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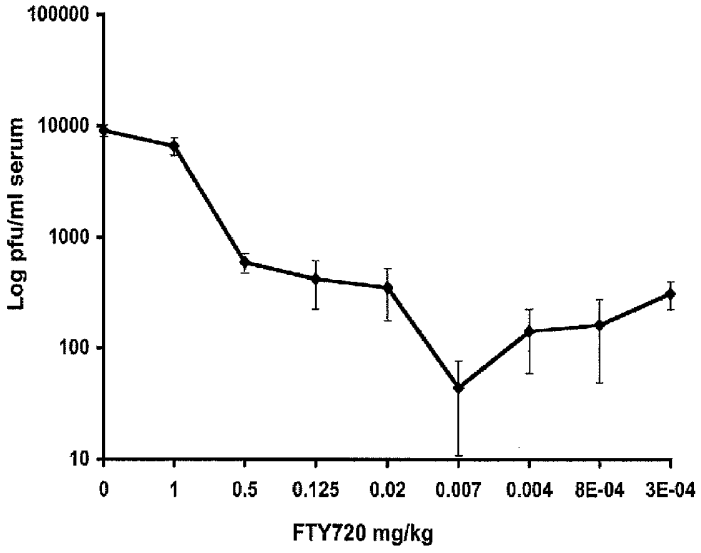
(54) Title: METHODS AND COMPOSITIONS FOR TREATING OR PREVENTING INFECTION USING LEUKOCYTE SEQUESTRATION AGENTS

Figure 1



(57) Abstract: Compositions and methods are provided for using leukocyte sequestration agents to treat infection. In particular, the methods and compositions comprise leukocyte sequestration agents that are lymphocyte sequestration agents such as sphingosine 1 -phosphate (SIP) receptor agonists (particularly FTY720), sphingosine kinase inhibitors, and SIP lyase inhibitors. Infections to be treated or prevented according to the present invention include bacterial, viral, fungal, or parasitic infection, particularly bacterial or viral infection.

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METHODS AND COMPOSITIONS FOR TREATING OR PREVENTING INFECTION USING LEUKOCYTE SEQUESTRATION AGENTS

FIELD OF THE INVENTION

The invention relates to compositions and methods for using leukocyte sequestration agents to treat or prevent infection. In particular, the present invention relates to the use of agents such as FTY720 to treat or prevent infection, in particular bacterial or viral infection.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

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BACKGROUND OF THE INVENTION

The innate capacity of microbial pathogens to respond to selective pressures has driven their evolution and enabled them to adapt to complex and variable environments. This same capacity, however, has led to the development of resistance to traditional anti-microbial agents. The resistance problem is compounded further by indiscriminate and inappropriate use of antibiotics and antiviral agents without compliance measures or public health policies to reduce disease burden.

Traditional drug treatments for infections typically target a distinguishing feature or characteristic of a specific pathogen. For example, acyclovir targets the replication stage of herpesvirus infection, zidovudine/AZT targets the reverse transcriptase of human immunodeficiency virus (HIV), and various protease inhibitors target HIV protease. Generally, however, these therapies have many disadvantages, including limited usefulness for only a specific pathogen, toxic side effects, and ineffectiveness due to pathogen variation and the development of drug resistance.

Therapeutic approaches directed at targeting host-immune cells rather than the pathogen would be expected to decrease the likelihood of generating resistant mutant viruses or other pathogens. As a consequence, such drugs would be predicted to be useful in both post-exposure prophylaxis and treatment of patients exposed to pathogens that establish chronic infections in humans, such as (but not limited to) HIV, HCV, or mycobacterium tuberculosis.

There is therefore an urgent need to develop compounds and methods effective for the prevention and treatment of infection, in particular bacterial or viral infection.

SUMMARY OF THE INVENTION

Methods and compositions for preventing or treating infection are provided. The methods comprise the use of leukocyte sequestration agents as immune enhancing agents. In one embodiment, the invention relates to the prevention or treatment of infection (*i.e.*, bacterial, viral, fungal, or parasitic infection), by administering at least one cycle of therapy to a subject in need thereof, where the cycle of therapy comprises administering at least one dose of a therapeutically effective amount of a leukocyte sequestration agent to the subject. In some embodiments, the treatment is transient and comprises one cycle of therapy while in other embodiments the treatment is chronic and comprises more than one cycle of therapy. In other embodiments, the treatment is initiated immediately following a subject's exposure to a pathogen, while in other embodiments treatment is initiated after a time delay following exposure. In some embodiments, the methods comprise use of leukocyte sequestration agents that include but are not limited to lymphocyte sequestration agents such as sphingosine 1-phosphate (S1P) receptor agonists (particularly FTY720), sphingosine kinase inhibitors, and S1P lyase inhibitors. In one embodiment, the infection is a bacterial or viral infection.

Methods are also provided for preventing or treating cancers with viral etiologies comprising administration of at least one dose of a therapeutically effective amount of a leukocyte sequestration agent to a subject in need thereof.

Immunogenic compositions and pharmaceutical kits are also provided. In one embodiment, an immunogenic composition is provided comprising an immunogen and an adjuvant composition that comprises a leukocyte sequestration agent as described above. In one embodiment, the immunogen is one or more antigenic polypeptides, immunogenic polypeptides or polysaccharides. In some embodiments, the immunogen comprises a bacterial or viral antigen. In other embodiments, pharmaceutical kits comprising the immunogenic compositions described above are provided, as are methods of using the immunogenic compositions for immunization or for treating or preventing infection, in particular a bacterial or viral infection, in a subject in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a dose response curve for various concentrations of FTY720 on LCMV clone 13 serum titers at 8 days post-infection.

Figure 2 shows that mice treated with FTY720 clear LCMV clone 13 from the serum by day 30. The data points represented by the squares, circles, and triangles are serum titers (pfu/g of tissue) of LCMV clone 13. Individual plot points represented by the squares at top and bottom right at day 60 are virus titers in kidneys of untreated and FTY720 treated mice, respectively.

Figure 3 shows that LCMV-specific CD8+ T cell responses at day 60 post infection were maintained in clone 13 infected mice treated with FTY720 at days 0, 1, and 2 of the infection. Splenocytes were stimulated in the presence of Golgistop (BD, Franklin Lakes, IL) by the indicated peptide in 96 well plates for 6 hours then washed and stained for surface CD8 followed by BD cytofix/perm (BD, Franklin Lakes, IL) and stained for intracellular cytokines IL-2, TNFα and IFNγ. All plots are gated on CD8+ T cells.

Figure 4 shows loss of PD-1 expression on LCMV tetramer positive CD8 T cells following FTY720 treatment. Results are shown for day 15 post infection. Shaded curve = LCMV Armstrong; solid line = LCMV cl-13; and dotted line = LCMV cl-13 FTY treated.

Figure 5 shows that GP33 and NP396 CD8 T cell responses at day 60 were preserved in clone 13 infected mice treated with FTY720 at days 0, 1, and 2 following infection. Splenocytes were collected and stimulated for 6 hours. The cells were surface stained then fixed with BD cytofix/perm (BD, Franklin Lakes, IL) and stained for intracellular cytokines. Results were collected on a BD LSRII flow cytometer (BD, Franklin Lakes, IL).

Figure 6 shows data demonstrating that treatment of mice chronically infected with clone 13 leads to viral clearance. Viral titers at 60d post infection (30d post FTY720 treatment) were measured in the kidneys. Similar results were seen in the brain.

Figure 7 shows data demonstrating that treatment of mice chronically infected with LCMV clone 13 leads to restoration of functional memory cells. CD62L and PD-1 (A and B respectively) expression was measured on LCMV-specific tetramer positive gp33 CD8+ T cells. (C) Splenocytes were stimulated *in vitro* and TNFα and IFNγ were measured.

Figure 8 shows a diagram of the experimental design of the GK1.5, $CD4^+$ depletion study.

Figure 9 shows data demonstrating that FTY720-mediated preservation of T cell responses in LCMV clone 13-infected mice is dependent upon CD4+ T cells. Mice were infected with LCMV cl-13 and administered 500 μg/day GK1.5 on days 0 and 1 post infection. Mice were then treated with FTY720 at different times post infection and GK1.5 treatment. (A) Shows the decline of CD4 T cells in the blood following GK1.5 treatment and the recovery of CD4 cells over time post treatment. (B) Intracellular cytokine staining for TNFα and IFNγ was performed on GP33 or NP396-stimulated cells. All panels are gated on CD8+ cells of CD8 T cells from cl-13 infected mice treated with FTY720 following GK1.5 treatment.

Figure 10 shows serum viral titers at up to 60 days post-infection with Cl-13 in mice with either no treatment or treated with GK1.5 or GK1.5 + FTY720. Results demonstrated that mice treated with GK1.5 and administered FTY720 at the same time were unable to control the infection.

Figure 11 shows data demonstrating that low dose FTY720 delays disease development following intranasal infection with vaccinia virus (Western Reserve). Groups of 10 C57BL/6J mice were intranasally infected with $5x10^5$ pfu (10 LD_{50}) of vaccinia virus (Western Reserve) and either treated i.v. once-a-day for three days with vehicle, 4 μg/kg FTY720, or 200 μg/kg FTY720. (A) Weight loss at d5 post infection. (B) Time-course of weight loss as a function of FTY720 treament in vvWR-infected mice. (C) The illness score was based upon the following criteria: loss of motility, ruffled fur, arched back, and weight loss ≥ 25% of original body weight, with each criteria adding 1 to the total score.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods of using leukocyte sequestration agents as immune enhancing agents to prevent or treat infection. As discussed in more detail below, the present invention relates to the use of leukocyte sequestration agents to prevent or treat bacterial, viral, fungal, or parasitic infection. In particular, the invention relates to the prevention or treatment of infection by administering at least one cycle of therapy to a subject in need thereof, wherein the cycle of therapy comprises administering at least one dose of a therapeutically effective amount of a leukocyte sequestration agent to the subject. The cycle of treatment may be initiated at any time following a subject's exposure to a pathogen (*i.e.*, immediately or after a time delay) and treatment may be transient (one cycle of therapy) or chronic (more than one cycle of therapy) in nature. The invention also relates to immunogenic compositions and pharmaceutical kits comprising leukocyte sequestration agents as adjuvants as well as methods of use thereof. Leukocyte sequestration agents for use within the methods and compositions of the present invention include but are not limited to lymphocyte sequestration agents such as sphingosine 1-phosphate (S1P) receptor agonists (particularly FTY720), sphingosine kinase inhibitors, and S1P lyase inhibitors.

The terms "treat" or "treatment" as used herein refers to the application or administration of a leukocyte sequestration agent, or pharmaceutical composition comprising a leukocyte sequestration agent, to a subject having an infection or symptom of infection (*e.g.*, a bacterial, viral, fungal, or parasitic infection), and where

the purpose is to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the infection or any associated symptoms of the infection. The terms "prevent" or "preventing" as used herein refers to the application or administration of a leukocyte sequestration agent, or pharmaceutical composition comprising a leukocyte sequestration agent, to a subject that has been exposed to a pathogen or otherwise has a predisposition toward development of infection, where the purpose is to inhibit or stop the development of infection.

The term "leukocyte sequestration agent" as used herein refers to an agent that induces or enhances removal of leukocytes from the general blood circulation of a subject for redistribution within the subject's body. The term "leukocyte" (or "white blood cell") refers to cell types that include dendritic cells, lymphocytes, granulocytes (including neutrophils, eosinophils, and basophils), macrophages, and monocytes. In one embodiment, agents for use within the methods and compositions of the invention are lymphocyte sequestration agents. The term "lymphocyte sequestration agent" as used herein refers to an agent that induces or enhances removal of lymphocytes from the general blood circulation of a subject for redistribution in any of the subject's primary or secondary lympohoid organs. This lymphocyte sequestration activity can be reversible, so that suspending treatment restores normal lymphocyte populations. The lymphocyte sequestration agent may also act to selectively decrease populations of certain lymphocytes in blood or lymph tissue, such as specifically decreasing populations of circulating lymphocytes or spleen lymphocytes.

The ability of lymphocyte sequestration agents to sequester lymphocytes within lymph nodes is generally viewed in the art as providing an immunosuppressive effect (see, *e.g.*, U.S. Patent No. 6,004,565); presumably because it keeps T cells away from sites of pathology. However, as described more fully below in the Experimental section, in the case of infection it is the secondary lymphoid organs that are often a significant site of pathology or of pathogen replication and that contribute to dissemination of the pathogen throughout the rest of the body. In this context, lymphocyte sequestration may provide an immune enhancing effect and is useful in the treatment or prevention of infection, in particular bacterial, viral, fungal, or parasitic infection. Specific compounds for use within the methods and compositions of the present invention are described more fully below.

In one embodiment, the present invention relates to a method for preventing or treating infection comprising administering at least one cycle of therapy to a subject in need thereof, where the cycle of therapy comprises administering at least one dose of a therapeutically effective amount of a leukocyte sequestration agent to the subject. The term "subject" as used herein refers to an animal, preferably a mammal, more preferably a human. In a particular embodiment, the cycle of therapy begins immediately following the subject's exposure to a pathogen (*e.g.*, within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours following exposure to a pathogen). In another embodiment, the cycle of therapy begins after a time delay following the subject's exposure to a pathogen (*e.g.*, more than 24, 36, 48, 60, or 72 hours following exposure to a pathogen, or more than 1, 2, 3, or 4 weeks following exposure to a pathogen). In a further embodiment, the method comprises transient treatment in which the subject receives only one cycle of therapy. In another embodiment, the method comprises chronic treatment in which the subject receives more than one cycle of therapy.

The term "cycle of therapy" as used herein refers to administration of at least one dose of a therapeutically effective amount of a leukocyte sequestration agent to a subject in need thereof for the prevention or treatment of infection. In one embodiment, the cycle of therapy comprises administering at least one dose of a therapeutically effective amount of a leukocyte sequestration agent to a subject in need thereof. In another embodiment, the cycle of therapy comprises one or more dose per day of a therapeutically effective amount of a leukocyte sequestration agent to a subject in need thereof for 1, 2, 3, 4, 5, or more days. In a particular embodiment, the infection is a bacterial or viral infection.

Dosage levels of a leukocyte sequestration agent such as FTY720 on the order of about 1 μg/kg to about 1 mg/kg may be useful in the treatment or prevention of infections in a subject as noted herein above. As described more fully in the Experimental section below, leukocyte sequestration may be achieved in subjects with infection using doses of these agents (in particular FTY720) that are lower than those required to achieve leukocyte sequestration in normal non-infected subjects. In one embodiment of the present invention, a therapeutically effective dose of a leukocyte sequestration agent as described herein is an amount between about 1 μg/kg to about 1

mg/kg, about 1 μg/kg to about 750 μg /kg, about 1 μg/kg to about 500 μg /kg, about 1 μg/kg to about 250 μg /kg, about 1 μg/kg to about 100 μg /kg, about 1 μg/kg to about 50 μg /kg, about 1 μg/kg to about 25 μg /kg, or about 1 μg/kg to about 10 μg /kg. In another embodiment, the therapeutically effective dose of a leukocyte sequestration agent is an amount of about 1 μg/kg, about 2 μg/kg, about 3 μg/kg, about 4 μg/kg, about 5 μg/kg, about 6 μg/kg, about 7 μg/kg, about 8 μg/kg, about 9 μg/kg, about 10 μg/kg, about 15 μg/kg, about 20 μg/kg, about 25 μg/kg, about 50 μg/kg, about 75 μg/kg, about 100 μg/kg, about 150 μg/kg, about 200 μg/kg, about 250 μg/kg, about 300 μg/kg, about 350 μg/kg, about 400 μg/kg, about 450 μg/kg, about 500 μg/kg, about 550 μg/kg, about 600 μg/kg, about 650 μg/kg, about 700 μg/kg, about 750 μg/kg, about 800 μg/kg, about 850 μg/kg, about 900 μg/kg, about 950 μg/kg, or about 1 mg/kg. Where the subject is a human subject, the dosage levels are based upon a body weight of approximately 70 kg. It will be understood, however, that the specific dose level and frequency of dosage for any particular subject may be varied and will depend upon a variety of factors including body weight, age, general health, sex, and diet of the subject, the metabolic stability and length of action of the administered compound, mode and time of administration, rate of excretion, drug combination, and severity of the particular condition.

The leukocyte sequestration agent can be formulated according to known methods to prepare pharmaceutically useful compositions, and may be administered to a subject by any mode of administration, including oral, rectal, topical, nasal, ophthalmic, or parenteral (including intraperitoneal, intravenous, subcutaneous, or intramuscular injection) administration. Suitable formulations and their appropriate carrier vehicles are described, for example, in *Remington's Pharmaceutical Sciences* (16th ed., Osol, A. (ed.), Mack, Easton PA (1980)).

As used herein, the term "infection" refers to a bacterial, viral, fungal, or parasitic (including helminthic and protozoan) infection.

In one embodiment, the invention relates to methods for treating or preventing bacterial infection. Bacterial infectious organisms include, but are in no way limited to, Streptococcus sp., *Haemophilus influenzae*, Klebsiella sp., Escherichia sp., Legionella sp., Mycoplasma sp., *Pneumocystis carinii*, Listeria, Corynebacterium sp., Staphylococcus sp., Serratia, Pseudomonas, Shigella, Vibrio, Hemophilus sp.,

Yersinia, and Enterobacter, and include also diseases due to Mycobacteria, such as tuberculosis (caused by *Mycobacterlum tuberculosis*) and leprosy (caused by *Mycobacterium leprae*). Infectious Chlamydiae organisms are also encompassed in this definition (such as *C. trachomatis*), as are rickettsiae organisms (such as those causing typhus and potted fever). In a particular embodiment, the invention relates to methods for treating or preventing bacterial infection caused by *Escherichia coli*, *Helicobacter pylori*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Shigella Flexneri*, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, or *Bacillus anthracis*.

In another embodiment, the invention relates to methods for treating or preventing viral infection. Viral infectious organisms include viruses of the following families: Poxviridae, Herpesviridae, Adenoviridiae, Papovaviridae, Hepadnaviridae, Parvoviridae, Reviridae, Togoviridae, Flaviviridae, Coronaviridae, Paramyxoviridae, Rhabdoviridae, Bunyaviridae, Arenaviridae, Retroviridae, Picornaviridae, and Caliciviridae. Viral diseases and viruses include, but are in no way limited to, smallpox, Vaccinia, herpes sp., Influenza sp., *Varicella-zoster*, cytomegalovirus sp., Epstein-Barr, rubella, yellow fever, rabies, measles, Ebola, polio, and HIV (the cause of AIDS). In a particular embodiment, the invention relates to methods for treating or preventing viral infection caused by a vaccinia virus, a variola virus, a polyomavirus, an arenavirus, a herpes virus, an influenza virus, a hepatitis virus, or a human immunodeficiency virus.

In another embodiment, the invention relates to methods for treating or preventing fungal infection. Fungal infectious organisms (mycoses) include, but are in no way limited to Aspergillus sp., Candida sp., *Cryptococcus neofonnans*, *Coccidiodes inmitis*, *Blastomyces dermatitidis*, Rhizopus sp., Mucor sp., and Fusariaum sp.

In another embodiment, the invention relates to methods for treating or preventing parasitic infection. Parasitic organisms include, but are in no way limited to, those organisms responsible for protozoan infections, e.g., toxoplasmosis (Toxoplasma sp.), malaria (Plasmodium sp.), sleeping sickness and Chagas disease (trypanosomiasis; Tryapanosoma sp.), leishmaniasis (Leishmania sp.), cryptosporidiosis (Cryptosporidium sp.), giardiasis (Giardia sp.), amebiasis (Entamoeba sp.), or trichomoniasis (Trichomonas sp.), cestode infections (tapeworms

(*Diphyllobothrium latum*), or echinococcosis (Echinococcosis sp.), worm (Schistosoma sp.), fluke or nematode infections, or filariasis (various filarial parasites including *Wuchereria bancrofti*, *Brugia malayi* and *Onchocerca volvulus*).

The present invention also relates to methods for preventing or treating cancers with viral etiologies comprising administration of at least one dose of a therapeutically effective amount of a leukocyte sequestration agent to a subject in need thereof. Cancers with viral etiologies include, but are not limited to, nasopharyngeal cancer, non-Hodgkin's lymphoma, cervical cancer, hepatocellular carcinoma, Kaposi's sarcoma, and adult T-cell leukemia.

In addition to methods for treating or preventing infection or cancers with viral etiologies, the present invention also relates to compositions and methods whereby leukocyte sequestration agents (in particular lymphocyte sequestration agents including FTY720) are used to make vaccines more effective. For example, it is well known that immunization of neonates with live viruses does not contribute to acquired immunity because maternal antibodies neutralize the vaccine (Bot and Bona (2002) *Microbes Infect.* 4: 511). In one embodiment, administration of a leukocyte sequestration agent of the present invention allows for safe administration of higher doses of virus to overcome antibody response and permit acquisition of cellular immunity. In another embodiment, leukocyte sequestration agents of the present invention facilitate immune clearance of the pathogen. For some chronic viruses (e.g., HIV and HCV), high viral loads have been found to compromise T cell function (Welsh (2001) *J. Exp. Med.* 193:F19). Thus, lowering the viral burden could permit recovery of T cell function and thereby facilitate clearance. In another embodiment, leukocyte sequestration agents of the present invention permit immunocompromised individuals to be vaccinated.

Accordingly, in one embodiment, the invention relates to an immunogenic composition comprising an immunogen and an adjuvant composition comprising a leukocyte sequestration agent. In a particular embodiment, the leukocyte sequestration agent is a lymphocyte sequestration agent, more particularly an S1P receptor agonist (such as FTY720), a sphingosine kinase inhibitor, or a S1P lyase inhibitor. The term "immunogen" as used herein refers to one or more antigenic polypeptides, immunogenic polypeptides or polysaccharides. As used herein, an

antigenic polypeptide or an immunogenic polypeptide is a polypeptide which, when introduced into a vertebrate, reacts with the immune system molecules of the vertebrate, *i.e.*, is antigenic, and/or induces an immune response in the vertebrate, *i.e.*, is immunogenic. It is quite likely that an immunogenic polypeptide will also be antigenic, but an antigenic polypeptide, because of its size or conformation, may not necessarily be immunogenic. Examples of antigenic and immunogenic polypeptides include, but are not limited to, polypeptides from infectious pathogens such as bacteria, viruses, parasites, or fungi. In a particular embodiment, the immunogenic composition comprises a bacterial or viral antigen as an immunogen.

In another embodiment, the invention relates to a method of immunization comprising administering to a subject in need thereof an immunogenic composition comprising: a) an immunogen in an amount sufficient to generate an immune response to the immunogen in the subject; and b) an adjuvant composition comprising a leukocyte sequestration agent; where the immunogen is one or more antigenic polypeptides, immunogenic polypeptides or polysaccharides one or more antigenic polypeptides, immunogenic polypeptides or polysaccharides. In a particular embodiment, the leukocyte sequestration agent is a lymphocyte sequestration agent, more particularly an S1P receptor agonist (such as FTY720), a sphingosine kinase inhibitor, or a S1P lyase inhibitor.

In another embodiment, the invention relates to a method for preventing or treating infection comprising administering to a subject in need thereof an immunogenic composition comprising: a) an immunogen in an amount sufficient to generate an immune response to the immunogen in the subject; and b) an adjuvant composition comprising a leukocyte sequestration agent; where the immunogen is one or more antigenic polypeptides, immunogenic polypeptides or polysaccharides one or more antigenic polypeptides, immunogenic polypeptides or polysaccharides. In a particular embodiment, the leukocyte sequestration agent is a lymphocyte sequestration agent, more particularly an S1P receptor agonist (such as FTY720), a sphingosine kinase inhibitor, or a S1P lyase inhibitor.

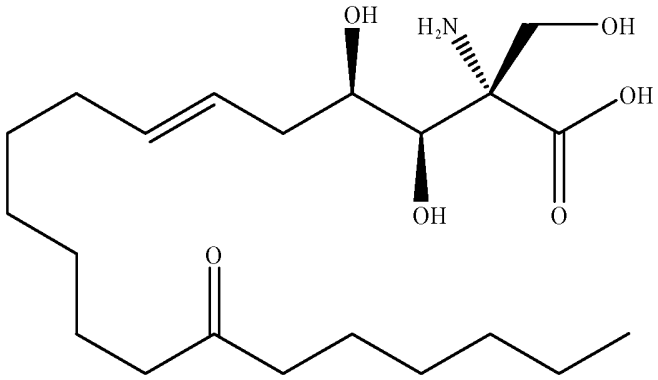
In one embodiment, the invention also relates to a pharmaceutical or packaged kit comprising: a) a container holding an immunogen; and b) an adjuvant composition comprising a leukocyte sequestration agent; where the immunogen is provided in a

therapeutically effective amount to prevent or treat infection in a subject in need thereof, and where the immunogen is one or more antigenic polypeptides, immunogenic polypeptides or polysaccharides. In a particular embodiment, the leukocyte sequestration agent is a lymphocyte sequestration agent, more particularly an S1P receptor agonist (such as FTY720), a sphingosine kinase inhibitor, or a S1P lyase inhibitor. The packaged or pharmaceutical kit may comprise a container, preferably sealed, for housing the immunogenic composition during storage and prior to use, and instructions for carrying out administration of the composition in a manner effective for preventing or treating infection in a subject. The instructions will typically be written instructions on a package insert and/or on a label. Depending on the type of immunogenic composition and the intended mode of administration, the kit may also include a device for administering the composition. The parts of the kit may be independently held in one or more containers, such as bottles, syringes, plates, wells, blister packs, or any other type of pharmaceutical packaging.

Leukocyte Sequestration Agents for Use in the Methods and Compositions of the Invention

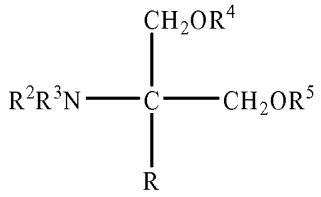
As described above, the present invention relates to methods of use and compositions comprising leukocyte sequestration agents. In a particular embodiment, the agents mimic the activity of S1P or increase the level or availability of endogenous S1P in a subject. Without being bound by theory, the interaction of S1P with its receptor $S1P_1$ is believed to be important in lymphocyte trafficking. S1P is a lysosphingophospholipid stored and secreted by platelets as well as other cells such as erythrocytes, neutrophils and mononuclear cells (31-35 - ML). S1P levels are regulated by its synthesis from sphingosine and sphingosine kinase (SK) and its catabolism by lipid phosphatases and by sphingosine lyase (SPL), a pyridoxal 5' phosphate dependent enzyme that resides in the ER and is responsible for the irreversible degration of S1P to ethanolamine phosphate and hexadecenal (36 – ML). S1P binds to five members of this receptor family $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$, previously referred to as endothelial differentiation gene (EDG)-1, -5, -3, -6 and -8. With respect to the immune system, $S1P_1$ is expressed on dendritic cells and on leukocytes such as lymphocytes and monocytes.

Thus, in one embodiment, the leukocyte sequestration agents (or, more particularly, lymphocyte sequestration agents) for use within the methods and compositions of the present invention are S1P receptor agonists. Such agents may be selective, meaning that they actively bind one S1P receptor subtypes, or non-selective, meaning that they actively bind more than one S1P receptor subtype. In a particular embodiment, the S1P receptor agonist is a homolog, analog, or derivative of the compound myriosin or ISP-1, a natural product of *Isaria sinclairii* (Fujita *et al.* (1994) *J. Antibiotics* 47:208-215). Myriocin is depicted below.



Homologs, analogs or derivatives of myriocin can be prepared and tested by one skilled in the art to ensure that they possess lymphocyte sequestration activity using assays and procedures known in the art (see, *e.g.*, Fujita *et al.* (1994) *J. Antibiotics* 47:208-215 and U.S. Patent No. 6,004,565).

In one embodiment, the S1P receptor agonist for use within the methods and compositions of the present invention is a 2-aminopropane-1,3-diol compound according to formula I:

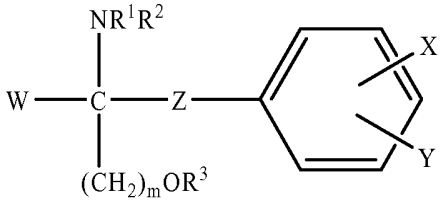


I

or a pharmaceutically acceptable salt thereof; wherein R is an optionally substituted straight or branched carbon chain, an optionally substituted aryl, an optionally substituted cycloalkyl or the like;

and R^2, R^3, R^4, and R^5 are the same or different and each is a hydrogen, an alkyl, an acyl, or an alkoxycarbonyl, or R^4 and R^5 may be bonded to form an alkylene chain, which may be substituted by alkyl, aryl, or an alkoxycarbonyl.

In another embodiment, the S1P receptor agonist for use within the methods and compositions of the present invention is a benzene compound according to formula II:



II

or a pharmaceutically acceptable salt thereof, wherein W is hydrogen; a straight or branched chain alkyl having 1 to 6 carbon atoms; a straight or branched chain alkenyl having 2 to 6 carbon atoms; a straight or branch chain alkynyl having 2 to 6 carbon atoms; a phenyl, which may be substituted by hydroxy; $R^4(CH_2)_n$; or a straight or branched chain C_1-C_6 alkyl substituted by 1 to 3 substituents selected from the group consisting of a halogen, a cycloalkyl, and a phenyl, which may be substituted by hydroxy;

X is hydrogen, a straight-chain alkyl having carbon atoms in the number of p or a straight-chain alkoxy having carbon atoms in the number of (p-1), wherein the straight-chain alkyl having carbon atoms in the number of p and the straight-chain alkoxy having carbon atoms in the number of (p-1) may have 1 to 3 substituents selected from the group consisting of an alkyl, hydroxy, an alkoxy, an acyloxy, amino, an akylamino, an acylamino, oxo, a haloalkyl, a halogen, and a phenyl, which may have a substituent, and wherein the phenyl, which may have a substituent, may have 1 to 3 substituents selected from the group consisting of an alkyl, hydroxy, an alkoxy, an acyl, an acyloxy, amino, an alkylamino, an acylamino, a holalkyl, and a halogen;

Y is hydrogen, an alkyl, hydroxy, an alkoxy, an acyl, an acyloxy, amino, an alkylamino,

an acylamino, a haloalkyl, or a halogen;

Z is a single bond or a straight-chain alkylene having carbon atoms in the number of q;

p and q are the same or different and each is an integer of 1 to 20, with the proviso that

$6 \leq p+q \leq 23$;

m is 1, 2, or 3;

n is 2 or 3;

R^1 and R^2 are the same or different and each is hydrogen, an alkyl or an acyl;

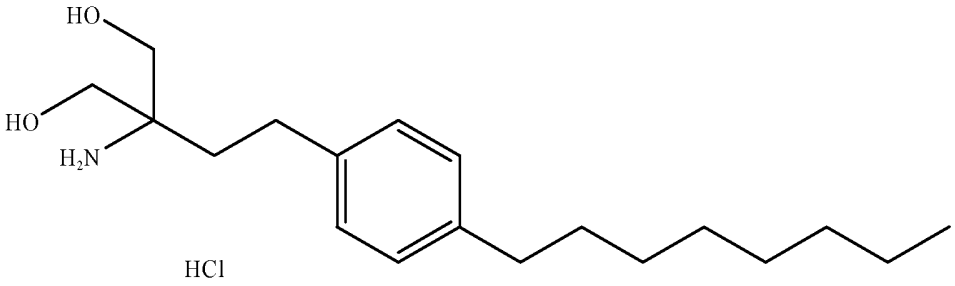
R^3 is hydrogen, an alkyl or an acyl; and

R^4 is hydrogen, an alkyl or an acyl.

For the 2-aminopropane-1,3-diol compounds and benzene compounds described above, a disclosure of specific compounds, substituent groups, and variations included in the S1P receptor agonists that may be used within the methods and compositions of this invention can be found in U.S. Patent Nos. 6,667,025, 6,004,565, and 5,604,229. These documents also describe methods to produce and isolate specific compounds that can be used according to this invention. Methods for producing these compounds are well known in the art (see, *e.g.*, Fujita *et al.* (1995) *BioMed. Chem. Lett.* 5:847-852; Fujita *et al.* (1996) *J. Med. Chem.* 39:4451-4459; Adachi *et al.* (1995) *BioMed. Chem. Lett.* 5:853-856). The entire contents of each of these patent documents and references are specifically incorporated by reference into this disclosure and can be relied on to make or isolate the compounds and practice this invention. The compounds can be prepared or isolated as any of a number of pharmaceutically or physiologically acceptable salts or be prepared as optically active isomers of any of the described compounds.

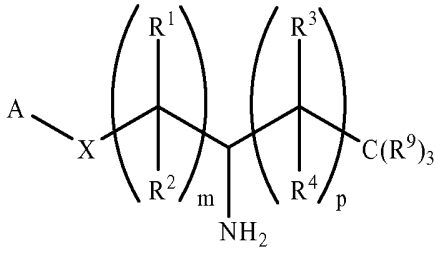
In one embodiment, the S1P receptor agonists for use in the methods and compositions of the invention share structural features with S1P such as a 2-substituted 2-aminoethanol group (see, *e.g.*, Kiuchi (1998) *Bioorg. Med. Chem. Lett.* 8:101-106), as well as a lipophilic tail region and a phosphate head group (see, *e.g.*, Clemens *et al.* (2003) *Bioorg. Med. Chem. Lett.* 13:3401-3404). One such preferred

structural embodiment of S1P receptor agonists is FTY720, 2-amino-2[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride, shown below, or a pharmaceutically acceptable salt thereof.



FTY720 is a prodrug that undergoes *O*-phosphorylation, and it is the phosphorylated FTY720 (phospho-FTY720) that exhibits S1P receptor agonist activity (see, *e.g.*, Clemens *et al.* (2003) *Bioorg. Med. Chem. Lett.* 13:3401-3404).

Selective $S1P_1$ receptor agonists for use in the methods and compositions of the invention include compounds according to formula III as disclosed and described in U.S. Patent App. Pub. No. 2005/0107345:



III

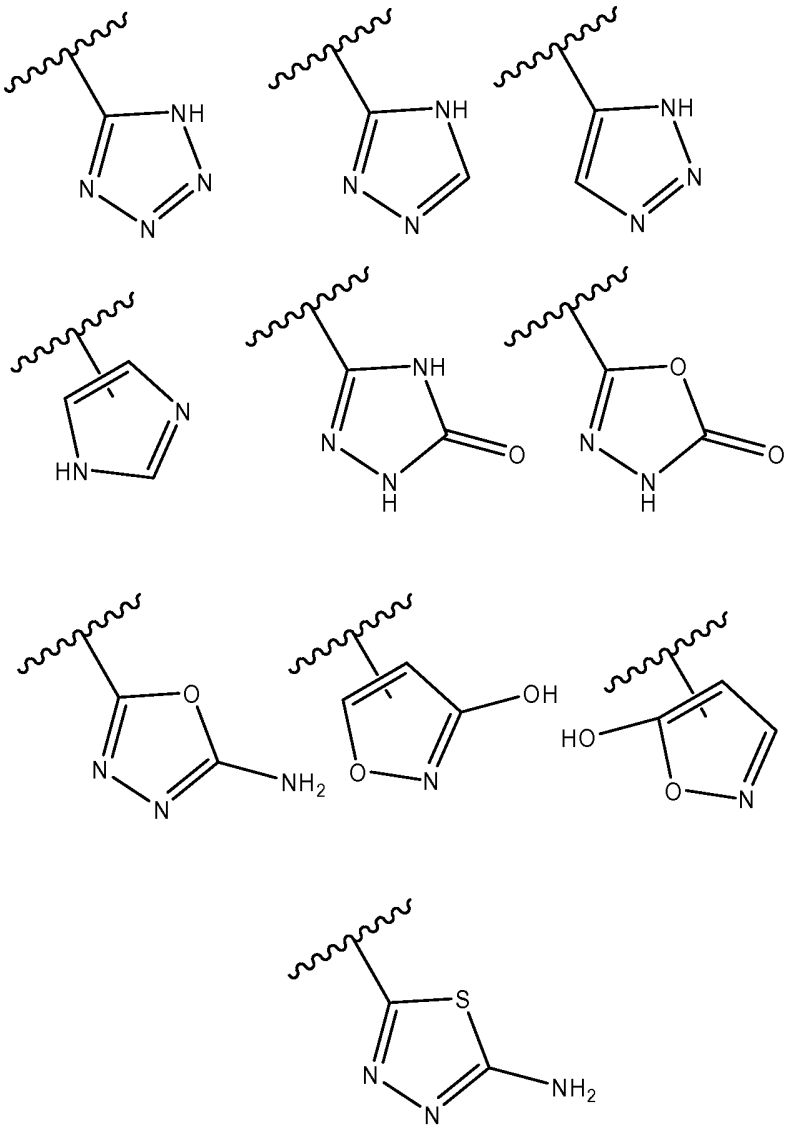
or a pharmaceutically acceptable salt thereof, wherein

m = 1, 2, 3, or 4;

p = 9 to 20;

X is a bond, O, NH, $S(O)_k$, wherein k is 0, 1 or 2;

A is selected from the group consisting of: $—CO_2H$, $—PO_3H_2$, $—PO_2H_2$, $—SO_3H$, $—PO(R_8)OH$,



each R^1 is independently selected from the group consisting of: hydrogen, halo, hydroxy,

—CO_2H, C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkylthio and aryl, wherein said C_{1-4}alkyl, C_{1-4}alkoxy and C_{1-4}alkylthio are each optionally substituted from one up to the maximum number of substitutable positions with halo and wherein said aryl is optionally substituted with 1-5 substituents independently selected from halo and C_{1-4}alkyl, or two R^1 groups on adjacent carbon atoms may be joined together to form a double bond;

each R^3 is independently selected from the group consisting of: hydrogen, halo, hydroxy,

—CO_2H, C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkylthio and aryl, wherein said C_{1-4}alkyl, C_{1-4}alkoxy and C_{1-4}alkylthio are each optionally substituted from one up to the maximum number of substitutable positions with halo and wherein said aryl is optionally substituted with 1-5 substituents independently selected from halo and C_{1-4}alkyl, or

two R^3 groups on adjacent carbon atoms may be joined together to form a double bond;

and

R^2 and R^4 are each independently selected from the group consisting of: hydrogen, halo,

hydroxy, —CO_2H, C_{1-4}alkyl, C_{1-4}alkoxy, C_{1-4}alkylthio and aryl, wherein said C_{1-4}alkyl, C_{1-4}alkoxy and C_{1-4}alkylthio are each optionally substituted from one up to the maximum number of substitutable positions with halo and wherein said aryl is optionally substituted with 1-5 substituents independently selected from halo and C_{1-4}alkyl;

or R^1 and R^2 or R^3 and R^4 residing on the same carbon atom may optionally be joined together to form a carbonyl group,

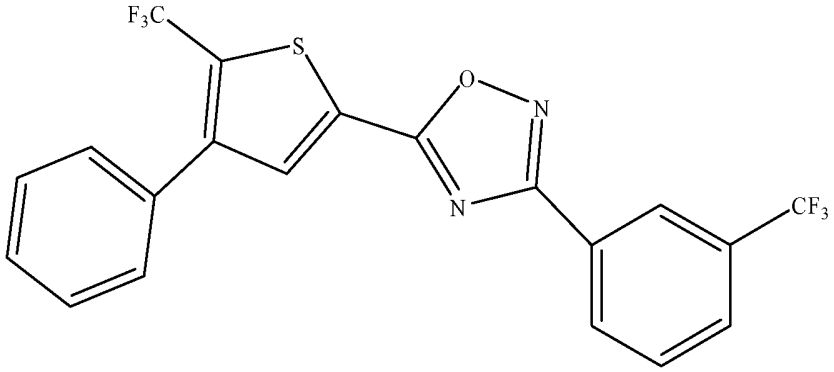
R^8 is selected from the group consisting of: C_{1-4}alkyl and aryl, wherein said C_{1-4}alkyl is

optionally substituted with 1-3 halo groups and aryl is optionally substituted with 1-5 substituents independently selected from the group consisting of: halo, C_{1-6}alkyl, C_{3-6}cycloalkyl, C_{1-6}alkoxy, C_{1-6}alkylthio and C_{3-6}cycloalkoxy, said C_{1-6}alkyl, C_{3-6}cycloalkyl, C_{1-6}alkoxy, C_{1-6}alkylthio and C_{3-6}cycloalkoxy optionally substituted from one up to the maximum number of substitutable positions with halo,

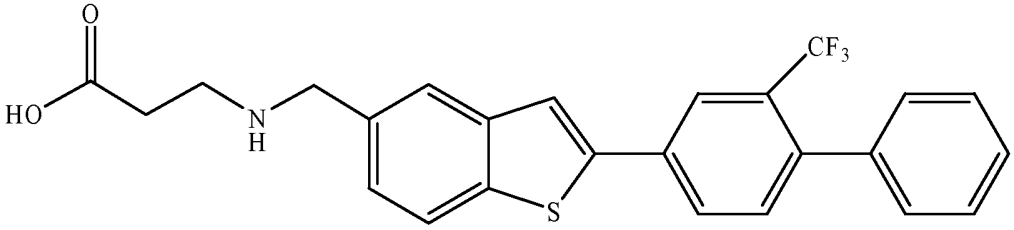
R^9 is selected from the group consisting of: hydrogen, halo, hydroxy, C_{1-4}alkoxy, C_{1-4}alkylthio and C_{3-7}cycloalkyl, wherein said C_{1-4}alkoxy, C_{1-4}alkylthio and C_{3-7}cycloalkyl are each independently optionally substituted from one up to the

maximum number of substitutable positions with halo and wherein said aryl is optionally substituted with 1-5 substituents independently selected from halo and C_{1-4}alkyl.

In one embodiment, a selective $S1P_1$ receptor agonist for use in the methods and compositions of the invention is 5-[4-phenyl-5-(trifluoromethyl)-2-thienyl]-3-[3-(trifluoromethyl)phenyl]-1,2,4-oxadiazole (SEW2871), shown below, or a pharmaceutically acceptable salt thereof.



In another embodiment, a selective $S1P_1$ receptor agonist for use in the methods and compositions of the invention is AUY954, shown below, or a pharmaceutically acceptable salt thereof (see Pan *et al.* (2006) *Chem. Bio.* 13:1227-1234).



Other selective and non-selective S1P receptor agonists for use in the methods and compositions of the invention include 3,5-diphenyl-1,2,4-oxadiazole based compounds disclosed in Li *et al.* (2005) *J. Med. Chem.* 48:6169-6173; *para*-alkyl aryl amide analogs of S1P disclosed in Clemens *et al.* (2003) *Bioorg. Med. Chem. Lett.* 13:3401-3404; selective S1P receptor agonists as described in Forrest (2004) *J. Pharmaol. Exp. Ther.* 309:758-768; 2,2-disubstituted 2-aminoethanols as disclosed in Kiuchi (1998) *Bioorg. Med. Chem. Lett.* 8:101-106; 2-substituted 2-aminopropane-1,3-diols as disclosed in Kiuchi (2000) *J. Med. Chem.* 43:2946-2961;

phosphothionates as disclosed in Foss *et al.* (2005) *Bioorg. Med. Chem. Lett.* 15:4470-4474; 3-(4-heterocyclyl-3-methyl-phenyl)propionic acid, 1,3,4-thiadiazole, and 3-cyano-4-isopropyloxyphenyl compounds disclosed in Vachal *et al.* (2006) *Bioorg. Med. Chem. Lett.* 16:3684-3687), 3-arylpropionic acids as disclosed in Yan *et al.* (2006) 16:3679-3683; and S1P receptor agonists as described in Rosen (2003) *PNAS* 100:10907-10912. Assays and methods for identifying and testing additional S1P receptor agonists for use in the methods and compositions of the invention are well known in the art as described in the references cited above (see also, *e.g.*, Jo *et al.* (2005) *Chem. Bio.* 12:703-715).

In another embodiment, the leukocyte sequestration agents (or, more particularly, lymphocyte sequestration agents) for use within the methods and compositions of the present invention are sphingosine kinase (SK) inhibitors. Such agents may be specific, meaning that they inhibit only SK and not other protein and lipid kinases, or non-specific, meaning that they inhibit SK but may also inhibit other protein and lipid kinases. SK inhibitors that may be used within the methods and compositions of the invention include the non-specific SK inhibitors N,N-dimethylsphingosine (DMS), D,L-*threo*-dihydrosphingosine, and N,N,N-trimethylsphingosine (see, *e.g.*, French *et al.* (2006) *J. Pharmacol. Exp. Ther.* 318:596-603 and Edsall (1998) *Biochem.* 37:12892-12898). Specific SK inhibitors that may be used within the methods and compositions of the invention include compounds disclosed in French *et al.* (2003) *Cancer Res.* 63:5962-5969 (*e.g.*, CAS Registry Nos. 306301-68-8, 312636-16-1, 359899-55-1, and 24388-08-7), azaphilone-type metabolites as described in Kono *et al.* (2001) *J. Antibiot. (Tokyo)* 54:415-420, and substituted adamantine compounds as described in U.S. Patent App. Pub. No. 2006/0287317. Assays and methods for identifying and testing additional SK inhibitors for use in the methods and compositions of the invention are well known in the art as described in the references cited above.

In another embodiment, the leukocyte sequestration agents (or, more particularly, lymphocyte sequestration agents) for use within the methods and compositions of the present invention are S1P lyase inhibitors. S1P lyase inhibitors

that may be used within the methods and compositions of the invention include the food colorant 2-acetyl-4-tetrahydroxybutylimidazole (THI; see Schwab *et al.* (2005) *Science* 309:1735-1739).

In yet another embodiment, the leukocyte sequestration agents (or, more particularly, lymphocyte sequestration agents) for use within the methods and compositions of the present invention include any drug or biological agent, such as S1P receptor antibodies (*e.g,* $S1P_1$ polyclonal antibodies such as those available from Cayman Chemical, Ann Arbor, MI) that induce or enhance redistribution of lymphocytes out of the blood and into secondary lymphoid organs.

In accordance with the methods of the present invention, the leukocyte sequestration agents described herein may be administered in combination with one another, or with other compounds, particularly antipathogenic compounds. Such antipathogenic compounds include conventional antimicrobials. Where the leukocyte sequestration agents of the present invention are administered as part of a combination therapy to treat or prevent infection, they may be administered concurrently or sequentially, in either order, with the additional compound(s).

It is to be understood that the present invention encompasses the use not only of the specific compounds described above, but also any pharmaceutically acceptable salts, enantiomers, analogs, esters, amides, prodrugs, metabolites, or derivatives thereof.

Having now generally described this invention, the same will be better understood by reference to certain specific examples which are included below, which are offered for purposes of illustration only and are not intended to be limiting of the invention.

EXPERIMENTAL

The following experiments examined the effects of leukocyte sequestration agents on infection, particularly bacterial and viral infection. Before describing these experiments in more detail, it will be helpful to provide the following background.

Background

Persisting pathogens and their evasion of the immune response: A functional adaptive immune response is necessary to clear intracellular pathogens such as viruses and bacterial infections. Factors that result in immune dysfunction and an inability to control disease progression are often attributed to the immune evasion properties of the pathogen and T cell exhaustion. Although in some cases a pathogen that causes a chronic illness in one host can be cleared in another and therefore it remains unclear the exact conditions that confer clearance or persistence for many viruses and whether our immune system itself is capable of eliminating immune suppressive agents such as HIV.

Pathogens that cause persistent infections such as HIV, hepatitis B and C (HBV, HCV) have been studied for their ability to evade or disarm the immune system. HIV for example evades the immune response by rapidly mutating its highly glycosylated envelope glycoprotein (Johnson (2002) *Annual Review of Medicine* 53:499-518) and high viral loads have been associated with T cell exhaustion, measured as an inability to T cells to proliferate. Functional CD8 T cells are important in the acute phase as well as long-term control of many persistent viruses such as Epstein-Barr virus (EBV), cytomegalovirus, HBV and HCV viruses. Potent HIV specific CD8 T cell response correlates with acute viral control and long-term no progression. However, during infections with HIV, HBV and HCV CD8 T cells fail to contain viral replication. Recently PD-1 expression has been found on the surface of both HIV specific and HCV specific T cells (Day *et al.* (2006) *Nature* 443:350-354; Urbani *et al.* (2006) *J. Virol.* 80:11398-11403). PD-1 expression has been linked to viral persistence and blocking of PD-L1 during LCMV cl-13 restored CD8 T cell responses (Barber *et al.* (2006) *Nature* 439:682-687). There is some evidence to suggest that PD-L1 blockade may reverse exhausted T cells during persistent human infections such as HIV (Day *et al.* (2006) *Nature* 443:350-354).

LCMV, the virus and a model for persistent viral infections: Lymphocytic choriomeningitis virus (LCMV) naturally infects mice and is a prototypic arenavirus important for studying both acute and persistent viral infections and their associated diseases. Important concepts in both immunology and viral pathogenesis have been

developed using this model (Mims and Blanden (1972) *Infect. Immun.* 6:695-698; Mims and Wainwright (1968) *J. Immunol.* 101:717-724; Oldstone *et al.* (1973) *J. Exp. Med.* 137:1201-1212). LCMV is an enveloped bisegmented negative-stranded RNA virus. There are numerous strains of LCMV. LCMV Armstrong (Arm) causes an acute infection that is cleared between days 8-10 post infection. During Arm there is expansion of LCMV specific $CD8^+$ T cells that peaks at day 8 and these cells contract of form a stable memory population (Matloubian *et al.* (1994) *J. Virol.* 68:8056-8063). In contrast LCMV isolate cl-13 (cl-13) (Ahmed *et al.* (1984) *J. Exp. Med.* 160:521-540) causes an infection that persists in several organs for the life of the mouse. During cl-13 infection LCMV specific $CD8^+$ initially expand but become exhausted (Wherry *et al.* (2003) *J. Virol.* 77:4911-4927; Zajac *et al.* (1998) *J. Exp. Med.* 188:2205-2213), unable to produce both TNFα and IFNγ upon in vitro stimulation and are unable to control virus. T cell dysfunction has been found during human diseases such as HIV, HBV and HCV (Borysiewicz and Sissons (1994) *Curr. Top. Microbiol. Immunol.* 189:123-150; Chisari and Ferrari (1995) *Springer Semin Immunopathol.* 17:261-281; Rickinson and Moss (1997) *Annu. Rev. Immunol.* 15:405-431; Walker (1999) *Hepatitis C virus* (John Wiley & Sons Ltd., New York)). CD8 T cell exhaustion during cl-13 has been linked to CD4 T cell dysfunction (Matloubian *et al.* (1994) *J. Virol.* 68:8056-8063), which occurs during the transition to viral persistence.

Studies have also shown that in contrast to Arm, cl-13 targets antigen presenting cells (APC), including dendritic cells (DCs). Cl-13 infection of DCs impairs the expression of MHC and costimulatory molecules on both splenic myeloid and lymphoid DCs (Borrow *et al.* (1995) *J. Virol.* 69:1059-1070; Sevilla *et al.* (2000) *J. Exp. Med.* 192:1249-1260). Additionally cl-13 effects the development and differentiation of DC $CD8\alpha^+$ and CD8 α^- bone marrow precursors (Sevilla *et al.* (2004) *J. Clin. Invest.* 113:737-745). DCs are infected and impaired in their ability to stimulate T cells during several human infections such as Measles virus (Hahm *et al.* (2005) *Immunity* 22:247-257), herpes simplex (Vollstedt *et al.* (2006) *Eur. J. Immunol.* 36:1231-1240), and vaccinia virus (Engelmayer *et al.* (1999) *J. Immunol.*

163:6762-6768). HIV exploits DCs for transmission to T cells (Geijtenbeek *et al.* (2000) *Cell* 100:587-597) and EBV induces apoptosis of monocytic precursors (Li *et al.* (2002) *Blood* 99:3725-3734).

In addition the use of LCMV cl-13 as a model for persistent infections and immune suppression (Borrow *et al.* (1995) *J. Virol.* 69:1059-1070; Tishon *et al.* (1993) *Virology* 195:397-405; Colonna (2004) *J. Clin. Invest.* 113:660-662; Althage *et al.* (1992) *Eur. J. Immunol.* 22:1803-1812) it has been used to discover potential therapeutic targets. Anti-PD-L1 treatment of cl-13 has restored CD8 T cell effector function (Barber *et al.* (2006) *Nature* 439:682-687), IL-10 receptor blockade has lead to the clearance of persistent viral infection (Brooks *et al.* (2006) *Nat. Med.* 12:1301-1309; Ejrnaes *et al.* (2006) *J. Exp. Med.* 203:2461-2472) and using this LCMV model it was found that by blocking CD27 the LCMV persistent strain Docile can be eliminated (Matter *et al.* (2006) *J. Exp. Med.* 203:2145-2155). Therefore LCMV is an excellent model for acute and persistent viral infection and in investigating potential therapeutic targets.

Immune modulating effects of Sphingosine-1-phosphate: Sphingosine-1-phosphate (S1P) is a lysosphingophospholipid stored and secreted by platelets (Zhang *et al.* (1999) *Blood* 93:2984-2990) and present in density gradient being high in the serum as compared to tissues (Schwab *et al.* (2005) *Science* 309:1735-1739; Cyster (2005) *Annu. Rev. Immunol.* 23:127-159; Rosen and Goetzl (2005) *Nat. Rev. Immunol.* 5:560-570). Though platelets are believed to be the major secreted source of S1P, other cells such as erythrocytes, neutrophils and mononuclear cells also secrete S1P (Dahm *et al.* (2006) *J. Thromb. Haemost.* 4:2704-2709; Hanel *et al.* (2007) *Faseb. J.*). S1P levels are regulated by its synthesis from sphingosine and sphingosine kinase (SK) and its catabolism by lipid phosphatases and by sphingosine lyase (SPL), a pyridoxal 5 phosphate dependent enzyme that resides in the ER and is responsible for the irreversible degration of S1P to ethanolamine phosphate and hexadecenal (Spiegel and Milstien (2000) *FEBS. Lett.* 476:55-57). S1P binds to five members of this receptor family S1P(1), S1P(2), S1P(3), S1P(4) and S1P(5), previously referred to as endothelial differentiation gene (EDG)-1, -5, -3, -6 and -8. S1P receptors are expressed in various tissues, and therefore S1P is an important lipid

mediator in various physiological processes such as vascular maturation, cardiac development, lymphocyte trafficking, and vascular permeability (Dahm *et al.* (2006) *J. Thromb. Haemost.* 4:2704-2709; Hanel *et al.* (2007) *Faseb. J.*).

With regards to the immune system, S1P1 is expressed on lymphocytes, DCs and monocytes. The receptor binds to S1P in a gradient dependent fashion (Schwab *et al.* (2005) *Science* 309:1735-1739) and the binding provides a mechanism for lymphocyte egress from lymph nodes (Matloubian *et al.* (2004) *Nature* 427:355-360). Recently it was found that a type I interferon (IFN) response induced the global up regulation of CD69 on the cell surface of lymphocytes (Shiow *et al.* (2006) *Nature* 440:540-544). This up regulation of CD69 caused the internalization of S1P1 and non-responsiveness to S1P gradient. Following type I IFN response lymphocytes became sequestered in lymph nodes. The interaction of S1P with its receptor S1P1 is important in lymphocyte trafficking and is the believed target of the immunotherapeutic drug FTY720.

Immunosuppressive effects of FTY720: FTY720 is an immunomodulatory agent that inhibits lymphocytes trafficking. FTY720 is derived from the fungus Isaria sinclairii ISP-1 (myiocin), a fungal metabolite that is an eternal youth nostrum in traditional Chinese herbal medicine (Fujita *et al.* (1994) *J. Antibiot. (Tokyo)* 47:208-215). FTY720 was designed by modification of myriocin by interrupting sphingolipid metabolism (Fujita *et al.* (1994) *J. Antibiot. (Tokyo)* 47:208-215). The compound FTY720 (2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol) targets G protein-coupled receptors (GPCR) and interacts with the heterotrimeric G proteins of the $\alpha_{i/o}$ subtype (Pabst *et al.* (2006) *J. Immunol.* 176:1474-1480). FTY720 is phosphorylated in vivo by SPK-1 (Paugh *et al.* (2003) *FEBS Lett.* 554:189-19) and is stable in the presence of active S1P lyase. The phosphorylated drug (FTY720P) is found predominantly in the Peyer's patches, spleen and LN; though the heart, liver and kidney also contain some of the phosphorylated drug (Brinkmann *et al.* (2002) *J. Biol. Chem.* 277:21453-21457).

FTY720P is a high potency agonist for S1P1, S1P3, S1P4 and S1P5. Both S1P1 and S1P4 are expressed at high levels on lymphocytes where as S1P1 and S1P3 are expressed on DCs (Czeloth *et al.* (2005) *J. Immunol.* 175:2960-2967) that are

maturing (though not on immature DCs in tissues or DCs that have migrated to draining LNs) and S1P1 is also expressed on monocytes (Singer *et al.* (2005) *J. Immunol.* 175:7151-7161). FTY720 induces a peripheral blood lymphopenia (Brinkmann *et al.* (2002) *J. Biol. Chem.* 277:21453-21457; Mandala *et al.* (2002) *Science* 296:346-349; Graler and Goetzl (2004) *Faseb. J.* 18:551-553; Morris *et al.* (2005) *Eur. J. Immunol.* 35:3570-3580) effecting T cells more then B cells (Halin *et al.* (2005) *Blood* 106:1314-1322). The drug enhances entry of lymphocytes into LNs by a cysteinyl leukotriene-dependent T cell chemotaxis and inhibits egress of both naïve and activated CD4, CD8 and B cells. FTY720 does not prevent the activation or function of T and B cells in response to antigen (Pinschewer *et al.* (2000) *J. Immunol.* 164:5761-5770).

FTY720 has been used as an immunosuppressive drug and is currently in phase II clinical trials to treat Multiple Sclerosis (MS) (Gardell *et al.* (2006) *Trends Mol. Med.* 12:65-75; Virley (2005) *NeuroRx* 2:638-649) and phase III trials prevent kidney transplant rejection (Gardell *et al.* (2006) *Trends Mol Med* 12:65-75, Kunzendorf *et al.* (2004) *Nephrol. Dial Transplant* 19:1677-1681; Park *et al.* (2005) *Braz. J. Med. Biol. Res.* 38:683-694; Vaessen *et al.* (2006) *Transpl. Immunol.* 15:281-288; Bohler *et al.* (2004) *Transplantation* 77:1424-1432; Budde *et al.* (2002) *J. Am. Soc. Nephrol.* 13:1073-1083; Skerjanec *et al.* (2005) *J. Clin. Pharmacol.* 45:1268-1278). As an immunosuppressive drug FTY720 is administered daily at an average dose of 1mg.

Without being bound by theory, it is believed that the FTY720 interactions with S1P1 are responsible for the drug effect on lymphocytes (Matloubian *et al.* (2004) *Nature* 427:355-360). It functions by acting as an S1P1 agonist, down regulating it on the surface of lymphocytes, altering the ability of lymphocytes to respond to the S1P gradient required for egress out of the lymph node. Down regulation of S1P1 is thought to be the major effect of FTY720 since lymphocytes lacking S1P1 expression emulates the effects of FTY720 on lymphocyte egress and trafficking (Graler and Goetzl (2004) *Faseb. J.* 18:551-553; Allende *et al.* (2004) *J. Biol. Chem.* 279:52487-52492). In doing so lymphocytes are sequestered in secondary lymphoid tissues and their numbers reduced at the site of chronic

inflammation. It is believed that for FTY720 to be and effective immune suppressive drug it must sequester at least 80% of circulating lymphocytes (Park *et al.* (2005) *Braz. J. Med. Biol. Res.* 38:683-694).

In the Examples provided below it has been shown that even transient administration of FTY720 can potentiate immune responses to a strain of LCMV (clone 13) that normally establishes a chronic infection that starts in lymphoid tissues and that persists in a variety of organs such as the kidney and the brain. FTY720 was used in two distinct modes: a) when used immediately after infection (on days 0, 1, and 2), it prevented establishment of a chronic infection (this may be called "post-exposure prophylaxis mode", or PEP); and b) when administered for 3 days one month after establishment of a chronic infection, FTY720 led to clearance of the infection.

Since FTY720 acts on host-immune cells and alters their trafficking patterns, it is unlikely to generate resistant mutant viruses or other pathogens. As a consequence, the drug is predicted to be useful in both post-exposure prophylaxis and treatment of patients exposed to pathogens that establish chronic infections in humans, such as (but not limited to) HIV, HCV, or mycobacterium tuberculosis.

Example 1. Development of FTY720 Post-Exposure Prophylaxis

Initial studies were conducted on a trace population of bystander T cell receptor transgenic cells (F5, specific for a T cell epitope in influenza) in mice infected with one of two strains of LCMV—the non-pathogenic Armstrong strain, which is rapidly cleared by immunocompetent mice, and the clone 13 strain, which establishes a chronic infection in the same mouse strains (Ahmed *et al.* (1984) *J. Exp. Med.* 160:521-540). Naïve T cells have been shown to exit the blood following infection with the non-pathogenic Armstrong strain (delivered intraperitoneally), but not following infection with the clone 13 strain (delivered intravenously). Sequestration of T cells away from the blood and into secondary lymphoid organs is now believed to be controlled by expression of CD69, which is upregulated in response to type I interferons that are typically produced in abundance in most viral infections (Shiow *et al.* (2006) *Nature* 440:540-544). CD69 inhibits the function of $S1P_1$, one of five receptors for sphingosine 1-phosphate; this is the principle pathway

that regulates T cell egress from secondary lymphoid organs (Cyster (2005) *Annu. Rev. Immunol.* 23:127-159). It is not currently understood why naïve T cells in a clone 13 infection failed to redistribute from the blood to secondary lymphoid organs, as preliminary data suggested that there were no defects in type I interferon production in clone 13 infected mice, nor was there a defect in upregulation of CD69.

Nevertheless, the lack of naïve T cell redistribution in clone 13 infections led to the question of whether forced redistribution of naïve T cells from the blood and into lymph nodes by treatment with FTY720 would have an effect on the ability of the immune system to control an otherwise chronic infection.

Dose Response: In studies in the mouse, the most commonly used dose of FTY720 has been about 0.3-1 mg/kg (Pinschewer *et al.* (2000) *J. Immunol.* 164:5761-5770; Halin *et al.* (2005) *Blood* 106:1314-1322). All of these studies were done in uninfected mice, so a dose-response experiment in the context of an LCMV infection was conducted to determine if there might be some unexpected synergy between the drug and some feature of the innate immune response (such as IFN-I-dependent upregulation of CD69).

We measured the sequestration of lymphocytes in infected and uninfected animals as well as viral titers on day 8 post infection. B6 mice were infected i.v. with $2x10^6$ pfu of LCMV clone 13 at day 0. Once a day, at days 0, 1, and 2 post-infection, FTY720 was given i.v. at various doses. Titers of LCMV in the serum were determined by plaque assay at 8 days post infection. At high doses (1 mg/kg) lymphocytes were sequestered but were prevented from re-circulating by day 4-post infection, whereas at the lower doses (67 μg/kg and 4 μg/kg) the lymphocytes were re-circulating on day 4. Figure 1 shows a 1.5-2.0 log reduction in viral titers over a broad range of FTY720, including concentrations below those capable of inducing substantial lymphopenia in uninfected mice. In addition, we saw that doses as low as 4 μg/kg could induce lymphopenia in clone 13-infected but not uninfected mice.

Based upon these data, all subsequent experiments were performed at 4 μg/kg of FTY720, unless otherwise noted. In addition, all subsequent experiments have been performed with a 3-day drug treatment regimen, and so far, we continue to give the drug i.v., though it has often been used orally.

Experimental Design: Testing began on the effects of FTY720 on the course of LCMV clone 13 infection with an FTY720-treatment regimen of daily doses at 0, 1, and 2 days post-infection (4 μg/kg i.v.). Regimens of a single dose given at 1 day post-infection, and two doses given at days 1 and 2 after infection, were also tested. These regimens are summarized in Table 1.

Table 1. FTY720 Dosing Regimens

Regimen Designation	Dosing
A	3 doses, daily, at 0, 1, and 2 days post infection
B	1 dose, at day 1 post-infection
C	2 doses, at days 1, and 2 post-infection
At each time point, FTY720 was given at a dose of 4 μg/kg i.v.	

Viral Loads: Figure 2 shows that mice that were infected with clone 13 and transiently treated with FTY720 on days 0, 1, and 2 following infection were able to clear clone 13 from the serum between days 22 and 30 post-infection and from the kidneys by 60 days post infection. Additionally, as early as day 5 post-infection there was a significant ½ log reduction in serum viral titers in clone 13-infected FTY720-treated mice as compared to LCMV clone 13 untreated mice.

Untreated mice eventually cleared clone 13 from the serum (as seen in the dramatic reduction in virus titers between days 30 and 60), but FTY720-treated mice did so significantly faster (though FTY720-induced clearance of clone 13 was slower than clearance of the Armstrong strain given at the same dose and by the same route). In addition, in untreated mice clone 13 is known to persist for much longer periods in the kidneys and the brain, and in our experiments, untreated mice had 10^5 pfu of virus in their kidneys at d60 post infection. In contrast, no virus was found at d60 post infection in the kidneys of FTY720-treated mice. As discussed below, clearance of the virus was immune mediated, and not due to any previously unknown anti-viral effects of FTY720 (Figures 9 and 10).

Protection of T Cell Function: As a result of its high level persistence, clone 13 induces a reproducible pattern of antigen-specific T cell depletion and/or dysfunction in C57Bl/6 mice (Zajac *et al.* (1998) *J. Exp. Med.* 188:2205-2213). T cells specific for the immunodominant $CD8^+$ T cell epitopes D^b/NP396 and K^b/GP34 are deleted, while T cells specific for D^b/GP33 and D^b/GP276 have a significantly reduced capacity to produce the cytokines IFNγ, TNFα, and IL-2 (Wherry *et al.* (2003) *J. Virol.* 77:4911-4927).

We used intracellular cytokine staining for IFNγ and TNFα to examine responses specific for the GP33 epitope and the NP396 epitope in C57BL/6J mice infected with LCMV Armstrong or clone 13 and treated with vehicle, or in clone 13-infected mice, treated with FTY720 (Figure 3). The key differences between the control clone 13 mice and the FTY720-treated clone 13 mice were most apparent at days 15 and 30 post infection, where the FTY720-treated mice had both greater frequencies of responding cells for each epitope, and of the responding cells, a greater proportion of them made TNFα in addition to IFNγ. However, the ability of day 15 and day 60 post-infection cells to produce both IFNγ and TNFα in response to both GP33 and NP36 was almost completely absent in control mice infected with clone 13 but otherwise untreated. The loss of TNFα production capacity is associated with an early exhausted phenotype (Wherry *et al.* (2003) *J. Virol.* 77:4911-4927), and FTY720 was able to prevent this.

T Cell Phenotypes: Clone 13 dependent induction of T cell dysfunction is associated with increased expression of the molecule PD-1 on the surface of antigen-specific cells (Barber *et al.* (2005) *Nature* 439:682-687). PD-1 expressing cells have an "exhausted" phenotype that is reversed upon antibody-mediated blockade of its ligand, PD-1L (Barber *et al.* (2005) *Nature* 439:682-687). In addition to its apparent function as a negative regulator of T cell function, expression of PD-1 can also be regarded as a sort of antigen sensor.

PD-1 expression of D^b/GP33- and D^b/GP276-specific cells was analyzed in various tissues at various times post infection. The most striking data were seen at day 15-post infection, where PD-1 expression on D^b/GP33- and D^b/GP276- specific cells were clearly coming down in FTY720 treated mice, but was maintained at high

levels in clone 13 infected but otherwise untreated mice (see Figure 4). This shift in PD-1 levels in the FTY720 treated mice correlated with their control of the infection having a one half log drop in serum LCMV titers on day 5 post infection (see Figure 1) and this drop continued until clearance by day 30 post infection. Therefore FTY720 prevented the establishment of a persistent infection.

Formation of Memory Cells: Evidence for the protection against CD8 T cell exhaustion that results from FTY720 treatment was even more pronounced at day 60 post-infection, as seen in Figure 5. Compared to untreated clone 13-infected controls, FTY720 treated mice, it was found that: (1) there were more GP33- and NP396-specific CD8 T cells (there were hardly any NP396-specific cells in untreated mice); (2) a greater proportion of the GP33- and NP396-specific cells produced TNFα in addition to IFNγ; and (3) a greater proportion of the GP-33 and NP396-specific cells produced IL2 in addition to IFNγ. These features were all associated with clearance of the virus, and were more pronounced at d60 than at d15 or d30 (Figure 3) probably because virus is not fully cleared until sometime around d30 (Figure 2).

The final feature of the T cell response that was associated with clearance of the virus was the cell surface phenotype of LCMV-specific cells (identified by MHC tetramer staining (Murali-Krishna *et al.* (1998) Immunity 8:177-187)). When antigen persisted, as in untreated clone 13-infected mice, LCMV-specific T cells expressed high levels of PD-1 and failed to regain expression of CD62L (Barber *et al.* (2006) *Nature* 439:682-687); in contrast, tetramer-positive, LCMV-specific cells at d60 in clone 13-infected mice that were treated with FTY720 at days 0, 1, and 2 were uniformly PD-1 low and many of them had regained CD62L expression. The clearance of antigen that was enabled by FTY720 treatment allowed the LCMV-specific CD8+ T cells to return to a resting memory state.

Example 2. Using FTY720 to Treat an Established Chronic Infection

Our success in using FTY720 to prevent establishment of a chronic infection prompted us to test whether FTY720 could promote clearance of an established chronic LCMV infection. During the course of persistent infection the innate immune system becomes compromised and dysfunctional during clone 13 infection. Dendritic

cell function is altered (Hahm *et al.* (2005) *Immunity* 22:247-257; Colonna (2004) *J. Clin. Invest.* 113:660-662; Montoya *et al.* (2005) *J. Immunol.* 174:1851-1861) and type I IFN responses are prevented (Louten *et al.* (2006) *J. Immunol.* 177:3266-3272). Therefore by administering FTY720 during an established infection, the question of whether we could enhance immunity in the presence of $CD8^+$ T cell exhaustion and a dysfunctional innate immune system was addressed. It was not expected that the experiment would work since the circumstances that provoked administering FTY720 during priming were not present during the chronic phase. Surprisingly, an established cl-13 infection was cleared following treatment with FTY720.

In Figure 6 and Figure 7, we show the results of a set of experiments in which we infected C57BL/6J mice with clone 13 and waited 30 days before beginning a 3 day treatment regimen with FTY720, also at a dose of 4 μg/kg; we call this treatment mode TCI, for "treating chronic infection". In the first set of experiments, we waited until d60 before analyzing the mice for viral titers and T cell responses.

Untreated mice often clear clone 13 from the serum and highly vascularized organs (spleen, liver, etc.) between days 40-60 post infection (Wherry *et al.* (2003) *J. Virol.* 77:4911-4927), but virus invariably persists at a high titer for much longer periods of time in the kidneys and the brain. While we have looked at a variety of tissues, the most dramatic results were seen in the kidneys (Figure 6) and brain; in untreated mice, both organs have at least 5 logs of virus per gram of tissue, while virus titers in both organs were below the limit of detection of the plaque assay in the FTY720 treatment group. We have not detected virus in any tissue in FTY720-treated mice in these experiments.

T cell data from d60 from the TCI experiment is shown in Figure 7. Figure 7A and Figure 7B show cell surface phenotypes of GP33-specific CD8+ T cells, defined by staining with anti-CD8 and the Db/GP33 tetramer (Murali-Krishna *et al.* (1998) Immunity 8:177-187). In these Figures, the predominant phenotype of D^b/GP33-specific cells in untreated mice is $CD62L^{lo}$ and PD-1^{hi}; the CD62L phenotype is typical of effector or so-called effector memory cells, while the PD-1 phenotype indicated the ongoing presence of antigen and in the clone 13 model has been associated with an exhausted response phenotype (Barber *et al.* (2006) *Nature* 439:682-687). In contrast, in mice that have been treated with FTY720 on d30-32,

most of the D^b/GP33-specific cells were $CD62L^{hi}$, a phenotype associated with so-called central memory cells (Wherry *et al.* (2003) *Nat. Immunol.* 4:225-234; Sallusto *et al.* (1999) *Nature* 401:708-712), and essentially all of them were PD-1^{lo}. In agreement with the cell-surface phenotypes of these cells, Figure 7D shows that the vast majority of the GP33-specific cells in the treated mice produced both TNFα and IFNγ, in contrast to the cells that produced only IFNγ that dominated in the untreated mice. Even more remarkably, FTY720-treated mice regained NP396- specific cells (Figure 7C), though a greater proportion of these produced only IFNγ and not IFNγ + TNFα compared to the GP33-specific cells.

Conclusions for Examples 1 and 2

Using two different treatment modes, our results showed that treatment of mice with FTY720 led to clearance of an otherwise chronic infection. These results are summarized in Table 2.

Table 2. Summary of Modes of FTY720 Treatment of LCMV Clone 13 Infection

Treatment mode	Regimen	Result
Post-exposure prophylaxis	3 days, once per day, i.v., begun immediately after infection	Virus clearance from all organs; preservation of T cell function.
Treatment of chronic infection	3 days, once per day, i.v., begun 30 days post-infection	Virus clearance from all organs; restoration of T cell function (perhaps recruiting new naïve T cells from recent thymic emigrants).

Without being bound by theory, it is believed that FTY720 is promoting T cell-mediated control of an otherwise chronic infection by sequestering virus-specific precursors and effector T cells at sites of significant viral replication. Ssequestering virus-specific cells in secondary lymphoid organs may also increase their potency and permit them to control the virus infection before the cells become overstimulated and dysfunctional. Effects on CD4 T cells, B cell and dendritic cells may also play a role, all of which express the S1P1 receptors and other receptors that FTY720 has been demonstrated to interact with.

Because FTY720 manipulates the host response in a non-specific way—both at the T cell level and at the pathogen level—and because data from clinical trials already suggests that FTY720 works the same way in humans as it does in mice, it is predicted that FTY720 treatment immediately following infection of humans with chronic persisting pathogens will also permit immunological control of the infections. Furthermore, because FTY720 is acting at the host level and does not interact with pathogens in a specific way, development of pathogen-specific resistance to the drug is highly unlikely. In this way, FTY720 constitutes a completely generalized treatment for pathogens such as HIV, HCV, mycobacterium tuberculosis, and others as described elsewhere herein. FTY720 is also predicted to be efficacious in post-exposure treatment of otherwise lethal acute infections such as pandemic influenza, where a faster effective immune response may reduce severe morbidity or mortality. A non-exhaustive list of human pathogens that might be treated with FTY720 is presented in Table 3.

Table 3. Human Pathogens Treatable With FTY720

Category	**Exemplary Pathogens**
Chronic viral infections	HIV
	HCV
Chronic bacterial infections	Mycobacterium tuberculosis
Acute viral infections	Pandemic influenza
Biological weapons	Smallpox, anthrax, arenaviruses

Example 3. Mechanism of FTY720 Treatment of Clone-13 Infected Mice

The goal of this set of experiments was to examine the mechanism through which FTY720 exerts its immune enhancing effects. As described below, experiments involving the anti-CD4 antibody GK1.5 showed that $CD4^+$ T cells appear to be necessary for $CD8^+$ T cell enhancement and viral clearance following FTY720 treatment of cl-13 infected mice.

Figure 8 shows the experimental design of the GK1.5, $CD4^+$ depletion study. As shown, in this study groups of mice were treated with FTY720 beginning at different times post cl-13 infection and GK1.5 treatment. Mice were infected with cl-13 and all were treated with GK1.5 at 500μg i.p. on days 0 and 1 post infection.

In all mice that are treated with GK1.5 (0.5 mg/mouse/day) on days 0 and 1, CD4 cells (measured in the spleen) did not start coming back until d15 and the frequency of CD4+ splenocytes continued to increase monotonically through d30 (Figure 9A); neither the infection nor the FTY720 treatment had a significant effect upon the return of CD4 cells in the spleen. Robust GP33-specific T cell responses, including cells that produced both IFNγ and TNFα, did not appear until 15 days after treatment with FTY720 that occurred from d15-17 (Figure 9B, plot j), and the two-week-post-treatment GP33 specific responses were even stronger when treatment with FTY720 is delayed (Figure 9B, plots k-l). When the analysis was restricted to one-week-post-treatment with FTY720, GP33-specific responses did not appear until FTY720-treatment was delayed until d30-32 post infection (Figure 9B, plot f); largely similar results were seen for NP396-specific responses. The return of efficacy of FTY720 treatment exactly corresponded to the return of CD4 cells in the GK1.5-treated mice.

The results in Figure 9 allowed two important conclusions: (1) the efficacy of FTY720 in treating a clone 13 infection was completely dependent upon CD4+ T cells; and (2) FTY720 was promoting clearance of clone 13 by enhancing the immune response to the virus, and not via a direct anti-viral effect (if there were a dominant anti-viral effect of the drug, it would not have been affected by CD4 depletion). Viral titers from serum also demonstrated no *in vivo* antiviral activity of FTY720 since mice treated with GK1.5 and administered FTY720 at the same time were unable to control the infection (Figure 10) as compared to WT mice cl-13 infected mice treated with FTY720 (Figure 2).

Example 4. Use of FTY720 in a Second Viral Infection Model

This experiment was conducted to assess whether FTY720 could influence the course of an otherwise lethal vaccinia infection. We used the Western Reserve strain (vvWR) at a dose of $5x10^5$ pfu given by the intranasal route, corresponding to 10 LD_{50}'s for our stock. Groups of 10 mice each were treated once a day for three days (0, 1, and 2) immediately following infection (as for the LCMV experiments above) with vehicle, 4 μg/kg FTY720 (low dose), or 200 μg/kg FTY720 (high dose); a fourth group of uninfected mice was used as a control. The primary endpoints were death and weight-loss; a composite clinical score was used as a secondary endpoint. The results are presented in Figure 11.

All of the untreated mice were found dead in their cages at d5 or d6 post-infection. Both doses of FTY720 provided some measure of protection, but by every measure, the low-dose was more efficacious. None of the mice in the low-dose treatment group died on their own, though all were euthanized on d7 because they had reached IACUC-approved endpoints, principally loss of motility and arched backs; only one of 10 had lost more than 25% of its original body weight. The differences in weight loss at d5 between all combinations of infected groups were significant (Figure 11A) by Fischer's exact test. These data suggest that transient use of FTY720 provides protection against a second virus (vaccinia vs. LCMV) delivered by a second route of infection (i.n. vs. i.v.).

Many modifications and other embodiments of the inventions set forth herein will come to mind to one skilled in the art to which these inventions pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the inventions are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation. Further, it must be noted that as used in this specification and the appended embodiments, the singular forms "a," an" and "the" include plural referents unless the context clearly dictates otherwise.

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

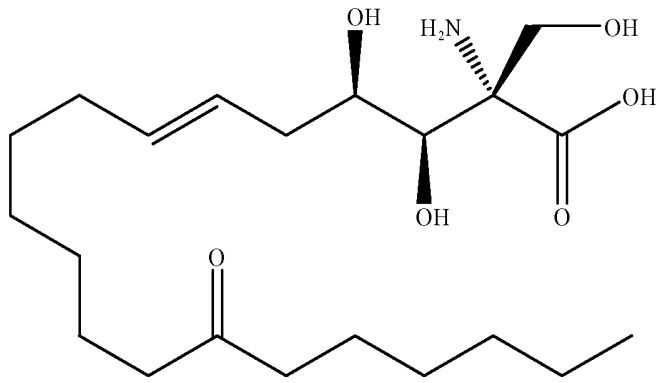
THAT WHICH IS CLAIMED:

1. A method for preventing or treating a bacterial infection or a viral infection comprising administering at least one cycle of therapy to a subject in need thereof, wherein said cycle of therapy comprises administering at least one dose of a therapeutically effective amount of a leukocyte sequestration agent to said subject.

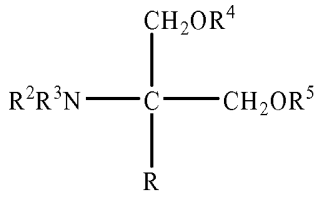
2. The method of claim 1, wherein said leukocyte sequestration agent is a lymphocyte sequestration agent.

3. The method of claim 2, wherein said lymphocyte sequestration agent is a sphingosine 1-phosphate (S1P) receptor agonist, a sphingosine kinase inhibitor, or a S1P lyase inhibitor.

4. The method of claim 3, wherein said lymphocyte sequestration agent is an S1P receptor agonist, further wherein said S1P receptor agonist is a homolog, analog, or derivative of the compound myriosin, depicted below.



5. The method of claim 4, wherein said S1P receptor agonist is a 2-aminopropane-1,3-diol compound according to formula I:



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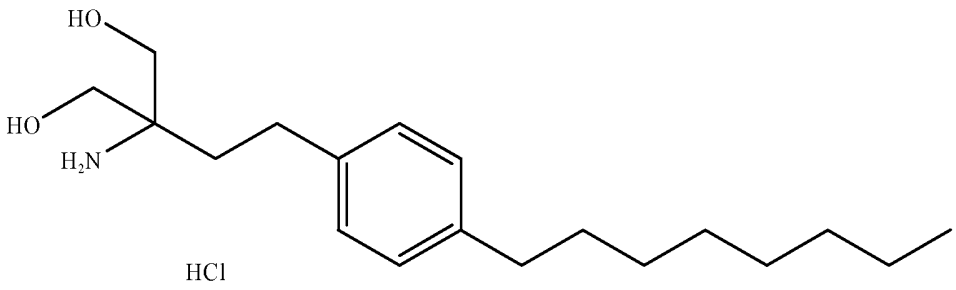
or a pharmaceutically acceptable salt thereof; wherein R is an optionally substituted straight or branched carbon chain, an optionally substituted aryl, an optionally substituted cycloalkyl or the like;

and R^2, R^3, R^4, and R^5 are the same or different and each is a hydrogen, an alkyl, an acyl, or an alkoxycarbonyl, or R^4 and R^5 may be bonded to form an alkylene chain, which may be substituted by alkyl, aryl, or an alkoxycarbonyl.

6. The method of claim 5, wherein said 2-aminopropane-1,3-diol compound is the compound 2-amino-2[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride (FTY720), depicted below, or a pharmaceutically acceptable salt thereof.



7. The method of claim 6, wherein said compound is administered to said subject in at least one dose of about 1 μg/kg to about 1 mg/kg.

8. The method of claim 7, wherein said compound is administered to said subject in at least one dose of about 4 μg/kg.

9. The method of claim 8, wherein said cycle of therapy begins immediately following exposure to a bacterium or virus.

10. The method of claim 8, wherein said cycle of therapy begins within 24 hours following exposure to a bacterium or virus.

11. The method of claim 8, wherein said cycle of therapy begins more than 24 hours following exposure to a bacterium or virus.

12. The method of claim 8, wherein said cycle of therapy begins more than 2 weeks following exposure to a bacterium or virus.

13. The method of claim 8, wherein said cycle of therapy begins more than 4 weeks following exposure to a bacterium or virus.

14. The method of claim 6, wherein said viral infection is caused by a vaccinia virus, a variola virus, a polyomavirus, an arenavirus, a herpes virus, an influenza virus, a hepatitis virus, or a human immunodeficiency virus.

15. The method of claim 6, wherein said bacterial infection is caused by *Escherichia coli*, *Helicobacter pylori*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Shigella Flexneri*, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, or *Bacillus anthracis*.

16. An immunogenic composition comprising:

(a) an immunogen; and

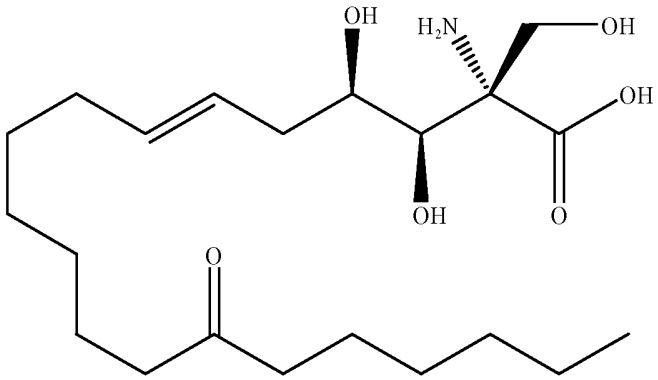
(b) an adjuvant composition comprising a leukocyte sequestration agent;

wherein said immunogen is one or more antigenic polypeptides, immunogenic polypeptides or polysaccharides.

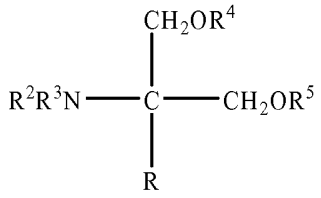
17. The immunogenic composition of claim 16, wherein said leukocyte sequestration agent is a lymphocyte sequestration agent.

18. The immunogenic composition of claim 17, wherein said lymphocyte sequestration agent is an S1P receptor agonist, a sphingosine kinase inhibitor, or a S1P lyase inhibitor.

19. The immunogenic composition of claim 18, wherein said lymphocyte sequestration agent is an S1P receptor agonist, further wherein said S1P receptor agonist is a homolog, analog, or derivative of the compound myriosin, depicted below.



20. The immunogenic composition of claim 19, wherein said S1P receptor agonist is a 2-aminopropane-1,3-diol compound according to formula I:



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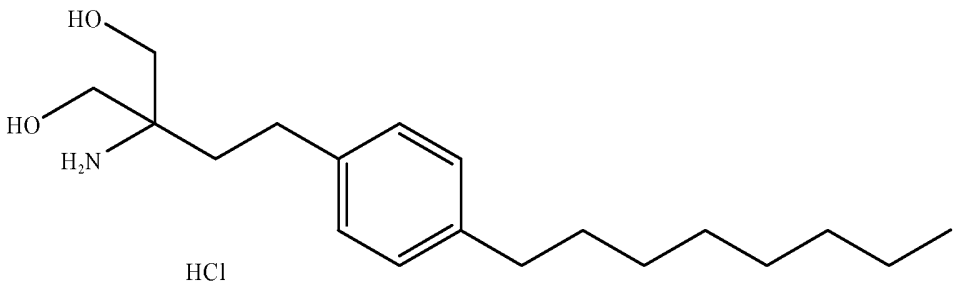
or a pharmaceutically acceptable salt thereof, wherein R is an optionally substituted straight or branched carbon chain, an optionally substituted aryl, an optionally substituted cycloalkyl or the like;

and R^2, R^3, R^4, and R^5 are the same or different and each is a hydrogen, an alkyl, an acyl, or an alkoxycarbonyl, or R^4 and R^5 may be bonded to form an alkylene chain, which may be substituted by alkyl, aryl, or an alkoxycarbonyl.

21. The immunogenic composition of claim 20, wherein said 2-aminopropane-1,3-diol compound is the compound 2-amino-2[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride (FTY720), depicted below, or a pharmaceutically acceptable salt thereof.



22. The immunogenic composition of claim 21, wherein said immunogen comprises a bacterial or viral antigen.

23. A method of immunization comprising administering to a subject in need thereof an immunogenic composition comprising:

(a) an immunogen in an amount sufficient to generate an immune response to said immunogen in said subject; and

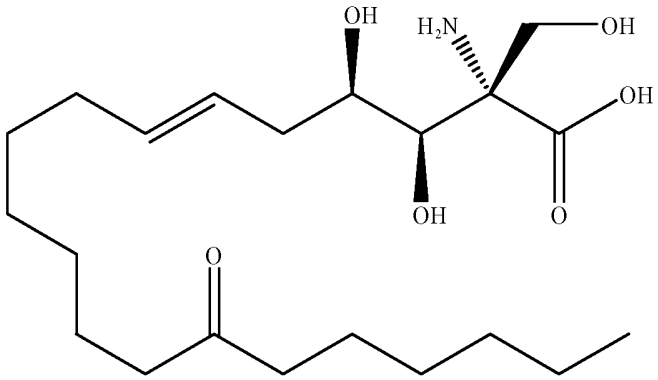
(b) an adjuvant composition comprising a leukocyte sequestration agent;

wherein said immunogen is one or more antigenic polypeptides, immunogenic polypeptides or polysaccharides one or more antigenic polypeptides, immunogenic polypeptides or polysaccharides.

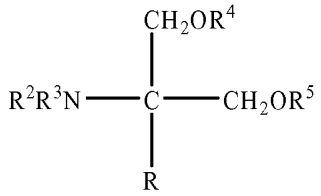
24. The method of claim 23, wherein said leukocyte sequestration agent is a lymphocyte sequestration agent.

25. The method of claim 24, wherein said lymphocyte sequestration agent is an S1P receptor agonist, a sphingosine kinase inhibitor, or a S1P lyase inhibitor.

26. The method of claim 25, wherein said lymphocyte sequestration agent is an S1P receptor agonist, further wherein said S1P receptor agonist is a homolog, analog, or derivative of the compound myriosin, depicted below.



27. The method of claim 26, wherein said S1P receptor agonist is a 2-aminopropane-1,3-diol compound according to formula I:



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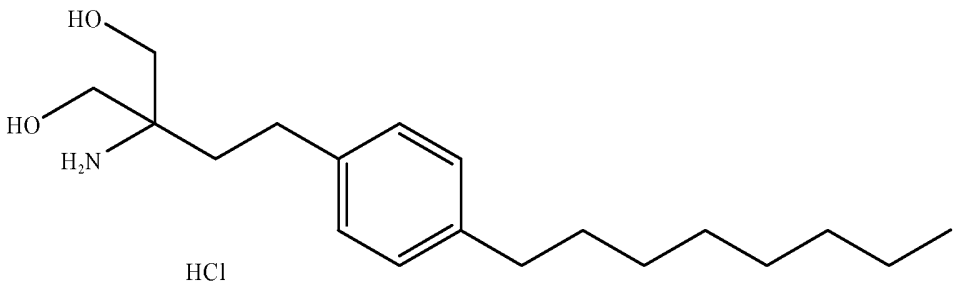
or a pharmaceutically acceptable salt thereof, wherein R is an optionally substituted straight or branched carbon chain, an optionally substituted aryl, an optionally substituted cycloalkyl or the like;

and R^2, R^3, R^4, and R^5 are the same or different and each is a hydrogen, an alkyl, an acyl, or an alkoxycarbonyl, or R^4 and R^5 may be bonded to form an alkylene chain, which may be substituted by alkyl, aryl, or an alkoxycarbonyl.

28. The method of claim 27, wherein said 2-aminopropane-1,3-diol compound is the compound 2-amino-2[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride (FTY720), depicted below, or a pharmaceutically acceptable salt thereof.



29. The method of claim 28, wherein said immunogen comprises a bacterial or viral antigen.

30. A method for preventing or treating a bacterial infection or a viral infection comprising administering to a subject in need thereof an immunogenic composition comprising:

(a) an immunogen in an amount sufficient to generate an immune response to said immunogen in said subject; and

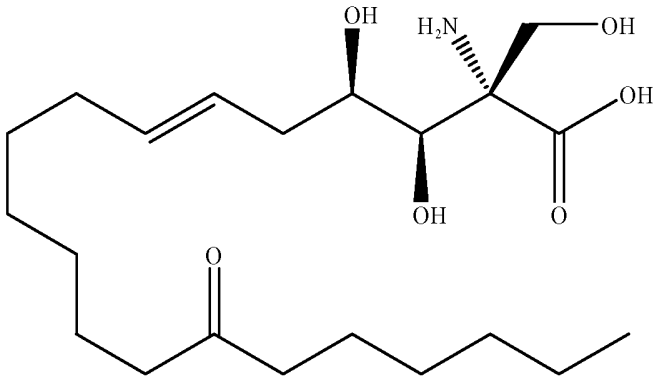
(b) an adjuvant composition comprising a leukocyte sequestration agent;

wherein said immunogen is one or more antigenic polypeptides, immunogenic polypeptides or polysaccharides one or more antigenic polypeptides, immunogenic polypeptides or polysaccharides.

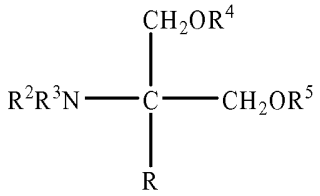
31. The method of claim 30, wherein said leukocyte sequestration agent is a lymphocyte sequestration agent.

32. The method of claim 31, wherein said lymphocyte sequestration agent is an S1P receptor agonist, a sphingosine kinase inhibitor, or a S1P lyase inhibitor.

33. The method of claim 32, wherein said lymphocyte sequestration agent is an S1P receptor agonist, further wherein said S1P receptor agonist is a homolog, analog, or derivative of the compound myriosin, depicted below.



34. The method of claim 33, wherein said S1P receptor agonist is a 2-aminopropane-1,3-diol compound according to formula I:

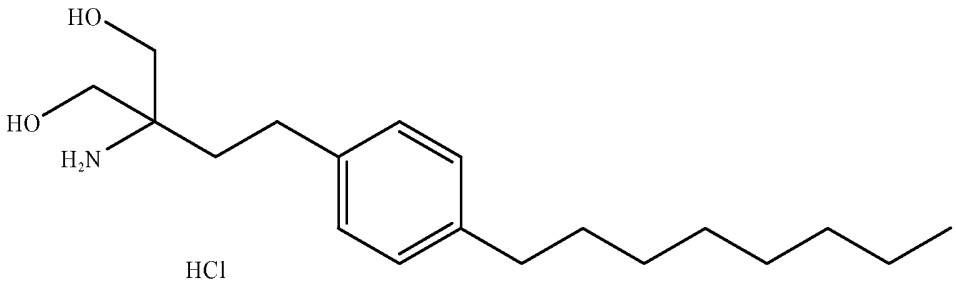


I

or a pharmaceutically acceptable salt thereof, wherein R is an optionally substituted straight or branched carbon chain, an optionally substituted aryl, an optionally substituted cycloalkyl or the like;

and R^2, R^3, R^4, and R^5 are the same or different and each is a hydrogen, an alkyl, an acyl, or an alkoxycarbonyl, or R^4 and R^5 may be bonded to form an alkylene chain, which may be substituted by alkyl, aryl, or an alkoxycarbonyl.

35. The method of claim 34, wherein said 2-aminopropane-1,3-diol compound is the compound 2-amino-2[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride (FTY720), depicted below, or a pharmaceutically acceptable salt thereof.



36. The method of claim 35, wherein said immunogen comprises a bacterial or viral antigen.

37. A pharmaceutical kit comprising:

(a) a container holding an immunogen; and

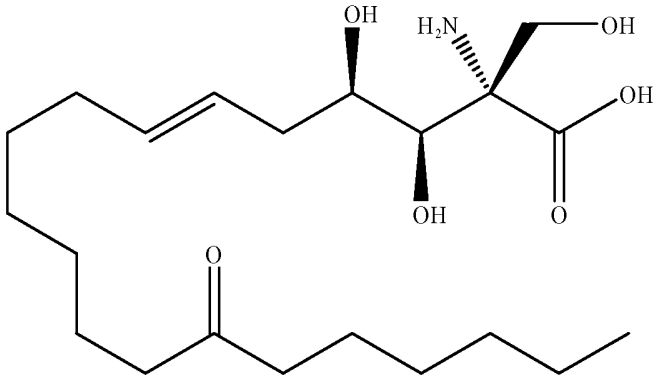
(b) an adjuvant composition comprising a leukocyte sequestration agent;

wherein said immunogen is provided in a therapeutically effective amount to prevent or treat a bacterial infection or a viral infection in a subject in need thereof, and wherein said immunogen is one or more antigenic polypeptides, immunogenic polypeptides or polysaccharides.

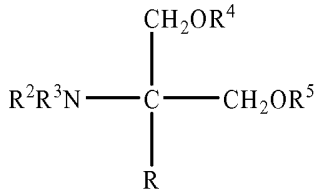
38. The pharmaceutical kit of claim 37, wherein said leukocyte sequestration agent is a lymphocyte sequestration agent.

39. The pharmaceutical kit of claim 38, wherein said lymphocyte sequestration agent is an S1P receptor agonist, a sphingosine kinase inhibitor, or a S1P lyase inhibitor.

40. The pharmaceutical kit of claim 39, wherein said lymphocyte sequestration agent is an S1P receptor agonist, further wherein said S1P receptor agonist is a homolog, analog, or derivative of the compound myriosin, depicted below.



41. The pharmaceutical kit of claim 40, wherein said S1P receptor agonist is a 2-aminopropane-1,3-diol compound according to formula I:

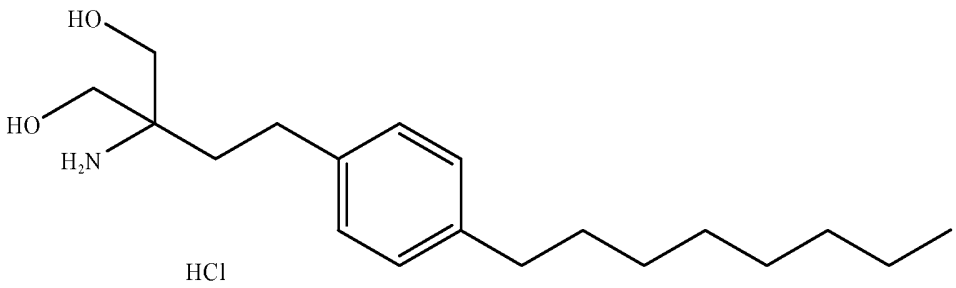


I

or a pharmaceutically acceptable salt thereof, wherein R is an optionally substituted straight or branched carbon chain, an optionally substituted aryl, an optionally substituted cycloalkyl or the like;

and R^2, R^3, R^4, and R^5 are the same or different and each is a hydrogen, an alkyl, an acyl, or an alkoxycarbonyl, or R^4 and R^5 may be bonded to form an alkylene chain, which may be substituted by alkyl, aryl, or an alkoxycarbonyl.

42. The pharmaceutical kit of claim 41, wherein said 2-aminopropane-1,3-diol compound is the compound 2-amino-2[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride (FTY720), depicted below, or a pharmaceutically acceptable salt thereof.



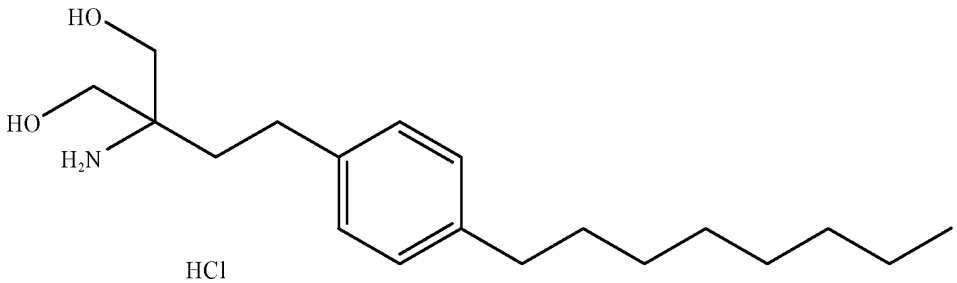
43. The pharmaceutical kit of claim 42, wherein said immunogen comprises a bacterial or viral antigen.

44. A method for preventing or treating a cancer with a viral etiology comprising administering at least one cycle of therapy to a subject in need thereof, wherein said cycle of therapy comprises administering at least one dose of a therapeutically effective amount of a leukocyte sequestration agent to said subject.

45. The method of claim 44, wherein said cancer with a viral etiology is nasopharyngeal cancer, non-Hodgkin's lymphoma, cervical cancer, hepatocellular carcinoma, Kaposi's sarcoma, or adult T-cell leukemia.

46. The method of claim 44, wherein said leukocyte sequestration agent is a S1P receptor agonist, a sphingosine kinase inhibitor, or a S1P lyase inhibitor.

47. The method of claim 46, wherein said S1P receptor agonist is 2-amino-2[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride (FTY720), depicted below, or a pharmaceutically acceptable salt thereof.



HCl

Figure 1

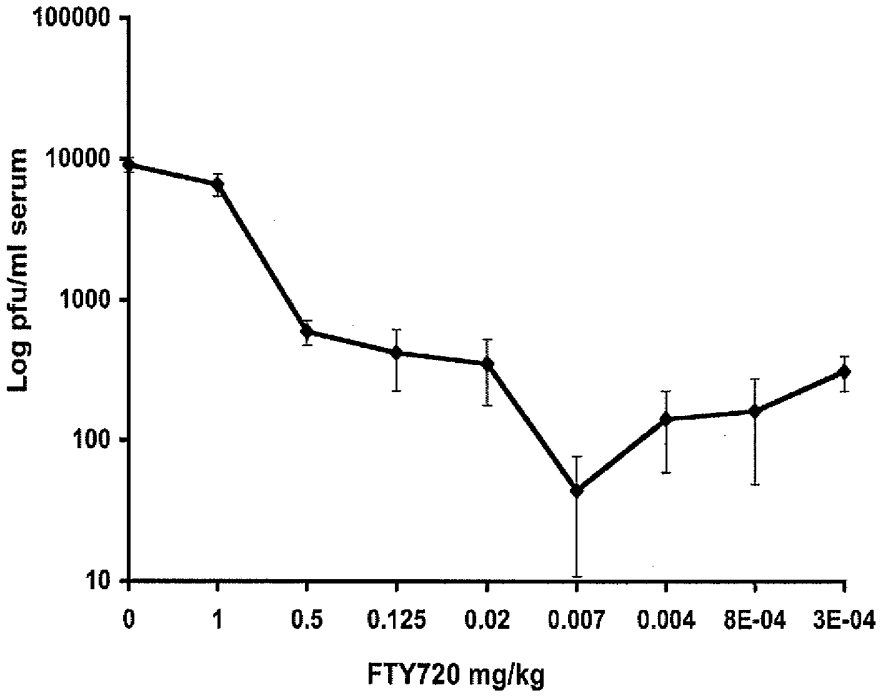

100000
10000
1000
100
10
Log pfu/ml serum
0
1
0.5
0.125
0.02
0.007
0.004
8E-04
3E-04
FTY720 mg/kg

Figure 2

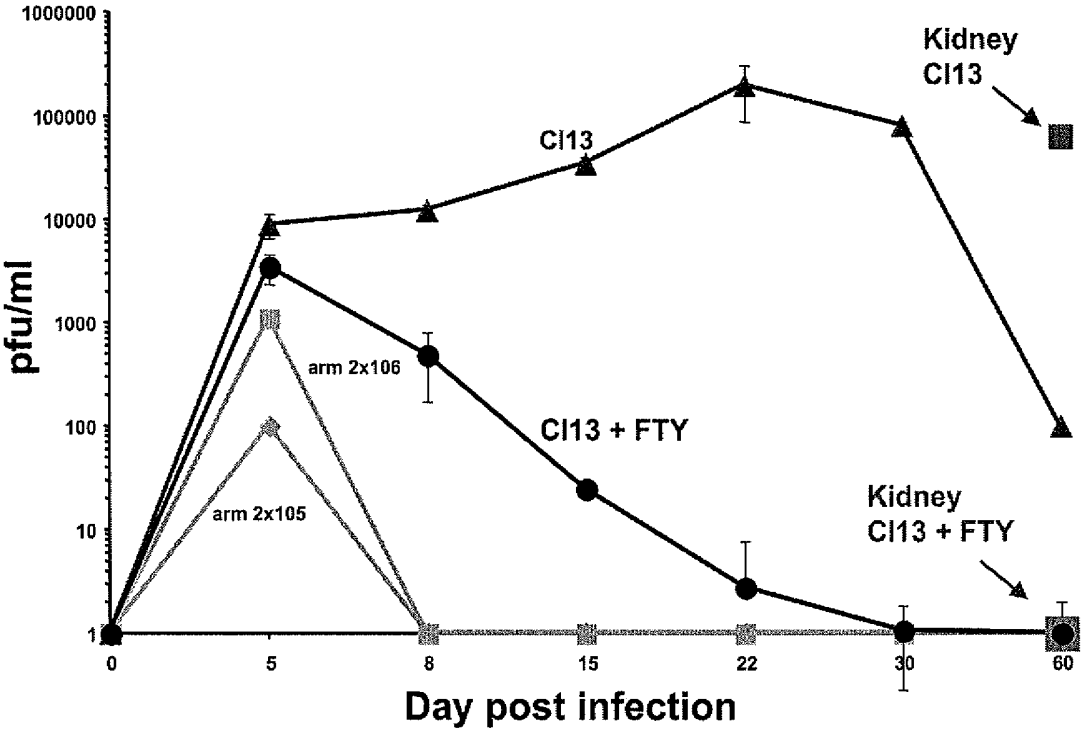

1000000
100000
10000
1000
100
10
1
pfu/ml
0
5
8
15
22
30
60
Day post infection
Cl13
arm 2x106
arm 2x105
Cl13 + FTY
Kidney
Cl13
Kidney
Cl13 + FTY

Figure 3

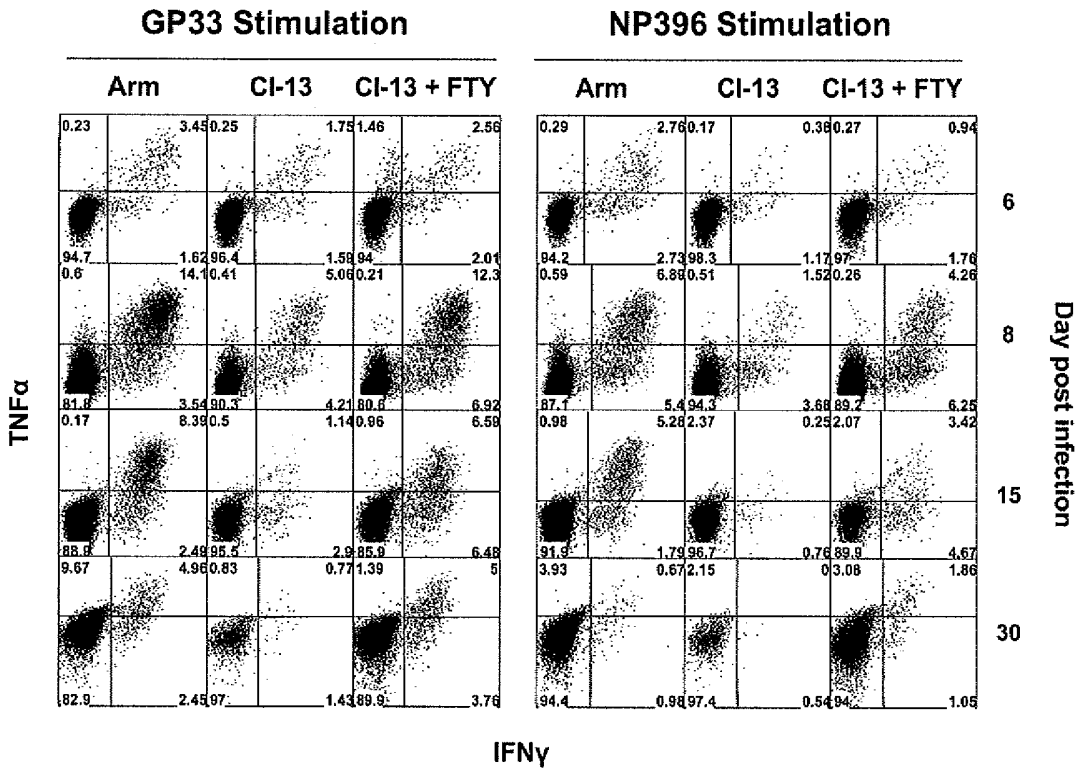

GP33 Stimulation
NP396 Stimulation
Arm
CI-13
CI-13 + FTY
Arm
CI-13
CI-13 + FTY
TNFα
IFNγ
Day post infection
6
8
15
30

Figure 4

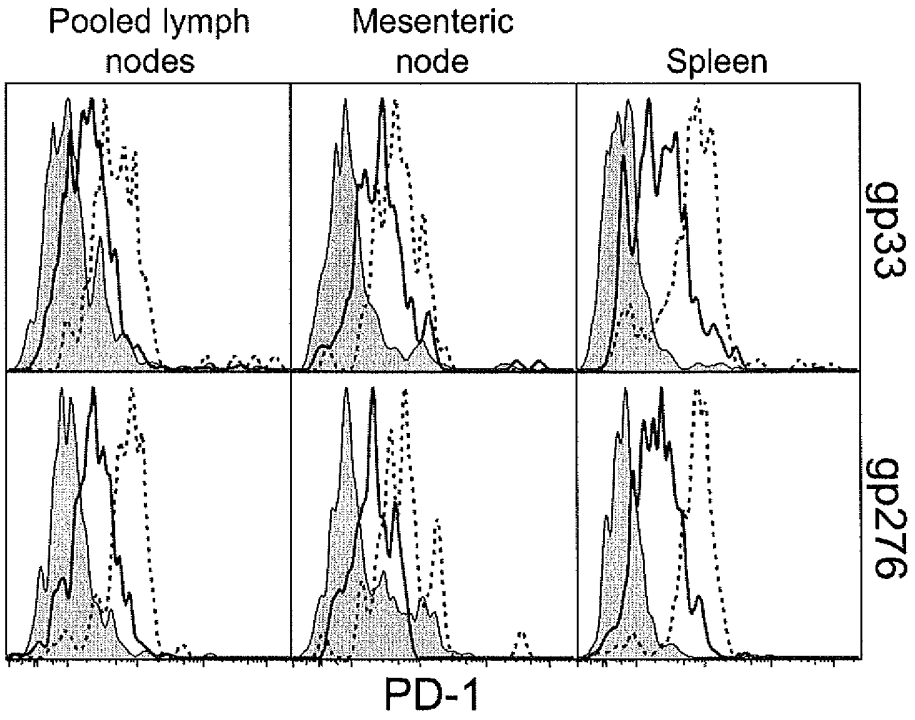


Figure 5

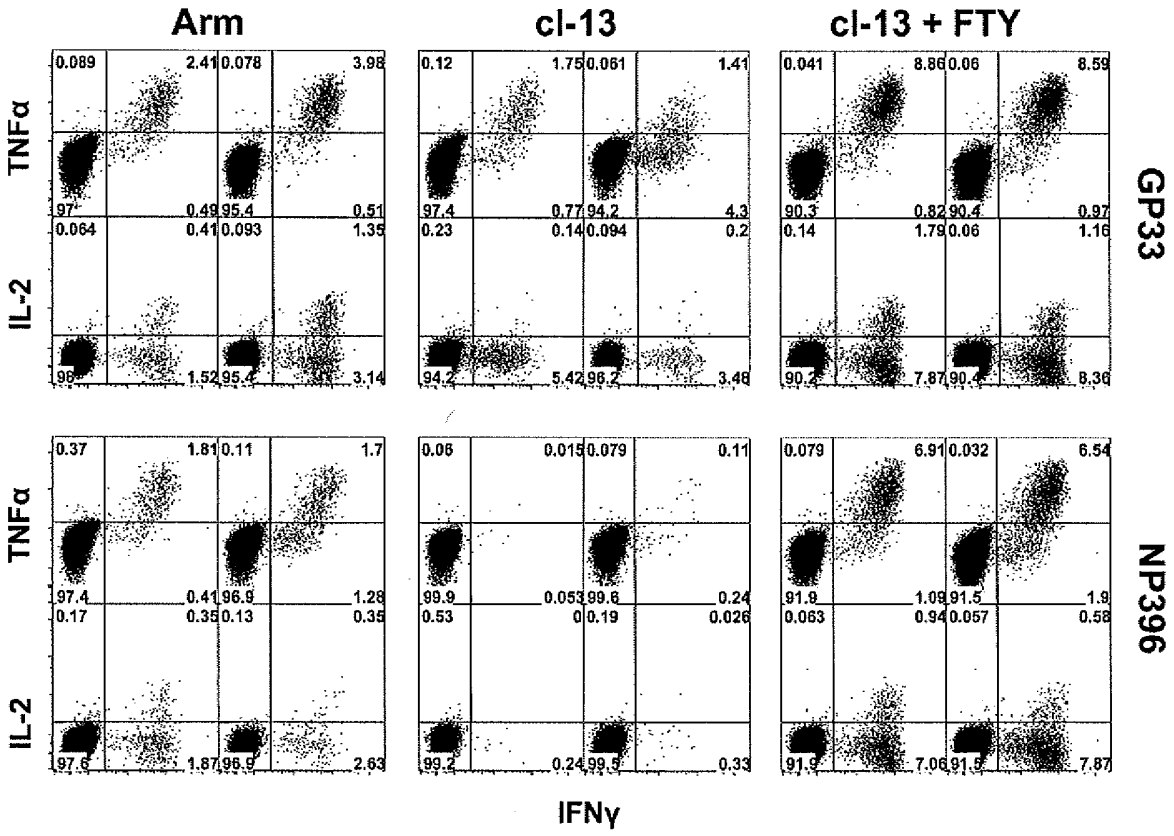

Arm
cl-13
cl-13 + FTY
TNFα
IL-2
GP33
NP396
IFNγ
0.089 2.41 0.078 3.98
97 0.49 95.4 0.51
0.064 0.41 0.093 1.35
98 1.52 95.4 3.14
0.12 1.75 0.061 1.41
97.4 0.77 94.2 4.3
0.23 0.14 0.094 0.2
94.2 5.42 96.2 3.48
0.041 8.86 0.06 8.59
90.3 0.82 90.4 0.97
0.14 1.79 0.06 1.16
90.2 7.87 90.4 8.36
0.37 1.81 0.11 1.7
97.4 0.41 96.9 1.28
0.17 0.35 0.13 0.35
97.6 1.87 96.9 2.63
0.06 0.015 0.079 0.11
99.9 0.053 99.6 0.24
0.53 0 0.19 0.026
99.2 0.24 99.5 0.33
0.079 6.91 0.032 6.54
91.9 1.09 91.5 1.9
0.063 0.94 0.057 0.58
91.9 7.06 91.5 7.87

Figure 6

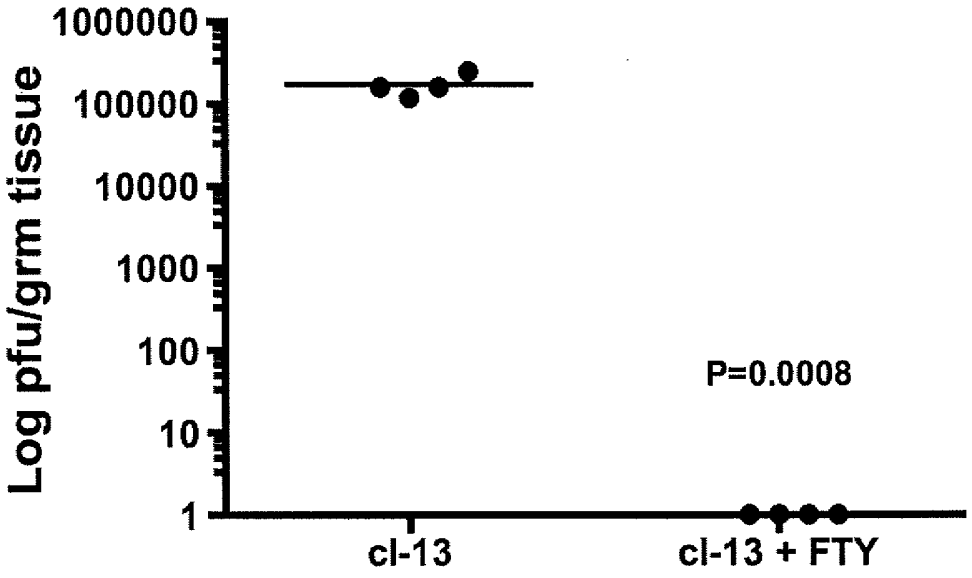

1000000
100000
10000
1000
100
10
1
Log pfu/grm tissue
P=0.0008
cl-13
cl-13 + FTY

Figure 7

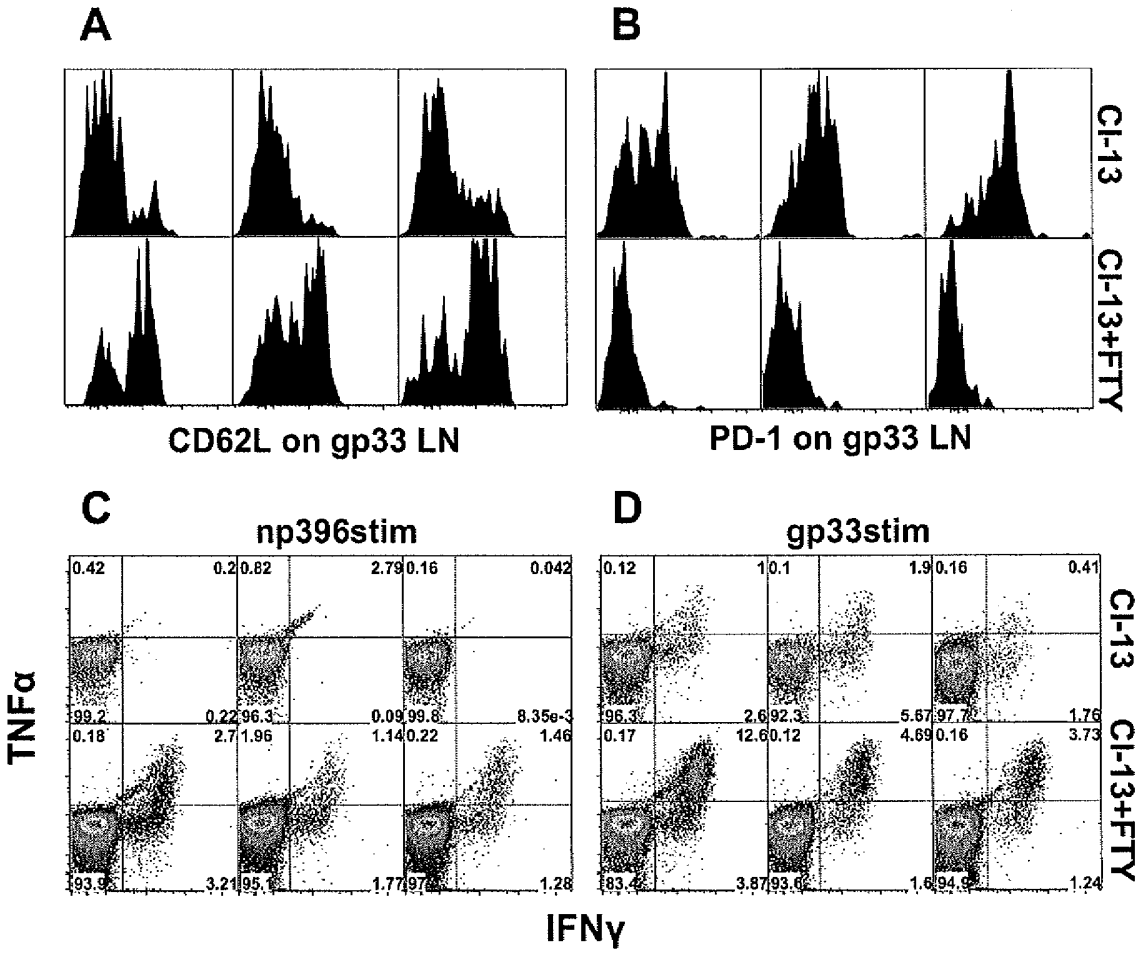

A
B
Cl-13
Cl-13+FTY
CD62L on gp33 LN
PD-1 on gp33 LN
C
np396stim
D
gp33stim
TNFα
IFNγ
0.42
0.2
0.82
2.79
0.16
0.042
99.2
0.22
96.3
0.09
99.8
8.35e-3
0.18
2.7
1.96
1.14
0.22
1.46
93.9
3.21
95.1
1.77
97.6
1.28
0.12
1
0.1
1.9
0.16
0.41
96.3
2.6
92.3
5.67
97.7
1.76
0.17
12.6
0.12
4.69
0.16
3.73
83.4
3.87
93.6
1.6
94.9
1.24

Figure 8

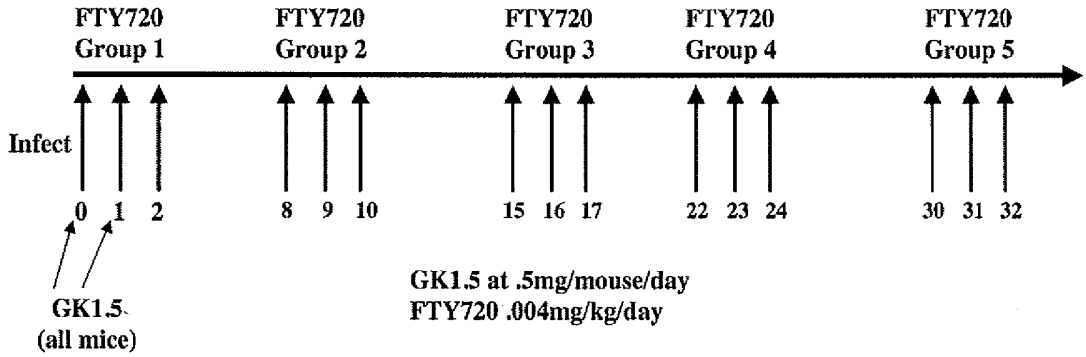

FTY720
Group 1
FTY720
Group 2
FTY720
Group 3
FTY720
Group 4
FTY720
Group 5
Infect
0 1 2
8 9 10
15 16 17
22 23 24
30 31 32
GK1.5
(all mice)
GK1.5 at .5mg/mouse/day
FTY720 .004mg/kg/day

Figure 9

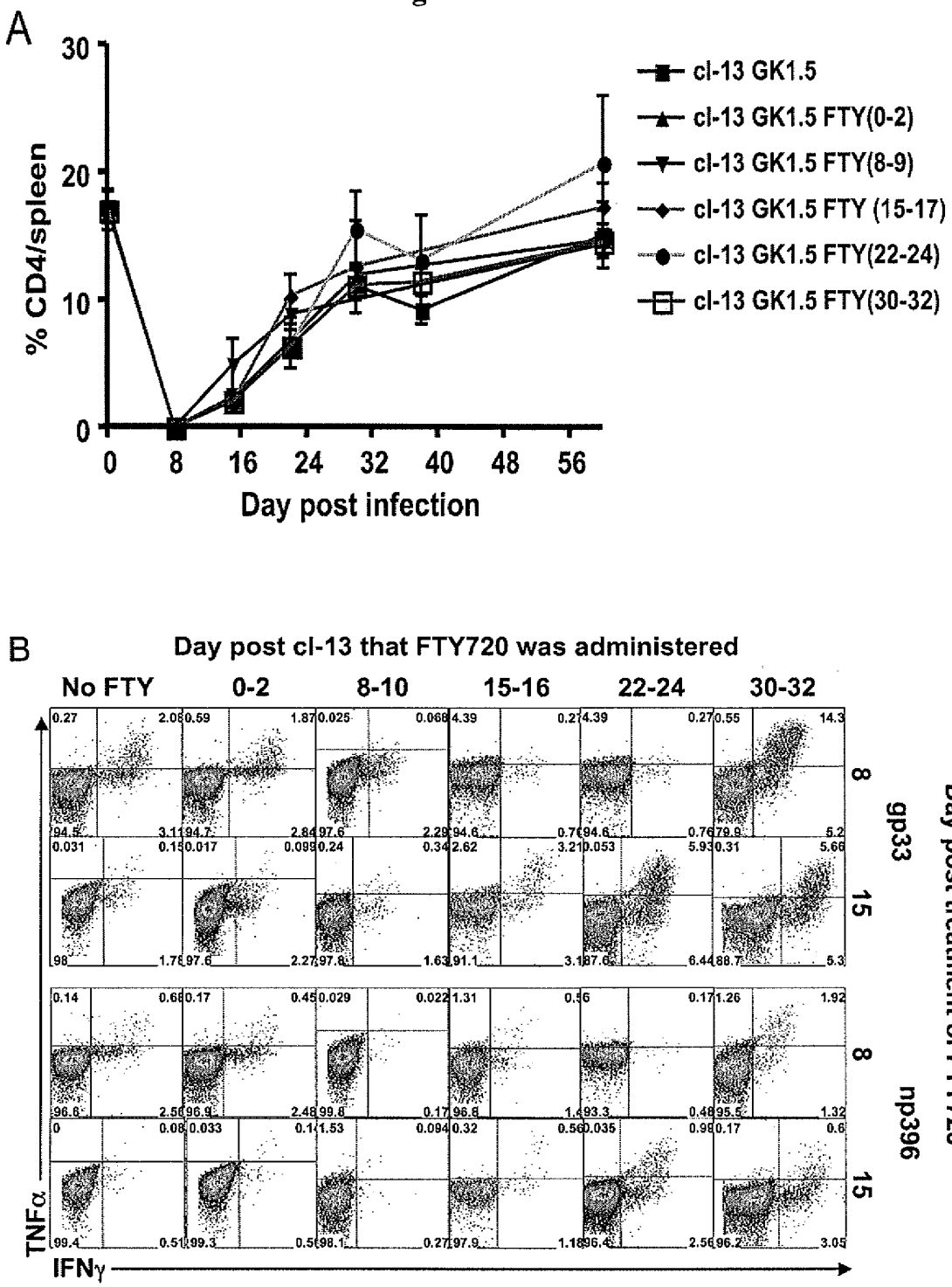

A
% CD4/spleen
Day post infection
cl-13 GK1.5
cl-13 GK1.5 FTY(0-2)
cl-13 GK1.5 FTY(8-9)
cl-13 GK1.5 FTY (15-17)
cl-13 GK1.5 FTY(22-24)
cl-13 GK1.5 FTY(30-32)
B
Day post cl-13 that FTY720 was administered
No FTY
0-2
8-10
15-16
22-24
30-32
gp33
np396
Day post treatment of FTY720
TNFα
IFNγ

Figure 10

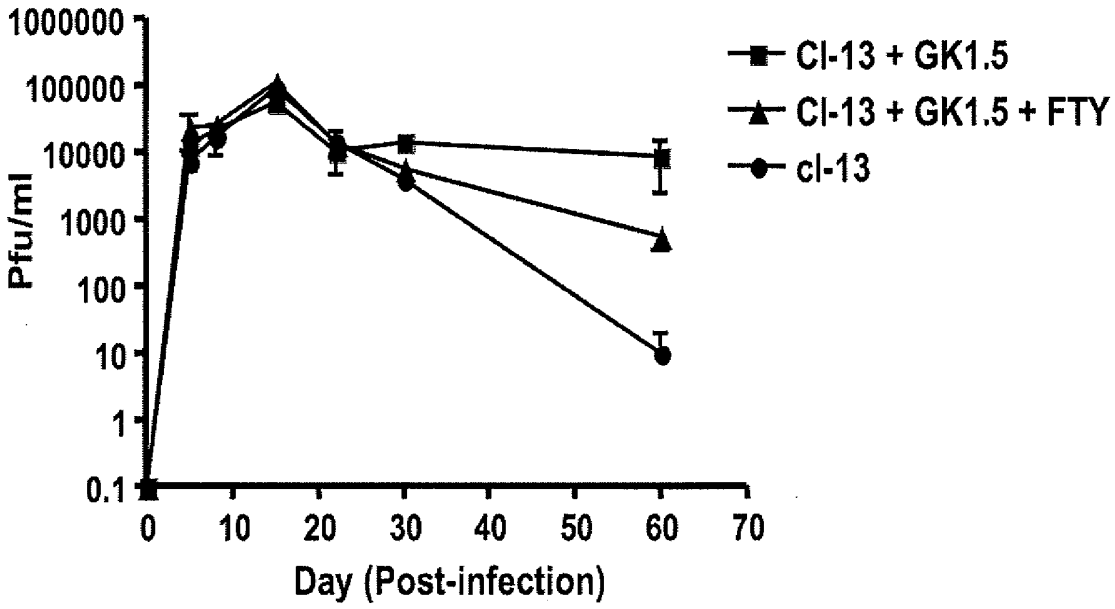
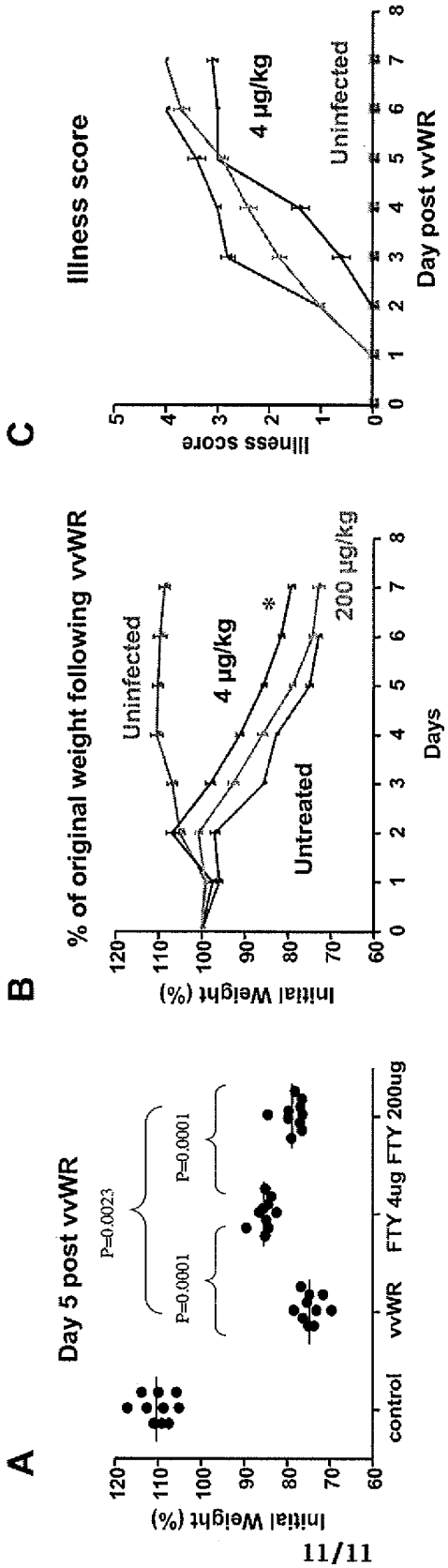

1000000
100000
10000
1000
100
10
1
0.1
Pfu/ml
0
10
20
30
40
50
60
70
Day (Post-infection)
Cl-13 + GK1.5
Cl-13 + GK1.5 + FTY
cl-13

Figure 11


A
Day 5 post vvWR
P=0.0023
P=0.0001
P=0.0001
Initial Weight (%)
120
110
100
90
80
70
60
control
vvWR
FTY 4ug
FTY 200ug
B
% of original weight following vvWR
Uninfected
4 µg/kg
Untreated
200 µg/kg
*
Initial Weight (%)
Days
C
Illness score
Illness score
4 µg/kg
Uninfected
Day post vvWR

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/053923

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/135 A61P31/04 A61P31/12 A61P31/18 A61P31/22 A61P31/16

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)
A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/072562 A (NOVARTIS AG [CH]; NOVARTIS PHARMA GMBH [AT]; BRINKMANN VOLKER [DE]; FE) 13 July 2006 (2006-07-13) page 14 page 21, paragraph 8 page 22, paragraph 2 page 13	1-47
X	EP 1 201 236 A (MITSUBISHI PHARMA CORP [JP]; MATSUMORI AKIRA [JP] MATSUMORI AKIRA [JP]) 2 May 2002 (2002-05-02) claims	1-47
X	GB 2 400 318 A (NOVARTIS AG [CH]; MITSUBISHI PHARMA CORP [JP]) 13 October 2004 (2004-10-13) page 1, paragraph 1 page 16, paragraph 5	1-47

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 September 2008	23/09/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Büttner, Ulf

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/053923

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/118523 A (NOVARTIS AG [CH]; NOVARTIS PHARMA GMBH [AT]; HINTERDING KLAUS [DE]; HO) 15 December 2005 (2005-12-15) pages 18-20	1-47
X	WO 2005/082089 A (IRM LLC [US]; PAN SHIFENG [US]; MARSILJE THOMAS H [US]; LU WENSHOU [US) 9 September 2005 (2005-09-09) paragraphs [0041], [0042]	1-47
X	MIYAMOTO TADASHI ET AL: "Therapeutic effects of FTY720, a new immunosuppressive agent, in a murine model of acute viral myocarditis" JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, vol. 37, no. 6, May 2001 (2001-05), pages 1713-1718, XP002494538 ISSN: 0735-1097 page 1715, column 1	1-47

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2008/053923

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. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-47 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

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.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an 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 reportcovers only those claims for which fees were paid, specifically claims Nos.:

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

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2008/053923

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006072562 A	13-07-2006	AU 2006204524 A1	13-07-2006
		CA 2592883 A1	13-07-2006
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		DE 102004016947 A1	21-10-2004
		EP 1613288 A1	11-01-2006
		WO 2004089341 A1	21-10-2004
		ES 2228282 A1	01-04-2005
		FR 2854073 A1	29-10-2004
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		HK 1071685 A1	04-08-2006
		HR 20050886 A2	30-11-2006
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		IS 8114 A	01-11-2005
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		MA 27729 A1	02-01-2006
		MX PA05010860 A	25-05-2006
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		EP 1753712 A1	21-02-2007
		JP 2008500302 T	10-01-2008
		KR 20070015439 A	02-02-2007
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		AU 2005216540 A1	09-09-2005
		BR PI0507988 A	31-07-2007
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		CN 1921863 A	28-02-2007
		EP 1718307 A2	08-11-2006
		JP 2007523910 T	23-08-2007