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(72) Inventeur/Inventor:
YOELIN, STEVEN, US

(73) Propriétaire/Owner:
YOELIN, STEVEN, US

(74) Agent: GOWLING WLG (CANADA) LLP

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(57) Abrégé/Abstract:
The present invention is directed to compositions and methods comprising the use of bimatoprost and cyclosporine, used concurrently and in combination, to grow hair and for the treatment of all types of hair loss conditions such as but not limited toalopecia areata. The present invention is also directed to compositions containing bimatoprost and cyclosporine A to grow hair on the scalp and other areas of the body and methods of administration of the compositions.

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(72) Inventor; and
(71) Applicant : **YOELIN, Steven** [US/US]; 300 Standard Avenue, Santa Ana, California 92701 (US).

(74) Agents: **WURST, John** et al.; ALLERGAN, INC., 2525 Dupont Drive, Irvine, California 92612 (US).

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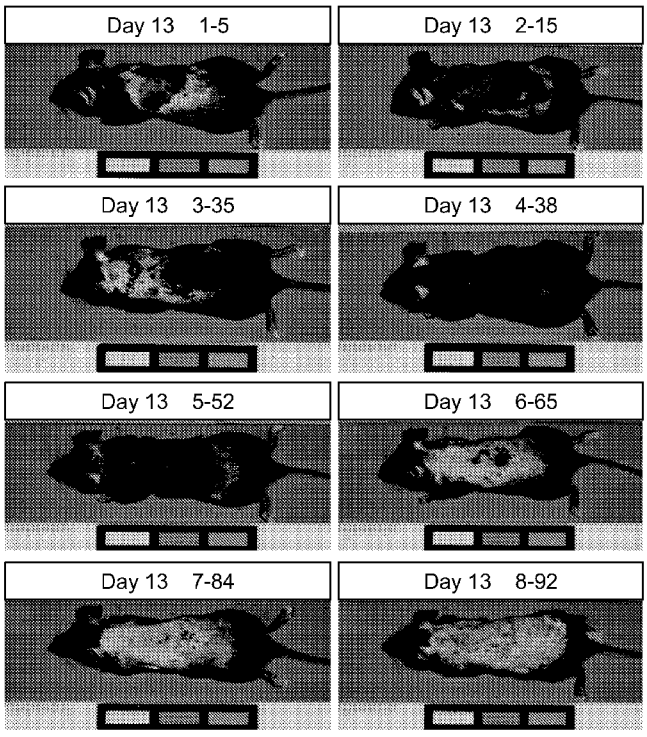

FIG. 1

(57) Abstract: The present invention is directed to compositions and methods comprising the use of bimatoprost and cyclosporine, used concurrently and in combination, to grow hair and for the treatment of all types of hair loss conditions such as but not limited toalopecia areata. The present invention is also directed to compositions containing bimatoprost and cyclosporine A to grow hair on the scalp and other areas of the body and methods of administration of the compositions.

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COMPOSITIONS AND METHODS FOR HAIR GROWTH

Inventor: Steven G. Yoelin

Field of the Invention:

The present invention is directed to the use of bimatoprost and cyclosporine, used concurrently and in combination, to grow hair. The present invention is also directed to compositions containing bimatoprost and cyclosporine to grow hair on the scalp for the treatment of all forms of hair loss not just alopecia .

Background of the invention:

Hypotrichosis or thinning hair of the scalp is a common medical problem that can often be socially debilitating. The mechanism of hair loss is unclear and is hypothesized to be caused by a variety of factors, including but not limited to hormonal imbalance, medications, infections and disease states. Current treatment options for hair loss can be lengthy with high rates of side effects or no effect at all, including steroid injection, minoxidil, and photochemotherapy. Oral cyclosporine A (CsA) has been shown to enhance hair growth in patients with moderate to severe alopecia, yet a high rate of discontinuation occurs due to systemic side-effects. Few studies have examined the efficacy and safety of topical cyclosporine A in animal models with hair loss. *In vivo* studies demonstrate that mice treated with 0.1% and 1% shift toward the anagen phase 21 weeks earlier than control mice, with significant hair growth observed after 2 weeks of treatment . Topical 0.5% cyclosporine A applied BID to Experimental Bald Rats exhibited hair growth one week after application for 6 weeks with a reduction in follicular inflammation . Topical cyclosporine A has been shown to reduce dermal inflammation associated with atopic dermatitis . The application of cyclosporine A as a topical to suppress T-cell function and enhance the anagen phase of the hair growth cycle is promising for patients with thinning hair.

Prostaglandins

Prostaglandins are a class of pharmacologically active hormone-like substances that mediate a wide range of physiological functions including blood pressure, smooth muscle contraction, inflammation and vascular permeability. A prostaglandin is any member of a group of lipid compounds that are derived enzymatically from fatty acids and have important functions in the animal body. Every prostaglandin contains 20 carbon atoms, including a 5-carbon ring. Prostaglandins are autocrine and paracrine lipid mediators that act upon platelets, endothelium, uterine and mast cells. They are synthesized in the cell from the essential fatty acids (EFAs). One example of an EFA is linoleic acid (LA). An LA derivative, gamma-linolenic acid (GLA) is known to reduce inflammation, and prostaglandins are known to regulate inflammatory mediation. The present invention recognizes that LA, GLA and prostaglandins may all play a role in hair health. In at least one embodiment of the present invention, the topical application of prostaglandins are used to enhance hair health and enhance hair growth.

There are nine types of prostaglandins, designated by the letters A to I, the degree of saturation of the side chain of each being designated by subscripts 1, 2, and 3. Examples of prostaglandins include, without limitation, prostaglandin E_1(alprostadil), prostaglandin E_2 (dinoprostone), latanoprost and travoprost. Latanoprost and travoprost are actually prostaglandin prodrugs (i.e. 1-isopropyl esters of a prostaglandin) however, they are referred to as prostaglandins because they act on the prostaglandin F receptor, after being hydrolyzed to the 1-carboxylic acid.

A prostamide (also called a prostaglandin-ethanolamide) is a prostaglandin analog, which is pharmacologically unique from a prostaglandin (i.e. because prostamides act on a different cell receptor [the prostamide receptor] than do prostaglandins), and is a neutral lipid formed as a product of cyclo-oxygenase-2 ("COX-2") enzyme oxygenation of an endocannabinoid (such as anandamide). Additionally, prostamides do not hydrolyze in-situ to the 1-carboxylic acid. Examples of prostamides are bimatoprost (the synthetically made ethyl amide of 17-phenyl prostaglandin $F_{2\alpha}$) and prostamide F_{2n}.

Bimatoprost, a prostaglandin analogue, has been shown to induce eyelash growth, resulting in increased length, thickness, and darkness in healthy patients .
However, LATISSE® was found to have less effectiveness in promoting eyelash growth in patients with alopecia areata . This may be due to the inflamed tissue

causing an inhibition or reduction in the ability of the drug to penetrate the follicle shaft, limiting its effectiveness.

Density and thickness of hair increased significantly after 3 months of treatment. US Patent Nos. 6,403,649; 5,688.819; 5,474,979; and 4,839,342.

Immunomodulators

An immunomodulator, also known as an immunotherapy is a substance (e.g. a drug) which has an effect on the immune system. Immunomodulators may be used in compositions of the present invention, and include immunosuppressants, immunostimulants and tolerogens. Immunosuppressants inhibit immune response, for example, in autoimmune diseases. Immunostimulants increase the immune response, and can be useful, for example, in infections, immunodeficiency and cancers. Tolerogens induce tolerance and make tissue non-responsive to antigen.

Immunomodulators that can be used in compositions of the present invention include and are not limited to: cyclosporine, tacrolimus, azathioprine, cyclophosphamide, methotrexate, chlorambucil, mycophenolate mofetil, prednisolone, muromonab CD3, antithymocyte globin (ATG), Rho (D) immuneglobin, efalizumab, levamisole, thalidomide, and mixtures thereof. In at least one embodiment, the immunomodulator used in the composition is cyclosporine.

Cyclosporine

As stated previously, the compositions of the present invention may contain cyclosporine or other active compounds. Cyclosporines are a group of nonpolar cyclic oligopeptides with known immunosuppressant activity. Cyclosporine A, along with several other minor metabolites, as well as cyclosporine B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y and Z, have been identified. In addition, derivatives, salts and the like of such cyclosporines and a number of synthetic analogs have been prepared and may be useful in the present invention. The use of cyclosporine-A and cyclosporine A derivatives to treat ophthalmic conditions has been the subject of various patents, for example Ding et al U.S. Patent 5,474,979; Garst U.S. Patent Nos. 6,254,860; 6,350,442, and 7,368,436.

In general, commercially available cyclosporines may contain a mixture of several individual cyclosporines which all share a cyclic peptide structure consisting of eleven amino acid residues with a total molecular weight of about 1,200, but with different substituents or configurations of some of the amino acids.

The term "cyclosporine component" as used herein is intended to include any individual member of the cyclosporine group, salts thereof, derivatives thereof, analogs thereof and mixtures thereof, as well as mixtures of two or more individual cyclosporines salts thereof, derivatives thereof, analogs thereof and mixtures thereof.

In certain embodiments, cyclosporine components include, without limitation, cyclosporine A, derivatives of cyclosporine A, salts of cyclosporine A and the like and mixtures thereof. Cyclosporine A is a useful cyclosporine component.

Brief Description of the Drawings:

Figure 1 shows pictures of hair re-growth after 13 days of treatment with either vehicle, Cyclosporine (CsA) (0.05%, 0.1%, 1%) alone and in combination with Bimatoprost (0.03%, 0.3%, 3%) and Bimatoprost alone (0.03% and 0.3%); and,

Figure 2 shows Cyclosporine (CsA) alone (0.05%, 0.1%) and in combination with Bimatoprost (Bpst) (0.03%) stimulates hair-regrowth in shaved C57BL/6 male mice at day 13.

Summary of the Invention:

One aspect of the present invention is directed to the co-application of bimatoprost (including analogs, prodrugs and derivatives) and cyclosporine, specifically cyclosporine A, to the scalp and other areas of the body to stimulate hair growth in normal patients (with or without hair loss) and in patients suffering from alopecia and other disorders resulting in hair loss including but not limited to androgen hormones including testosterone dihydrotestosterone, telogen,effluvium, poor nutrition, hair loss after surgery, hair loss after pregnancy, certain medications, hot oil treatments (perms etc.), excessive brushing, stress, traction alopecia, androgenetic alopecia, triangular alopecia, non-scarring alopecia, alopecia aerate, all other forms of alopecia.

The present invention is also directed to compositions and/or formulations containing both bimatoprost and cyclosporine together, particularly cyclosporine A (or another immune modulators or immunosuppressant such as Protopic [Tacrolimus], a calineurin inhibitor), to

the scalp and other areas of the body to stimulate hair growth in normal patients (with or without hair loss) and in patients suffering from alopecia and other disorders resulting in hair loss including but not limited to androgen hormones including testosterone, dihydrotestosterone, telogen,effluvium, poor nutrition, hair loss after surgery, hair loss after pregnancy, certain medications, hot oil treatments (perms etc.), excessive brushing, stress, traction alopecia, androgenetic alopecia, triangular alopecia, non-scarring alopecia, alopecia aerate, all other forms of alopecia. The present invention is also directed to novel methods of application of compositions containing cyclosporine and bimatoprost to the scalp and other parts of the body to grow hair or for treatment of hair loss. The present invention is also directed to compositions and methods to prevent graying of hair or loss of melanin or pigment.

Some embodiments of the invention are enclosed in the following paragraphs:

1) A composition for growing hair in a patient wherein the composition comprises bimatoprost from 0.03 – 0.3% by w/v and cyclosporine by 0.05 – 0.5% w/v.
2) The composition of paragraph 1 wherein the composition comprises 0.03% w/v bimatoprost and 0.05% w/v cyclosporine A.
3) The composition of paragraph 1 wherein the composition comprises 0.3% w/v bimatoprost and 0.05% w/v cyclosporine A.
4) The composition of paragraphs 1 – 3 wherein the composition further includes ethanol, propylene glycol, D-limonene and water.
5) The composition of paragraph 4 wherein the composition further includes at least one excipient selected from the group consisting of Transcutol®, Labrasol®, cineole, Cremophor RH-40, DMSO, oleic acid, isopropyl myristate, propylene glycol, oxybutynin and monolaurate.
6) The compositions of paragraphs 1 – 5 wherein the composition is in the form of one selected from the group consisting of a liquid, suspension, emulsion, reverse emulsion, micro-emulsion, foam, semi-solid, solution, dispersion, capsule, gel, lotion, cream, paste, and polish.
7) The composition of paragraph 4 wherein the composition is selected from the group consisting of a solution, foam, emulsion and gel.
8) The composition of paragraphs 1 – 7 wherein the composition is applied by an applicator.

9) The composition of paragraph 5 wherein the composition consists essentially of 0.3% bimatoprost, 0.5% cyclosporine A, ethanol, propylene glycol, D-limonene and water.

10) The composition of paragraph 9 wherein the composition further includes transcutol.

11) A method of treating hair loss in a patient by applying a composition comprising 0.03% w/v bimatoprost and 0.05% w/v cyclosporine A.

12) The method of paragraph 11 wherein the method includes applying a foam of 0.3% w/v bimatoprost and 0.05% w/v cyclosporine A at least once a day to the scalp.

13) The method of paragraphs 10 – 12 wherein prior to application of the foam, the scalp is pretreated by application to the scalp of at least one of the following selected from the group consisting of heat, mechanical stimulation, sonophoresis, vibration and massage.

14) The method of paragraphs 10 – 13 wherein the composition is applied on the scalp by application of a roll-on applicator.

15) A method of growing hair on a human scalp comprising application of a composition comprising bimatoprost from 0.03 – 0.3% by w/v and cyclosporine by 0.05 – 0.5% w/v.

16) The method of paragraph 15 wherein the composition comprises 0.03% w/v bimatoprost and 0.05 % w/v cyclosporine A.

17) The method of paragraph 15 wherein the composition comprises 0.3% w/v bimatoprost and 0.1 % w/v cyclosporine A

18) The method of paragraphs 15 - 17 wherein the composition is applied at least once a day.

19) The method of paragraph 18 wherein the composition is applied as one selected from the group consisting of a shampoo and conditioner.

20) The method of paragraph 18 wherein the composition is in the form of a liquid, suspension, emulsion, reverse emulsion, micro-emulsion, foam, semi-solid, solution, dispersion, capsule, gel, lotion, cream, paste, and polish.

Detailed Description of the Invention:

1) Application of Latisse® and Restasis® alone in comparison to combined bimatoprost and cyclosporine A formulations:

One aspect of the present invention are combined compositions or formulations of cyclosporine ("CsA") and bimatoprost ("Bpst") on hair growth. Such formulations will be compared for their ability to stimulate hair growth to application of Latisse® and Restasis® applied individually to patients with thinning hair and with conditions resulting in hypertrichosis and other hair growth disorders.

1. Compositions containing both cyclosporine and bimatoprost

The present invention is directed in part to compositions or formulations containing both cyclosporine, preferably cyclosporine A, and bimatoprost in a single composition for use in growing hair and their methods of application. The combination of cyclopsporine A and bimatoprost is believed to be a safe and effective treatment in suppressing the inflammatory component of alopecia and other hair growth disorders h and in enhancing the amount of hair growth on the scalp in normal patients (patients without suffering from a hair loss disorder).

In accordance with the present invention, cyclosporine, cyclosporine A or cyclosporine derivatives may be applied in an efficacious concentration, e.g., 0.01 % w/v to saturation (e.g. greater than 20% w/v) together with bimatoprost in pharmaceutically acceptable carrier.

a. Cyclosporine component

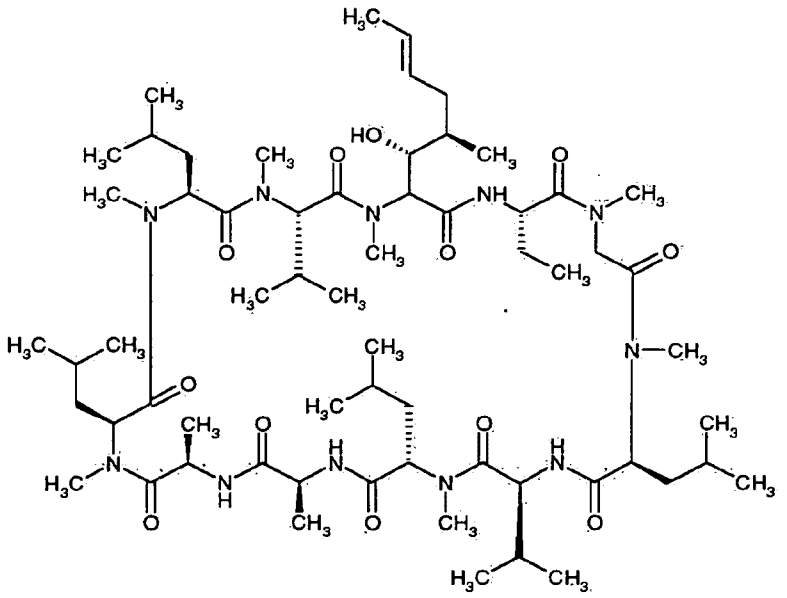
Cyclosporines are a group of nonpolar cyclic oligopeptides with known immunosuppressant activity. Cyclosporine A, along with several other minor metabolites, as well as cyclosporine B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y and Z, have been identified. In addition, derivatives, salts and the like of such cyclosporines and a number of synthetic analogs have been prepared and may be useful in the present invention. The use of cyclosporine A and cyclosporine A derivatives to treat various ophthalmic conditions, has been the subject of various patents, for example US Patent Nos. 5,474,979; 6,254,860; 6,350,442; and 7,368,436.

In general, commercially available cyclosporines may contain a mixture of several individual cyclosporines which all share a cyclic peptide structure consisting of eleven amino acid residues with a total molecular weight of about 1,200 Dalton, but with different substituents or configurations of some of the amino acids. Thus the present invention also contemplates mixtures of different types of cyclosporine or cyclosporine components. The term "cyclosporine component" as used herein is intended to include any individual member of the cyclosporine group, salts thereof, derivatives thereof, analogs thereof and mixtures thereof, as well as mixtures of two or more individual cyclosporines salts thereof, derivatives thereof, analogs thereof and mixtures thereof.

Particularly preferred cyclosporine components include, without limitation, cyclosporine A, derivatives of cyclosporine A, salts of cyclosporine A and the like and mixtures thereof. Cyclosporine A is an especially useful cyclosporine component.

The chemical structure for cyclosporine A is represented by Formula 1:

Formula I

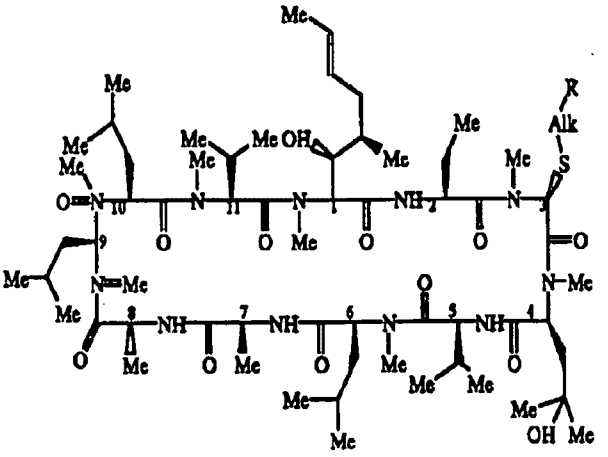


As used herein, the term "derivatives" of a cyclosporine refer to compounds having structures sufficiently similar to the cyclosporine so as to function in a manner substantially similar to or substantially identical to the cyclosporine, for example, cyclosporine A, in the present compositions and methods. Included, without limitation, within the useful cyclosporine A derivatives are those selected from ((R)-methylthio-Sar)^3-(4'-hydroxy-MeLeu) cyclosporine A, ((R)-(Cyclo)alkylthio-Sar)^3-(4'-hydroxy-MeLeu)^4-cyclosporine A, and ((R)-(Cyclo)alkylthio-Sar)^3-cyclosporine A derivatives described below.

These cyclosporine derivatives are represented by the following general formulas (II) and (III), respectively:

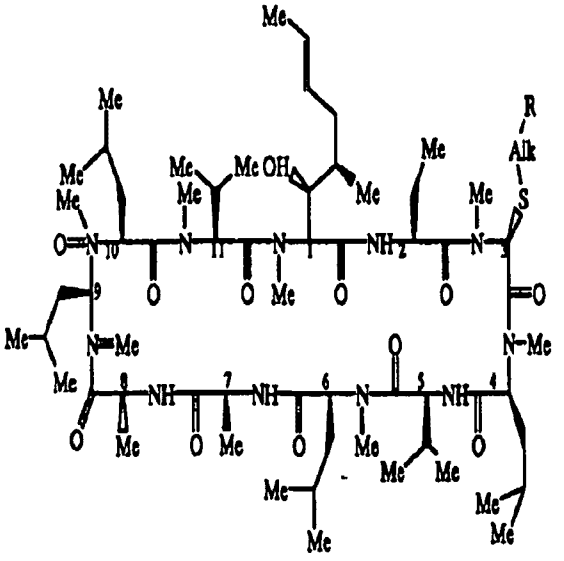
Formula II

(II)



Formula III

(III)



wherein Me is methyl; Alk is 2-6C alkylene or 3-6C cycloalkylene; R is OH, COOH, alkoxycarbonyl, $-NR_1R_2$ or $N(R_3)—(CH_2)—NR_1R_2$; wherein R_1,R_2 is H, alkyl, 3-6C

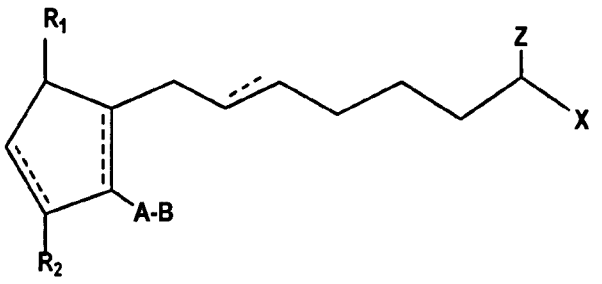
cycloalkyl, phenyl (optionally substituted by halo, alkoxy, alkoxycarbonyl, amino, alkylamino or dialkylamino), benzyl or saturated or unsaturated heterocyclyl having 5 or 6 members and 1-3 heteroatoms; or NR_1R_2 is a 5 or 6 membered heterocycle which may contain a further N, O or S heteroatom and may be alkylated; R_3 is H or alkyl and n is 2-4; and the alkyl moieties contain 1-4C.

The present compositions and methods may be practiced employing any suitable compositions or combinations of compositions including therapeutically effective amounts of cyclosporine component in conjunction with bimatoprost useful to promote hair growth. The cyclosporine component is present in an amount and/or concentration effective enough to provide the desired therapeutic effect when the cyclosporine-containing composition is administered to a human or animal in accordance with the present invention. Mixtures of cyclosporine components are contemplated. In one embodiment of the invention, the cyclosporine component advantageously is present in the compositions in amounts ranging from about 0.01-0.05% w/v, 0.05 – 0.1% w/v, 0.1% to about 0.5% w/v, 0.5% - 5% w/v, 5% - 15% w/v, and 15% or about 20% or 25% w/v of the composition.

In another embodiment, the cyclosporine component is present in an amount of about 0.01% to about 5% or about 10% or about 15% by weight of the composition. Other concentrations of the cyclosporine component(s) contemplated are 0.1 to 20 % w/v, 0.1 – 10 % w/v, 0.1 – 5% w/v, 0.1 – 1.0% w/v, 0.09% - 0.1% w/v, 0.08% - 0.1% w/v, 0.07% - 0.1% w/v, 0.06% - 0.1% w/v, 0.05% - 0.1% w/v, 0.04% - 0.1% w/v, 0.03% - 0.1% w/v, 0.02% - 0.1% w/v, 0.01% - 0.1% w/v, 0.01 – 0.09%, 0.01 – 0.08% 0.01 – 0.07% w/v, 0.01 - 0.06 % w/v, 0.01 – 0.05% w/v, 0.01 – 0.04% w/v, 0.01 – 0.03% w/v, 0.01 - 0.02% w/v, 0.01 – 0.0125% w/v and most preferably 0.01% to 0.05% w/v, 0.0125% w/v, 0.02 % w/v, 0.03% w/v, 0.04 % w/v, 0.05% w/v, 0.06 % w/v, 0.07 % w/v, 0.08 % w/v, 0.09 % w/v or 0.1% w/v, 0.2% w/v, 0.3% w/v, 0.4% w/v and 0.5% w/v of cyclosporine, cyclosporine A and its derivatives. It is intended, however, that the choice of a particular amount of cyclosporine component is to be made in accordance with factors well known in the medicinal arts, including mode of administration, the size and condition of the human or animal and the type and severity of the condition to be treated.

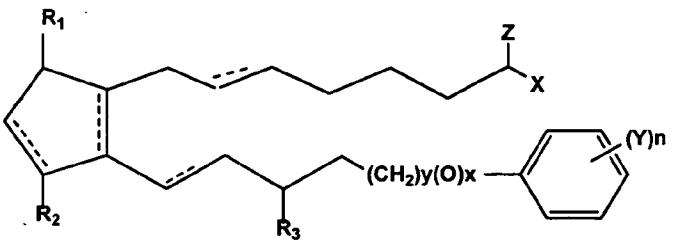
b. Bimatoprost

The present invention also involves the use of cyclosporine A in conjunction with bimatoprost, which may be represented generally by the formula I:



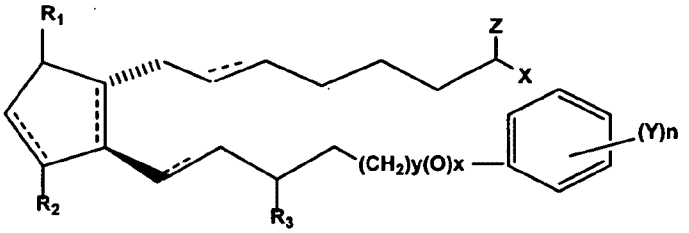
wherein the dashed bonds represent a single or double bond which can be in the cis or trans configuration, A is an alkylene or alkenylene radical having from two to six carbon atoms, which radical may be interrupted by one or more oxide radicals and substituted with one or more hydroxy, oxo, alkyloxy or akylcarboxy groups wherein said alkyl radical comprises from one to six carbon atoms; B is a cycloalkyl radical having from three to seven carbon atoms, or an aryl radical, selected from the group consisting of hydrocarbyl aryl and heteroaryl radicals having from four to ten carbon atoms wherein the heteroatom is selected from the group consisting of nitrogen, oxygen and sulfur atoms; X is a radical selected from the group consisting of $-OR^4$ and $-N(R^4)_2$ wherein R^4 is independently selected from the group consisting of hydrogen, a lower alkyl radical having from one to six carbon atoms, R^5-C- or R^5-O-C-- wherein R^5 is a lower alkyl radical having from one to six carbon atoms; Z is =O or represents 2 hydrogen radicals; one of R_1 and R_2 is =O, -OH or a $-O(CO)R_6$ group, and the other one is -OH or $-O(CO)R_6$, or R_1 is =O and R_2 is H, wherein R_6 is a saturated or unsaturated acyclic hydrocarbon group having from 1 to about 20 carbon atoms, or $-(CH_2)mR_7$ wherein m is 0 or an integer of from 1 to 10, and R_7 is cycloalkyl radical, having from three to seven carbon atoms, or a hydrocarbyl aryl or heteroaryl radical, as defined above, or a pharmaceutically-acceptable salt thereof, provided, however, that when B is not substituted with a pendant heteroatom-containing radical, and Z is =O, then X is not $-OR^4$. (That is, the cycloalkyl or hydrocarbyl aryl or heteroaryl radical is not substituted with a pendant radical having an atom other than carbon or hydrogen.)

Bimatoprost may also be represented by the following general formula II:



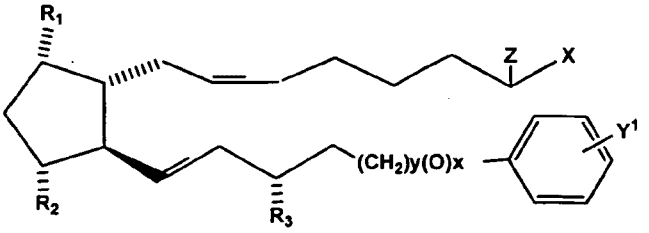
wherein y is 0 or 1, x is 0 or 1 and x and y are not both 1, Y is a radical selected from the group consisting of alkyl, halo, e.g. fluoro, chloro, etc., nitro, amino, thiol, hydroxy, alkyloxy, alkylcarboxy, halo substituted alkyl wherein said alkyl radical comprises from one to six carbon atoms, etc. and n is 0 or an integer of from 1 to about 3 and R_3 is =O, -OH or -O(CO)R_6 wherein R_6 is as defined above . Preferably, n is 1 or 2.

Bimatoprost may also be represented by the general formula (III).

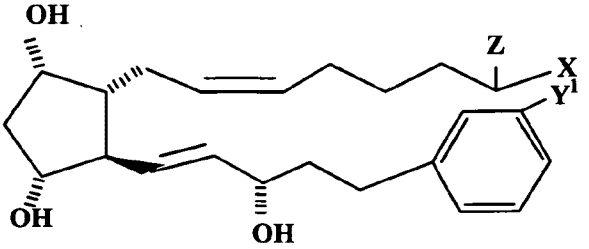


wherein hatched lines indicate a configuration, solid triangles are used to indicate □ configuration

Bimatoprost may also be represented by the general Formula (IV):



Or represented by the general Formula V:



In all of the above formulae, as well as in those provided hereinafter, the dotted lines on bonds between carbons 5 and 6 (C-5), between carbons 13 and 14 (C-13), between carbons 8 and 12 (C-8), and between carbons 10 and 11 (C-10), indicate a single or a double bond which can be in the cis or trans configuration. If two solid lines are used that indicates a specific configuration for that double bond. Hatched lines at positions C-9, C-11 and C-15 indicate the α configuration. If one were to draw the β configuration, a solid triangular line would be used.

In the compounds used in accordance with the present invention, compounds having the C-9 or C-11 or C-15 substituents in the α or β configuration are contemplated. As hereinabove mentioned, in all formulas provided herein broken line attachments to the cyclopentane ring indicate substituents in the a configuration. Thickened solid line attachments to the cyclopentane ring indicate substituents in the α configuration. Also, the broken line attachment of the hydroxyl group or other substituent to the C-11 and C-15 carbon atoms signifies the α configuration.

For the purpose of this invention, unless further limited, the term "alkyl" refers to alkyl groups having from one to ten carbon atoms, the term "cycloalkyl" refers to cycloalkyl groups having from three to seven carbon atoms, the term "aryl" refers to aryl groups having from four to ten carbon atoms. The term "saturated or unsaturated acyclic hydrocarbon group" is used to refer to straight or branched chain, saturated or unsaturated hydrocarbon groups having from one to about 6, preferably one to about 4 carbon atoms. Such groups include alkyl, alkenyl and alkynyl groups of appropriate lengths, and preferably are alkyl, e.g. methyl, ethyl, propyl, butyl, pentyl, or hexyl, or an isomeric form thereof.

The definition of R_6 may include a cyclic component, $-(CH_2)_mR_7$, wherein n is 0 or an integer of from 1 to 10, R_7 is an aliphatic ring from about 3 to about 7 carbon atoms, or an aromatic or heteroaromatic ring. The "aliphatic ring" may be saturated or unsaturated, and preferably is a saturated ring having 3-7 carbon atoms, inclusive. As an aromatic ring, R_7 preferably is phenyl, and the heteroaromatic rings have oxygen, nitrogen or sulfur as a heteroatom, i.e. R_7 may be thienyl, furanyl, pyridyl, etc. Preferably m is 0 or an integer of from 1 to 4.

Z is =O or represents two hydrogen atoms.

X may be selected from the group consisting of $-OR^4$ and $-N(R^4)_2$ wherein R^4 is selected from the group consisting of hydrogen, a lower alkyl radical having from one to six

carbon atoms, R^5-C- or R^5-O-C- wherein R^5 is a lower alkyl radical having from one to six carbon atoms.

Preferred representatives of the compounds within the scope of the present invention are the compounds of formula V wherein X is -OH, i.e. cyclopentane heptenoic acid, 5-cis-2-(3-α hydroxy-4-m-chlorophenoxy-1-trans-butenyl)-3,5-dihydroxy, [1_α, 2_β, 3_α, 5_α] and cyclopentane methylheptenoate-5-cis-2(3-αhydroxy-4-m-chlorophenoxy-1-trans-butenyl)-3, 5 dihydroxy, [1_α,2_β,3_α,5_α] and the 9- and/or 11- and/or 15-esters of this compound. (The numbered designations in brackets refer to the positions on the cyclopentane ring.)

The following novel compounds may be used in the pharmaceutical compositions and the methods of treatment of the present invention.

(1) cyclopentane heptenol-5-cis-2-(3α–hydroxy-5-phenyl-1-trans-pentenyl)-3, 5-dihydroxy, [1_α, 2_β, 3_α, 5_α]

(2) cyclopentane heptenamide-5-cis-2-(3α–hydroxy-5-phenyl-1-trans-pentenyl)-3, 5-dihydroxy, [1_α, 2_β, 3_α, 5_α]

(3) cyclopentane N,N-dimethylheptenamide-5-cis-2-(3α–hydroxy-5-phenyl-1-trans-pentenyl)-3, 5-dihydroxy, [1_α, 2_β, 3_α, 5_α]

(4) cyclopentane heptenyl methoxide-5-cis-2-(3α–hydroxy-5-phenyl-1-trans-pentenyl)-3, 5-dihydroxy, [1_α, 2_β, 3_α, 5_α]

(5) cyclopentane heptenyl ethoxide-5-cis-2-(3α–hydroxy-4-meta-chlorophenoxy-1-trans-pentenyl)-3, 5-dihydroxy, [1_α, 2_β, 3_α, 5_α]

(6) cyclopentane heptenylamide-5-cis-2-(3α–hydroxy-4-meta-chlorophenoxy-1-trans-pentenyl)-3, 5-dihydroxy, [1_α, 2_β, 3_α, 5_α]

(7) cyclopentane heptenylamide-5-cis-2-(3α–hydroxy-4-trifluoromethylphenoxy-1-trans-pentenyl)-3, 5-dihydroxy, [1_α, 2_β, 3_α, 5_α]

(8) cyclopentane N-isopropyl heptenamide-5-cis-2-(3α–hydroxy-5-phenyl-1-trans-pentenyl)-3, 5-dihydroxy, [1_α, 2_β, 3_α, 5_α]

(9) cyclopentane N-ethyl heptenamide-5-cis-2-(3α–hydroxy-5-phenyl-1-trans-pentenyl)-3, 5 dihydroxy, [1_α, 2_β, 3_α, 5_α]

(10) cyclopentane N-methyl heptenamide-5-cis-2-(3α–hydroxy-5-phenyl-1-trans-pentenyl)-3, 5-dihydroxy, [1_α, 2_β, 3_α, 5_α]

(11) cyclopentane heptenol-5-cis-2-(3α–hydroxy-4-meta-chlorophenoxy-1-trans-butenyl)-3, 5-dihydroxy, [1_α, 2_β, 3_α, 5_α]

(12) cyclopentane heptenamide-5-cis-2-(3α–hydroxy-4-meta-chlorophenoxy-1-trans-butenyl)-3, 5-dihydroxy, [1_α, 2_β, 3_α, 5_α]

(13) cyclopentane heptenol-5-cis-2-(3α–hydroxy-5-phenyl-1-trans-pentenyl)3, 5-dihydroxy, [1_α, 2_β, 3_α, 5_α]

A pharmaceutically acceptable salt is any salt which retains the activity of the parent compound and does not impart any deleterious or undesirable effect on the subject to whom it is administered and in the context in which it is administered. Such salts are those formed with pharmaceutically acceptable cations, e.g., alkali metals, alkali earth metals, etc.

In one embodiment, the bimatoprost component is present in an amount of about 0.01% to about 5% or about 10% or about 15% by weight of the composition in conjunction with the cyclosporine component(s). Other concentrations of the bimatoprost component contemplated are 0.1 to 20 % w/v, 0.1 – 10 % w/v, 0.1 – 5% w/v, 0.1 – 1.0% w/v, 0.09% - 0.1% w/v, 0.08% - 0.1% w/v, 0.07% - 0.1% w/v, 0.06% - 0.1% w/v, 0.05% - 0.1% w/v, 0.04% - 0.1% w/v, 0.03% - 0.1% w/v, 0.02% - 0.1% w/v, 0.01% - 0.1% w/v, 0.01 – 0.09%, 0.01 – 0.08% 0.01 – 0.07% w/v, 0.01 - 0.06 % w/v, 0.01 – 0.05% w/v, 0.01 – 0.04% w/v, 0.01 – 0.03% w/v, 0.01 - 0.02% w/v, 0.01 – 0.0125% w/v and most preferably 0.01 to 0.05% or 0.01% w/v, 0.0125% w/v, 0.02 % w/v, 0.03% w/v, 0.04 % w/v, 0.05% w/v, 0.06 % w/v, 0.07 % w/v, 0.08 % w/v, 0.09 % w/v or 0.1% w/v, 0.2% w/v, 0.3% w/v of bimatoprost. It is intended, however, that the choice of a particular amount of bimatoprost is to be made in accordance with factors related to efficacy and factors well known in the medicinal arts, including mode of administration, the size and condition of the human or animal and the type and severity of the condition to be treated.

It is intended that the various listed concentrations of cyclosporine can be combined with the various listed concentrations of bimatoprost such as, and not limited to, 0.05% w/v cyclosporine and 0.03 % w/v bimatoprost, 0.05% w/v cyclosporine and 0.02% w/v bimatoprost, 0.05% w/v cyclosporine and 0.01% w/v bimatoprost, 0.04% w/v cyclosporine and 0.03% w/v bimatoprost, 0.04% w/v cyclosporine and 0.02% w/v bimatoprost, 0.04% w/v cyclosporine and 0.01% w/v bimatoprost, 0.03% w/v cyclosporine and 0.03% bimatoprost, 0.03% w/v cyclosporine and 0.02 % w/v bimatoprost, 0.03% w/v cyclosporine and 0.01 % w/v bimatoprost, 0.02% w/v cyclosporine and 0.03 % w/v bimatoprost, 0.02% w/v cyclosporine and 0.02 % w/v bimatoprost, 0.02% w/v cyclosporine and 0.01 % w/v

bimatoprost, 0.01% w/v cyclosporine and 0.03 % w/v bimatoprost, 0.01% w/v cyclosporine and 0.02 % w/v bimatoprost, 0.01% w/v cyclosporine and 0.01 % w/v bimatoprost; 0.01% w/v cyclosporine and 0.01 % w/v bimatoprost combined with the excipients and vehicles listed herein, such as, but not limited to, the vehicle in Example 1.

Useful compositions or formulations for practicing the invention may be in the form of liquids, suspensions, emulsions, reverse emulsions, micro-emulsions, semi-solids, solutions, dispersions, capsules, gels, lotions, creams, patch, foams, spray, pastes, polishes and the like. Those skilled in the art of pharmaceutical formulation are able to formulate suitable compositions including cyclosporine and bimatoprost components in a suitable form, such as those forms noted herein, for example, including one or more pharmaceutically acceptable excipients, such as those conventionally used in similar compositions.

C. Excipients

Specific examples of pharmaceutically acceptable excipients included in the compositions of the present invention may be olive oil, arachis oil, castor oil, mineral oil, petroleum jelly, dimethyl sulphoxide, chremophor, Miglyol 182 (commercially available from Dynamit Nobel Kay-Fries Chemical Company, Mont Vale, N.J.), an alcohol (e.g. ethanol, n-propyl alcohol, or iso-propyl alcohol), liposomes or liposome-like products or a silicone fluid. Preferred excipients are dimethyl sulphoxide and olive oil. Mixtures of any suitable excipients are contemplated.

Excipients combinations which may be included in the compositions of the present invention, include, but are not limited to ethanol, propylene glycol: water (60:20:20). Other possible excipient combinations may include a ETOH: lipid: and water combination. Various penetrants/penetration enhancers may be used including but not limited to D-limonene, Transcutol®, Labrasol®, cineole, Cremophor RH-40, DMSO, oleic acid, isopropyl myristate, propylene glycol, oxybutynin and monolaurate. Botanicals included but are not limited to Aloe Vera (this may also be used as a pre-treatment product).

The compositions may be in the form of micro emulsions (water, oil and surfactants) with various surfactants including but not limited to labrasol, transcutol, soy bean lecithin and cineole. Certain botanicals may also be used including but not limited to aloe vera which may be used as a pre-treatment product. Various Non-phosphorylated fatty acid oils may also be included, for example, but not limited emu oil and others,

and various polyphenols including, but not limited to, epigallocatechin-3-gallate (EGCG) a major constituent of polyphenols (found in green tea) also may be included. Accelerants may be included in the compositions including 8M-urea and dimethylsulphoxide (DMSOSome embodiments of the present invention include (these really need to be made much clearer):

Various excipients, including but not limited to Ethanol: propylene glycol: water (60:20:20). Other possible excipients may include but are not limited to a ETOH: lipid: water combination. Grice et al. Relative Uptake of Minoxidil into Appendages and Stratum Corneum and Permeation through Human Skin In Vitro. *J Pharm Sci* 2010; Vol 99 .

Various penetrants/penetration enhancers including but not limited to D-limonene. Fang et al. In Vitro and in vivo evaluations of the efficacy and safety of skin permeation enhancers using flurbiprofen as a model drug. *Int J Pharmaceutics* 2003; 255:153-166 .

Various types of micro emulsions (water, oil and surfactants) including but not limited to Labrasol, Transcutol and cineole. Mura et al. Penetration enhancer-containing vesicles (PEVs) as carriers for cutaneous delivery of minoxidil. *Int J Pharmaceutics* 2009; 380: 72-79. Kreilgaard M et al. Influence of microemulsions of cutaneous drug delivery. Adv *Drug Delivery Rev* 2002; 1: S77-S98. Verma DD et al. Treatment of alopecia areata in the DEBR model using Cyclosporin A lipid vesicles. *EJD* 2004; 14:332-8 .

Various Non-phosphoralated fatty acid oils including but not limited to Emu oil are known in the art.

Various Polyphenols including but not limited to epigallocatechin-3-gallate (EGCG) a major constituent of polyphenols (found in green tea), which can be used in a pretreatment solution as well are known in the art.

Various nano-structure lipid carriers including but not limited to soybean lecithin are known in the art.

Various accelerants including but not limited to "8M-urea and dimethylsulphoxide (DMSO). MECHANISM OF ACTION OF ACCELERANTS ON SKIN PENETRATION ,C. ALLENBY, N.H. CREASEN, J.A.G. EDGINTON, J.A. FLETCHER, and C. SCHOCK Article first published online: 29 JUL 2006 DOI: 10.1111/j.1365-2133. 1969.tb16061.x Issue British Journal of Dermatology Volume 81, pages 47–55, August 1969 .

Various free base and acid addition salt forms. Skin penetration enhancement using free base and acid addition salt combination of active agents. US patent 4888354..

D. Pretreatment Modalities

Various pretreatment modalities may be used to improve the efficacy of the present invention or increase penetration of the active compounds into the scalp. This includes varying the skin/scalp temperatures, including but not limited increasing the pre-treatment temperature of the area to be treated. Various forms of skin/scalp stimulation may also be employed including but not limited to mechanical stimulation, vibration and massage. Sound waves may also be utilized including but not limited to low frequency sonophoresis (ultrasound). This could be incorporated into the delivery device, for example a "roll-on" type of applicator could have a built in ultrasound device. Various forms of electrical stimulation including but not limited to sonophoresis/ultrasound and iontophoresis (for example Power Paper which is a iontophoresis type of product).

E. Applicators

The compositions of the present invention may be applied to the treatment areas by various types of applicators including but not limited to spray pump bottles, aerosol bottles, and roll-on devices similar to "roll-on" antiperspirant applicators. The compositions may also be applied by shampoos or conditioners as well as other pretreatment products that would allow the actives that follow the shampooing/conditioning/pre-treating process to penetrate the scalp to the greatest degree possible. Also included are medicated shampoos that contain active pharmaceutical agents including but not limited to shampoos that contain ketoconazole. The shampoo known as Nizoral (ketoconazole) blocks the effects of DHT at the scalp, which may prevent hair loss. DHT is a testosterone metabolite that is believed to cause hair loss.

Example I

Rapid Induction of Hair Growth by Penetration of Bimatoprost ("Bpst") and Cyclosporine A ("CsA") on Shaved Backs of Rodents.

Objective

The objective of this non GLP study is to evaluate hair regrowth on male C57BL6 mice after single and combination therapy of cyclosporine A and bimatoprost every day for the first 20 days and ten every other day until Day 40 using a photography system. Documentation of observed skin pigmentation (correlating to hair follicle cycle) and skin irritation to detect dermal tolerability (dermatitis) every day for 40 days will also be evaluated. Mice weight will be recorded weekly to monitor health and appearance. Blood plasma will be collected for the pharmacokinetic determination of compounds via HPLC-MS for serum levels of interleukin-2 (IL-2) by ELISA. Tissue will be collected to determine hair follicle cycle (anagen, telogen, catagen) by histological evaluation at day 40.

Dose Preparation

Test articles will be prepared once at the beginning of the study and will be prepared in a vehicle of 10% w/v EtOH, 2% w/v propylene glycol, 0.75% w/v carboxy methylcellulose, PBS (or ddw with pH adjustment). Cyclosporine A will be prepared at 0.05% (wt/v), 0.1% (wt/v), and 1% (wt/v) for groups 2, 4, 5, 7, and 8, respectively. Bimatoprost will be prepared at 0.03% (wt/v), 0.3% (wt/v), and 3% (wt/v), for dose groups 3, 4, 6, 7, and 8, respectively.

Based on stability information from the Sponsor, dose solutions will be prepared once at the beginning of the study and will be stored at 2-8 °C prior to administration. The bottles will be wrapped in aluminum foil to prevent exposing the test article to light.

Test System and Husbandry

General

Species:	Mouse
Strain:	C57BL/6NCrlBR
Gender:	Male
Source:	Charles River
Initial Weight:	20-30 g
Age:	7 weeks
Number:	120
Identification:	Cage card and/or ear mark
Acclimation:	≥ 7 days

Justification for Use of the Test System

Justification for the use of mice in this study is based on the premise that animal testing is an appropriate and ethical prerequisite to testing new drugs in humans, and that data obtained from nonclinical animal models will have relevance to the behavior of the test material in humans. Because of the complex interactions that occur *in vivo*, an *in vitro* system does not provide sufficient information for evaluation of a compound's *in vivo* activities . Mice have been used extensively to assess the nonclinical behavior (e.g., pharmacology, toxicity and pharmacokinetics) of a wide variety of pharmacological and toxicological (including environmental) agents. It is expected that the number of animals used in this study will provide a large enough sample for scientifically-meaningful results.

Husbandry

Housing and Enrichment. Animals are maintained and monitored for good health . During acclimation, animals will be group housed in polycarbonate rodent boxes containing absorbent, bedding shavings. During study, animals will be individually housed in rodent boxes containing absorbent, bedding shavings.

Acclimation Period. Animals placed on study will have been acclimated to the testing facility for at least seven days prior to initiation of the study. Health observations will be performed periodically during acclimation to ensure acceptability for study; animals are placed on study at the discretion of the Study Director.

Environment. Animals will be maintained in a controlled environment with a temperature of 20 to 26°C, humidity of 50 ± 20%, and a light/dark cycle of 12 hours. The 12-hr lighting cycle may be interrupted to accommodate study procedures. The animals will be maintained in rooms with at least ten room air changes per hour. Vivarium facility records are kept on file at Pacific BioLabs.

Diet and Feeding. Animals will receive *ad libitum* certified (Laboratory Rodent Diet, etc), unless specified otherwise for dose administration. Analysis of food is provided by the manufacturer and representative reports of analyses are archived at Pacific BioLabs. There are no known contaminants in the dietary materials at levels expected to interfere with the conduct of this study.

Drinking Water. Fresh, potable drinking water will be available to all animals, ad libitum, via water bottle and sipper tubes. Water is supplied by the local utility and is analyzed two times per year by Pacific BioLabs for potential contaminants; results of water analyses are archived at Pacific BioLabs. There are no known contaminants in the water at levels expected to interfere with the conduct of this study.

Identification. Animals will be identified by cage cards and/or ear mark.

No animal will be used in more than one major operative procedure from which it is allowed to recover, unless scientifically justified or required as a veterinary procedure. Paralytics will not be used without appropriate anesthesia.

Veterinary Care. The purpose of the study is to establish the pharmacological and toxicological behavior of the test article, including adverse effects that may include pain or distress to the animal. The Study Director has reviewed the literature, and alternative tests that would avoid pain or distress are not currently available. Therefore, the withholding of palliative care, unless pain is severe (or chronic) or the animal is otherwise moribund, is justified.

Veterinary care will be available throughout the study, and animals found in severe distress may be treated to alleviate pain and suffering at the discretion of the consulting veterinarian. Such treatments will be noted and the Sponsor will be notified of the additional veterinary care. Animals in severe distress or moribund may be euthanized at the discretion of the

consulting veterinarian and the Study Director. The Sponsor will be consulted prior to euthanasia, if possible.

Animals removed from the study may be replaced at the discretion of the Study Director, if replacement does not adversely affect study conduct.

TEST PROCEDURES

Experimental Design

Animals will receive daily topical application of the test article as summarized in Table 1. Doses will be administered to male C57BL/6 mice, with 12/mice group, and a total of 8 treatment groups for 40 days.

Table 1. Group Designations and Dose Levels

Group #	Test Article	Gender	n	Dose Route	Dose Concentration or Amount (% w/v)	Dose Volume (ml/mouse/day)	Dose frequency
1	Vehicle	M	12	Topical	0	0.2	single daily
2	0.05% CsA	M	12	Topical	0.05% CsA	0.2	single daily
3	0.03% Bpst	M	12	Topical	0.03% Bpst	0.2	single daily
4	0.05% CsA 0.03% Bpst	M	12	Topical	0.05% CsA 0.03% Bpst	0.2	single daily
5	0.1% CsA	M	12	Topical	0.1% CsA	0.2	single daily
6	0.3% Bpst	M	12	Topical	0.3% Bpst	0.2	single daily
7	0.1% CsA 0.3% Bpst	M	12	Topical	0.1% CsA 0.3% Bpst	0.2	single daily
8	1% CsA 3% Bpst	M	12	topical	1% CsA 3% Bpst	0.2	single daily

Group Assignment

Animals will be assigned to treatment groups by the Study Director without apparent bias, but without randomization.

Dose Administration

All animals will be shaved from the base of the ears to the base of the tail; care will be taken to avoid abrading the skin. The skin will be evaluated for pigmentation on the day prior to beginning treatment. All animals will be weighed weekly. Doses will be administered daily as a topical application.

IN-LIFE OBSERVATIONS

Body Weight Measurement

Body weights will be measured weekly (Day 1, 7, 14, 21, 28, 36, 40) and recorded.

Moribundity/Mortality

General morbidity and mortality checks (e.g., cage-side observations) will be performed once daily.

Clinical Observations

Clinical observations will be performed daily after dosing. Characteristics to be observed include general appearance of animal health and behavior.

Other measures

Photos will be taken from the tip of the mouse nose to the base of the tail on a daily basis for the first 20 days of the study and then every other day until the conclusion of the study. Additionally, each mouse will be labeled (on cage card and tail) and each group will be color coded. Mice will be anesthetized with Isofluorane and the back skin of each mouse will be clipped and shaved carefully with electric clippers, so as not to damage the skin on study day 1. The hair removal will extend from the base of the ears to the base of the tail. Skin color will be assessed on a four point scale (P-1: pale pink (telogen), P-2: dark pink, P-3: light gray, P-4: dark grey/black (anagen)) and a photo will be taken after shaving. The skin should appear pale pink in color due to telogen phase of cycle (note: animals will not be used if skin is darker than pale pink). Photos will be downloaded and stored on computer and/or memory stick. Three rodents will be sacrificed for histological studies at Day 1 time point, prior to treatment application.

On day 39, 700 µl of blood will be collected from 6 animals from each treatment group by terminal cardiac puncture for pharmacokinetic analysis. 700 µl of blood will be dispensed into a green top tube containing sodium heparin and centrifuged at 2500g for 15 minutes to collect plasma. The plasma will be transferred to a microfuge tube and stored at -70 °C. An additional 700 µl of blood from three animals from each treatment group will be collected by cardiac puncture and transferred to serum separator tubes and centrifuged at 2500g for 15 minutes to collect serum for IL-2 analysis. The serum will be transferred to fresh tubes and stored at -70 °C until analysis.

On day 40, three mice/treatment group will be sacrificed by CO_2 asphyxiation and the skin will be excised from the treatment area and the skin will be fixed in 10% neutral buffered formalin.

Table 2. Study Activities and Timetable

Day	Activity	Body Weights (Day)
1	Shave, Pictures, Score, Dose, Histology (3 rodents)	1
1-20	Score Pigmentation, Pictures, Dose	7, 14
21-40	Score Pigmentation, Pictures every other day, Dose	21, 28, 36
39	Cardiac Puncture (6 animals/group) for PK Cardiac Puncture (3 animals/group) for serum IL-2	n/a
40	Histology tissue collection (3 animals/group)	40

TERMINAL OBSERVATIONS

Post mortem Examinations

Early Deaths. Animals found dead will not be subject to a gross necropsy; organs and tissues from animals found dead will not be collected for histopathology.

Scheduled Deaths. Animals will not be subject to a gross necropsy at the scheduled termination; skin from the treatment area of these animals will be collected for histopathology.

Euthanasia. Animals will be euthanized with CO_2.

Histopathology

The skin from the treatment area of three animals/group will be collected for subsequent histological evaluation on day 40 and fixed in 10% neutral buffered formalin.

Collection Time Points

Pharmacokinetic samples will be obtained from study animals (6/group) on Day 39. Blood (700 µl) will be collected from animals by terminal cardiac puncture and transferred to potassium EDTA tubes, centrifuged for 15 minutes at 2500g, and plasma will be transferred to fresh tubes. Plasma samples will be stored at -70 °C and any analysis will be the responsibility of the sponsor.

Pharmacology samples for IL-2 analysis will be obtained from study animals (3/group) on Day 39. Blood (700 µl) will be collected from animals by terminal cardiac puncture and transferred to serum separator tubes, centrifuged at 2500g, and the serum will be transferred to fresh tubes and stored at -60 to -80 °C. Analysis of the serum samples will be the responsibility of the sponsor.

Collection and Processing

Blood samples will be collected from anesthetized animals via terminal cardiac puncture in an appropriately sized tube containing potassium EDTA as an anticoagulant for pharmacokinetic analysis or serum separator tubes for IL-2 analysis. Samples will be kept on wet ice until centrifugation at approximately 2800 rpm at room temperature for approximately 15 min. The plasma will be separated and stored at -60 to -80°C.

Bioanalysis

Bioanalysis for test article plasma concentrations will be the responsibility of the Sponsor .

DATA ACQUISITION AND ANALYSIS

Major computer software systems used on this study may include, but are not limited to, Microsoft Excel®, Microsoft Word®, and the Rees Scientific Environmental Monitoring System® for study room environmental control.

Descriptive Statistics

Descriptive statistics (mean, standard deviations, and number of replicates) will be presented for all measurement data and will be shown in summary tables. Descriptive statistics will be calculated with Microsoft Excel®.

Results

On Day 13, which is not the endpoint of the study (Day 40), hair growth of groups in Table 1 were evaluate as shown in Figure 1. As stated previously, 8 week old male C57BL/6 mice were shaved on the dorsal surface to remove the hair and the skin was scored for skin pigmentation as an indicator of telogen (pink) or anagen (dark gray). Only the mice with pink skin (telogen phase) on day one after shaving were included in the study. The mice were administered a daily topical application of 200 µl of either vehicle, CsA (0.05%, 0.1%, and 1%) alone and in combination with Bpst (0.03%, 0.3%, and 3%), or bimatoprost alone (0.03%,0.3%) in a vehicle of 10% ethanol, 2% propylene glycol, 0.75% carboxymethylcellulose, and distilled de-ionized water with the pH adjusted to 7.4. On Day 13, the mice were anesthetized with Isofluorane and photos of the dosing area on each mouse were taken daily with a Nikon D90 digital camera mounted on a copy stand. From top left to right and down (group 1 animal 5, control), (group 2 animal 15, 0.05% CsA), (group 3 animal 35, 0.03% Bpst), (group 4 animal 38, 0.05% CsA/0.03% Bpst), (group 5 animal 52, 0.1% CsA), (group 6 animal 65, 0.3% Bpst), (group 7 animal 84, 0.1% CsA/0.3% Bpst), (group 8, animal 92, 1% CsA/3% Bpst). These photos are shown in Figure 1.

Figure 2 shows a rectangle (852x436 arbitrary units) was created using Image J software to encompass the shaved area on the dorsum of each mouse. The mean gray value of the rectangular area was measured using Image J software as an indicator of skin darkening and hair re-growth. * indicates $p<0.05$ vs. control by t-test analysis using Microsoft Excel software. # indicates $p<0.05$ vs. 0.03% bimatoprost alone by t-test analysis using Microsoft Excel software. N=12/group ± S.E.M. The combination of 0.05% CsA and 0.03% Bpst demonstrated superiority over the control and 0.05% CsA treatment groups by stimulating skin darkening and hair re-growth by 46% over control treated mice and by 3% over 0.05% CsA alone. CsA alone at 0.05% and 0.1% increased skin darkening and hair re-growth by 42% and 45% over controls, respectively. The combination treatment of 0.05% CsA and 0.03% bimatoprost increased skin darkening and hair re-growth by 0.4% over the 0.1% CsA alone treated group. The 0.05% CsA/0.03% Bpst group demonstrated superiority to 0.03% bimatoprost alone by stimulating skin darkening and hair re-growth by 73% over 0.03% bimatoprost alone at day 13 of the study.

Example II

Purpose/Hypothesis: To demonstrate that the combination of cyclopsporine A ("CsA") and bimatoprost ("Bpst") are a safe and effective treatment in enhancing the length of hair growth on the scalp in patients with thinning hair and conditions resulting in hypotrichosis.

Primary Outcome Measure:

To determine the penetration of single and combination therapy of cyclosporine A and bimatoprost for the treatment of hypotrichosis of the scalp in patients with thinning hair and conditions resulting in hypotrichosis.

Secondary Outcome Measures:

Comparison of length, thickness, and amount of hair regrowth between the treatment groups:

1) To determine the change in growth of hair length at baseline and after application of single and combination therapy with cyclosporine A and bimatoprost;
2) To determine the safety of cyclosporine A by analysis of blood serum, specifically creatinine levels, thyroid hormone levels and immune cells, such as T-cell and interleukin counts, measured by laboratory testing;
3) To determine the safety of cyclosporine A by monitoring blood pressure measured by pressure cuff; and,

4) To determine the safety of bimatoprost by observation of changes in pigmentation of skin or dermatitis of the skin subjected to drug as measured by AOI software via Canfield photography.

Hair loss will be determined by questionnaire assessment and observation of scalp, where ≥1 square inch of the scalp must be thinning or without hair. The hair follicle must be determined to be active. On initial assessment, each patient will fill out a questionnaire, sign informed consent and have blood drawn. Selected patients will have photos taken of affected scalp area prior to treatment on Day 0.

Four groups of 10 patients will be assessed (total n=40). The four groups of patients will apply formulations (vehicle will have about 10% w/v ethanol, about 2% w/v propylene glycol, 0.75% w/v carboxymethylcellulose in distilled deionized water with the pH adjusted to 7.4 and further and may optionally including a penetration enhancer and/or preservative) of: 1) 0.05% CsA (Restasis®) alone (C0); 2) 0.03% Bpst (Latisse®) alone (B0); 3) a 0.05% CsA and 0.03% Bpst formulation (CB1); and 4) 0.1% CsA and 0.3% Bpst formulation (CB2). "About" refers to variations in concentration of actives or excipients that a regulatory agency such as the FDA or EMEA would find bioequivalent.

All patients will clean and dry their scalp in either the morning or evening, followed by a single treatment of product to treatment area of scalp 1 time/evening (qhs). Treatment should remain on the scalp for at least 8 hours. Patients will keep a diary with weekly entries regarding hair growth and any adverse events. The office visits will be conducted on the initial day of the trial, Day 0, at 2 weeks, followed by monthly visits for a duration of 6 months. A total of 8 visits will be required and at each visit, photographs of the treatment area will be taken with AOI software. This will be calculated by the percent of affected area per cm2 demonstrating growth, as measured by AOI software via Canfield photography. At Day 0 and Month 6, there will be assessment of blood pressure via pressure cuff and a blood draw followed by blood laboratory analysis, with attention to creatinine and thyroid levels, and immune cell count of T-cells and interleukins.

Subject Characteristics (Inclusion criteria):

1) Subjects must give written informed consent and must authorize release and use of health information;

2) Male and female patients must have a diagnosis of hair loss or thinning hair with patches or over entire scalp as determined by the study investigator;
3) Male and female patients ≥18 years to 65 years of age; and,
4) Subjects must have CD4+ T-lymphocyte counts at lower limit of normal as determined by local laboratory.

Subject Characteristics (Exclusion criteria):

1) Autoimmune disorders, immune suppression or evidence of immunocompromise;
2) Abnormal T-lymphocyte count and/or liver function tests or serum hemoglobin;
3) Pregnant or breastfeeding women;
4) Hypertension, taking hypertensive medication or unstable cardiovascular disease;
5) IOP lowering medications;
6) Currently taking steroid medications or steroid derivatives, or hormone supplements;
7) History of male pattern baldness ;
8) History of tricotillomania or patients with damaged hair follicles; and,
9) Other unspecified reasons that contraindicate enrollment in the study, as determined by the investigator

Statistical Analysis Plan:

The primary and secondary outcome measures will first be determined using 3-way ANOVA, with patient, pretreatment (bimatoprost or placebo), and treatment (cyclosporine or placebo) as the between-subjects factors. Subsequent analyses of variance using 2-way ANOVA will be used to determine the effects of pretreatment and treatment within the patient group. If there are main effects of pretreatment or treatment, or an interaction effect, post hoc analyses using Bonferroni correction will be used to determine the source of these effects. A p-value of 0.05 is significant.

Results will demonstrate that combined formulations of cyclosporine and bimatoprost alone are superior in enhancing hair growth in patients with thinning hair and in patients suffering from hypertrichosis or other hair-loss disorders.

Example III

A 47 year old Caucasian male suffering from alopecia areata applies a 0.03%/0.05% w/v bimatoprost/cyclosporine A solution to his scalp by applying the solution twice a day with an

applicator, once in the morning and once at night for a period of 60 days. Hair growth is measured once a week by calculating the percent of affected area per cm2 demonstrating growth, as measured by AOI software, via Canfield photography. After 60 days of twice daily application of the 0.03%/0.05% w/v bimatoprost/cyclosporine A solution, a 27% increase in hair growth will be measured.

Example IV

A 35 year old Caucasian female with thinning hair applies a 0.3%/0.5% w/v bimatoprost/cyclosporine A emulsion to her hair once a day with a roll-on applicator for a period of 90 days. Hair growth is measured weekly by calculating the percent of affected area per cm2 demonstrating growth, as measured by AOI software, via Canfield photography. After 90 days of daily application of the 0.3%/0.5% w/v bimatoprost/cyclosporine A solution, a 31% increase in hair growth and fullness will be measured.

Example V

A 57 year old African American female with alopecia areata and thinning eyebrows applies a 0.1%/0.3% bimatoprost/cyclosporine A foam to her scalp and eyebrows twice a day, once in the morning and once at night, for a period of 90 days. The foam is applied and allowed to remain on the skin for 15 minutes after pretreatment of the skin with a warmed, moist towel to increase the temperature of the treatment area. After 90 days of application of the 0.1%/0.3% bimatoprost/cyclosporine A foam, a 26% increase in hair growth is observed on the scalp and an increase of 21% of eyebrow growth will be observed using AOI software and Canfield photography.

Example VI

A 27 year old Hispanic male suffering from male pattern baldness and alopecia areata applies a 0.2%/0.4% w/v bimatoprost/cyclosporine A gel to his hair once daily for a period of 120 days. The gel is applied to the scalp and is allowed to dry. Hair growth is measured weekly by calculating the percent of affected area per cm2 demonstrating growth, as measured by AOI software, via Canfield photography. After 120 days of daily application of the 0.2%/0.4% w/v bimatoprost/cyclosporine A gel, a 24% increase in hair growth will be measured.

Example VII

An 18 year old Middle Eastern female complaining of post chemo hair thinning and thinning eyebrows uses a specified pretreatment shampoo on her scalp and eyebrows (this shampoo

allows compounds that follow shampoo treatment to penetrate the hair follicles to a greater degree). The patient sprays 0.3%/.01% w/v bimatoprost/cyclosporine solution to her hair and eyebrows at bedtime for 90 days. Hair growth is measured weekly by calculating the percent of affected area per cm2 demonstrating growth, as measured by AOI software, via Canfield photography. After 120 days of daily application of the 0.2%/0.4% w/v bimatoprost/cyclosporine A solution, a 31% increase in scalp growth and eyebrow growth will be measured.

Example VIII

A 58 year old post menopausal female suffering from diffuse thinning hair throughout her scalp applies iontorphoresis patches at night on top of the areas of diffuse hair thinning (for a specified period of time) impregnated with a combination of 0.3%/0.5% w/v bimatoprost/cyclosporine solution. Hair growth is measured weekly by calculating the percent of affected area per cm2 demonstrating growth, as measured by AOI software, via Canfield photography. After 60 days of nightly application of the impregnated iontorphoresis patches 0.3%/0.5% w/v bimatoprost/cyclosporine A solution, a 41% increase in scalp growth will be measured.

Example IX

A 25 year old Asian male suffering from diffuse hair loss uses a specially formulated conditioner designed to allow better follicular penetration of the bimatoprost/cyclosporine formulation followed by application of 0.3%/0.5% w/v bimatoprost/cyclosporine A emulsion to his hair once a day with a roll-on applicator for a period of 90 days. Hair growth is measured weekly by calculating the percent of affected area per cm2 demonstrating growth, as measured by AOI software, via Canfield photography. After 90 days of daily application of the 0.3%/0.5% w/v bimatoprost/cyclosporine A emulsion a 38% increase in hair growth and fullness will be measured.

Example X

A 34 year old Latin female, 6 weeks post partum suffers from patchy hair loss following the delivery of her twins. This patient shampoos and conditions her hair each night (the shampoo and conditioner that is being used allows the product/compound that follows the shampoo/conditioner combination to be absorbed to a greater degree). She than applies a gel that contains a variety penetrants as well as 0.2%/0.4% w/v bimatoprost/cyclosporine A to her hair once nightly for a period of 120 days. The gel is applied to the scalp and is allowed to dry. Hair growth is measured weekly by calculating the percent of affected area per cm2 demonstrating growth, as measured by AOI software, via Canfield photography. After 120

days of daily application of the 0.2%/0.4% w/v bimatoprost/cyclosporine A gel, a 36% increase in hair growth will be measured.

Example XI

A 32 year old Caucasian male suffers from alopecia totalis applies a 0.1%/0.3% bimatoprost/cyclosporine A foam to his scalp once in the morning and once at night, for a period of 120 days. The foam is applied and allowed to remain on the entire scalp for 15 minutes after pretreatment of the skin with a warmed, vibratory device to increase the ability of the scalp to absorb compounds that follow this type of pretreatment regiment. After 90 days of application of the 0.1%/0.3% bimatoprost/cyclosporine A foam, sustained hair growth will bel noted to occur on the scalp. This is documented by using AOI software and Canfield photography. Additionally, hair growth is documented to take place at twice the rate in normal individuals that are not using the regiment noted above.

Claims:

1. A composition for growing hair in a patient wherein the composition comprises 0.03% w/v bimatoprost and 0.05% w/v cyclosporine A.

2. The composition of claim 1 wherein the composition further comprises ethanol, propylene glycol, D-limonene and water.

3. The composition of claim 2 wherein the composition further comprises Transcutol®, Labrasol®, cineole, Cremophor RH-40, DMSO, oleic acid, isopropyl myristate, oxybutynin or monolaurate.

4. The compositions of claim 3 wherein the composition is in the form of a liquid, suspension, emulsion, reverse emulsion, microemulsion, foam, semi-solid, solution, dispersion, capsule, gel, lotion, cream, paste, or polish.

5. The composition of claim 2 wherein the composition is a solution, foam, emulsion or gel.

6. The composition of claim 5 wherein the composition is for application by an applicator.

7. The composition of claim 1 wherein the composition consists essentially of 0.03% bimatoprost, 0.05% cyclosporine A, ethanol, propylene glycol, D-limonene and water.

8. The composition of claim 7 wherein the composition further comprises transcutol.

9. Use of a composition comprising 0.03% w/v bimatoprost and 0.05% w/v cyclosporine A for treatment of hair loss.

10. Use of a composition comprising 0.03% w/v bimatoprost and 0.05% w/v cyclosporine A for preparation of a medicament for treatment of hair loss.

11. The use of claim 10 wherein the medicament is a foam of 0.03% w/v bimatoprost and 0.05% w/v cyclosporine A for at least once a day use on a scalp.

12. The use of claim 11 wherein the composition is a foam and the composition is for application following, heat, mechanical stimulation, sonophoresis, vibration or massage of a scalp.

13. The use of claim 12 wherein the composition is for application by a roll-on applicator.

14. Use of a composition comprising 0.03% w/v bimatoprost and 0.05% w/v cyclosporine A for growing hair on a human scalp.

15. Use of a composition comprising 0.03% w/v bimatoprost and 0.05% w/v cyclosporine A for preparation of a medicament for growing hair on a human scalp.

16. The use of claim 14 or 15 wherein the composition is for at least once a day application.

17. The use of claim 14 or 15 wherein the composition is for application as a shampoo or conditioner.

18. The use of claim 14 wherein the composition is in the form of a liquid, suspension, emulsion, reverse emulsion, micro-emulsion, foam, semi-solid, solution, dispersion, capsule, gel, lotion, cream, paste, or polish.

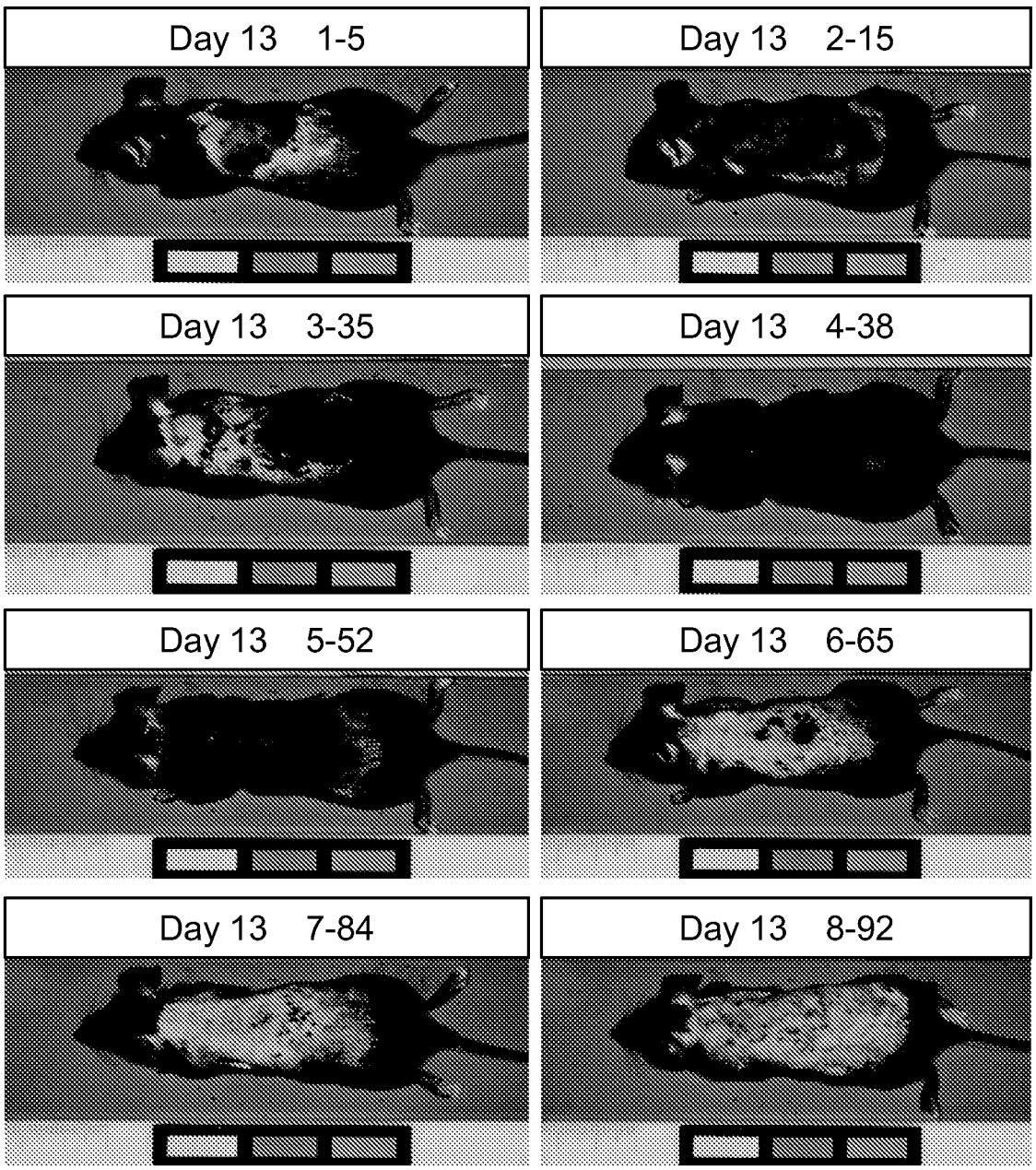

Day 13 1-5
Day 13 2-15
Day 13 3-35
Day 13 4-38
Day 13 5-52
Day 13 6-65
Day 13 7-84
Day 13 8-92

FIG. 1

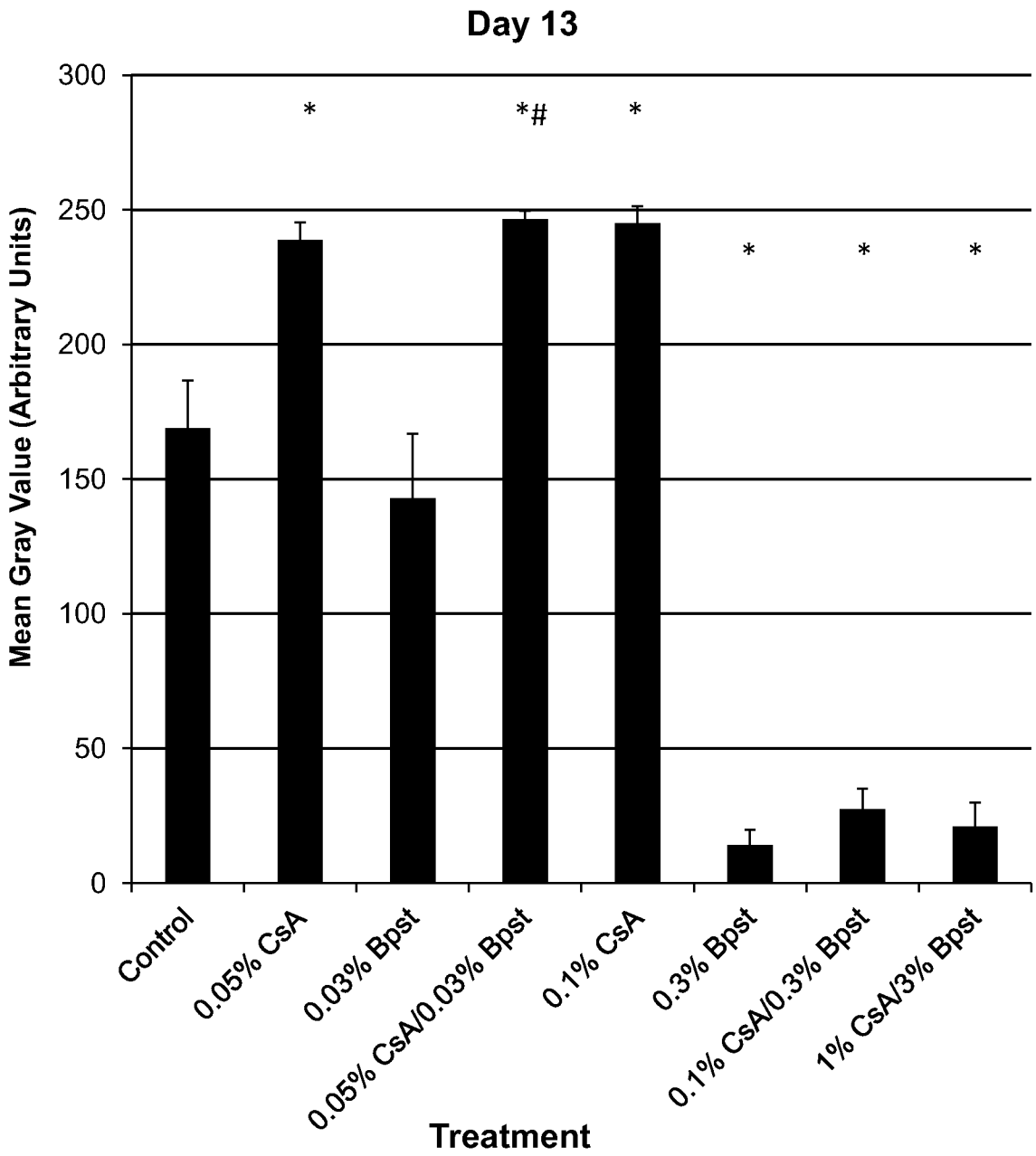

Day 13
Mean Gray Value (Arbitrary Units)
300
250
200
150
100
50
0
*
*#
*
*
*
*
Control
0.05% CsA
0.03% Bpst
0.05% CsA/0.03% Bpst
0.1% CsA
0.3% Bpst
0.1% CsA/0.3% Bpst
1% CsA/3% Bpst
Treatment

FIG. 2