Photo(chemo)therapy for vitiligo

Alessia Pacifico & Giovanni Leone

Photodermatology Unit, San Gallicano Dermatological Institute, Roma, Italy

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Correspondence:
Giovanni Leone, Photodermatology Unit, San Gallicano Dermatological Institute, Via Elio Chianesi, 53, Roma 00144, Italy.
Tel: +39 065 266 5982
Fax: +39 065 266 5343
e-mail: gleone@ifo.it

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Summary

Vitiligo is a common skin disease characterized by loss of normal melanin pigments in the skin and its pathogenesis is still unclear. Treatment modalities include psoralen plus ultraviolet A, narrow-band ultraviolet B (NB UVB) phototherapy, topical and systemic steroids, topical calcineurin inhibitors, topical vitamin D analogues in monotherapy or in association with phototherapy, and surgical treatment. NB UVB (310–315 nm) radiation is now considered as the 'gold standard' for the treatment of diffuse vitiligo, and treatment with two recently introduced UVB sources that emit 308 nm wavelengths, the 308 nm xenon chloride (XeCl) excimer laser and the 308 nm XeCl excimer light, has also been reported to be effective and might be the treatment of choice for localized disease: this treatment modality has been defined as 'targeted phototherapy.'

Vitiligo is an acquired cutaneous depigmentation disorder affecting approximately 1–2% of the world population, with no predilection of age, gender or racial background. Familial occurrence is found in about 30% of the patients (1). Although not life-threatening, vitiligo is a disfiguring disorder and can have deep psychological consequences (2). The progressive depigmentation of the skin that characterizes vitiligo is associated with loss of melanocytes from the basal layer of the epidermis.

A variety of pathogenic mechanisms have been proposed: alterations of cellular and humoral immunity (3, 4), oxidative stress (5, 6), cathecholamines (7), a pigment cell structural aberration (8), melanocytorrhagia (9) and epidermal cytokines (10); a multifactor theory integrating the various hypotheses could be the outcome. Genetically affected individuals are prone to damage, affecting melanocytes. In vitiligo, DNA sequence variants in the NALP1 region are associated with the risk of several epidemiologically associated autoimmune and auto-inflammatory diseases (11, 12).

Current therapies require many months to years of treatment and sometimes result in disappointing outcomes. Photochemotherapy with the photosensitizer psoralen plus ultraviolet A (PUVA) is an effective treatment but carries the potential risk of skin carcinomas such as squamous cell carcinoma and malignant melanoma (13, 14). An alternative for PUVA therapy is narrow-band ultraviolet B (NB UVB) phototherapy using lamps that have a maximum emission at 311–312 nm.

PUVA therapy

Until a few years ago, this was the most popular treatment for vitiligo worldwide. This therapy was first used in ancient Egypt and in India, more than 3000 years ago, when topical applications and ingestion of extracts from the plants Psoralea corylifolia Linnaeus in India and Ammi majus Linnaeus in Egypt were used to treat vitiligo. PUVA was first introduced in 1948. Its usefulness was later reviewed by various authors and it has been the mainstay for vitiligo management for a long period.

Photochemotherapy or PUVA therapy consists of the patient receiving total body irradiation with UVA (320–400 nm) several times a week after taking a photosensitizer. The photosensitizer used most often is 8-methoxypsoralen (methoxsalen, 8-MOP), which is also used for treating psoriasis. It is taken 2 h before irradiation, generally at a dose of 0.6 mg/kg body weight. Other photosensitizers have also been used but none of them conferred an additional therapeutic advantage. 5-methoxypsoralen or 5-MOP, which is only used in some European countries and is less erythemogenic and not associated with gastrointestinal intolerance, and trimethyl-psoralen (TMP) are used in modern photochemotherapy regimens in the form of topical agents (creams, gels and solutions) or orally, followed by exposure to natural sunlight (so-called PUVASOL) (15–18).

The exact mechanism of action of methoxsalen is not known. The best-known biochemical reaction of methoxsalen is with

DNA. Methoxsalen, upon photoactivation, conjugates and forms covalent bonds with DNA, which leads to the formation of both monofunctional (addition to a single strand of DNA) and bifunctional adducts (crosslinking of psoralen to both strands of DNA). It is generally assumed that this effect may be the therapeutic mechanism in psoriasis (19–21).

However, DNA cross-linking does not appear to be a prerequisite for all the therapeutic effects of PUVA, and the successful treatment of other skin diseases is unlikely to be due directly to this molecular reaction. Psoralens also interact with RNA, proteins and other cellular components and indirectly modify proteins and lipids via oxygen-mediated reactions or by generating free radicals. Although not proven, it is possible that these could be the therapeutic mechanisms in non-hyperproliferative diseases. Psoralens also stimulate melanogenesis. This involves the photoconjugation of psoralens to DNA in melanocytes, followed by mitosis and the subsequent proliferation of melanocytes, increased formation and melanization of melanosomes, increased transfer of melanosomes to keratinocytes, and activation and increased synthesis of tyrosinase via the stimulation of cAMP activity (20–23).

Oral PUVA

For vitiligo, the irradiation is usually administered twice a week with at least 1 day, preferably 48 h, between treatments. Only patients with extensive vitiligo are considered suitable for this kind of treatment (24). For all psoralen preparations, the time interval required to achieve peak blood levels can vary with the intake of food, especially fatty foods. The peak blood levels also show a wide interindividual variation from patient to patient. The kind of pharmaceutical preparation also plays an important role in absorption (25). Interpatient variability in the peak plasma concentration after an oral dose of methoxsalen ranges from six to 15 fold (26). We found different blood levels of 8-MOP, after 2 h, in patients consuming 8-MOP preparations from different chemists (unpublished data). It is important to instruct patients to be consistent with the amount, type and time of intake of food and the drug. For all oral PUVA protocols, the initial dose should be based on the skin type and can vary from approximately 0.5 and 1 J/cm^2, with increments of 0.5 J/cm^2 given for each subsequent treatment or every other treatment until asymptomatic mild erythema is observed in the vitiligo lesions (19, 27). Further increments can be given just to maintain this erythema and usually 0.2–0.3 J/cm^2 every other treatment is suitable for this purpose. Alternatively, the initial dose can also be based on the minimum phototoxic dose (MPD), which is defined as the UVA dose that produces barely perceptible erythema with well-defined borders at 48–72 h in a patient who has ingested the requisite dose of psoralen at the appropriate time interval before exposure (28). The initial dose often given is 70% of the MPD and treatments are typically administered twice per week.

The results with oral PUVA vary and complete repigmentation is achieved in only a few patients, while cosmetically acceptable improvement is achieved in a majority of the patients. A recent retrospective study has pointed out that this treatment is only moderately effective in widespread vitiligo (29).

The total number of treatments required is between 50 and 300. Darker skin types show maximal responses to PUVA and repigmented areas can remain stable during decades, but if therapy is stopped, partial repigmentation may reverse (17, 30). In all patients on oral PUVA, the recommendation is to wear protective sunglasses for 12–24 h after the treatment. Sunscreens can be used to cover the vitiligo lesions to avoid inadvertent excessive exposure to sunlight. Repigmentation with PUVA, as with other modes of treatment, is usually observed around the hair follicles and/or from the periphery of the lesions. PUVA is contraindicated in children. In addition, the need for oculo-cutaneous protection can be cumbersome in young patients for whom long-term carcinogenesis and photoaging issues are more of concern. Male genital areas respond poorly to treatment and have an increased susceptibility to skin cancer, and these areas should be shielded during PUVA treatments. Protection also while exposed to sunlight traveling to and from a center for treatment should be mandatory.

Patient education and selection is important, especially with regard to avoidance of sunlight after the treatments. In the guidelines for the treatment of vitiligo performed by Njoo *et al.* (31), PUVA is considered a second-line therapy for generalized vitiligo in adults, although in the past, it was considered the gold standard treatment.

The most common PUVA short-term side effects include cutaneous phototoxicity, ocular phototoxicity, nausea and symptoms such as insomnia, headache and lightheadedness after the ingestion of psoralens (15, 19, 32). PUVA therapy is associated with significant long-term adverse effects that are dose related, being most common in patients receiving the highest exposure to treatment and mainly affecting patients with skin types I and II (15, 19):

- Photoaging: wrinkling, telangiectasia, dyspigmentation and actinic keratoses on exposed skin.
- Non-melanoma skin cancer: the risk of SCC is highly increased. The risk of BCC may be slightly increased (33–35).
- Melanoma skin cancer: starting 15 years after the first exposure to PUVAtherapy, the risk of melanoma was observed to increase in one study (14). The finding has not been confirmed in other follow-up studies but this may be due to shorter observation periods (33).

The incidence of squamous cell carcinoma is higher in patients with cumulative doses >1000–1500 J/cm^2. The incidences of basocellular carcinoma and melanoma also increase with cumulative treatments (14, 33–37). The main prevention strategies are limiting the number of annual sessions, restricting maintenance treatments and performing an annual clinical evaluation of patients that exceed the number of recommended sessions (BPG guidelines, AAD guidelines) (38, 39).

PUVA is contraindicated in patients with a history of skin cancer (melanoma or non-melanoma), pre-malignant skin lesions, cataracts, alteration of liver function (40), skin type I, pregnancy

and lactation, obesity (increased risk of erythema), concomitant immunosuppressive therapy or associated phototoxic treatments. In order to avoid the potentially dangerous side effects, PUVA is being replaced increasingly more frequently with NB UVB.

Apart from these more serious side effects that have been described mainly in patients treated for psoriasis, a 'confetti-like' depigmentation occurred in a patient treated with PUVA for vitiligo (41), and a case has also been reported by Falabella (42) in one of his patients. We have also observed a few cases of a similar depigmentation that mimics guttate idiopathic hypomelanosis in our patients treated with long-lasting PUVA and also after a long course of NB UVB, and the aspect can be similar to 'eruptive' new vitiligo lesions.

PUVAsol (psoralen and solar exposure)

PUVAsol, which is commonly used in countries where sunlight is in abundance and where the facilities for artificial sources of light are often lacking, works on the same principle, except that natural sunlight is used instead of UVA. PUVAsol can be systemic or topical: the same types of oral and topical preparations are used as for PUVA. The initial exposure is of a short duration and subsequent exposures are gradually increased until satisfactory erythema is achieved. In sunnier climates, treatments can be performed throughout the year and this kind of treatment is still quite popular. Usually, TMP, at a dose of 0.3 mg/kg, is used, being administered 2–4 h before the initial 10–15-min sun exposure, increasing 5 min per treatment until developing perceptible erythema (43). The results reported in the past were fairly good (44). In a retrospective study on a large series of patients (45), good results were reported with both systemic and topical PUVAsol, but phototoxicity was frequently observed with systemic PUVAsol. It was reported recently that the combination of PUVAsol and calcipotriol is highly effective and works faster than PUVA alone (46). In the case of natural phototherapy or sun exposure, variable factors including the compliance of the patient, the degree of exposure and the country where the trial is conducted can limit the interpretation and applicability of results. Nevertheless, when artificial sources for PUVA and narrow-band UVB are readily available, this rather empiric procedure should be discouraged due to its potentially dangerous and frequent side effects.

Topical PUVA

Topical PUVA is a good therapeutic approach for localized vitiligo in adults and children over 2 years old and may provide a wider margin of safety because of a lesser cumulative UVA dose and negligible systemic absorption of psoralen. In the guidelines published by Njoo *et al.* (31), it is considered a second-line therapy that should be used if no response is obtained after 6 months of class 3 topical corticosteroids combined with UV-A radiation. Topical PUVA has to be carried out cautiously to avoid phototoxicity and koebnerization (appearance of new lesions at blistered sites) (47). Nonetheless, treatments can, in fact, be performed very carefully, using lower concentrations of methoxsalen (0.1% and below). The preparation, which is usually in solution or cream form, is applied directly to the lesions, which are then exposed to UVA after 20 min (48). Physicians and patients should be aware that solutions can produce hyperpigmented lines. One or two weekly sessions are recommended. Better results are obtained with topical preparations containing TMP, which is more commonly used for topical PUVA in countries in northern Europe. The initial doses are about 0.25 J/cm^2, with weekly increments ranging from 0.12 to 0.25 J/cm^2, according to the patient's skin phototype, until mild erythema is achieved in the lesions (47, 48). Treatments with sunlight are best avoided due to the risk of phototoxic reactions. Even if topical PUVA is correctly performed, in most cases, perilesional hyperpigmentation can be observed and can represent an obstacle for continuing the treatment.

Bath PUVA may be an alternative, and interesting results have been reported in children (49) even if it remains rather impractical as compared with NB UVB phototherapy.

Khellin + UV

Khellin, a furanochromone extracted from the plant *Amni visnaga* with structural similarities to 8-MOP, and with similar photochemical and phototherapeutic properties, was initially used as a vasodilator in the treatment of coronary disease. It has since been used along with UVA (KUVA) in the treatment of vitiligo by several investigators.

There have been conflicting reports on this drug, which can be used orally and topically (50). The oral administration of Khellin, followed by exposure to UVA was described as being fairly effective in one study, but up to 200 treatments were necessary (51). But there is also a concern regarding increased levels of hepatic transaminases, and the possibility of permanent liver damage should discourage the routine use of oral Khellin. Topical Khellin, followed by UVA exposure or sun exposure (KUVA-sun), has also been widely used, especially in our country. The side effects are minor as compared with local PUVA but it is also less effective. Topical Khellin 4% ointment is applied on vitiliginous skin 20 min before UVA or sun exposure. Evidence from a study of topical Khellin, in combination with sunlight or UVA, showed that its effect was no different than that of UVA alone (52). Controlled trials evaluating Khellin, followed by sun exposure vs. sun exposure alone have not been performed; thus, the efficacy might be that of UV exposure on unsensitized skin.

Phenylalanine + UV

This chemical is not phototoxic, but the combination of UV light and phenylalanine seems to result in some repigmentation and, with sunlight, it stimulates the migration of melanocytes (53). Melanin is derived from L-tyrosine, which in turn is derived from L-phenylalanine, and in vitiligo, there seems to be an impaired turnover of L-phenylalanine. Phenylalanine has been tried for the treatment of vitiligo along with UVA and the initial reports were optimistic (54). Phenylalanine is given orally at a dose of 50 mg/

kg, 30 min to 1 h before 2–12 J/cm^2 UVA exposure (PheUVA) (43). A large retrospective study was performed on 193 patients who were treated with a combination of topical and oral phenylalanine, followed by midday sun exposure (55). The authors reported an overall improvement rate of 83.1%, with the maximum response on the face. The results obtained in other studies (56) do not confirm these good results: lower success rates are between 14% and 44%. Nevertheless, there are many contraindications, which include phenylketonuria, impaired liver and kidney function, malignant skin disease, pregnancy, breast-feeding, previous arsenic exposure or radiotherapy and autoimmune disorders. More research is warranted before the widespread use of phenylalanine in patients with vitiligo.

Phototherapy

Broadband UVA (320–400 nm)

Ultraviolet A has been used in association with fluticasone propionate (FP) in vitiligo patients and compared with UVA alone and FP alone (57): in this large study on 135 adults, the combination of FP with UVA appeared to be equally effective as FP alone, and both options were more effective than UVA alone. The authors concluded that UVA alone was scarcely effective in vitiligo. Ultraviolet A without psoralen has also been used three times weekly in a controlled, comparative and randomized trial in 20 selected patients: after 48 sessions, spread over 16 weeks with 15 J/cm^2, $>$ 60% repigmentation was observed in 50% of the patients, suggesting UVA as an alternate therapy for vitiligo (58). The effectiveness of UVA alone has not been confirmed in other studies and this does not seem to be a popular approach in vitiligo phototherapy.

Broad-band UVB (BB UVB)

Wavelengths from 290 to 320 nm (BB UVB), were widely used in the past for the treatment of a variety of skin disorders. There is only one report on the efficacy of BB UVB in vitiligo (59). Fourteen patients were treated and eight patients (57.1%) achieved 75% repigmentation in 12 months; in this series, the patients showing the best results were those with facial lesions and skin types V and VI. These results have never been confirmed. BB UVB phototherapy is being replaced by NB UVB in other indications and also in vitiligo.

NB UVB

In 1981, Parrish and Jaenicke found that 311 nm wavelength UVB radiation was most effective for the treatment of psoriasis (60). This finding provided the impetus for developing the Philips TL 01 fluorescent bulb (Royal Philips Electronics, Eindhoven, The Netherlands), the NB UVB light source. Currently, there are several clinical indications for NB UVB phototherapy including psoriasis, atopic dermatitis, desensitization therapy for photodermatoses and patch-stage cutaneous T cell lymphomas.

The use of NB UVB phototherapy for vitiligo was first reported by Westerhof and Nieuweboer-Krobotova (61), who compared twice-weekly topical PUVA with twice-weekly NB UVB phototherapy. They showed that after 4 months of therapy, 67% of patients undergoing NB UVB phototherapy developed repigmentation compared with 46% of patients receiving topical PUVA. The extent and rates of repigmentation among patients receiving NB UVB were further examined in a separate subset of patients. In this subset, 8% of patients repigmented >75% after 3 months of treatment and 63% did so after 12 months. The authors concluded that NB UVB was slightly, but not significantly, more effective than topical PUVA. The lower cumulative dose and the fewer side effects were considered to be the major advantages of the use of NB UVB over PUVA. It was concluded that compared with topical PUVA therapy, NB UVB was equally effective and was associated with fewer side effects as well. As no photosensitizer is used, ocular or gastrointestinal side effects are non-existent.

After this study, the same authors conducted an open trial (62) on the treatment of children with generalized vitiligo with NB UVB. In this study, 51 children were treated on a twice-weekly schedule for up to 1 year and 53% achieved $>$ 75% repigmentation and stabilization of disease was reported in 80%.

A retrospective analysis (63) of seven adult patients treated with NB UVB thrice weekly revealed a $>$ 75% repigmentation in five patients after a mean number of 19 exposures. The mean disease duration among these five patients was 13 months. Two other patients showed 50% and 40% repigmentation, after 46 and 48 treatments, respectively. Their mean disease duration was 132 months, suggesting that patients with long-standing disease have a poorer prognosis. In a subsequent paper, the same group of authors (64) summarized the response rate of 71 vitiligo patients who had been treated with 15–123 sessions of NB UVB. Significant (66–100%) repigmentation occurred in 39% patients, moderate (26–65%) or mild (10–25%) repigmentation in 22% and 21%, respectively, and $<$ 10% repigmentation in 10% of the patients. Patients who showed better improvement tended to have had higher numbers of treatments, indicating that the length of treatment is positively correlated with the degree of repigmentation. Again, patients who showed significant improvement had shorter disease duration than those who showed a less favorable response.

A large open trial (65) conducted so far included 110 evaluable patients (97 non-segmental, 13 segmental) who were treated with NB UVB twice weekly for 2.5–19.5 months (mean 7.8 months). In the non-segmental vitiligo group, 48% of patients showed a marked response ($>$ 75% repigmentation), 27%, a moderate response (25–75% repigmentation) and 25%, a mild response ($<$ 25% repigmentation). When analyzed by site, the best results were achieved on the face, followed by the trunk and limbs. The poorest outcome was noted for acral lesions that, at best, showed a moderate response. As reported in previous studies, the duration of the disease was inversely correlated with the degree of treatment-induced repigmentation. In the segmental vitiligo group, all but one patient showed no more than a mild response, whatever the site of the lesion was.

Irradiation protocols for NB UVB. NB UVB is currently the first-choice treatment for inducing repigmentation in generalized vitiligo affecting multiple or large areas of the body and it may stop disease progression in active vitiligo. However, the adoption of a personalized irradiation protocol for each patient is necessary to achieve optimum treatment results. There is no universally accepted protocol for NB-UVB; hence, treatment protocols differ from site to site. There is a widespread belief that vitiligo patients, in terms of photosensitivity, behave as skin type I and, consequently, have been treated with very low initial NB UVB doses ranging from 150 to 250 mJ/cm^2 (61) to avoid sunburn. In a recent study, however, this approach has been challenged. It was shown that the erythemal sensitivity in vitiliginous skin depends on the skin type, with darker skin types tolerating higher UVB doses than subjects with a fair complexion. In addition, the minimal erythemal dose (MED) values in vitiligo skin were, on an average, only 35% (95% CI = 31–39%) lower than those in the normal skin of the same individual (66). The importance of stratum corneum thickening, as a protective mechanism against UV (67), has been emphasized in vitiliginous skin. Recently, it has been shown that vitiliginous skin, treated with targeted phototherapy, undergoes progressive photoadaptation with a corresponding increase in the MED (68); a positive correlation was also found between skin phototype and MED, which reflects the increased NB UVB tolerance shown by the higher skin types. In normal skin, the same finding may be attributable to the increase in melanogenesis; in vitiliginous skin, which is deficient in melanin, this may be due to photoprotective mechanisms other than melanin. The data published by El-Khateeb (69) confirm that, in vitiliginous skin, photoprotective mechanisms other than melanin, including epidermal layer thickness, optical properties and chromophores, may play a major role. This has to be kept in mind when starting a phototherapy course in an individual with a dark skin type, where vitiliginous patches show a proportionally higher resistance to NB UVB irradiation as compared with that of a lower skin type. Apart from the photoadaptation phenomenon and the efficacy of defense mechanisms other than melanin in vitiliginuous skin, another study showed that doses close to the MED are the most effective in determining the induction, *in vitro*, of soluble mediators like endothelin 1 (ET-1) (70) (see the following section on mechanisms of action). On the basis of these data and our personal experience, we initiate vitiligo treatment with higher NB UVB doses, as compared with the phototherapy protocols that were earlier described, e.g., 70% of the MED determined on normally pigmented skin, or 50% in the case of lighter skin types (I or II). We adopt the twice-weekly schedule: dose increments at each treatment session are adjusted following the photoadaptation of the irradiated skin by dose increments of 10–40% with the aim to induce and maintain a faint erythemal reaction in lesional skin. In the majority of the studies, the dose is stabilized when mild erythema develops. Generally, after the first few sessions, the rate of increase in the UVB dose is individualized for each patient.

In practice, we increase by 10–20% for skin type I-II, 30% for skin type III, 40% for skin type IV and eventually 50–60% for the darker skin types (Fig. 1). Skin type I usually does not require

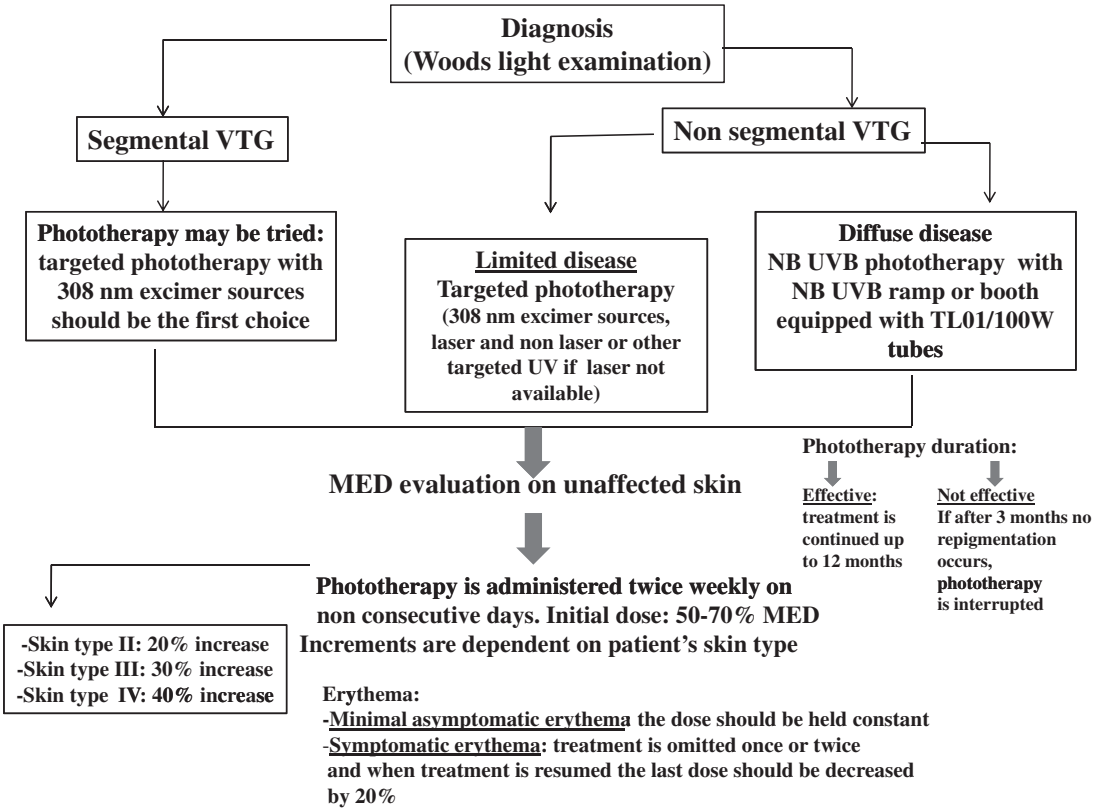

Fig. 1. Suggested diagnostic and therapeutic approach when treating vitiligo with phototherapy.

treatment and, on the other hand, phototherapy is hardly tolerated, the potential side effects being more numerous than the benefits. If symptomatic erythema or blistering develops, treatment is omitted (once or twice) and, when treatment is resumed, the last dose is decreased by 20%.

Treatment is continued as long as there is ongoing repigmentation. We interrupt the treatment if no repigmentation is achieved within 3 months of treatment, or, if after 6 months, only minimal repigmentation is obtained ($< 25\%$) with continuous NB UVB exposure.

In some cases, where the response to phototherapy is slow and scarce, an interruption of the treatment, followed by resumption after 2 months may be tried. This is based on the concept that intermittent irradiation might induce a more effective stimulation of melanocyte proliferation than a continuous treatment schedule. There is currently a trial that is investigating the effects of such a protocol (stop and go) and the preliminary data seem to be encouraging (71).

Adverse effects. The acute side effects of NB UVB phototherapy include erythema, which has been shown to have characteristics similar to those induced by BB UVB. The incidence of erythema with NB UVB varies according to the treatment regimen. Reactivation of herpes simplex may occur with UVB treatment and precautionary measures should be adopted in those patients with a history of this condition. No data are available to date for the effect of NB UVB on human deficiency virus promoter expression, although, it is already known to be activated by BB UVB. The potential effects of NB UVB on the eyes, in particular exposure-related conjunctivitis, or keratitis need to be taken into account when treating patients with periocular disease: compliance of the patient is a must and treatment of the periocular area in children has to be performed under close supervision.

The long-term risks of NB UVB remain unclear and the question as to the carcinogenicity of UVB still remains unanswered. The induction of photodegenerative changes by UVB is well established. The action spectrum for the induction of photodamage and photocarcinogenesis in animals is maximal in the UVB region. Ultraviolet B is a complete carcinogen and NB UVB has been shown in human skin, cell and animal models to induce DNA damage and to be more carcinogenic than BB UVB (72, 73). However, a recent study (74) did not provide evidence for an increased skin cancer risk in patients with psoriasis treated with either BB UVB or NB UVB. In other studies (75, 76), no increased risk for melanomas or squamous cell carcinomas was detected in patients receiving NB UVB for diseases other than vitiligo, but an increased risk was evident for basal cell carcinomas. Hearn *et al.* (77) have shown on 3867 patients that there is no significant increase in SCC or malignant melanoma in those patients treated with NB UVB and only a small increase in basal cell carcinoma among patients who had also been treated with PUVA before receiving NB UVB. No data are available so far regarding carcinogenesis in patients with vitiligo treated with NB-UVB and, paradoxically, skin cancer appears to occur rarely in vitiliginous skin despite the lacking protection by melanin from ultraviolet radiation (78, 79). This phenomenon could be related to epidermal upregulation of wild-type p53 in vitiligo (80). Keratoacanthoma in a vitiligo lesion after NB UVB phototherapy has been described as a rare event by Brazzelli *et al.* (81).

There is currently no evidence that patients with vitiligo could have an increased risk compared with other patients receiving NB UVB. There is no doubt that NB UVB should be considered less carcinogenic than PUVA, but all efforts should be made to keep the cumulative dose at the lowest possible levels and patients receiving long-term therapy should be followed up regularly.

Duration of repigmentation and predictors of response. Several variables significantly influence repigmentation in patients with vitiligo. These include age, patient motivation, number of treatments, maintenance of adequate lesional erythema, location of lesions, type of vitiligo, skin type and the presence of residual lesional melanocytes. Usually, maximal repigmentation occurs in children with vitiligo. Two to three months of treatment with NB UVB are usually required before new pigment becomes evident; however, it is not uncommon to observe patients with rapid responses (in our series, $> 50\%$ of the patients have experienced repigmentation within the first three months of treatment). Follow-up data of patients after termination of a NB UVB phototherapy course are limited. In their series, Nicolaidou *et al.* (82) followed up 25 patients for up to 4 years after stopping phototherapy. Relapse was observed in 44% of them within 1 year after treatment cessation. In 14.3% of patients, no new vitiligo lesions appeared within the repigmented areas 4 years after treatment. Sitek *et al.* (83) followed up 11 patients with more than 75% overall repigmentation after a course of NB UVB and reported that 45% of patients were in full remission 2 years after the end of treatment. Natta *et al.* (84) followed up nine patients for up to 2 years and reported 25% and 43% relapse rates in 1 year and 18 months, respectively, whereas in the series by Kanwar *et al.* (85), four of out of eight patients relapsed within 3 months. Recently, Kumar *et al.* (86) reported the results of a study on 150 Indian patients treated with NB UVB with a follow-up period of 6 months after the cessation of therapy for stability of repigmentation; only three patients developed depigmentation of repigmented sites during follow-up. There is general agreement on the fact that those who relapse usually respond to a second course of treatment. The duration of the disease is inversely correlated with repigmentation percentage in the face, trunk and limb lesions but not for acral lesions, which could be explained by the exhaustion of the melanocyte storage present in the outer root sheath of the hair follicle that occurs progressively with time in areas other than the extremities, where the follicular reservoir is insufficient from the beginning. This is in agreement with the findings of Njoo *et al.* (31), who reported that early lesions respond better than old ones, suggesting that the follicular melanocytes are also destroyed by the disease process, and Scherschun *et al.* (63), who observed that the longer duration of disease correlated with less successful repigmentation. Also, in our patients undergoing treatment with NB UVB, we have noticed that vitiligo of recent onset responds better to UV treatment (87). This was not confirmed by the study of Natta *et al.* (84), who found that the duration of the disease had no

impact on the response. In general, facial and small areas of vitiligo involvement are more responsive to NB UVB phototherapy than larger areas of vitiligo (88) or disease at acral sites, like hand skin, where the poor response is related to low hair follicle density (89). An open, uncontrolled study from Greece (82) reported on 70 patients with non-segmental vitiligo who were treated over a maximum period of 1.5 years. Cosmetically acceptable repigmentation ($>$ 75%) was achieved in 34.4% of patients with lesions on the face after a mean treatment period of 6 months but in only 7.4% of patients with lesions on the body after a mean treatment period of 9.2 months. Hand and feet vitiligo showed minimal or no repigmentation and lesions on the elbows and knees responded less than lesions on the trunk but better than acral vitiligo. Predictors of a good response, in this study, were darker skin types (III–V) and early initial repigmentation. Twenty-five patients were followed for up to 4 years: seven patients (28%) remained stable over 1–4 years, whereas 18 patients (72%) relapsed after 1–3.5 years. In vitiligo, repigmentation may spread inwards from the borders of the lesion or from the hair follicles in the lesion. Kim *et al.* (90) demonstrated that the involved site and the duration of vitiligo are important determining factors in the manifestation of repigmentation patterns in vitiliginous patches. Some (91) have noticed that old lesions showed white hairs and no response to phototherapy contrary to the lesions that appeared more recently. It could be hypothesized that, the longer the duration of vitiligo, the higher the possibility of having white hairs. Because the melanocytes in the hair follicles are a major source of repigmentation, the existence of white hairs in vitiligo lesions suggests that vitiligo may be resistant to phototherapy (92). Thus, the poor response to phototherapy in old vitiligo may be related to white hairs, suggesting that in vitiligo, early treatment is required before these white hairs appear. The evaluation of white hairs using a portable digital microscope may predict the response to phototherapy (93).

Non-segmental vs. segmental vitiligo. It has been shown that in patients with segmental vitiligo, NB UVB phototherapy is less effective (65). The existence of white hairs in the lesion of segmental vitiligo indicates a poor response to phototherapy, suggesting that segmental vitiligo with white hairs may require surgical treatment such as suction blister epidermal grafting (93). In our hands, phototherapy with the excimer laser and the excimer lamp proved to be slightly more effective than NB UVB (unpublished data). Surgery remains the best choice in segmental vitiligo.

Mechanism of action. The mechanism of action of NB UVB phototherapy in vitiligo has not been completely understood. Vitiligo is characterized by the selective destruction of the active melanocytes in the epidermis, whereas the inactive (dopa-negative) melanocytes in the outer root sheaths of hair follicles are not affected (89); the cause is unknown, but is generally believed to be an autoimmune process. As T-lymphocytes seem to participate in the pathogenesis of the disease, the induction of T-lymphocyte apoptosis by NB UVB, which can be observed in psoriasis (94), may play also a role in repigmentation of vitiligo lesions. Repigmentation, when it occurs, begins at the hair follicle, where dopa-negative, amelanotic melanocytes in the outer root sheaths are somehow activated to proliferate, produce melanins and migrate outward to surrounding depigmented skin.

Melanogenic paracrine cytokine networks are observed *in vitro* between melanocytes and other types of skin cells, including keratinocytes and fibroblasts. Melanocytes' mitogenesis, melanogenesis and melanocyte migration have been shown to be induced by various cytokines and inflammatory mediators, including interleukin (IL-1), tumor necrosis factor-α, leukotriene C4, basic fibroblast growth factor, membrane-type stem cell factor, growth-related oncogene-a, hepatocyte growth factor, alfa-MSH, granulocyte–macrophage colony-stimulating factor and ET-1. IL 1α stimulates the synthesis of ET-1, a potent vasoconstrictive peptide that has mitogenic and melanogenic properties. Imokawa (95) found that the expressions of ET-1, IL-1α and tyrosinase in human keratinocytes *in vitro* and *in vivo* were increased after UVB irradiation, suggesting a possible mechanism of UVB-induced repigmentation. ET-1 is associated with UVB-induced migration of melanocytes and stimulates DNA synthesis in melanocytes. In the keratinocyte supernatant, NB UVB irradiation induced a significant increase in the ET-1 levels, suggesting that UVB increases ET-1 secretion (96). In a recent study (70), NB UVB, excimer light and excimer laser irradiation were found to significantly increase ET-1 levels in the culture supernatant, while the increase induced by BB UVB was not significant. The authors used a multilayered human epidermal tissue model (EPI-MODEL TM) with a morphology that is comparable with that of the human epidermis and this is the first study demonstrating that ET-1 secretion is induced by various UVB radiation sources, including BB UVB, NB UVB, excimer light and excimer laser, in a dose-dependent manner, especially at a dose close to the MED of each UVB radiation source. This last finding, concerning the dose–effect relationship, may justify the use of fairly aggressive phototherapy protocols, confirming that the induction of moderate erythema is correlated to the induction of repigmentation. NB UVB may also act through the stimulation of the expression of phosphorylated focal adhesion kinase (p125 FAK) on melanocytes and through increased expression of matrix metalloproteinase-2 activity from melanocytes (96).

NB UVB vs. PUVA. After the establishment of NB UVB phototherapy for vitiligo, an obvious challenge was to assess its therapeutic efficacy relative to that of other phototherapeutic modalities, in particular, photochemotherapy. To this end, several trials have been performed so far. In a first bilateral comparison study, NB UVB was compared with PUVA in 15 adult patients with symmetrical vitiligo. After 60 sessions, the clinical response to both treatments did not differ significantly (97). A retrospective comparison of 38 patients on oral PUVA and 31 patients on NB UVB showed a significantly better outcome for NB UVB. In the PUVA group, marked to complete improvement was observed in 23.6%, moderate improvement in 36.8%, no to mild repigmentation in 32.6% and worsening in 7% of patients. The respective figures for the NB UVB group were 41.9%, 32.2%, 25.9% and 0% (98). Another study compared randomly allocated treatment with thrice-weekly NB UVB and oral TMP-

UVA in 50 non-segmental vitiligo patients. The mean treatment duration was 6.3 months for NB UVB and 5.6 months for TMP UVA. Both in terms of stability achieved and efficacy in active and stable disease, NB UVB was found to be superior to TMP-UVA (99). Recently, the first randomized, double-blind trial was published on the efficacy of NB UVB vs. oral 8-MOP (or 5-MOP) UVA in 50 patients with non-segmental vitiligo. Treatment was given twice weekly and assessments were performed after every 16 sessions. At the end of the study, the PUVA group had received a mean number of 47 treatments as opposed to 97 treatments in the NB UVB group. This difference was suspected to be due to differences in efficacy and adverse effects; 64% of patients in the NB UVB group showed $>50\%$ improvement compared with 36% of patients in the PUVA group. Also, when only those patients who completed ≥ 48 sessions were considered, the reduction of the affected body surface area was significantly greater for NB UVB than for PUVA. The color match of repigmented skin was excellent in all patients treated with NB UVB but in only 44% of those treated with PUVA. The clear conclusion of this study was that NB UVB is superior to oral PUVA in non-segmental vitiligo (99). A small, open, four-quarter comparison study evaluated NB UVB vs. BB UVB in combination with topical calcipotriol vs. placebo in nine patients with generalized symmetrical vitiligo. NB UVB was delivered to the upper part of the body until the navel and BB UVB to the lower part of the body. Irradiations were performed thrice weekly during the first months and twice weekly thereafter; additionally, calcipotriol was applied once in the evening on vitiligo lesions on the right side of the body and placebo ointment on lesions on the left side. After 6 months of treatment, none of the patients showed repigmentation on the lower part of the body, indicating that neither BB UVB and calcipotriol alone nor their combination had been therapeutically effective. BB UVB was then discontinued and NB UVB was applied entire the whole body. At the end of the treatment, after 12 months, no difference in repigmentation was apparent between calcipotriol- and placebo-treated sites, indicating that calcipotriol failed to enhance the response to NB UVB (100).

In summary, the majority of comparison studies have shown that NB UVB is more effective than other phototherapeutic modalities. Therefore, most treatment centers nowadays consider NB UVB phototherapy as the first-line treatment for generalized vitiligo. Its distinct advantages over PUVA include the lack of psoralen-related side effects and precautions, cosmetically better color match and its safety in children. However, the relative stability of NB UVB-induced repigmentation over PUVA, its maximum safe duration and the cumulative dose allowed still remain to be determined.

Studies comparing NB UVB with the excimer laser or the excimer light are discussed below in the section on targeted phototherapy in vitiligo.

NB UVB in combination with systemic treatments. Folic acid, vitamin B12 and sun exposure were used in an open-label study for a minimal period of 3–6 months in 100 patients with vitiligo during summer and together with UVB irradiation in winter: improvement occurred in 52 patients and total repigmentation was observed in six others (102). This finding was not confirmed in a parallel group study in which the addition of folic acid and vitamin B12 to NB UVB was assessed: 27 patients with stable vitiligo were randomized to receive either NB UVB alone or NB UVB combined with vitamin B12 and folic acid for 1 year. Although repigmentation was notable on the face, trunk and extremities, it was minimal on the hands and feet, and vitamin B12 and folic acid did not seem to improve the effect of NB UVB (103).

Because oxidative stress has been implicated in the pathogenesis of vitiligo, the combination of NB UVB with agents that have antioxidative properties has been suggested to increase the effectiveness of phototherapy. Recently, we have treated 35 patients with NB UVB and an oral antioxidant pool (AP) containing α-lipoic acid, vitamins C and E and polyunsaturated fatty acids, in a randomized, double-blind, placebo-controlled multicenter trial (103). The therapeutic response with the AP and NB UVB revealed 47% of the patients achieving $>75\%$ repigmentation against 18% in the placebo group; the average number of treatments required to induce 50% repigmentation was 18 in the AP group and 23 in the placebo group. We hypothesize that therapy with the AP may significantly have improved the clinical effectiveness of NB UVB by reducing oxidative stress. Another study (104) focused on the effect of vitamin E in association with NB UVB phototherapy. Twenty-four patients were treated either with NB UVB plus oral vitamin E or with NB UVB alone in a randomized study. Vitamin E increased the effectiveness of NB UVB, and this was attributed to its ability to prevent lipid peroxidation in the cellular membrane of melanocytes. The antioxidative and photoprotective plant *Polypodium leucotomos* (PL) extract has also been found to have immune modulator properties: it is able to modulate the immune response after trauma, inhibiting Th2 pathway activation (105). Given the pathogenic role of oxidative stress and autoimmunity in vitiligo, the therapeutic potential of PL in combination with NB UVB has been evaluated in a double-blind, placebo-controlled trial. Fifty patients were randomized to receive either 250 mg PL capsules or placebo three times daily in conjunction with twice-weekly NB UVB. At week 26, there was a body area-dependent trend towards more repigmentation in the PL+NB UVB group. The mean cumulative NB UVB dose was similar for both groups. Patients with skin type II and III appeared to benefit more from PL than darker skin types (106).

Currently, antioxidants should be used as a co adjuvant rather than as a first-line therapy.

NB UVB in combination with topical treatments. Corticosteroids, calcineurin inhibitors, vitamin D analogues and preparations containing pseudocatalase or a combination of catalase and superoxide dismutase have all been used as topical treatments for vitiligo with different and sometimes equivocal results. In addition to their use as a monotherapy, a number of studies have investigated the combination of these agents with NB UVB with the aim of accelerating and increasing the therapeutic response to phototherapy.

A case report from India first described the use of thrice-weekly NB UVB in combination with calcipotriol cream on the right and placebo cream on the left lower limb. At the end of 6 months, repigmentation was almost complete over the right limb, whereas it was less than 50% on the placebo-treated side (107). Subsequently, several other trials assessed the usefulness of a once- or twice-daily combination of NB UVB with calcipotriol (calcipotriene) or tacalcitol in small patient cohorts. Most of these trials were open and uncontrolled and did not extend over more than 6 months. In one of these, NB UVB thrice weekly in combination with calcipotriol twice daily applied to all vitiligo lesions on the left side of the body yielded better results in six out of 17 patients. The study period was not clearly specified; apparently, up to 116 treatments (or more) of NB UVB were received by some patients (108). In another open trial on 24 patients, twice-daily application of calcipotriol was found to potentiate the efficacy of NB UVB. About two-thirds of the patients had an earlier onset of repigmentation with the combination. After 6 months of treatment, the overall response rate was 51% for the combination and 39% for NB UVB alone (109). A greater extent of repigmentation and an increase in the response rate was also reported for NB UVB in combination with tacalcitol. We performed a randomized, investigator-blinded bilateral comparison study on 32 pairs of symmetrical vitiligo lesions that were exposed twice weekly to NB UVB. In addition, a standard dose of tacalcitol was applied once daily in the evening on one of the paired lesions. Throughout the entire observation period, the combination led to significantly higher repigmentation scores than NB UVB alone. Lesions treated with combination regimen repigmented both earlier and to a greater extent (110). In contrast, no effect of twice-daily calcipotriol cream in addition to twice- or thrice-weekly NB UVB was observed in an investigator-blinded study on 20 patients after 6 to 12 months of treatment (111). Two further studies also reported negative findings. Calcipotriol once daily did not enhance NB UVB-induced repigmentation when given over a period of 1 year (100). Another trial compared monotherapy with NB UVB (24 patients) with NB UVB plus twice-daily calcipotriol (13 patients). No significant difference in repigmentation was found between the two groups after 30 sessions of phototherapy (112).

A small, randomized, placebo-controlled, double-blind trial compared NB UVB plus tacrolimus vs. NB UVB plus placebo in the treatment of generalized vitiligo: paired vitiligo lesions in nine patients were treated thrice weekly with NB UVB plus a twice-daily application of either 0.1% tacrolimus or petrolatum, over a total period of 12 weeks. Overall, both sides improved without a statistically significant difference between tacrolimus and placebo (113). In an open, uncontrolled trial including 110 patients with 403 lesions, tacrolimus ointment once daily was combined with twice-weekly NB UVB for 16 weeks. Greater than 50% repigmentation was observed in 42% of the lesions. Because of the uncontrolled nature of the study, it is not possible to assess the additional effect, if any, of tacrolimus to NB UVB in this trial (114). A recent prospective single blind study on 80 patients with generalized, symmetrically distributed, vitiligo has demonstrated that addition of topical tacrolimus increases the extent of overall repigmentation achieved with NB UVB therapy and reduces the cumulative NB UVB dose needed to achieve a therapeutic benefit in affected patients (115).

There has been a wide debate in the last few years about the possible risk of this association and, namely, the possibility that its use might increase the carcinogenic risk of phototherapy. In a recent paper, Lerche and Wulf (116) reviewed the available data and concluded that the risks have been overestimated. Other publications confirm this position. The principal objections are as follows: the scarce ability of these molecules to penetrate into vitiligo skin, due to their high molecular weight, and the fact that all the data on carcinogenesis have been collected, so far, in animals that underwent massive applications of these topical drugs (117). Also, in our hands, calcineurin inhibitors have proven to be effective together with phototherapy in vitiligo, but further studies are needed to exclude definitely the long-term side effects and validate a therapeutic protocol that may minimize the risks (application frequency, dosage, etc.), and, for the moment, this association should not be routinely recommended.

There is evidence that the vitiligo epidermis shows oxidative stress that is believed to have a fundamental role in the pigment cell degeneration found in vitiligo (118). On the basis of such findings, Schallreuter *et al.* (5) have argued that excess hydrogen peroxide in the vitiligo epidermis leads to the inactivation of catalase and also identified calcium deficiency in vitiligo keratinocytes (119), suggesting that the correction of catalase deficiency could be a valid therapeutic approach. After an early report on the benefit of pseudocatalase in combination with short-term UVB exposure, no effect of a combination of pseudocatalase with NB UVB was found in a later investigation. Both studies were small, open and uncontrolled (120, 121). Bakis-Petsoglou *et al.* (122) report a clinical trial of a pseudocatalase cream vs. placebo over a 24-week period, with patients in both arms of the study receiving NB UVB. The authors found that the use of pseudocatalase cream with NB UVB was not superior to the use of placebo cream with NB UVB. The therapeutic role of pseudocatalase, alone or in combination with UV, is controversial (123). A small, double-blind, intraindividual comparison study on NB UVB in combination with either a gel containing catalase and superoxide dismutase (Vitix®, Laboratoire ACM, Clichy-La Garenne, France) or placebo (the excipient only) suggested some effect of the verum preparation (124). A recent small study (125) also described the use of this product in combination with NB UVB. Because of the open, uncontrolled design of the study, the relevance of the data cannot be interpreted.

There is only one report (126) on the combination of NB UVB with topical corticosteroids: the combination of topical clobetasol together with NB UVB was tested against placebo with NB UVB and no significant differences could be shown.

In summary, there is some evidence that concurrent treatment with topical vitamin D analogues and calcineurin inhibitors might accelerate and increase the response to NB UVB in a proportion of patients. However, controlled, larger scale trials with longer treatment periods are required to corroborate this contention. Available data on the combination of NB UVB with other topical treatments are scarce and of low evidence level, thus precluding a well-founded assessment.

Phototherapy as an adjuvant to surgical treatment in vitiligo

With cultured melanocyte grafts, repigmentation is attained several months after surgery, and PUVA contributes to provide faster and deeper repigmentation (42).

A recent report (127) on 17 vitiligo patients with stable focal or segmental vitiligo has demonstrated that combination treatment with split-skin-thickness grafting and post-surgical exposure to a 308 nm excimer laser can lead to fast, cosmetically good, long-lasting results. PUVA, NB UVB phototherapy and excimer laser phototherapy may be used after surgical procedures in vitiligo to accelerate repigmentation and improve the esthetic results.

Targeted phototherapy

Phototherapy, conventionally with PUVA, or the more recently reported NB UVB, with its wavelength of 311–313 nm, is the mainstay for generalized vitiligo. These treatments, however, are associated with burning and skin aging when administered long term. In conventional NB UVB, as in any other type of phototherapy, ultraviolet radiation is delivered using a stand-up, whole-body unit: in this manner, the normal, uninvolved, skin is unavoidably exposed to UV radiation, resulting in several adverse effects. Recently, efforts have been made to develop therapeutic devices that deliver light, be it laser or incoherent, selectively to the lesions. With newer phototherapy units capable of emitting light in a more targeted manner and also with higher fluencies, the lesions can be selectively treated while the normal skin is spared.

Lasers

Helium–neon laser. Low-energy helium–neon (He–Ne) lasers (632.8 nm) have been used in a variety of clinical treatments including vitiligo management. The light-mediated reaction to low-energy laser irradiation is referred to as a biostimulation rather than a thermal effect. A first report was published in 2003 (128). This study investigated the effect of a He–Ne laser both *in vitro* and *in vivo*. *In vitro* studies revealed a significant increase in basic fibroblast growth factor release from both keratinocytes and fibroblasts and a significant increase in nerve growth factor release from keratinocytes (129). It has also been shown that melanocyte migration was enhanced either directly by He–Ne laser irradiation or indirectly by the medium derived from He–Ne laser-treated keratinocytes. Furthermore, 30 patients with segmental-type vitiligo on the head and/or the neck were enrolled in this study. He–Ne laser light was administered locally at 3.0 J/ cm^2 with point stimulation once or twice weekly. After an average of 16 treatment sessions, $>$ 50% repigmentation was observed in 60% of patients (128). A recent study by the same group (130) demonstrated that a He–Ne laser induced a growth-stimulatory effect on functional melanocytes via mitochondria-related pathways and suggested that other minor pathways including DNA damage may also be inflicted by laser treatment on irradiated cells. Despite the interesting results obtained in cell cultures and the clinical improvement noted in patients with segmental vitiligo, further clinical studies are required on larger series, also including non-segmental vitiligo, to confirm the indication for the use of a He–Ne laser in vitiligo.

Excimer laser 308 nm. The excimer laser represents the latest advance in the concept of selective phototherapy. It emits a wavelength of 308 nm and shares the physical properties of lasers: a monochromatic and coherent beam of light, selective treatment of the target and the ability to deliver high fluences. The 308 nm excimer laser was first used in dermatology for treating psoriasis. Since then, many studies have evaluated this new device in a number of dermatologic disorders. Psoriasis and vitiligo have each been further investigated, and the use of excimer lasers for both conditions is now approved by the US Food and Drug Administration.

The excimer laser emits a wavelength of 308 nm produced using xenon and chlorine gases. Transmission of the beam of light is achieved using a liquid light guide (LLG). The spot size is variable from 14 to 30 mm in diameter depending on the model used. These technical characteristics provide this laser with many advantages over conventional phototherapies. High fluencies can be emitted, which can be useful in thick plaques of psoriasis but not in vitiligo, where only low fluencies are used. It is also possible to selectively turn the beam of light and thus to treat the specific area involved, sparing healthy skin. In vitiligo, this selectivity limits the unsightly tanning of perilesional skin, which is commonly observed with other phototherapies. The LLG also makes it easier to reach areas that are usually difficult to treat, such as folds and mucosa. The disadvantages include the fact that the limited size of spots can be impractical for treating large surfaces ($>$ 20% of total surface body area) and that the purchase and maintenance costs of these devices are rather high. Because of its selectivity and pro-pigmentary properties, the 308 nm excimer laser represents an interesting new approach for treating vitiligo. The use of the 308 nm excimer laser in treating vitiligo was first reported by Baltas *et al.* (131). Since then, many studies have shown the efficacy of this laser for repigmenting vitiligo lesions. Spencer *et al.* (132) recently reported that the 308 nm excimer laser may represent a new therapeutic option for the management of vitiligo, resulting in repigmentation of vitiligo patches in less time than that required with current modalities. With this treatment, pigmentation can start after only five sessions and increase with the continuation of treatment. Low fluencies (from 50 to 200 mJ/cm^2) have been used in one to three sessions a week for 1–6 months, depending on the study. Among the factors that can influence the clinical response to treatment, localization of the lesions seems to play a crucial role (133). In their study, Taneja *et al.* (134) report repigmentation of at least 75% in all the lesions located on the face vs. none on the hands and feet. The variability of some results reported certainly depends on the localization of target lesions. Sessions can be performed once, twice or three times a week. The repigmentation rate seems to be linked to the total number of sessions and not to their frequency (135). It is difficult to know whether repigmentation is stable with time because the follow-up of existing series is short or non-existent. A recent study reports no depigmentation 1 year after the end of

sessions (136). On the other hand, Passeron and Ortonne (137), in a recent review article, report that in their series about 15% of new depigmentation is observed 1–3 years after the end of treatment. Tolerance of treatment is usually very good, and immediate side effects are limited to erythema and in rare cases blistering. Phototherapy with the excimer laser may represent a valuable therapeutic option in children with vitiligo. In a group of children with vitiligo, Hui-Lan *et al.* (138) showed that the combination of topical pimecrolimus and excimer laser is statistically better than excimer laser alone: combined therapy may lead to faster repigmentation than excimer laser monotherapy for facial lesions only. In the same study, the authors concluded that the 308 nm excimer laser was effective, safe and had minimal side-effects for childhood vitiligo. Topical tacrolimus has been shown to increase the efficacy of excimer laser phototherapy in vitiligo. Two pilot prospective studies (139, 140) have compared the efficacy of the excimer laser combined with 0.1% tacrolimus ointment with excimer laser monotherapy or laser associated with a placebo. In the first series, two sessions per week were performed vs. three in the second. In both cases, a total of 24 sessions were carried out and 0.1% tacrolimus ointment was applied twice a day. The results were comparable and clearly showed a greater efficacy and a shorter response time to treatment with combined therapy as compared with the excimer laser alone. Such a combination might not be used routinely as there is an ongoing debate regarding whether tacrolimus might increase the risk for UV-induced cutaneous cancers. Another possible association could be with topical corticosteroids. In a recent study, Sassi and colleagues showed that twice as many patients had more than 75% repigmentation when using hydrocortisone 17-butyrate during 308 nm excimer laser treatment as compared with patients treated with the laser alone (141).

The possible mechanisms of action of NB UVB radiation in vitiligo have already been discussed, but additive actions can be advocated in the case of the new potent monochromatic light sources used for targeted phototherapy. Photobiologically, the wavelengths of the excimer laser (308 nm) and NB UVB (311 nm) are very close to one another, and the therapeutic effects may well be similar. The biological effects of coherent and collimated laser light may differ from those of incoherent light of the same wavelength. Conventional UVB sources emit polychromatic, continuous, incoherent light, whereas the excimer laser emits coherent, monochromatic, UVB light in short pulses. These photophysical properties of the excimer laser could account for its greater effectiveness as compared with conventional NB UVB in the treatment of vitiligo, namely, the possibility to deliver high doses in a short interval of time may account for the differences between the biological effects of conventional NB UVB and those of the monochromatic excimer sources. Even if the Bunsen–Roscoe law of reciprocity states that a certain biological effect is directly proportional to the total energy dose, irrespective of the regimen administered, in some cases, it has been shown not to hold, and this could also be the case for the effects of the excimer laser. Recently, the mechanism of the excimer laser's high efficacy in psoriasis treatment has been investigated; on the other hand, data on the autoimmune origins of vitiligo underline the probable implications of the immunosuppressive action of UV in treating vitiligo and thus the intense pro-apoptotic effect of the 308 nm wavelength on T cells, which has been demonstrated in psoriasis, could also play a role in vitiligo. Among UVB light sources, the XeCl excimer laser is the strongest inducer of apoptosis in T cells (142). Novak *et al.* (143) also compared seven spectral distributions of UVB light in the spectral region of 290–311 nm and found that the 308 nm XeCl excimer laser induced apoptosis more strongly at a dose six times less than that of NB UVB. Another study on psoriasis patients (144), based on an immunohistochemical evaluation of T cells and the expression of various molecules associated with apoptosis, demonstrated that the 308 nm excimer lamp effectively depleted T cells in psoriatic hyperproliferative skin after treatment. In the previously cited study by Noborio and Morita (69) on the ability of different light sources in the UVB range to induce the secretion of ET-1, the excimer laser irradiation induced high levels of ET-1 secretion compared with the other sources.

Non-laser light sources

Monochromatic excimer lamp/excimer light 308 nm (MEL 308 nm). The 308 nm monochromatic radiation can also be delivered by excimer lamps. The effectiveness of a new 308 nm monochromatic excimer source, with emission close to that used in NB UVB phototherapy, has been initially described in the treatment of recalcitrant palmoplantar psoriasis (145, 146).

A pilot study (147) that we have carried out, using such a device, reports that 18 out of 37 vitiligo patients achieved 75% or more repigmentation after 6 months of treatment. The source in this study was a 308 nm XeCl MEL device with a power density of 48 mW/cm^2 at a distance of 15 cm from skin and the irradiation field covered an area of 504 cm^2 with a rectangular shape (36 cm × 14 cm). Interestingly, a satisfactory response on the hands was noted: two patients achieved grade 3 repigmentation. This finding is not significant because of the limited number of patients who received treatment on the hands in this study ($n = 3$), but it may suggest the efficacy of higher NB UVB fluences on these locations that usually respond poorly to conventional treatment. The 308 nm MEL may present some advantages over the laser: lower power density and consequently reduced risk of accidents due to overexposure and the larger irradiation field that allows to treat larger areas at a time, with a shorter treatment duration. More recently, devices that deliver 308 nm MEL to the skin by means of an optic fiber or with a smaller hand piece have been introduced. This offers the possibility to treat both small and large lesions.

Apart from calcineurine inhibitors that proved to enhance the efficacy of UV treatment in the majority of the published studies, other topical treatments have been associated with 308 nm MEL phototherapy in vitiligo and may variably increase its effectiveness Good results have been reported with topical tacalcitol (148) in a half-body comparison study; topical khellin has been evaluated in association with 308 nm MEL, and the results were better than those with excimer light alone (149). The cost of these devices is lower than that of the lasers and the maintenance is less frequently required, with a favorable cost–benefit ratio. The short- and long-term side effects should be the same as conventional NB UVB. Never-

theless, the carcinogenic risk of MEL 308 nm has been questioned in a recent paper (150) due to the presence of erythemogenic and carcinogenic wavelengths, and the use of appropriate filters to improve the risk–benefit ratio has been suggested; the data were obtained on irradiated cultured T cells on which apoptosis and cyclobutane pyrimidine dimers were measured. These findings need to be confirmed with clinical studies on humans, and, in any case, the risks are counterbalanced by the fact that the irradiation strength of the excimer lamp is stronger, and thus, less irradiation time is required for treatment, with the comparative advantages of fewer treatments and a lower cumulative UVB dose.

Excimer laser/lamp vs. NB UVB, excimer laser vs. /excimer lamp. In the last few years, studies have been published that compare the excimer laser or light with conventional NB UVB phototherapy. A first paper (151) compared the efficacy of the laser with that of the lamp and also with NB UVB in psoriasis. There were no significant differences between the excimer lamp and the laser, but both were more effective than NB UVB, as long as an aggressive protocol was used and also the cumulative UV dose was reduced as compared with NB UVB. In vitiligo, a first study demonstrated the greater efficacy of the 308 nm excimer laser over NB UVB, with more rapid and profound repigmentation induced by the laser. The comparison was made on 23 patients with symmetrical vitiligo patches treated with the 308 nm laser or NB UVB on a 2-week session schedule for a maximum of 20 treatments (152). A similar, but multicenter study, has been published by our group comparing the therapeutic effectiveness of MEL 308 nm and conventional NB UVB phototherapy in 21 vitiligo patients. Symmetrical vitiligo lesions on one body side were treated twice weekly for 6 months, while NB UVB was used to treat lesions on the opposite site. At the end of the study, 37.5% of lesions treated with 308 nm MEL and only 6% of lesions treated with NB UVB achieved an excellent degree of repigmentation (153).

Both these comparative studies suggest that treatment with the excimer laser or MEL 308 nm may allow repigmentation within a shorter period of time than NB UVB phototherapy does, while limiting exposure to only selected areas. Also, the rapid onset of repigmentation may play an important role in supporting patients' motivation and compliance, and, importantly, the cumulative UV dose at the end of the treatment is significantly lower than with conventional NB UVB. Le Duff *et al.* (154) published, in 2010, a study on 20 vitiligo patients where the aim was to compare the effects of 308 nm excimer laser treatment and the excimer lamp treatment: the design was that of a randomized monocentric study, where one lesion was treated with the 308 nm excimer laser and its counterpart with the 308 nm excimer lamp. The results showed that the 308 nm excimer lamp and laser showed a similar efficacy in treating vitiligo. For the same fluence, the lamp induced more erythema, suggesting photobiological differences between the two devices. This is not a surprise if the output of the sources is compared from a photophysical point of view, looking at the spectrum: the laser exhibits more 'pure' monochromaticity; nevertheless, these differences do not seem to influence the biological effect and the therapeutic outcome. The types of repigmentation pattern obtained with NB UVB phototherapy or targeted phototherapy using a 308 nm excimer laser were compared in a recent study (89) These patterns, according to the location, age, duration of lesions and speed of response, showed similarities in both the NB UVB- and the excimer laser-treated groups, the most frequent being the perifollicular type in both groups treated with NB UVB or excimer laser, followed by marginal, diffuse and combined; nevertheless, the marginal pattern was more frequent in the early response group. The authors did not find significant differences in the repigmentation pattern according to the location of lesions, patient's age or duration of lesions.

Mercury arc lamps. Different devices equipped with high-pressure mercury arc lamps are now available for targeted phototherapy. Usually, the light is delivered to the skin by means of an optic fiber. Because of the emission spectrum of these lamps, this is also referred to as 'targeted broad-band UVB'. Interesting results have been reported in vitiligo, with a high-pressure mercury lamp capable of emitting either UVB or UVA (Dua-Light, Thera Light Inc., Carlsbad, CA, USA). The UVB spectral output of this light source includes peaks at 302 and 312 nm, with an average weighted erythemal wavelength of 304 nm. The high output of this device allows irradiation of 100 mJ/cm^2 of UVB to take place within approximately 0.7 s. Ultraviolet radiation is delivered through a square aperture sized 1.9 cm × 1.9 cm. Asawanonda *et al.* (155) reported their experience on six patients. Twenty-nine vitiligo lesions were treated with targeted, BB UVB phototherapy. The treatments were carried out twice weekly for 12 weeks. Some degree of repigmentation occurred in all subjects. The onset of repigmentation was as early as 3 weeks of treatment in some subjects. According to these results, targeted BB UVB could be an efficacious and safe modality for the treatment of localized vitiligo. The same authors, in a subsequent study, reported that there were no differences between targeted BB UVB and targeted NB UVB (non-laser), obtained with the adjunctive filtration of the beam (156). In the case of non-laser devices emitting UVB, the results may differ from those that can be obtained with a monochromatic source such as an excimer laser. A possible advantage is that these mercury arc lamps are less expensive and require minor maintenance as compared with the excimer devices. Another device, similar to that mentioned above, has been used, with interesting results in psoriasis (157), and a recent report has been published on its use in vitiligo (158). The B Clear-Targeted PhotoClearing System (Lumenis Inc., Santa Clara, CA, USA) uses a UVB lamp to deliver targeted BB UVB, filtered incoherent pulsed or continuous UVB light at 290–320 nm. Peak irradiance occurs between 310 and 315 nm. The 16 mm × 16 mm spot size emits a pulse width of 0.5–2.0 s with a fluence range of 50–800 mJ/cm^2. The authors reported on a group of 12 patients with good results on vitiligo lesions located on the face, to a lesser degree on the trunk and with no response in acral lesions; there were minimal adverse effects that did not require discontinuation of treatment. A system, named 'UVB microphototherapy' (300–320 nm, peak at 311 nm), with a focused beam of BB UVB light with a 311 nm peak, two to three times per week, has been reported as an alternative to NB UVB and PUVA for localized vitiligo. From 734 patients, 510 (69.48%) achieved > 75%

repigmentation of treated areas and 112 were totally repigmented; the other 155 patients (21.12%) achieved 50–75% repigmentation and 69 (9.40%) showed < 50% repigmentation (159).

Plasma lamps. A phototherapy device, the MultiClear® system (Cure Light Ltd, Gladstone, NJ, USA), has been introduced recently (86). It is based on Selective Photo Clearing (SPC™, Cure Light Ltd, Gladstone, NJ, USA), a proprietary technology generating, by means of high-power plasma light source, emission of different wavelengths: 296–315, 360–370 and 405–420 nm (blue light PDT). The system allows to select a high intensity of UVB, UVA and a blend of targeted UVB and UVA1 delivered by means of a flexible light guide, with a treatment spot of 23 mm × 23 mm: UVA1 and UVB could act synergistically to induce repigmentation in vitiligo according to the producer's specifications (unpublished data). There is a report on a small number of vitiligo patients (160), where the use of the device led to repigmentation, but phototherapy was combined with topical fluticasone. The efficacy of this device has to be demonstrated with clinical studies in vitiligo and in other indications.

Clearly, all these sources do not have the peculiar advantages of the high-intensity monochromatic NB UVB peaking at 308 nm; nevertheless, light is selectively targeted to the skin, and this is an advantage as compared with whole-body NB UVB. The spectra of the different sources may be slightly different from case to case, with a variable presence of the shorter more erythemogenic UVB wavelengths. In some of these devices, the emission is filtered to eliminate erythemogenic radiation, but this can considerably reduce the power density.

Conclusions

Phototherapy remains an essential treatment option for vitiligo. The treatment of vitiligo with phototherapeutic modalities may halt disease progression and induce significant repigmentation in vitiligo. Most treatment centers nowadays consider NB UVB phototherapy as the first-line treatment for generalized vitiligo because of its relatively good efficacy and excellent tolerance as compared with systemic and topical PUVAtherapy. The efficacy of NB UVB, in vitiligo, can be further enhanced with associated topical or systemic treatments. Many investigations have documented the benefits of targeted phototherapy systems, including 308 nm xenon chloride excimer lasers, which offer several advantages over conventional NB UVB units that irradiate both diseased and normal skin, whereas targeted sources deliver high-intensity light exclusively to depigmented areas. Rapid therapeutic responses have been reported after targeted phototherapy that may contribute to the reduction of the cumulative UV dose.

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