

## A Double Blind Randomized Phase IV Clinical Trial of basic Fibroblast Growth Factor Related Deca-peptide in Vitiligo

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### Abstract

**Back ground:** Topical application of bFGF related deca peptide followed many hours after with sun /UVA exposure was found effective in the earlier conducted clinical trials to treat Non-segmental, segmental, PUV-A resistant and fast spreading vitiligo either by itself or in combination with PUV-A, or oral steroids. The deca peptide lotion is marketed in India since 2004 to treat vitiligo.

**Objectives:** A multicentre randomized double blind phase IV clinical trial on bFGF related deca peptide to treat vitiligo was conducted with the following twin objectives. One was to evaluate the efficacy and safety of topically applied deca peptide in vehicle in combination with narrow band ultra violet light (NBUVB) in patients with vitiligo. The second objective was whether deca peptide is effective to repigment vitiligo macules on sun protected areas without sun exposure.

**Methods:** This was a multi-centre study. Two chosen macules on each patient that were non-sun exposed areas on patients with stable non segmental vitiligo were selected. The duration of treatment of volunteers was for 3 months starting with or without the topical application of deca peptide in vehicle on 30 volunteer patients with NBUVB and on 32 volunteers with deca peptide in vehicle.

**Results:** The results demonstrated that with NB-UVB plus deca peptide in vehicle group, 9 patches out of 30 repigmented by more than 40% compared to only 5 out of 62 patches treated with NBUVB alone and with peptide alone groups indicating that NBUVB and peptide act synergistically in repigmentation of the macules. In addition, the mean value of repigmentation at the end of 3 months in all the 30 macules of 30 patients with NBUVB+ peptide was 229 mm square compared to only 90 only with NBUVB in vehicle alone. More repigmentation occurred with NBUVB + peptide at all-time points of evaluation.

**Conclusions:** The results demonstrated that NB-UVB plus deca peptide act synergistically and produced more repigmentation of the macules right from the start compared to control even though deca peptide application was in effect for 3 days in a week. NBUVB+ peptide was superior to NBUVB alone in repigmenting vitiligo macules. The deca peptide in absence of sun exposure of vitiligo macule may not be as effective to repigment vitiligo macules as with sun exposure. NBUVB and peptide were well tolerated.

**Keywords** Vitiligo; peptide; Fibroblast Growth Factor; repigmentation

### Introduction

The etiopathology of vitiligo is far from clear [1]. Traditional therapies mainly include Photo chemotherapy with topical/oral Psoralens along with ultraviolet radiation (PUVA) and topical/oral Corticosteroids [2-7]. NB-UVB therapy pioneered by Westerhof et al. is more acceptable than PUVA therapy [5,8]. It has been postulated that the basic defect in vitiligo lies within the melanocytes [9].

Based on the studies of Puri et al. and Ramaiah et al. we hypothesized that growth factors for melanocytes like basic Fibroblast Growth Factor (bFGF) or other growth factors may be involved in repigmentation [10-13]. This hypothesis was supported by the fact that

growth defects of melanocytes from the untreated vitiligo patients were corrected when they were grown in the presence of bFGF [14] bFGF or its short active peptides increase the proliferation of melanocytes obtained from the peri-lesional areas of untreated vitiligo lesions or in the mixed cultures of melanocytes and keratinocytes in a dose dependent manner [14]. Other growth factors may also be involved in repigmentation [15,16]. We proposed that active peptides derived from b-FGF could be potential therapeutic agents for managing vitiligo since bFGF or its active short peptides under in vitro conditions are mitogenic to melanocytes and stimulates melanogenesis [14,17]. bFGF derived peptides (bFGFRP) were effective in repigmentation of depigmented patches in experimental animal models [18-20,14]. Based on the above studies, clinical trials were conducted to study the efficacy and safety of the bFGFRP in India [21].

The drug controller general (India) approved the results of the trials and listed deca peptide as a drug to treat vitiligo.

The present multicentre randomized double blind phase IV clinical trial on bFGFRP to treat vitiligo was conducted with the idea of getting this product in to western markets based on licensing the foreign patents on bFGFRP to a western company for which a double blind study was necessary. This study was done with the following twin objectives.

One was to evaluate the efficacy and safety of topically applied deca peptide in vehicle in combination with Narrow band ultra violet light (NBUVB) in patients with vitiligo.

The second objective was whether deca peptide is as effective to repigment vitiligo macules on sun protected areas without sun exposure as with sun exposure.

## Materials and Methods

bFGF derived peptide is a deca peptide that was approved by the drug controller general (India) in 2001 to treat vitiligo and is in the market by the name Melgain since 2004. In the present clinical trial bFGRP was custom made by Hemmo pharma Pvt Ltd a European approved peptide synthesis facility located at Mumbai. The peptide was custom made by automated solid phase synthesis method. N terminal is blocked with Para hydroxyl phenyl prop ionic acid to make it hydrophobic for better permeability in to the epidermis. The molecular weight of the peptide with the N terminal block is 1542.7Da. The peptide is acetate salt and contain four acetate residues. Decapeptide (0.1%) in the formulation after correction for 4 acetate residues was used in the study.

## Methods

This trial was a multicenter, randomized double blind study of bFGFRP in vehicle or vehicle administered control or in combination with full body NBUVB for treatment of vitiligo macules on sun protected sites in patients with stable non-segmental vitiligo for duration of 12 weeks. The recruitment period of volunteers was from April 2009-October 2009 but the duration of treatment of each volunteer is for 3 months starting from the time of topical application of deca peptide. The patients were recruited by four different treatment centers with 62 subjects (30 subjects for Peptide in vehicle +NBUVB or NBUVB+ vehicle (Group 1) or 32 subjects with peptide in vehicle or vehicle (Group 2). The four different centers were 1. Ram Manohar Lohia Hospital Delhi under the direction of Prof H.K Kar, Maulana Azad Medical college Delhi under direction of Prof Vijay Kumar Garg, Guru Nanak Skin Clinic (3773 Main Rd Kanhaiya Nagar New Delhi) under the direction of Dr Neeraj Bajaj, and Skin Clinic Leucoderma and Laser centre (101 Rishabh Corporate Tower Community Centre Karkardooma Delhi) under the direction of Dr. Mukesh Girdhar. This number is fixed on assumption that standard deviation using one sample t test (Paired difference) with 20% difference of repigmentation in the paired macules will be less than 30%.

## Patient selection

Patients between 18 to 60 years of age with non-segmental stable vitiligo with disease duration of less than 5 years were included. Patients of Fitzpatrick skin type IV, V and VI were included. Only those patients were recruited who had not received more than 12

consecutive NB-UVB sessions in the past 12 months with no previous exposure to Melgain/decapeptide. Stable vitiligo was defined as  $\leq 10\%$  increase in any lesion, no new significant macules and no progression of existing macules for  $\geq 6$  months. Other inclusion criteria included: body surface area  $\leq 25\%$ , two target macules of similar size measuring  $\geq 9 \text{ cm}^2$  but  $\leq 200 \text{ cm}^2$  with no leucotrichia located on sun protected skin (e.g. trunk) separated by at least 10 cm of uninvolved skin at their nearest point. Patients on systemic therapies in the last 2 months on topical therapy(s) e.g. steroids Class III or stronger, PUVA within 1 month were excluded. Patients with other medical issues, pregnant and lactating women were also excluded.

Subjects were randomized by Interactive Voice Responsive System (IVRS) by Kythera Biopharmaceuticals, Los Angeles, USA. The randomization occurred at two levels. Each subject was randomized to receive either NBUVB or no NBUVB. Each target depigmented macule (2 macules per patient) was randomized to receive either deca peptide in vehicle or vehicle alone. A subject's NBUVB assignment and assignment of peptide/vehicle did not change during the course of study. The patients, investigators and technical personnel were blinded to treatment assignments and only authorized staff could un-blind when essential via opening un-blinding envelopes. The trial was conducted in accordance with the International Conference Harmonization (ICH) and the principles enunciated in the Declaration of Helsinki (Ethical Principles for Medical Research) involving Human subjects revised in 2008.

The investigator selected two non-contiguous macules on sun protected body area for each subject successfully screened and enrolled. Subjects randomized to receive NB-UVB (Group 1) received full body NBUVB commencing at baseline visit and subsequently thrice weekly on non-consecutive days. The radiation dose was  $0.3 \text{ J/cm}^2$  at baseline and increased by 10% weekly based on the subject's erythematic response and investigator's discretion. Subjects in Group 1 on the days of NBUVB administration were allowed 15 minutes time interval before they received bFGFRP (0.1% bFGFRP in vehicle at 5 microliters/cm<sup>2</sup>) on one target macule and vehicle on the corresponding paired macule in a blinded fashion six days per week for 12 weeks. Subjects randomized to Group 2 received bFGFRP (drug) in vehicle on one target macule with no NBUVB and vehicle on the corresponding paired macule with no NBUVB in a blinded fashion six days per week for 12 weeks. Study drug/vehicle was applied via finger cot by a trained study coordinator, investigator or sub investigator at the clinic. The patient was never allowed to administer the drug/vehicle himself/herself to insure the compliance of the protocol. If any patient missed 10 non consecutive days of study drug administration during the 12 week treatment period or the patient missed 7 consecutive days of study drug, the patient is removed from the trial.

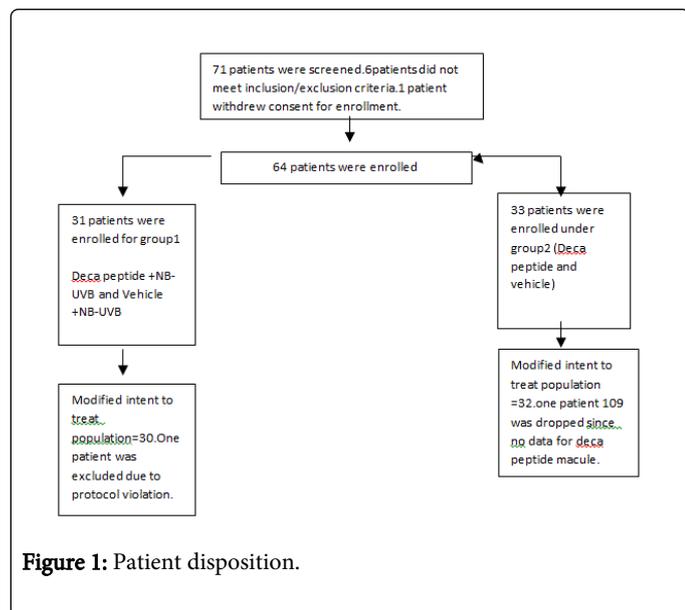
## Efficacy assessment

Macule area measurements were conducted using computer assisted measurements of photographs performed by Canfield Scientific Inc; New York USA (Canfield Scientific, Inc, Fairfield, NJ 07004-2524 USA). Macule colorimetric changes were also considered programmatically using Canfield proprietary software. This method concurred with the investigator's subjective assessment evaluation of target macules as well as that of the concerned patient.

This was comparable to what Hamzvi et al. did [22]. The photographs were taken at base line, at periods of 2 weeks, 4 weeks, 8 and 12 weeks.

### Statistical evaluation of data

Paired t test was used for evaluating the data presented in Tables 1-3 and Chi square test for the data in Table 4 for significance of synergy between the NBUVB and peptide. The p value was calculated by Chi square test for two group proportions at 95% confidence interval at more than 20% and 40% level of repigmentation [23,24].



**Figure 1:** Patient disposition.

### Results

The median age of patients was 30 years of whom 35.9% were females in NBUVB group and in non NBUVB group. Disposition of patients was summarized in (Figure 1). Of the 31 patients enrolled in Group 1 (NBUVB + bFGFRP/vehicle) 30 patients with one exclusion after protocol violation were included for the modified intent to treat (mITT). In Group 2 (no NBUVB + b-FGFRP/vehicle) 32 of 33 patients enrolled were included with one exclusion for lack of valid baseline data. mITT included all randomized subjects receiving topical bFGFRP and who had at least one post base line observation taken at 4, 8 and 12 weeks.

The areas measured for specificity described as threshold 1 method included only the white area of the macules as described in the 2nd

picture in Figure 2. The areas measured for high sensitivity described as threshold 2 method included the area in the macule lighter in color than the color of the border of the macule along with white area of the macules as shown in picture 3 of Figure 2. Both methods produced similar results. In this communication only the data obtained by threshold method 1 were presented.

The decrease/increase from base line macule area over time for macules treated with bFGFRP in vehicle or placebo (vehicle), or bFGFRP +NB-UVB or NB-UVB +vehicle were measured by threshold method1 in this communication.

The area of vitiligo macule repigmented expressed in mm square with any treatment method employed here at the end of 4, 8, or 12 weeks of treatment was obtained by subtracting from the base line area of the macule the area of the same macule at the end of 4, 8 or 12 weeks treatment. The macule location varied anywhere from trunk to arms, legs, hands and feet. The results of treatment with NB-UVB + bFGFRP or with NB-UVB+vehicle at all-time points of evaluation were shown in Table 1. Out of 30 volunteers in group 1, the data of volunteer 112 was erratic at some intervals and hence excluded in the data presented in Table 1. The volunteer 102 did not have the 4<sup>th</sup> week data for the macule treated with NBUVB+ peptide. Therefore the data at 4th week is for 28 volunteers and 29 for the rest of the intervals.

Out of 30 patients in this group, Patient 102 did not have data at 4th week and data of patient 112 was erratic at some intervals and so excluded from this evaluation. Hence the number of patients is 28 at 4th week and 29 at other points. For patients 317 and 319 data at week 2 was used as base line. In cases of patients 102, 213, 215, and 302, last observation was carried forward.

The data on the area of macules of all 30 patients included in mITT group repigmented with NB-UVB+ bFGFRP treatment or the corresponding controls at the end of 12 weeks of treatment were shown in Table 2. 26 macules out of 30 of patients (87%) treated with NB-UVB + bFGFRP repigment compared to 18 macules out of 30 of patients (60%) treated with NBUVB+ vehicle (Data not shown). The mean value of repigmentation of the macules or the percentage repigmentation of the mean base line area of the macules treated with NBUVB+ peptide is 2.5 times more than the NBUVB+ vehicle treated macules.

Time from start	4 weeks	4 weeks	8 weeks	8 weeks	12 weeks	12 weeks
Treatment	NBUVB+Peptide in vehicle	NBUVB +Vehicle	NBUVB+Peptide in vehicle	NBUVB+vehicle	NBUVB +Peptide	NBUVB+vehicle
N0. of patients	28	28	29	29	29	29
Mean base line area of macules mm square	962.5	1027.6	954.5	1021.9	954.5	1021.9
Mean area of macules repigmented	75.63 (SD=163.76)	-13 (SD=206.12)	116.5 (SD=216.12)	-0.37 (SD=260.38)	240.27 SD=296.8	90.84 SD=239.29
%base line area repigmented	7.8	-1.28	12.2	-0.03	25.17	8.9
Paired t test 2 tail P value	0.028		0.015		0.031	

**Table 1:** Area of macules repigmented that were treated with NBUVB with or without deca peptide at various time intervals of treatment.

Macules of 23 patients out of 30 patients in mITT group treated with NB-UVB + bFGFRP for 12 weeks repigment more than the corresponding NB-UVB+ vehicle treated macules. The mean value of repigmentation of the macules of 23 patients treated with NBUVB+ peptide is about 5.5 times more than the corresponding NBUVB+ vehicle treated macules while the percentage repigmentation of the mean base line area of the NBUVB+ peptide treated macules is about 5 times more than NBUVB+ vehicle treated macules. These results were presented in Table 3.

Treatment	NBUVB+Peptide in vehicle	NBUVB+vehicle
No. of Macules	30	30
Mean base line area of macules in mm square	996.9	998.8
Mean value of repigmentation	229.11	90.12
Standard deviation of repigmentation (S.D)	298.09	318.54
Median Repigmentation	100.3	59.6
Minimal Repigmentation	-121.63	-1046
Maximal Repigmentation	1047	967
%Repigmentation of mean baseline area	23	9.02
Paired t test 2 tail P value	0.04	

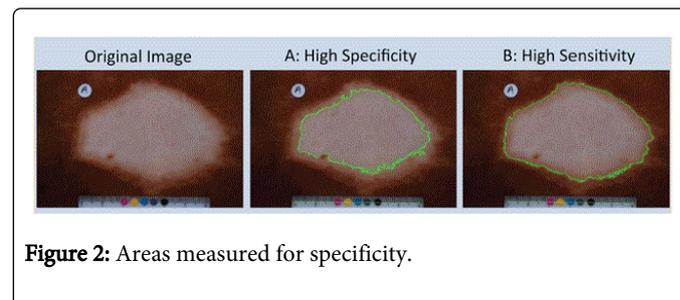
**Table 2:** The data on the area of macules of all 30 patients included in mITT group repigmented with NB-UVB+ bFGFRP treatment or the corresponding controls at the end of 12 weeks of treatment.

Treatment	NBUVB+Peptide in vehicle	NBUVB+vehicle
No of Macules	23	23
Mean base line area of 23 macules mm square	1057.6	956.2
Mean value of repigmentation	286.2	51.33
Standard deviation of repigmentation (S.D)	316.75	347.41
Median repigmentation	195.4	-3.475
Minimal repigmentation	-121.63	-1046.44
Maximal repigmentation	1040.66	967.39
% repigmentation	27.05	5.36
Paired t test two tail P value	0.00333	

**Table 3:** Statistical evaluation of repigmentation of 23 patients who had more repigmentation with NBUVB +peptide compared to NBUVB +vehicle.

Some representative photographs before and after 12 weeks of treatment with NB-UVB+ peptide compared with NBUVB+ vehicle were presented in Figures 3 and 4.

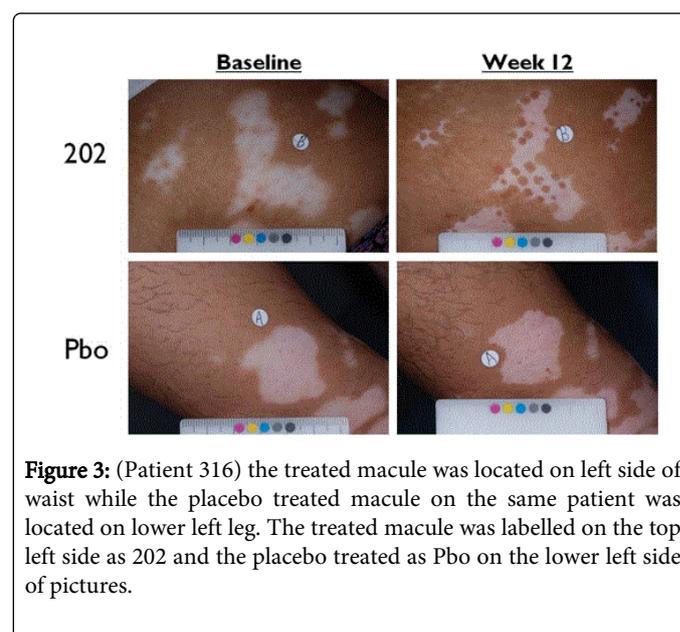
In the Figure 3, the treated macule was located on left side of waist while the placebo treated macule on the same patient was located on lower left leg. In the Figure 4, the treated macule was located on the back right side while the placebo treated macule of the same patient was on the left cubital fossa. In the case of Figure 5, the treated macule was located on the left side waist while the placebo treated macule of the same patient was on the right hip.



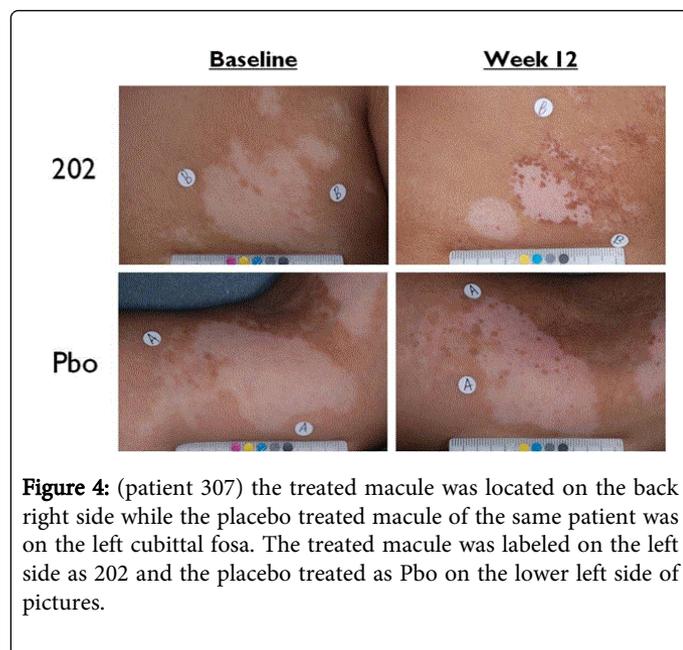
**Figure 2:** Areas measured for specificity.

Treatment Regimen	No of patients with more than 20% Repigmentation of base line area of the macules by 12 weeks of treatment	No of patients with more than 40% repigmentation of base line area of macules by 12 weeks of treatment
Peptide Alone Group (N=32)	5	1
NBUVB +Vehicle Group (N=30)	11	4
NBUVB +Peptide Group (N=30)	15	9
Chi square test at 95% confidence interval 2 tail P value	P=0.039	P=0.03

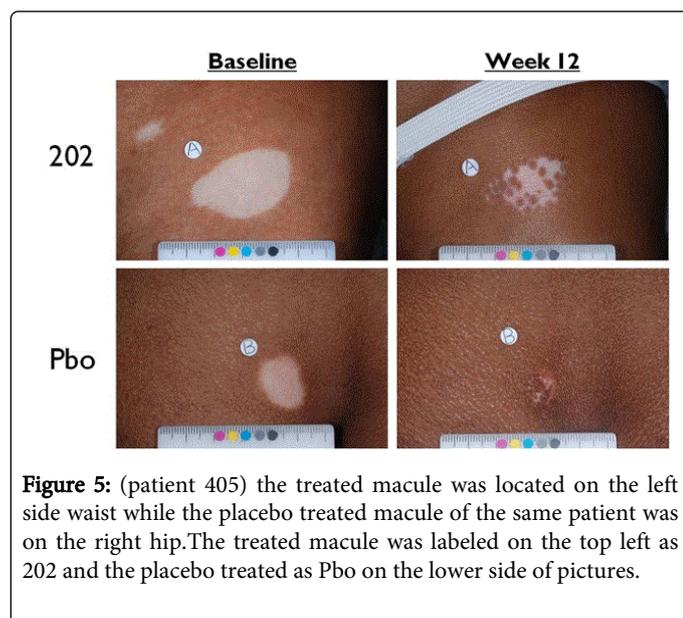
**Table 4:** Chi square test for two group proportions to show synergy between deca peptide and NB-UVB in repigmenting vitiligo macules.



**Figure 3:** (Patient 316) the treated macule was located on left side of waist while the placebo treated macule on the same patient was located on lower left leg. The treated macule was labelled on the top left side as 202 and the placebo treated as Pbo on the lower left side of pictures.



**Figure 4:** (patient 307) the treated macule was located on the back right side while the placebo treated macule of the same patient was on the left cubital fossa. The treated macule was labeled on the left side as 202 and the placebo treated as Pbo on the lower left side of pictures.



**Figure 5:** (patient 405) the treated macule was located on the left side waist while the placebo treated macule of the same patient was on the right hip. The treated macule was labeled on the top left as 202 and the placebo treated as Pbo on the lower side of pictures.

The percentage of the mean base area of macules repigmented with NB-UVB+ vehicle at the end of 12 weeks as shown in Table 2 is 9% compared to 2.6% for the vehicle treated macules but this difference was not statistically significant by the paired t test (data not shown). The mean area of macules repigmented in the group 2 patients treated with peptide in the vehicle was about the same as the mean area repigmented in the controls under present protocol conditions (data not shown) in contrast to earlier observations where the macules treated with peptide about 10-12 hours later were exposed to sun /UV-A produced significant repigmentation [21]. However, peptide in combination with NB-UVB repigmented with statistical significance from the controls (Tables 1-3) indicating synergy between them. The statistical significance of synergistic interaction between peptide and NB-UVB was evaluated by taking in to consideration the individual effects of NB-UVB alone, deca peptide alone and the effects seen in

repigmentation when they were present together as shown in Table 4. The p value was calculated by Chi square test for two group proportions at 95% confidence interval as given by Graph pad software at more than 20% and 40% level of repigmentation.

## Discussion

The repigmented areas of vitiligo macules at various intervals of treatment vary widely both in the group 1 and group 2 treated patients. This is inherent in any treatment of vitiligo mainly because the responses of vitiligo macules to any treatment vary greatly depending on their body location in addition to other unknown factors [25]. For instance it is not clear why 7 macules treated with NBUVB+ bFGFRP repigment similar or worse than the NBUVB treated macules in contrast to 23 macules which repigment more than the NBUVB treated macules with high statistical significance (Table 3). Often the repigmentation is from all sides of the borders and from the expansion of islands of pigment within the macule (Figures 3-5).

This study clearly demonstrated that NBUVB in combination with topical bFGFRP produced statistically significant repigmentation than NBUVB+ vehicle at all-time points (Tables 1 and 2). And that peptide acted synergistically with NBUVB as shown in Table 4. This is in concordance to the synergistic effect of PUVA in combination with bFGFRP therapy [21] our results are in agreement with the present understanding on the mode of action of NBUVB in repigmenting vitiligo macules and the role of growth factors/bFGF [10-13,24-27]. The decrease in macule size due to repigmentation was seen maximally between 8th and 12th week in both NBUVB plus decapeptide and with NBUVB treated volunteers (Table 1). If the trial was allowed beyond 3 months, the difference between the two groups was likely to persist based on the observation of Puri et al. that treatment of vitiligo macules with PUVA in combination with bFGFRP for a period of 6-8 months resulted in 100% more marked repigmentation compared to PUVA alone [28-31].

It is possible that a more dramatic outcome would have emerged even within 12 weeks of treatment if the protocol involved topical application of bFGFRP and exposure to sun on the other 3 days of the week when no NBUVB exposure to macule was done. It is because it was observed in the present studies that topical application of bFGFRP was ineffective by itself to repigment vitiligo macules in absence of exposure to sun. Thus in effect the results obtained with topical application of bFGFRP in combination with NBUVB should be taken to be the results of topical application of bFGFRP only three times a week rather than 6 times as the protocol indicates since the other three applications of bFGFRP without sun exposure could not have resulted with any repigmentation as shown in Group 2.

Various theories were proposed to explain the patho-mechanisms of vitiligo [1,27]. Repigmentation of vitiligo macules should involve not only elimination/reduction of the negative effectors on melanocytes as often emphasized, but also growth factors/ mitogens for the melanocytes for their multiplication and migration [10-13,32-35]. Wu et al. identified that serum levels of growth factors namely bFGF, Stem Cell Growth Factor, Hepatocyte Growth Factor and ET-1 were raised after successful PUVA therapy [32]. The bFGF level in the serum was shown to increase by three folds compared to the untreated vitiligo subjects after PUVA [32]. It was hypothesized in 1989 that increase in growth factor(s) in serum of successfully repigmenting vitiligo patients after PUVA therapy was perhaps the mechanism of action of PUVA in repigmenting vitiligo macules by

correcting the growth defects in the melanocytes in untreated vitiligo subjects [11]. The external application of peptides mitogenic to melanocytes as for instance bFGRP which was shown to be partially agonist peptide of bFGF [28] thus further augment the internally elevated mitogens to repigment faster when administered in combination with UVB or PUV-A or with helium laser [26,36,37]. No serious adverse side effects of the regimens were reported during the trial. No adverse events leading to early withdrawal were reported during the study.

## Conclusion

The study indicated that NBUVB in combination with bFGFRP was safe and repigment vitiligo macules faster than NBUVB alone and that they act synergistically. The deca peptide in absence of sun exposure of vitiligo macule after its application may not be as effective to repigment vitiligo macules as with sun exposure.

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