Abstract: The present invention provides oral pharmaceutical formulations comprising a VLA-4 antagonist and a surfactant. In one preferred embodiment, the VLA-4 antagonist is the mono-sodium salt of (3S)-3-(((1-(2-chlorobenzyl)-4-hydroxy-5-methyl-2-oxo-1,2-dihydropyridin-3-yl)amino)carbonyl)amino)-3-(4-methylphenyl)propanoic acid. The present invention also provides methods for treating and/or preventing and/or inhibiting progress of an inflammatory disease using such pharmaceutical formulations.

Figure 1
Declarations under Rule 4.17:

- as to the applicant’s entitlement to claim the priority of the earlier application (Rule 4.17(Hi))

Published:

- without international search report and to be republished upon receipt of that report (Rule 48.2(g))
ORAL PHARMACEUTICAL FORMULATIONS OF VLA-4 ANTAGONISTS

FIELD OF THE INVENTION

The present invention relates to novel oral pharmaceutical formulations comprising a VLA-4 antagonist and a surfactant. The invention also relates to methods of using such pharmaceutical formulations for the treatment and/or prevention of inflammatory disease.

BACKGROUND OF THE INVENTION

Citation of or reference to any application or publication in this Section or any Section of this application is not an admission that such document is available as prior art to the present invention.

Very late antigen-4 (VLA-4) is an alpha4betal integrin dimer. Primarily, integrin receptor vascular cell adhesion molecule-1 (VCAM-1) is an endothelial ligand for VLA-4 and for integrin alpha4beta7. VLA-4 integrin, normally expressed on leukocyte plasma membranes, does not adhere to its appropriate ligands until the leukocytes are activated by chemotactic agents or other stimuli which are often produced by the endothelium or other cells at the site of injury. Only then do the integrins undergo the conformational change necessary to confer high binding affinity for the endothelial adhesion molecules.

When a tissue has been invaded by a microorganism or has been damaged, white blood cells, also called leukocytes, play a major role in the inflammatory response. One of the most important aspects of the inflammatory response involves the cell adhesion event. Generally, white blood cells are found circulating through the bloodstream. However, when a tissue is infected or becomes damaged, the white blood cells recognize the invaded or damaged tissue, bind to the wall of the capillary and migrate through the capillary into the affected tissue. These events are mediated by a family of proteins called cell adhesion molecules.

There are three main types of white blood cells: granulocytes, monocytes and lymphocytes. The integrin VLA-4 (also called alpha4beta1) is a heterodimeric cell surface receptor protein composed of CD49d (alpha) and CD29 (beta). VLA-4 is predominantly distributed on the surface of monocytes, lymphocytes and two subclasses of granulocytes: eosinophils and basophils. This
protein plays a key role in cell adhesion through its ability to recognize and bind VCAM-I and fibronectin, proteins associated with the endothelial cells that line the interior wall of capillaries.

Following infection or damage of tissue surrounding a capillary, endothelial cells express a series of adhesion molecules, including VCAM-I, that are critical for binding the white blood cells that are necessary for fighting infection. Prior to binding to VCAM-I or fibronectin, the white blood cells initially bind to certain adhesion molecules to slow their flow and allow the cells to "roll" along the activated endothelium. Monocytes, lymphocytes, basophils and eosinophils are then able to firmly bind to VCAM-I or fibronectin on the blood vessel wall via the VLA-4 integrin. There is evidence that such interactions are also involved in transmigration of these white blood cells into the damaged tissue as well as the initial rolling event itself.

Although white blood cell migration to the site of injury helps fight infection and destroy foreign material, in many instances this migration can become uncontrolled, with white blood cells flooding to the scene, causing widespread tissue damage as found in chronic inflammation and autoimmune disorders. VLA-4 inhibition has been shown to decrease the accumulation of white blood cells and eosinophils at the site of inflammation, subsequently lessening the severity of inflammatory diseases.

VLA-4 antagonists and methods of making the same, including (3S)-3-[[{[(1S)-(2-chlorobenzyl)-4-hydroxy-5-methyl-2-oxo-1,2-dihydropyridin-3-yl]amino}{carbonyl}amino]-3-(4-methylphenyl)propanoic acid (referred to herein as Compound I and pharmaceutically acceptable salts thereof, such as its mono-sodium salt referred to herein as Compound IA, are described in United States Patent No. 6,972,296, the entire disclosure of which is incorporated herein by reference. United States Patent No. 6,972,296 also generally describes pharmaceutical formulations of VLA-4 antagonists, including Compound I or pharmaceutically acceptable salts thereof, in combination with a pharmaceutically acceptable carrier. United States Patent Publication No. 2007/0060608 generally describes pharmaceutical formulations of VLA-4 antagonists, including Compound I or pharmaceutically acceptable salts thereof, in combination with one or more other therapeutically active compounds, and a pharmacologically acceptable diluent. United States Patent No. 6,972,296 and United States Patent Publication No. 2007/0060608 also describe methods of using the same to treat and/or prevent disease states in which VLA-4 is involved.

Nevertheless, there is a need for pharmaceutical formulations of VLA-4 antagonists, including Compound I or a pharmaceutically acceptable salt thereof, that provide acceptable drug loading, dissolution, stability, and bioavailability for a treatment regimen wherein the number of
doses administered per day to achieve the desired therapeutic plasma concentration could be reduced. Such formulations would reduce the dose, reduce the cost of goods for the product, and/or reduce the dosing regimen. Solid dosage forms of such VLA-4 antagonists would also provide greater convenience for patients and hence promote patient compliance. These and other objectives are provided by the novel pharmaceutical formulations of the present invention.

SUMMARY OF THE INVENTION

The pharmaceutical formulations of the present invention address the aforementioned needs. In particular, the pharmaceutical formulations of the present invention comprising a surfactant provide enhanced dissolution of a VLA-4 antagonist compared to that of a VLA-4 antagonist in the absence of surfactant. Surprisingly, such pharmaceutical formulations also provide a favorable pharmacokinetic profile with less variability in humans for VLA-4 antagonists that are BCS class IV. In addition, wet granulation surprisingly provided a dosage form of VLA-4 antagonist with advantageous bioavailability that provides modest increases in peripheral lymphocyte counts at a single dose ≥ 150 mg Compound IA. These granulation pharmaceutical formulations thereby provide relatively higher drug loading compared to dry-blended formulations and thus reduce number of doses administered per day to achieve the desired therapeutic plasma concentration.

In one embodiment, the present invention provides pharmaceutical formulations suitable for oral administration comprising a granulate comprising a VLA-4 antagonist that is Compound I or a pharmaceutically acceptable salt thereof and a surfactant. In certain embodiments, the granulate further comprises a binder, a disintegrant, a diluent, or a combination of two or more thereof. In certain preferred embodiments, the VLA-4 antagonist comprises about 5% to 50% by weight of the pharmaceutical formulation.

In another embodiment, the present invention provides pharmaceutical formulations suitable for oral administration comprising a granulate comprising a VLA-4 antagonist that is Compound I or a pharmaceutically acceptable salt thereof, a surfactant, a binder, a disintegrant, and a diluent, wherein the VLA-4 antagonist comprises about 5% to 50% by weight of the pharmaceutical formulation. In preferred embodiments, the VLA-4 antagonist is the mono-sodium salt of (3S)-3-[(1-(2-chlorobenzyl)-4-hydroxy-5-methyl-2-oxo-1,2-dihydropyridin-3-yl)amino]carbonyl]amino]1-3-(4-methylphenyl)propanoic acid, referred to herein as Compound IA, the structure of which is:
Pharmaceutically acceptable salts of Compound I, including the sodium salt of Compound IA are described in United States Patent No. 6,972,296 at column 52, line 48 to column 53, line 38, incorporated herein by reference.

In certain preferred embodiments, the surfactant is a non-ionic surfactant, an anionic surfactant, or a combination thereof. In one preferred embodiment, the surfactant is a polyoxyalkylene sorbitan fatty acid ester, a sorbitan fatty acid ester, an alkylene glycol fatty acid ester, a polyoxyalkylene fatty acid ester, a fatty acid ester, a polyoxyalkylene fatty acid ether, a C_{6-24} fatty acid, a fatty acid mono-, di-, or poly-glyceride, a polyoxyalkylene alkyl phenol, an alkyl phenyl ether, a polyoxyethylene polyoxypropylene block copolymer, a fatty amine oxide, a fatty acid alkanolamide, an alkyl cellulose, a carboxyalkyl cellulose, a polyoxyalkylene castor oil derivatives, an alkyl sulfate, an olefin sulfate, an ether sulfate, a monoglyceride sulfate, an alkyl sulfonate, an aryl sulfonate, an olefin sulfonate, an alkyl sulfosuccinate, an aryl sulfosuccinate, or a combination of two or more thereof. In one preferred embodiment, the surfactant is sodium lauryl sulfate. Preferably, the surfactant is present at about 0.5% to about 2% by weight. In one preferred embodiment, the surfactant is present at about 1% by weight.

In certain preferred embodiments, the binder is hydroxyethyl cellulose, hydroxypropyl cellulose, pregelatinized starch, povidone, or a combination of two or more thereof. In one preferred embodiment, the binder is pregelatinized starch.

In certain preferred embodiments, the disintegrant is sodium starch glycolate, crospovidone, croscarmellose sodium, or a combination of two or more thereof. In one preferred embodiment, the disintegrant is croscarmellose sodium.

In certain preferred embodiments, the diluent is lactose, microcrystalline cellulose, mannitol, dibasic calcium phosphate, starch, or a combination of two or more thereof. In one preferred embodiment, the diluent is lactose.

In certain preferred embodiments, the surfactant is sodium lauryl sulfate and the pharmaceutical formulation further comprises pregelatinized starch, croscarmellose sodium, and lactose.
In certain embodiments, the pharmaceutical formulation further comprises an extragranular excipient. In one preferred embodiment, the extragranular excipient is a disintegrant, a flow aid, a lubricant, or a combination of two or more thereof.

In certain preferred embodiments, the pharmaceutical formulation further comprises an extragranular disintegrant which is sodium starch glycolate, crospovidone, croscarmellose sodium, or a combination of two or more thereof. In one preferred embodiment, the extragranular disintegrant is croscarmellose sodium. In another preferred embodiment, the pharmaceutical formulation further comprises an extragranular flow aid which is silicon dioxide, talc, or a combination thereof. In certain preferred embodiments, the extragranular flow aid is silicon dioxide. In still other preferred embodiments, the pharmaceutical formulation further comprises an extragranular lubricant which is magnesium stearate, calcium stearate, glyceryl monostearate, sodium stearyl fumarate, stearic acid, talc, or a combination of two or more thereof. In certain preferred embodiments, the extragranular lubricant is magnesium stearate.

In one preferred embodiment, the pharmaceutical formulation is contained in a capsule. In one preferred embodiment, the VLA-4 antagonist comprises 50% by weight of the pharmaceutical formulation.

In another aspect the present invention provides pharmaceutical formulations comprising a granulate of Compound IA which provides a mean steady-state AUC of Compound IA that is about 45 microgram-hr/ml when administered at a 200 mg dose of Compound IA once-a-day to a patient. The present invention also encompasses pharmaceutical formulations which are similarly bioavailable such that the relative mean steady-state AUC of Compound IA is within 80% to 125% of 45 microgram-hr/ml, that is within the range from about 36 microgram-hr/ml to about 57 microgram-hr/ml, when administered at a 200 mg dose of Compound IA once-a-day to a patient. In one embodiment, the pharmaceutical formulation provides a mean steady-state AUC of Compound IA which is at least 80% of 45 microgram-hr/ml, that is at least 36 microgram-hr/ml, when administered at a 200 mg dose of Compound IA once-a-day to a patient. In one embodiment, the pharmaceutical formulation provides a mean steady-state AUC of Compound IA which is at least 45 microgram-hr/ml when administered at a 200 mg dose of Compound IA once-a-day to a patient.

In another aspect the present invention provides pharmaceutical formulations comprising a granulate of Compound IA which provides a mean steady-state Cmin of Compound IA that is at least 0.2 microgram/ml when administered at a 200 mg dose of Compound IA once-a-day to a patient. In one embodiment, the pharmaceutical formulation further comprises sodium lauryl sulfate, pregelatinized starch, croscarmellose sodium, and lactose.
In one embodiment, the pharmaceutical formulation comprising a granulate of Compound IA provides a median Tmax that is in the range from about 1 hour to about 4 hours when administered at a 200 mg dose of Compound IA once-a-day to a patient. In one embodiment, the pharmaceutical formulation comprising a granulate of Compound IA provides a mean steady-state Cmax of Compound IA that is about 6 microgram/ml when administered at a 200 mg dose of Compound IA once-a-day to a patient. The present invention also encompasses pharmaceutical formulations which are similarly bioavailable such that the relative mean steady-state Cmax of Compound IA is within 80% to 125% of 6 microgram/ml, that is within the range from about 4.8 microgram/ml to about 7.6 microgram/ml, when administered at a 200 mg dose of Compound IA once-a-day to a patient. In a certain embodiment, the pharmaceutical formulation comprising a granulate of Compound IA which is at least 80% of 6 microgram/ml, that is at least 4.8 microgram/ml, when administered at a 200 mg dose of Compound IA once-a-day to a patient. In one embodiment, the pharmaceutical formulation provides a mean steady-state Cmax of Compound IA which is at least 6 microgram/ml when administered at a 200 mg dose of Compound IA once-a-day to a patient.

Notably, steady state exposure of Compound IA was attained by Day 11 when the pharmaceutical formulations of the present invention were given once-a-day.

In one embodiment, a pharmaceutical formulation of the present invention comprising Compound IA provides a mean steady-state AUC of Compound IA that is at least 36 microgram hr/mL when administered at a dose that is 200 mg once-a-day to a patient and assessed as described infra herein (See Examples).

In an embodiment, a pharmaceutical formulation of the present invention provides a mean steady-state Cmax of Compound IA that is at least 4.8 microgram/mL as measured as described infra herein (See Examples).

In an embodiment, a pharmaceutical formulation of the present invention comprising a granulate of Compound IA provides a mean steady-state Cmin of Compound IA that is at least 0.2 microgram hr/mL when administered at a dose that is 200 mg once-a-day to a patient and measured as described infra herein (see Examples).

In another aspect, the present invention provides methods for the treatment or prevention or inhibition of progression of an inflammatory disease, which comprises administering to a patient in need thereof a therapeutically effective amount of any of the pharmaceutical formulations of the present invention. In one preferred embodiment, the inflammatory disease is multiple sclerosis.
DETAILED DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph of the lymphocyte change from time-matched baseline over a time period up to 12 hours post-dose in subjects given a single dose of either placebo, or Compound IA that is 50 mg, 150 mg, 300 mg, 600 mg, or 1000 mg.

Figure 2 is a bar graph of the lymphocyte change from time-matched baseline at various time points (specifically, 4 hours, maximum, and day average) post-dose in subjects given a single dose of either placebo, or Compound IA that is 50 mg, 150 mg, 300 mg, 600 mg, or 1000 mg.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as those commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. The materials, methods and examples are illustrative only, and are not intended to be limiting. All publications, patents and other documents mentioned herein are incorporated by reference in their entirety.

As used herein, the term "AUC" is the area under the plasma concentration-time curve from time zero to a certain time period of the sample. For example, AUC (4h) means the area under the plasma concentration-time curve from time zero to 4 hours.

As used herein, the term "CL/F" is the apparent total clearance of the drug from plasma after oral administration. CL/F is calculated by dividing the dose administered by the AUC.

The term "treating" or "treatment" is intended to mean mitigating or alleviating the symptoms of the recited condition, disease or disorder in a mammal such as a human.

As used herein, the term "t1/2" refers to the half-life of the drug.

PHARMACEUTICAL FORMULATIONS

In most preferred embodiments, the present invention provides pharmaceutical formulations of VLA-4 antagonist Compound I or a pharmaceutically acceptable salt thereof suitable for oral administration that meet the aforementioned need for acceptable drug loading, dissolution, stability, and bioavailability of Compound I. In preferred embodiments, the VLA-4
antagonist is Compound IA. The pharmaceutical formulations of the present invention comprising
a surfactant enhance the dissolution of VLA-4 antagonist Compound IA at pH 7 relative to a
pharmaceutical formulation lacking a surfactant. Likewise, the pharmaceutical formulations of the
present invention provide favorable dissolution profiles and bioavailability of the Compound IA.

Compound I can be prepared according to Example 25, at columns 75-76, of U.S. Pat. No.
6,972,296; also see Scheme 25 at columns 41-42, incorporated herein by reference.
Pharmaceutically acceptable salts can be formed with an appropriate base. The mono-sodium salt
of Compound I, herein referred to as Compound IA, has the following structure:

\[ \text{structure image} \]

and can be formed from Compound I by reacting the free acid with an appropriate base, e.g.,
sodium hydroxide. The development of Compound IA necessitates overcoming several
physicochemical and pharmacokinetic challenges. Notably, Compound IA has low solubility at
pH = 6.8 (< 1 microgram/mL). In addition, according to the Biopharmaceutics Classification
System, Compound IA is a Class IV compound, that is, a compound having low solubility and low
permeability. Consequently, Compound IA has relatively low bioavailability. Furthermore,
Compound IA has very low bulk density (0.08 g/cc) and very poor flow properties as the
crystalline form is needle-like and thus not readily micronized on a commercial scale. Thus,
pharmaceutical formulations that are sufficiently stable and provide enhanced bioavailability of
Compound IA suitable for commercialization are needed.

In one embodiment, the present invention provides pharmaceutical formulations suitable
for oral administration comprising a granulate comprising a VLA-4 antagonist that is Compound I
or a pharmaceutically acceptable salt thereof and a surfactant. In certain embodiments, the
granulate further comprises a binder, a disintegrant, a diluent, or a combination of two or more
thereof. In certain preferred embodiments, the VLA-4 antagonist comprises about 5% to 50% by
weight of the pharmaceutical formulation.

In another embodiment, the present invention provides pharmaceutical formulations
suitable for oral administration comprising a granulate comprising a VLA-4 antagonist that is
Compound I or a pharmaceutically acceptable salt thereof, a surfactant, a binder, a disintegrant,
and a diluent, wherein the VLA-4 antagonist comprises about 5% to 50% by weight of the pharmaceutical formulation. Preferably, the VLA-4 antagonist is Compound IA.

Binders are generally used to impart cohesive qualities to the solid dosage form. Examples of binders include, without limitation, hydroxyethyl cellulose, hydroxypropyl cellulose, pregelatinized starch, povidone, or a combination of two or more thereof. Binders may make up about 1% to about 15% by weight. In certain preferred embodiments, the binder is present at about 15% by weight. In one preferred embodiment, the binder is pregelatinized starch and is present in an amount that is at least 15% by weight.

Examples of disintegrants include, without limitation, sodium starch glycolate, crospovidone, croscarmellose sodium, or a combination of two or more thereof. Disintegrants may make up about 1% to 25% by weight. In one preferred embodiment, the disintegrant is croscarmellose sodium present at about 15% by weight. In another preferred embodiment, the disintegrant is sodium starch glycolate present in an amount that is up to about 10% by weight. In yet another preferred embodiment, the disintegrant is crospovidone present in an amount that is up to about 5% by weight.

Suitable surfactants for use in the present invention include nonionic surfactants, anionic surfactants, or a combination thereof. Examples of nonionic surfactants include, without limitation, polyoxyalkylene sorbitan fatty acid esters, sorbitan fatty acid esters, alkylene glycol fatty acid esters, polyoxyalkylene fatty acid esters, fatty acid esters, polyoxyalkylene fatty acid alkyl ethers, Ci6 to C24 fatty acids, fatty acid mono-, di- or poly-glycerides, polyoxyalkylene alkyl phenols, alkyl phenyl ethers, polyoxyethylene polyoxypropylene block copolymers, fatty amine oxides, fatty acid alkanolamides, alkyl cellulose, carboxyalkyl cellulose, and polyoxyalkylene castor oil derivatives. For example, nonionic surfactants include polyoxyethylene(20) sorbitan monolaurate (Tween® 20), polyoxyethylene(4) sorbitan monolaurate (Tween® 21), polyoxyethylene(20) sorbitan monopalmitate (Tween® 40), polyoxyethylene(20) sorbitan monostearate (Tween® 60), polyoxyethylene(20) sorbitan tristearate (Tween® 65), polyoxyethylene(20) sorbitan monooleate (Tween® 80 or polysorbate 80), or polyoxyethylene(20) sorbitan trioleate (Tween®85), lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, glycerol monooleate, polysorbate 40, 60, 65 and 80, sucrose fatty acid ester, sorbitan laurate, sorbitan oleate, sorbitan palmitate, sorbitan stearate, sorbitan tristearate, sorbitan sesquioleate, sorbitan trioleate, sorbitan istostearate, propylene glycol monostearate, polyoxyethylene monostearate, polyoxyethylene distearate, glycerclyl monostearate, polyoxyethylene lauryl ether, polyoxyethylene cetyl ether, polyoxyethylene stearyl ether, polyoxyethylene oleyl ether, palmitic acid, stearic acid, oleic acid, ethyl oleate,
isopropyl myristate, sodium palmitate, sodium stearate, sodium oleate, nonylphenol polyethoxylates, tributylphenoxy-polyethoxylate, octylphenoxy-polyethoxylate, poloxylene glycerol tricinoleate or polyoxy 35 castor oil (Cremophor® EL, BASF Corp.), poloxylene glycerol oxystearate (Cremophor® RH 40), polyethylene glycol 60 hydrogenated castor oil (Cremophor® RH 60), Poloxamer® 124, Poloxamer® 188, Poloxamer® 237, Poloxamer® 388, Poloxamer® 407 (BASF Wyandotte Corp.), methylcellulose and carboxymethyl cellulose. Examples of anionic surfactants include, without limitation, alkyl sulfates, olefin sulfates, ether sulfates, monoglyceride sulfates, alkyl sulfonates, aryl sulfonates, olefin sulfonates, alkyl sulfosuccinates, and aryl sulfosuccinates. For example, anionic surfactants include sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. In one preferred embodiment, the surfactant is a poloxylene sorbitan fatty acid ester, a sorbitan fatty acid ester, an alkylene glycol fatty acid ester, a poloxylene fatty acid ester, a fatty acid ester, a poloxyalkylene fatty acid ether, a C16 to C24 fatty acid, a fatty acid mono-, di- or poly-glyceride, a poloxyalkylene alkyl phenol, an alkyl phenyl ether, a poloxylene polyoxypropylene block copolymer, a fatty amine oxide, a fatty acid alkanolamide, an alkyl cellulose, a carboxyalkyl cellulose, a poloxylene castor oil derivative, an alkyl sulfate, an olefin sulfate, an ether sulfate, a monoglyceride sulfate, an alkyl sulfonate, an aryl sulfonate, an olefin sulfonate, an alkyl sulfosuccinate, an aryl sulfosuccinate, or a combination of two or more thereof. In a preferred embodiment, the surfactant is sodium lauryl sulfate. Surfactants may make up about 0.5% to about 2% by weight. In one preferred embodiment, the surfactant is present at about 1% by weight. In one preferred embodiment, the surfactant is sodium lauryl sulfate present at about 1% by weight.

Examples of diluents include, without limitation, lactose, microcrystalline cellulose, mannitol, dibasic calcium phosphate, starch, or a combination of two or more thereof. In one preferred embodiment, the diluent is lactose. In other preferred embodiments, the diluent is microcrystalline cellulose in combination with lactose.

The pharmaceutical formulation of the present invention may further comprise an extragranular excipient which is a glidant, lubricant, disintegrant, or a combination of two or more thereof.

Examples of glidants, also known as flow agents, include, without limitation, silicon dioxide (including colloidal silicon dioxide), talc, or a combination thereof. Glidants may make up about 0.1% to about 0.5% by weight. In certain preferred embodiments, the glidant is present at about 0.5% by weight. In one preferred embodiment, the glidant is silicon dioxide.

Examples of lubricants include, without limitation, magnesium stearate, calcium stearate, glyceryl monostearate, sodium stearyl fiimarate, stearic acid, talc, or a combination of two or more
thereof. Lubricants may make up about 0.1% to about 1%. In certain preferred embodiments, the lubricant is present at about 0.5% by weight. In one preferred embodiment, the lubricant is magnesium stearate.

Suitable oral pharmaceutical formulations include, but are not limited to, capsules, tablets, granules, powders, and unit dose packets. In one embodiment, the oral pharmaceutical formulation is a capsule. In another embodiment, the oral pharmaceutical formulation is a tablet.

**METHODS OF PREPARING PHARMACEUTICAL FORMULATIONS**

Pharmaceutical formulations of the present invention can be prepared by a variety of suitable wet granulation, including high shear wet granulation, techniques. For example, such pharmaceutical formulations can be prepared using the process diagramed in Scheme I below:

**Scheme I**

(a) Dry-blend:
   (i) Compound I or a pharmaceutically acceptable salt thereof;
   (ii) a granular excipient (e.g., surfactant, diluent, disintegrant, binder)
   to provide a first dry-blended powder

(b) Agglomerate the first dry-blended powder from Step "a" in a high shear granulator using a granulating solution comprising water and/or an organic solvent

(c) Form a wet milled granulate by wet-milling the agglomerate prepared in Step "b"

(d) Form a dried granulate by drying the wet milled granulate from Step "c"

(e) Dry mill the dried granulate from Step "d"

(f) Dry-blend to homogeneity:
   (i) Dried milled granulate from Step "e"; and
   (ii) optionally, one or more extragranular excipient(s) (e.g., lubricant, glidant, disintegrant)

Encapsulate or Tablet
Note that the binder may be added to the dry-blend in step "a" or dissolved in the granulating solution used in step "b". Also note that the wet granulate in step "d" can be dried in any one of the following ways: a) within the high-shear granulator, using microwave power as the source of energy to evaporate the granulating liquid and vacuum to remove the vapor; b) in a fluidized bed dryer using heated air to dry the granules and remove the vapor; or c) in a tray dryer, in which the wet granules are spread on trays in a heated and ventilated drying oven.

METHODS OF TREATMENT AND/OR PREVENTION

Another aspect of the invention provides methods for the treatment and/or prevention of disease states in which VLA-4 is involved in a patient comprising administering to the patient a pharmaceutical formulation according to the present invention. In preferred embodiments, the pharmaceutical formulations comprise Compound I or a pharmaceutically acceptable salt thereof, preferably Compound IA.

Representative diseases which can be treated using the formulations of the present invention include, but are not limited to, atherosclerosis, rheumatoid arthritis, asthma, allergy, multiple sclerosis, lupus, inflammatory bowel disease, graft rejection, contact hypersensitivity, and type I diabetes. In addition to being found on some white blood cells, VLA-4 is also found on various cancer cells, including leukemia, melanoma, lymphoma and sarcoma cells. Cell adhesion involving VLA-4 may be involved in the metastasis of certain cancers. Inhibitors of VLA-4 binding may, therefore, also be useful in the treatment of some forms of cancer.

In one embodiment, Compound I or a pharmaceutically acceptable salt thereof, preferably Compound IA, is administered for the treatment of an inflammatory disease. In another embodiment, Compound I or a pharmaceutically acceptable salt thereof, preferably Compound IA, is administered for the prevention or inhibition of an inflammatory disease. In one preferred embodiment, the inflammatory disease is multiple sclerosis.

The amount and frequency of administration of Compound I or a pharmaceutically acceptable salt thereof will be regulated according to the judgment of the attending clinician considering such factors as age, condition, size of the patient as well as severity of the symptoms being treated. A typical recommended daily dosage regimen for the treatment of an inflammatory disease can range from about 25 mg/day to about 1000 mg/day. In certain preferred embodiments, the recommended daily dosage regimen for the treatment of an inflammatory disease can range from 25 mg/day to 400 mg/day. In one preferred embodiment, the daily dosage regimen for the treatment of an inflammatory disease is about 400 mg/day administered orally in a single daily dose.
Similarly, a typical recommended daily dosage regimen for the prevention or inhibition of an inflammatory disease can range from about 25 mg/day to about 1000 mg/day. In certain preferred embodiments, the recommended daily dosage regimen for the prevention or inhibition of an inflammatory disease can range from 25 mg/day to 400 mg/day. In one preferred embodiment, the daily dosage regimen for the prevention or inhibition of an inflammatory disease is about 400 mg/day administered orally in a single daily dose.

Other features and embodiments of the invention will become apparent by the following examples which are given for illustration of the invention rather than limiting its intended scope.

EXAMPLES

**PREPARATION OF EXEMPLARY FORMULATIONS**

Exemplary formulations of the present invention are detailed in Tables IA and IB.

<table>
<thead>
<tr>
<th>Table 1A: Exemplary formulations of Compound 1A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation</td>
</tr>
<tr>
<td>(mg/capsule)</td>
</tr>
<tr>
<td>Compound 1A</td>
</tr>
<tr>
<td>Granular ingredients</td>
</tr>
<tr>
<td>Lactose Monohydrate Impalpable</td>
</tr>
<tr>
<td>Pregelatinized starch</td>
</tr>
<tr>
<td>Croscarmellose Sodium</td>
</tr>
<tr>
<td>Sodium Lauryl Sulfate</td>
</tr>
<tr>
<td>Extragranular ingredients</td>
</tr>
<tr>
<td>Croscarmellose Sodium</td>
</tr>
<tr>
<td>Silicon dioxide</td>
</tr>
<tr>
<td>Magnesium Stearate</td>
</tr>
<tr>
<td>Total Capsule Fill Weight (mg)</td>
</tr>
</tbody>
</table>
Table IB: Comparative formulations of Compound IA

<table>
<thead>
<tr>
<th>Formulation</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound IA</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>Microcrystalline cellulose PH102</td>
<td>161</td>
<td>114</td>
</tr>
<tr>
<td>Croscarmellose Sodium</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Sodium Lauryl Sulfate</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Silicon dioxide</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Total Capsule Fill Weight (mg)</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

Exemplary formulations A-D were made by wet granulation. Specifically, the formulations were manufactured by first wet-granulating Compound IA and the granular excipients to make a granulate. The granules were then dried, mixed with the extragranular excipients and then encapsulated in size 0 capsules. The outline of the method of manufacture is presented below.

- Weigh Compound IA (active pharmaceutical ingredient (API)) and granular excipients including binder.
- Pass granular excipients through an 18 mesh sieve.
- Add screened granular excipients and API to granulator (e.g., Fielder Granulator) alternating between granular excipients and API.
- Mix for about 5 minutes dry.
- Add water slowly while mixing until well blended.
- Pass the wet granulate through a Comil 4 mesh screen.
- Dry the granulate on the Glatt fluid bed drier until a loss-on-drying (i.e., moisture content) of 1-3% is obtained.
- Pass the dried granulate through a Comil screen of about 16 mesh.
- Blend the dried granulation along with pre-screened extragranular excipients (other than lubricant, e.g., magnesium stearate) in an appropriate sized blender for about 15 minutes.
- Add the screened lubricant, e.g., magnesium stearate, and blend for another 3 minutes.
- Encapsulate the final blend into size 0 capsules on a Bosch capsule filling machine using a 16 mm dosing disk.
In contrast, comparative formulations E and F were made by dry-blending Compound IA with the excipients to form a homogenous mixture and then encapsulating the mixture in size 0 capsules.

STABILITY DATA FOR EXEMPLARY FORMULATIONS

Stability studies were conducted on capsules of exemplary formulation D. Specifically, capsules were packaged as 10 capsules per bottle in induction-sealed HDPE bottle with child-resistant caps. Samples were stored at 25 °C and 60% relative humidity up to 1 year or 40 °C and 75% relative humidity up to 6 months. Following storage under the aforementioned conditions, samples were pulled at various time points (e.g., 1 month, 3 months, 6 months, 9 months, 12 months) and assessed for moisture content (using volumetric Karl Fischer titration method), label strength, and the presence of Compound IA degradation products.

The presence of Compound IA and determination of degradation products in capsules following storage was assessed using reverse-phase High Performance Liquid Chromatography. In brief, samples for assay and degradation product testing were prepared as follows. Capsules were emptied, contents ground using mortar and pestle; an aliquot of powder weighed and diluted with 1:1 water:85% acetonitrile, then shaken to dissolve drug and subsequently filtered to remove insoluble formulation components. The filtrate was then further diluted with 1:1 water:85% acetonitrile; and the sample analyzed using reverse-phase High Performance Liquid Chromatography. Specifically, an ACE 3 C18 column (4.6 mm x 150 mm, 3.0 μm particles), a gradient elution with mobile phases consisting of acetonitrile, water and 85% phosphoric acid (60:40:0.05, v:v:v). Quantitation of Compound IA was performed by UV detection at 220 nm against an external Compound I reference standard. Notably, the detection limit for the presence of Compound IA degradation products using this assay is less than 0.05%, and the quantitation limit of 0.1%.

Samples stored under both conditions had acceptable moisture contents between 3.6% and 4.7%, as compared to an initial moisture content of 3.6%. Likewise, samples stored under both conditions displayed acceptable label strength between 97.6% and 99.1% that was comparable to the initial label strength of 95.9%. Lastly, the presence of Compound IA degradation products was neither quantifiable up to the 1 year time point examined following storage at 25 °C and 60% relative humidity nor up to the 6 months time point following storage at 40 °C and 75% relative humidity.
These results indicate that Compound IA is stable within the formulation tested for at least 1 year.

**Dissolution Profile of Exemplary Formulations**

The dissolution profile of Compound IA in exemplary formulations Capsule A and Capsule B was determined at pH 7 to simulate intestinal pH. In brief, a sample dissolution profile was obtained for the release rate of Compound IA from capsules using a USP-II dissolution apparatus, with paddles operated at 50 rpm. Each formulation was tested in 900 mL of 0.02 M potassium phosphate buffer (pH 7) as dissolution medium. Aliquots of 10 ml were removed periodically (e.g., 15, 30, 45, and 60 minutes) and filtered through a 0.45 micrometer Millipore Millex-HV PVDF filter. The first 3 mL of filtrate was discarded and a 2.5 mL portion of the remaining filtrate was diluted 1:4 by volume with a mobile phase solution containing acetonitrile:water: 85% phosphoric acid (60:40:0.05, v:v:v). The samples were analyzed by reverse-phase HPLC maintained at 30 °C ± 0.5 °C utilizing an ACE 3 C18 column (4.6 mm x 150 mm, 3.0 µm particles).

Quantitation of Compound IA was performed by UV detection at 265 nm against an external Compound I reference standard. The dissolution profiles of Compound IA in capsules of exemplary formulations A (0% SLS) and B (0.5% SLS) are illustrated in Table 2.

<p>| Table 2: Dissolution profile of Compound IA in exemplary formulations A and B |
|-----------------------------------|----------------------------------|----------------------------------|</p>
<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Dissolution of Exemplary Formulation A</th>
<th>Dissolution of Exemplary Formulation B</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>36</td>
<td>94</td>
</tr>
<tr>
<td>30</td>
<td>73</td>
<td>104</td>
</tr>
<tr>
<td>45</td>
<td>86</td>
<td>105</td>
</tr>
<tr>
<td>60</td>
<td>94</td>
<td>106</td>
</tr>
</tbody>
</table>

Exemplary formulation B provides enhanced dissolution of Compound IA in the presence of surfactant compared to that of Compound IA in the absence of surfactant. Consequently, the present inventors believe that formulations of Compound I or a salt thereof comprising surfactant will be beneficial to increase the extent of Compound I absorption in the gastrointestinal tract thereby leading to enhanced bioavailability of Compound I.

Similarly, dissolution studies were carried out for capsules of exemplary formulations C and D at pH 5.7. In brief, a sample dissolution profile was obtained for the release rate of Compound IA from capsules using a USP-II dissolution apparatus, with paddles operated at 100 rpm, maintained at 37 °C ± 0.5 °C. Each formulation was tested in 900 mL of 0.02 M ammonium
acetate buffer with 1.5% Cetyl Trimethyl Ammonium Bromide (CTAB) (pH 5.7) as dissolution medium. Aliquots of 10 ml were removed periodically (e.g., 15, 30, 45, and 60 minutes) and filtered through a 0.45 micrometer Millipore Millex-HV PVDF filter. The first 3 mL of filtrate was discarded and a 2.5 mL portion of the remaining filtrate was diluted 1:4 by volume with a mobile phase solution containing acetonitrile:water: 85% phosphoric acid (60:40:0.05, v:v:v). The samples were analyzed by reverse-phase HPLC maintained at 30 ± 2 °C utilizing an ACE 3 C18 column (4.6 mm x 150 mm, 3.0 µm particles). Quantitation of Compound IA was performed by UV detection at 220 nm against an external Compound I reference standard. The dissolution profiles for Compound IA in capsules=of exemplary formulations C (25 mg per capsule granulation formulation), D (200 mg per capsule granulation formulation), and E (5 mg per capsule dry-blended formulation) are illustrated in Tables 3, 4, and 5 respectively.
The dissolution profiles of capsules of exemplary formulations C, D, and comparative formulation E demonstrated 90% or greater release of Compound IA within 30 minutes. As such, all three pharmaceutical formulations provide favorable dissolution profiles for release of Compound IA.

Further dissolution studies were carried out for capsules of exemplary formulation D at pH 7 (as described above) to ascertain whether the dissolution profile displayed uniformity across the
batch produced. Specifically, a sample from the beginning, middle, and end of the batch were examined. The resultant dissolution profiles of this study are illustrated in Table 6.

<table>
<thead>
<tr>
<th>Time point</th>
<th>Dissolution (%) Compound IA Released</th>
<th>n=2</th>
<th>n=2</th>
<th>n=2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beginning Sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 minutes</td>
<td>83.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 minutes</td>
<td>90.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45 minutes</td>
<td>93.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 minutes</td>
<td>94.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Middle Sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 minutes</td>
<td>89.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 minutes</td>
<td>97.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45 minutes</td>
<td>99.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 minutes</td>
<td>100.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>End Sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 minutes</td>
<td>89.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 minutes</td>
<td>95.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45 minutes</td>
<td>98.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 minutes</td>
<td>99.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The dissolution profile obtained from samples at the beginning, middle, and end of the batch reflected adequate dissolution, and comparison of the 60-minutes percent-dissolution indicate acceptable content uniformity across the batch.

**CLINICAL STUDIES OF COMPOUND IA PHARMACEUTICAL FORMULATIONS**

**Rising single-dose assessment in healthy adult subjects**

**Objective:** (i) Evaluate the safety and tolerability of Compound IA when administered orally at single doses of 50, 150, 300, 600, and 1000 mg. (ii) Determine the single dose pharmacokinetic profile of Compound IA in healthy volunteers. Explore the pharmacological activity of Compound IA. Assess food effect on the exposure to Compound IA in a preliminary manner.

**Methodology:** Single-center, randomized, third-party blind (within dose-level), placebo-controlled, rising, single-dose study conducted in conformance with Good Clinical Practices. Five dose groups were administered a single dose of 50, 150, 300, 600, and 1000 mg of Compound IA as described below. Within each dose group, six subjects were to receive the active drug, and two subjects were to receive placebo. The first dose level was 50 mg. Each of the higher doses in the progression was not administered until the safety and tolerability data of the previously administered lower dose, up to 72 hours postdose, had been evaluated. The decision to progress to high dose levels was determined by the review of safety laboratory tests, vital signs, electrocardiograms (ECGs), and adverse events and agreed to by sponsor representatives and the principal investigator. Multiple time-matched ECGs were obtained on Days -1 and 1, and these ECGs were machine read with over-reading by the investigator. For pharmacokinetic analyses, blood samples were collected predose (0 hour), 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, and 72 hours postdose. For metabolite profiling, blood samples were collected predose (0 hour), 2 and
6 hours postdose, and urine was collected from 0 to 24 hours postdose. Whole blood was collected predose (0 hour), 2 and 6 hours postdose to explore the effect of Compound IA on the expression of cell surface markers and the relative abundance of lymphocyte subtypes in peripheral blood using FACS analyses. Multiple blood samples were collected on Days -1 and 1 in a time-matched manner to determine the peripheral lymphocyte counts.

**Food Effect on Pharmacokinetics of Compound IA:** Subjects receiving 600 mg Compound IA or placebo returned to the study site after 7 days of washout to receive a second dose of Compound IA or placebo within 5 minutes after consuming a standardized high-fat breakfast. Blood samples were collected predose (0 hour), 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, and 72 hours postdose for pharmacokinetic analyses.

**Number of Subjects:** A total of 40 healthy volunteers participated in this study. Thirty subjects (6 at each dose level) received a single dose of Compound IA at a dose of 50, 150, 300, 600, or 1000 mg. Ten subjects (2 at each dose level) received a single dose of matching placebo. The determination of sample size was based on empirical rather than statistical consideration.

**Diagnosis and Criteria for Inclusion:** A total of 40 healthy adult subjects (16 men and 24 women) between the ages of 18 and 44 years were treated. All of the subjects were Caucasian. Body weights ranged from 50.2 kg to 89.6 kg, and heights ranged from 151 cm to 187 cm. The subjects BMI ranged from 18.9 to 27.1. All but one subject completed the study. A subject who was administered a 1000 mg dose of Compound IA was discontinued from the study due to adverse events (nausea, vomiting). This subject vomited immediately following dosing and all of the capsules were intact in the vomitus.

**Test Product, Dose, Mode of Administration:** Compound IA, 50 mg capsules (specifically, capsules of comparative formulation F described in Table 1B above) and 200 mg capsules (specifically, capsules of exemplary formulation D described in Table 1A above). Doses administered were: 1 x 50 mg (total 50 mg), 3 x 50 mg (total 150 mg), 6 x 50 mg (total 300 mg), 3 x 200 mg (total 600 mg), and 5 x 200 mg (total 1000 mg). The study drug was administered orally.

**Reference Therapy, Dose, Mode of Administration:** Matching placebo 50 mg and 200 mg capsules. The number of placebo capsules was the same as the number of Compound IA capsules required for each treatment (dose-level) group. The placebo capsule(s) were administered orally.

**Duration of Treatment:** After a screening phase of up to 3 weeks, subjects were administered a single dose of Compound IA or matching placebo and were followed for 3 days.
after dosing. Subjects from the 600 mg dose group (Compound IA or matching placebo) returned to the study site, after a washout period of 7 days, to receive a second dose of Compound IA or matching placebo within 5 minutes after consuming a standardized high-fat meal.

**Criteria for Evaluation:** Safety and tolerability of Compound IA were assessed by collecting adverse events, vital signs, ECGs, physical examinations, and safety laboratory tests. Adverse event, laboratory test results, and ECGs have been reviewed for signs of any serious adverse events.

**Pharmacokinetics:** The following pharmacokinetic parameters were to be determined. Cmax, Tmax, AUC, t1/2, Vd/F, and CL/F.

**Pharmacodynamics:** The effect of Compound IA on lymphocyte count, the expression of cell surface markers, and the relative abundance of lymphocyte subtypes in peripheral blood were also explored by conventional hematology and FACS analyses.

Safety: Adverse events were tabulated by treatment. ECG parameters, vital signs, and safety laboratory tests were summarized using descriptive statistics.

**Pharmacokinetics:** Multiple plasma samples were collected for pharmacokinetic analyses, but the final pharmacokinetic parameters were not reported. Based on preclinical animal data, no significant amount of acyl glucuronide metabolite was anticipated in humans; therefore, the plasma samples were not collected under optimal condition (i.e., acidified condition) to accurately quantify the circulating acyl glucuronide metabolite. However, metabolite profiling from this study suggested an appreciable amount of circulating acyl glucuronide metabolite, which could be readily converted to parent drug during the process of sample handling. The plasma concentration determined under the non-optimized sampling/storage condition could lead to an overestimation of circulating parent drug and an underestimation of acyl glucuronide metabolite. Despite the above-mentioned limitations, the following conclusion about the pharmacokinetics of Compound IA can be drawn: Compound IA pharmaceutical formulations described herein provided sufficient bioavailability following oral administration at a single dose >150 mg to elicit a modest increase in peripheral circulating lymphocytes. The exposure appeared to increase as dose increased, and a high-fat meal appeared to increase exposure to Compound IA. An appreciable amount of acyl glucuronide metabolite was observed in plasma.

**Pharmacodynamics:** Lymphocyte counts from Day -1 and up to 24 hours postdose were listed and the change from time-match baseline count were summarized using descriptive statistics. Both florescence intensity (CD62L, CD49d, and CD29) for subtypes of lymphocytes and the relative abundance of subtypes of lymphocytes and monocytes were also reported.
Descriptive statistics for the change of mean fluorescence intensity from predose (Day 1) for CD62L, CD49d, and CD29 for subtypes of lymphocytes were provided. Results from FACS analyses were also listed for each subject. The relative abundance (expressed as a percentage) of subtypes of lymphocytes and monocytes and its change from predose baseline were summarized using descriptive statistics, the results of which are illustrated graphically in Figures 1 and 2. As reflected in Figures 1 and 2, Compound IA administered at a single dose >150 mg results in a modest increase in peripheral circulating lymphocytes.

**Conclusions:** Based on this rising single-dose study of Compound IA pharmaceutical formulations, the following conclusions were drawn:

- Compound IA is safe and well tolerated at single doses up to and including 1000 mg.
- Compound IA pharmaceutical formulations described herein provided sufficient bioavailability following oral administration at a single dose >150 mg to elicit a modest increase in peripheral circulating lymphocytes.

**Rising multiple-dose assessment in healthy adult subjects**

**Objectives:** (i) Evaluate the safety and tolerability of Compound IA when administered orally once daily for 14 days at doses of 200, 400, and 800 mg. (ii) Determine the single dose and multiple-dose pharmacokinetic profile of Compound IA in healthy volunteers. Explore the pharmacological activities of Compound IA.

**Methodology:** This was a randomized, placebo-controlled, third-party blind (within dose-level), rising multiple-dose study to evaluate the safety, tolerability, and pharmacokinetics of Compound IA in healthy volunteers and in a group of otherwise healthy subjects with known allergy as evidenced by a positive skin-prick test (>5 mm to house dust mite or grass pollen extract). This study consisted of two parts. The first part assessed the safety, tolerability, pharmacokinetics, and pharmacological activity of rising multiple doses of Compound IA. The pharmacological activity of Compound IA was assessed primarily by delayed-type hypersensitivity (DTH) with skin biopsy. Three dose groups were treated for 14 days with once-daily doses of either 200, 400, or 800 mg Compound IA. The second part assessed the safety, tolerability, pharmacokinetics, and pharmacological activity of Compound IA at one dose level (maximum tolerated dose or highest dose from Part 1 of this study) in otherwise healthy subjects with known allergy as evidenced by a positive skin-prick test. In addition to a DTH response, the effect of Compound IA on allergen-induced late cutaneous response was assessed in Part 2. For Part 1 of the study, 8 subjects were randomized to active drug and 4 subjects to placebo within each dose.
group. The first dose level was 200 mg. Each of the higher doses in the progression was assessed after the safety and tolerability data from the previously administered lower dose had been evaluated.

The decision to progress to higher dose levels was determined following review of safety laboratories, vital signs, electrocardiograms (ECGs) and adverse events. Subjects in each cohort were challenged with three different concentrations/dilutions of Candin® (antigen extracts from Candida albicans) for a DTH response at Baseline (Day - 3) and approximately at 1 hour postdose on Day 11. Subjects were vaccinated with hepatitis A on Day 2 and antibody titers determined at Baseline, Day 17 and Day 42. For Part 2 of the study, subjects with a positive skin-prick test to either dust mite or grass pollen were treated with the maximum tolerated dose of Compound IA or matching placebo for 14 days. An intradermal allergen (either house dust mite or rye grass pollen) challenge was performed at Baseline (Day - 2) and approximately at 1 hour postdose on Day 11.

Blood samples were collected into EDTA tubes predose (0 hour) and at 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours postdose on Days 1 and 14, predose on Days 11, 12, and 13, and at 36 hours (Day 15), 48 hours (Day 16), and 72 hours (Day 17) postdose on Day 14 for Compound IA analyses. All plasma samples were analyzed for Compound IA using a validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) assay method with a LLOQ of 25 ng/mL.

Blood samples were collected predose (0 hour) and 2, 6 and 24 hours postdose on Day 1 and predose (0 hour) and 2, 6, and 24 hours postdose on Day 14 for metabolite profiling. Urine was collected predose on Day 1 and from 0 to 24 hours postdose on Days 1 and 14, for metabolite profiling including analysis of Compound IA.

**Number of Subjects:** A total of 47 healthy adult subjects (30 men and 17 women) between the ages of 18 and 48 years of age (mean, 25.4 years of age) were treated. Thirty-nine (83%) of the subjects were white; 7 (15%) were Asians; and one was multi-racial. Two subjects were withdrawn from the study; one was due to lab abnormalities and the other was due to a personal reason.

**Test Product, Dose, Mode of Administration:** Compound IA, 200 mg capsules (specifically, capsules of exemplary formulation D described in Table IA above). Doses administered included: 1 x 200 mg, 2 x 200 mg, and 4 x 200 mg.

**Reference Product, Dose, Mode of Administration:** Matching placebo capsules. The number of placebo capsules were the same as Compound IA for each treatment group.

**Duration of Treatment:** Subjects were treated with multiple doses of Compound IA or matching placebo (14 doses, once per day) and were followed for 3 days as inpatients after the end of treatment. The subjects were followed up as outpatients until 28 days post-last-dose of Compound IA or matching placebo.
Criteria for Evaluation  

Primary Endpoints: Safety and tolerability of Compound IA were assessed by collecting adverse events, vital signs, ECGs, physical examinations, and safety laboratory tests.

Criteria for Evaluation Secondary Endpoints: 1) The following pharmacokinetic parameters were determined: Cmax, Tmax, AUC, K, θ, Vd/F, CI/F, and accumulation factor. 2) The following pharmacodynamic endpoints were evaluated: the DTH score and numbers of T cell infiltrate in the skin biopsy; peripheral lymphocyte counts, the expression of cell surface markers, and the relative abundance of lymphocyte subtypes determined by FACS analysis in peripheral blood. Scores of allergen-induced late cutaneous response and eosinophil abundance in the skin biopsy were determined. Serum antibody titers to hepatitis A were assessed at different times following vaccination. In addition, plasma JC virus was analyzed using PCR assay.

Statistical Methods Pharmacokinetics: Plasma Compound IA concentrations and pharmacokinetic parameters were listed and summarized using descriptive statistics. The primary pharmacokinetic parameters for Compound IA were the AUC and Cmax values. Log-transformed AUC and Cmax were statistically analyzed using a one-way analyses of variance model. Ninety percent confidence interval estimates of the mean concentrations and pharmacokinetic parameters for each dose were computed. The dose normalized Cmax and AUC were analyzed in a similar fashion and 90% confidence intervals for difference were computed for a preliminary assessment of dose proportionality. Trough concentrations at Days 11, 12, 13, 14, and 15 were used to assess steady-state for each dose level.

Pharmacokinetics: Following oral administration, Compound IA was absorbed with a median Tmax of 3 hours. Steady-state concentration of Compound IA was achieved by Day 11. Pharmacokinetics parameters of Compound IA and its acyl glucuronide metabolite, Compound IB, from the rising multiple-dose study are shown in Table 7 and Table 8.
<table>
<thead>
<tr>
<th>Day</th>
<th>Dose (mg)</th>
<th>Group</th>
<th>Cmax (μg/mL)</th>
<th>Tmax (hr)</th>
<th>AUC(0-24 hr) (μg hr/mL)</th>
<th>AUC/Dose (μg hr/mL)</th>
<th>CLss/F (L/hr)</th>
<th>T½ (hr)</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>1</td>
<td>4.89 (28)</td>
<td>3.50 (2-4)</td>
<td>33.4 (30)</td>
<td>0.175</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>(n=8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>2</td>
<td>8.31 (30)</td>
<td>3.00 (2-4)</td>
<td>52.5 (40)</td>
<td>0.138</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>(n=8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>3.6</td>
<td>13.5 (34)</td>
<td>3.00 (2-4)</td>
<td>107 (52)</td>
<td>0.140</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
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<tr>
<td></td>
<td>(n=15)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>14</td>
<td>200</td>
<td>1</td>
<td>6.04 (31)</td>
<td>3.00 (1-4)</td>
<td>45.5 (41)</td>
<td>0.238</td>
<td>4.73 (34)</td>
<td>9.60b</td>
<td>1.36</td>
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<td></td>
<td>(n=8)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>2</td>
<td>8.60 (32)</td>
<td>3.00 (1-4)</td>
<td>56.0 (42)</td>
<td>0.147</td>
<td>7.76 (32)</td>
<td>11.7c</td>
<td>1.10</td>
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<td></td>
<td>(n=8)</td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>800</td>
<td>3.6</td>
<td>13.6 (42)</td>
<td>3.00 (1-4)</td>
<td>114 (51)</td>
<td>0.149</td>
<td>8.45 (50)</td>
<td>13.7c</td>
<td>1.12</td>
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<td></td>
<td>(n=13)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NC = not calculable.

a: Median (range).
b: Excluding 2 subjects with r² value less than 0.9 (n=6).
c: Excluding 1 outlier and 1 subject with r² value less than 0.9 (n=6).
d: Excluding 2 subjects with r² value less than 0.9 (n=11).

dose value on Day 14
Compound I had a terminal half-life of approximately 10 to 14 hours. There was a dose-related, but apparently less than dose-proportional, increase of exposure (Table 9). Both Cmax and AUC increased when the dose was increased from 200 to 800 mg. Compound IB plus its isomers resulting from acyl migration and/or, perhaps, anomerization, were observed in plasma (Table 8), and the ratio of acyl glucuronide to parent drug in plasma was approximately 0.3 to 0.4. The elimination half-life of Compound IB paralleled that of Compound IA, suggesting that the

### Table 8: Mean (%CV) Pharmacokinetic Parameters of Acyl Glucuronide Metabolite of Compound IA (Compound IB) Following Daily Oral Administration of 200, 400, or 800 mg Compound IA to Healthy Adult Subjects

<table>
<thead>
<tr>
<th>Day</th>
<th>Dose (mg)</th>
<th>Group</th>
<th>Cmax (µg/mL)</th>
<th>Tmax (hr)</th>
<th>AUC$_{24}$ (µg hr/mL)</th>
<th>T½ (hr)</th>
<th>R</th>
<th>% Metabolite/Parent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200 (n=8)</td>
<td>1</td>
<td>1.24 (48)</td>
<td>5.00 (2-6)</td>
<td>10.8 (49)</td>
<td>NC</td>
<td>NC</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>2</td>
<td>2.59 (38)</td>
<td>3.00 (2-4)</td>
<td>20.0 (32)</td>
<td>NC</td>
<td>NC</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>3,6</td>
<td>3.96 (40)</td>
<td>3.00 (2-4)</td>
<td>37.5 (38)</td>
<td>NC</td>
<td>NC</td>
<td>35</td>
</tr>
<tr>
<td>14</td>
<td>200 (n=8)</td>
<td>1</td>
<td>1.48 (57)</td>
<td>3.00 (2-6)</td>
<td>13.3 (49)</td>
<td>8.63b</td>
<td>8.29</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>2</td>
<td>2.44 (38)</td>
<td>4.00 (2-6)</td>
<td>21.7 (46)</td>
<td>10.3</td>
<td>(27)</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>3,6</td>
<td>3.84 (51)</td>
<td>4.00 (2-6)</td>
<td>40.5 (54)</td>
<td>15.2c</td>
<td>1.03</td>
<td>36</td>
</tr>
</tbody>
</table>

NC = not calculable.

a: Median (range).
b: Excluding 2 subjects with r² value less than 0.9 (n=6).
c: Excluding 1 subject with r² value less than 0.9 (n=12).

### Table 9: Ninety Percent Confidence Interval for the Log-Transformed, Dose Normalized Cmax and AUC (0-24 hr) of Compound IA (ANOVA)

<table>
<thead>
<tr>
<th>Day</th>
<th>Comparison (Sample Size)</th>
<th>Parameter</th>
<th>Ratio Estimate (%)</th>
<th>90% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>400 mg/200 mg (n=8/n=8)</td>
<td>Cmax</td>
<td>85</td>
<td>65 – 110</td>
</tr>
<tr>
<td></td>
<td>800 mg/200 mg (n=15/n=8)</td>
<td>AUC(0 – 24h)</td>
<td>77</td>
<td>54 – 110</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cmax</td>
<td>68</td>
<td>54 – 86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AUC(0 – 24h)</td>
<td>76</td>
<td>56 – 103</td>
</tr>
<tr>
<td>14</td>
<td>400 mg/200 mg (n=8/n=8)</td>
<td>Cmax</td>
<td>72</td>
<td>53 – 96</td>
</tr>
<tr>
<td></td>
<td>800 mg/200 mg (n=13/n=8)</td>
<td>AUC(0 – 24h)</td>
<td>61</td>
<td>42 – 89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cmax</td>
<td>55</td>
<td>42 – 71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AUC(0 – 24h)</td>
<td>59</td>
<td>42 – 83</td>
</tr>
</tbody>
</table>

Doses expressed as free acid were used.
elimination of Compound IB was formation rate-limited. The variability of pharmacokinetics is mild to moderate.

**Statistical Methods**

Pharmacodynamics: Lymphocyte counts from Day -1 and at different times postdose were listed and the change from time-matched baseline counts was summarized using descriptive statistics. Results from FACS analyses were listed for each subject. Both fluorescence intensity (CD62L, CD49d, and CD29) for subtypes of lymphocytes and the relative abundance of subtypes of lymphocytes and monocytes were reported. Descriptive statistics for the change of mean fluorescence intensity from predose (Day 1) for CD62L, CD49d, and CD29 for subtypes of lymphocytes were provided. The relative abundance (expressed as a percentage) of subtypes of lymphocytes and monocytes and its change from predose baseline were summarized using descriptive statistics. Skin tissue lymphocytes from biopsy of the DTH test were quantified and descriptive statistics were provided. According to the protocol, eosinophils from biopsy of allergen induced late cutaneous responses were to be quantified, but the analysis was not performed due to lack of effect of Compound IA on induction of late cutaneous response.

Pharmacodynamics: To assess the effect of Compound IA on peripheral lymphocyte counts, multiple blood samples were collected both prior to (Day -1) and at steady state (on Day 14) exposures to Compound IA. As reflected in Table 10, Compound IA caused a modest increase on peripheral lymphocyte counts.

<table>
<thead>
<tr>
<th>Table 10</th>
<th>Mean Change of Peripheral Blood Lymphocytes on Day 14 From Time-Matched Baseline: Day Average, 4 Hours Postdose, and Maximal Changes (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 mg (n=8)</td>
</tr>
<tr>
<td>Day Average Change (10^7 Counts/L)</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>90% CI</td>
<td>0.07 – 0.35</td>
</tr>
<tr>
<td>Change at 4 Hours (10^7 Counts/L)</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>90% CI</td>
<td>-0.06 – 0.51</td>
</tr>
<tr>
<td>Maximum Change (10^7 Counts/L)</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>90% CI</td>
<td>0.28 – 0.62</td>
</tr>
</tbody>
</table>

* Two subjects discontinued from the study treatment prior to Day 14.

Once daily administration of Compound IA at a dose up to 800 mg for 14 days had no apparent effect on the subpopulation of lymphocytes and cell surface markers such as CD29, CD49d, and CD62L. Even though Compound IA appeared to cause a subtle increase of CD34 positive cells in the circulating blood, these data should be interpreted with caution due to small changes (mean 11% to 30% increase at 6 hours postdose on Day 14 in Compound IA-treated...
subjects vs a mean 17% decrease in placebo-treated subjects) and small sample size and variability in data.

The potential effect of Compound IA on cellular and humoral immunity was assessed using the DTH response and antibody production to hepatitis A vaccination in this rising multiple-dose study. The DTH response to Candida albicans (Candin®) were assessed either before or following 11 days of Compound IA. Compound IA at doses up to 800 mg had no significant effect on the DTH response. Immunohistochemical analysis did not reveal any apparent effect of Compound IA on CD29 and/or CD49d expressing lymphocytes from tissue biopsy of Candin-induced DTH response. The potential effect on allergen-induced late cutaneous response was also assessed in a small number of subjects at the 800 mg dose level; no apparent effect was observed at this dose level. In addition, no apparent effect on antibody production against hepatitis A vaccination was observed with treatment of Compound IA at doses up to 800 mg.

**Conclusions:** Based on this rising multiple-dose study of Compound IA pharmaceutical formulations, the following conclusions were drawn:

- Oral administration of Compound IA at daily doses up to 800 mg for 14 days was safe and generally well tolerated.
- Compound IA had no clinically significant effect on blood pressure, pulse rate, or ECGs.
- Compound IA was not associated with clinically significant effects on safety laboratory tests, including liver function tests, electrolytes, and hematology.
- Compound IA pharmaceutical formulations described herein provided sufficient bioavailability following oral administration at a single dose to elicit modest lymphocytosis.
- Compound IA was orally bioavailable and eliminated with a half-life of 10 to 14 hours.
- Exposure to Compound IA increased as the dose of Compound IA increased.
- Acyl glucuronide metabolite (Compound IB) was observed as a major circulating metabolite of Compound IA and was eliminated in parallel with its parent drug.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.
Claims:

1. A pharmaceutical formulation comprising a granulate comprising a VLA-4 antagonist that is Compound I or a pharmaceutically acceptable salt thereof and a surfactant, wherein the formulation is suitable for oral administration.

2. The pharmaceutical formulation of claim 1 wherein the granulate further comprises a binder, a disintegrant, a diluent, or a combination of two or more thereof.

3. The pharmaceutical formulation of claim 1, wherein the VLA-4 antagonist comprises about 5% to 50% by weight of the pharmaceutical formulation.

4. The pharmaceutical formulation of claim 1, wherein the VLA-4 antagonist is Compound IA.

5. The pharmaceutical formulation of claim 1, wherein the surfactant is a polyoxyalkylene sorbitan fatty acid ester, a sorbitan fatty acid ester, an alkylene glycol fatty acid ester, a polyoxyalkylene fatty acid ester, a fatty acid ester, a polyoxyalkylene fatty acid ether, a C16 TO C24 fatty acid, a fatty acid mono-, di- or poly-glyceride, a polyoxyalkylene alkyl phenol, an alkyl phenyl ether, a polyoxyethylene polyoxypropylene block copolymer, a fatty amine oxide, a fatty acid alkanolamide, an alkyl cellulose, a carboxyalkyl cellulose, a polyoxyalkylene castor oil derivatives, an alkyl sulfate, an olefin sulfate, an ether sulfate, a monoglyceride sulfate, an alkyl sulfonate, an aryl sulfonate, an olefin sulfonate, an alkyl sulfosuccinate, an aryl sulfosuccinate, or a combination of two or more thereof.

6. The pharmaceutical formulation of claim 1, wherein the surfactant is sodium lauryl sulfate.

7. The pharmaceutical formulation of claim 1, wherein the surfactant is present at about 0.5% to about 2% by weight.

8. The pharmaceutical formulation of claim 2, wherein the binder is hydroxyethyl cellulose, hydroxypropyl cellulose, povidone, pregelatinized starch, or a combination of two or more thereof.

9. The pharmaceutical formulation of claim 2, wherein the binder is pregelatinized starch.
The pharmaceutical formulation of claim 2, wherein the disintegrant is sodium starch glycolate, crospovidone, croscarmellose sodium, or a combination of two or more thereof.

The pharmaceutical formulation of claim 2, wherein the disintegrant is croscarmellose sodium.

The pharmaceutical formulation of claim 2, wherein the diluent is lactose, microcrystalline cellulose, mannitol, dibasic calcium phosphate, starch, or a combination of two or more thereof.

The pharmaceutical formulation of claim 2, wherein the diluent is lactose.

The pharmaceutical formulation of claim 1, further comprising an extragranular excipient.

The pharmaceutical formulation of claim 1, further comprising an extragranular disintegrant which is sodium starch glycolate, crospovidone, croscarmellose sodium, or a combination of two or more thereof.

The pharmaceutical formulation of claim 1, further comprising an extragranular disintegrant which is croscarmellose sodium.

The pharmaceutical formulation of claim 1, further comprising an extragranular flow aid which is silicon dioxide, talc, or a combination thereof.

The pharmaceutical formulation of claim 1, further comprising an extragranular flow aid which is silicon dioxide.

The pharmaceutical formulation of claim 1, further comprising an extragranular lubricant which is magnesium stearate, calcium stearate, glyceryl monostearate, sodium stearyl fumarate, stearic acid, talc, or a combination of two or more thereof.

The pharmaceutical formulation of claim 1, further comprising an extragranular lubricant which is magnesium stearate.
21. The pharmaceutical formulation of claim 1, wherein the VLA-4 antagonist comprises 50% by weight of the pharmaceutical formulation.

22. A pharmaceutical formulation comprising a granulate of Compound IA which provides a median Tmax that is in the range of about 1 to about 4 hours post-dose.

23. A method for the treatment of an inflammatory disease, comprising administering to a patient in need thereof a therapeutically effective amount of the pharmaceutical formulation of claim 1.

24. The method of claim 23, wherein the inflammatory disease is multiple sclerosis.

25. A method for inhibiting the progression of an inflammatory disease, comprising administering to a patient in need thereof a therapeutically effective amount of the pharmaceutical formulation of claim 1.

26. The method of claim 25, wherein the inflammatory disease is multiple sclerosis.

27. The pharmaceutical formulation of claim 4 wherein the surfactant is sodium lauryl sulfate and the formulation further comprises pregelatinized starch, crocarmellose sodium, and lactose.
Figure 1
Figure 2