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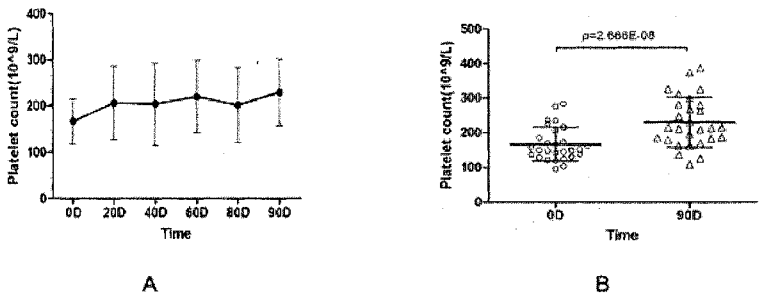
(54) **Title**: MOGROSIDE IV AND MOGROSIDE V AS AGONIST/STIMULATOR/UN-BLOCKING AGENT FOR TOLL-LIKE RECEPTOR 4 AND ADJUVANT FOR USE IN HUMAN/ANIMAL VACCINE AND TO STIMULATE IMMUNITY AGAINST DISEASE AGENTS.

Figure 4



(57) **Abstract**: This patent presents evidence that Mogroside IV.Mogroside V and combinations thereof act as a Toll -Like Receptor -4 agonist and immune stimulant that can be utilized for both therapeutic vaccine design in cancer and for other pathogenic agents. Therapy with Mogroside IV. Mogroside V and combinations thereof are also presented to created immune clearance of a viral infection.

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TITLE

Mogroside IV and Mogroside V as Agonist/Stimulator/un-blocking agent for Toll-Like Receptor 4 and Adjuvant for use in human/animal vaccine and to stimulate immunity against disease agents.

FIELD OF THE INVENTION

This application relates generally to selectively stimulating toll-like receptors (specifically TLR-4) utilising Mogroside IV and Mogroside V and methods of therapeutically using such compounds as adjuvant and in the therapy of pathogens which undermine the patients immune system by attenuating the full functions of TLR-4.

BACKGROUND OF THE INVENTION

Luo Han Guo (also known as Luo Han Kuo) is a fruit of the *Siraitia grosvenori Swingle* (formally *Momoridca grosvenori Swingle*) belonging to the Cucurbitaceae species. These fruits are cultivated in parts of China and extract of the Luo Han Guo plant is currently manufactured and marketed as a natural low-calorie sweetener, for example, PURELO™. US Patent No. 5,411,755 describes a process for preparing a sweet juice from Luo Han Guo fruit. the sweetness of the extract from Luo Han Guo is due to the presence of highly stable triterpene glycosides, known as mogrosides, which are about 250 to 300 times sweeter than sucrose. These compounds possess a triterpene backbone with two to six glucose units attached. Examples of cucurbitane glycosides purified from *Siraitia grosvenori* include 20-hydroxy-11-oxomogroside IA_1, 11-oxomogroside IIE, 11-oxomogroside IA_1, Mogroside IIE, Mogroside III, Mogroside IV, Mogroside V, Siamenoside I and Neogroside. We in this patent present the molecule Mogroside IV and Mogroside V for the first time as Toll-Like Receptor 4 agonists and immune vaccine adjuvants and stimulators.

The innate immune system provides the body with a first line defence against invading pathogens. In an innate immune response, an invading pathogen is recognised by a germ line-encoded receptor, the activation of which initiates a signalling cascade that leads to the induction of cytokine expression. Innate immune system receptors have broad specificity, recognising molecular structures that are highly conserved among different pathogens. One family of these receptors is known as Toll-like receptors (TLRs), due to their homology with receptors that were first identified and named in Drosophila, and are present in cells such as macrophages, dendritic cells, and epithelial cells.

There are at least ten different TLRs identified in mammals. Ligands and corresponding signalling cascades have been identified for some of these receptors. For example, TLR2 is activated by the lipoprotein of bacteria (e.g., E. coli), TLR3 is activated by double-stranded RNA, TLR4 is activated by lipopolysaccharide (i.e., LPS or endotoxin) of Gram-negative bacteria (e.g., Salmonella and E. coli O157:H7), TLR5 is activated by flagellin of motile bacteria (e.g., Listeria), TLR7 recognises and responds to imiquimod and TLR9 is activated by unmethylated CpG sequences of pathogen DNA. The stimulation of each of these receptors leads to activation of the transcription factor NF-.kappa.B, and other signalling molecules that are involved in regulating the expression of cytokine genes, including those encoding tumour necrosis factor-alpha (TNF-.alpha.), interleukin-1 (IL-1), and certain chemokines.

There are a number of diseases, disorders, and conditions linked to TLRs dysfunctions, such therapies using a TLR-4 stimulant would enhance therapy options, these including but not limited to melanoma, non-small cell lung carcinoma, hepatocellular carcinoma, basal cell carcinoma, renal cell carcinoma, myeloma, hepatic fibrosis, and viral infections such as HBV, Flaviviridae viruses, HCV, HPV, RSV, SARS, or influenza.

The treatment of Flaviviridae virus infections with TLR-4 Agonist is particularly promising. Viruses of the Flaviviridae family comprise at least three distinguishable

genera including pestiviruses, flaviviruses, and hepaciviruses (Calisher, et al., J. Gen. Virol., 1993, 70, 37-43). While pestiviruses cause many economically important animal diseases such as bovine viral diarrhea virus (BVDV), classical swine fever virus (CSFV, hog cholera) and border disease of sheep (BDV), their importance in human disease is less well characterized (Moennig, V., et al., Adv. Vir. Res. 1992, 48, 53-98). Flaviviruses are responsible for important human diseases such as dengue fever and yellow fever while hepaciviruses cause hepatitis C virus infections in humans. Other important viral infections caused by the Flaviviridae family include West Nile virus (WNV) Japanese encephalitis virus (JEV), tick-borne encephalitis virus, Jungian virus, Murray Valley encephalitis, St Louis enchaplitis, Omsk hemorrhagic fever virus and Zika virus. Combined, infections from the Flaviviridae virus family cause significant mortality, morbidity and economic losses throughout the world.

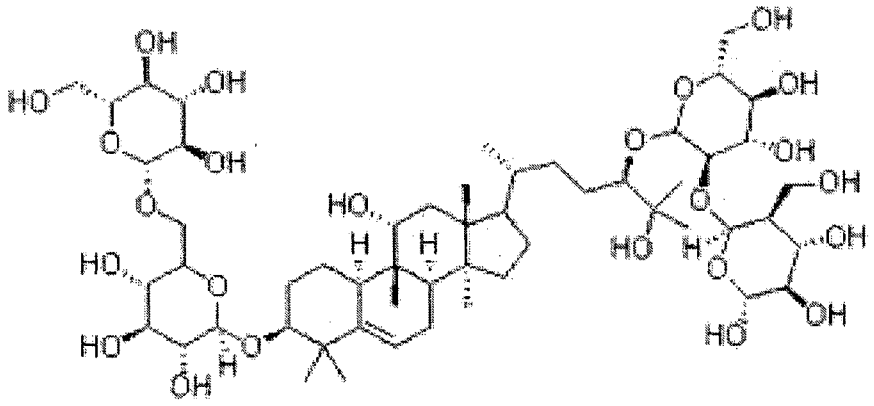
The hepatitis C virus (HCV) is the leading cause of chronic liver disease worldwide (Boyer, N. et al. J Hepatol. 32:98-112, 2000) so a significant focus of current antiviral research is directed toward the development of improved methods of treatment of chronic HCV infections in humans (Di Besceglie, A. M. and Bacon, B. R., Scientific American, October: 80-85, (1999); Gordon, C. P., et al., J. Med. Chem. 2005, 48, 1-20; Maradpour, D.; et al., Nat. Rev. Micro. 2007, 5(6), 453-463). A number of HCV treatments are reviewed by Bymock et al. in Antiviral Chemistry & Chemotherapy, 11:2; 79-95 (2000). Currently, there is primarily a group of antiviral compounds, known as Direct Antiviral Agents. Eg (Telaprevir and Boceprevir) together with ribavirin, a nucleoside analog, and interferon-alpha (.alpha.) (IFN), that are used for the treatment of chronic HCV infections in humans. Ribavirin alone is not effective in reducing viral RNA levels, has significant toxicity, and is known to induce anemia. The combination of IFN and ribavirin has been reported to be effective in the management of chronic hepatitis C (Scott, L. J., et al. Drugs 2002, 62, 507-556) but less than half the patients given this treatment show a persistent benefit. Recently there is documented proof that Hep C virus attenuated Toll-Like Receptor -4 functions to allow it achieve chronic status in infected patients. This discovery has

driven research to identify and utilise molecules such as Mogroside IV and mogroside V and those disclosed in this patent to create immune clearance of this virus in patients by reactivating TLR-4 function.

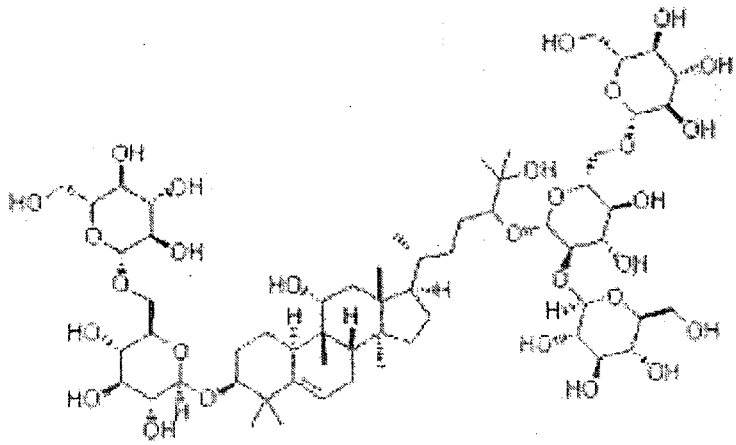
SUMMARY OF THE INVENTION

While not wishing to be bound by theory, the inventor currently has proved that the compounds of Mogroside IV and Mogroside V are agonists of TLR-4 and may also be agonists of other TLRs.

Another aspect of the present invention includes a method for treating a viral infection comprising administering a therapeutically effective amount of a compound of Mogroside IV and /or Mogroside V.The compound is administered to a human subject in need thereof, such as a human being who is infected with a virus of the Flaviviridae family, such as hepatitis C virus. In one embodiment, the viral infection is acute or chronic HCV infection. In one embodiment the Mogroside IV and/or mogroside V acting as immune stimulator creates a cytotoxic cell immune clearance of the Hep C virus when administered alone or in combination with a known antiviral.



Mogroside IV



Mogroside V

Another aspect of the present invention includes the use of a compounds of Mogroside IV and/or Mogroside V for the manufacture of a medicament for the treatment of a viral infection. Another aspect of the present invention includes a compound of Mogroside IV and/or Mogroside V for the use in treating a viral infection. In one embodiment, the viral infection is acute or chronic HCV infection. In one embodiment, the Mogroside IV and/or Mogroside V treatment results in a reduction in viral load as a result of the stimulation of a cytotoxic cell response to Hep C infected cells.

In another aspect, a method for treating Flaviviridae viral infections is provided comprising administering an therapeutically effective amount of a compound of Mogroside IV and/or Mogroside V to a patient in need thereof. The compound of Mogroside IVand/or Mogroside V is administered to a human subject in need thereof, such as a human being who is infected with viruses of the Flaviviridae family. In another embodiment, the compounds of Mogroside IV and/or Mogroside V is administered to a human subject in need thereof, such as a human being who is infected with a HCV virus. In one embodiment, the treatment results in the reduction of one or more of the in viral loads or clearance of virus RNA in the patient.

In another embodiment, provided is a method of treating and/or preventing a disease caused by a viral infection wherein the viral infection is caused by a virus selected

from the group consisting of dengue virus, yellow fever virus, West Nile virus, Japanese encephalitis virus, tick-borne encephalitis virus, Junjin virus, Murray Valley encephalitis virus, St Louis encephalitis virus, Omsk hemorrhagic fever virus, bovine viral diarrhea virus, Zika virus and Hepatitis C virus; by administering to a subject in need thereof a therapeutically effective amount of compounds of Mogroside IV and/or Mogroside V or a pharmaceutically acceptable salt thereof.

In another aspect, provided is the use of compounds of Mogroside IV and/or Mogroside V for the manufacture of a medicament for the treatment of Flaviviridae viral infections. In another aspect, provided are compounds of Mogroside IV and/or Mogroside V for use in treating a Flaviviridae viral infection. In one embodiment, the Flaviviridae viral infection is acute or chronic HCV infection. In one embodiment of each aspect of use and compounds the treatment results in the reduction of one or more of the viral loads or clearance of viruses in the patient by generating a cytotoxic cells stimulated by the agonist activity of Mogroside V! and/or V to T-LR-4.

In another aspect, provided is a method for treating or preventing HCV comprising administering an effective amount of compounds of Mogroside IV and/or Mogroside V to a patient in need thereof. In another aspect, provided is the use of the compounds of the present invention for the manufacture of a medicament for the treatment or prevention of viral infections.

Pharmaceutical Formulations

The compounds of this invention are formulated with conventional carriers and excipients, which will be selected in accord with ordinary practice. Tablets will contain excipients, fillers, binders and the like. Aqueous formulations are prepared in sterile form, and when intended for delivery by other than oral administration generally will be isotonic. All formulations will optionally contain excipients such as those set forth in the Handbook of Pharmaceutical Excipients (1986), herein incorporated by reference in its entirety. Excipients include ascorbic acid and other antioxidants,

chelating agents such as EDTA, carbohydrates such as dextrin, hydroxyalkylcellulose, hydroxyalkylmethylcellulose, stearic acid and the like. The pH of the formulations ranges from about 3 to about 11, but is ordinarily about 7 to 10.

While it is possible for the active ingredients to be administered alone it may be preferable to present them as pharmaceutical formulations. The formulations of the invention, both for veterinary and for human use, comprise at least one active ingredient, together with one or more acceptable carriers and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and physiologically innocuous to the recipient thereof.

The formulations include those suitable for the foregoing administration routes. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Techniques and formulations generally are found in Remington's Pharmaceutical Sciences (Mack Publishing Co., Easton, Pa.), herein incorporated by reference in its entirety. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be administered as a bolus, electuary or paste.

A tablet is made by compression or molding, optionally with one or more accessory ingrediens. Compressed tablets may be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered active ingredient moistened with an inert liquid diluent. The tablets may optionally be coated or scored and optionally are formulated so as to provide slow or controlled release of the active ingredient.

For administration to the eye or other external tissues e.g., mouth and skin, the formulations are preferably applied as a topical ointment or cream containing the active ingredient(s) in an amount of, for example, 0.075 to 20% w/w (including active ingredient(s) in a range between 0.1% and 20% in increments of 0.1% w/w such as 0.6% w/w, 0.7% w/w, etc.), preferably 0.2 to 15% w/w and most preferably 0.5 to 10% w/w. When formulated in an ointment, the active ingredients may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredients may be formulated in a cream with an oil-in-water cream base.

If desired, the aqueous phase of the cream base may include, for example, at least 30% w/w of a polyhydric alcohol, i.e. an alcohol having two or more hydroxyl groups such as propylene glycol, butane 1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol (including PEG 400) and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active ingredients through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethyl sulphoxide and related analogs.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While the phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic

emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil and fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

Emulgents and emulsion stabilizers suitable for use in the formulation of the invention include Tween.RTM. 60, Span.RTM. 80, cetostearyl alcohol, benzyl alcohol, myristyl alcohol, glyceryl mono-stearate and sodium lauryl sulfate.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties. The cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils are used.

Pharmaceutical formulations according to the present invention comprise compounds of Mogroside IV and/or MogrosideV of the invention together with one or more pharmaceutically acceptable carriers or excipients and optionally other therapeutic agents. Pharmaceutical formulations containing the active ingredients may be in any form suitable for the intended method of administration. When used for oral use for example, tablets, troches, lozenges, aqueous or oil suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixirs may be prepared. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents including sweetening agents, flavoring agents,

coloring agents and preserving agents, in order to provide a palatable preparation. Tablets containing the active ingredi(s) in admixture with non-toxic pharmaceutically acceptable excipient which are suitable for manufacture of tablets are acceptable. These excipients may be, for example, inert diluents, such as calcium or sodium carbonate, lactose, lactose monohydrate, , povidone, calcium or sodium phosphate; granulating and disintegrating agents, such as maize starch, or alginic acid; binding agents, such as cellulose, microcrystalline cellulose, starch, gelatin or acacia; and lubricating agents, such as magnesium stearate, stearic acid or talc. Tablets may be uncoated or may be coated by known techniques including microencapsulation to delay disintegration and adsorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

Formulations for oral use may be also presented as hard gelatine capsules where the active ingredient is mixed with an inert solid diluent, for example calcium phosphate or kaolin, or as soft gelatine capsules wherein the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions of the invention contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include a suspending agent, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropyl methylcelluose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethyleneoxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan monooleate). The aqueous suspension may also contain one or more preservatives such as ethyl or n-propyl p-hydroxy-benzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose or saccharin.

Oil suspensions may be formulated by suspending the active ingredient in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oral suspensions may contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth herein, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid.

Dispersible powders and granules of the invention suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient(s) in admixture with a dispersing or wetting agent, a suspending agent, and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those disclosed above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, such as olive oil or arachis oil, a mineral oil, such as liquid paraffin, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan monooleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and flavoring agents. Syrups and elixirs may be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring or a coloring agent.

The pharmaceutical compositions of the invention may be in the form of a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using

those suitable dispersing or wetting agents and suspending agents which have been mentioned herein. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,3-butanediol or prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables.

The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a time-release formulation intended for oral administration to humans may contain approximately 1 to 1000 mg of active material compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95% of the total compositions (weight:weight). The pharmaceutical composition can be prepared to provide easily measurable amounts for administration. For example, an aqueous solution intended for intravenous infusion may contain from about 3 to 500 .micro.g of the active ingredient per milliliter of solution in order that infusion of a suitable volume at a rate of about 30 mL/hr can occur.

Formulations suitable for administration to the eye include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the active ingredient. The active ingredient is preferably present in such formulations in a concentration of 0.5 to 20%, advantageously 0.5 to 10% particularly about 1.5% w/w.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin

and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

Formulations suitable for intrapulmonary or nasal administration have a particle size for example in the range of 0.1 to 500 .micro.m (including particle sizes in a range between 0.1 and 500 .micro.m in increments such as 0.5 .micro.m, 1 .micro m, 30 .micro .m, 35 .micro m, etc.), which is administered by rapid inhalation through the nasal passage or by inhalation through the mouth so as to reach the alveolar sacs. Suitable formulations include aqueous or oily solutions of the active ingredient(s). Formulations suitable for aerosol or dry powder administration may be prepared according to conventional methods and may be delivered with other therapeutic agents such as compounds heretofore used in the treatment or prophylaxis of infections as described herein.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient(s) such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostatics and solutes which render the formulation isotonically compatable with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents.

The formulations are presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for

injection, immediately prior to use. Extemporaneous injection solutions and suspensions are prepared from sterile powders, granules and tablets of the kind previously described. Preferred unit dosage formulations are those containing a daily dose or unit daily sub-dose, as herein above recited, or an appropriate fraction thereof, of the active ingredient(s).

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavoring agents.

Compounds of the invention can also be formulated to provide controlled release of the active ingredient to allow less frequent dosing or to improve the pharmacokinetic or toxicity profile of the active ingredient(s). Accordingly, the invention also provides compositions comprising one or more compounds of the invention formulated for sustained or controlled release.

The effective dose of an active ingredient depends at least on the nature of the condition being treated, toxicity, whether the compound is being used prophylactically (lower doses) or against an active disease or condition, the method of delivery, and the pharmaceutical formulation, and will be determined by the clinician using conventional dose escalation studies. The effective dose can be expected to be from about 0.0001 to about 10 mg/kg body weight per day, typically from about 0.001 to about 1 mg/kg body weight per day, more typically from about 0.01 to about 1 mg/kg body weight per day, more typically from about 0.05 to about 0.5 mg/kg body weight per day. For example, the daily candidate dose for an adult human of approximately 70 kg body weight will range from about 0.5 mg to about 500 mg, .

In yet another embodiment, the present application discloses pharmaceutical compositions comprising a compound of Mogroside IV or V or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or excipient.

Routes of Administration

One or more compounds of the invention (herein referred to as the active ingredient(s) are administered by any route appropriate to the condition to be treated. Suitable routes include oral, rectal, nasal, topical (including buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural), and the like. It will be appreciated that the preferred route may vary with for example the condition of the recipient. An advantage of the compounds of this invention is that they are orally bioavailable and can be dosed orally.

Combination Therapy

In one embodiment, the compounds of the present invention are used in combination with an additional active therapeutic ingredient or agent.

In one embodiment, combinations of the compound of Mogroside IV and /or Mogroside V and additional active agents may be selected to treat patients with a viral infection, for example, HBV, HCV, or HIV infection.

Useful active therapeutic agents for HBV include reverse transcriptase inhibitors, such as lamivudine (Epivir.RTM.), adefovir (Hepsera.RTM.), tenofovir (Viread.RTM.), telbivudine (Tyzeka.RTM.), entecavir (Baraclude.RTM.), and Clevudine.RTM.. Other useful active therapeutic agents include immunomodulators, such as interferon alpha-2b (Intron A.RTM.), pegylated interferon alpha-2a (Pegasys.RTM.), interferon alpha 2a (Roferon.RTM.), interferon alpha N1, prednisone, predinisolone, Thymalfasin.RTM., retinoic acid receptor agonists, 4-methylumbelliferone, Alamifovir.RTM., Metacavir.RTM., Albuferon.RTM., agonists of TLRs (e.g., TLR-7 agonists), and cytokines.

With regard to treatment for HCV, other active therapeutic ingredients or agents are interferons, ribavirin or its analogs, HCV NS3 protease inhibitors, alpha-glucosidase

1 inhibitors, hepatoprotectants, nucleoside or nucleotide inhibitors of HCV NS5B polymerase, non-nucleoside inhibitors of HCV NS5B polymerase, HCV NS5A inhibitors, TLR-7 agonists, cyclophillin inhibitors, HCV IRES inhibitors, pharmacokinetic enhancers, and other drugs for treating HCV, or mixtures thereof.

Combinations of the compounds are typically selected based on the condition to be treated, cross-reactivities of ingredients and pharmaco-properties of the combination. For example, when treating an infection (e.g., HCV), the compositions of the invention are combined with other active agents (such as those described herein).

Suitable active agents or ingredients which can be combined with the compounds of Mogroside IV and/or Mogroside V and salts thereof, can include one or more compounds selected from the group consisting of:

(1) interferons selected from the group consisting of pegylated rIFN-alpha 2b (PEG-Intron), pegylated rIFN-alpha 2a (Pegasys), rIFN-alpha 2b (Intron A), rIFN-alpha 2a (Roferon-A), interferon alpha (MOR-22, OPC-18, Alfaferone, Alfanative, Multiferon, subalin), interferon alfacon-1 (Infergen), interferon alpha-n1 (Wellferon), interferon alpha-n3 (Alferon), interferon-beta (Avonex, DL-8234), interferon-omega (omega DUROS, Biomed 510), albinterferon alpha-2b (Albuferon), IFN alpha-2b XL, BLX-883 (Locteron), DA-3021, glycosylated interferon alpha-2b (AVI-005), PEG-Infergen, PEGylated interferon lambda-1 (PEGylated IL-29), belerofon, and mixtures thereof;

(2) ribavirin and its analogs selected from the group consisting of ribavirin (Rebetol, Copegus), taribavirin (Viramidine), and mixtures thereof;

(3) HCV NS3 protease inhibitors selected from the group consisting of boceprevir (SCH-503034, SCH-7), telaprevir (VX-950), TMC435350, BI-1335, BI-1230, MK-7009, VBY-376, VX-500, BMS-790052, BMS-605339, PHX-1766, AS-101, YH-5258, YH5530, YH5531, ITMN-191, and mixtures thereof;

(4) alpha-glucosidase 1 inhibitors selected from the group consisting of celgosivir (MX-3253), Miglitol, UT-231B, and mixtures thereof;

(5) hepatoprotectants selected from the group consisting of IDN-6556, ME 3738, LB-84451, silibilin, MitoQ, and mixtures thereof;

(6) nucleoside or nucleotide inhibitors of HCV NS5B polymerase selected from the group consisting of R1626, R7128 (R4048), IDX184, IDX-102, BCX-4678, valopicitabine (NM-283), MK-0608, and mixtures thereof;

(7) non-nucleoside inhibitors of HCV NS5B polymerase selected from the group consisting of PF-868554, VCH-759, VCH-916, JTK-652, MK-3281, VBY-708, VCH-222, A848837, ANA-598, GL60667, GL59728, A-63890, A-48773, A-48547, BC-2329, VCH-796 (nesbuvir), GSK625433, BILN-1941, XTL-2125, GS-9190, and mixtures thereof;

(8) HCV NS5A inhibitors selected from the group consisting of AZD-2836 (A-831), A-689, and mixtures thereof;

(9) TLR-7 agonists selected from the group consisting of ANA-975, SM-360320, and mixtures thereof;

(10) cyclophillin inhibitors selected from the group consisting of DEBIO-025, SCY-635, NIM811, and mixtures thereof;

(11) HCV IRES inhibitors selected from the group consisting of MCI-067,

(12) pharmacokinetic enhancers selected from the group consisting of BAS-100, SPI-452, PF-4194477, TMC-41629, roxythromycin, and mixtures thereof; and

(13) other drugs for treating HCV selected from the group consisting of. Incivek(Telaprevir). Victrelis (Boceprevir) thymosin alpha 1 (Zadaxin), nitazoxanide (Alinea, NTZ), BIVN-401 (virostat), PYN-17 (altirex), KPE02003002, actilon (CPG-10101), KRN-7000, civacir, GI-5005, XTL-6865, BIT225, PTX-111, ITX2865, TT-033i, ANA 971, NOV-205, tarvacin, EHC-18, VGX-410C, EMZ-702, AVI 4065, BMS-650032, BMS-791325, Bavituximab, MDX-1106 (ONO-4538), Oglufanide, VX-497 (merimepodib), and mixtures thereof.

In addition, the compounds of the invention may be employed in combination with other therapeutic agents for the treatment or prophylaxis of AIDS and/or one or more other diseases present in a human subject suffering from AIDS (e.g., bacterial and/or fungal infections, other viral infections such as hepatitis B or hepatitis C, or cancers such as Kaposi's sarcoma). The additional therapeutic agent(s) may be coformulated with one or more salts of the invention (e.g., coformulated in a tablet).

Examples of such additional therapeutic agents include agents that are effective for the treatment or prophylaxis of viral, parasitic or bacterial infections, or associated conditions, or for treatment of tumors or related conditions, include 3'-azido-3'-deoxythymidine (zidovudine, AZT), 2'-deoxy-3'-thiacytidine (3TC), 2',3'-dideoxy-2',3'-didehydroadenosine (D4A), 2',3'-dideoxy-2',3'-didehydrothymidine (D4T), carbovir (carbocyclic 2',3'-dideoxy-2',3'-didehydroguanosine), 3'-azido-2',3'-dideoxyuridine, 5-fluorothymidine, (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU), 2-chlorodeoxyadenosine, 2-deoxycoformycin, 5-fluorouracil, 5-fluorouridine, 5-fluoro-2'-deoxyuridine, 5-trifluoromethyl-2'-deoxyuridine, 6-azauridine, 5-fluoroorotic acid, methotrexate, triacetyluridine, 1-(2'-deoxy-2'-fluoro-1-.beta.-arabinosyl)-5-iodocytidine (FIAC), tetrahydro-imidazo(4,5,1-jk)-(1,4)-benzodiazepin-2(1H)-thione (TIBO), 2'-nor-cyclicGMP, 6-methoxypurine arabinoside (ara-M), 6-methoxypurine arabinoside 2'-O-

valerate; cytosine arabinoside (ara-C), 2',3'-dideoxynucleosides such as 2',3'-dideoxycytidine (ddC), 2',3'-dideoxyadenosine (ddA) and 2',3'-dideoxyinosine (ddI); acyclic nucleosides such as acyclovir, penciclovir, famciclovir, ganciclovir, HPMPC, PMEA, PMEG, PMPA, PMPDAP, FPMPA, HPMPA, HPMPDAP, (2R,5R)-9.fwdarw.tetrahydro-5-(phosphonomethoxy)-2-furanyladenine, (2R,5R)-1.fwdarw.tetrahydro-5-(phosphonomethoxy)-2-furanylthymine; other antivirals including ribavirin (adenine arabinoside), 2-thio-6-azauridine, tubercidin, aurintricarboxylic acid, 3-deazaneoplanocin, neoplanocin, rimantidine, adamantine, and foscarnet (trisodium phosphonoformate); antibacterial agents including bactericidal fluoroquinolones (ciprofloxacin, pefloxacin and the like); aminoglycoside bactericidal antibiotics (streptomycin, gentamicin, amicacin and the like); .beta.-lactamase inhibitors (cephalosporins, penicillins and the like); other antibacterials including tetracycline, isoniazid, rifampin, cefoperazone, claithromycin and azithromycin, antiparasite or antifungal agents including pentamidine (1,5-bis(4'-aminophenoxy)pentane), 9-deaza-inosine, sulfamethoxazole, sulfadiazine, quinapyramine, quinine, fluconazole, ketoconazole, itraconazole, Amphotericin B, 5-fluorocytosine, clotrimazole, hexadecylphosphocholine and nystatin; renal excretion inhibitors such as probenicid; nucleoside transport inhibitors such as dipyridamole, dilazep and nitrobenzylthioinosine, immunomodulators such as FK506, cyclosporin A, thymosin .alpha.-1; cytokines including TNF and TGF-.beta.; interferons including IFN-.alpha., IFN-.beta., and IFN-.gamma.; interleukins including various interleukins, macrophage/granulocyte colony stimulating factors including GM-CSF, G-CSF, M-CSF, cytokine antagonists including anti-TNF antibodies, anti-interleukin antibodies, soluble interleukin receptors, protein kinase C inhibitors and the like.

Examples of suitable active therapeutic agents or ingredients which can be combined with the compound of the invention, and which have activity against HIV, include 1) HIV protease inhibitors, e.g., amprenavir, atazanavir, fosamprenavir, indinavir, lopinavir, ritonavir, lopinavir+ritonavir, nelfinavir, saquinavir, tipranavir, brecanavir, darunavir, TMC-126, TMC-114, mozenavir (DMP-450), JE-2147 (AG1776), AG1859, DG35, L-756423, RO0334649, KNI-272, DPC-681, DPC-684, and GW640385X, DG17, PPL-100, 2) a HIV non-nucleoside inhibitor of reverse transcriptase, e.g.,

capravirine, emivirine, delaviridine, efavirenz, nevirapine, (+) calanolide A, etravirine, GW5634, DPC-083, DPC-961, DPC-963, MIV-150, and TMC-120, TMC-278 (rilpivirine), efavirenz, BILR 355 BS, VRX 840773, UK-453,061, RDEA806, 3) a HIV nucleoside inhibitor of reverse transcriptase, e.g., zidovudine, emtricitabine, didanosine, stavudine, zalcitabine, lamivudine, abacavir, amdoxovir, elvucitabine, alovudine, MIV-210, racivir (-FTC), D-d4FC, emtricitabine, phosphazide, fozivudine tidoxil, fosalvudine tidoxil, apricitibine (AVX754), amdoxovir, KP-1461, abacavir+lamivudine, abacavir+lamivudine+zidovudine, zidovudine+lamivudine, 4) a HIV nucleotide inhibitor of reverse transcriptase, e.g., tenofovir, tenofovir disoproxil fumarate+emtricitabine, tenofovir disoproxil fumarate+emtricitabine+efavirenz, and adefovir, 5) a HIV integrase inhibitor, e.g., curcumin, derivatives of curcumin, chicoric acid, derivatives of chicoric acid, 3,5-dicaffeoylquinic acid, derivatives of 3,5-dicaffeoylquinic acid, aurintricarboxylic acid, derivatives of aurintricarboxylic acid, caffeic acid phenethyl ester, derivatives of caffeic acid phenethyl ester, tyrphostin, derivatives of tyrphostin, quercetin, derivatives of quercetin, S-1360, zintevir (AR-177), L-870812, and L-870810, MK-0518 (raltegravir), BMS-707035, MK-2048, BA-011, BMS-538158, GSK364735C, 6) a gp41 inhibitor, e.g., enfuvirtide, sifuvirtide, FB006M, TRI-1144, SPC3, DES6, Locus gp41, CovX, and REP 9, 7) a CXCR4 inhibitor, e.g., AMD-070, 8) an entry inhibitor, e.g., SP01A, TNX-355, 9) a gp120 inhibitor, e.g., BMS-488043 and BlockAide/CR, 10) a G6PD and NADH-oxidase inhibitor, e.g., immunitin, 10) a CCR5 inhibitor, e.g., aplaviroc, vicriviroc, INCB9471, PRO-140, INCB15050, PF-232798, CCR5 mAb004, and maraviroc, 11) an interferon, e.g., pegylated rIFN-alpha 2b, pegylated rIFN-alpha 2a, rIFN-alpha 2b, IFN alpha-2b XL, rIFN-alpha 2a, consensus IFN alpha, infergen, rebif, locteron, AVI-005, PEG-infergen, pegylated IFN-beta, oral interferon alpha, feron, reaferon, intermax alpha, r-IFN-beta, infergen+actimmune, IFN-omega with DUROS, and albuferon, 12) ribavirin analogs, e.g., rebetol, copegus, levovirin, VX-497, and viramidine (taribavirin) 13) NS5a inhibitors, e.g., A-831 and A-689, 14) NS5b polymerase inhibitors, e.g., NM-283, valopicitabine, R1626, PSI-6130 (R1656), HIV-796, BILB 1941, MK-0608, NM-107, R7128, VCH-759, PF-868554, GSK625433, and XTL-2125, 15) NS3 protease inhibitors, e.g., SCH-503034 (SCH-7), VX-950 (Telaprevir), ITMN-191, and BILN-2065, 16) alpha-glucosidase 1 inhibitors, e.g., MX-3253 (celgosivir) and UT-231B,

17) hepatoprotectants, e.g., IDN-6556, ME 3738, MitoQ, and LB-84451, 18) non-nucleoside inhibitors of HIV, e.g., benzimidazole derivatives, benzo-1,2,4-thiadiazine derivatives, and phenylalanine derivatives, 19) other drugs for treating HIV, e.g., zadaxin, nitazoxanide (alinea), BIVN-401 (virostat), DEBIO-025, VGX-410C, EMZ-702, AVI 4065, bavituximab, oglufanide, PYN-17, KPE02003002, actilon (CPG-10101), KRN-7000, civacir, GI-5005, ANA-975 (isatoribine), XTL-6865, ANA 971, NOV-205, tarvacin, EHC-18, and NIM811, 19) pharmacokinetic enhancers, e.g., BAS-100 and SPI452, 20) RNAse H inhibitors, e.g., ODN-93 and ODN-112, 21) other anti-HIV agents, e.g., VGV-1, PA-457 (bevirimat), ampligen, HRG214, cytolin, polymun, VGX-410, KD247, AMZ 0026, CYT 99007, A-221 HIV, BAY 50-4798, MDX010 (iplimumab), PBS119, ALG889, and PA-1050040.

Again by way of example, the following list discloses exemplary HIV antivirals, with their corresponding U.S. Patent numbers, incorporated by reference with regard to the preparation of such antivirals, which can be combined with the compounds of the present invention.

Exemplary HIV Antivirals and Patent Numbers

Ziagen (Abacavir sulfate, U.S. Pat. No. 5,034,394)

Epzicom (Abacavir sulfate/lamivudine, U.S. Pat. No. 5,034,394)

Hepsera (Adefovir dipivoxil, U.S. Pat. No. 4,724,233)

Agenerase (Amprenavir, U.S. Pat. No. 5,646,180)

Reyataz (Atazanavir sulfate, U.S. Pat. No. 5,849,911)

Rescriptor (Delavirdine mesilate, U.S. Pat. No. 5,563,142)

Hivid (Dideoxycytidine; Zalcitabine, U.S. Pat. No. 5,028,595)

Videx (Dideoxyinosine; Didanosine, U.S. Pat. No. 4,861,759)

Sustiva (Efavirenz, U.S. Pat. No. 5,519,021)

Emtriva (Emtricitabine, U.S. Pat. No. 6,642,245)

Lexiva (Fosamprenavir calcium, U.S. Pat. No. 6,436,989)

Virudin; Triapten; Foscavir (Foscarnet sodium, U.S. Pat. No. 6,476,009)

Crixivan (Indinavir sulfate, U.S. Pat. No. 5,413,999)

Epivir (Lamivudine, U.S. Pat. No. 5,047,407)

Combivir (Lamivudine/Zidovudine, U.S. Pat. No. 4,724,232)

Aluviran (Lopinavir)

Kaletra (Lopinavir/ritonavir, U.S. Pat. No. 5,541,206)

Viracept (Nelfinavir mesilate, U.S. Pat. No. 5,484,926)

Viramune (Nevirapine, U.S. Pat. No. 5,366,972)

Norvir (Ritonavir, U.S. Pat. No. 5,541,206)

Invirase; Fortovase (Saquinavir mesilate, U.S. Pat. No. 5,196,438)

Zerit (Stavudine, U.S. Pat. No. 4,978,655)

Truvada (Tenofovir disoproxil fumarate/emtricitabine, U.S. Pat. No. 5,210,085)

Aptivus (Tipranavir)

Retrovir (Zidovudine; Azidothymidine, U.S. Pat. No. 4,724,232)

Where the disorder is cancer, combination with at least one other anticancer therapy is envisaged. In particular, in anti-cancer therapy, combination with other anti-neoplastic agent (including chemotherapeutic, hormonal or antibody agents) is envisaged as well as combination with surgical therapy and radiotherapy. Combination therapies according to the present invention thus comprise the administration of at least one compound of formula (I)and/or formula (2) or a salt or solvate thereof, and the use of at least one other cancer treatment method. Preferably, combination therapies according to the present invention comprise the administration of at least one compound of formula (I)and/formula(2) or a salt or solvate thereof, and at least one other pharmaceutically active agent, preferably an anti-neoplastic agent. The compound(s) of formula (I)) and/or formula (2) the other pharmaceutically active agent(s) may be administered together or separately and, when administered separately this may occur simultaneously or sequentially in any order (including administration on different days according to the therapy regimen)

and by any convenient route. The amounts of the compound(s) of formula (II) and/or Formuls (2) the other pharmaceutically active agent(s) and the relative timings of administration will be selected in order to achieve the desired combined therapeutic effect.

In one embodiment, the further anti-cancer therapy is at least one additional anti-neoplastic agent. Any anti-neoplastic agent that has activity versus a susceptible tumor being treated may be utilized in the combination. Typical anti-neoplastic agents useful include, but are not limited to, anti-microtubule agents such as diterpenoids and vinca alkaloids; platinum coordination complexes; alkylating agents such as nitrogen mustards, oxazaphosphorines, alkylsulfonates, nitrosoureas, and triazenes; antibiotic agents such as anthracyclins, actinomycins and bleomycins; topoisomerase II inhibitors such as epipodophyllotoxins; antimetabolites such as purine and pyrimidine analogues and anti-folate compounds; topoisomerase I inhibitors such as camptothecins; hormones and hormonal analogues; signal transduction pathway inhibitors; nonreceptor tyrosine kinase angiogenesis inhibitors; immunotherapeutic agents; proapoptotic agents; and cell cycle signaling inhibitors.

Anti-microtubule or anti-mitotic agents are phase specific agents active against the microtubules of tumor cells during M or the mitosis phase of the cell cycle. Examples of anti-microtubule agents include, but are not limited to, diterpenoids and vinca alkaloids.

Diterpenoids, which are derived from natural sources, are phase specific anti-cancer agents that operate at the G.sub.2/M phases of the cell cycle. It is believed that the diterpenoids stabilize the .beta.-tubulin subunit of the microtubules, by binding with this protein. Disassembly of the protein appears then to be inhibited with mitosis being arrested and cell death following. Examples of diterpenoids include, but are not limited to, paclitaxel and its analog docetaxel.

Paclitaxel, 5.beta.,20-epoxy-1,2.alpha.,4,7.beta.,10.beta.,13.alpha.-hexa-hydroxytax-- 11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)--N-benzoyl-3-phenylisoserine; is a natural diterpene product isolated from the Pacific yew tree Taxus brevifolia and is commercially available as an injectable solution TAXOL.RTM.. It is a member of the taxane family of terpenes. Paclitaxel has been approved for clinical use in the treatment of refractory ovarian cancer in the United States (Markman et al., Yale Journal of Biology and Medicine, 64:583, 1991; McGuire et al., Ann. Intern, Med., 111:273, 1989) and for the treatment of breast cancer (Holmes et al., J. Nat. Cancer Inst., 83:1797, 1991.) It is a potential candidate for treatment of neoplasms in the skin (Einzig et. al., Proc. Am. Soc. Clin. Oncol., 20:46) and head and neck carcinomas (Forastire et. al., Sem. Oncol., 20:56, 1990). The compounds also shows potential for the treatment of polycystic kidney disease (Woo et. al., Nature, 368:750. 1994), lung cancer and malaria. Treatment of patients with paclitaxel results in bone marrow suppression (multiple cell lineages, Ignoff, R J. et. al, Cancer Chemotherapy Pocket Guide.sub.A 1998) related to the duration of dosing above a threshold concentration (50 nM) (Kearns, C M. et. al., Seminars in Oncology, 3(6) p. 16-23, 1995).

Docetaxel, (2R,3S)--N-carboxy-3-phenylisoserine,N-te/f-butyl ester, 13-ester with 5.beta.-20-epoxy-1,2.alpha.,4,7.beta.,10.beta.,13.alpha.-hexahydroxytax-1- 1-en-9-one 4-acetate 2-benzoate, trihydrate; is commercially available as an injectable solution as TAXOTERE.RTM.. Docetaxel is indicated for the treatment of breast cancer. Docetaxel is a semisynthetic derivative of paclitaxel q.v., prepared using a natural precursor, 10-deacetyl-baccatin III, extracted from the needle of the European Yew tree.

Vinca alkaloids are phase specific anti-neoplastic agents derived from the periwinkle plant. Vinca alkaloids act at the M phase (mitosis) of the cell cycle by binding specifically to tubulin. Consequently, the bound tubulin molecule is unable to polymerize into microtubules. Mitosis is believed to be arrested in metaphase with

cell death following. Examples of vinca alkaloids include, but are not limited to, vinblastine, vincristine, and vinorelbine.

Vinblastine, vincaleukoblastine sulfate, is commercially available as VELBAN.RTM. as an injectable solution. Although, it has possible indication as a second line therapy of various solid tumors, it is primarily indicated in the treatment of testicular cancer and various lymphomas including Hodgkin's Disease; and lymphocytic and histiocytic lymphomas. Myelosuppression is the dose limiting side effect of vinblastine. Vincristine, vincaleukoblastine, 22-oxo-, sulfate, is commercially available as ONCOVIN.RTM. as an injectable solution. Vincristine is indicated for the treatment of acute leukemias and has also found use in treatment regimens for Hodgkin's and non-Hodgkin's malignant lymphomas. Alopecia and neurologic effects are the most common side effect of vincristine and to a lesser extent myelosupression and gastrointestinal mucositis effects occur.

Vinorelbine, 3',4'-didehydro-4'-deoxy-C'-norvincaleukoblastine [R--(R*,R*)-2,3-dihydroxybutanedioate (1:2)(salt)], commercially available as an injectable solution of vinorelbine tartrate (NAVELBINE.RTM.), is a semisynthetic vinca alkaloid. Vinorelbine is indicated as a single agent or in combination with other chemotherapeutic agents, such as cisplatin, in the treatment of various solid tumors, particularly non-small cell lung, advanced breast, and hormone refractory prostate cancers. Myelosuppression is the most common dose limiting side effect of vinorelbine.

Platinum coordination complexes are non-phase specific anti-cancer agents, which are interactive with DNA. The platinum complexes enter tumor cells, undergo, aquation and form intra- and interstrand crosslinks with DNA causing adverse biological effects to the tumor. Examples of platinum coordination complexes include, but are not limited to, oxaliplatin, cisplatin and carboplatin. Cisplatin, cis-diamminedichloroplatinum, is commercially available as PLATINOL.RTM. as an injectable solution. Cisplatin is primarily indicated in the treatment of metastatic

testicular and ovarian cancer and advanced bladder cancer. Carboplatin, platinum, diammine [1,1-cyclobutane-dicarboxylate(2-)-O,O'], is commercially available as PARAPLATIN.RTM. as an injectable solution. Carboplatin is primarily indicated in the first and second line treatment of advanced ovarian carcinoma.

Alkylating agents are non-phase anti-cancer specific agents and strong electrophiles. Typically, alkylating agents form covalent linkages, by alkylation, to DNA through nucleophilic moieties of the DNA molecule such as phosphate, amino, sulfhydryl, hydroxyl, carboxyl, and imidazole groups. Such alkylation disrupts nucleic acid function leading to cell death. Examples of alkylating agents include, but are not limited to, nitrogen mustards such as cyclophosphamide, melphalan, and chlorambucil; alkyl sulfonates such as busulfan; nitrosoureas such as carmustine; and triazenes such as dacarbazine. Cyclophosphamide, 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate, is commercially available as an injectable solution or tablets as CYTOXAN.RTM.. Cyclophosphamide is indicated as a single agent or in combination with other chemotherapeutic agents, in the treatment of malignant lymphomas, multiple myeloma, and leukemias. Melphalan, 4-[bis(2-chloroethyl)amino]-l-phenylalanine, is commercially available as an injectable solution or tablets as ALKERAN.RTM.. Melphalan is indicated for the palliative treatment of multiple myeloma and non-resectable epithelial carcinoma of the ovary. Bone marrow suppression is the most common dose limiting side effect of melphalan. Chlorambucil, 4-[bis(2-chloroethyl)amino]benzenebutanoic acid, is commercially available as LEUKERAN.RTM. tablets. Chlorambucil is indicated for the palliative treatment of chronic lymphatic leukemia, and malignant lymphomas such as lymphosarcoma, giant follicular lymphoma, and Hodgkin's disease. Busulfan, 1,4-butanediol dimethanesulfonate, is commercially available as MYLERAN.RTM. TABLETS. Busulfan is indicated for the palliative treatment of chronic myelogenous leukemia. Carmustine, 1,3-[bis(2-chloroethyl)-1-nitrosourea, is commercially available as single vials of lyophilized material as BiCNU.RTM.. Carmustine is indicated for the palliative treatment as a single agent or in combination with other agents for brain tumors, multiple myeloma, Hodgkin's disease, and non-Hodgkin's lymphomas. Dacarbazine,

5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide, is commercially available as single vials of material as DTIC-Dome.RTM.. Dacarbazine is indicated for the treatment of metastatic malignant melanoma and in combination with other agents for the second line treatment of Hodgkin's Disease.

Antibiotic anti-neoplasties are non-phase specific agents, which bind or intercalate with DNA. Typically, such action results in stable DNA complexes or strand breakage, which disrupts ordinary function of the nucleic acids leading to cell death. Examples of antibiotic anti-neoplastic agents include, but are not limited to, actinomycins such as dactinomycin, anthrocyclins such as daunorubicin and doxorubicin; and bleomycins. Dactinomycin, also know as Actinomycin D, is commercially available in injectable form as COSMEGEN.RTM.. Dactinomycin is indicated for the treatment of Wilm's tumor and rhabdomyosarcoma. Daunorubicin, (8S-cis-)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-.alpha.-l-lyxo-hexopyranos- yl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12 naphthacenedione hydrochloride, is commercially available as a liposomal injectable form as DAUNOXOME.RTM. or as an injectable as CERUBIDINE.RTM.. Daunorubicin is indicated for remission induction in the treatment of acute nonlymphocytic leukemia and advanced HIV associated Kaposi's sarcoma. Doxorubicin, (8S,10S)-10-[(3-amino-2,3,6-trideoxy-.alpha.-l-lyxo-hexopyranosyl)oxy]-8-- glycoloyl, 7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12 naphthacenedione hydrochloride, is commercially available as an injectable form as RUBEX.RTM. or ADRIAMYCIN RDF.RTM.. Doxorubicin is primarily indicated for the treatment of acute lymphoblastic leukemia and acute myeloblasts leukemia, but is also a useful component in the treatment of some solid tumors and lymphomas. Bleomycin, a mixture of cytotoxic glycopeptide antibiotics isolated from a strain of Streptomyces verticillus, is commercially available as BLENOXAN E.RTM.. Bleomycin is indicated as a palliative treatment, as a single agent or in combination with other agents, of squamous cell carcinoma, lymphomas, and testicular carcinomas.

Topoisomerase II inhibitors include, but are not limited to, epipodophyllotoxins. Epipodophyllotoxins are phase specific anti-neoplastic agents derived from the

mandrake plant. Epipodophyllotoxins typically affect cells in the S and G.sub.2 phases of the cell cycle by forming a ternary complex with topoisomerase II and DNA causing DNA strand breaks. The strand breaks accumulate and cell death follows. Examples of epipodophyllotoxins include, but are not limited to, etoposide and teniposide. Etoposide, 4'-demethyl-epipodophyllotoxin 9[4,6-0-(R)-ethylidene-.beta.-D-glucopyranoside], is commercially available as an injectable solution or capsules as VePESID.RTM. and is commonly known as VP-16. Etoposide is indicated as a single agent or in combination with other chemotherapy agents in the treatment of testicular and non-small cell lung cancers. Teniposide, 4'-demethyl-epipodophyllotoxin 9[4,6-0-(R)-thenylidene-.beta.-D-glucopyranoside], is commercially available as an injectable solution as VUMON.RTM. and is commonly known as VM-26. Teniposide is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia in children.

Antimetabolite neoplastic agents are phase specific anti-neoplastic agents that act at S phase (DNA synthesis) of the cell cycle by inhibiting DNA synthesis or by inhibiting purine or pyrimidine base synthesis and thereby limiting DNA synthesis. Consequently, S phase does not proceed and cell death follows. Examples of antimetabolite anti-neoplastic agents include, but are not limited to, fluorouracil, methotrexate, cytarabine, mercaptopurine, thioguanine, and gemcitabine. 5-fluorouracil, 5-fluoro-2,4-(1H,3H) pyrimidinedione, is commercially available as fluorouracil. Administration of 5-fluorouracil leads to inhibition of thymidylate synthesis and is also incorporated into both RNA and DNA. The result typically is cell death. 5-fluorouracil is indicated as a single agent or in combination with other chemotherapy agents in the treatment of carcinomas of the breast, colon, rectum, stomach and pancreas. Other fluoropyrimidine analogs include 5-fluoro deoxyuridine (floxuridine) and 5-fluorodeoxyuridine monophosphate.

Cytarabine, 4-amino-1-.beta.-D-arabinofuranosyl-2(1H)-pyrimidinone, is commercially available as CYTOSAR-U.RTM. and is commonly known as Ara-C. It is believed that cytarabine exhibits cell phase specificity at S-phase by inhibiting DNA chain

elongation by terminal incorporation of cytarabine into the growing DNA chain. Cytarabine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. Other cytidine analogs include 5-azacytidine and 2',2'-difluorodeoxycytidine (gemcitabine). Mercaptopurine, 1,7-dihydro-6H-purine-6-thione monohydrate, is commercially available as PURINETHOL.RTM.. Mercaptopurine exhibits cell phase specificity at S-phase by inhibiting DNA synthesis by an as of yet unspecified mechanism. Mercaptopurine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. A useful mercaptopurine analog is azathioprine. Thioguanine, 2-amino-1,7-dihydro-6H-purine-6-thione, is commercially available as TABLOID.RTM.. Thioguanine exhibits cell phase specificity at S-phase by inhibiting DNA synthesis by an as of yet unspecified mechanism. Thioguanine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. Other purine analogs include pentostatin, erythrohydroxynonyladenine, fludarabine phosphate, and cladribine. Gemcitabine, 2'-deoxy-2',2'-difluorocytidine monohydrochloride (.beta.-isomer), is commercially available as GEMZAR.RTM.. Gemcitabine exhibits cell phase specificity at S-phase and by blocking progression of cells through the G1/S boundary. Gemcitabine is indicated in combination with cisplatin in the treatment of locally advanced non-small cell lung cancer and alone in the treatment of locally advanced pancreatic cancer. Methotrexate, N-[4[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-l-glutamic acid, is commercially available as methotrexate sodium. Methotrexate exhibits cell phase effects specifically at S-phase by inhibiting DNA synthesis, repair and/or replication through the inhibition of dyhydrofolic acid reductase which is required for synthesis of purine nucleotides and thymidylate. Methotrexate is indicated as a single agent or in combination with other chemotherapy agents in the treatment of choriocarcinoma, meningeal leukemia, non-Hodgkin's lymphoma, and carcinomas of the breast, head, neck, ovary and bladder.

Camptothecins, including, camptothecin and camptothecin derivatives are available or under development as Topoisomerase I inhibitors. Camptothecins cytotoxic activity is believed to be related to its Topoisomerase I inhibitory activity. Examples of

camptothecins include, but are not limited to irinotecan, topotecan, and the various optical forms of 7-(4-methylpiperazino-methylene)-10,11-ethylenedioxy-20-camptothecin described below. Irinotecan HCl, (4S)-4,11-diethyl-4-hydroxy-9-[(4-piperidinopiperidino) carbonyloxy]-1H-pyrano[3',4',6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-- dione hydrochloride, is commercially available as the injectable solution CAMPTOSAR.RTM.. Irinotecan is a derivative of camptothecin which binds, along with its active metabolite SN-38, to the topoisomerase I-DNA complex. It is believed that cytotoxicity occurs as a result of irreparable double strand breaks caused by interaction of the topoisomerase I: DNA: irintecan or SN-38 ternary complex with replication enzymes. Irinotecan is indicated for treatment of metastatic cancer of the colon or rectum. Topotecan HCl, (S)-10-[(dimethylamino)methyl]-4-ethyl-4,9-dihydroxy-1H-pyrano[3',4',6,7]- indolizino[1,2-b]quinoline-3,14-(4H,12H)-dione monohydrochloride, is commercially available as the injectable solution HYCAMTIN.RTM.. Topotecan is a derivative of camptothecin which binds to the topoisomerase I-DNA complex and prevents religation of singles strand breaks caused by Topoisomerase I in response to torsional strain of the DNA molecule. Topotecan is indicated for second line treatment of metastatic carcinoma of the ovary and small cell lung cancer.

Hormones and hormonal analogues are useful compounds for treating cancers in which there is a relationship between the hormone(s) and growth and/or lack of growth of the cancer. Examples of hormones and hormonal analogues useful in cancer treatment include, but are not limited to, adrenocorticosteroids such as prednisone and prednisolone which are useful in the treatment of malignant lymphoma and acute leukemia in children; aminoglutethimide and other aromatase inhibitors such as anastrozole, letrazole, vorazole, and exemestane useful in the treatment of adrenocortical carcinoma and hormone dependent breast carcinoma containing estrogen receptors; progestrins such as megestrol acetate useful in the treatment of hormone dependent breast cancer and endometrial carcinoma; estrogens, androgens, and anti-androgens such as flutamide, nilutamide, bicalutamide, cyproterone acetate and 5.alpha.-reductases such as finasteride and dutasteride, useful in the treatment of prostatic carcinoma and benign prostatic

hypertrophy; anti-estrogens such as tamoxifen, toremifene, raloxifene, droloxifene, iodoxyfene, as well as selective estrogen receptor modulators (SERMS) such those described in U.S. Pat. Nos. 5,681,835, 5,877,219, and 6,207,716, useful in the treatment of hormone dependent breast carcinoma and other susceptible cancers; and gonadotropin-releasing hormone (GnRH) and analogues thereof which stimulate the release of leutinizing hormone (LH) and/or follicle stimulating hormone (FSH) for the treatment prostatic carcinoma, for instance, LHRH agonists and antagagonists such as goserelin acetate and luprolide.

Signal transduction pathway inhibitors are those inhibitors, which block or inhibit a chemical process which evokes an intracellular change. As used herein this change is cell proliferation or differentiation. Signal transduction inhibitors useful in the present invention include inhibitors of receptor tyrosine kinases, non-receptor tyrosine kinases, SH2/SH3 domain blockers, serine/threonine kinases, phosphotidyl inositol-3 kinases, myo-inositol signaling, and Ras oncogenes.

Several protein tyrosine kinases catalyse the phosphorylation of specific tyrosyl residues in various proteins involved in the regulation of cell growth. Such protein tyrosine kinases can be broadly classified as receptor or non-receptor kinases.

Receptor tyrosine kinases are transmembrane proteins having an extracellular ligand binding domain, a transmembrane domain, and a tyrosine kinase domain. Receptor tyrosine kinases are involved in the regulation of cell growth and are generally termed growth factor receptors. Inappropriate or uncontrolled activation of many of these kinases, i.e. aberrant kinase growth factor receptor activity, for example by over-expression or mutation, has been shown to result in uncontrolled cell growth. Accordingly, the aberrant activity of such kinases has been linked to malignant tissue growth. Consequently, inhibitors of such kinases could provide cancer treatment methods. Growth factor receptors include, for example, epidermal growth factor receptor (EGFr), platelet derived growth factor receptor (PDGFr), erbB2, erbB4, ret, vascular endothelial growth factor receptor (VEGFr), tyrosine kinase with

immunoglobulin-like and epidermal growth factor homology domains (TIE-2), insulin growth factor-I (IGFI) receptor, macrophage colony stimulating factor (cfms), BTK, ckit, cmet, fibroblast growth factor (FGF) receptors, Trk receptors (TrkA, TrkB, and TrkC), ephrin (eph) receptors, and the RET protooncogene. Several inhibitors of growth receptors are under development and include ligand antagonists, antibodies, tyrosine kinase inhibitors and anti-sense oligonucleotides. Growth factor receptors and agents that inhibit growth factor receptor function are described, for instance, in Kath, John C, Exp. Opin. Ther. Patents (2000) 10(6):803-818; Shawver et at DDT Vol 2, No. 2 Feb. 1997; and Lofts, F. J. et al, "Growth factor receptors as targets", New Molecular Targets for Cancer Chemotherapy, ed. Workman, Paul and Kerr, David, CRC press 1994, London.

Tyrosine kinases, which are not growth factor receptor kinases are termed nonreceptor tyrosine kinases. Non-receptor tyrosine kinases useful in the present invention, which are targets or potential targets of anti-cancer drugs, include cSrc, Lck, Fyn, Yes, Jak, cAbl, FAK (Focal adhesion kinase), Brutons tyrosine kinase, and Bcr-Abl. Such non-receptor kinases and agents which inhibit non-receptor tyrosine kinase function are described in Sinh, S, and Corey, S. J., (1999) Journal of Hematotherapy and Stem Cell Research 8 (5): 465-80; and Bolen, J. B., Brugge, J. S., (1997) Annual review of Immunology. 15: 371-404. SH2/SH3 domain blockers are agents that disrupt SH2 or SH3 domain binding in a variety of enzymes or adaptor proteins including, PI3-K p85 subunit, Src family kinases, adaptor molecules (She, Crk, Nek, Grb2) and Ras-GAP. SH2/SH3 domains as targets for anti-cancer drugs are discussed in Smithgall, T. E. (1995), Journal of Pharmacological and Toxicological Methods. 34(3) 125-32.

Inhibitors of Serine/Threonine Kinases including MAP kinase cascade blockers which include blockers of Raf kinases (rafk), Mitogen or Extracellular Regulated Kinase (MEKs), and Extracellular Regulated Kinases (ERKs); and Protein kinase C family member blockers including blockers of PKCs (alpha, beta, gamma, epsilon, mu, lambda, iota, zeta). 1 kB kinase family (IKKa, IKKb), PKB family kinases, akt kinase

family members, and TGF beta receptor kinases. Such Serine/Threonine kinases and inhibitors thereof are described in Yamamoto, T., Taya, S., Kaibuchi, K., (1999), Journal of Biochemistry. 126 (5) 799-803; Brodt, P, Samani, A., and Navab, R. (2000), Biochemical Pharmacology, 60. 1101-1107; Massague, J., Weis-Garcia, F. (1996) Cancer Surveys. 27:41-64; Philip, P. A., and Harris, A. L. (1995), Cancer Treatment and Research. 78: 3-27, Lackey, K. et al Bioorganic and Medicinal Chemistry Letters, (10), 2000, 223-226; U.S. Pat. No. 6,268,391; and Martinez-Iacaci, L., et al, Int. J. Cancer (2000), 88(1), 44-52.

Inhibitors of Phosphotidyl inositol-3 Kinase family members including blockers of PI3-kinase, ATM, DNA-PK, and Ku are also useful in the present invention. Such kinases are discussed in Abraham, R T. (1996), Current Opinion in Immunology. 8 (3) 412-8; Canman, C. E., Lim, D. S. (1998), Oncogene 17 (25) 3301-3308; Jackson, S. P. (1997), International Journal of Biochemistry and Cell Biology. 29 (7):935-8; and Zhong, H. et al, Cancer res, (2000) 60(6), 1541-1545.

Also useful in the present invention are Myo-inositol signaling inhibitors such as phospholipase C blockers and Myoinositol analogues. Such signal inhibitors are described in Powis, G., and Kozikowski A., (1994) New Molecular Targets for Cancer Chemotherapy ed., Paul Workman and David Kerr, CRC press 1994, London.

Another group of signal transduction pathway inhibitors are inhibitors of Ras Oncogene. Such inhibitors include inhibitors of farnesyltransferase, geranyl-geranyl transferase, and CAAX proteases as well as anti-sense oligonucleotides, ribozymes and immunotherapy. Such inhibitors have been shown to block ras activation in cells containing wild type mutant ras, thereby acting as antiproliferation agents. Ras oncogene inhibition is discussed in Scharovsky, O. G., Rozados, V. R., Gervasoni, S. I. Matar, P. (2000), Journal of Biomedical Science. 7(4) 292-8; Ashby, M. N. (1998), Current Opinion in Lipidology. 9 (2) 99-102; and BioChim. Biophys. Acta, (1989) 1423(3):19-30.

As mentioned above, antibody antagonists to receptor kinase ligand binding may also serve as signal transduction inhibitors. This group of signal transduction pathway inhibitors includes the use of humanized antibodies to the extracellular ligand binding domain of receptor tyrosine kinases. For example Imclone C225 EGFR specific antibody (see Green, M. C. et al, Monoclonal Antibody Therapy for Solid Tumors, Cancer Treat. Rev., (2000), 26(4), 269-286); Herceptin.RTM. erbB2 antibody (see Tyrosine Kinase Signalling in Breast cance.pi.erbB Family Receptor Tyrosine Kniases, Breast cancer Res., 2000, 2(3), 176-183); and 2CB VEGFR2 specific antibody (see Brekken, R. A. et al, Selective Inhibition of VEGFR2Activity by a monoclonal Anti-VEGF antibody blocks tumor growth in mice, Cancer Res. (2000) 60, 5117-5124).

Anti-angiogenic agents including non-receptorkinase angiogenesis inhibitors may also be useful. Anti-angiogenic agents such as those which inhibit the effects of vascular edothelial growth factor, (for example the anti-vascular endothelial cell growth factor antibody bevacizumab [Avastin.TM.], and compounds that work by other mechanisms (for example linomide, inhibitors of integrin .alpha.v.beta.3 function, endostatin and angiostatin).

Agents used in immunotherapeutic regimens may also be useful in combination with the compounds of formula (I).and/or Formula (2) Immunotherapy approaches, including for example ex-vivo and in-vivo approaches to increase the immunogenecity of patient tumour cells, such as transfection with cytokines such as interleukin 2, interleukin 4 or granulocyte-macrophage colony stimulating factor, approaches to decrease T-cell anergy, approaches using transfected immune cells such as cytokine-transfected dendritic cells, approaches using cytokine-transfected tumour cell lines and approaches using anti-idiotypic antibodies.

Agents used in proapoptotic regimens (e.g., bcl-2 antisense oligonucleotides) may also be used in the combination of the present invention.

Cell cycle signalling inhibitors inhibit molecules involved in the control of the cell cycle. A family of protein kinases called cyclin dependent kinases (CDKs) and their interaction with a family of proteins termed cyclins controls progression through the eukaryotic cell cycle. The coordinate activation and inactivation of different cyclin/CDK complexes is necessary for normal progression through the cell cycle. Several inhibitors of cell cycle signalling are under development. For instance, examples of cyclin dependent kinases, including CDK2, CDK4, and CDK6 and inhibitors for the same are described in, for instance, Rosania et al, Exp. Opin. Ther. Patents (2000) 10(2):215-230.

For the treatment or prophylaxis of pulmonary disorders, anticholinergics of potential use in treating asthma, COPD, bronchitis, and the like, and therefore useful as an additional therapeutic agent include antagonists of the muscarinic receptor (particularly of the M3 subtype) which have shown therapeutic efficacy in man for the control of cholinergic tone in COPD (Witek, 1999); 1-{4-Hydroxy-1-[3,3,3-tris-(4-fluoro-phenyl)-propionyl]-pyrrolidine-2-car- bonyl}-pyrrolidine-2-carboxylic acid (1-methyl-piperidin-4-ylmethyl)-amide; 3-[3-(2-Diethylamino-acetoxy)-2-phenyl-propionyloxy]-8-isopropyl-8-methyl-8-azonia bicyclo[3.2.1]octane(Ipratropium-N,N-diethylglycinate); 1-Cyclohexyl-3,4-dihydro-1H-isoquinoline-2-carboxylic acid 1-aza-bicyclo[2.2.2]oct-3-yl ester (Solifenacin); 2-Hydroxymethyl-4-methanesulfinyl-2-phenyl-butyric acid 1-aza-bicyclo[2.2.2]oct-3-yl ester (Revatropate); 2-{1-[2-(2,3-Dihydro-benzofuran-5-yl)-ethyl]-pyrrolidin-3-yl}-2,2-dipheny- l-acetamide (Darifenacin); 4-Azepan-1-yl-2,2-diphenyl-butyramide (Buzepide); 7-[3-(2-Diethylamino-acetoxy)-2-phenyl-propionyloxy]-9-ethyl-9-methyl-3-o- xa-9-azonia-tricyclo[3.3.1.02,4]nonane (Oxitropium-N,N-diethylglycinate); 7-[2-(2-Diethylamino-acetoxy)-2,2-di-thiophen-2-yl-acetoxy]-9,9-dimethyl-- 3-oxa-9-azonia-tricyclo[3.3.1.02,4]nonane (Tiotropium-N,N-diethylglycinate); Dimethylamino-acetic acid 2-(3-diisopropylamino-1-phenyl-propyl)-4-methyl-phenyl ester (Tolterodine-N,N-dimethylglycinate); 3-[4,4-Bis-(4-fluoro-phenyl)-2-oxo-imidazolidin-1-yl]-1-methyl-1-(2-oxo-2- -pyridin-2-yl-ethyl)-pyrrolidinium; 1-[1-(3-Fluoro-benzyl)-piperidin-4-yl]-4,4-bis-(4-fluoro-phenyl)-imidazol- idin-2-one; 1-Cyclooctyl-3-(3-methoxy-1-aza-bicyclo[2.2.2]oct-3-yl)-1-phenyl-prop-2-y- n-1-ol; 3-[2-(2-Diethylamino-acetoxy)-2,2-di-thiophen-2-yl-acetoxy]-1-(3-p-henoxy-propyl)-1-azonia-bicyclo[2.2.2]octane

(Aclidinium-N,N-diethylglycinate); or (2-Diethylamino-acetoxy)-di-thiophen-2-yl-acetic acid 1-methyl-1-(2-phenoxy-ethyl)-piperidin-4-yl ester; beta-2 agonist used to treat broncho-constriction in asthma, COPD and bronchitis include salmeterol and albuterol; anti-inflammatory signal transduction modulators for asthma.

With regard to the pulmonary condition of asthma, those skilled in the art appreciate that asthma is a chronic inflammatory disease of the airways resulting from the infiltration of pro-inflammatory cells, mostly eosinophils and activated T-lymphocytes into the bronchial mucosa and submucosa. The secretion of potent chemical mediators, including cytokines, by these proinflammatory cells alters mucosal permeability, mucus production, and causes smooth muscle contraction. All of these factors lead to an increased reactivity of the airways to a wide variety of irritant stimuli (Kaliner, 1988). Targeting signal transduction pathways is an attractive approach to treating inflammatory diseases, as the same pathways are usually involved in several cell types and regulate several coordinated inflammatory processes, hence modulators have the prospect of a wide spectrum of beneficial effects. Multiple inflammatory signals activate a variety of cell surface receptors that activate a limited number of signal transduction pathways, most of which involve cascades of kinases. These kinases in turn may activate transcription factors that regulate multiple inflammatory genes. Applying "anti-inflammatory signal transduction modulators" (referred to in this text as AISTM), like phosphodiesterase inhibitors (e.g. PDE-4, PDE-5, or PDE-7 specific), transcription factor inhibitors (e.g. blocking NF.kappa.B through IKK inhibition), or kinase inhibitors (e.g. blocking P38 MAP, JNK, PI3K, EGFR or Syk) is a logical approach to switching off inflammation as these small molecules target a limited number of common intracellular pathways--those signal transduction pathways that are critical points for the anti-inflammatory therapeutic intervention (see review by P. J. Barnes, 2006).

Additional therapeutic agents include: 5-(2,4-Difluoro-phenoxy)-1-isobutyl-1H-indazole-6-carboxylic acid (2-dimethylamino-ethyl)-amide (P38 Map kinase inhibitor ARRY-797); 3-Cyclopropylmethoxy-N-(3,5-dichloro-pyridin-4-yl)-4-difluorormethoxy-

ben- zamide (PDE-4 inhibitor Roflumilast); 4-[2-(3-cyclopentyloxy-4-methoxyphenyl)-2-phenyl-ethyl]-pyridine (PDE-4 inhibitor CDP-840); N-(3,5-dichloro-4-pyridinyl)-4-(difluoromethoxy)-8-[(methylsulfonyl)amino-]-1-dibenzofurancarboxamide (PDE-4 inhibitor Oglemilast); N-(3,5-Dichloro-pyridin-4-yl)-2-[1-(4-fluorobenzyl)-5-hydroxy-1H-indol-3--yl]-2-oxo-acetamide (PDE-4 inhibitor AWD 12-281); 8-Methoxy-2-trifluoromethyl-quinoline-5-carboxylic acid (3,5-dichloro-1-oxy-pyridin-4-yl)-amide (PDE-4 inhibitor Sch 351591); 4-[5-(4-Fluorophenyl)-2-(4-methanesulfinyl-phenyl)-1H-imidazol-4-yl]-pyri- dine (P38 inhibitor SB-203850); 4-[4-(4-Fluoro-phenyl)-1-(3-phenyl-propyl)-5-pyridin-4-yl-1H-imidazol-2-y- l]-but-3-yn-1-ol (P38 inhibitor RWJ-67657); 4-Cyano-4-(3-cyclopentyloxy-4-methoxy-phenyl)-cyclohexanecarboxylic acid 2-diethylamino-ethyl ester (2-diethyl-ethyl ester prodrug of Cilomilast, PDE-4 inhibitor); (3-Chloro-4-fluorophenyl)-[7-methoxy-6-(3-morpholin-4-yl-propoxy)-quinazo- lin-4-yl]-amine (Gefitinib, EGFR inhibitor); and 4-(4-Methyl-piperazin-1-ylmethyl)-N-[4-methyl-3-(4-pyridin-3-yl-pyrimidin- -2-ylamino)-phenyl]-benzamide (Imatinib, EGFR inhibitor).

Moreover, asthma is a chronic inflammatory disease of the airways produced by the infiltration of pro-inflammatory cells, mostly eosinophils and activated T-lymphocytes (Poston, Am. Rev. Respir. Dis., 145 (4 Pt 1), 918-921, 1992; Walker, J. Allergy Clin. Immunol., 88 (6), 935-42, 1991) into the bronchial mucosa and submucosa. The secretion of potent chemical mediators, including cytokines, by these proinflammatory cells alters mucosal permeability, mucus production, and causes smooth muscle contraction. All of these factors lead to an increased reactivity of the airways to a wide variety of irritant stimuli (Kaliner, "Bronchial asthma, Immunologic diseases" E. M. Samter, Boston, Little, Brown and Company: 117-118. 1988).

Glucocorticoids, which were first introduced as an asthma therapy in 1950 (Carryer, Journal of Allergy, 21, 282-287, 1950), remain the most potent and consistently effective therapy for this disease, although their mechanism of action is not yet fully understood (Morris, J. Allergy Clin. Immunol., 75 (1 Pt) 1-13, 1985). Unfortunately, oral glucocorticoid therapies are associated with profound undesirable side effects

such as truncal obesity, hypertension, glaucoma, glucose intolerance, acceleration of cataract formation, bone mineral loss, and psychological effects, all of which limit their use as long-term therapeutic agents (Goodman and Gilman, 10th edition, 2001). A solution to systemic side effects is to deliver steroid drugs directly to the site of inflammation. Inhaled corticosteroids (ICS) have been developed to mitigate the severe adverse effects of oral steroids. While ICS are very effective in controlling inflammation in asthma, they too are not precisely delivered to the optimal site of action in the lungs and produce unwanted side effects in the mouth and pharynx (candidiasis, sore throat, dysphonia). Combinations of inhaled .beta.2-adrenoreceptor agonist bronchodilators such as formoterol or salmeterol with ICS's are also used to treat both the bronchoconstriction and the inflammation associated with asthma and COPD (Symbicort.RTM. and Advair.RTM., respectively). However, these combinations have the side effects of both the ICS's and the .beta.2-adrenoreceptor agonist because of systemic absorption (tachycardia, ventricular dysrhythmias, hypokalemia) primarily because neither agent is delivered to the optimal sites of actions in the lungs. In consideration of all problems and disadvantages connected with the adverse side effect profile of ICS and of .beta.2-agonists it would be highly advantageous to provide mutual steroid-.beta.2-agonist prodrug to mask the pharmacological properties of both steroids and .beta.2-agonists until such a prodrug reaches the lungs, thereby mitigating the oropharyngeal side effects of ICS and cardiovascular side-effects of .beta.2-agonists. In one aspect, such a mutual steroid-.beta.2-agonist prodrug would be effectively delivered to the endobronchial space and converted to active drugs by the action of lung enzymes, thereby delivering to the site of inflammation and bronchoconstriction a therapeutic amount of both drugs. An anti-inflammatory agent for combination therapy includes dexamethasone, dexamethasone sodium phosphate, fluorometholone, fluorometholone acetate, loteprednol, loteprednol etabonate, hydrocortisone, prednisolone, fludrocortisones, triamcinolone, triamcinolone acetonide, betamethasone, beclomethasone diproprionate, methylprednisolone, fluocinolone, fluocinolone acetonide, flunisolide, fluocortin-21-butylate, flumethasone, flumetasone pivalate, budesonide, halobetasol propionate, mometasone furoate, fluticasone propionate, ciclesonide; or a pharmaceutically acceptable salt thereof.

The immune response to certain antigens be they cancer or bacterial or viral which can be enhanced through the use of immune potentiators, known as vaccine adjuvants such as the compounds of Mogroside IV and/or Mogroside V of this patent. A discussion of immunological adjuvants can be found in "Current Status of Immunological Adjuvants", Ann. Rev. Immunol., 1986, 4, pp. 369-388 and "Recent Advances in Vaccine Adjuvants and Delivery Systems" by D. T. O'Hagan and N. M. Valiante. The disclosures of U.S. Pat. Nos. 4,806,352; 5,026,543; and 5,026,546 describe various vaccine adjuvants appearing in the patent literature. Each of these references is hereby incorporated by reference in their entireties.

In one embodiment of this invention is provided are methods of administering a vaccine by administering a compounds of Mogroside IVand/or Mogroside V alone or in combination with antigens and/or other agents. In another embodiment, immune responses to vaccines using antigenic epitopes from sources such as synthetic peptides, bacterial, or viral antigens are enhanced by co-administration of the compounds of Mogroside IVand/or Mogroside V In other embodiments, the instant invention provides immunogenic compositions comprising one or more antigens and a compound(s) of Mogroside IV and/or Mogroside V effective to stimulate a cell mediated response to said one or more antigens.

In another embodiment, compounds of Mogroside IVand/or Mogroside V can be used in the manufacture of a medicament for enhancing the immune response to an antigen. Other embodiments provide the use of the compound(s) of Mogroside IVand/or Mogroside V in the manufacture of a medicament for immune stimulation, and another agent, such as an antigen, for simultaneous, separate or sequential administration.

In another embodiment, provided is a pharmaceutical preparation comprising (a) compounds of Mogroside IV and/or Mogroside V (b) an antigen, wherein (a) and (b) are either in admixture or are separate compositions. These embodiments are for simultaneous, separate or sequential administration. When in separate compositions,

the compound(s) of Mogroside IVand/or Mogroside V may be administered enterally, orally, parenterally, sublingually, intradermally, by inhalation spray, rectally, or topically in dosage unit formulations that include conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. For example, suitable modes of administration include oral, subcutaneous, transdermal, transmucosal, iontophoretic, intravenous, intramuscular, intraperitoneal, intranasal, subdermal, rectal, and the like. Topical administration may also include the use of transdermal administration such as transdermal patches or ionophoresis devices. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection, or infusion techniques. orally, topically, nasally, rectally, by inhalation or by injection.

In another embodiment, compound(s) of Mogroside IV and/or MogrosideV are used as polyclonal activators for the production of antigens. More particularly the invention relates to a method of preparing monoclonal antibodies with a desired antigen specificity comprising contacting a compound(s) of Mogrosides IV and/or Mogroside V with immortalized memory B cells. The monoclonal antibodies produced therefrom, or fragments thereof, may be used for the treatment of disease, for the prevention of disease or for the diagnosis of disease.

Vaccines or immunogenic compositions of the present invention comprising compound(s) of Mogroside IV and/or Mogroside V may be administered in conjunction with one or more immunoregulatory agents. In particular, compositions can include another adjuvant. Adjuvants for use with the invention include, but are not limited to, mineral containing compositions such as calcium or aluminium salts, for example AlK(SO.sub.4).sub.2, Al(OH).sub.3, AlPO.sub.4, or combinations thereof. Other adjuvants include oil-emulsions, particularly submicron oil-in-water emulsions such as those described in WO90/14837, U.S. Pat. No. 6,299,884 and U.S. Pat. No. 6,452,325. Other adjuvants include saponin formulations such as QS7, QS17, QS18, QS21, QH-A, QH-B and QH-C, see U.S. Pat. No. 5,057,540 and Barr, et al. Advanced Drug Delivery Reviews (1998), 32:247-271. Other adjuvants include

virosomes and virus like particles (VLPs) (Gluck, et al., Vaccine (2002) 20:B10-B16, US 20090263470); bacterial or microbial derivatives, Lipid A derivatives, immunostimulartory oligonucleotides, ADP-ribosylating toxins and detoxified derivaties thereof, bioadhesives and mucoadhesives, microparticles, liposomes, polyphasphazene (PCPP), and other small molecule immunopotentiators. One or more of the above named adjuvants may be used in a vaccine combination with a compound(s) of Mogroside IVand/or Mogroside V.
The invention is also directed to methods of administering the immunogenic compositions of the invention, wherein the immunogenic composition includes in one embodiment one or more adjuvants and antigens as described herein in combination with compound(s) of Mogroside IV and/or Mogroside V. In some embodiments, the immunogenic composition is administered to the subject in an amount effective to stimulate an immune response. The amount that constitutes an effective amount depends, inter alia, on the particular immunogenic composition used, the particular adjuvant compound being administered and the amount thereof, the immune response that is to be enhanced (humoral or cell mediated), the state of the immune system (e.g., suppressed, compromised, stimulated), and the desired therapeutic result. Accordingly it is not practical to set forth generally the amount that constitutes an effective amount of the immunogenic composition. Those of ordinary skill in the art, however, can readily determine the appropriate amount with due consideration of such factors.

The immunogenic compositions of the present invention can be used in the manufacture of a vaccine. Suitable vaccines include, but are not limited to, any material that raises either or both humoral or cell mediated immune response. Suitable vaccines can include live viral and bacterial antigens and inactivated viral, tumor-derived, protozoal, organism-derived, fungal, and bacterial antigens, toxoids, toxins, polysaccharides, proteins, glycoproteins, peptides, and the like.

Compositions of compound(s) of Mogroside IV and/or Mogroside V may be administered in conjunction with one or more antigens for use in therapeutic,

prophylactic, or diagnostic methods of the present invention. In another aspect of this embodiment, these compositions may be used to treat or prevent infections caused by pathogens. In another aspect of this embodiment, these compositions may also be combined with an adjuvant as described previously.

Antigens for use with the invention include, but are not limited to, one or more of the antigens comprising bacterial antigens, viral antigens, fungal antigens, antigens from sexually transmitted diseases (STD), respiratory antigens, pediatric vaccine antigens, antigens suitable for use in elderly or immunocompromised individuals, antigens suitable for use in adolescent vaccines, and tumor antigens.

In yet another embodiment, the present application discloses pharmaceutical compositions comprising a compound(s) of the present invention, or a pharmaceutically acceptable salt thereof, in combination with at least one additional active agent, and a pharmaceutically acceptable carrier or excipient. In yet another embodiment, the present application provides a combination pharmaceutical agent with two or more therapeutic agents in a unitary dosage form. Thus, it is also possible to combine any compound(s) of the invention with one or more other active agents in a unitary dosage form.

The combination therapy may be administered as a simultaneous or sequential regimen. When administered sequentially, the combination may be administered in two or more administrations.

Co-administration of compound(s) of the invention with one or more other active agents generally refers to simultaneous or sequential administration of compound(s) of the invention and one or more other active agents, such that therapeutically effective amounts of the compound(s) of the invention and one or more other active agents are both present in the body of the patient.

Co-administration includes administration of unit dosages of the compound(s) of the invention before or after administration of unit dosages of one or more other active

agents, for example, administration of the compound(s) of the invention within seconds, minutes, or hours of the administration of one or more other active agents. For example, a unit dose of compound(s) of the invention can be administered first, followed within seconds or minutes by administration of a unit dose of one or more other active agents. Alternatively, a unit dose of one or more other active agents can be administered first, followed by administration of a unit dose of compound(s) of the invention within seconds or minutes. In some cases, it may be desirable to administer a unit dose of compound(s) of the invention first, followed, after a period of hours (e.g., 1-12 hours), by administration of a unit dose of one or more other active agents. In other cases, it may be desirable to administer a unit dose of one or more other active agents first, followed, after a period of hours (e.g., 1-12 hours), by administration of a unit dose of compound(s) of the invention.

The combination therapy may provide "synergy" and "synergistic effect", i.e. the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect may be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when the compounds are administered or delivered sequentially, e.g., in separate tablets, pills or capsules, or by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e. serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.

Methods of Treatment

As used herein, an "agonist" is a substance that stimulates its binding partner, typically a receptor. Stimulation is defined in the context of the particular assay, or may be apparent in the literature from a discussion herein that makes a comparison

to a factor or substance that is accepted as an "agonist" or an "antagonist" of the particular binding partner under substantially similar circumstances as appreciated by those of skill in the art. Stimulation may be defined with respect to an increase in a particular effect or function that is induced by interaction of the agonist or partial agonist with a binding partner and can include allosteric effects.

As used herein, an "antagonist" is a substance that inhibits its binding partner, typically a receptor. Inhibition is defined in the context of the particular assay, or may be apparent in the literature from a discussion herein that makes a comparison to a factor or substance that is accepted as an "agonist" or an "antagonist" of the particular binding partner under substantially similar circumstances as appreciated by those of skill in the art. Inhibition may be defined with respect to a decrease in a particular effect or function that is induced by interaction of the antagonist with a binding partner, and can include allosteric effects.

As used herein, a "partial agonist" or a "partial antagonist" is a substance that provides a level of stimulation or inhibition, respectively, to its binding partner that is not fully or completely agonistic or antagonistic, respectively. It will be recognized that stimulation, and hence, inhibition is defined intrinsically for any substance or category of substances to be defined as agonists, antagonists, or partial agonists.

As used herein, "intrinsic activity" or "efficacy" relates to some measure of biological effectiveness of the binding partner complex. With regard to receptor pharmacology, the context in which intrinsic activity or efficacy should be defined will depend on the context of the binding partner (e.g., receptor/ligand) complex and the consideration of an activity relevant to a particular biological outcome. For example, in some circumstances, intrinsic activity may vary depending on the particular second messenger system involved. Where such contextually specific evaluations are relevant, and how they might be relevant in the context of the present invention, will be apparent to one of ordinary skill in the art.

As used herein, modulation of a receptor includes agonism, partial agonism, antagonism, partial antagonism, or inverse agonism of a receptor.

As will be appreciated by those skilled in the art, when treating a viral infection such as HCV, HBV, or HIV or dengue, such treatment may be characterized in a variety of ways and measured by a variety of endpoints. The scope of the present invention is intended to encompass all such characterizations.

In one embodiment, the method can be used to induce an immune response against multiple epitopes of a viral infection in a human or animal. Induction of an immune response against viral infection can be assessed using any technique that is known by those of skill in the art for determining whether an immune response has occurred. Suitable methods of detecting an immune response for the present invention include, among others, detecting a decrease in viral load or antigen in a subject's serum, detection of IFN-gamma-secreting peptide specific T cells, and detection of elevated levels of one or more liver enzymes, such as alanine transferase (ALT) and aspartate transferase (AST). In one embodiment, the detection of IFN-gamma-secreting peptide specific T cells is accomplished using an ELISPOT assay. Another embodiment includes reducing the viral load associated with HBV dengue infection, including a reduction as measured by PCR testing.

In another aspect, the present invention provides methods for treating a hepatitis B viral infection or a hepatitis C viral infection, wherein each of the methods includes the step of administering to a human subject infected with dengue or hepatitis B virus or hepatitis C virus a therapeutically effective amount a compound of Mogroside IV, Mogroside V or a combination thereof or a pharmaceutically acceptable salts thereof. Typically, the human subject is suffering from a chronic hepatitis B infection or a chronic hepatitis C infection, or dengue infection, although it is within the scope of the present invention to treat people who are acutely infected with HBV , HCV.or dengue.

Treatment in accordance with the present invention results in the stimulation of an immune response against HBV , HCV or dengue in a human infected with HBV ,

HCV or Dengue respectively, and a consequent reduction in the viral load of HBV HCV or dengue in the infected person. Examples of immune responses include production of antibodies (e.g., IgG antibodies) and/or production of cytokines, such as interferons, that modulate the activity of the immune system. The immune system response can be a newly induced response, or can be boosting of an existing immune response. In particular, the immune system response can be seroconversion against one or more HBV ,HCV or Dengue antigens.

The viral load can be determined by measuring the amount of HBV DNA or HCV DNA or dengue virus DNA present in the blood. For example, dengue can be monitored by DENV-1-4 . Real Time RT-PCR assay. Blood serum HBV DNA can be quantified using the Roche COBAS Amplicor Monitor PCR assay (version 2.0; lower limit of quantification, 300 copies/mL [57 IU/mL]) and the Quantiplex bDNA assay (lower limit of quantification, 0.7 MEq/mL; Bayer Diagnostics, formerly Chiron Diagnostics, Emeryville, Calif.). The amount of antibodies against specific HBV or HCV antigens (e.g., hepatitis B surface antigen (HBsAG)) or dengue can be measured using such art-recognized techniques as enzyme-linked immunoassays and enzyme-linked immunoabsorbent assays. For example, the amount of antibodies against specific HBV or HCV antigens can be measured using the Abbott AxSYM microparticle enzyme immunoassay system (Abbott Laboratories, North Chicago, Ill.).

Compound(s) of Mogroside IV,,Mogroside V or combinations thereof can be administered by any useful route and means, such as by oral or parenteral (e.g., intravenous) administration. Therapeutically effective amounts of Mogroside IV, Mogroside V and combinations thereof are from about 0.00001 mg/kg body weight per day to about 10 mg/kg body weight per day, such as from about 0.0001 mg/kg body weight per day to about 10 mg/kg body weight per day, or such as from about 0.001 mg/kg body weight per day to about 1 mg/kg body weight per day, or such as from about 0.01 mg/kg body weight per day to about 1 mg/kg body weight per day, or such as from about 0.05 mg/kg body weight per day to about 500 mg/kg body weight

per day, or such as from about 0.3mu.g to about 30 mg per day, or such as from about 30mu.g to about 300m.g per day.

The frequency of dosaging with Mogroside IV,Mogroside V and combinations thereof will be determined by the needs of the individual patient and can be, for example, once per day , twice, or more times, per day. Administration of Mogroside IV ,Mogroside V or combinations thereof continues for as long as necessary to treat the HBV,HCV or Dengue infection. For example, Mogroside IV,Mogroside V or combinations thereof can be administered to a human infected with HBV, HCV or Dengue for a period of from 20 days to 180 days or, for example, for a period of from 20 days to 90 days or, for example, for a period of from 30 days to 60 days.

Administration can be intermittent, with a period of several or more days during which a patient receives a daily dose of Mogroside IV,Mogroside V or combinations thereof followed by a period of several or more days during which a patient does not receive a daily dose of Mogroside IV,Mogroside V and combinations thereof/. For example, a patient can receive a dose of Mogroside IV ,Mogroside V or combinations thereofevery other day, or three times per week. Again by way of example, a patient can receive a dose of Mogroside IV,Mogroside V or combination thereof each day for a period of from 1 to 14 days, followed by a period of 7 to 21 days during which the patient does not receive a dose of Mogroside IV,Mogroside V or combinations thereof followed by a subsequent period (e.g., from 1 to 14 days) during which the patient again receives a daily dose of Mogroside IV,Mogroside V or combinations thereof Alternating periods of administration of Mogroside IV,Mogroside V or combinations thereof followed by non-administration of Mogroside IVMogrosideV or combination thereof , can be repeated as clinically required to treat the patient.

As described more fully herein, Mogroside IV,Mogroside V or combination thereof can be administered with one or more additional therapeutic agent(s) to a human being infected with HBV, HCV.or dengue. The additional therapeutic agent(s) can be administered to the infected human at the same time as Mogroside IV,Mogroside V

or a combination thereof or before or after administration of Mogroside IV,Mogroside V and combination thereof.

In another aspect, the present invention provides a method for ameliorating a symptom associated with an HBV infection,HCV or dengue infection, wherein the method comprises administering to a human infected with hepatitis B virus or hepatitis C virus or dengue virus a therapeutically effective amount of Mogroside IV,Mogroside V or a combination thereof or pharmaceutically acceptable salst thereof, wherein the therapeutically effective amount is sufficient to ameliorate a symptom associated with the HBV , HCV or dengue infection. Such symptoms include the presence of HBV virus particles (or HCV virus particles) or dengue viral particles in the blood, liver inflammation, jaundice, muscle aches, weakness and tiredness.

In a further aspect, the present invention provides a method for reducing the rate of progression of a hepatitis B viral infection, or a hepatitis C virus infection,or dengue in a human wherein the method comprises administering to a human infected with hepatitis B virus or hepatitis C virus or dengue a therapeutically effective amount of Mogroside IV,Mogroside V and combinations of same or pharmaceutically acceptable salts thereof, wherein the therapeutically effective amount is sufficient to reduce the rate of progression of the hepatitis B viral infection , hepatitis C viral infection or dengue.. The rate of progression of the infection can be followed by measuring the amount of HBV virus particles or HCV virus particles or dengue viral DNA in the blood.

In another aspect, the present invention provides a method for reducing the viral load associated with HBV , HCV or Dengue infection, wherein the method comprises administering to a human infected with HBV HCV or dengue a therapeutically effective amount of Mogroside IV ,Mogroside V or combinations thereof or pharmaceutically acceptable salts thereof, wherein the therapeutically effective amount is sufficient to reduce the HBV , HCV and Dengue viral load in the human.

In a further aspect, the present invention provides a method of inducing or boosting an immune response against Hepatitis B ,Hepatitis C or Dengue virus in a human . wherein the method comprises administering a therapeutically effective amount of Mogroside IVMogroside V or a combination thereof,, or pharmaceutically acceptable salst thereof, to the human , wherein a new immune response against Hepatitis B, Hepatitis C or dengue virus is induced in the human , or a preexisting immune response against Hepatitis B , Hepatitis C or Dengue virus is boosted in the human Seroconversion with respect to HBV, HCV or Dengue can be induced in the human . Examples of immune responses include production of antibodies, such as IgG antibody molecules, and/or production of cytokine molecules that modulate the activity of one or more components of the human immune system.

Induction of seroconversion against HCV , HBV or Dengue in patients chronically infected with any of these viruses is an unexpected property of Mogroside IV,Mogroside V or a combination of both,. In clinical practice, an HBVr,HCV or Dengue patient, is treated with Mogroside IV,Mogroside V and combinations thereof alone or in combination with one or more other therapeutic agents, until an immune response against HBV, HCV or dengue is induced or enhanced and the viral load of HBV , HCVor dengue is reduced. Thereafter, although the HBV, HCV or dengue virus may persist in a latent form in the patient's body, treatment with Mogroside IV,Mogroside V or combinations thereof can be stopped, and the patient's own immune system is capable of suppressing further viral replication. In patients treated in accordance with the present invention and who are already receiving treatment with an antiviral agent that suppresses replication of the HBV ,HCV or Dengue virus, there may be little or no detectable viral particles in the body of the patient during treatment with the antiviral agent(s). In these patients, seroconversion will be evident when the antiviral agent(s) is no longer administered to the patient and there is no increase in the viral load of HBV, HCV.or DENGUE.

In the practice of the present invention, an immune response is induced against one or more antigens of HBV ,HCV.or Dengue. For example, an immune response can

be induced against the HBV surface antigen (HBsAg), or against the small form of the HBV surface antigen (small S antigen), or against the medium form of the HBV surface antigen (medium S antigen), or against a combination thereof. Again by way of example, an immune response can be induced against the HBV surface antigen (HBsAg) and also against other HBV-derived antigens, such as the core polymerase or x-protein.

Induction of an immune response against HCV ,or dengue HBV can be assessed using any technique that is known by those of skill in the art for determining whether an immune response has occurred. Suitable methods of detecting an immune response for the present invention include, among others, detecting a decrease in viral load in a subject's serum, such as by measuring the amount of HBV DNA o, HCV DNA or Dengue DNA in a subject's blood using a PCR assay, and/or by measuring the amount of anti-HBV antibodies, anti-HCV antibodies,or dengue antibodies in the subject's blood using a method such as an ELISA.

Additionally, the compounds of this invention are useful in the treatment of cancer or tumors (including dysplasias, such as uterine dysplasia). These includes hematological malignancies, oral carcinomas (for example of the lip, tongue or pharynx), digestive organs (for example esophagus, stomach, small intestine, colon, large intestine, or rectum), liver and biliary passages, pancreas, respiratory system such as larynx or lung (small cell and non-small cell), bone, connective tissue, skin (e.g., melanoma), breast, reproductive organs (uterus, cervix, testicles, ovary, or prostate), urinary tract (e.g., bladder or kidney), brain and endocrine glands such as the thyroid. In summary, the compounds of this invention are employed to treat any neoplasm, including not only hematologic malignancies but also solid tumors of all kinds.

Hematological malignancies are broadly defined as proliferative disorders of blood cells and/or their progenitors, in which these cells proliferate in an uncontrolled manner. Anatomically, the hematologic malignancies are divided into two primary

groups: lymphomas--malignant masses of lymphoid cells, primarily but not exclusively in lymph nodes, and leukemias--neoplasm derived typically from lymphoid or myeloid cells and primarily affecting the bone marrow and peripheral blood. The lymphomas can be sub-divided into Hodgkin's Disease and Non-Hodgkin's lymphoma (NHL). The later group comprises several distinct entities, which can be distinguished clinically (e.g. aggressive lymphoma, indolent lymphoma), histologically (e.g. follicular lymphoma, mantle cell lymphoma) or based on the origin of the malignant cell (e.g. B lymphocyte, T lymphocyte). Leukemias and related malignancies include acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL). Other hematological malignancies include the plasma cell dyscrasias including multiple myeloma, and the myelodysplastic syndromes.

DESCRIPTION OF THE INVENTION

Maturation of dendritic cells (DCs) is usually attenuated in the tumour microenvironment and In certain viral infections such as Hep.C , HIV and dengue,which is an major immunological problem in DC-based immunotherapy of cancer and viral vaccine development.

In this patent we present the effect of Mogroside V on DC maturation. When we treated dendritic cells generated from mouse BM cells with Mogroside V, Mogroside V induced a phenotypic maturation of Dendritic cells as proven by the increased expression of CD40, CD80/86, and MHC-I/II molecules. Mogroside V induced a functional maturation of dendritic cells, in that Mogroside V increased the expression of the following cytokines: IL-12, IL-1b, TNF- a , and IFN-b, treatment with Mogroside Vdecreased antigen capture capacity, and enhanced allogenic T cell stimulation.

Also Mogroside V efficiently induced maturation of dendritic cells from C3H/HeN mice having normal toll-like receptor4 (TLR4), but no maturation of dendritic cells were formed which were isolated from C3H/HeJ mice having a mutated TLR4 receptor,

suggesting that TLR4 is one of the membrane receptors utilised by mogroside V in its mechanism of action in stimulating dendritic cell maturation. Mogroside V increased phosphorylation of mitogen-activated protein kinase (MAPKs), and nuclear translocation of NF- j B p65 subunit, which are both important signal molecules downstream from the toll-like receptor4(TLR4.). The data presented in this patent proves that Mogroside V induces DC maturation through TLR4 and that Mogroside V can be used as outlined in this patent an adjuvant in DC-based cancer immunotherapy and as an adjuvant combination with direct acting antivirals (DAA's) in the treatment of viral infections such as Hepatitis Band C and HIV. Also Mogroside V activity at inducing Dendritic cell maturation and enhanced immune T-cell activity can be utilised as a component in cancer vaccine and infectious organism vaccine development.

Dendritic cells are the most potent antigen-presenting cells and initiate the majority of immune responses in the body, including tumour or viral infected specific T cell responses.

Dendritic cells are generated from either lymphoid or myeloid bone marrow progenitor cells through intermediate dendritic precursors.

Dendritic cells are positioned at different portals of the human body, such as the mucosal surface and blood, where they can encounter any invading pathogens .After the uptake of antigen from a pathogen or tumour and exposure to inflammatory cytokines, immature dendritic cells mature and migrate to the T cell areas of secondary lymphoid organs. Mature dendritic cells down-regulate antigen uptake and processing capacity, but go on to sensitise naïve T cells through major histocompatibility (MHC) and co-stimulatory molecules .

The human body's natural immune defences are attenuated (weakened) during neoplastic disease development and infection with certain pathogens and dendritic

cell maturation is inhibited by tumour-derived factors, such as vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-b), Interleukin 10 (IL-10), prostaglandin E2 (PGE2), and gangliosides.

Natural immune defences are attenuated in the early stages of certain viral infections as with Hepatitis C when certain viral proteins are incorporated into dendritic cells and undermine their total function of creating and properly stimulating especially cytotoxic T cells that target viral infected cells and allow chronic infection to be established.

Immature Dendritic cells usually accumulate in tumor-bearing mice as in cancer patients. Also, several key deficiencies in maturation, migration to lymph nodes, and T cell activation by dendritic cells have been documented in the tumour microenvironment and in the immune dysfunction of Hepatitis C patients and patients suffering from other pathogenic infections.

Defective immature dendritic cells in the tumour micro-environment cause important immunological deficits that limits the success of cancer therapy and immunotherapy that we propose mogroside V will alleviate or nullify.

This patent presents evidence to prove that overcoming defective maturation of dendritic cells in cancer patients is an important advance in cancer cell clearance from the body and similarly in chronic viral infections to aid viral infected cells to be cleared from the body.

Experimentally over the years many studies attempted to find inducers of dendritic cell maturation using monocyte-conditioned medium, PGE2, toll-like receptor (TLR) ligands, and inflammatory cytokines, such as TNF- a , IL-6, and IL-1b that did not comprehensively achieve immunological clearance of either the tumour cell population or clearance of virally infected cells or pathogens.

In this patent we provide evidence through in-vitro,animal model studies and patient studies that mogroside V administration increases Dendritic cell maturation and cytotoxic and other immune cells attack of virally infected cell and tumour cells .

Materials Utilized for animal and in-vitro experiment on Mogroside V activity

Female C57BL/6, BALB/c, C3H/HeN, and C3H/HeJ mice (6–8 weeks old) were obtained.

Mice were housed in specific pathogen-free conditions at 21–24 °C and 40–60% relative humidity under a 12 h light/dark cycle. All animals were acclimatized for at least 1 week prior to the experiments. Anti-mouse antibodies against CD11c, CD40, CD80, CD86, and MHC-I/II were utilised and those against extracellular signal-regulated kinase (ERK), C-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinases (MAPKs) were purchased .Lipopolysaccharide (LPS), polymyxin B (PMB), and propidium iodide (PI) were acquired from Sigma.

Mogroside V was isolated from the Luo Han Guo fruit and provided by Chengdu BioPurify China specification attached.

Generation of bone marrow-derived DCs

Dendritic cells (DC) were generated from bone marrow (BM) cells obtained from 6–7-week-old female mice. Briefly, bone marrow cells were flushed out from femurs and tibias. After lysing red blood cells, whole bone marrow cells (2 Â 10 5 cells/ml) were cultured in 100-mm 2 culture dishes in 10 ml/dish of complete medium containing 2 ng/ml GM-CSF. On culture day 3, another 10 ml of fresh complete medium containing 2 ng/ml GM-CSF was added, and on day 6 half of the medium was changed. On day 8 non-adherent and adherent DCs were harvested by vigorous pipetting and used as immature dendritic cells (iDCs). iDCs recovered from these cultures were generally >85% CD11c + , but not CD3 + and B220 + positive.

Phenotypic maturation of DCs was analyzed by flow cytometry. Cell staining was performed using a combination of FITC-conjugated anti-CD40, anti-CD80, anti-CD86, or anti-MHC plus PE-conjugated CD11c antibodies. Cells were analyzed using a FACS Calibur flow cytometer and data was analyzed using CellQuest Pro. Forward and side scatter parameters were used to gate the live cells.

To analyse the endocytosis of dendritic cells, 4 Â 10 5 dendritic cells were incubated at 37 °C for 1 h with 0.4 mg/ml FITC-dextran (42,000 Da). After incubation, cells were washed twice with cold washing buffer (PBS containing 0.5% BSA) and stained using PE-conjugated anti-CD11c antibody. Double stained dendritic cells were analysed by flow cytometry. In addition, parallel experiments were performed at 4 °C to determine the nonspecific binding of FITC-dextran to dendritic cells.. Mogroside IV or LPS were pretreated with 2,000 U/ml of polymyxin B (PMB) for 1 h and then used to treat Dendritic cells.

Total RNA was isolated using TRIZOL TM Reagent .For RT-PCR, single-strand cDNA was synthesised from RNA. The primer sequences used were as synthesised in-house. PCR products were fractionated on 1% agarose gels and stained with 5 l g/ml ethidium bromide. After analysing band areas using a Chemi Doc TM XRS + (BIO RAD, USA) and Image Lab Software. Cytokine levels of IL-1b, IL-12, and TNF-a in culture supernatants were measured using commercial immunoassay kits.

Lysates were prepared from total cells or nuclear fraction as previously reported in the literature.Detergent-insoluble materials were removed, and equal amounts of protein were fractionated by 10% sodium duodecyl sulphate–polyacrylamide gel electrophoresis and transferred to pure nitrocellulose membranes. Membranes were blocked with 5% skim milk in Tween 20 plus Tris-buffered saline for 1 h and then incubated with an appropriate dilution of primary antibody in 5% bovine serum

albumin (in Tris-buffered saline containing Tween 20) for 2 hours. Blots were incubated with biotintilated antibody for 1 h and further incubated with horseradish peroxidase–conjugated streptavidin for 1 h. Signals were detected by enhanced chemiluminescence.

Mixed leukocyte reaction:

Responder T cells were purified from the spleen of BALB/c mice by negative depletion using biotinylated antibodies for CD45R , GR-1, and CD11c and Dynabeads M-280 streptoavidin , as previously described in the literature. Purity was typically more than 90%. Dendritic cells were generated from the BM cells of C57BL/6 mice and were treated with 40 I g/ml mitomycin C (MMC) for 1 h. MMC-treated DCs were added to 1 Â 10 5 T cells in U-bottom 96-well plates. Allogenic T cells were pulsed with 3 H-thymidine (113 Ci/nmol)at a concentration of 1 I Ci/well for the last 18 h and harvested on day 3 using an automated cell harvester . The amount of 3 H-thymidine incorporated into cells was measured using a Wallac Microbeta scintillation counter. Cytokine levels of IL-2 and IFN- c in culture supernatants were measured using commercial immunoassay kits.

Mogroside V induces. A. phenotypic maturation of Dendritic cells.

Dendritic cells were generated from bone marrow precursors by using 2ng/ml of granulocyte macrophage colony stimulating factor (GM-CSF). On day 8 of culture, non-adherent and loosely adherent cells (i.e.,immature dendritic cells) were harvested from cultures and further activated with lipopolysaccharide (LPS) or Mogroside V for 24 h. An analysis of cell surface makers showed that more than 85% of cells were CD11c +, but not CD3 + or B220 +. Mogroside V dose-dependently increased the expression of CD40, CD80, CD86, and MHC-I/II which are known maturation markers of dendritic cells.

Mogroside V-treated dendritic cells showed a mature morphology with long dendritis, but untreated dendritic cells showed short dendritis . Mogroside V or LPS did not

affect cell viability during the incubation these results are consistent with this patents assertion that Mogroside V induces the phenotypic maturation of Dendritic cells.

Mogroside V induces functional maturation of Dendritic cells.

Immature dendritic cells efficiently capture antigens and show a high level of endocytosis, but mature dendritic cells have reduced antigen capture capacities. To determine the effect of Mogroside V on endocytosis of dendritic cells we treated dendritic cells with fluorescein-isothiocyanate (FITC)-coupled dextran and observed that antigen uptake was dose-dependently reduced in Mogroside V-treated dendritic cells .A similar result was obtained with LPS-treated dendritic cells. Parallel experiments were performed at low temperature to examine nonspecific binding of FITC-coupled dextran to dendritic cells. Antigen uptake by dendritic cells was inhibited at low temperature, which suggested that dendritic cells endocytosis was a specific active process. To examine and exclude LPS contamination of Mogroside V Mogroside V was treated with polymyxin B (PMB), which inhibits the biological effects of LPS by binding to the lipid A moiety that may have been present in the Mogroside V material used. As PMB recovered the lowered endocytosis of LPS-treated dendritic cells whereas it did not affect Mogroside V endocytosis activity .These results demonstrated that the Mogroside V used was free of LPS contamination.

Mature dendritic cells secrete IL-12 and other inflammatory cytokines.

Utilizing Mogroside V we demonstrated significantly increased IL-12 gene expression by Dendritic cells which is a major factor in the induction of Th1 immune response. In addition we demonstrated Mogroside V increased the gene expression of inflammatory cytokines, such as IL-1b, TNF- a , and interferon-beta . Protein production of IL-1b, IL-12, and TNF- a by Mogroside V -treated dendritic cells was determined quantitatively and measured by enzyme-linked immunosorbant assay (ELISA). The results obtained were consistent and confirmed the claim in this patent that Mogroside V induces the functional maturation of Dendritic Cells and stimulates a Th1 immune reaction.

Mogroside V-treated Dendritic Cells demonstrate enhanced allogenic T cell proliferation

Mature dendritic cells have the capacity to induce proliferation of allogenic T cells. The phenotypic and functional maturations of dendritic cells by Mogroside V suggested that Mogroside V -treated Dendritic cells are superior as stimulator cells for the allogenic T cell responses. To confirm this claim we induced mixed leukocyte response using C57BL/6 mouse-derived dendritic cells and BALB/c mouse-derived T cells after 3 days, Mogroside V -treated dendritic cells were able to stimulate very efficiently allogenic T cells, but immature dendritic cells only slightly affected this response. T cell or mitomycin C-treated dendritic cells alone could not proliferate. We also examined the production of T cell–derived cytokines in culture medium 2 days after mixing dendritic cells and T cells.

The Mogroside V -treated dendritic cells could Strongly activate T cells to produce IL-2 and IFN's.

However, Mogroside V-untreated dendritic cells showed weak stimulation activity. These results allow us to claim in this patent that Mogroside V-treated dendritic cells stimulate Allogenic T cells to proliferate and produce cytokines.

Identification of membrane receptor for Mogroside V

Since Mogroside V molecule cannot penetrate dendritic cells due to its hydrophilic molecular nature, Dendritic cell maturation may be caused by surface binding of Mogroside V to a membrane receptors. We presumed that Mogroside V might activate dendritic cells by using LPS receptor TLR4, because both compounds showed similar effect on dendritic cells. To confirm this fact, we generated immature dendritic cells from two different mice: C3H/HeN having normal TLR4 and C3H/HeJ having mutated TLR4.

Mogroside V treatment increased the surface expression of CD86 molecule and TNF-a. cytokine production in dendritic cells generated from C3H/HeN mice, but not from C3H/HeJ mice lacking the normal TLR4. We also evaluated the effect of Mogroside V on endocytosis by dendritic cells (DCs) As was claim FITC-coupled dextran uptake was dose dependently reduced in Mogroside V-treated DCs from C3H/HeN mice, but not from C3H/HeJ mice .TLR3 ligand Poly (I:C) could enhance maturation of DCs from C3H/HeJ mice. From these results we can claim that Mogroside V induces DC maturation at least in part through attachment to the extra-cellular receptor TLR4.

Mogroside V activates TLR4-downstream signalling in Dendritic cells.

Upon binding with LPS, TLR4 activated both MyD88-dependent and TIR-domain-containing adapter-inducing IFN-b (TRIF)-dependent signalling pathways. Although the detailed events downstream of MyD88 and TRIF differ, mitogen-activated protein kinases (MAP-Ks) and nuclear factor-kappa B (NF- j B) signalling are commonly activated. Signalling pathways involving NF- j B and MAPK play a major role in dendritic cell maturation. Thus, we performed Western blotting to detect the level of activation of MAPKs and NF-j B complex. As demonstrated herein, basal levels of phosphorylated extracellular signal-regulated kinase (ERK), c-Jun, N-terminal kinase(JNK), and p38 MAPKs in immature dendritic cells were low, whereas phosphorylation of the three MAPKs were significantly increased over basal levels in dendritic cells upon exposure of Mogroside.V induced the nuclear translocation of NF- j B p65, as demonstrated by the increased levels of nuclear p65 protein. These results demonstrate that Mogroside IV induces DC maturation through MAPKs and NF- j B signaling downstream from TLR4.

The immuno- modulating functions of dendritic cells are usually attenuated in tumour and viral infection micro environments ,which is one of the critical hurdles to the success of dendritic cell -based immunotherapy of cancers and viral infections.

Mogroside V a natural isolate from Luo Han Guo is claimed as adjuvant dendritic cells, and can as demonstrated induce maturation of immature dendritic cells. Several changes were observed in Mogroside IV- treated dendritic cells

(i) increased expression of co-stimulatory molecules (CD40, CD80, and CD86), and MHC-I/II;

(ii) decreased endocytosis;

(iii) increased cytokine gene expression and production;

(iv) an increased capacity to induce T cell proliferation and cytokine production.

Overall this generated data allows us to claim in this patent that Mogroside V induces phenotypic and functional maturation of dendritic cells.

The first evidence of dendritic activation by Mogroside V is presented in this patent and was observed on the surface of Dendritic cells. Immature Dendritic cells are usually poor activators of T cells due to relatively low expression of CD40, CD80/86, and MHC-I/II. Upon receiving the maturation signals from exposure to Mogroside V Dendritic cells increased the expression of MHC-I/II and co-stimulator molecules of CD40 and CD80/CD86. Dendritic cells present antigenic peptides to CD4 + and CD8 + T cells via MHC-I/II.

These molecules amplify T cell receptor (TCR) signalling and promote T cell activation. In addition, sufficient immune response depends on efficient T cell activation by co-stimulators molecules, such as CD40 and CD80/CD86 .The interaction of CD40, CD80, and CD86 in dendritic cells with their counter-receptors (CD40L, CD28, and CTLA-4) on T cells provides a particularly potent co-stimulatory signal, which amplifies the response of T cells.

The second line of evidence of Dendritic activation by Mogroside V was demonstrated inside the Dendritic cell. Mogroside V treated Dendritic cells could produce large amount of several cytokines. Mogroside V increased IL-12, IL-1b, and TNF- a cytokine production by Dendritic cells. IL-12 can promote Th1 cell activation, which induces the secretion of other cytokines such as IL-2, IFN- c, and TNF-b.

Eventually, IL-12 might be one of the key cytokines that stimulate Th1 cell - mediated immune responses, the major defence mechanisms against infection and tumours. In addition, dendritic cells activate natural killer cells through IL-12 .Other cytokines, such as TNF- a and IL-1b, produced by dendritic cells up-regulate adhesion molecules by the endothelium and these molecules contribute to the recruitment of monocytes and other cell types to the tumour micro-environment and to locations of virally infected cells within the body for lysis and destruction.

The third line of evidence of dendritic cells being activated by Mogroside V was observed in the changes of antigen-degrading ability of dendritic cells Immature dendritic cells are recruited to sites of inflammation in peripheral tissues following exposure to pathogen. Then, immature dendritic cells capture antigens by phagocytosis or macro pinocytosis, or via interaction with a variety of cell surface receptors and endocytosis. However, TNF-α and IL-1b added to immature dendritic cells induced a coordinate series of changes resulting in the loss of endocytic activity, up-regulation of adhesion, costimulatory molecules, and MHC-I/II. We demonstrate here that immature dendritic cells show a high uptake of antigen, but antigen uptake is low in Mogroside V -treated mature Dendritic cells.

The fourth line of evidence and proof that DC (dendritic cells) are activation by Mogroside V was observed in the changes of T cell activation ability of Dendritic cells. Mogroside V -treated dendritic cells could make T cells proliferate and produce cytokines.

Dendritic cells (DC) present antigen to both CD4 + and CD8 + T cells. This appears to be the most prominent role for Dendritic cells since dendritic cells present antigen to T cells more efficiently by 10- to 100-fold than B cells and monocytes ,Activated Th1 cells can produce IFN's and IL-2, which can activate anti-tumour and anti-viral cells, such as cytotoxic T cells and NK cells as demonstrated in our primate Hep. C studies and human Hep C studies utilizing Mogroside V. (data attached)

An Open-label, Efficacy and Safety Study to Evaluate the Effects of Mogroside IV and Ribavirin on Viral Load in Treatment Naïve, Genotype 1 Patients with Chronic Hepatitis C

Aim: To evaluate efficacy and safety of Mogroside IV plus ribavirin therapy for treatment treatment naïve genotype 1 chronic hepatitis C patients.

Methods: From June 2012 to December 2012, 28 HCV patients were enrolled. All patients were treatment naïve and fulfilled the inclusion criteria: HCV genotype 1 and HCV RNA >1.0×104 copies/ml. Mogroside IV 1000mg were administrated twice daily orally for 4 days with a 6 days break before resuming next cycle. Repeat the cycle for 9 times, total 90 days. Meanwhile, patients were required to take ribavirin orally for 90 days (weight ≤60kg, 800mg/d ; weight=60-80 kg, 1000mg/d ; weight≥80 kg,1200 mg/d). Then continue to observe patients for another 90 days. During the totally 180 days study duration, collect blood sample at Day 0, D20, D40, D60, D80, D90, D120, D150 and D180, to obverse serum HCV RNA viral load and liver function, clinical symptoms and physical signs and collect adverse events.

Results:

① We have screened 29 HCV patients, 28 subjects fulfilled the inclusion criteria (male:17; female:11) with mean 51.68±6.53 years old. Twenty five subjects completed the treatment as protocol design. One female 50 years subject was diagnosed as superficial gastritis, she discontinued both Mogroside IV and ribavirin at day 62 and resumed 2 days later; one male subject with 61 years old was diagnosed as duodenal ulcer (A2) at day 81, ribavirin was stopped for 9 days, but Mogroside IV was used as protocol predesigned; another female subject with 58 years old discontinued ribavirin at day 61 for personal reason then resumed 9 days later.

② Clinical symptoms and physical signs: Compared to baseline, the overall patients' clinical symptoms and physical signs improved significantly at day 90, they were malaise, food intake, abdominal distension, sleep problems and hepatic face.

③ Biochemical response: Compared to baseline data, at day 90 the last day of therapy, serum ALT decreased significantly from (97.281±65.88 (IU/L)) to (68.82± 53.58 (IU/L) (P=0.022), in addition serum AST decreased significantly from (63.78 ±35.22 (IU/L)) to (40.54±25.51 (IU/L)) (P=0.002). However, direct bilirubin increased at day 90 compared with baseline data, and albumin decreased at day 90 4 compared with baseline data.

④ Virological response: Compared to baseline HCV RNA viral load (5.66±1.37) ×106 copies/ml, viral load showed a obvious decrease tendency at Day 90 ((5.07± 1.11) ×106 copies/ml) but no statistical significantly. However, compared with day 90, HCV RNA viral load increased to (7.56±1.43)×106 copies/ml at day 180.

⑤ Blood platelet counts: During the treatment period, patients' blood platelet counts increased gradually. Compared with baseline (166.43±48.871)×109 /L, platelet counts increased significantly at day 90 (229.64±72.258)×109 /L (P<0.0000).

⑥ Adverse events: Two no investigational medication related serious adverse events reported. Total 18 patients reported 21 adverse events. All the non-serious events resolved without receiving treatment, they were fatigue (8), dizzy (5), abdominal distension (4), dry mouth and mouth bitterness (2) and rash (2). Conclusions: Mogroside IV plus ribavirin therapy for HCV genotype 1 presented a significant effect to improve liver function and increase blood platelet counts and had a tendency to inhibit of HCV RNA reproduction. The therapy was well tolerated for study patients.

Introduction

Current standard therapy for chronic hepatitis C is a combination of pegylated interferon plus ribavirin.For all kinds of HCV genotype, genotype 1 is the most difficult one to treat, with sustained virological response (SVR) rates only around 50% in north America and Europe. Currently a growing of new antiviral agents has been developed, such as direct antiviral agents (DAAs), including protease inhibitor, HCV RNA polymerase inhibitor et al. Although the preliminary study results were very effective in treating HCV patients, but due to HCV gene lacks of proofreading mechanism, HCV mutates rapidly due to a high error rate on the part of the virus' RNA-dependent RNA polymerase. As protease inhibitor, HCV RNA polymerase

inhibitor widely used in clinics, it can be speculated that higher frequency of HCV viral mutations will occur accordingly. Therefore, it is necessary to explore and develop antiviral drug having 5 other antiviral mechanism as DAA, two drugs or three drugs therapy might acquire high efficiency antiviral effect. Mogroside IV is a extract from plant, Luo Han Guo, a natural material, the percentage of it is between 2.2%-8.2% in PureloTM. Previous preclinical study and phase II study demonstrated that Mogroside IV showed an effect to decrease HCV RNA viral load and was well tolerance. The present study is to explore the efficacy and safety of Mogroside IV plus ribavirin to treat treatment naïve HCV patients with genotype 1.

Part I the preparation of study

Study protocol design

2.1 Inclusion and exclusion criteria Inclusion criteria

1. The patients can be contacted by phone or other ways. The compliance of patients can be promised.
2. Subjects volunteered to join the study and sign informed consent
3. Age ≥ 18 years and ≤ 70 years, male or female
4. Serologic evidence of Hepatitic C infection by an anti-HCV antibody test. The patient is confirmed as genotype I by PCR method. Serum HCV RNA quantifiable at > 1 ×104 copies/mL at screening period.
5. Not receive any antiviral treatment including Interferon (IFN) with or without RBV therapy within one year (Interview will be sufficient)
6. No clinical suspicion or radiological evidence of primary hepatocarcinoma and a serum AFP < 50ng/mL 6
7. Negative urine pregnancy test (for women of childbearing potential) documented within the 24-hour period prior to the first dose for test drug, and no planned pregnancy within one year
8. Male and female subjects agree to take contraceptive measures during the period of taking part in the study till after 6 months of the last administration time
9. Genotype of HCV RNA is genotype-I.

Exclusion criteria

1. Patients who have previously taken any antiviral therapy including Interferon (IFN) and/or RBV within one year of entry
2. History or other evidence of a medical condition associated with alcohol liver disease (Diagnosed according to the Guidelines of Alcoholic Liver Disease)
3. History of epilepsy poorly controlled on prescribed medications (Interview will be sufficient)
4. Women with ongoing pregnancy or breast feeding
5. Neutrophil count < 1000 cells/mm3, Hgb 2 times the upper limit of normal at screening
6. Evidence of alcohol and/or drug abuse within one year of entry;
7. History of severe psychiatric disease, especially depression. Severe psychiatric disease is defined as treatment with an antidepressant medication or a major tranquillizer at therapeutic doses for major depression or psychosis, respectively, for at least 4 months at any previous time or any history f the following: a suicidal attempt, hospitalization for psychiatric disease, or a period of disability due to a psychiatric disease. (Interview will be sufficient)
8. History of immunologically mediated disease (e.g., inflammatory bowel disease, idiopathic thrombocytopenic purpura, lupus erythematous, autoimmune haemolytic anemia, scleroderma,severe psoriasis, sarcoidosis, etc.). (Interview will be sufficient)
9. History or other evidence of bleeding from esophageal varices or hepatic 7 encephalopathy (Interview will be sufficient)
10. History of diabetes poorly controlled on prescription medications;
11. History of malignant tumor or suspected malignant tumor (Interview will be sufficient)
12. History or organ transplantation (Interview will be sufficient)
13. History of thyroid disease poorly controlled on prescribed medications
14. Hemoglobinopathy(severe mediterranean anemia)
15. Patients who have previously taken didanosine

16. History of peripheral neuropathy, pancreatitis, and hyperlactatemia/lactic acidosis
17. Patients who have previously taken azathioprine
18. Inability or unwillingness to provide informed consent
19. History or other evidence of severe illness or any other conditions which would make the patient, in the opinion of the investigator, unsuitable for the study

2.2 Study medication administration and follow up

Mogroside IV: 1000mg/day, 250mg/capsules, twice daily (Morning: 500mg/2capsules, evening: 500mg/2capsule) for 4 days, stop for 6 days, which is one cycle. Repeat for total 9 cycles.

Ribavirin : Weight ≤60kg: 800mg/day (8 tablets/day orally; morning:4 tablets, evening: 4 tablets); weight 60-80kg: 1000mg/day(10tablets/day; Morning: 5tablets, evening: 5tablets) weight≥80 kg : 1200 mg/day (12tablets/day) (4 tablets, BID)

Ribavirin was administrated from baseline till to day 90.

Keep observing patients for another 90 days.

During study duration, patients visited hospital at day 0, 20, 40, 60, 80, 90, 120, 150, 180. Collect patients' blood samples at hospital visits and perform physical examination. HCV RNA viral load, liver function and biochemical variables were tested.

2. 3 Case report form and informed consent design

Since Mogroside IV is an extract of plant, Luo Han Guo, the study was conducted under compassionate used program

Part II the conduction of clinical study

1 The kick-off meeting and patients screening

The study kick-off meeting was held in May, 2012. From Jun 7 th to 9 th , we have screened 29 chronic hepatitis C patients from Dingxi County, Gansu province. Total

28 patients with genotype 1b fulfilled the inclusion criteria with 17 male and 11 female and mean 51.68±6.53 years old. One patient was excluded due to HCV RNA<1.0 ×104 copies/ml.

2 Observation periods

2.1 Study medication distributed and administration training

To ensure patients followed protocol correctly, we trained patients with detailed medication administration instruction before the beginning of the study. A detailed calendar of administration note was on one side of each envelop and reminder note of two different medication administration was on the other side of each envelop;

Follow up patients by phone to instruct administration and collect adverse events At each hospital visit we distributed two cycles of medications, and collected the previous medication envelop from each patient, and asked every patient whether he or she had any complain, conducted physical examination and collected the record of medication administration history.

3 Blood sample collection

At protocol designed times, collect fast blood from patients, separated serum, conduct liver function test, meanwhile stored serum at −80°C.

4 Fill in CRF and data input

Completed CRF and data input in excel file.

5 Statistical Analyses

Data are analyzed by SPSS13 software packet. The results were measured by mean± standard deviation. If the continuous variable obeys normal distribution, paired t test will be used.

Part III Study results

1 The Baseline Characteristics of Study Subjects (n=28) (Table 1).

Twenty nine patients were screened, 28 patients fulfilled the inclusion criteria. Seventeen male and 11 female with mean 51.68±6.53 years old. Twenty five patients completed the treatment as protocol design.

Three patients discontinued study medication during study duration.

One female patient with 50 years old was diagnosed as superficial gastritis, discontinued Mogroside IV and ribavirin at day 62 and resumed 2 days later; one male patient with 61 years old was diagnosed as duodenal ulcer (A2) at day 81, ribavirin was stopped for 9 days, but Mogroside IV was used as protocol predesigned; the detailed description of this two adverse events was discussed in part III study results, 5 adverse events. One female patient with 58 years old discontinued ribavirin at day 61 with for personal reason then resumed 9 days later.

Table 1 The Baseline Characteristics of Study Subjects (n=28)

Indices	Value
Age (years), Mean ± SD	52.96±7.41
Gender, n(%)	
Male:	17(60.7)
Female:	11(39.3)
BMI (kg/ m^2) , Mean ± SD	21.91±2.19
Body surface area (m^2) , Mean ± SD	1.65±0.16
ALT (U/L), Mean ± SD	97.29±65.88
AST(U/L) , Mean ± SD	63.79±35.22
Tbil (μmol/L) , Mean ± SD	21.90±7.61
Dbil (μmol/L) , Mean ± SD	13.69±15.36
ALB (g/L) , Mean ± SD	53.75±4.21
GLU (mmol/L) , Mean ± SD	4.71±0.54
Cr (μmol/L) , Mean ± SD	62.43±11.78

RBC(10^{12}/L) , Mean ± SD	4.80±0.65
AFP (ng/mL) , Mean ± SD	14.51±62.09
HCV RNA(IU/ml): Mean (range)	5.66×10^{6} (2.84×10^{5}-3.24×10^{7})

2 The improvement of clinical symptoms and physical signs

As showed in Figure 1, compared to baseline, significant improvements were observed for patients in malaise, anorexia, food intake, abdominal distension, sleep, facies hepatica at day 90.

3 The response to biochemical variables As Figure 2 showed, compared to baseline, at day 90, serum ALT decreased significantly from (97.28±65.88 (IU/L)) to (68.82±53.58 (IU/L) (P=0.022), in addition serum AST decreased significantly from (63.78±35.22 (IU/L)) to (40.54±25.511 (IU/L)) (P=0.002). However, direct Bilirubin increased at day 90 compared with baseline and albumin decreased at day 90 compared with baseline data.

4 Virological response: Compared to baseline viral load (5.66±1.37) ×106 copies/ml, HCV RNA viral load showed a decrease tendency at day 90 (5.07±1.11)×106copies/ml but no statistical significantly, however, HCV RNA viral load rebounded to (7.56±1.43)×106copies/ml at day 180.

Figure 3 HCV RNA during study duration.

Data was presented as mean ±SEM. # P=0.628 by paired-sample T test performing between 0D and 90D & P=0.115 by paired-sample T test performing between 0D and 180D

5 Blood platelet counts: During the treatment period, blood platelet counts increased gradually (Figure 4A). Compared with baseline (166.43±48.871×109/L), platelet counts increased significantly at day 90 (229.64±72.258)×109/L (P<0.0000 (Figure 4B),

Figure 4 Platelet count during study duration.

Data was presented as mean ±SEM.
14 A: Platelet counts increased gradually during treatment period. B: P<0.000 by paired-sample T test performing between 0D and 90D

6 Adverse events Total 18 patients reported 21 adverse events. All the non-serious events resolved without receiving treatment, they were fatigue (8), dizzy (5), abdominal distension (4), dry mouth and mouth bitterness (2) and rash (2).One patient discontinued Ribavirin due to personal reason for 9 days, and then resumed.

Two serious adverse events reported. ① Patient No. 21, 50 years old, female, had history of gastric disorder. At the 2nd day of the 7th administration, she was hospitalized due to acute abdominal pain. The diagnosis was superficial gastritis, and she was discharged with prescription of Anisodamine tablets and Sucralfate Chewable Tablets 2 days later. The study medication of Mogroside IV capsule and Ribavirin tablets was stopped during hospitalized and resumed after she was discharged. At day 90, no adverse event reported. ② Patient No. 23, 61 years old, male. At the last day of the 8th administration, just after took Mogroside IV capsules, patient experienced diarrhoea and vomiting, so he took norfloxacin tablets, then diarrhoes resolved partially, but he felt fatigue, dazzle and tinnitus. The next day he called us and said his skin was yellowed. At the 1st day of the 9th administration he visited hospital, the lab test found he experienced anemia. Gastroscopy showed duodenal ulcer (A2), we gave him anti ulcer medication. At the same time, Ribavirin tablets were stopped, but Mogroside IV capsules was used as study protocol. At day 90, no adverse event reported.

Part IV Discussion

One interesting finding of present study was that Mogroside IV plus ribavirin therapy had a significant effect to protect liver function. Actually similar results from study conducted at Brooke Army Medical Center in the USA have been observed previously. However, the mechanisms were unclear present.

Another interesting finding was that during the Mogroside IV plus ribavirin therapy, patients' blood platelet counts had been increasing steadily, and compared with

baseline at the end of administration, blood platelet counts showed statistically significantly increased (P<0.0000), however, the mechanisms were largely unknown presently.

Meanwhile, we also noticed that serum direct bilirubin levels increased and album levels decreased after patients completed Mogroside IV plus ribavirin therapy. As previously reported Ribavirin can lead to red blood cell breakage and further may induce to indirect bilirubin level increased. It is hard to conclude that direct bilirubin level increasing was caused by Mogroside IV plus ribavirin, or Mogroside IV only. Therefore, to clarify of this problem, a large size and controlled trial need be designed, and histopathological examination results of liver tissues may provide clear and definite explanation. Meanwhile, since ribavirin is known to produce stomach discomfort side reaction, which may lead to food intake less and poor nutrition status, which further lead to albumin level decrease. However, at presently we cannot rule out other possibility.

On the other hand, the study results also showed a tendency of antivirus effects. Many studies demonstrated that Toll-like receptor may recognize pathogen and activate body innate immune response. Canopus found that Mogroside IV may activate immune response, the mechanism maybe due to reactive with host receptor, then up-regulation of immune system. Mogroside IV active with Toll-like receptor then induces inner interferon production which further inhibits virus reproduction. But, 16 whether the antiviral effect was by Mogroside IV plus ribavirin or Mogroside IV along need controlled study validated in the future. In conclusion, patients were well tolerated for Mogroside IV plus Ribavirin therapy; no investigational medication related serious adverse effects reported.

Part V Inclusion and expectation

1. Mogroside IV plus Ribavirin treatment of genotype 1 chronic C hepatitis C patients had significantly effects on decreasing ALT levels and increasing blood platelet counts, and showed a tendency inhibition of HCV RNA. Mogroside IV was well tolerated with no study medication related serious adverse events.

2. In present study, the mechanism of decreasing of ALT and increasing blood platelet counts needs to be explored and clarify, the increasing of direct bilirubin and decreasing of albumin need further confirmed as well.
3. The mechanisms of anti-HCV for Mogroside IV plus Ribavirin therapy need to be further investigated. For example, the anti-HCV effect is by Mogroside IV only or ribavirin only or both of them together?
4. Controlled, longer study duration study and with liver histological examination validation study are need to be designed to clarify our results.

CLAIMS

1. A sterile vaccine composition comprising an antigen combined with Mogroside IV or its analogues as Immune Stimulatory Adjuvant.

2. A sterile vaccine composition comprising an antigen combined with Mogroside V or its analogues as Immune Stimulatory Adjuvant.

3. A sterile vaccine composition comprising an antigen combined with Mogroside IV and Mogroside V or their analogues as Immune Stimulatory Adjuvant.

4. A vaccine composition as claimed in Claim 1 and/or in Claim 2, or Claim 3 in which the antigen is an intact viral particle or a viral protein or composition or complex of viral peptides

5. A vaccine composition as claimed in Claim 1 and/or in Claim 2 or Claim 3 in which the antigen comprises a viral antigen.

6. A vaccine composition as claimed in Claim 1 and/or Claim 2 or Claim 3 wherein the antigen comprises a Hepatitis A antigen.

7. A vaccine composition as claimed in claim 6 wherein the Hepatitis A antigen is derived from the HM-I75 strain.

8. A vaccine composition as claimed in claim 1 and/or Claim 2 or Claim 3 wherein the antigen comprises a hepatitis B antigen.

9. A vaccine composition as claimed in Claim 8 wherein the antigen comprises Hepatitis B surface antigen (HBsAg) or a variant thereof.

10. A vaccine composition according to Claim 1 and/or Claim 2 or Claim 3 additionally comprising a Hepatitis A antigen.

11. A vaccine composition as claimed in Claim 1 and/or Claim 2 or Claim 3 wherein the antigen is HBsAg and comprises the S antigen of HBsAg.

12. A vaccine composition as claimed in Claim 1 and/or Claim 2 or Claim 3 wherein the antigen is a surface antigen from Hepatitis B virus and comprises a pre-S sequence and the S antigen.

13. A vaccine composition as claimed in Claim 1 and/or Claim 2 or Claim 3 wherein the antigen comprises a composite particle which contains a modified L protein of hepatitis B virus having an amino acid sequence comprising residues 12-52 followed by residues 133-145 followed by residues 175-400 of the L protein and the S-protein of HBsAg.

14. A vaccine composition as claimed in Claim 1 and/or Claim 2 or Claim 3 comprising one or more hepatitis antigens and at least one other component selected from a non-hepatitis antigen which affords protection against one or more of the following: diphtheria. tetanus. pertussis. *Haemophilus influenzae* b (Hib). and polio.

15. A vaccine composition according to Claim 1 and/or Claim 2 or Claim 3 comprising antigens selected from a DTP (diphtheria-tetanus pertussis) HBsAg combination. an Hib-HBsAg combination. a DTP-Hib-I-IBsAg combination and an IPV (inactivated polio vaccine)-UI'P-Hib~HBsAg combination.

16. A vaccine composition as claimed in Claim 1 and/or Claim 2 or Claim 3 comprising an HSV glycoprotein D or an immunological fragment thereof.

17. A vaccine composition as claimed in Claim 1 and/or Claim 2 or Claim 3 wherein the glycoprotein D is a truncated protein.

18. A vaccine composition as claimed in Claim 1 and/or Claim 2 or Claim 3 wherein the antigen comprises HSVgD2 antigen and is devoid of the C terminal anchor region.

19. A method of treating a human subject suffering from or susceptible to infection comprising administering an effective amount of Mogroside IV ,Mogroside V or a combination of both .

20. A vaccine composition as claimed in Claim 1 and/or Claim 2 or Claim 3 wherein the antigen is a soluble NSI-OspA fusion protein, where N51 is influenza NS1 and OspA is *Borrelia burgdorferi* OspA.

21. A compound of Mogroside IV or a pharmaceutically acceptable salt or analogue of Mogroside IV as outlines in the structures of Mogroside IV. As an agonist, unblocking the activity of Toll-Like Receptor 4 in immune compromised states generated by neoplasm or infection by a pathogen.

22. A compound of Mogroside V or a pharmaceutically acceptable salt or analogue of Mogroside V as outlines in the structures of Mogroside V. As an agonist, unblocking the activity of Toll-Like Receptor 4 in immune compromised states generated by neoplasm or infection by a pathogen.

23. A combination of Mogroside IV and Mogroside V or a pharmaceutical acceptable salts or analogues thereof, As an agonist unblocking the activity of Toll-Like-Receptor 4 in immune compromised states generated by neoplasm or infection by pathogen.

24. A pharmaceutical composition comprising a therapeutically effective amount of Mogroside IV ,Mogroside V or a combination of both or a pharmaceutically acceptable carrier or excipient for the treatment of any bacterial infections (eg Tuberculosis) fungal and or viral infections (eg Dengue Hepatitis B/C or

coronoviral infections) which result from infectious organism interference with the immune system of the patient causing defective functioning of immune system.

25. The pharmaceutical composition of Mogroside IV, Mogroside V or a combination of both further comprising at least one additional therapeutic agent selected from the group consisting of interferons, ribavirin or its analogs, HCV NS3 protease inhibitors, alpha-glucosidase 1 inhibitors, hepatoprotectants, nucleoside or nucleotide inhibitors of HCV NS5B polymerase, non-nucleoside inhibitors of HCV NS5B polymerase, HCV NS5A inhibitors, TLR-7 agonists, cyclophilin inhibitors, HCV IRES inhibitors, pharmacokinetic enhancers, and other drugs for treating HCV, or drugs for treating Hep B or mixture of virus infection.

26. The pharmaceutical composition of Claims 24 further comprising at least one additional therapeutic agent selected from the group consisting of lamivudine, adefovir, tenofovir, telbivudine, entecavir, interferon alpha-2b, pegylated interferon alpha-2a, interferon alpha 2a, interferon alpha N1, prednisone, predinisolone, Thymalfasin, retinoic acid receptor agonists, 4-methylumbelliferone, Alamifovir, Metacavir, Albuferon, cytokne.t

27. A method for treating a Hepatitis C viral infection comprising administering to a human subject infected with Hepatitis C virus a therapeutically effective amount of Mogroside IV, Mogroside V or a combination of both alone or in combination with one or more antiviral drugs.

28. The pharmaceutical composition of claims 21, 22, 23 further comprising at least one additional therapeutic agent selected from the group consisting of anti-cancer chemo therapeutic agents and/ or radiation for the therapy of heptocellular carcinoma HCC) this Toll-Like Receptor 4 agonist of this patent

offers an effective molecular-targeted treatment against HCC, which has proved notoriously resistant to systemic conventional therapies, often recurring even after aggressive therapy.

29. The pharmaceutical composition of claims 1,2 and 3 further comprising at least one additional therapeutic agent selected from the group consisting of anti-cancer chemo therapeutic agents and/ or radiation for the therapy of carcinoma Or neoplasm this offers an effective molecular-targeted treatment against cancerous tumours which is notoriously resistant to systemic therapies, often recurring even after aggressive therapy because the tumour is undermining the patients immune system by inhibiting the activity of Toll Like Receptor -4

30. A vaccine composition as claimed in claims, 1 ,2and 3 in which a cancer cell or cancer marker molecule is included as vaccine antigen.

31. A vaccine composition as claimed in claim 30 in which the cancer cell or marker is obtained from human breast neoplastic cells and included as vaccine antigen

32. A vaccine composition as claimed in claim 30 in which the cancer cell or marker is from human small cell lung cancer and included as vaccine antigen.

33. A vaccine composition as claimed in claim 30 in which the cancer cell or marker molecule is from a prostate cancer cell line or tissue and included as vaccine antigen.

34. A vaccine composition as claimed in claim 30 in which the cancer cell or marker molecule is from any human or animal cell line or tissue

35. A therapeutic formulation of MICRONIZED Mogroside V for administration to stimulate denritic cell maturation in ta patient and combined with viral or other pathogenic antigens.

36. A therapeutic formulation of MICRONIZED Mogroside IV for administration to stimulate denritic cell maturation in ta patient and combined with viral or other pathogenic antigens.

37. A therapeutic formulation of MICRONIZED Mogrosides V and IV for administration to stimulate denritic cell maturation in ta patient and combined with viral or other pathogenic antigens

38. A vaccine composition as claimed in claims 1, 2 and 3 in which the antigen is any or all four strains of dengue virus.

Figure 1

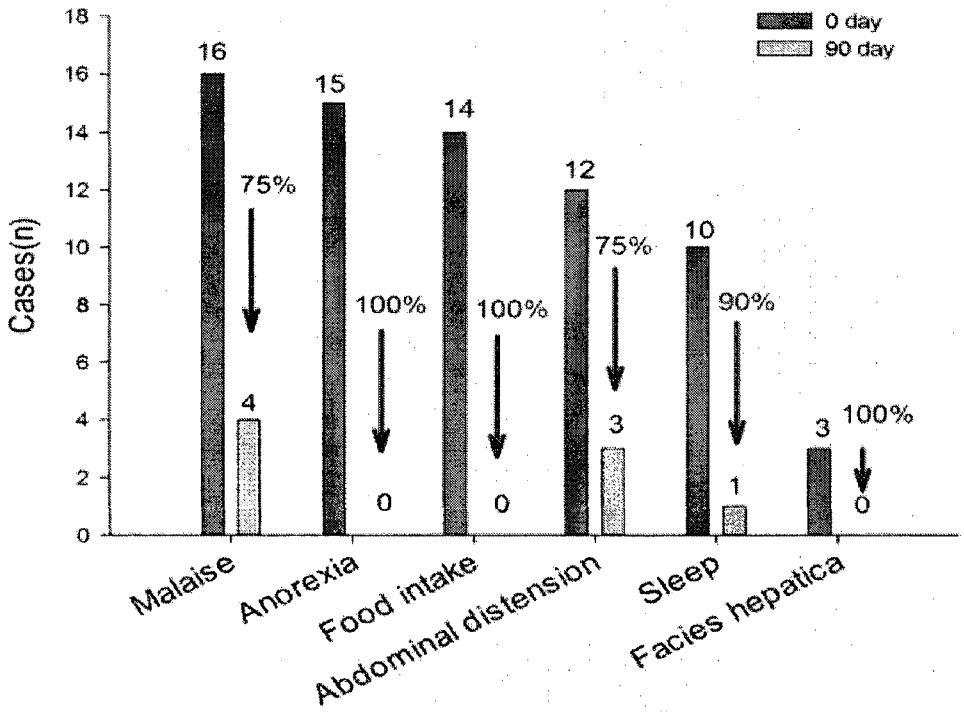


Figure 2

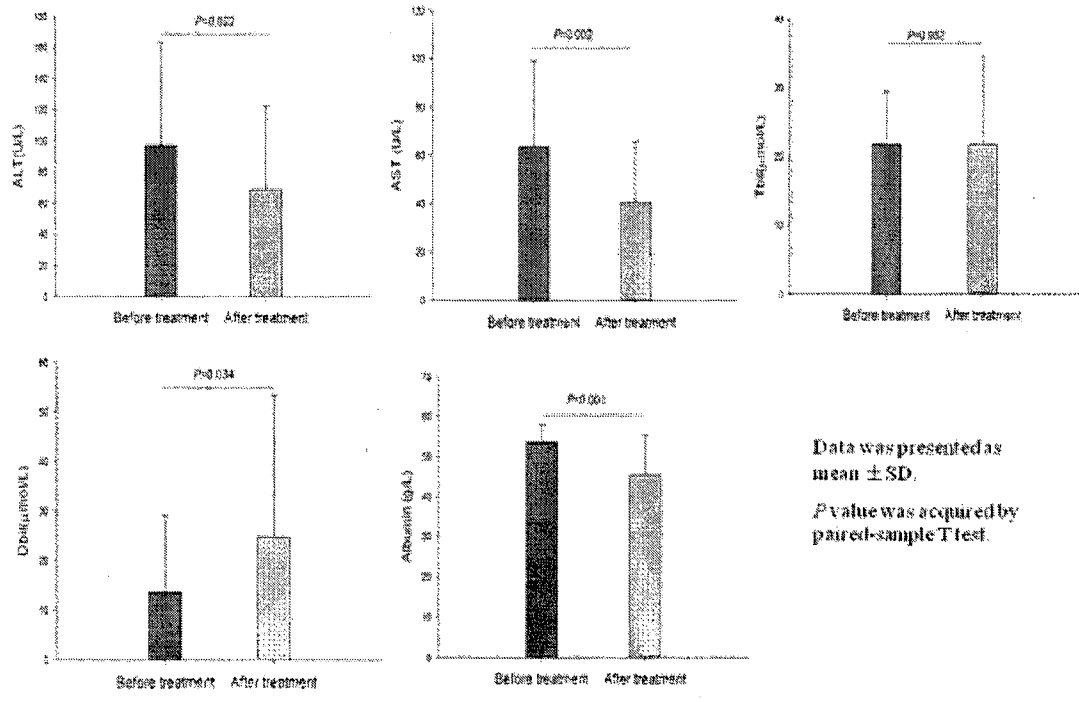


Figure 3

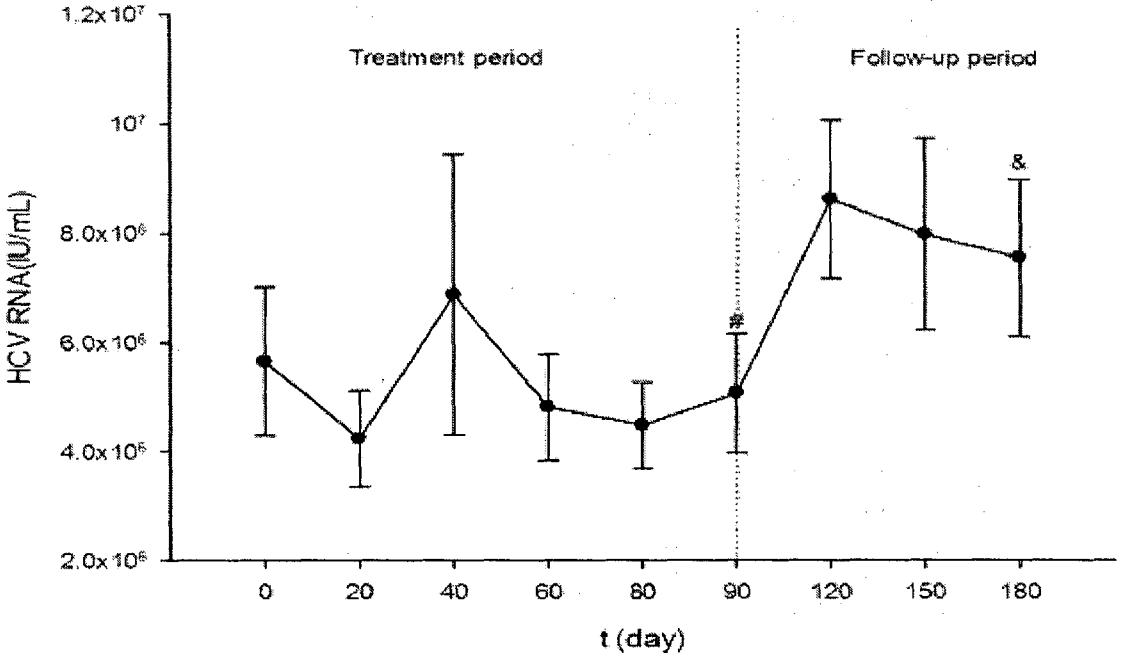
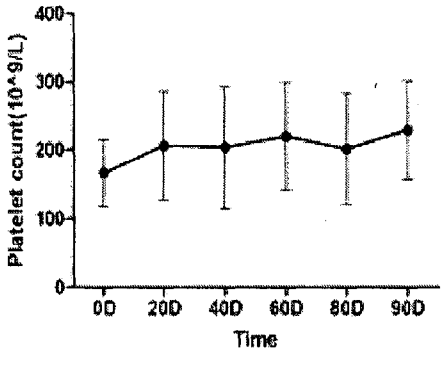


Figure 4



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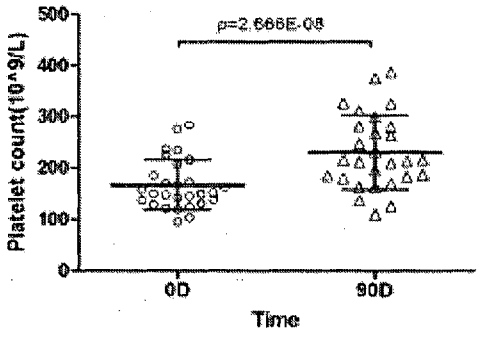

B

INTERNATIONAL SEARCH REPORT

International application No
PCT/IE2015/000007

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00 A61K39/39
ADD.

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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, PAJ, 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	CN 102 146 116 A (UNIV GUILIN MEDICAL) 10 August 2011 (2011-08-10) abstract, paragraphs [0004] - [0006], paragraphs [0010]-[0013] and claims ----- -/--	1-38

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

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
20 November 2015	27/11/2015

Name and mailing address of the ISA/	Authorized officer
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DONGFANG SHI ET AL: "Protective effects and mechanisms of mogroside V on LPS-induced acute lung injury in mice", PHARMACEUTICAL BIOLOGY, SWETS AND ZEITLINGER, LISSE, NL, vol. 52, no. 6, 1 January 2014 (2014-01-01), pages 729-734, XP009187197, ISSN: 1388-0209, DOI: 10.3109/13880209.2013.867451 abstract, p. 730 right-hand column first full paragraph, p. 731 paragraph bridging the left- to the right-hand column - p. 732 right-hand column first full paragraph, Fig. 2-7	1-38
A	US 6 080 725 A (MARCIANI DANTE J [US]) 27 June 2000 (2000-06-27) the whole document	1-38
A	WO 2007/052059 A2 (NOVARTIS VACCINES & DIAGNOSTIC [IT]; O'HAGAN DEREK [IT]) 10 May 2007 (2007-05-10) abstract, p. 10 line 22 - p. 11 line 4	1-38
A	WO 2010/146414 A1 (UNIV SINGAPORE [SG]; PASTEUR INSTITUT [FR]; INST NAT SANTE RECH MED [F) 23 December 2010 (2010-12-23) abstract, p. 16 paragraph [0078] - p. 17 paragraph [0081]	1-38
A	HIROE HONDA ET AL: "Molecular basis of regulatory effect of medicinal plant components on the innate immune response through TLR4 signaling", THE JOURNAL OF IMMUNOLOGY, vol. 184, no. 1001, 1 April 2010 (2010-04-01), page 89.1, XP055229680, the whole document	1-38
A	RONG DI ET AL: "Anti-inflammatory Activities of Mogrosides from Momordica grosvenori in Murine Macrophages and a Murine Ear Edema Model", JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, vol. 59, no. 13, 13 July 2011 (2011-07-13) , pages 7474-7481, XP055229517, US ISSN: 0021-8561, DOI: 10.1021/jf201207m the whole document	1-38

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MASAO TAKEI ET AL: "Dendritic cells maturation promoted by M1 and M4, end products of steroidal ginseng saponins metabolized in digestive tracts, drive a potent Th1 polarization", BIOCHEMICAL PHARMACOLOGY, vol. 68, no. 3, 1 August 2004 (2004-08-01) , pages 441-452, XP055229760, US ISSN: 0006-2952, DOI: 10.1016/j.bcp.2004.04.015 Abstract, p. 442 right-hand column first full paragraph - p. 443 left-hand column line 6, p. 444 paragraph bridging the left- to the right-hand column, p. 447 right-hand column first full paragraph, Fig. 1-7 and Table 1 -----	1-38

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Information on patent family members

International application No
PCT/IE2015/000007

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