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ORIGINAL ARTICLE

Effects of autologous micrografts on stable bilateral vitiligo: A focus on hand lesions

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ABSTRACT

Vitiligo is an autoimmune skin disorder characterized by depigmented patches of the skin associated with, among several factors, dysregulation and death of melanocytes. Currently, the treatment of vitiligo is based both on the arrest of the progression of active disease and on the stimulation of the skin repigmentation. The aim of this study was to assess the effects of autologous micrografts and narrowband ultraviolet B (NBUVB) phototherapy for skin repigmentation of patients with bilateral stable vitiligo. Autologous micrografts are derived from mechanical disaggregation of small pieces of the patient's own skin, while phototherapy is a strategy treatment already used. Twenty patients with stable bilateral vitiligo were treated, showing a mean percentage rate of 59.1% at baseline. Combined treatment by autologous micrografts and NBUVB was performed only on the lesions of the hands, and the clinical follow up was performed after 3 and 6 months by photographs taken under Wood's light. After 6 months, we classed 100% of patients as responders. We also reported a mean of repigmentation rate of 36.7% after 3 months and 64.6% after 6 months of treatment. In particular, six of the 20 patients reached a marked repigmentation rate (75–100%), four moderate (51–75%) and 10 mild (26–50%). No adverse effects were observed and no drugs were administrated as co-adjuvant therapy. These results are suggestive of a potential wide use of autologous micrografts associated with NBUVB phototherapy for the treatment of stable vitiligo.

Key words: autologous micrografts, hand lesions, narrowband ultraviolet B phototherapy, skin repigmentation, vitiligo.

INTRODUCTION

Vitiligo is a skin disorder characterized by depigmented patches of skin and, among dermal diseases, one of the most difficult to cure.[1] The pathogenesis of vitiligo is not exactly known and it is considered to be correlated with genetic and immunity failures, or even their combination. Despite not being life-threatening, patients have to deal with psychological discomfort, often leading to additional mental disorders.[1]

Usually, it is possible to distinguish two main types of vitiligo: segmental pattern vitiligo involving less than 10% body surface area (BSA), and generalized vitiligo, which typically involves 10% or more BSA. Commonly, the first type is considered stable, while the second form appears bilaterally with a symmetrical distribution and follows a relapsing and remitting disease course.[2] To date, the treatment of vitiligo is related to clinical classification/characteristics of the disease and usually based on two different strategies. The first strategy aims to arrest the progression of active disease, thus limiting the area involved by depigmentation, while the second aims to induce repigmentation of the injured area.[1] Some conventional therapies, including medical and surgical approaches, or unconventional therapies, such as phototherapy, are used in the management of this pathological condition.[3–5] Additionally, many tissue grafting techniques are available, but suction blister epidermal grafting and mini-punch grafting are the most widely utilized.[6]

In recent years, the Rigenera® micrografting technology (Human Brain Wave, Turin, Italy) has been developed, by using the Rigeneracons® approved medical device, a new clinical approach to obtain autologous micrografts from mechanical disaggregation of small fragments of the patient's own tissue. This medical device is already used for several different clinical applications, such as plastic surgery, oral surgery and dermatology, showing successful dermal, bone and cartilage regeneration.[7–12] In particular, regarding the dermatological applications, previous studies reported that micrografts are effective in the treatment of androgenetic alopecia and hypertrophic scars[13–15].

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Considering the clinical and preclinical evidence already reported in the published work for many years, we assessed the role of autologous micrografts and narrowband ultraviolet B (NBUVB) phototherapy in skin repigmentation treatment of patients affected by stable vitiligo. Clinical follow up was performed between baseline and 3 and 6 months post-treatment.

METHODS

All patients gave informed consent before participating in the study. We treated 20 patients (female : male ratio, 17:3, mean age, 37.6 ± 12.8 and 37 ± 9 years, respectively) affected by bilateral stable vitiligo with a mean percentage rate at baseline of 59.1%. Inclusion criteria were the following: bilateral vitiligo for at least 5 years and stable for at least 2 years; no use of drugs or other therapies for at least 6 months; adult patients; and female patients who were not pregnant.

In all of these patients, we only treated the lesions on the hands because the other skin surfaces normally show a good response to conventional treatments, while (as commonly known) the response of the hands and feet is often slow and poor.

Autologous micrografts and NBUVB phototherapy

The first step to obtain the autologous micrografts suspension was the collection of four pieces of dermal tissue from the mastoid hairy area containing hair follicles through a biopsy punch of 4-mm diameter. Before collecting the samples, the hair and the stratum corneum of the epidermis were removed with a scalpel by gentle scaping (Fig. 1a). The pieces of tissue were inserted entirely into a Rigeneracons® medial device (Human Brain Wave), which mechanically disaggregates the samples with 600 microblades (Fig. 1b). To do so, the Rigeneracons was preloaded with 1.5 mL sterile saline solution and the samples processed for 2 min through the activation of the Rigenera machine which enables the rotation of the 600 microblades and subsequent disaggregation of the sample. Finally, the micrografts suspension was collected with a syringe and immediately injected into the dermis of the depigmented hand area with a volume of 0.1 mL/cm^2 (Fig. 1c). One week after the micrograft application, the patients underwent NBUVB phototherapy three times a week for a total of 6 months, while the micrografts treatment was performed only once.

The clinical follow up was performed 3 and 6 months after micrografts application and NBUVB phototherapy by photographs taken under Wood's light. Repigmentation was graded based on a quartile scale with at least mild (≥25% repigmentation), moderate (≥50% repigmentation) and marked (≥75% repigmentation) responses. The degree of repigmentation was evaluated based on the hand lesions in each participant.

Statistical analysis

Data are expressed as mean ± standard deviation. Statistical significance was set at $P \leq 0.05$ and calculated by GraphPad Prism software (GraphPad Software, La Jolla, CA, USA).

RESULTS

Surprisingly, after 6 months from the micrografts application and NBUVB treatment, 100% of patients were classed as responders, meaning that all patients had reached at least 25% repigmentation rate. As showed in Fig. 2, we reported a mean percentage of vitiligo area of 59.1% at baseline, 42.1% after 3 months and 27.7% after 6 months of treatment (reduction of 28.8% and 53.1%, respectively; $P \leq 0.05$ and 0.01). Furthermore, we reported a mean of repigmentation rate of 36.7% after 3 months and 64.6% after 6 months of treatment ($P \leq 0.001$) (Fig. 2). No adverse effects were observed and no drugs were administrated as co-adjuvant therapy. The results in terms of repigmentation rate and degree are summarized in Table 1.

Figures 3–5 and Figure S1 provide images of the clinical outcomes for all patients treated with micrografts and NBUVB in this study, classed as marked (≥75% repigmentation rate; Fig. 3), moderate (≥50% repigmentation rate; Fig. 4) and mild (≥25% repigmentation rate; Fig. 4, and Fig. S1).

Figure 1. Procedure of micrografts collection. (a) A dermal punch is used to collect a small sample of autologous tissue from the patient. (b) The autologous tissue is disaggregated in the disposable device to generate micrografts. (c) The micrografts suspension is injected directly into the depigmented area of the patient.

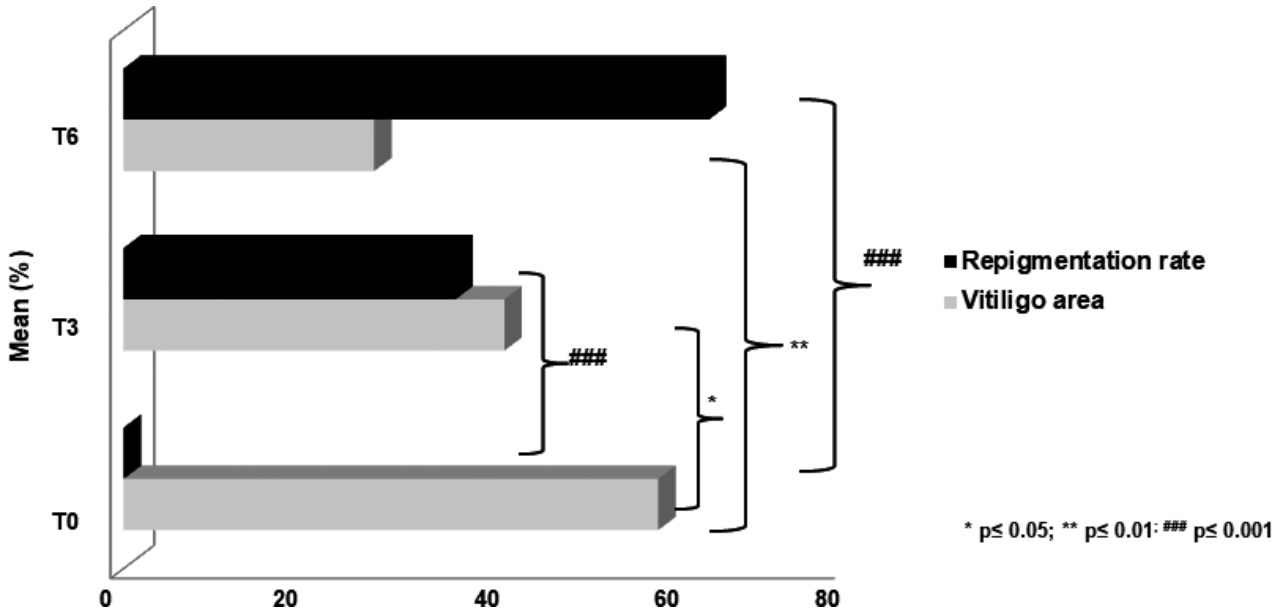

Figure 2. Mean percentage of vitiligo area (gray) and repigmentation rate (black) of the hands at baseline and after 3 and 6 months of treatment with autologous micrografts and narrowband ultraviolet B phototherapy. The data are expressed as mean of percentage. *P*-values were calculated by Student's *t*-test. $*P \leq 0.05$; $**P \leq 0.01$; $^{###}P \leq 0.001$.

Table 1. Summary of hand repigmentation rates

Group of patients	% of repigmentation rate	Repigmentation degree
6/20 (30%)	75–100%	Marked
4/20 (20%)	51–75%	Moderate
10/20 (50%)	26–50%	Mild

Mean percentage of repigmentation rates for patient groups is based on individual clinical response.

DISCUSSION

In this study, for the first time, we provide evidence that the combination of autologous micrografts (mechanically obtained) and NBUVB phototherapy result in a good clinical outcome, showing a significant repigmentation rate after 3 and 6 months of treatment, in patients affected by bilateral stable vitiligo of the hands.

Phototherapy has been the principal treatment for vitiligo for decades and includes psoralen and ultraviolet A (PUVA) and NBUVB phototherapy.[6,16] Although PUVA is an effective therapy, it has several limitations, including phototoxic effects, nausea and a potential risk of skin cancer. On the contrary, NBUVB phototherapy has gradually taken the place of PUVA phototherapy due to the lack of a photosensitizer, lower cumulative dose and fewer adverse effects, and is now considered the standard therapy for generalized vitiligo.[6,16] NBUVB phototherapy was reported to be highly effective in restoring pigmentation in patients affected by vitiligo, showing no side-effects,[17] and a recent meta-analysis confirmed that a long duration of phototherapy should be encouraged to enhance the treatment response in vitiligo, mainly for the face and neck where a greater response is commonly observed.[16]

The repigmentation of extremities is very difficult, and from the published work data it is evident that only 17.3% patients are responders to NBUVB on the extremities, while the treatment responses on the hands and feet were extremely low, and a mild response was observed in only 11.0% of patients.[16]

To confirm this, a more recent study has reported that the face–neck show the best effect and hands–feet show poor effect when also using a filiform fire needle assisted by 308-nm excimer laser therapy.[18]

In a more recent study, the transplantation of melanocytes derived from human hair follicles in patients with stable vitiligo showed skin repigmentation within 8 weeks, even if early skin repigmentation was not uniform,[19] possibly due to the use of enzyme-assisted disaggregation of the tissue which made this approach unsafe in clinical practice.

Another approach commonly used by dermatologists is the autologous non-cultured melanocyte–keratinocyte transplantation procedure (MKTP), a grafting technique which offers a 50–100% repigmentation rate with 1:3 up to 1:10 donor : recipient ratio and very good color matching in most of the treated cases;[20–22] however, the limiting step is the need for a wider tissue sample to treat the depigmented area.

The autologous micrografts approach, challenged in this stusy, is based on the same concepts of the melanocytes derived from human hair follicles and MKTP procedures; however, it has several advantages. Different to the clinical procedures involving melanocytes derived from the hair follicle, the use of the Rigeneracons device does not require any enzymes (e.g. trypsin), making its use clinically safer.

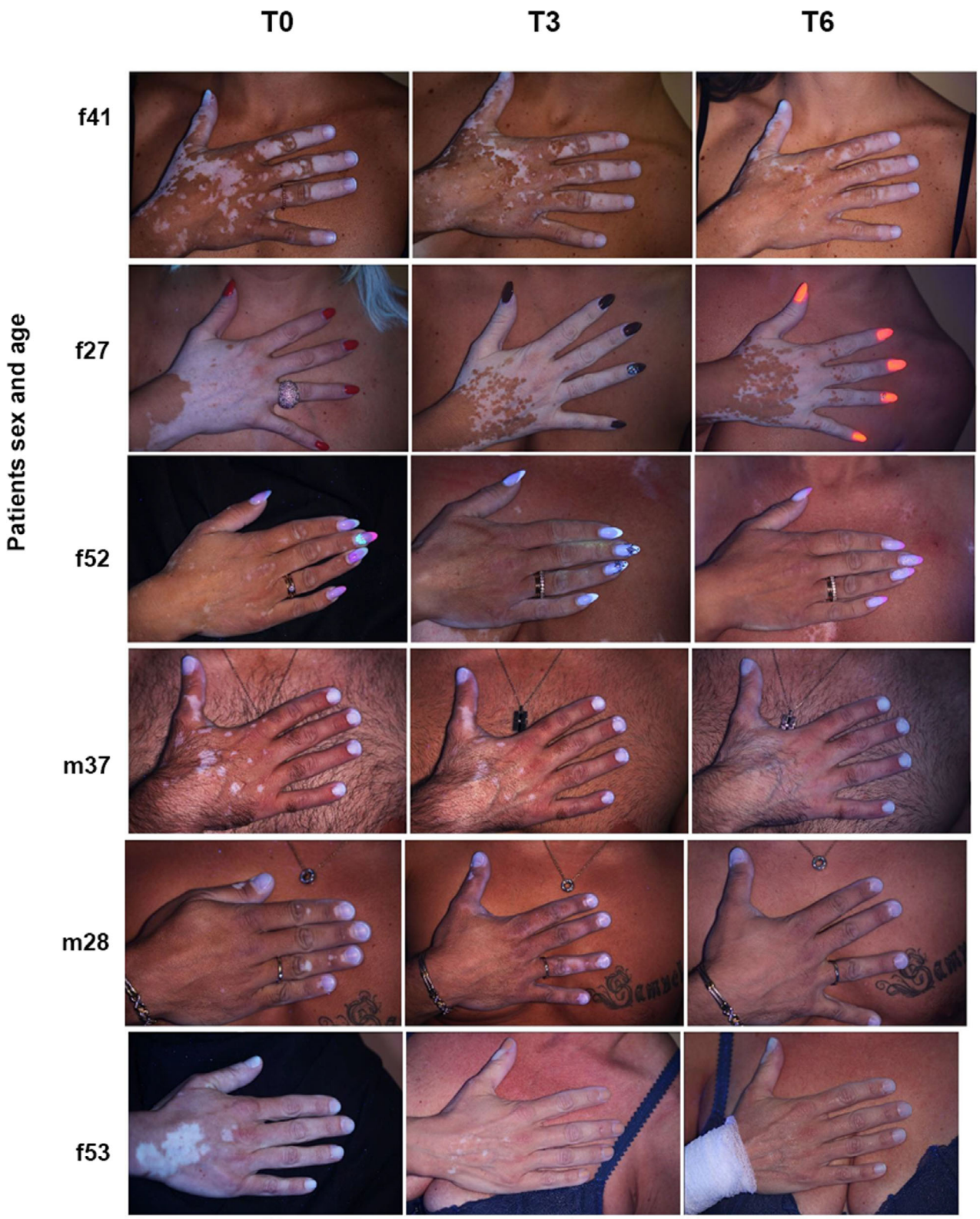

Figure 3. Marked repigmentation rate is evident between baseline and 3 and 6 months of treatment with autologous micrografts and narrowband ultraviolet B phototherapy.

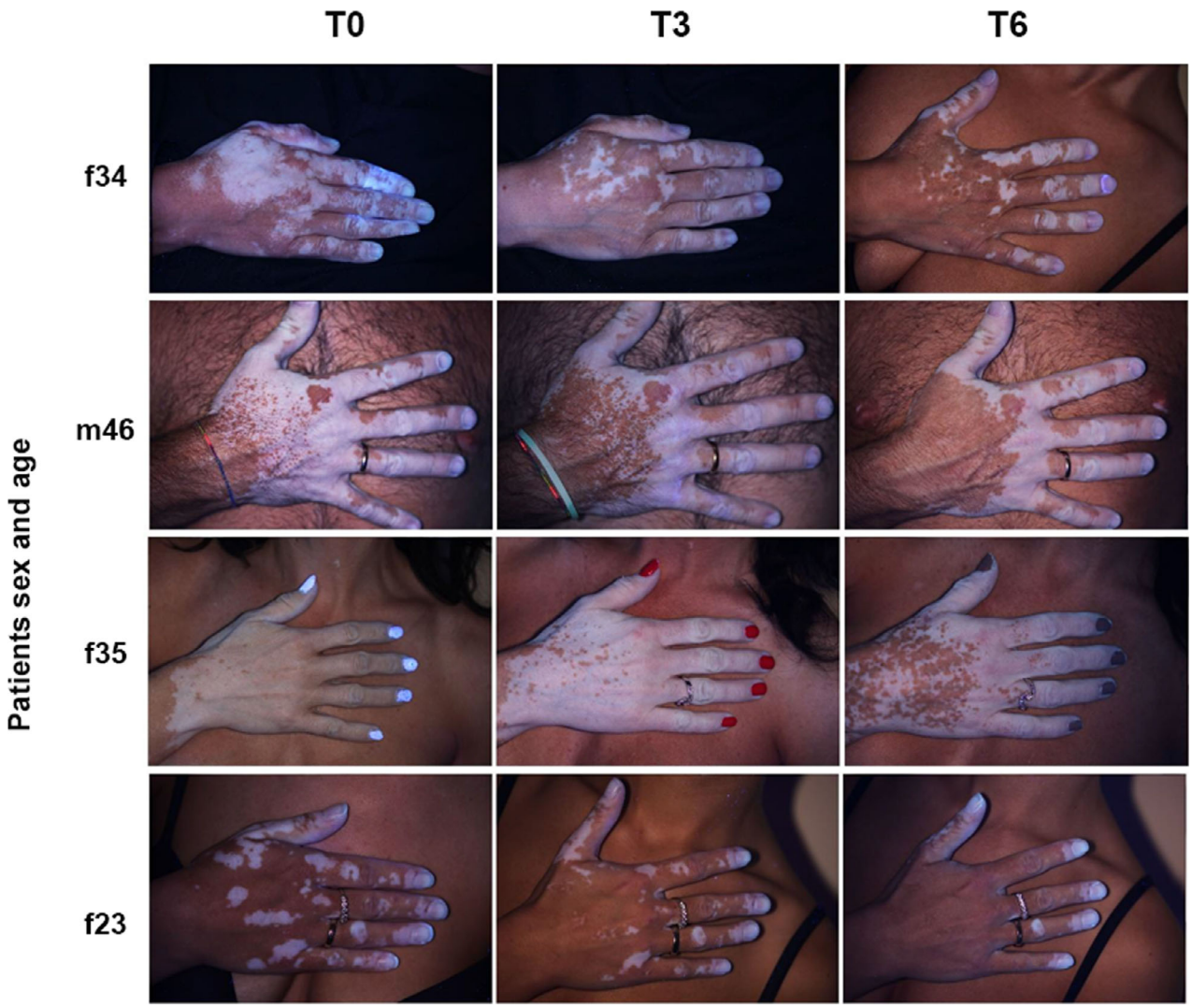

Figure 4. Moderate repigmentation rate is evident between baseline and 3 and 6 months of treatment with autologous micrografts and narrowband ultraviolet B phototherapy.

For MKTP procedures, there is a technical limit: the more the tissue is expanded, the more the cells inside die from mechanical damage, which is why the highest ratio achievable is 1:10. The Rigenera device allows disaggregation of the tissue to a much smaller dimension (80 μm) while assuring high cellular viability;[23] this is an important concept as it allowed us to reach a ratio of 1:200 (ratio chosen following the published work for this device) while still achieving very good clinical outcomes.

The Rigenera disaggregation process allows the collection of any component of the tissue tissue,[24] hair roots included, which are known to contain a pool of undifferentiated melanocyte progenitors cells;[25] we can thus speculate that the mechanism responsible for the repigmentation is due to the grafting of the melanocytes collected by the hair follicles, and secondarily, because *in vitro* characterization of the micrografts showed that those are enriched in progenitor cells expressing mesenchymal stem cell-like markers such as CD90, CD73 and CD105, but also growth factors and extracellular matrix,[23,26] accounting for the immunomodulatory and regenerative outcome.[27]

To summarize, this specific micrografts technology has different advantages: (i) the use of autologous sample which are almost immediately (2 min) disaggregated/used; and (ii) differing from other protocols described in the published work, the amount of sample collected is extremely smaller (1:10 vs 1:200 ratio).

Taken together, these data are suggestive of a wider use of the autologous micrografts in the treatment of stable vitiligo in combination with NBUVB phototherapy. Further studies will be performed to confirm this evidence, possibly with more patients and with a control group, which is missing in this specific paper.

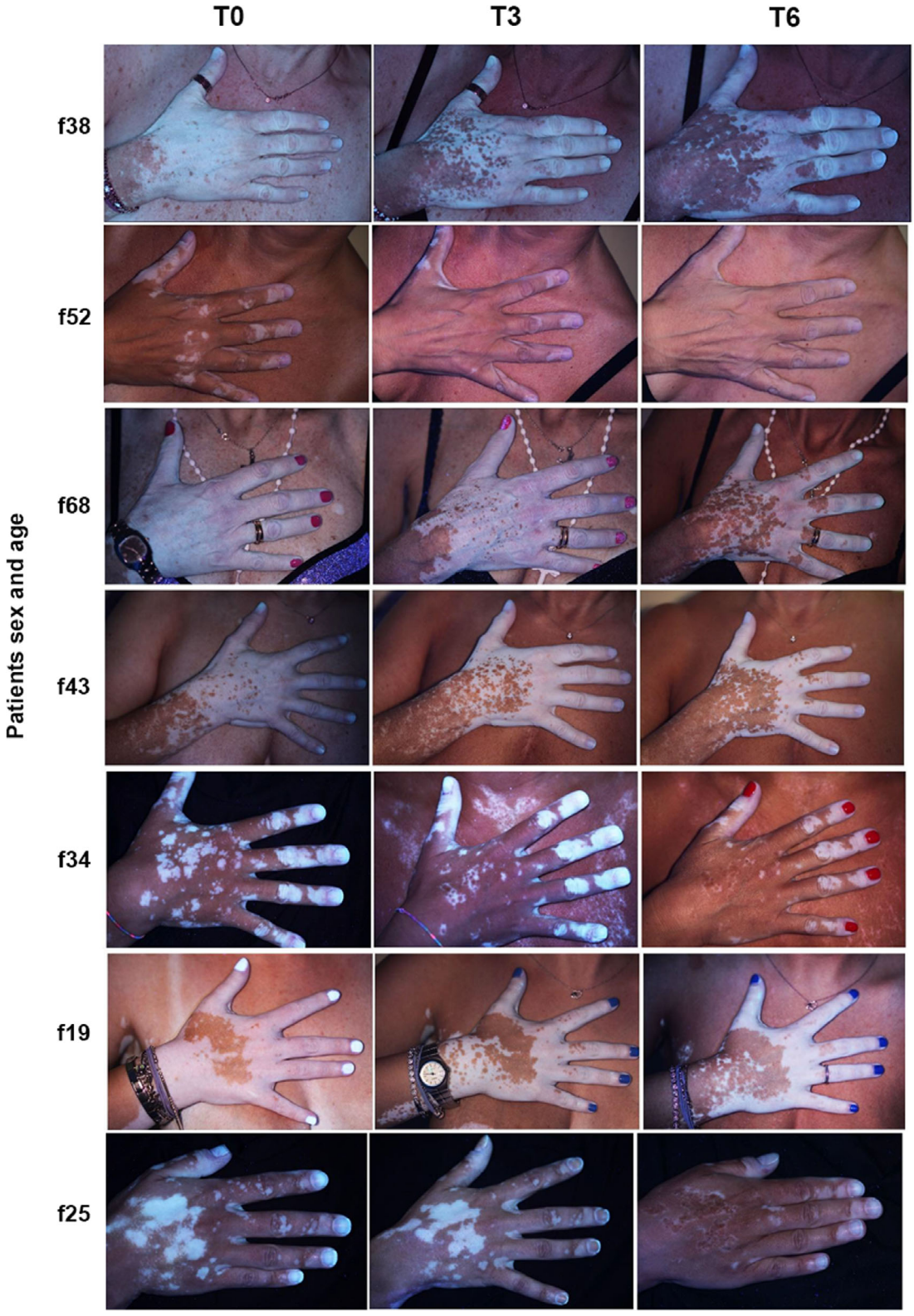

Figure 5. Mild repigmentation rate is evident between baseline and 3 and 6 months of treatment with autologous micrografts and narrowband ultraviolet B phototherapy.

CONFLICT OF INTEREST: C. A. is employed by the R&D Division of Human Brain Wave Srl, the company owner of Rigenera micrografting technology. No conflict of interest is declared for the other author.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Clinical outcomes of patients who achieved a mild repigmentation rate from baseline and after 3 and 6 months of treatment with autologous micrografts and narrowband ultraviolet B phototherapy.

