Maintenance Therapy of Adult Vitiligo with 0.1% Tacrolimus Ointment: A Randomized, Double Blind, Placebo–Controlled Study

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The risk of relapse after successful repigmentation in vitiligo is estimated to 40% within the first year. It has been shown in atopic dermatitis that continuous low-level use of topical corticosteroids and calcineurin inhibitors in previously affected skin can prevent new flares. We hypothesized that a twice-weekly application of 0.1% tacrolimus ointment might be effective for maintaining repigmentation in therapeutically repigmented lesions of vitiligo patients. After randomization, sixteen patients with 31 patches were assigned to the placebo group and 19 patients with 41 patches were assigned to the tacrolimus group. In the intention-to-treat analysis, 48.4% of lesions showed depigmentation in the placebo group, whereas 26.8% did in the tacrolimus group ($P=0.059$). The intention-to-treat results did not remain significant after adjustment for within-patient clustering, odds ratio (OR) 2.55; 95% confidence interval (CI; 0.65–9.97); $P=0.1765$. The per-protocol analysis ($n=56$) showed that 40% of lesions had some depigmentation in the placebo group, whereas only 9.7% did in the tacrolimus group ($P=0.0075$). The per-protocol results remained significant after adjustment for within-patient clustering: OR 6.22; 95% CI (1.48–26.12); $P=0.0299$. Our study shows that twice-weekly application of 0.1% tacrolimus ointment is effective in preventing the depigmentation of vitiligo patches that have been previously successfully repigmented.

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INTRODUCTION

Vitiligo is an acquired depigmentation of the skin and sometimes hair follicle affecting 0.5% to 1% of the world's population. The pathophysiology is complex and involves several possible cellular mechanisms. There is now strong evidence for a role of both oxidative stress and immune systems in genetically predisposed individuals (Passeron and Ortonne, 2012). Although there is no cure for vitiligo, treatments such as excimer light, topical tacrolimus or pimecrolimus, and combination approaches (with phototherapy and topical steroids or calcineurin inhibitors) can provide cosmetically acceptable repigmentation (>75%), in particular on the face and neck (Ostovari *et al.*, 2004; Passeron *et al.*, 2004; Whitton *et al.*, 2010; Felsten *et al.*, 2011; Taieb *et al.*, 2013; Ezzedine *et al.*). However, one of the major concerns in patients who achieve repigmentation of vitiligo lesions is the risk of relapse estimated to be almost 40% within the first year after stopping treatment (Nicolaidou *et al.*, 2007). To date, there is no treatment to prevent relapses. Of note, the chronic and unpredictable course of the disease has a strong impact on the quality of life of affected individuals (Parsad *et al.*, 2003). It has been shown in atopic dermatitis that between flares the skin has a subclinical inflammatory infiltrate. Proactive treatment consisting of continuous low-level use of topical anti-inflammatory agents (topical steroids or topical calcineurin inhibitors) to previously affected skin areas has shown to be effective for preventing new flares of atopic dermatitis (Schmitt *et al.*, 2011). We therefore hypothesized that a twice-weekly application of 0.1% tacrolimus ointment might be effective for maintaining repigmentation and preventing relapses of previously repigmented lesions in vitiligo patients.

RESULTS

A total of 78 consecutive patients from the clinics of vitiligo of Bordeaux and Nice university hospitals, who received treatment for vitiligo, were assessed for eligibility. Of these, 37 came back for routine follow-up visit with a repigmentation rate of >75% in at least one lesion. For each patient, one to

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Abbreviations: CI, confidence interval; ITT, intention-to-treat; OR, odds ratio; PGA, Physician Global Assessment

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four lesions had successfully repigmented. As two patients denied participating in this maintenance therapy study, 35 patients with 72 lesions were finally enrolled. Sixteen patients with 31 patches were assigned to undergo placebo and 19 with 41 patches were assigned to undergo topical tacrolimus. Five patients were lost to follow-up. The flow diagram of the study is summarized in Figure 1, and localizations of the lesions and the treatments that allowed the initial repigmentation rate of more than 75% are presented in Figure 2. Characteristics of patients and clinical data about their vitiligo were comparable at baseline. They are presented in Table 1 and Supplementary Table 1 online.

In the intention-to-treat (ITT) analysis ($n=72$), 48.4% of lesions showed some depigmentation in the placebo group, whereas 26.8% did in the tacrolimus group ($P=0.059$). In per-protocol analysis ($n=56$), we observed that 40.0% of lesions showed some depigmentation in the placebo group, whereas only 9.7% did in the tacrolimus group ($P=0.0075$; Figure 3). A depigmentation superior to 50% was observed in 12% of the lesions in the placebo group and 0% in the tacrolimus group (Figure 3).

Accounting for within-patient clustering in generalized estimation equations models resulted in the ITT analysis, in a nonsignificant association, OR 2.55; 95% CI (0.65–9.97); $P=0.1765$, whereas in per-protocol analysis tacrolimus maintenance therapy was significantly associated with lesions pigmentation, OR 6.22; 95% CI (1.48–26.12); $P=0.0299$, consistently with previous results.

The localizations of treated lesions in the placebo group are face and neck 86% and rest of the body 14%. In the face and neck group, 40% showed a depigmentation and 60% no depigmentation. In the rest of the body group, 46% showed a depigmentation and 54% no depigmentation. The localizations of treated lesions in the tacrolimus group are face and neck 73% and rest of the body 27%. In the face and neck group, 4% showed a depigmentation and 96% no depigmentation. In the rest of the body group, 22% showed a depigmentation and 78% no depigmentation (Supplementary Figure 1A online). This clearly shows the benefits of a twice-weekly tacrolimus maintenance therapy, especially in face/neck areas but also on the rest of the body. The depigmentation rate according to lesions that were initially repigmented following tacrolimus treatment to those that repigmented with any other treatment is reported in Supplementary Figure 1B online. Further adjustment of the association between depigmentation and treatment use on these parameters, lesion localization, and treatment modality used to repigment the lesion did not change the results of the univariate models,OR 2.59; 95% CI (0.62–10.79), $P=0.1909$ and OR 6.84, 95% CI (1.23–38.10), $P=0.028$, respectively, in the ITT and per-protocol analyses.

The Physician Global Assessment (PGA) score showed, in the placebo and tacrolimus groups, respectively, depigmentation in 48.2% versus 10.4%, no change in 40.7% versuss 58.6%, and repigmentation in 11.1% versus 31% of the lesions ($P=0.0053$).

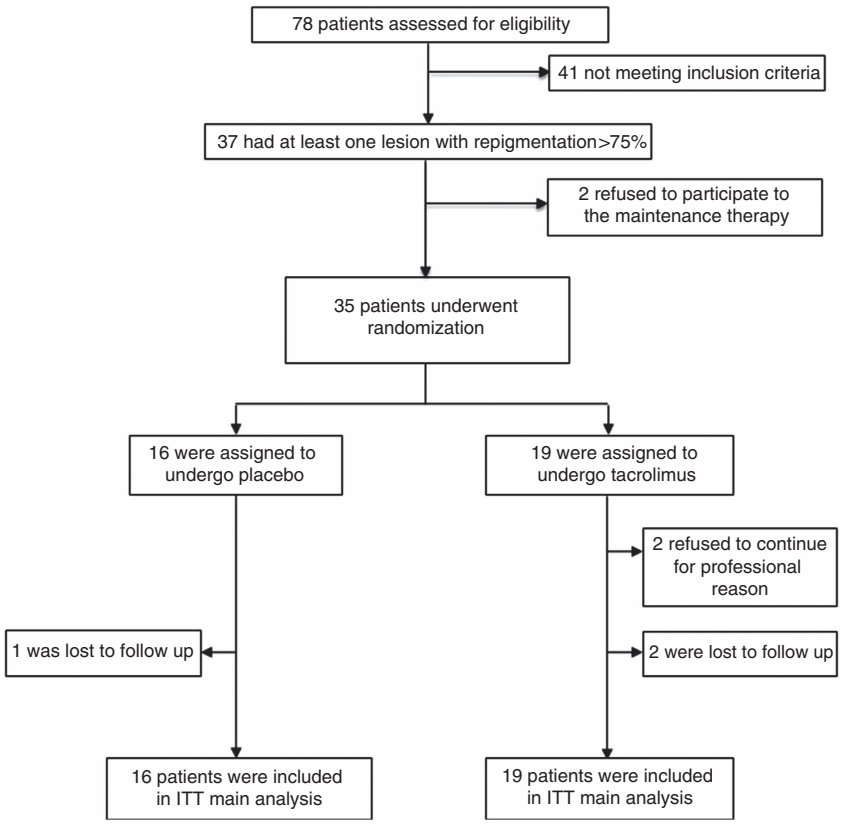

Figure 1. Flow diagram of the study.

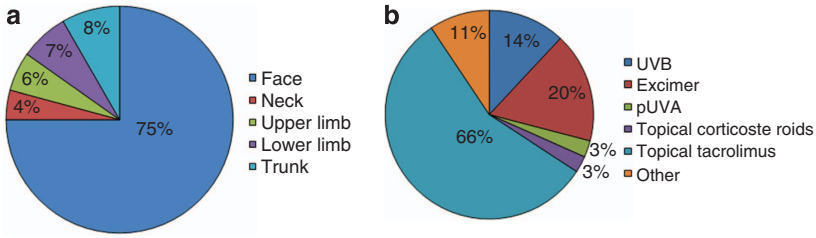

Figure 2. Localization of the lesions and therapies that allow their repigmentation. (**a**) Description of treated areas. (**b**) Treatments that allowed the repigmentation before the patients entered the study.

Table 1. Characteristics of the population

	Placebo group ($n=16$)		Tacrolimus 0.1% group ($n=19$)	
	n	**%**	***n***	**%**
Sex	16		19	
Male	10	62.5	11	57.9
Female	6	37.5	8	42.1
Age (years) median (interquartile range)	16	43.0 (38.0–46.5)	19	44.0 (33.0–52.0)
Skin type				
II	0	0.0	3	15.8
III	11	68.8	13	68.4
IV	4	25.0	2	10.5
V	1	6.2	0	0.0
VI	0	0.0	1	5.3
Age at diagnosis	16	32.8 (17.0–40.8)	19	32.4 (25.2–43.2)
Previous repigmentation episodes, n *(%)*	10	62.5	10	52.6
Under treatment	4	40.0	7	70.0
After sun exposure	6	60.0	3	30.0
Type of repigmentation				
Marginal	6	37.5	6	31.6
Perifollicular	7	43.8	7	36.8
Both	3	18.7	6	31.6
Koebner's phenomenon	6	37.5	6	31.6
Halo nevi	0	0.0	4	21.1
Vitiligo activity, n *(%)*				
Active vitiligo	7	43.8	6	31.6
Stable vitiligo	9	56.2	13	68.4
Season of inclusion, n *(%)*				
Winter	14	87.5	16	84.2
Summer	2	12.5	3	15.8
Associated auto-immune disorder, *n* (%)	2	12.5	4	21.0

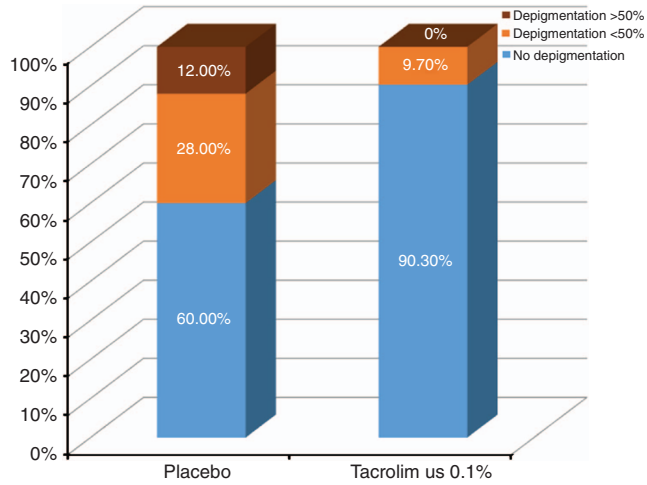

Figure 3. Depigmentation category.

The quality of life slightly improved in both groups from 4.79 (± 3.58) to 3.54 (±2.91) in the tacrolimus group versus 6.48 (±2.80) to 4.59 (±3.53) in the placebo group, $P=0.6112$. Four patients (three in the tacrolimus group and one in the placebo group) reported side effects with mild and transient erythema, stinging and burning sensations that did not require any specific intervention. In all cases, the treatment could be continued.

DISCUSSION

Our results show that a maintenance treatment using twice-weekly applications of tacrolimus 0.1% can reduce the recurrences of previously repigmented vitiligo lesions. In atopic dermatitis, both topical steroids and tacrolimus ointment were shown to be effective for preventing the relapses, but a meta-analysis suggests that topical steroids might be the more effective (Schmitt *et al.*, 2011). In vitiligo, topical steroids and tacrolimus ointment were shown to be as effective for repigmenting the lesions, but side effects were fewer with tacrolimus, especially on facial lesions (Lepe *et al.*, 2003). We thus decided to use tacrolimus for this study. However, it might be hypothesized that twice-weekly applications of topical steroids could also be effective for the maintenance therapy in vitiligo. The low frequencies of the applications and the absence of the need for concomitant sun or UV exposure facilitated a good compliance in both groups. In atopic dermatitis, some authors proposed 2 or 3 consecutive days for convenience of the patients so they can do a 'week-end' therapy. The rationale of using tacrolimus twice a week is to inhibit the low-grade immune reaction against melanocytes, and to this respect it sounds more relevant to us to apply the

treatment at more regular intervals. However, we cannot assess whether this treatment schedule is optimal or not. Further studies will have to determine whether other protocols would be able to achieve even better results.

A limitation of this study is the number of patients lost to follow-up. Unfortunately, four out of the five patients lost to follow-up were in the tacrolimus group, and thus this had a strong impact on the ITT results as the imputation performed was considered a failure in the treatment of all lesions of patients lost to follow-up. However, two patients in the tacrolimus group withdrew from the study for professional reasons and not for reasons linked to the treatment arm, minimizing the risk of bias induced by study withdrawal specifically associated with a treatment regimen. Nevertheless, even with such an imputation, in the ITT analysis three-fourths of the lesions remained stable or continued to repigment in the tacrolimus group as compared with only 52% in the placebo group.

The results showed no significant variation in the Dermatology Life Quality Index in both groups. This is explained by the fact that even though their treated lesions did not recur, most of the patients still have several other stable vitiligo lesions that continue to alter their quality of life.

A potential bias could have resulted from the differences between groups in terms of vitiligo activity and season of inclusion (with a potential beneficial action of sun exposure). However, the two groups were comparable at baseline for these parameters. Localization of the lesions has been shown for affecting the response to repigmenting therapies in vitiligo (Ostovari *et al.*, 2004; Passeron *et al.*, 2004). We did not include very resistant areas such as hands and feet in either the placebo or the tacrolimus group. However, face and neck are known usually to have the best response rate. It is confirmed here as more than three out of four of the lesions that were initially repigmented, and thus included in the study, were located on these localizations. Also, maintenance therapy appeared to be more effective to maintain pigmentation on face and neck as compared with the rest of the body (96% and 78% of the lesions treated, respectively). Hence, one could have thought that a potential limitation arose from the fact that the modality of the treatment that allowed for initial repigmentation (i.e, tacrolimus or not) may have influenced the response to the tacrolimus maintenance therapy. Hence, as these parameters were potential important ones to account for, in despite of randomization, we adjusted our analyses on these variables. We observed no association of these parameters (localization and treatment that allowed the repigmentation) with the pigmentation outcome making very unlikely their impact on our results. Thus, as the success rate of maintenance therapy is independent of the modality of the initial treatment, clinicians may choose to use this maintenance therapy regardless of whether topical tacrolimus, topical steroids, or phototherapy was used for initial treatment. However, further studies would be warranted to determine formally whether localization influences or not the efficacy of the maintenance therapy.

The relapse rate of 40% observed in the present study is consistent with that reported in the literature (Nicolaidou *et al.*, 2007). Although the aim of the study was the maintenance of the repigmentation, we observed that one-third of the lesions completed their repigmentation under twice-weekly tacrolimus maintenance treatment, as the PGA score shows. This suggests that tacrolimus 0.1% applied twice weekly can achieve ongoing repigmentation in addition to prevent the depigmentation. Interestingly, we observed that four patients who completed the study and who did not have recurrences during the study period had depigmentation within the six months following the end of their participation. After the unblinding procedure, we noticed that all of these four patients belonged to the tacrolimus group. This is in favor of the effectiveness of tacrolimus for preventing depigmentation as its discontinuation resulted, at least in some patients, in a depigmentation.

Taken together, our data show that twice-weekly application of 0.1% tacrolimus ointment is effective in preventing the depigmentation of vitiligo patches that have been previously successfully repigmented. Taking into account the data gathered for atopic dermatitis, the effectiveness of such a maintenance approach in vitiligo suggests that a low-grade of inflammation might also be observed in vitiligo lesions and be responsible for the recurrences. This hypothesis is supported by transcriptional analysis of non-depigmented skin of vitiligo patients that also shows low-level of inflammation in these areas compared with healthy controls (personal data, manuscript in preparation).

These results have a strong implication in terms of daily care of vitiligo patients. Further studies should determine the optimal topical therapy and schedule of applications along with the duration of such a maintenance treatment.

MATERIALS AND METHODS

Type of study

We conducted a bi-centric prospective randomized double-blind placebo-controlled study. Prior to any procedures, the investigation was approved by the local ethics committee and was registered at the US National Institutes of Health Clinical Trial Register (NCT01841008). Patients provided written informed consent before randomization.

Settings

Departments of Dermatology of Nice and Bordeaux University Hospitals between December 2011 and October 2013.

Patients

We recruited in a consecutive manner, patients with vitiligo for whom lesions had been successfully treated (at least 75% of repigmentation). Exclusion criteria were segmental vitiligo, spontaneous repigmentation, and contraindication to the use of topical tacrolimus. For each patient, all the lesions that achieved at least 75% of repigmentation were included.

Randomization

Patients were randomly assigned, in a 1:1 ratio, to either tacrolimus ointment 0.1% or placebo (centralized randomization at methodological center). All the patient' lesions included in the study were treated accordingly to the patient randomization arm.

Interventions

After repigmentation, patients were immediately (or within a maximum of 2 weeks) transitioned to the maintenance protocol. Patients had to apply twice weekly, in non-consecutive days, the ointment on the repigmented areas over 24 weeks. If a patient has more than one lesion to treat, all his lesions received the same product. We choose to randomize by patient and not by lesions to be able to assess the effect on the quality of life at the end of the study and to avoid any confusion or misuse by the patient during the 6-month study duration.

Evaluation

The efficacy of the treatment was evaluated on standardized pictures with direct light and Wood lamp by two physicians blinded to treatment received. They made a comparative evaluation of the previously repigmented areas. The main criteria of evaluation was the level of depigmentation assessed in three categories (0: no depigmentation compared with the initial picture; 1: $<50\%$ depigmentation compared with the initial picture; 2: $>50\%$ depigmentation compared with the initial picture). In addition, PGA assessed the evolution of vitiligo between initial and final pictures in three categories: stable; repigmentation, and depigmentation. For depigmentation assessment and PGA, in case of discordance, a third evaluation blinded to previous evaluations was asked to perform the definitive evaluation. The quality of life was assessed using the Dermatology Life Quality Index score. Evaluation was performed at maintenance therapy initiation and at M6 (end of the study). Data were collected using a standardized questionnaire, followed by a complete clinical examination. Data on age, sex, skin type, age at the diagnosis of vitiligo, family history of vitiligo, associated auto-immune disorders including thyroiditis, Koebner phenomenon, episodes of repigmentation in the past, localizations treated, treatment that allowed to achieved the repigmentation, and activity of the vitiligo were collected. The vitiligo was considered as active if there were modifications of lesions or appearance of new ones in the past 3 months.

Sample size calculation

On the basis of the literature, we anticipated a depigmentation rate of 40% in the placebo group (Nicolaidou *et al.*, 2007). Assuming an expected depigmentation rate of 10% in the tacrolimus group, with a 80% power and 5% type I error, we calculated that 23 lesions by arm were needed. Taking into account 10% of lost to follow-up, we decided to include at least 52 lesions. According to the design of our study, all the patches of a patient were treated in the same arm. We assumed that, in a low hypothesis, around one-third of the selected patients would present two lesions that achieved 75% of repigmentation, and hence would be eligible for the study, and the others one lesion. We then estimated the number of patients to include around 36 (21 patients with 1 lesion and 15 patients with 2 lesions, resulting in a total of 52 lesions).

Statistical analyses

Analysis of the primary objective was performed on the ITT principle in all patients who underwent randomization. Missing values were imputed as failure to the treatment considering lost to follow-up patients as having had relapse of their vitiligo. In the analyses, the principal outcome, depigmentation, was considered as binary, regardless of the percentage. A per-protocol analysis was also performed considering only patients who completed the entire study without violation to the protocol.

First, we compared the rate of depigmentation between treatment groups, with the lesion as the statistical unit, using the χ^2 test. Then, to account for within-patient clustering, we reanalyzed the data using generalized estimation equations logistic models. Additional adjustment of these models on the localizations treated (head and neck vs. others) and the treatment that allowed to achieve the repigmentation (tacrolimus yes/no) was performed.

We used the χ^2 test to compare PGA score between the groups and an analysis of covariance to study the quality of life allowing adjusting the analysis on the baseline value of the Dermatology Life Quality Index score.

Two-sided *P*-values <0.05 were considered statistically significant. Statistical analyses were performed using SAS version 9.1 software (SAS institute, Cary, NC).

CONFLICT OF INTEREST

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Disclaimer

The sponsor had no role in the collection, interpretation and analysis of the data, and in the drafting or the revision of the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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