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(71) Applicant: **CADILA HEALTHCARE LIMITED**
[IN/IN]; Zydus Tower, Satellite Cross Roads, Ahmedabad,
Gujarat, 380015 (IN).

(72) Inventors: **JAIN, Mukul R.**; Cadila Healthcare Limited,
Zydus Tower, Satellite Cross Roads, Ahmedabad, Gujarat,
380015 (IN). **CHATTERJEE, Abhijit**; Cadila Healthcare
Limited, Zydus Tower, Satellite Cross Roads, Ahmedabad,
Gujarat 380015 (IN). **SINGH, Jaideep**; Cadila Health-

care Limited, Zydus Tower, Satellite Cross Roads, Ahmed-
abad, Gujarat, 380015 (IN). **BANERJEE, Kaushik**; Cadi-
la Healthcare Limited, Zydus Tower, Satellite Cross Roads,
Ahmedabad, Gujarat, 380015 (IN). **MOHAPATRA, Jo-
geswar**; Cadila Healthcare Limited, Zydus Tower, Satellite
Cross Roads, Ahmedabad, Gujarat, 380015 (IN). **SHAR-
MA, Manoranjan**; Cadila Healthcare Limited, Zydus Tow-
er, Satellite Cross Roads, Ahmedabad, Gujarat, 380015
(IN). **PATEL, Dinesh**; Cadila Healthcare Limited, Zydus
Tower, Satellite Cross Roads, Ahmedabad, Gujarat, 380015
(IN).

(74) Agent: **GUPTA, Rashmi** et al.; Subramaniam & Asso-
ciates, 7th Floor, M3M Cosmopolitan, Sector 66, Golf
Course Extension Road, Gurugram, National Capital Re-
gion 122001 (IN).

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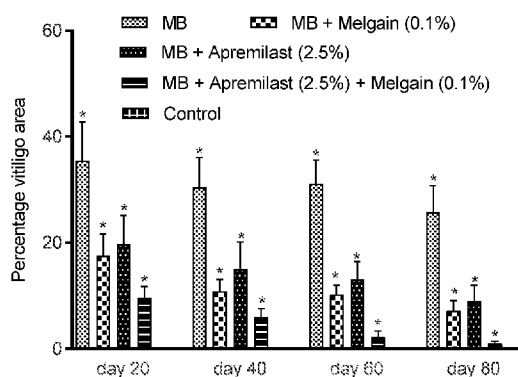


Figure 1

Data are mean $\pm$ SEM (n = 8 for each group). Significance was determined by two-Way ANOVA followed by Tukey's Multiple Comparisons Test. *: p < 0.05 when compared with MB group.

(57) Abstract: The present invention describes a combination of a compound from Basic Fibroblast Growth Factor (bFGF) and at least one additional therapeutic agent. The formulation is preferably in a topical form. The topical pharmaceutical composition comprises a therapeutically effective amount of the Basic Fibroblast Growth Factor (bFGF) of formula (I) and at least one additional therapeutic agent, along with at least one suitable pharmaceutically acceptable carrier, diluents, vehicle or excipient. The present application relates to use of the topical pharmaceutical composition for the treatment or prevention of a suitable disease or condition, such as Vitiligo. The invention also describes the preparation of such compositions.

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“PHARMACEUTICAL COMPOSITIONS FOR TREATMENT OF VITILIGO”

FIELD OF THE INVENTION

The present invention describes a combination of a compound from Basic Fibroblast Growth Factor (bFGF) class and at least one additional therapeutic agent. The formulation is preferably in a topical form. The topical pharmaceutical composition comprises a therapeutically effective amount of the Basic Fibroblast Growth Factor (bFGF) of formula (I) which is a decapeptide of the Basic Fibroblast Growth Factor (bFGF) class and at least one additional therapeutic agent, optionally with at least one suitable pharmaceutically acceptable agent selected from suitable carriers, diluents, vehicle or other excipient. The present application relates to use of the pharmaceutical composition for the treatment or prevention of a suitable disease or condition, such as Vitiligo. The invention also describes the preparation of such compositions.

BACKGROUND OF THE INVENTION

Vitiligo is an autoimmune disease presenting with progressive loss of skin pigmentation. Vitiligo is a cutaneous disease in which melanocytes are destroyed in discrete patches, resulting in lightened areas of variable size and location distributed throughout the skin of the body. It is a disorder that is characterized by white spots typically first noted on the fingers, knuckles, around the eyes and mouth, and on the feet and genitalia (Nordlund et al., Oxford: Blackwell Scientific, Inc; 2006. pp. 591–8).

Melanin, the compound primarily responsible in humans for hair, eye and skin pigmentation, is produced by melanocytes through a complicated process called melanogenesis that is catalyzed by tyrosinase and other tyrosinase-related proteins. The abnormal loss of melanin causes dermatological problems such as Vitiligo (Aisa HA et al., Molecules 2017; 4:22(8)). Melanogenesis is primarily regulated by α -melanocyte-stimulating hormone that binds to the Melanocortin-1 receptor (MC1R) on melanocytes leading to adenylate cyclase activation, elevation of the intracellular cyclic adenosine monophosphate (cAMP) content, and activation of protein kinase A (PKA). Increased PKA activity leads to increased tyrosinase activity (the enzyme responsible for the initiation of melanogenesis), dendrite formation and proliferation in melanocytes. In vitro, the melanogenic effects of α -melanocyte-stimulating hormone can be

mimicked by pharmacologic agents that activate cAMP levels in melanocytes. Two major types of melanin are produced within the melanosomes of human melanocytes eumelanin (brown/black pigments) and pheomelanin (yellow/red pigments). The type of melanin produced depends on the enzyme profile of melanocytes and the prevalent metabolism of the cells.

5 Melanogenesis begins with the hydroxylation of the amino acid L-Tyrosine to form L- DOPA, which is catalyzed by the enzyme Tyrosinase. In the next step of the cascade, Tyrosinase rapidly oxidizes L-DOPA to form Dopakinone which initiates Eu- or Pheo- melanogenesis. Once L-DOPA is formed, the further steps of melanogenesis occur spontaneously. Variations in the activity of melanocytes and the production of melanin are a primary determinant of human skin
10 color. (Askarian-Amiri ME et al., Int. J. Mol. Sci. 2016; 17:1144)

Vitiligo is a condition that causes skin depigmentation due to loss of function and/or death of melanocytes (pigment cells) in the epidermis producing milky-white patches on affected skin. The condition of Vitiligo can also affect eye pigmentation and ear function, as melanin is expressed in both the ear and the uveal tract of the eye. The lightened lesions of the skin
15 generally have greater susceptibility to the damaging effects of the sun, premature aging and possibly skin cancer. The disease occurs in up to 1% of the world population, generally during teenage years.

The exact etiology of Vitiligo is unknown but the most widely accepted view is that it is an autoimmune disease involving immune attack of melanocytes by both T and B cell dependent
20 mechanisms. The progressive loss of melanocytes from depigmenting vitiliginous skin is typically associated with cellular infiltrates containing T lymphocytes. Infiltrating cytotoxic T cells with high affinity T cell receptors have likely escaped clonal deletion in the thymus, allowing such T cells to enter the circulation. It is thought that through the expression of cutaneous lymphocyte antigen, these T cells home to the skin where they express type 1 -
25 cytokines and mediate melanocyte apoptosis via the granzyme/perforin pathway. In addition, the pathogenesis of Vitiligo involves B-cells and T-cells, and cytokines like IL-6, TNF-alpha, and IL-1 (Birol A et al., Int J Dermatol 2006; 45:992-3, Moretti S et al., Pigment Cell Res 2002; 15:87-92), apart from genetic and environmental factors. At the cutaneous level in active lesions level an imbalance in cytokine expression is observed; the perilesional area is immunologically
30 active, recording high levels of IL-1 and IL-17 (markers of increased Th1/Th17 cellular subsets

activation) which demonstrate a chronic pro-inflammatory shift of the immune system response. Oxidative stress is also considered one of the possible pathogenic events in the loss of melanocytes (Schallreuter KU. *Skin Pharmacol Appl Skin Physiol* 1999; 12:132-138).

Vitiligo can be cosmetically disfiguring and is a stigmatizing condition, often leading to psychological problems in daily life. Hence, there is a great need for continuing development of treatments that can be used to minimize the visible consequences of a condition such as Vitiligo, as well as other conditions which manifest themselves as discolorations of the skin (aging spots, liver spots, etc.).

Traditional therapies for Vitiligo mainly include photo chemotherapy with topical/oral psoralens followed by exposure to ultra violet A radiation (PUV-A) or topical/oral steroids. PUV-A therapy is perhaps the main stay in the treatment of Vitiligo. However only about 50% of cases get repigmentation. More over in response to PUV-A, many Vitiligo patches may repigment partially only and the rest of the patches may remain unresponsive to PUV-A therapy even after long duration of treatment. The repigmentation in the above therapies is a result of multiplication of melanocytes, the cells, which produce the pigment melanin in the skin. The multiplication of melanocytes in response to the above therapies occurs from the margins of the Vitiligo patch or at the pigmented hair follicles and their migration/spread to the Vitiligo patch.

Apremilast is a phosphodiesterase-4 enzyme (PDE-4) inhibitor and is used for the treatment of patients with moderate to severe plaque psoriasis who are candidates for phototherapy or systemic therapy, as well as for use in psoriatic arthritis. PDE-4 normally degrades cyclic adenosine monophosphate (cAMP) into 5'-adenosine monophosphate. By inhibiting the PDE-4 enzyme specific for cAMP degradation, this results in increased intracellular cAMP levels and thereby regulation of numerous inflammatory mediators through the cAMP second messenger effect (e.g., decreased expression of TNF- α , and interleukin- (IL-) 17, IL-23, and interferon gamma, as well as increased IL-10) (Schafer P, *Biochemical Pharmacology*; 2012. pp. 1583-1590). When intracellular cAMP is elevated, inflammatory signaling and cytokines are suppressed and anti-inflammatory modulators, such as IL-10, are increased. Vitiligo is linked to a dysregulated immune system governed by a proinflammatory cytokine network, involving local and systemic chronic inflammatory processes (Schafer P, *Cellular Signalling*; 2014. pp. 2016–2029). Melanocytes cultured from vitiligo patient skin samples have been shown to

express high levels of cytokines including IL-6 and IL-17. Positive correlations between levels of IL-17 and disease extent and activity have been found. IL-6 and IL-8 can attract immune components to the skin and may be the link between the triggering event and the initiation of the autoimmune response that results in Vitiligo progression (Manga P et al., F1000Research, 2016, Vol. 5). Apremilast at 30 mg/kg, twice daily, orally has shown good effect in patients of Vitiligo. These patients who were treated with Apremilast 30 mg twice daily achieved significant repigmentation in the presence of initial steroid bolstering and continued repigmentation without steroids. Common side effects related to oral Apremilast are diarrhea, headache, nausea, vomiting, weight loss, and depression. These side effects are dose-limiting and thus the therapeutic potential of Apremilast may not be fully realized. To overcome these obstacles, we propose the topical route of administration of Apremilast with potent and long-lasting local effect and minimal systemic exposure.

Basic Fibroblast Growth Factor (bFGF), also known as FGF2 is involved in repigmentation of Vitiligo macules, a pigmentary disorder characterized by patchy depigmentation of skin (Ramaiah A et al., Acta Derma Venereol (Stochhom) 1989; 69:323-327).

bFGF has been found to be expressed in a wide variety of tissue types including pituitary, brain and adrenal gland corpus luteum, retina, kidney, placenta and keratinocytes, fibroblasts.

Keratinocytes-melanocytes crosstalk is mainly modulated by basic Fibroblast Growth Factor (bFGF) which induces melanocytes growth and differentiation and melanin synthesis; it is also involved in the ROS detoxifying processes by inhibiting them (and related damages) via activation of PI3K/Akt and consequent block of NF-kB nuclear translocation. (Lotti et al., Pigmentary Disorders 2015; S3:001)

The local application of the bFGF or its agonist peptides in the formulation described in the U.S. Pat. No. 6,143,723 and Indian Pat. No. 186467 is effective in more than 80% of cases of stable generalized Vitiligo or segmental Vitiligo. However, bFGF peptide lotion therapy is not effective to prevent the fast spread of Vitiligo and a synergistic combinatorial therapy may emerge if bFGF peptide lotion therapy is used in combination with another therapy to be used in the case of fast spreading Vitiligo. Similarly, the local application the bFGF peptide(s) lotion therapy may

be advantageously used with another therapy including in combination with suitable immuno modulators such as topical Apremilast.

The current therapies for Vitiligo such PUV-A, steroids, oral PDE4 inhibitors has lot of side effects, longer duration of treatment and these therapies though are capable of stopping Vitiligo but are not very useful in reversing the effects of Vitiligo. Therefore, the topical combination of compound of formula (I) with Apremilast has significant advantages over other therapies for Vitiligo and may show significant improvement in Vitiligo especially in reversing the effects of Vitiligo.

SUMMARY OF THE INVENTION

The present invention provides a pharmaceutical composition comprising a compound of formula (I) which is a decapeptide of the Basic Fibroblast Growth Factor (bFGF) class in combination with a second therapeutic agent for the treatment of Vitiligo. In a preferred embodiment, the combination is applied topically.

The treatment of Vitiligo typically begins with monotherapy. For many patients however, monotherapy is not sufficiently effective, leading to a requirement for combination therapy. However, co-prescription of two or more drugs may result in treatment regimens that are complex and difficult for many patients to follow.

It has now surprisingly been found that the formulation of compound of formula (I), which is a Basic Fibroblast Growth Factor (bFGF), in combination with suitable PDE4 inhibitors provides a particularly beneficial effect in stopping Vitiligo but also shows beneficial effect on repigmentation with no observed adverse effects. Such combination is therefore particularly useful for the treatment of Vitiligo..

DESCRIPTION OF FIGURES

Figure 1:- The effect of Apremilast (2.5%) alone, Melgain® (0.1%) alone and their combination on depigmentation area of Monobenzene induced Vitiligo in mice

Figure 2:- The effect of Apremilast (2.5%) alone, Melgain® (0.1%) alone and their combination on melanin levels of Monobenzene induced Vitiligo in mice

Figure 3:- The effect of Apremilast (5%) alone, Melgain® (0.1%) alone and their combination on depigmentation area of Monobenzene induced Vitiligo in mice

Figure 4:- The effect of Apremilast (5%) alone, Melgain® (0.1%) alone and their combination on melanin levels of monobenzene induced Vitiligo in mice

5 EMBODIMENTS OF THE PRESENT INVENTION

In an embodiment is provided a combination comprising, compound of formula (I) which is a decapeptide of the Basic Fibroblast Growth Factor (bFGF) class and at least one additional therapeutic agent.

The compound of formula (I) is commercially known as Melgain®/Melbild® etc. The term
10 Melgain® and compound of formula (I) has been interchangeably throughout the specification.

In one embodiment of the present invention is provided a pharmaceutical combination comprising compound of formula (I) and a second therapeutic agent selected from suitable PDE4 inhibitors, methotrexate or derivatives thereof, cyclosporins or derivatives thereof, vitamins D, E and A or derivatives thereof, glucocorticoid or derivatives thereof, anti-inflammatory agents,
15 immunosuppressive agents, antihistamines, monoclonal antibodies and fusion proteins, cytokines and mediators, antibiotic or derivatives thereof, antifungal agents or derivatives thereof, hormones, peroxisome proliferator-activated receptor gamma activators or derivatives thereof, anti-viral agents or derivatives thereof, vegetable and/or animal extracts, phytochemicals or derivatives thereof, zinc pyrithione and/or other essential minerals, phototherapy and cell hyper
20 proliferation modulators for the treatment of Vitiligo.

In an embodiment, the combination is provided in a topical form.

In another embodiment is provided a topical pharmaceutical composition comprising Melgain® with one or more therapeutic agents from those described above for the treatment of humans and other mammals in need thereof.

25 In a preferred embodiment is provided a topical pharmaceutical composition comprising Melgain® and Apremilast along with one or more suitable pharmaceutical excipients.

In a preferred embodiment is provided a topical pharmaceutical composition comprising Melgain® and Apremilast for the treatment of Vitiligo.

In another embodiment is provided a topical pharmaceutical composition for use in the prevention or delay of progression of Vitiligo.

- 5 In another embodiment is provided a topical pharmaceutical composition for use in reversal of depigmentation and increasing melanin.

DETAILED DESCRIPTION OF THE INVENTION

As used herein the term 'MB' refers to Monobenzene.

10 As used herein the term 'pharmaceutically acceptable' use embraces both human and veterinary use.

The term "therapeutically effective amount" or "effective amount" is used herein to denote any amount of a topical formulation which will cause a substantial improvement in a disease condition when applied to the affected area. A single application can be sufficient, or the formulation can be applied repeatedly over a period of time. The amount will vary with the condition being treated, the stage of advancement of the condition, and the type and concentration of formulation applied. As used herein the term 'pharmaceutically acceptable' use embraces both human and veterinary use.

20 The term 'topical composition' or 'topical formulation' means a composition in which the drug may be placed for direct application to a skin surface and from which an effective amount of the drug is released. Such formulation may include creams, ointments, gels, lotions or any other dosage form suitable for topical application and the like. In some aspects, such formulation may be applied to the skin in an unoccluded form with/without additional backing, structures or devices.

25 The term 'skin' or 'skin surface' is meant to include the outer skin of a subject comprising one or more of epidermal layers to which a drug composition may be administered.

The term 'treating' or 'treatment of a state, disorder or condition as used herein means: (I) preventing or delaying the appearance of clinical symptoms of the state, disorder or condition developing in a mammal.

The topical formulation of the present invention include those suitable for topical, transdermal, rectal and buccal (e.g, sub-lingual) administered etc. preferably the formulation of the present invention are administered topically and are provided in the form of semisolid dosage forms. Suitable dosage forms include hydrous or anhydrous semisolids such as creams, ointments, gels, lotions or any other dosage form suitable for topical application and the like.

The present invention describes a combination comprising, compound of formula (I) which is a decapeptide of the Basic Fibroblast Growth Factor (bFGF) class and at least one additional therapeutic agent.

The compound of formula (I) is a decapeptide having a sequence



(Formula 1)

This compound is known commercially as Melgain®.

In an embodiment, the present invention provides a topical pharmaceutical composition comprising a compound of formula (I) and at least one additional therapeutic agent and pharmaceutically acceptable excipients, for use as an active therapeutic substance.

In an embodiment, the additional therapeutic agent used is selected from a PDE4 inhibitor, methotrexate or derivatives thereof, cyclosporine or derivatives thereof, vitamins D, E and A or derivatives thereof, glucocorticoid or derivatives thereof, anti-inflammatory agents, immunosuppressive agents, antihistamines, monoclonal antibodies and fusion proteins, cytokines and mediators, antibiotic or derivatives thereof, antifungal agents or derivatives thereof, hormones, peroxisome proliferator-activated receptor gamma activators or derivatives thereof, anti-viral agents or derivatives thereof, vegetable and/or animal extracts, phytochemicals or derivatives thereof, zinc pyrithione and/or other essential minerals, phototherapy and cell hyper proliferation modulators

In an embodiment, the additional therapeutic agent is PDE4 inhibitors. In a preferred embodiment the PDE4 inhibitor is Apremilast.

In another preferred embodiment the present application is directed to a topical pharmaceutical composition comprising (i) compound of formula (I), (ii) at least one additional therapeutic agent and (iii) optionally one or more pharmaceutically acceptable excipients.

5 The compound of formula (I) can be prepared by the general processes and examples disclosed in US6143723. Apremilast can be prepared by the general processes and examples disclosed in WO 2000025777 and WO2001034606.

In the treatment of the invention, the active medicaments are preferably administered in topical pharmaceutical composition form. The active agents may be applied separately or may be in the form of a pharmaceutical formulation comprising the two actives.

10 The compositions are preferably in topical form in an amount appropriate for the relevant daily dosage.

The compositions are prepared and formulated according to conventional methods, such as those disclosed in standard reference texts and are well within the scope of a skilled person. Thus the composition can be in the form of individual components delivered as a kit or may comprise of
15 suitable co-formulation of the two ingredients using suitable excipients. A skilled person is aware of the alternate ways to deliver a topical composition.

No adverse toxicological effects were seen for the topical compositions or methods of the invention in the above mentioned strength. Further the topical composition of the present invention was found suitable for the treatment of Vitiligo.

20 The present invention discloses a topical formulation comprising compound of formula (I) and Apremilast present at a concentration of 0.001 to 10% w/w and 0.01 to 25% w/w respectively. Topical formulation includes gel, ointment, creams, solution and foams and process for the preparation thereof. The topical formulations of this invention are suitable for the treatment of Vitiligo.

25 In a further another embodiment is provided the use of different formulations such as gel, ointment, creams, lotion, solution and foams comprising compound of formula (I) and Apremilast.

In an embodiment is provided a formulation comprising compound of formula (I) and Apremilast present at a concentration of 0.01 to 1% w/w and 0.5 to 10% w/w respectively when prepared as gel formulation and process for the preparation thereof.

5 In an embodiment is provided a formulation comprising compound of formula (I) and Apremilast present at a concentration of 0.01 to 1% w/v and 0.5 to 10% w/v respectively when prepared as a liquid formulation and process for the preparation thereof.

In a preferred embodiment is provided a formulation comprising compound of formula (I) and Apremilast present at a concentration of 0.01 to 1% w/v and 0.5 to 10% w/v respectively wherein liquid formulation are selected from lotion or solution formulation.

10 In an embodiment is provided a topical formulation comprising a compound of formula (I) and Apremilast, thickening agents, neutralizing agent for polymer and other pharmaceutically acceptable carriers.

Thickening agents used may be selected from the group of hydrophilic polymers such as carbomers, carbomer polymer, carbomer derivative, cellulose derivatives, anionic polymers, polyvinyl alcohol, water soluble polysaccharide may be at least one selected from the group
15 consisting of hyaluronic acid, hyaluronate, poly- γ -glutamic acid, poly- γ -glutamate, agar, alginic acid, alginate, carrageenan, furcellaran, pectin, arabic gum, karaya gum, tragacanth gum, ghatti gum, guar gum, locust bean gum, psyllium seed gum, glectin, chitin, dextran, xanthan gum, chitosan, chondroitin-4-sulfate, chondroitin-6-sulfate and starch and mixtures thereof.

20 Solvent used may be selected from the group of pharmaceutically acceptable Glycols (for example, polyethylene glycol, propyleneglycol and hexylene glycol), alcohols, polysorbate 40 (a polyhydroxy organic compound), Poloxamer.

Neutralisation agent for polymer used may be selected from the group of polyfunctional amine such as triethanolmine, poly (ethyleneimine), diisopropanolamine, triisopropanolamine, arginine, aminomethyl propanol, tetrahydroxypropyl ethylenediamine, tromethamine; alkali and alkaline
25 earth metal hydroxides such as sodium and potassium hydroxides and lithium hydroxide, ammonium hydroxide and the like. The epoxy cross-linking agent may be at least one selected from the group consisting of epichlorohydrin, 1,4-butandiol diglycidyl ether, ethylene glycol

diglycidyl ether, 1,6-hexanediol diglycidyl ether, propylene glycol diglycidyl ether, polyethylene glycol diglycidyl ether, polypropylene glycol diglycidyl ether, polytetramethylene glycol diglycidyl ether, neopentyl glycol diglycidyl ether, polyglycerol polyglycidyl ether, diglycerol polyglycidyl ether, glycerol polyglycidyl ether, trimethylpropane polyglycidyl ether, 1,2-
5 (bis(2,3-epoxypropoxy)ethylene, pentaerythritol polyglycidyl ether and sorbitol polyglycidyl ether.

The suitable pharmaceutical carrier are selected from conventional excipients such as binding agents, fillers, lubricants, glidants, disintegrants and wetting agents.

Vehicle is selected from water, suitable solvents as are known in the art and mixture of thereof.

10 The invention is further exemplified by the following non-limiting examples, which are illustrative representing the preferred modes of carrying out the invention. The invention's scope is not limited to these specific embodiments only but should be read in conjunction with what is disclosed anywhere else in the specification together with those information and knowledge, which are within the general understanding of a person skilled in the art.

15 **Study-1**

The 4-week old male and/or female c-57 mice were used for the study and mice received a daily topical dose of monobenzone (20-40 %) on the shaved back for 35-60 consecutive days to establish a model of monobenzone induced Vitiligo. To examine the efficacy of compound of formula (I) (as Melgain®) and Apremilast, mice were randomly divided into five groups:
20 vehicle-treated monobenzone-induced model group (MB), 2.5% Apremilast-treated monobenzone-induced model group (MB + 2.5% Apremilast), 0.1% Melgain®-treated monobenzone-induced model group (MB + 0.1% Melgain®), Combination (MB + 2.5% Apremilast + 0.1% Melgain®) and Plain propylene treated group (Control).

Monobenzone was applied till day 40 and further application of MB was stopped. At day 40,
25 depigmentation area was measured and animals were randomized based on depigmentation area. Treatment started on day 41 (day 0) after the topical application of MB and continued upto day 120 (0-80). All animals received daily treatment of vehicle, Apremilast, Melgain® and their combination topically. All animals were assessed for the severity of the depigmentation area on

day 20, 40, 60 and 80-post withdrawal of MB application. At day 80 (from the day of initiating treatment), animals were sacrificed, skin melanin levels were determined. Skin was collected and processed for histology.

Results

5 **Apremilast, Melgain® and their combination have shown re-pigmentation effect in MB induced depigmentation model in mice**

After topical application of Monobenzone, small white patches appeared on the drug-exposed area. These white patches expanded over time, with the possibility of ultimately resulting in substantial depigmentation over most parts of the body. In all groups, Monobenzone was
10 withdrawn on day-40 and observation continued till 120 days.

These white patches were significantly reduced in Apremilast, Melgain® and their combination when compared with vehicle control. The percentage improvement in depigmentation area (Vitiligo) was significantly higher in the combination treatment group (96%) when compared with Apremilast (65%) and Melgain® (72%) alone, indicating re-pigmentation or reversal of
15 depigmentation (Figure 1). Application of vehicle alone did not significantly affect Monobenzone induced vitiligo in mice.

As shown in figure 2, MB group exhibited less melanin levels when compared with control group due to the destruction of melanocytes.

Conclusion: Overall, these results indicate that a topical combination of Apremilast (2.5%) with
20 Melgain® (0.1%) has shown reversal in vitiligo condition. The combination has shown high improvement in vitiligo when compared with Apremilast and Melgain® individually. The combination of Apremilast (2.5%) with Melgain® (0.1%) has shown increase in melanin levels, which indicates improvement in damaged melanocytes or their functions. Therefore, this combination can be a valuable tool in the treatment in the vitiligo.

25 **Study -2**

The 4-week old male and/or female c-57 mice were used for the study and mice received a daily topical dose of monobenzone (20-40 %) on the shaved back for 35-60 consecutive days to

establish a model of monobenzene induced Vitiligo. To evaluate the efficacy of compounds, mice were randomly divided into five groups: vehicle-treated monobenzene-induced model group (MB), 5% Apremilast-treated monobenzene-induced model group (MB + 5% Apremilast), 0.1% Melgain®-treated monobenzene-induced model group (MB + 0.1% Melgain®),
5 Combination (MB + 5% Apremilast + 0.1% Melgain®) and Plain propylene treated group (Control).

Monobenzene was applied for 40 days and Monobenzene application was stopped at day 40. Depigmentation area was measured at day 40 and animals were randomized based on depigmentation area. Treatment started on day 41 (day 0) post the topical application of MB and
10 continued until day 120 (0-80). All animals received daily treatment of vehicle, Apremilast, Melgain® and their combination topically. All animals were assessed for the severity of the depigmentation area on days 20, 40, 60 and 80-post withdrawal of MB application. At day 80, animals were sacrificed, skin melanin levels were determined. Skin was collected and processed for histology.

15 Results

Apremilast, Melgain® and their combination have shown re-pigmentation effect in MB induced depigmentation model in mice

Post topical application of monobenzene, small white patches appeared on the drug-exposed area. These white patches expanded over time, with the possibility of ultimately resulting in
20 substantial depigmentation over most parts of the body. In all groups, Monobenzene was withdrawn on day-40 and observation continued until 120 days.

These white patches were significantly reduced in Apremilast, Melgain® and their combination when compared with vehicle control. The percentage improvement in depigmentation area (Vitiligo) was significantly higher in combination group (87%) when compared with Apremilast
25 (76%) and Melgain® (68%) alone group indicating re-pigmentation or reversal of depigmentation (Figure 3). Application of vehicle alone did not significantly affect MB induced vitiligo in mice.

As shown in figure 4, MB group exhibited less melanin levels when compared with control group due to the destruction of melanocytes.

Conclusion:

5 Topical combination of Apremilast (5%) with Melgain® (0.1%) has shown improvement in vitiligo condition. The combination of Apremilast with Melgain® has shown improvement in melanin levels and depigmentation, which might be due to the repairment of damaged melanocytes or improvement in their functions. As there is no complete cure of vitiligo and this combination has advantage over current therapy in terms of duration of treatment and side effects of current therapies, this can be a potential therapy for vitiligo.

We claim:

1. A pharmaceutical composition comprising (i) a decapeptide of formula (I)



(Formula 1)

- 5 (ii) atleast one additional therapeutic agent; and

(iii) optionally one or more pharmaceutically acceptable excipient

2. The pharmaceutical composition as claimed in claim 1, wherein additional therapeutic agent is selected from a PDE4 inhibitor, methotrexate or derivatives thereof, cyclosporine or derivatives thereof, vitamins D, E and A or derivatives thereof, glucocorticoid or derivatives thereof, anti-inflammatory agents, immunosuppressive agents, antihistamines, monoclonal antibodies and fusion proteins, cytokines and mediators, antibiotic or derivatives thereof, antifungal agents or derivatives thereof, hormones, peroxisome proliferator-activated receptor gamma activators or derivatives thereof, anti-viral agents or derivatives thereof, vegetable and/or animal extracts, phytochemicals or derivatives thereof, zinc pyrithione and/or other essential minerals, phototherapy and cell hyper proliferation modulators
- 10
3. The pharmaceutical composition as claimed in claim 2, wherein the additional therapeutic agent is selected from suitable PDE4 inhibitor.
4. The pharmaceutical composition as claimed in claim 3, wherein PDE4 inhibitor is Apremilast.
- 20
5. The pharmaceutical composition as claimed in claims 1-4 wherein the composition is in topical form.
6. The topical pharmaceutical composition as claimed in any one of claims 1 to 5, wherein the compound of formula (I) is present in an amount of from 0.001 to 10% and Apremilast is present in an amount of from 0.01 to 25 %.
- 25
7. The pharmaceutical composition as claimed in any one of claims 1 to 6, wherein the compound of formula (I) is present in an amount of from 0.01 to 1% and the Apremilast is present in an amount of from 0.5 to 10 %.

8. The pharmaceutical composition as claimed in any one of claims 1 to 7, when applied topically, can be in the form of a gel, ointment, creams, lotion, solution and foams.
9. The pharmaceutical composition as claimed in claims 1 to 8 for use as an active therapeutic substance.
- 5 10. The pharmaceutical composition as claimed in claim 9 for use in the treatment of Vitiligo.
11. The topical pharmaceutical composition as claimed in claim 9 for use in the prevention or delay of progression of Vitiligo.
12. The topical pharmaceutical composition as claimed in claim 9 for use in reversal of depigmentation and increasing melanin of patients suffering from Vitiligo.
- 10 13. The topical pharmaceutical composition as claimed in claim 1-12, wherein pharmaceutical acceptable excipients is selected from suitable thickening agents, neutralizing agents, binding agents, fillers, lubricants, glidants, disintegrants and wetting agents.
14. The topical composition of claim 13 wherein the excipients are those described in the specification.

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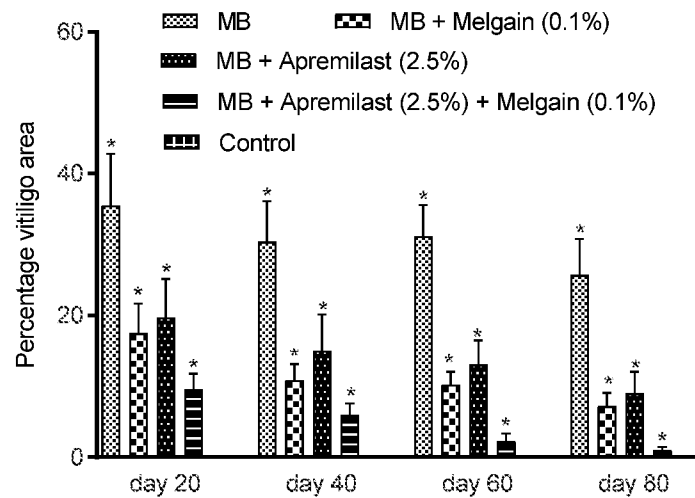
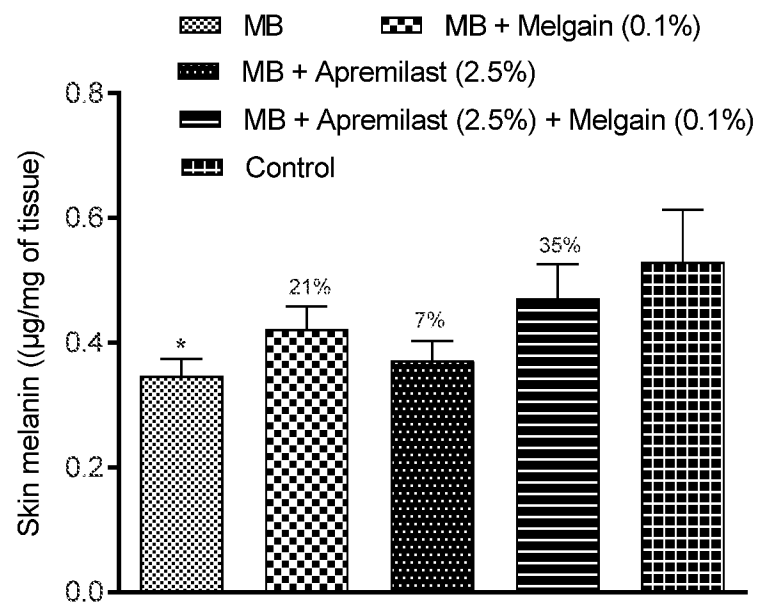


Figure 1

Data are mean $\pm$ SEM (n = 8 for each group). Significance was determined by two-Way ANOVA followed by Tukey's Multiple Comparisons Test. *: $p < 0.05$ when compared with MB group.

**Figure 2**

Data are mean $\pm$ SEM ($n=8$ for each group). Significance was determined by two-Way ANOVA followed by Tukey's Multiple Comparisons Test. *: $p < 0.05$ when compared with MB group.

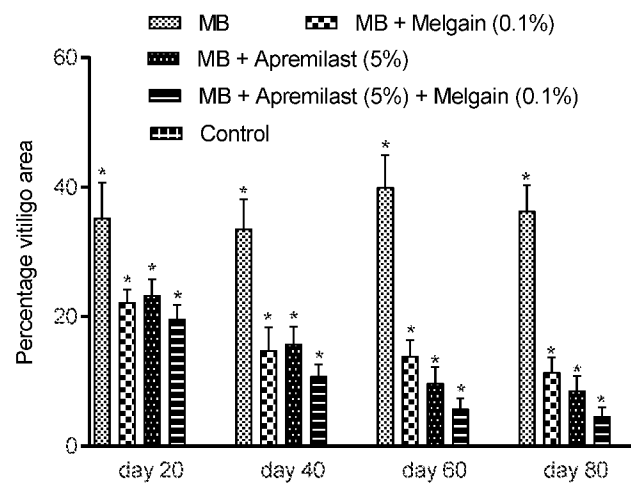


Figure 3

Data are mean $\pm$ SEM (n =8 for each group). Significance was determined by two-Way ANOVA followed by Tukey's Multiple Comparisons Test. *: p < 0.05 when compared with MB group.

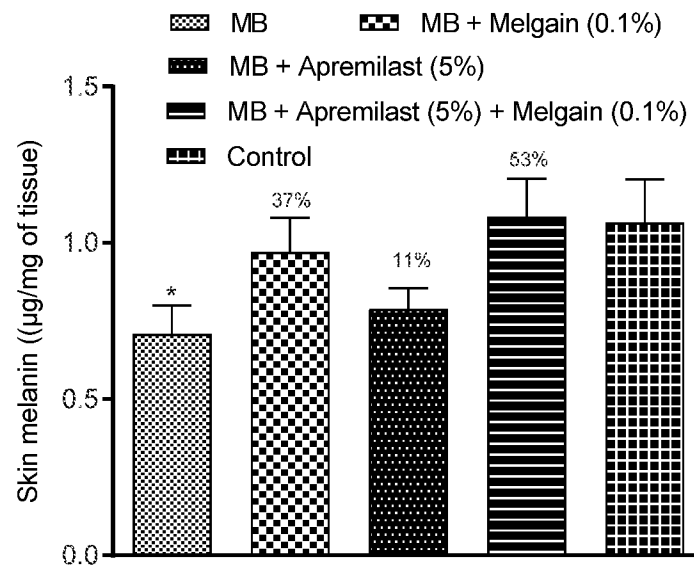


Figure 4

Data are mean $\pm$ SEM (n =8 for each group). Significance was determined by two-Way ANOVA followed by Tukey's Multiple Comparisons Test. *: p < 0.05 when compared with MB group.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2019/050384

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K38/18 A61K9/00 A61K31/4035 A61P17/00 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) A61K A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, WPI Data, BIOSIS, EMBASE, CHEM ABS Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/032029 A1 (RAMAIAH ABBURI [IN]) 22 March 2007 (2007-03-22)	1,2,5, 8-14
Y	claims; examples	1-14
X	AU 2005 203 228 A1 (RAMAIAH ABBURI) 8 February 2007 (2007-02-08)	1,2,5, 8-14
Y	claims; examples	1-14
Y	RAMAIAH A KAR HK: "A Double Blind Randomized Phase IV Clinical Trial of basic Fibroblast Growth Factor Related Deca-peptide in Vitiligo", JOURNAL OF PIGMENTARY DISORDERS, vol. s3, 1 January 2015 (2015-01-01), XP055584776, DOI: 10.4172/2376-0427.S3-004 abstract; tables	1-14
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☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
30 April 2019		17/05/2019
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Langer, Astrid

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2019/050384

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HUFF SARA B ET AL: "Repigmentation of Tenacious Vitiligo on Apremilast.", CASE REPORTS IN DERMATOLOGICAL MEDICINE 2017, vol. 2017, 2017, page 2386234, XP055584785, ISSN: 2090-6463 abstract; figures -----	1-14
A	EZZEDINE KHALED ET AL: "Vitiligo", LANCET (NORTH AMERICAN EDITION), vol. 386, no. 9988, 4 July 2015 (2015-07-04), pages 74-84, XP055584810, the whole document -----	1-14

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