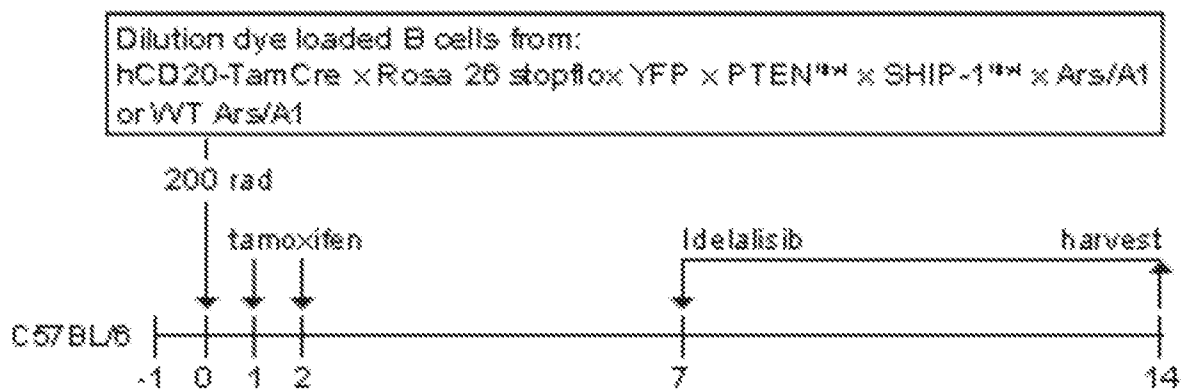
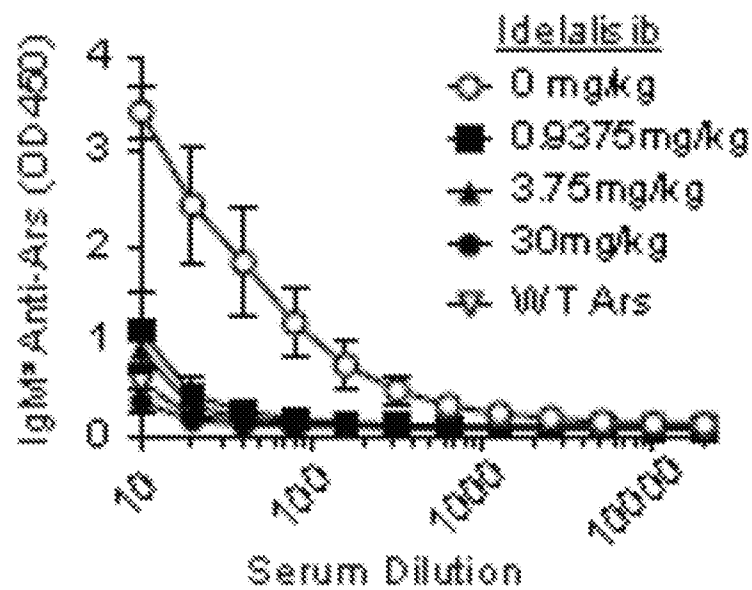


FIG. 1E

**FIG. 1D****FIG. 2A****FIG. 2B**

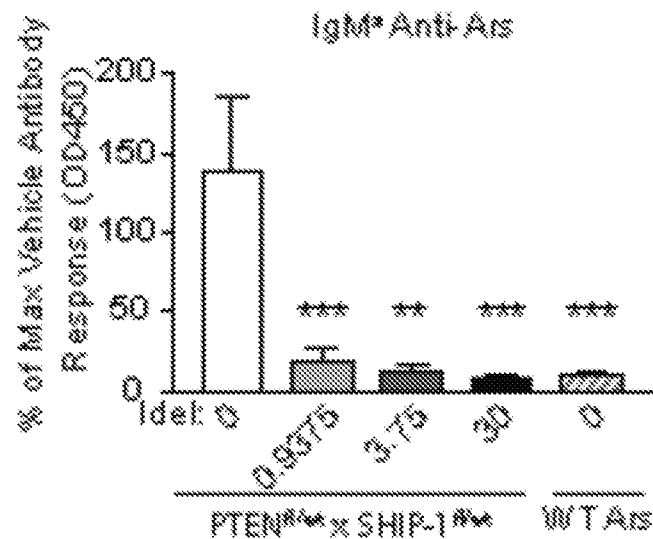


FIG. 2C

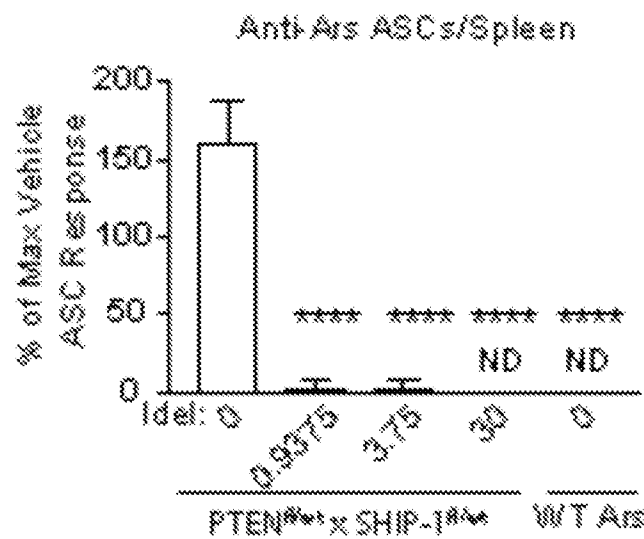


FIG. 2D

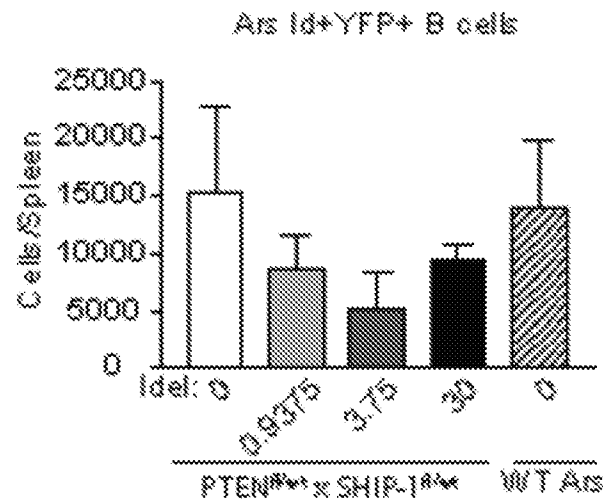


FIG. 2F

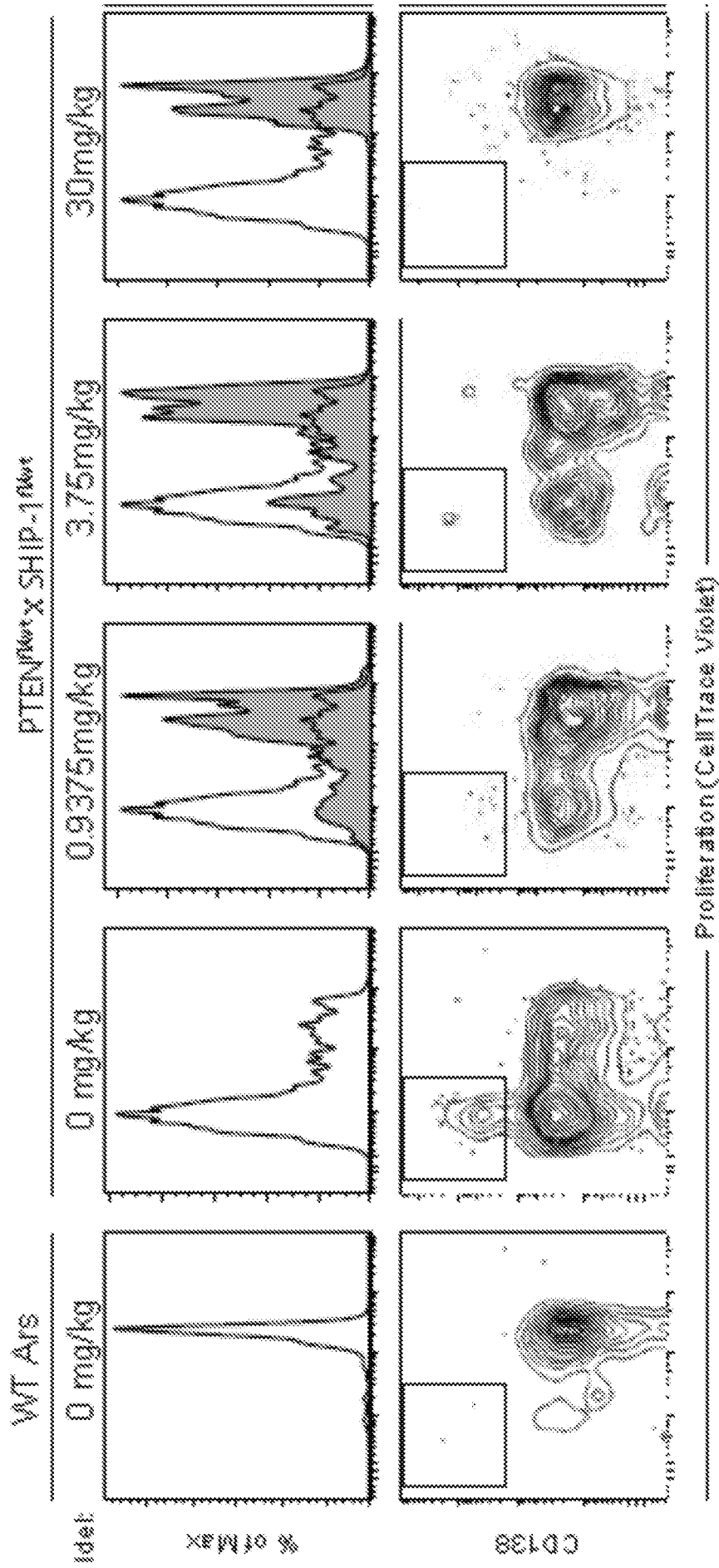
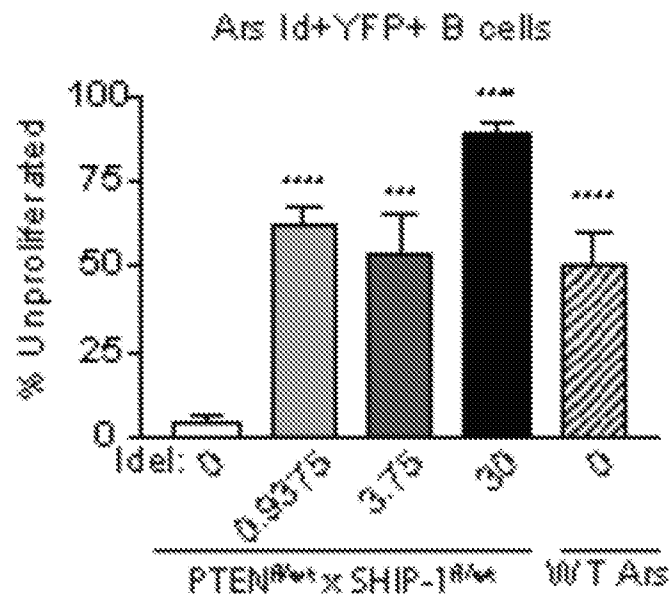
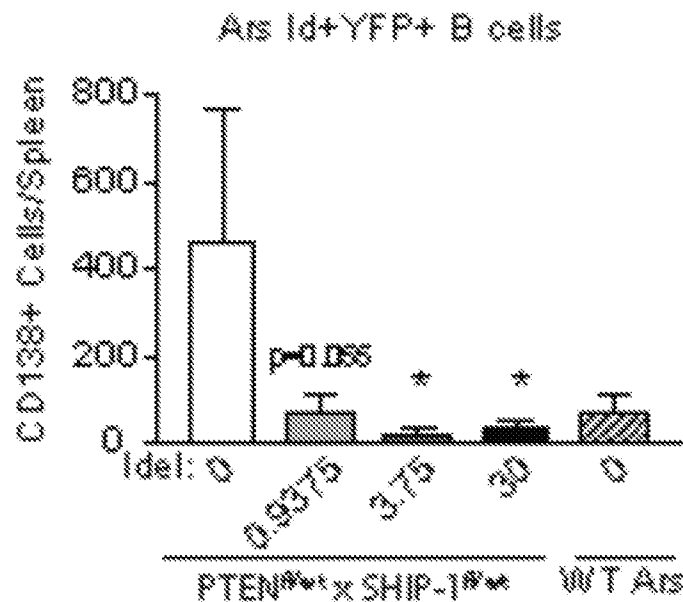
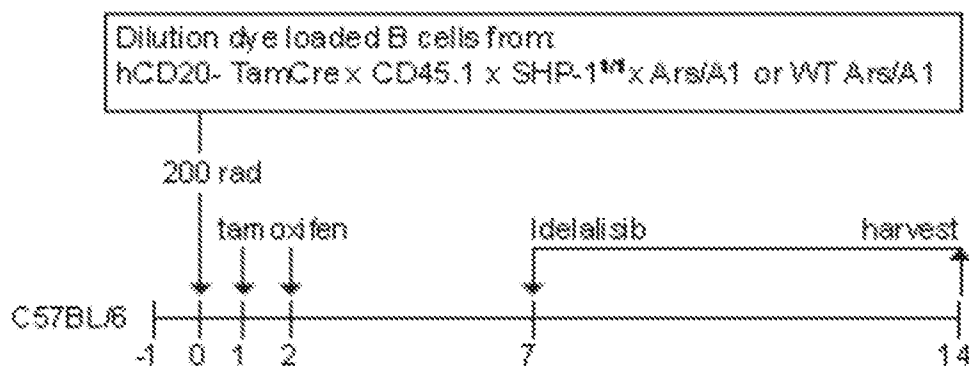
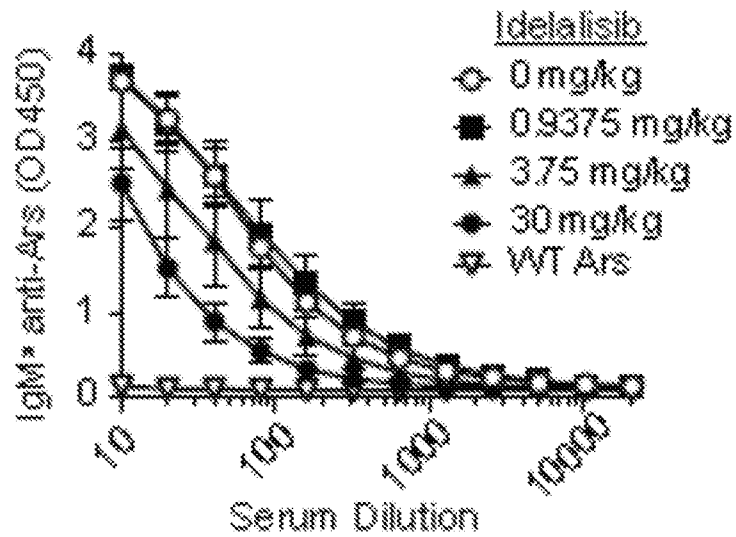
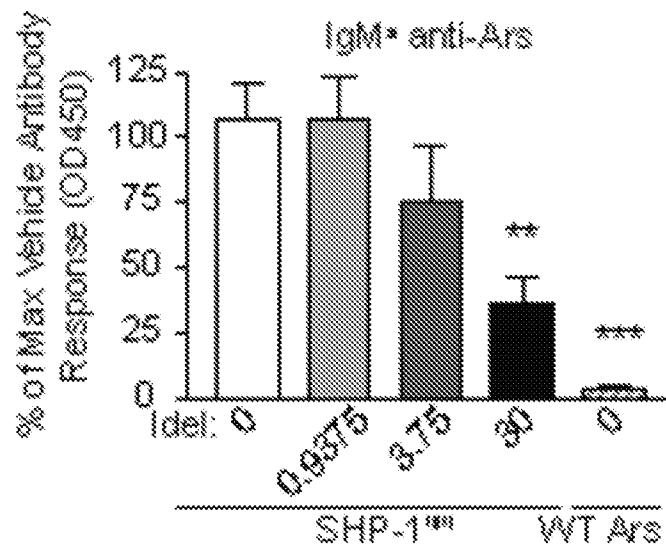
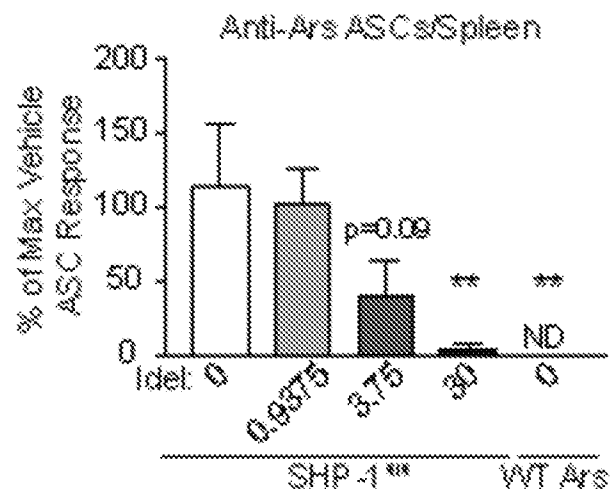


FIG. 2E

**FIG. 2G****FIG. 2H****FIG. 3A**

**FIG. 3B****FIG. 3C****FIG. 3D**

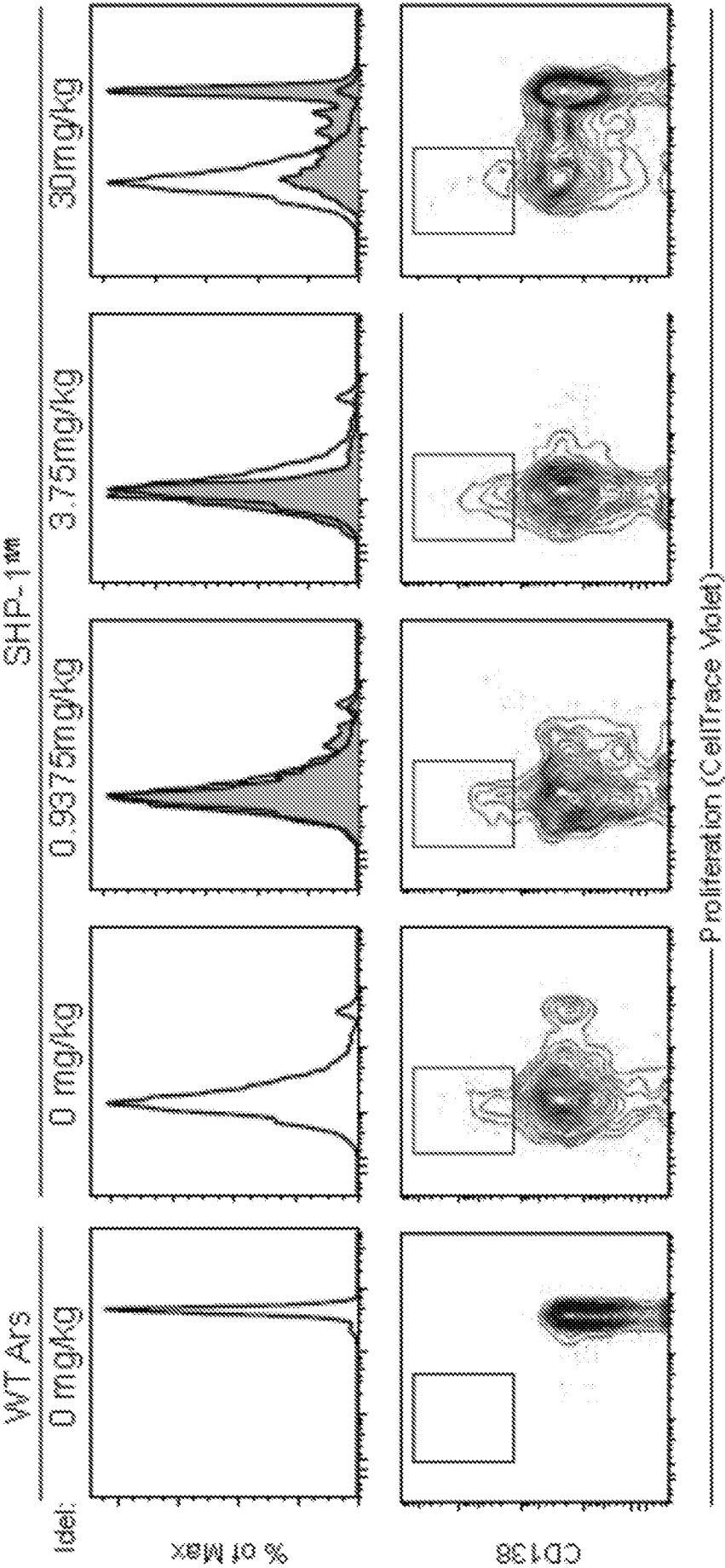


FIG. 3E

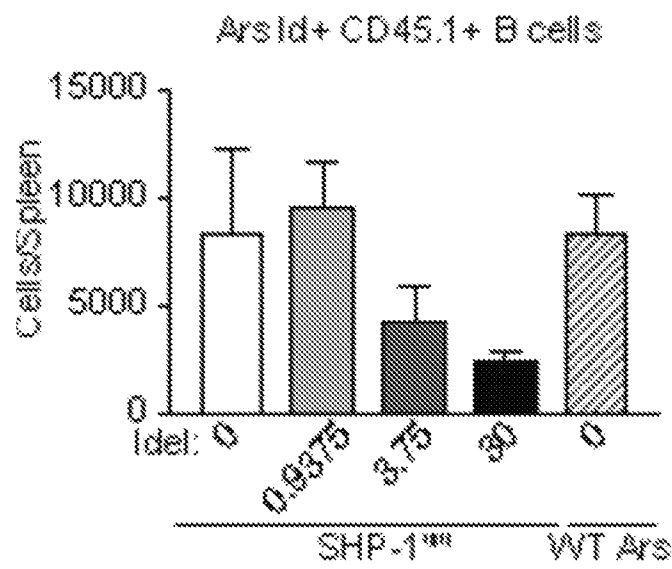


FIG. 3F

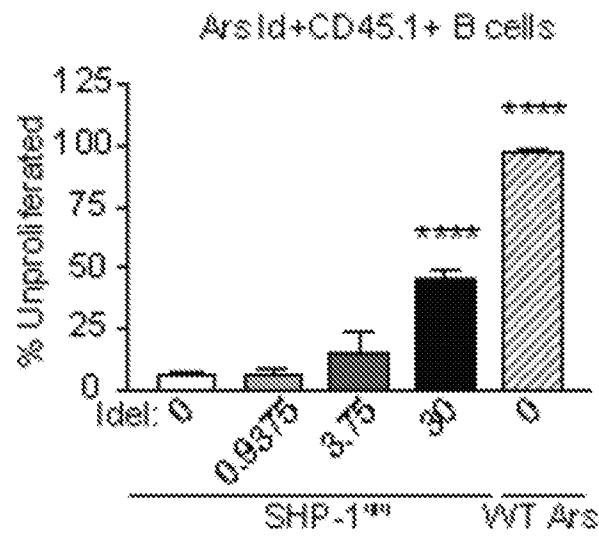


FIG. 3G

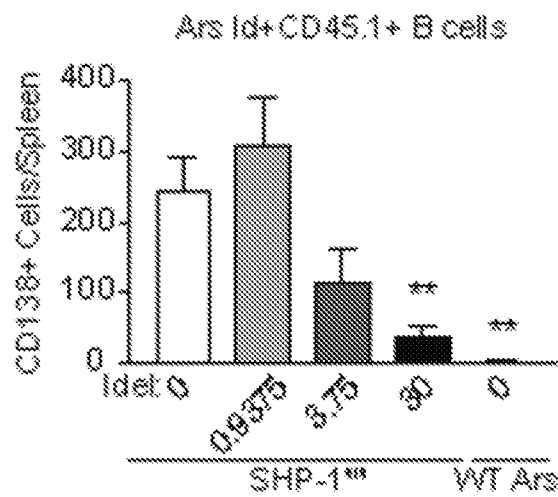
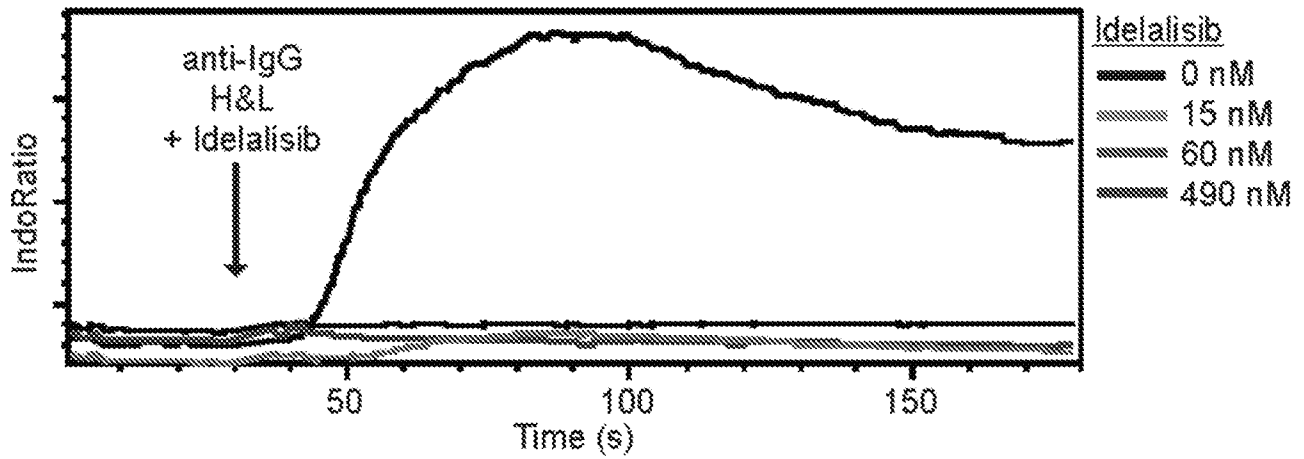
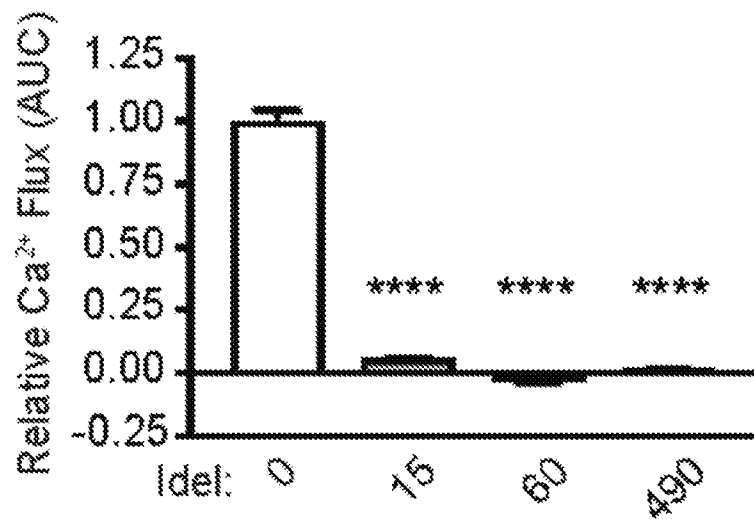
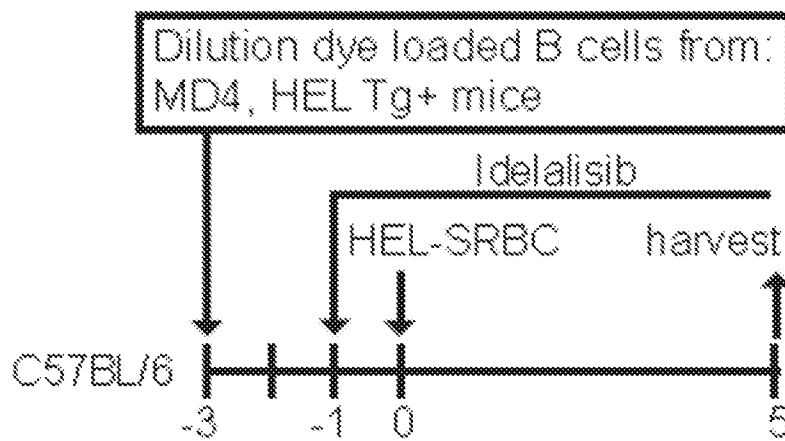


FIG. 3H

**FIG. 4A****FIG. 4B****FIG. 5A**

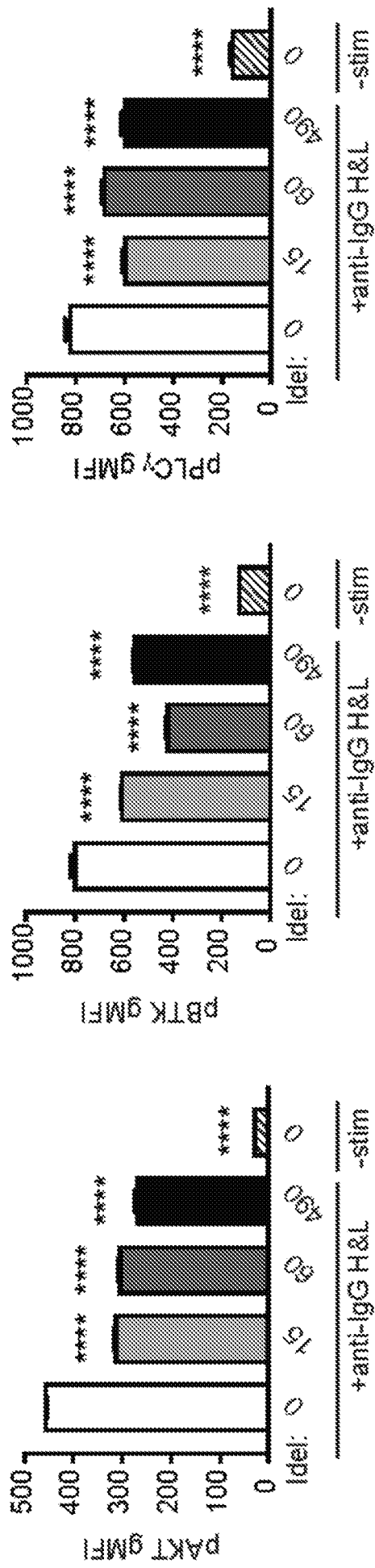


FIG. 4C

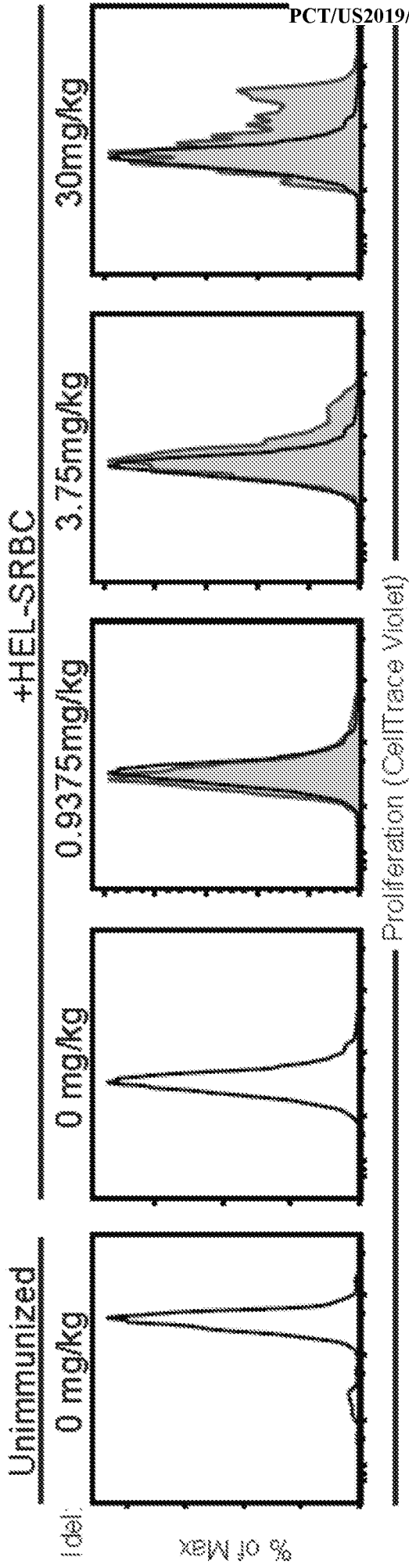
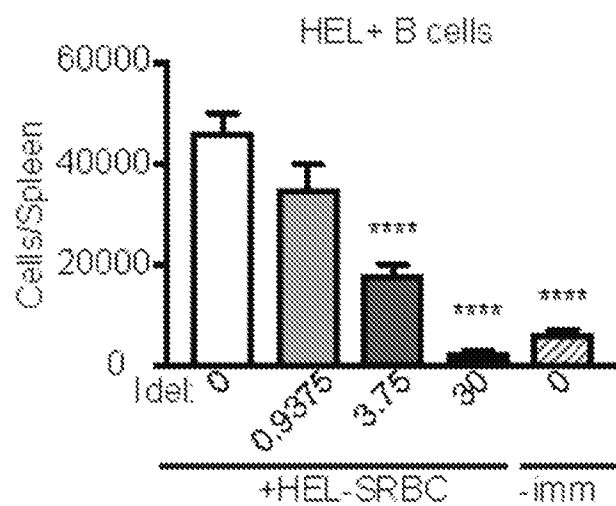
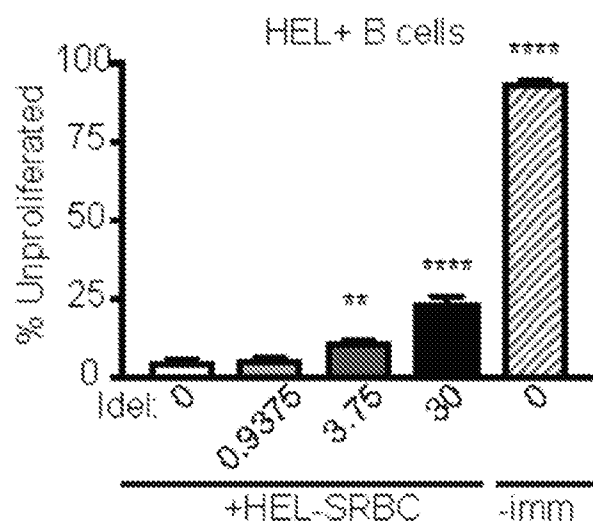
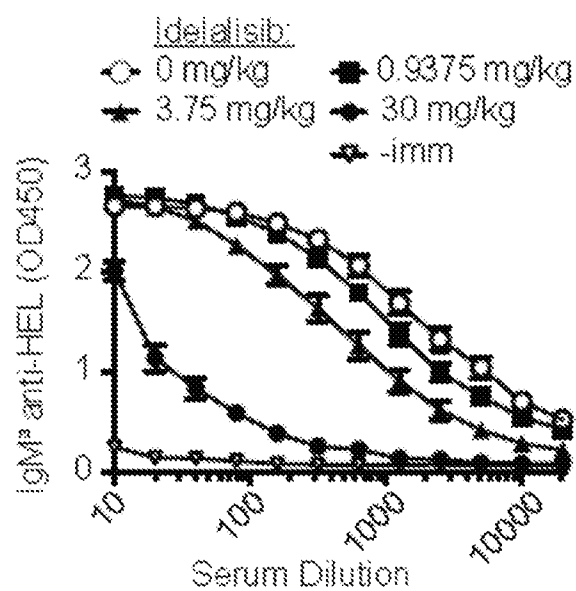
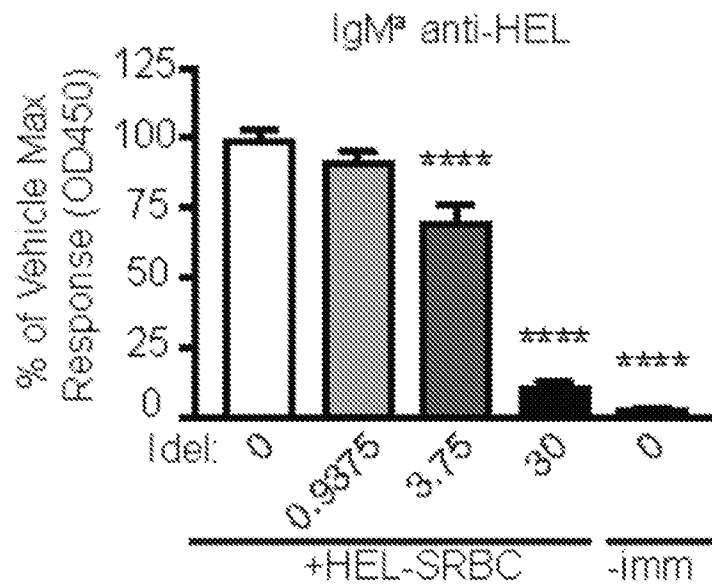
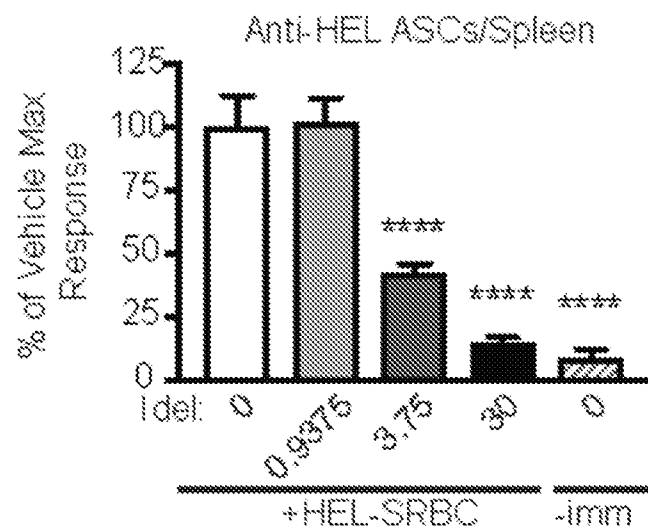
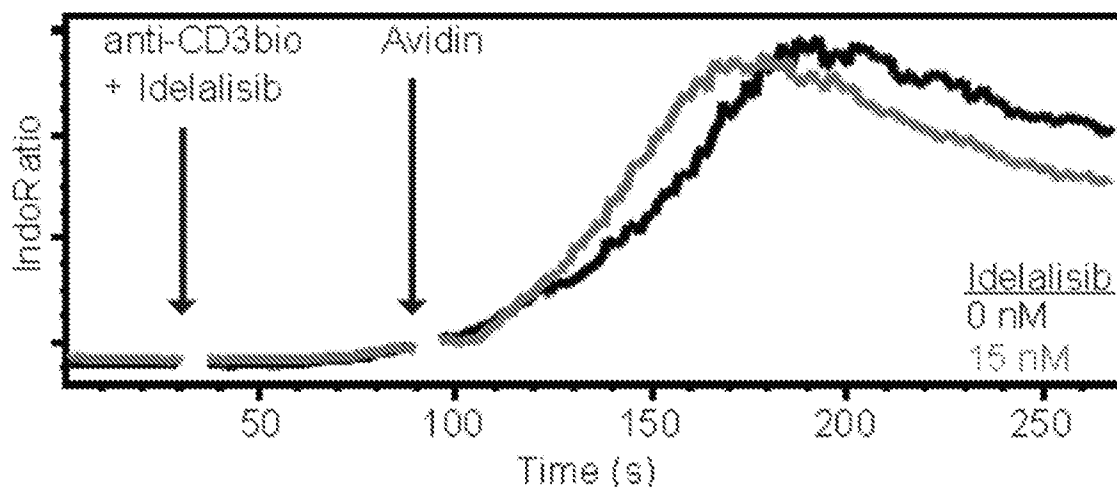
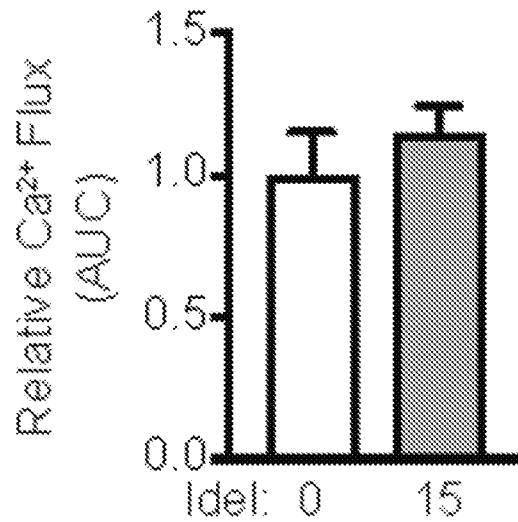
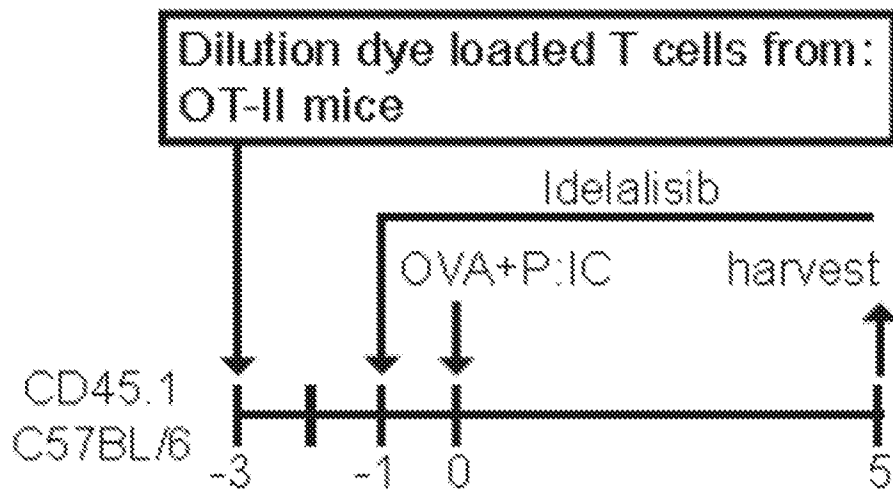
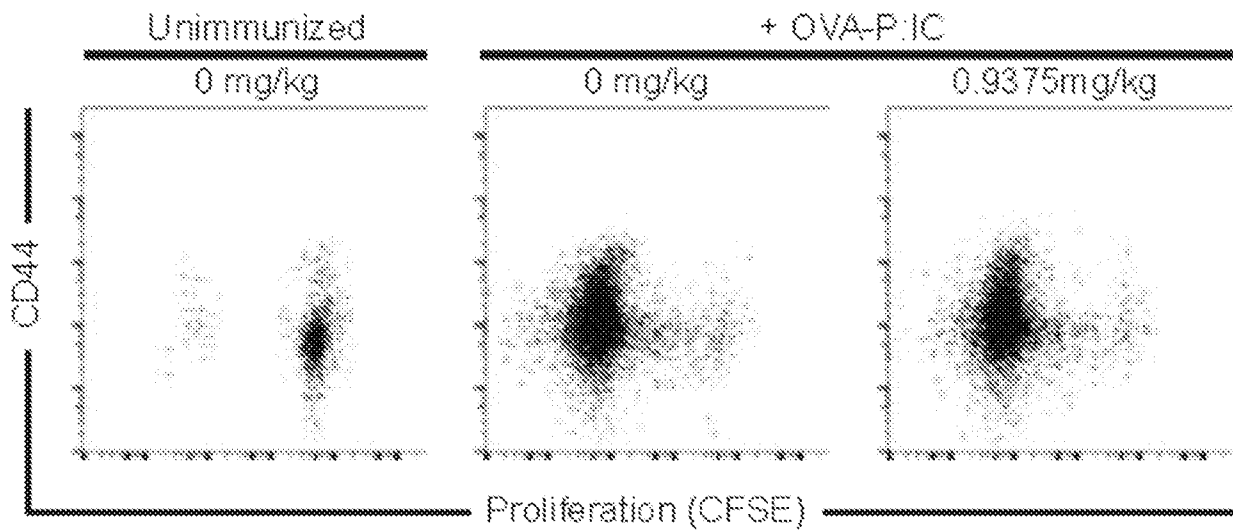
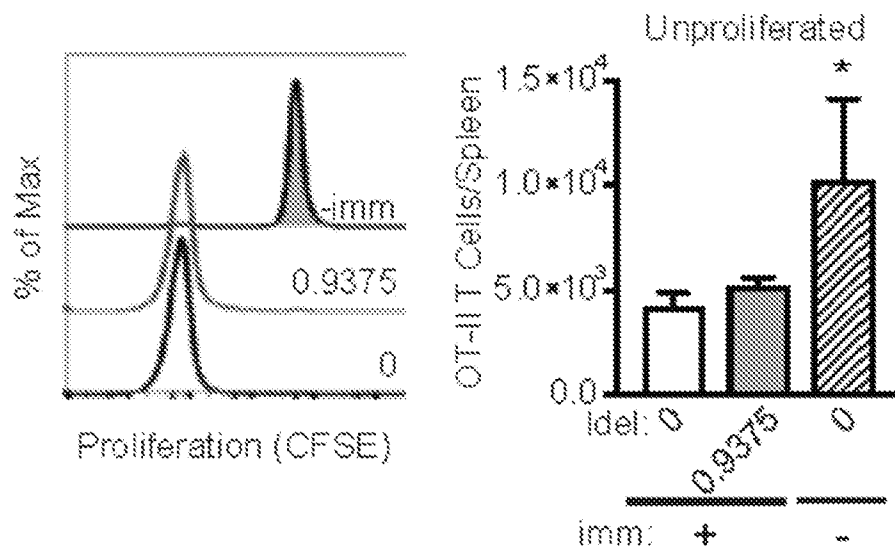
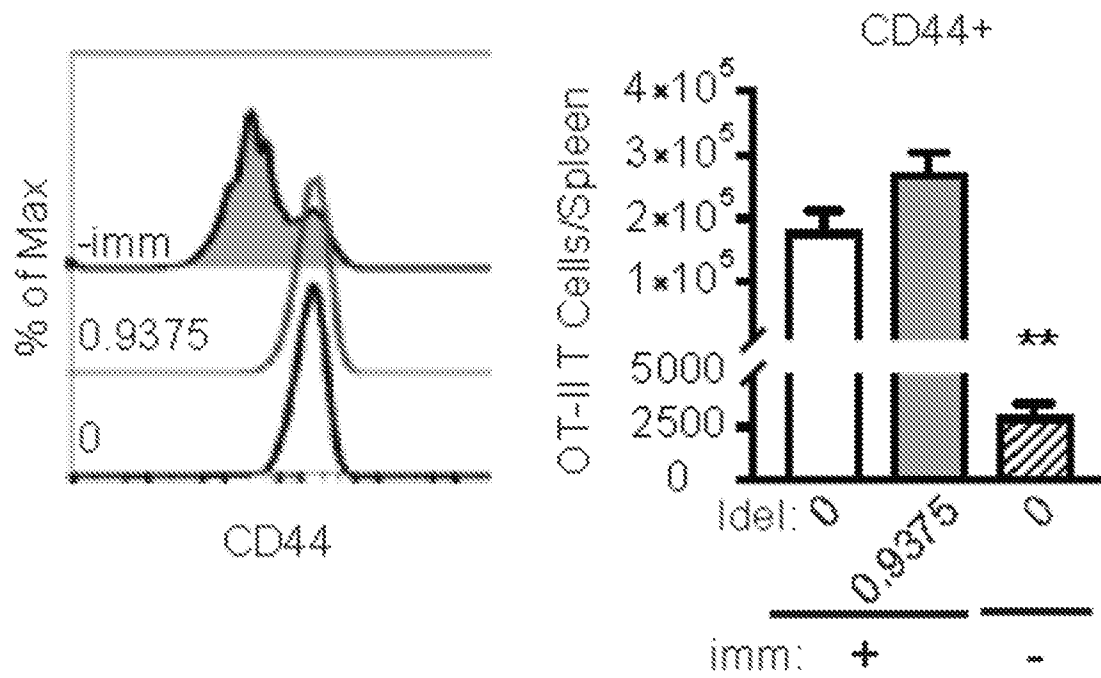


FIG. 5G

**FIG. 5B****FIG. 5C****FIG. 5D**

**FIG. 5E****FIG. 5F****FIG. 6A**

**FIG. 6B****FIG. 6C****FIG. 6D**

**FIG. 6E****FIG. 6F**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/012532

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

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

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

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

3. ☒ Claims Nos.: 35, 36
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

See extra sheet(s).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-10

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/012532

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/517; A61K 31/00; A61K 31/505 (2019.01)

CPC - A61K 31/517; A61K 31/00; A61K 31/505 (2019.02)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/0029693 A1 (DOUANGPANYA et al) 04 February 2010 (04.02.2010) entire document	1-10
A	US 2016/0075705 A1 (ICOS CORPORATION) 17 March 2016 (17.03.2016) entire document	1-10
A	WU et al. Defective PTEN regulation contributes to B cell hyperresponsiveness in systemic lupus erythematosus, Science Translational Medicine, Vol. 6, No. 246, 23 July 2014 [retrieved on 28 February 2019]. Retrieved from the Internet: <URL: https://www.ncbi.nlm.nih.gov/pubmed/25101889/ >. abstract	1-10

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

20 April 2019

Date of mailing of the international search report

07 MAY 2019

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

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Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/012532

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I: Claims 1-10 are drawn to methods of treating or delaying the onset or slowing the progression of an autoimmune disease or disorder in a subject comprising administering a therapeutically effective amount of a phosphoinositide 3-kinase p110-delta (P13K-p110δ) inhibitor to the subject at a dosage that does not reduce immune response to exogenous immunogens in the subject.

Group II: Claims 11-22 are drawn to methods of treating or delaying the onset or slowing the progression of an autoimmune disease or disorder in a subject, comprising administering a therapeutically effective amount of a P13K-p110δ inhibitor to a subject displaying reduced activity of phosphatase and tensin homolog (PTEN) and/or the SH2-containing inositol lipid phosphatase (SHIP1) in B cells.

Group III: Claims 23-34, 37, and 38 are drawn to P13K-p110δ inhibitors, and uses thereof.

The inventions listed in Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I, methods of treating or delaying the onset or slowing the progression of an autoimmune disease or disorder in a subject comprising administering a therapeutically effective amount of a phosphoinositide 3-kinase p110-delta (P13K-p110δ) inhibitor to the subject at a dosage that does not reduce immune response to exogenous immunogens in the subject, are not present in Groups II and III; the special technical features of Group II, methods of treating or delaying the onset or slowing the progression of an autoimmune disease or disorder in a subject, comprising administering a therapeutically effective amount of a P13K-p110δ inhibitor to a subject displaying reduced activity of phosphatase and tensin homolog (PTEN) and/or the SH2-containing inositol lipid phosphatase (SHIP1) in B cells, are not present in Groups I and III; and the special technical features of Group III, P13K-p110δ inhibitors, and uses thereof, are not present in Groups I and II.

Groups I-III share the technical features of a method of treating or delaying the onset or slowing the progression of an autoimmune disease or disorder in a subject, comprising administering a therapeutically effective amount of a phosphoinositide 3-kinase p110-delta (P13K-p110δ) inhibitor to a subject displaying reduced activity of phosphatase and tensin homolog (PTEN) in B cells; use of a P13K-p110δ inhibitor for treating or delaying the onset or slowing the progression of an autoimmune disease or disorder in a subject with reduced activity of PTEN in B cells; and idelalisib ((S)-5-fluoro-3-phenyl-2-[1-(9H-purin-6-ylamino)-propyl]-3H-quinazolin-4-one). However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2016/0075705 A1 to Icos Corporation teaches a method of treating or delaying the onset or slowing the progression of an autoimmune disease or disorder in a subject (Abstract, Compounds that inhibit P13Kδ activity, including compounds that selectively inhibit P13Kδ activity, are disclosed. Methods of inhibiting phosphatidylinositol 3-kinase delta isoform (P13Kδ) activity, and methods of treating diseases, such as disorders of immunity and inflammation in which P13Kδ plays a role in leukocyte function, using the compounds also are disclosed; Para. [0135], "Treating" as used herein refers to preventing a disorder from occurring in an animal that can be predisposed to the disorder, but has not yet been diagnosed as having it; inhibiting the disorder, i.e., arresting its development; relieving the disorder, i.e., causing its regression; or ameliorating the disorder, i.e., reducing the severity of symptoms associated with the disorder; Para. [0145], The present invention enables methods of treating such diseases as... autoimmune diseases...), comprising administering a therapeutically effective amount of a phosphoinositide 3-kinase p110-delta (P13K-p110δ) inhibitor to a subject (Para. [0020], One aspect of the present invention is to provide compounds capable of inhibiting the biological activity of human P13Kδ. Another aspect of the present invention is to provide compounds that inhibit P13Kδ selectively compared to the other P13K isoforms; Para. [0023], In the present method, it is preferred that the P13Kδ inhibitor is selective. It is preferred that the P13Kδ inhibitor is at least about 100-fold selective for inhibition of p110δ relative to p110α, at least about 40-fold selective relative to p110β, and at least about 10-fold selective relative to p110γ in a biochemical assay; Para. [0145]); use of a P13K-p110δ inhibitor for treating or delaying the onset or slowing the progression of an autoimmune disease or disorder in a subject (Abstract, Compounds that inhibit P13Kδ activity, including compounds that selectively inhibit P13Kδ activity, are disclosed. Methods of inhibiting phosphatidylinositol 3-kinase delta isoform (P13Kδ) activity, and methods of treating diseases, such as disorders of immunity and inflammation in which P13Kδ plays a role in leukocyte function, using the compounds also are disclosed; Para. [0135], "Treating" as used herein refers to preventing a disorder from occurring in an animal that can be predisposed to the disorder, but has not yet been diagnosed as having it; inhibiting the disorder, i.e., arresting its development; relieving the disorder, i.e., causing its regression; or ameliorating the disorder, i.e., reducing the severity of symptoms associated with the disorder; Para. [0145], The present invention enables methods of treating such diseases as... autoimmune diseases...; Para. [0020], One aspect of the present invention is to provide compounds capable of inhibiting the biological activity of human P13Kδ. Another aspect of the present invention is to provide compounds that inhibit P13Kδ selectively compared to the other P13K isoforms; Para. [0023], In the present method, it is preferred that the P13Kδ inhibitor is selective. It is preferred that the P13Kδ inhibitor is at least about 100-fold selective for inhibition of p110δ relative to p110α, at least about 40-fold selective relative to p110β, and at least about 10-fold selective relative to p110γ in a biochemical assay); and idelalisib ((S)-5-fluoro-3-phenyl-2-[1-(9H-purin-6-ylamino)-propyl]-3H-quinazolin-4-one) (Para. [0080], The methods can be practiced using, for example, the following compounds of the invention, but the invention is not limited to these compounds. Exemplary compounds include... 5-fluoro-3-phenyl-2-[1-(9H-purin-6-ylamino)-propyl]-3H-quinazolin-4-one...; see Formula above Para. [0353]; (S)-5-fluoro-3-phenyl-2-[1-(9H-purin-6-ylamino)-propyl]-3H-quinazolin-4-one (107)).

Further, "Defective PTEN regulation contributes to B cell hyperresponsiveness in systemic lupus erythematosus" to Wu et al. teach a subject displaying reduced activity of phosphatase and tensin homolog (PTEN) in B cells (Abstract, PTEN regulates normal signaling through the B cell receptor (BCR). In systemic lupus erythematosus (SLE), enhanced BCR signaling contributes to increased B cell activity, but the role of PTEN in human SLE has remained unclear. We performed fluorescence-activated cell sorting analysis in B cells from SLE patients and found that all SLE B cell subsets, except for memory B cells, showed decreased expression of PTEN compared with B cells from healthy controls).

The inventions listed in Groups I-III therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.