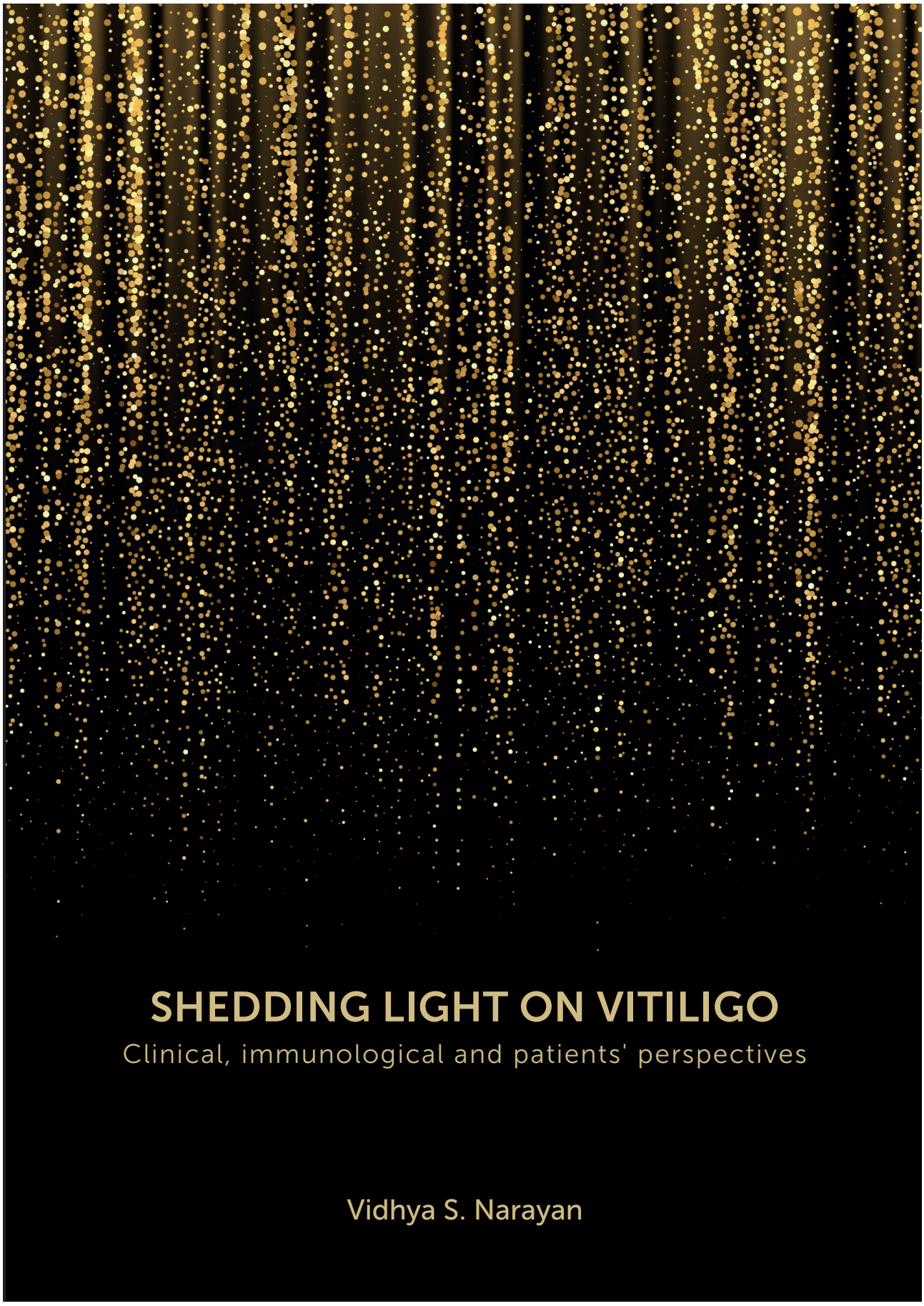

SHEDDING LIGHT ON VITILIGO

Clinical, immunological and patients' perspectives

Vidhya S. Narayan

Shedding light on vitiligo

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Vidhya Shankar Narayan

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Shedding light on vitiligo

Clinical, immunological and patients' perspectives

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Promotor:	prof. dr. R.M. Luiten	AMC-UvA
Copromotores:	dr. A. Wolkerstorfer	AMC-UvA
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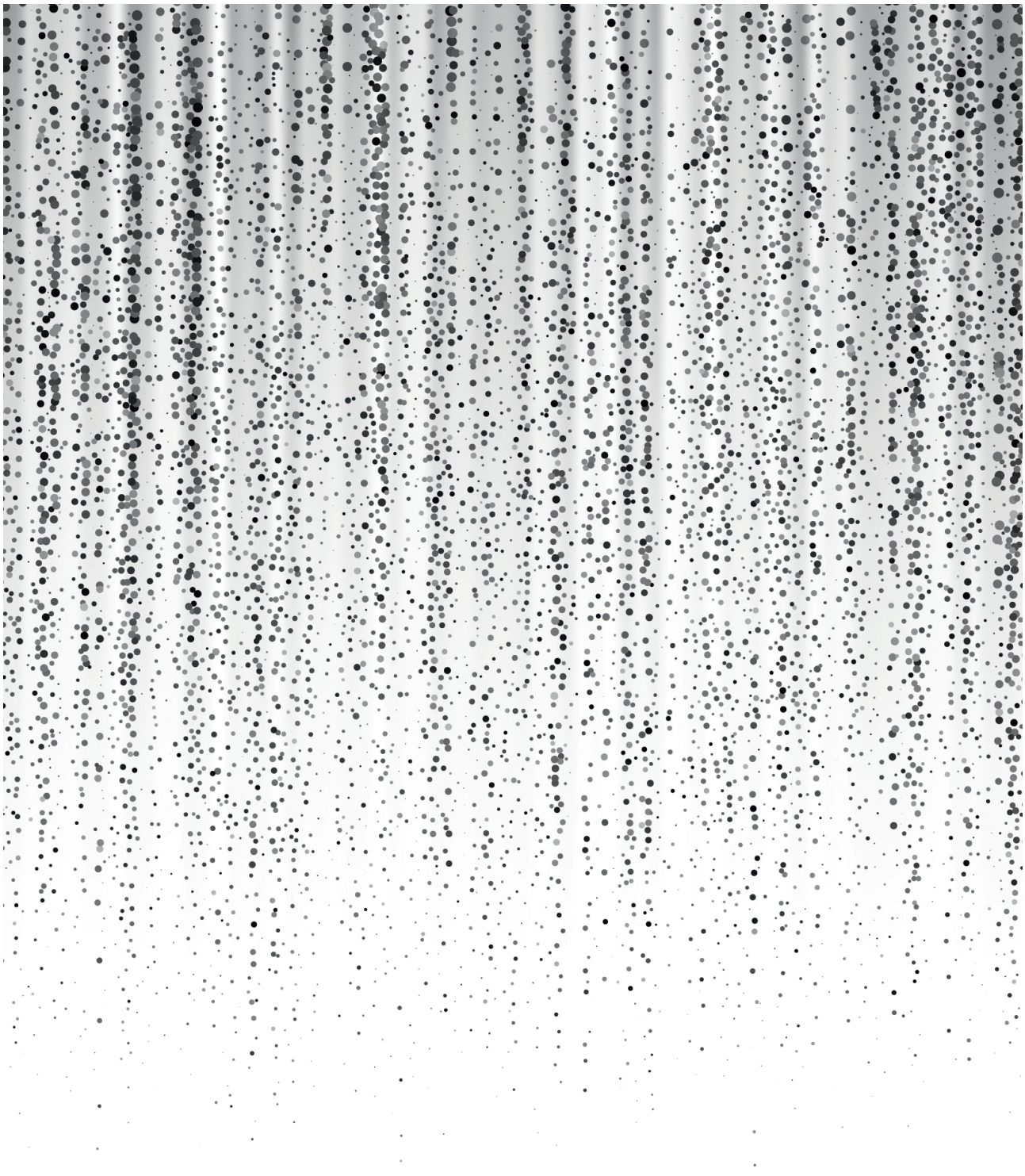


CHAPTER 1

General introduction

1

GENERAL INTRODUCTION

Vitiligo is the most common depigmenting skin disease affecting approximately 0.5-1% of the world population, regardless of sex, ethnicity or skin type.[1] The oldest records of this disease were found in ancient Indian books (1500 to 1000 before Christ) with descriptions of the Sanskrit word 'kilas' meaning 'which throws away colour'.[2] As mentioned in the ancient description, vitiligo is characterised by white patches due to loss of pigment caused by the destruction of melanocytes. The disease may appear at any age, however the average onset is usually before the age of 30 years.[2] Although problems like itch and sunburns are common, vitiligo generally does not lead to physical complaints.[3]

Quality of life impairment and disease burden

Vitiligo is sometimes considered to be merely a cosmetic problem, however its psychosocial impact and social stigmatization in various countries must not be neglected [4]. The disease is strongly associated with an impaired quality of life, as many of the patients feel stressed and stigmatized by their condition.[3,5] In addition, multiple studies have investigated this significant negative effect on the quality of life[4–6] describing several contributing factors such as dark skin type[7,8], vitiligo extent[9,10] and localisation on exposed areas.[11] Furthermore, other studies have shown that vitiligo is associated with a substantial disease burden[8,12], which also may be related to the disease extent and localization of the vitiligo lesions. The importance of treatment is often underestimated due to the lack of recognition of the impact of the disease for patients.

Diagnosis and measurement instruments

The diagnosis of vitiligo is usually straightforward, due to the typical clinical presentation with depigmented lesions. However, sometimes vitiligo is confused with other hypo- or depigmenting disorders, such as naevus depigmentosus, pityriasis alba or piebaldism. This last-mentioned disease, is an autosomal dominant depigmenting disorder, characterised by stable white patches usually located on the forehead (with poliosis) and the shins, present at birth. A distinction between hypopigmented and depigmented lesion can be made with aid of a (handheld) Wood's lamp. This lamp emits ultraviolet A (UVA) light and can be used to identify depigmentations that may not be visible to the bare eye, especially in lighter skin types.[13] In addition, Wood's light can be useful to improve the contrast between normally pigmented skin and depigmented skin when the lesional borders are not clear. Moreover, to monitor therapy response in daily practice or for study purposes, the Wood's lamp is used to capture the lesions on photograph. If needed, the UVA camera can additionally be used, to determine the areas of pigment loss more accurately.

Measurement of the degree of depigmentation is done in clinical trials as well as in daily practice, to assess the disease severity, disease progression and/or treatment response. Currently, no consensus exists as to which standardized outcome measure should be used to measure the degree of depigmentation in vitiligo. Several instruments exist for measuring the body surface area (BSA) of the lesional depigmented skin. One of the most frequently used measuring tools is the Vitiligo Area Scoring Index (VASI).[14,15] Komen et al. demonstrated that this is a reliable and responsive instrument to assess the degree of depigmentation.[16] However, authors describe that caution is needed for its use in daily practice, as the smallest detectable change (SDC) in depigmentations is relatively large. In 2016, the Vitiligo Extent Score (VES) was introduced as a new user-friendly clinical scoring instrument.[17] This tool is a validated and feasible instrument that can be used to assess the vitiligo involvement in 19 different areas of the body, with a slightly better SDC than the VASI.[17]

Classification

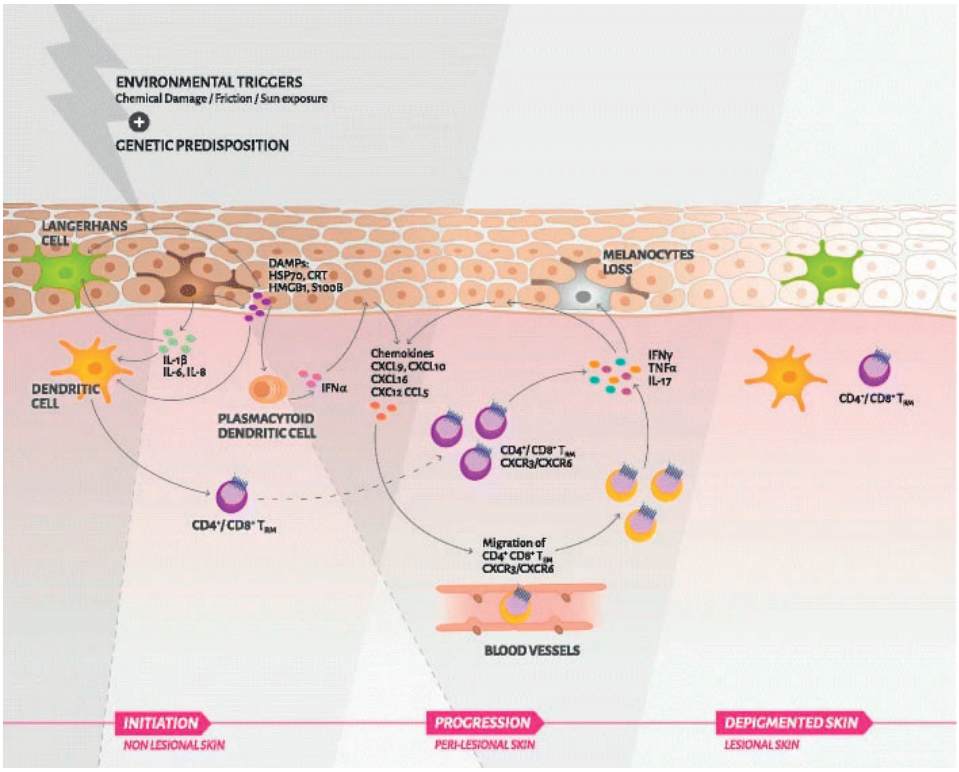
Two major subtypes of the disease can be distinguished: non-segmental and segmental vitiligo.[18] Non-segmental vitiligo (NSV) is the most common subtype, characterised by bilateral and symmetrical distribution of the depigmented macules. NSV often follows a slowly progressive course, with unpredictable periods of disease progression and remission. On the contrary, segmental vitiligo (SV) usually develops in a limited area and has a unilateral distribution, which does not cross the midline.[19] After an initial period of rapid spreading, SV generally spontaneously stabilizes.[20] Altogether, based on the significant clinical differences, this suggests that different underlying pathophysiology pathways are involved in both subtypes.

Pathophysiology

The pathophysiology of both subtypes is considered to be different and is complex, however the driving mechanisms have been gradually clarified over the past years. NSV is an autoimmune disease, that involves destruction of melanocytes due to attack of auto-reactive melanocyte specific cytotoxic CD8 T cells.[21] It is found to be closely associated with other autoimmune diseases such as thyroid disease, rheumatoid arthritis and alopecia areata.[22] In addition, the auto-immune pathophysiology has been further confirmed by large genome-wide association studies.[23,24] These studies identified multiple genetic loci that contribute to the risk of developing vitiligo. The vast majority of these loci are associated with immune regulation and several others regulate functions of melanocytes.

The initial cause of the melanocyte destruction in NSV is not certain, however many aspects including genetic predisposition and environmental factors may contribute to triggering oxidative stress (Figure 1). This in turn leads the release of danger-associated molecular patterns, inflammatory cytokines and melanocyte auto antigens.[25–27] Subsequently, this results in activation of the innate immune system[28]; attracting inflammatory dendritic cells (DCs).[29,30] These DCs then take up melanocyte antigens (i.e. antigen- presenting cells), migrate to the lymph nodes and present the antigens, which not only primes (melanocyte specific) T cells, but also induces B cell and antibody responses against melanocytes. After priming of the naïve T cells into melanocyte specific CD8 T cells, these cells migrate towards the skin.[27] A subpopulation of these CD8 T cells does not recirculate, but differentiates to resident memory T cells (T_{RM}) and remains in the epidermis of the skin.[31] Eventually, these T_{RMs} can yield an immune based destruction of melanocytes resulting in vitiligo.[27,32] Since these cells stay present in the skin, they can prevent repigmentation by continuously eliminating melanocytes, possibly explaining why relapses after treatment occur at same location as those previously affected.[33] In addition, these T_{RMs} can produce pro-inflammatory cytokines such as interferon gamma, Tumour Necrosis Factor alpha and interleukin-17, which affect the keratinocytes (Figure 1).[34] This induces chemokine (i.a. CXCL9 and CXCL10) production by keratinocytes, via the intracellular Janus kinase – signal transducer and activator of transcription proteins pathway, resulting in more recruitment of cytotoxic T (effector memory, T_{EM}) cells towards the skin.[34] Subsequently, these T_{EM} cells fulfil a similar function, resulting in amplification of the recruitment loop and maintaining melanocyte destruction. Furthermore, regulatory T cells (Tregs), seem to play an important role as well. Tregs inhibit proliferation and activity of CD8 cytotoxic T cells, generally resulting in suppression of autoimmunity.[35] It however appears that these cells are impaired in vitiligo, allowing an auto-immune response.[36] Summarizing, many different factors contribute to the onset and progression of NSV.

Figure 1. **Schematic reproduction of the immunopathophysiology of vitiligo** (figure derived from Boniface et al. 2017)[32]



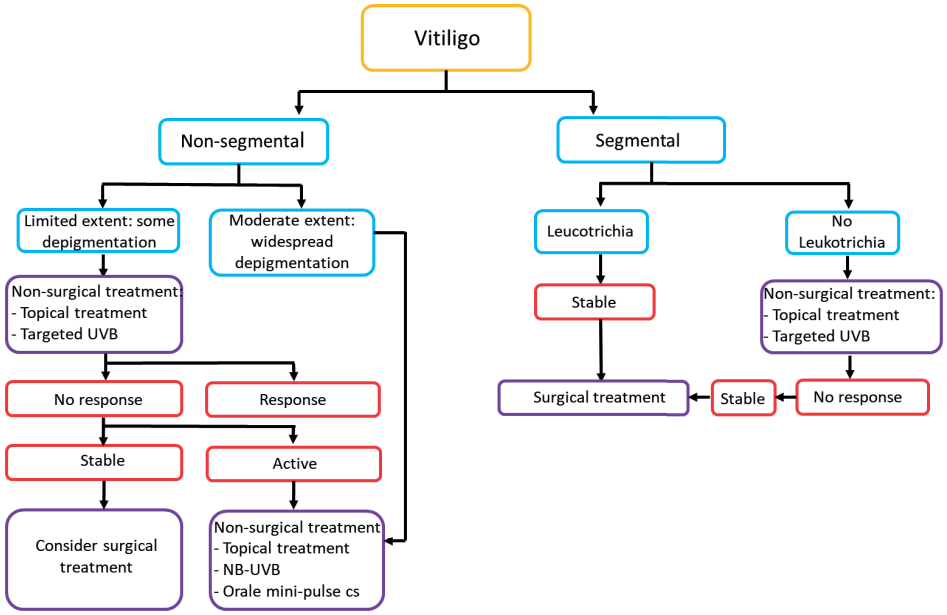
In SV, several underlying causes have been hypothesized to play a role in the pathogenesis, including neurogenic mechanisms, somatic mosaicism and microvascular skin homing.[37,38] Associated autoimmune diseases in patients with SV are reported less frequently than in NSV.[39] The primary explanation for the 'neural hypothesis' is based on the statement that SV has a dermatomal distribution.[40] However, close observation has shown that the distribution is generally not dermatomal[37,38], leaving this theory without adequate evidence. Another postulated clarification is the somatic mosaicism, involving a mutation in an embryonic melanocyte, resulting in differentiating of this melanocyte into functional epidermal melanocytes. Subsequently this leads to a unilateral distribution with distinct patterns of cutaneous mosaicism (e.g. along the Blaschkoid lines). Furthermore, it has been suggested that the midline delineation of unilateral lesions could be based on the migration pattern of cytotoxic T cells that possess homing receptors with a unilateral homing code.[41] However, a combination of the abovementioned hypotheses, could also be the case. In summary, the exact underlying causes of SV still remain to be fully elucidated.

1

Treatments

The choice of treatment is dependent on several factors, including the subset of vitiligo, the disease extent, the location, the skin type, the burden of disease and disease activity. In Figure 2 the treatment algorithm for vitiligo is presented, based on the European guideline developed by the Vitiligo Guideline Subcommittee of the European Dermatology Forum in 2013.[42] Current treatments include topical agents, phototherapy and surgical techniques which aim to arrest progression, improve melanocyte proliferation and stimulate repigmentation.

Figure 2. Treatment algorithm of vitiligo (figure derived from Narayan et al. 2020)[43]



Topical treatment

Topical treatment consists of potent topical corticosteroids (such as fluticasone propionate 0.05% cream or mometasone furoate) and calcineurin inhibitors (tacrolimus 0.1% ointment or pimecrolimus 1% cream). These topical therapies are widely used as first-line treatment for a limited vitiligo extent due to its local anti-inflammatory and immunomodulating effects.[42] Topical corticosteroids (TCS) are predominantly used for non-facial lesions and show the best treatment responses in face/neck region[44,45] and in recent lesions.[46] Well-known side effects include skin atrophy, striae and acneiform eruptions.[42] To minimize the risk of side effects, daily application in a discontinuous scheme (e.g. 4

consecutive days, followed by 3 days off) is recommended.[47] As first-line approach for lesions on the head and neck topical calcineurin inhibitors (TCI) are proposed, due to its proven efficacy in these regions and favourable side effect profile.[48,49]

Phototherapy

Currently, narrow-band ultraviolet B (NB-UVB, 311 nanometre) phototherapy is the main treatment modality for inducing repigmentation in active and/or widespread NSV.[50] In addition to its stimulating effect on melanocyte proliferation, NB-UVB can inhibit disease activity via immunosuppression.[51] Its use was first reported by Westerhof et al.[52] and so far, it is preferred over psoralen ultraviolet A (PUVA) based on its increased efficacy and more favourable side-effect profile.[48,53] For smaller areas of involvement UVB treatment can be given with targeted phototherapy (such as with excimer lasers or lamps, 308 nanometre). Side effects of both whole-body NB-UVB and targeted UVB include skin burning, sensitivity and skin thickening.

In daily practice, treatment with UVB phototherapy is usually given twice or three times a week, starting with a safe (i.e. 200 mJ) low dose which is gradually increased when asymptomatic pink erythema occurs and continued as long as there is ongoing repigmentation over a period of maximum 1-2 years.[42] To date however, there are no established guidelines as to what the optimal treatment regimen, maximum dosing and duration is for NB-UVB phototherapy, and practice varies widely.

Furthermore, the overall treatment responses to NB-UVB also varies between patients. In addition, variable repigmentation responses depending on the affected body region can be found. Facial vitiligo is known to have a better response to treatment than vitiligo lesions on other body parts.[54] Moreover, acral areas, such as lesions on the hands or feet, are considered to be most resistant to therapy. These differences in NB-UVB response between specific anatomical areas of the body have not been fully characterized.

Surgical treatment

Several methods of autologous skin transplantation are available as treatment for repigmenting stable (non-segmental and segmental) vitiligo lesions.[55,56] These surgical methods can roughly be divided into tissue grafting and cellular grafting. In tissue grafting the donor skin is directly transplanted into the depigmented area. The three major techniques of tissue grafting include punch grafting, epidermal blister grafting and split-thickness grafting.[57] Punch grafting is a commonly used and simple technique, with an average donor-recipient expansion ratio of 1:5[58], however it is time consuming for larger lesional areas and often results in a cobblestone appearance of the recipient site.[59] Suction blister grafting and split-thickness

grafting are reported to have the highest repigmentation and a good color match.[59] However, these techniques are not suitable for larger lesions, due to the low expansion ratio of the donor skin (expansion ratio 1:1).[60,61] In addition, repeated surgical operations are needed for treating large surface areas.

Contrastingly, in cellular grafting the donor skin is dissociated into single cells through enzymatic digestion, resulting in a suspension which in turn is transplanted onto the recipient site. Cellular grafting techniques include non-cultured cell suspension transplantation (NCST) and cultured melanocyte transplantation (CMT). These methods are preferred when treating larger surface areas due to the small amount of donor skin needed.[62] The donor to recipient (DR) expansion ratios and outcomes reported in studies with cellular grafting vary widely and to date, no overview or guideline exists on which ratio to use. Furthermore, little evidence is available on the correlation of expansion ratios with the repigmentation success rate. In summary, the above-mentioned current tissue and cellular grafting methods have various limitations concerning the size of transplanted skin and the ease of use. Table 1 shows an overview of the current transplantation methods, each with its specific advantages and disadvantages. There is an unmet need for a simple, reliable and effective transplantation technique for large skin surface areas. The Meek micrografting technique is a tissue grafting method, which has been used for large areas in acute burns surgery.[63] This technique could be effective as well in the surgical treatment of vitiligo, however this has never been performed before.

Table 1. Donor-recipient expansion ratios, repigmentation response percentages, advantages and disadvantages of current transplantation techniques.

Transplantation method	Donor : recipient expansion ratio	Repigmentation > 90% achieved	Advantages	Disadvantages
Punch grafting	1:5	45.76%	Simple in use, not expensive	Cobblestone appearance, milia and colour mismatch
Suction blister grafting	1:1	61.68%	Not expensive, no scarring donor site	Perigraft halo, colour mismatch
Splits skin graft	1:1	72.08%	Good colour match not expensive	Graft contracture, perigraft halo, scarring donor site
Non-cultured cell suspension transplantation	Variable from 1:1 to 1:10	47.51%	Uniform repigmentation, large expansion ratio	Scarring donor site, expensive kit, longer lasting pain and erythema
Cultured melanocyte transplantation	Variable from 1:1 to 1:100	56.82%	Large expansion ratio	Specialised laboratory required, time consuming

Oral steroid mini-pulse therapy

Oral mini-pulse (OMP) therapy is considered in patients with rapidly spreading NSV, since these patients may show an unsuccessful response to topical treatment or phototherapy.[42,64,65] OMP refers to the discontinuous administration (cyclical pulsed dose) of corticosteroids in lower doses than usually given with pulsed therapy.[42,66] The most commonly given treatment regimens consist of low-dose (i.e. 2 -5mg) dexamethasone or betamethasone for 3 to 6 months on 2 consecutive days of the week.[20] Potential side effects include acne, weight gain and hypertrichosis. These low dose OMP steroids are often given to arrest disease activity and progression, but are generally not effective as monotherapy in repigmenting stable vitiligo.[42]

Combination therapies

Several therapies have been combined with the aim to enhance repigmentation by means of targeting different pathogenic aspects of the disease. In cases of rapidly spreading vitiligo, OMP steroids are often added to the NB-UVB treatment, demonstrating an earlier arrest of depigmentation progression than OMP monotherapy.[67]

Moreover, in the Amsterdam University Medical Centre the previous treatment for NSV was NB-UVB twice a week, but this had changed to an intensified schedule with NB-UVB three times a week in combination with topical treatment. This change was implemented in 2011 due to recent studies showing that the combination of topical therapy and NB-UVB was superior to monotherapy.[68,69] Another recent study supports this finding as well.[70] Moreover, combination therapies of TCS, TCI and NB-UVB are recommended in current clinical guidelines.[71]

In general, the earliest onset of repigmentation after starting therapy takes approximately 3-4 months to become apparent since it takes time for melanocytes to proliferate, migrate and produce pigment. Aside from the delay in visible repigmentation during therapy, it is uncertain whether a patient will ultimately respond to therapy. Biomarkers of disease activity can be of added value to determine whether it is useful to start therapy for each individual patient. In addition, an early on treatment biomarker would help to predict if continuation of therapy is beneficial or not. Furthermore, it may also lead to discovering possible targets for future treatment.

Treatment outcomes and patient perspectives

To date, many different outcomes have been measured in vitiligo research leading to difficulties in comparing data and performing meta-analyses.[15] In 2015, an international consensus has been reached on a core outcome set for vitiligo

research.[72] This core outcome set includes measurement of repigmentation, side effects and maintenance of gained repigmentation. Since vitiligo also is a psychosocially devastating disease, subjective perception of the disease, such as patients' perspective on treatment outcome, is considered important as well. For interpretation of this treatment outcome, little evidence is available for example on the thresholds of successful repigmentation. In previous Cochrane reviews on vitiligo, success was arbitrarily defined as more than 75% repigmentation.[48] But why was 75% chosen as the limit? Until now, little is known on patients' perspective regarding successful treatment in terms of repigmentation. In addition, these success percentages could differ depending on the location.

Furthermore, it is known that a considerable number of patients does not sufficiently benefit from treatment. To improve these suboptimal results several new therapies are being developed and investigated. However, for the clinical implementation in daily practice, it is essential to understand the patients' need for these new treatment modalities. What are these new therapies worth to patients? And how satisfied are patients with the current treatments? More knowledge of these factors could be of substantial value to physicians, policy-makers and pharmaceutical companies. Moreover, if there is a demand for novel therapies from patients, this could be dependent on various factors, such as disease burden and location of the vitiligo lesions.

AIMS AND OUTLINE OF THIS THESIS

The overall aim of this thesis is to *shed light on* different clinical aspects of vitiligo in order to obtain a better understanding of the current state of affairs which may ultimately contribute to future improvement of vitiligo therapy and research. We aimed to:

- Assess the responses to topical treatment and NB-UVB phototherapy from clinical, cellular and patients' point of view.
- Provide an overview of the current surgical treatment results and explore new surgical possibilities
- Evaluate patient perspectives on current therapies, treatment outcome and disease burden.

In **chapter 2**, we conducted a prospective exploratory trial in order to gain more insight on potential biomarkers for treatment response in NSV patients. We evaluated the association between the changes within cell types and the clinical response to standard of care treatment with a potent topical corticosteroid alone and in combination with NB-UVB therapy.

Since current treatment results often vary between patients and variable repigmentation responses between different body regions are seen, we performed a study to provide an overview of these repigmentation responses. In **chapter 3** we evaluate the clinical response to standard of care vitiligo therapy consisting of NB-UVB treatment (with or without topical therapy and/or OMP systemic steroids) in several specific body regions as predefined by the VES. Furthermore, we aimed to assess demographic features such as sex, age, disease duration, disease activity and skin type in relation to the treatment response.

To date, no consensus exists as to what the optimal treatment regimen is for NB-UVB therapy. In order to determine what the most satisfactory and effective treatment regimen is for the patient, we explored whether a combination of topical therapy and NB-UVB three times weekly would be superior to NB-UVB alone two times weekly by using patient reported outcomes in **chapter 4.**

In the surgical treatment of stable vitiligo, several donor to recipient expansion ratios are used with the cellular grafting techniques. In **chapter 5** we performed a systematic review to provide an overview of the various expansion ratios used during NCST and CMT. Furthermore, we aimed to identify whether expansion ratios affect the repigmentation success rates and colour matching to the non-lesional

surrounding skin.

Due to current surgical therapies lacking the capacity of easily treating large depigmented areas with high success rates, we explored the possibility of using a novel transplantation technique for depigmented lesions. In **chapter 6A** we present the results of the first patient that we treated with the Meek micrografting technique. Since successful results were found in this patient, we continued to evaluate the outcomes of this novel technique, in a case-series study in **chapter 6B.** Currently patient perspectives are increasingly important in the medical field, which is why the last part of my thesis focusses on patients' view on treatment and outcomes. In **chapter 7** we aimed to evaluate the definition of successful repigmentation for facial and non-facial lesions from patients' perspective by carrying out a prospective cross-sectional questionnaire study.

And lastly, in **chapter 8,** we address the need for novel therapies for vitiligo. Our aim was to assess patients' view on current treatments and evaluate their demand for novel future therapies. Moreover, we aimed to assess demographic features (i.e. gender, skin type and location of lesions) in relation to the primary outcomes on satisfaction of current treatments, disease burden and need for new therapies.

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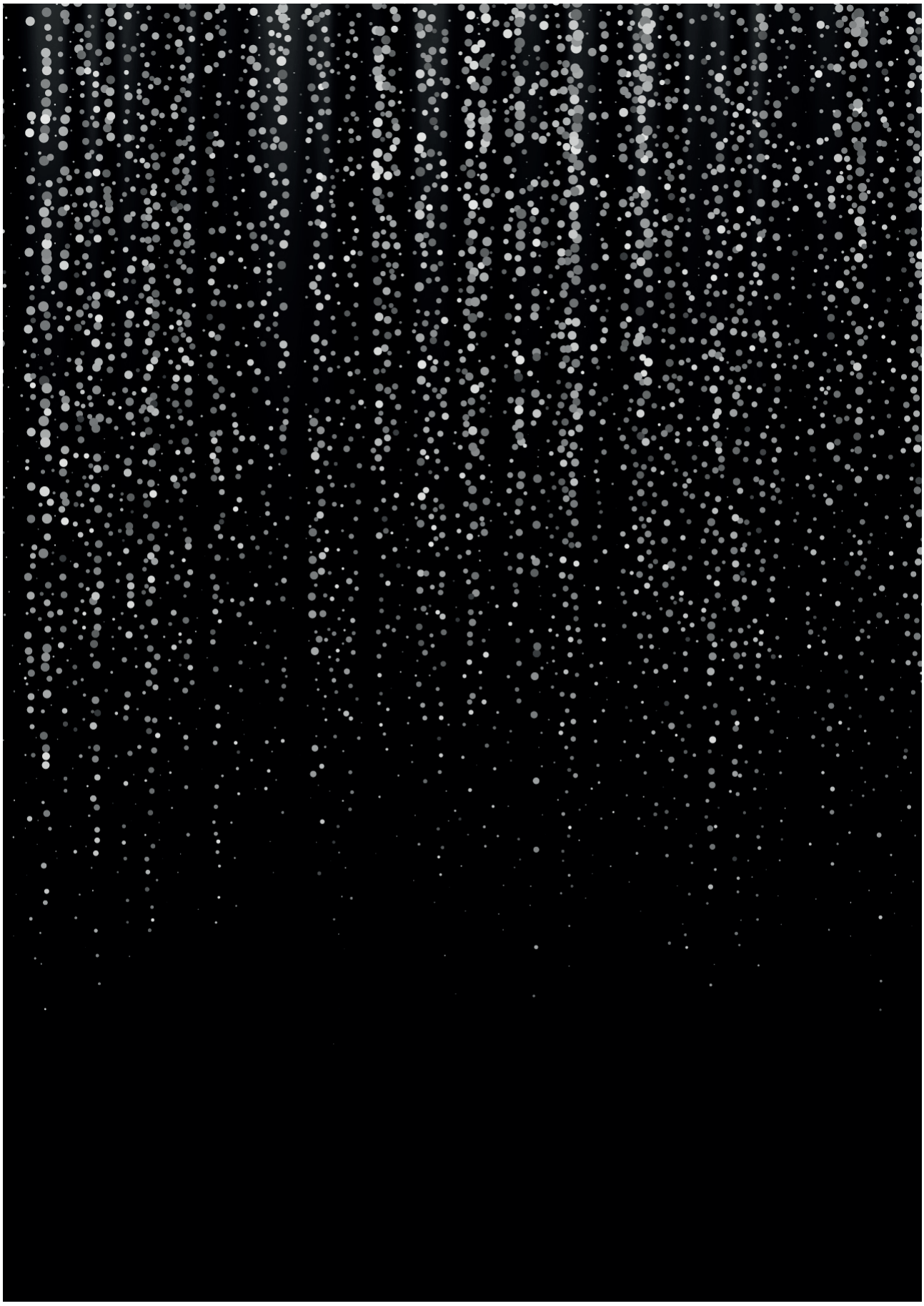
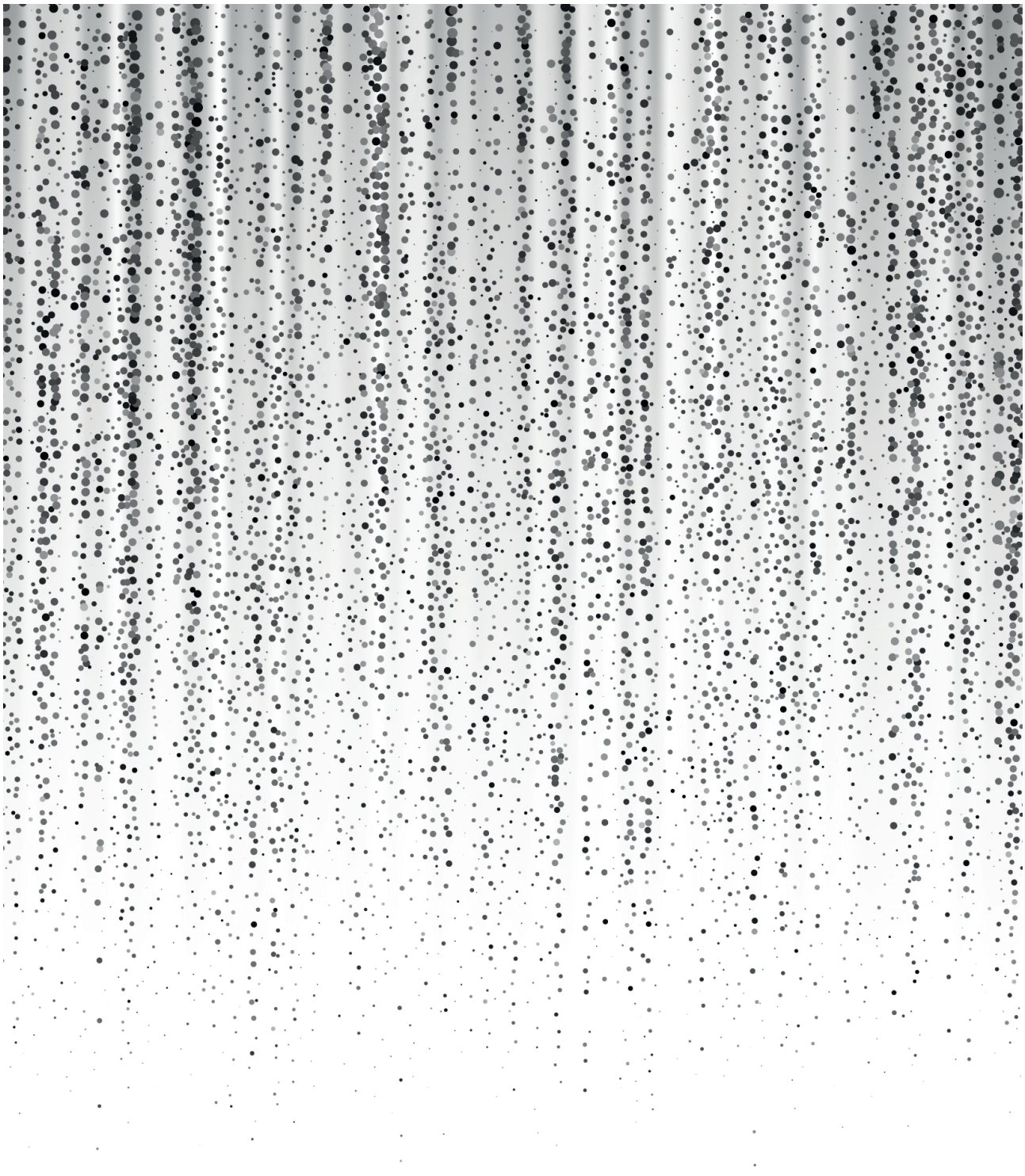


PART I

EVALUATION OF TOPICAL AND PHOTOTHERAPY RESPONSES



CHAPTER 2

Early cellular and clinical responses to treatment in non-segmental vitiligo

Vidhya S. Narayan
Saskia Chielie
Nathalie O.P. van Uden
Wouter Ouwerkerk
Martin-Alexander Schneider
Rasmus B. Kjellerup
Christian Loesche
Malika Hanser
Elizabeth McNamara
Marcel W. Bekkenk
A. Wolkerstorfer
Rosalie M. Luiten

Manuscript in preparation

ABSTRACT

Background

The treatment of non-segmental vitiligo (NSV) remains a challenge. Aside from a delay in visible repigmentation during therapy, it is uncertain whether a patient will ultimately respond to treatment. An early on treatment biomarker could help to predict the response to therapy.

Objective

The aim of this study was to evaluate the association between early changes within various immuno-cell types (T cell subsets and NK cells in blood and skin) and the clinical response to treatment.

Methods

This prospective exploratory study was conducted in a cohort of NSV patients starting with standard of care treatment consisting of topical therapy alone or in combination with narrowband UVB phototherapy. Evaluation of the depigmented surface area was conducted with the Vitiligo Extent Score at baseline (V1), 3 months (V2) and 6 months (V3) after starting therapy. Tissue sampling of blood and lesional skin biopsies were performed at baseline and 3 months after therapy. Tissues were analysed for changes in CD3, CD4, Granzyme B^+ $(GrB)^+CD4$, CD8, GrB^+CD8, GrB^+CD8^-, T_{RM}, Treg, NK and GrB^+NK cells at V2 in relation to the repigmentation at V3.

Results

A total of 30 patients completed the study. Our results showed that an early increase of GrB^+CD8^- T cells in skin was significantly correlated to a higher repigmentation after 6 months of treatment. In blood, the interaction of this same cell type (GrB^+CD4 T cells) between V1 and V2 showed significant relations with repigmentation at V3. Moreover, similar relations with repigmentation were found in skin between GrB^+NK cells percentages at V1 and V2. Other early changes in the analysed cell types did not show any significant relations with the treatment outcome.

Conclusions

This study evaluated early changes in cellular immunity in relation to repigmentation after standard of care therapy. Both adaptive (GrB^+CD4 T cells) and innate (GrB^+NK cells) early detectable immuno-changes could play a role in indicating the degree of treatment response after 6 months of therapy. For future implementations of an early on treatment biomarker, however, larger prospective studies are required to confirm these findings and to gain more insights on other potential biomarkers.

INTRODUCTION

Vitiligo is an auto-immune disease of the skin characterized by the development of white macules due to CD8 T cell mediated loss of melanocytes. It is the most common depigmenting disorder affecting approximately 1% of the world population regardless of sex, ethnicity or skin type.[1] The disease usually follows an unpredictable course with periods of disease progression and remission.[2] Even though not life threatening, multiple studies have shown that vitiligo can have a severe negative impact on the psychosocial wellbeing and the quality of life.[3,4]

So far, no curative therapies are available for vitiligo. Current treatments include topical agents, phototherapy and surgical techniques which aim to improve melanocyte proliferation and stimulate repigmentation.[2] To date, the standard of care (SoC) treatment of non-segmental vitiligo consists of localized topical therapy with corticosteroids and/or immunomodulators sometimes combined with narrow-band ultraviolet B (NB-UVB) phototherapy.[5] These therapies are predominantly used for their anti-inflammatory and immunomodulating properties. The earliest clinical changes take approximately 3-4 months to become apparent since it takes time for melanocytes to proliferate, migrate and produce pigment.[6]

There is a crucial need to identify biomarkers, due to the delayed response to therapy and unpredictable nature of the disease. In order to identify these markers, a better understanding is required of the driving mechanisms in vitiligo. The pathophysiology is complex, although the key factors seem to be gradually elucidated over the last decades. The hallmark event is the destruction of melanocytes due to attack of auto-reactive melanocyte specific cytotoxic CD8 T cells.[7] The initial cause of this is not certain, however several aspects including genetic and environmental factors may contribute to triggering oxidative stress. This in turn leads to a melanocyte cell stress response, causing the release of melanocyte auto antigens and danger-associated molecular patterns.[8–10] Consequently, this results in activation of the innate immune system[11]; a higher activity and infiltration of natural killer (NK) cells, type-1 innate lymphoid cells and inflammatory dendritic cells (DCs) are seen in active vitiligo lesions.[12,13] These DCs take up melanocyte antigens (i.e. antigen- presenting cells), migrate to the lymph nodes and present the antigens, which not only primes (melanocyte specific) T cells, but also induces B cell and antibody responses against melanocytes. After priming of the naïve T cells into melanocyte specific CD8 T cells, these cells migrate towards the skin.[10] A subpopulation of these CD8 T cells does not recirculate but differentiates to resident memory T cells (T_{RM}). These T_{RMs} express markers associated with tissue residency (i.e. CD69, CD103, CD49a) causing them to remain in the epidermis

of the skin.[14] Subsequently, these T_{RMs} can yield an immune based destruction of melanocytes resulting in vitiligo.[10,15] Since these cells stay present in the skin, they can prevent repigmentation by constantly eliminating melanocytes, possibly explaining why relapses after treatment occur at same location as those previously affected.[16] In addition, these T_{RMs} can produce pro-inflammatory cytokines such as interferon gamma (IFN-γ), tumour necrosis factor alpha (TNFα) and interleukin-17 (IL-17), which affect the keratinocytes.[17] This induces chemokine (i.a. CXCL9 and CXCL10) production by keratinocytes, via the intracellular Janus kinase – signal transducer and activator of transcription proteins pathway, resulting in more recruitment of cytotoxic effector memory T cells (T_{EM}) towards the skin.[17] Subsequently, these T_{EM} cells fulfil a similar function, resulting in amplification of the recruitment loop and maintaining melanocyte destruction. Furthermore, regulatory T cells (Tregs), a subset of CD4 T cells often indicated as $CD25^+$ $FoxP3^+$ or $CD127^{low/-}$ cells, seem to play an important role as well. Tregs inhibit proliferation and activity of CD8 cytotoxic T cells, generally resulting in suppression of autoimmunity.[18] It however appears that these cells are impaired in vitiligo, allowing an auto-immune response.[19] Summarizing, many different factors contribute to the onset and progression of vitiligo.

Throughout the past several years, different cell types in blood and skin have been reported to be linked to the disease activity in vitiligo.[20] Regarding circulating cell types (in blood), two studies have shown that increases in frequency of activated CD8 cytotoxic T cells correlate with disease activity.[21,22] On the contrary, no significant correlations were found between circulating memory T cells and disease activity.[23] A decreased number of peripheral Tregs is found during the inflammatory phase of vitiligo, [19,24] although contrasting data have been reported as well.[25,26] One study evaluated activated and inhibitory NK cell receptors by flow cytometric analysis, showing no differences in NK cell related proteins between patients with active an stable stages of the disease.[27]

Concerning cellular infiltrate in vitiligo skin, the number of CD3, CD4 and CD8 T cells in (peri)lesional active vitiligo skin are found to be increased in comparison to stable vitiligo, which could serve as an indicator of active melanocyte destruction.[28,29] In addition, results of suction blister fluid analysis revealed that active lesional sites had a larger infiltrate of CD8 T cells than non-lesional sites did.[6] The number of CD8 T cells and the CXCL-9 protein concentration in blister fluid seem to be sensitive and specific markers to assess disease activity.[6] Contrarily, the frequencies of $CD8^+$ T_{EM} and $CD69^+CD103^+$ $CD8^+$ T_{RM} measured in (perilesional) skin were independent of disease activity.[30] Interestingly, Lili et al. found increased numbers of both CD8 cytotoxic T cells and Tregs in perilesional skin of patients with active disease. The imbalance in frequencies between these two cell types

and an impaired suppressive effect of Tregs are suggested to be involved in an unrestrained active immune response causing vitiligo progression.[21]

While these studies focused on changes in relation to disease activity, others have reported differences in cellular immunity after treatment with NB-UVB phototherapy. Lin et al. demonstrated that NB-UVB treatment reduces a subset of the circulating memory T cell, i.e. $CD4^+$ and $CD8^+$ central memory T cell (T_{CM}) amounts, suggesting a possible systemic immunosuppressive effect of NB-UVB.[31] In addition, another study has reported an increased number of peripheral Tregs after repeated exposure to NB-UVB, confirming this systemic immunosuppressive capacity.[32] Furthermore, Hegazy et al. examined the effects on Tregs and T_h17 in (peri)lesional vitiligo skin after NB-UVB, resulting in a significant increase of Tregs after treatment and a decrease of IL-17 levels.[33] Moreover, NB-UVB is known to induce the production of the anti-inflammatory cytokine IL-10 in skin[34,35], which may also partly be responsible for its immunosuppressive effects.

To summarize, these studies demonstrate the involvement of various cell types in blood and skin that could be potential indicators of disease activity or treatment response in vitiligo. Aside from the delay in visible repigmentation during therapy, it is uncertain whether a patient will ultimately respond to therapy. Biomarkers of disease activity can be of added value to determine whether it is useful to start anti-inflammatory therapy in case of activity, or transplantation approaches in case of stability for each individual patient. In addition, an early on treatment biomarker would help to predict if continuation of therapy is beneficial or not. Furthermore, it may also lead to discovering possible targets for future treatment.

In order to gain more insight on potential biomarkers involved in local and systemic cellular immunity during treatment in vitiligo, this prospective exploratory study was performed. The aim was to evaluate the association between the changes (with-)in different cell types and the clinical response to treatment.

METHODS

Patients and treatment

This prospective study was conducted in a cohort of vitiligo patients at the Netherlands Institute for Pigment Disorders (NIPD) of the Amsterdam University Medical Centre. The study was performed in accordance with the Declaration of Helsinki and approved by the Institutional medical Ethics Review Committee (NL66309.018.18).

Consecutive eligible adult patients visiting our outpatient clinic between January 2019 and January 2020 were given information about the study. Eligibility criteria included patients with non-segmental vitiligo (NSV), aged ≥ 18 years, with active disease and commencing SoC treatment. Active disease was defined as new lesions and/or signs of disease progression within the past 6 months. Patients were excluded if they had been treated with topical therapy, phototherapy or systemic therapy for vitiligo within the preceding 4-6 weeks. A full overview of the in- and exclusion criteria can be found in the Supplements Table S1.

Patients were recruited only after SoC was prescribed by the treating physician. The decision to prescribe a certain treatment scheme was regardless of study participation. The SoC therapy that patients received consisted of topical immune modulators (tacrolimus 0.1% ointment or pimecrolimus 1% cream once daily for lesions on the face) and/or potent topical corticosteroids (such as fluticasone propionate 0.05% cream 4 times a week for lesions on the body) alone or combined with NB-UVB phototherapy 3 times a week for at least 6 months. Patients starting with NB-UVB treatment, received the equipment comprised of Waldmann UV-100 units with TL-01 lamps (irradiating light with a 311 nm wavelength) at home. The nursing staff of the home care institution (Linde) cared for guidance and explanation of the NB-UVB equipment. This study did not include an investigation medicinal product or (placebo) treatment. Written informed consent was obtained from all patients.

Clinical treatment response

An assessment of the degree of depigmentation was done at baseline (Visit 1 before starting treatment), at 3 months (Visit 2) and 6 months (Visit 3) after starting SoC treatment. The clinical treatment response was expressed as a percentage of repigmentation of the total depigmented body surface area (BSA) at baseline. The overall repigmentation and repigmentation per specific body region were calculated by using the VES (https://www.vitiligo-calculator.com).[36] The VES is a validated and feasible clinical scoring instrument that expresses the extent of the vitiligo lesions in percentage of the BSA.[36] Photographs were taken of all vitiligo lesions at three time points by a professional medical photographer. The photographs were assessed by 2 independent blinded physicians (AW, MB) to determine the VES scores at Visit 1 (V1), Visit 2 (V2) and Visit 3 (V3). Using the VES on photographs has shown to give similar results compared to a live evaluation.[36] The repigmentation percentage after 6 months was calculated by subtracting the V3 VES score from the V1 VES score, followed by a division with the baseline VES score, multiplied by 100.

Sample handling procedures and flow cytometry

Peripheral blood samples were obtained from all patients at V1 and V2. Peripheral Blood Mononuclear Cells (PBMC) were purified from the sampled peripheral blood by density gradient centrifugation (Axis-Shield density gradient media, Fresenius Kabi Norge AS, Halden, Norway). Serum and plasma were separated from the sampled peripheral blood samples by a twostep centrifugation. One four-millimetre skin punch biopsy was collected from the border of a vitiligo lesion at V1 and V2 (adjacent to the pre-treatment biopsies at the same lesion). These biopsies were dissociated into single cells through enzymatic digestion, by using the Whole Skin Dissociation Kit and mechanical dissociation with the gentleMACS™ Dissociator (both Miltenyi Biotec, Germany). Subsequently, a 70 µm cell strainer was used for filtering and collecting cells.

To detect the various T cell and NK cell subsets that seem to be involved in the vitiligo pathophysiology, cells (of blood and skin) were stained for flow cytometry analysis. Flow cytometry was used to identify the following cell types and their activation status: T cells (CD3), T helper cells (CD4), cytotoxic T cells (CD8), Activated T helper and cytotoxic T cells (Granzyme B^+ $CD4^+$ and Granzyme B^+ $CD8^+$), T_{RM} cells ($CD3^+$, $CD8^+$, $CD62L^-$, $CD69^+$), $CD103^+T_{RM}$ cells ($CD3^+$, $CD8^+$, $CD62L^-$, $CD69^+$, $CD103^+$)[37], NK cells ($CD3^-$, $CD56^+$) and activated NK cells ($CD3^-$, $CD56^+$, Granzyme B^+). In skin, the direct $CD4^+$ could not be measured due to the digestion of the surface marker by the isolation protocol', thus $CD8^-$ cells (gated for CD3) were assessed instead. Tregs were initially defined as $CD3^+$, $CD4^+$, $CD25^+$, $FoxP3^+$ cells. However, solely FoxP3 was not evaluable due to technical issues, which is why the Tregs were detected by CD3+, CD4+, $CD25^+$, $CD127^{low/-}$ in blood[38] and $CD3^+$, $CD8^-$, $CD25^+$, $CD127^+$ in skin.[39]

The fluorochrome-conjugated antibodies that were used (BioLegend, San Diego, California) to detect the abovementioned markers for the cell types can be found in the Supplements (Table S2). Cell surface staining was done with flow cytometry buffer (Phosphate-Buffered Saline supplemented with 1% Bovine Serum Albumin and 0.05% Sodium azide). Thereafter, cells were fixed in True-Nuclear™ Fix (BioLegend) and intranuclear staining was performed using True Nuclear Perm Wash buffer (BioLegend), according to the manufacturer's instructions. Single-cell measurement and flow cytometry acquisition were performed on a Sony Spectral Analyzer (Sony SP6800) and data were analysed using FlowJo software (BD Biosciences, version 10.7.1). PBMC reanalysis was necessary with frozen samples for 4 patients, since a machine malfunction occurred while collecting the fresh samples for these patients. Reanalysis of the skin samples of the same donors was not possible as no frozen material was available and therefore these four patients were excluded from the analyses from the skin.

Statistical analysis

The statistical analysis was performed using SPSS version 26.0 (IBM, Armonk, NY), GraphPad Prism version 8.3.0. (Graphpad Software Inc, San Diego, CA) and R software version 4.0.2 (R Foundation, Vienna, Austria). Descriptive statistics were used for summarizing patient demographics and repigmentation percentages. Not normally distributed data were statistically assessed by using the Mann-Whitney U (*U)* test for unpaired data and Wilcoxon signed-rank test (*Wx*) for paired data. Spearman's rank correlation (r) was calculated to correlate quantitative variables. Linear regression analysis, including the interaction between V1 and V2 for each cell type was performed to estimate VES repigmentation. P-values < 0.05 were considered statistically significant.

RESULTS

Patient features and treatment

A total of 36 patients were included in the study, out of whom 6 patients dropped out. Two patients were excluded due to the lockdown measures of the COVID-19 pandemic, another two patients discontinued because they complained of sampling procedures, one patient was not compliant and therefore excluded, and one patient could not complete the study due to personal reasons. In total, 30 patients completed the study and were included in the analyses. More than half of these 30 patients were female (63.3%) and the mean disease duration was 17 years (SD ± 12 years). Light skin type (skin type I, II, and III) was the dominant skin type (77%). All 30 patients received standard of care therapy with either topical therapy alone or in combination with NB-UVB phototherapy for a minimum of 6 months**.** Eleven patients (37%) were treated with topical therapy alone (cream group) and 19 patients (63%) received topical therapy in combination with NB-UVB phototherapy (UVB+cream group). Patient characteristics are illustrated in Table 1. Both treatment groups were comparable and did not significantly differ in sex, skin type, age, age of onset, vitiligo duration, Vitiligo Disease Activity (VIDA) score[40] and baseline affected BSA percentages (Table 1). The affected BSA percentages were assessed with the VES at baseline (V1), after 3 months (V2) and after 6 months (V3) of therapy. An overview of the overall affected BSA per visit per patient is shown in Figure 1.

Figure 1. Overall affected body surface area percentage before and during treatment. The affected body surface area (BSA) percentage is shown per patient at each visit. The x-axis shows the patient ID numbers and is arranged by patients with a low affected BSA (left) to patients with a high affected BSA (right) at visit 1. The y-axis displays total depigmented BSA percentage measured with the Vitiligo Extent Score (VES). * Patient 9 has a very low affected BSA ranging from 0.17% (at visit 1) to 0.18% (at visit 3), however this cannot be seen in the graph due to the scale size.

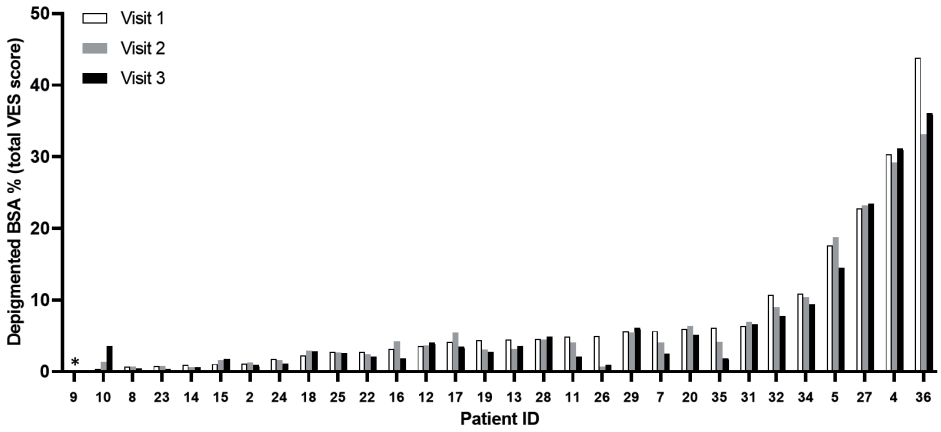


Table 1. Patient and vitiligo characteristics

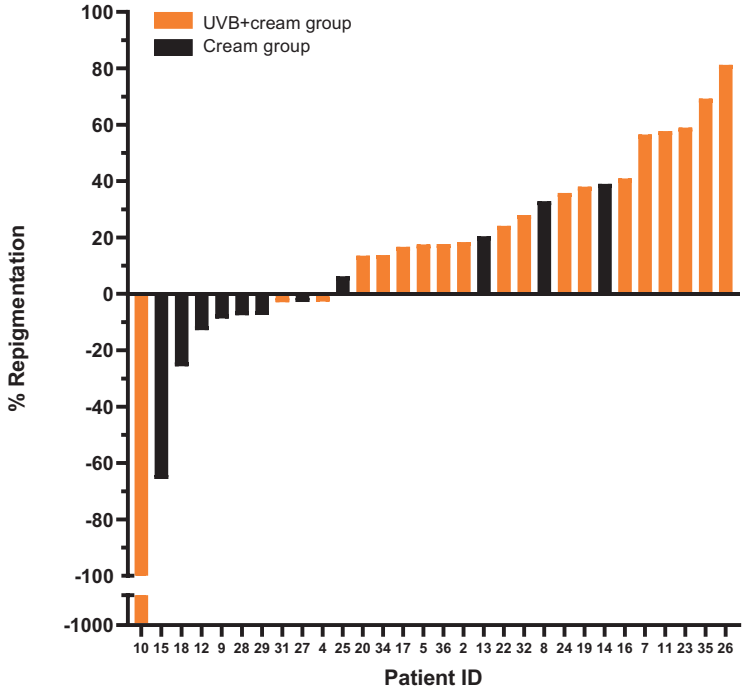
Patient ID	**Sex**	**Skin type**^	**Age at baseline** (yr)	**Age of onset** (yr)	**Vitiligo duration** (yr)	**Vitigo activity** (VIDA score)	**Vitiligo extent** (BSA%) at baseline	**Location of biopsied lesion**
Cream group (topical therapy)								
8	F	2	60	53	7	2	0.7	Hand
9	F	2	49	43	6	2	0.2	Axilla
12	F	3	41	19	22	2	3.6	Axilla
13	F	5	48	34	14	2	4.5	Abdomen
14	M	2	58	22	36	2	1.0	Leg
15	M	2	44	35	9	2	1.1	Axilla
18	F	5	33	29	3	3	2.3	Leg
25	M	2	67	40	27	2	2.8	Arm
27	F	2	41	15	26	4	22.8	Abdomen
28	F	2	46	28	18	2	4.6	Arm
29	F	2	30	8	22	2	5.7	Waist
Mean ± SD (min-max) **(n=11)**			47 ± 11[t] (30-67)	30 ± 13[t] (8-53)	17 ± 10[t] (3-36)		*2.8*[#,µ] [1-4.6]	
UVB+cream group (topical therapy + UVB)								
2	M	6	37	32	5	4	1.1	Arm
4	M	5	47	7	40	2	30.4	Leg
5	F	3	57	38	19	4	17.6	Arm
7	F	3	46	37	9	4	5.7	Axilla
10	F	4	19	18	1	4	0.3	Waist
11	F	3	49	17	32	2	4.9	Abdomen
16	F	3	57	52	5	2	3.2	Axilla
17	M	2	23	20	3	3	4.2	Arm
19	F	4	63	53	10	3	4.4	Leg
20	F	2	43	30	13	2	6.0	Waist
22	M	5	52	37	15	4	2.8	Arm
23	M	3	22	18	4	2	0.8	Arm
24	F	2	27	13	14	2	1.8	Leg
26	M	2	68	58	10	2	5.0	Abdomen
31	F	2	31	5	26	2	6.4	Arm
32	M	2	48	15	33	2	10.8	Leg
34	M	2	44	6	38	2	10.9	Arm
35	F	2	45	38	7	2	6.1	Waist
36	F	2	63	27	36	2	43.8	Abdomen
Mean ± SD (min-max) **(n=19)**			44 ± 15[t] (19-68)	27 ± 16[t] (5-58)	17 ± 13[t] (1-40)		*5*[#,µ] [2.8-10]	
Total Mean ± SD (min-max) **(n=30)**			45 ± 13 (19-68)	28 ± 15 (5-58)	17 ± 12 (1-40)		*4.5*[#] [1.6-6.2]	

M, male: F, female; Vitiligo Disease Activity (VIDA)[40] score 2, active in past 6 months; VIDA score 3, active in past 3 months; VIDA score 4, active in the past 6 weeks; ^Skin type according to Fitzpatrick skin scale[58]; [#]Median [Interquartile range]; [t] unpaired t-test shows no significant differences between treatment groups (p>0.05); [µ] Mann-Whitney-U test shows no significant difference between treatment groups (p>0.05).

Treatment response

The overall treatment response (repigmentation) at V3 varied between patients. Five patients showed ≥50% repigmentation, 6 patients repigmentation between 25-49%, 9 patients repigmentation between 1-24% and 10 patients were unresponsive (repigmentation <0%) (Figure 2). Repigmentation <0% means that the lesions increased in size or number during therapy. The overall affected BSA of all patients, however, did decrease from median 4.5% [IQR 1.6-6.2%] at V1 to 2.8% [IQR 1.6-6.2%] at V3. The UVB+cream group showed a significant better treatment response after 6 months compared to the cream group (median repigmentation 24.1% [IQR 13.8-56.5] vs -7.5% [IQR -12.8-20.5], respectively; *P*=0.008).

Figure 2. Repigmentation percentage after 6 months of therapy. The repigmentation percentage is shown per patient at visit 3 (6 months after therapy). The x-axis shows the patient ID numbers arranged by patients with a low response to therapy (left) to patients with a high response to therapy (right). The y-axis displays the repigmentation. Repigmentation was calculated as a percentage of the total affected BSA% at visit 1 measured with the Vitiligo Extent Score. Patients with a (-) negative repigmentation were unresponsive, meaning that their vitiligo lesions increased in size or number during therapy.



Cellular changes in blood and skin

We determined the frequencies of 10 immune cell types in blood and skin at V1 and V2 to establish changes in cellular immunity involved in vitiligo. The cell percentages in blood and skin at V1 did not significantly differ between the treatment groups, indicating comparable patient groups at V1. Furthermore, no significant differences were found at V2 between the treatment groups in blood and skin. Since we focused on repigmentation as an outcome (regardless of how this was achieved), we combined the treatment groups for further analyses on the association of changes in immune cells and treatment response.

In Figure 3 and 4 the changes in cell percentages are presented per treatment group at V1 and V2 in blood and skin, respectively. Changes in cell percentages within individual patients between V1 and V2 in blood were found, although these were not significant and did not differ between the treatment groups. In skin, however, significant decreases in CD3 and CD8 cells and a significant increase in $CD8^-$ cells were found at V2 in the UVB+cream group (p=0.044, p=0.006 and p=0.005, respectively; Fig. 4).

Cellular changes correlated to treatment response

In order to examine the association between the changes within cell types and the clinical response to treatment, the fold change of each cell type (V2/V1) was correlated (r) to the VES repigmentation at V3. In blood, the changes in the CD3, CD4, Granzyme B^+ $(GrB)^+CD4$, CD8, GrB^+CD8, T_{RM}, Treg, NK and GrB^+NK cells did not significantly correlate to the treatment response at V3 (Figure 5A), however, trends were seen towards a positive correlation between GrB^+CD8 cells and repigmentation (Figure 5B). Likewise, similar correlation analyses were performed in skin, demonstrating that the changes in CD3, $CD8^-$, CD8, GrB^+CD8, T_{RM}, Treg, NK and GrB^+NK cells did not significantly correlate to the repigmentation at V3 (Figure 5C). The GrB^+CD8^- cells, however, did show a significant positive correlation (p=0.014) to repigmentation (Figure 5D). Furthermore, the increase in Tregs in the skin showed a trend towards a positive correlation with repigmentation (Figure 5C).

Finally, the interaction for each cell type between V1, V2 and the repigmentation at V3 (based on linear regression) was analyzed, showing a significant relation only between GrB^+CD4 cell percentages in blood and repigmentation (p=0.001, Figure 6A). This analysis demonstrates that an above average percentage of GrB^+CD4 cells at V1 and a higher percentage of these cells at V2, result in more repigmentation at V3. Similarly, a below average GrB^+CD4 cell percentage at V1 and a lower cell percentage at V2 (compared to V1), also leads to more repigmentation at V3. In addition, if a high GrB^+CD4 cell percentage is found at V1, a decrease of these cell

percentages at V2 (compared to V1) is associated with more depigmentation (Fig. 6A, red arrow). However, more repigmentation is seen when % GrB^+CD4 cells remain high (green arrow).

Remarkably, in the skin, activated NK (GrB^+NK) cells at V1 and V2 were found to have a significant relation with repigmentation ($p=0.03$, Figure 6B). This analysis showed that an above average percentage of GrB^+NK cells at V1 and a higher percentage (>3%) of these cells at V2, result in more repigmentation at V3. Likewise, a below average GrB^+NK cell percentage at V1 and a lower cell percentage (<9%) at V2, also leads to more repigmentation at V3. Furthermore, when a below average GrB^+NK cell percentage is found at V1, an increase of these cell percentages at V2 (compared to V1) is associated with a negative repigmentation. No other significant relations in the other analyzed immune cell types were found upon the interaction analyses in blood and skin.

Figure 3. Change in cell percentages between visit 1 and 2 after treatment in blood. Blood samples were taken from patients at visit 1 (baseline) and visit 2. Visit 2 took place after 3 months of treatment with either topical therapy alone (cream) or treatment with NB-UVB in combination with topical therapy (UVB+cream). The percentage of CD3 T cells (**A**), CD4 T cells (**B**), GrB^+CD4 T cells (**C**), CD8 T cells (**D**), GrB^+CD8 T cells (**E**), NK cells (**I**) and GrB^+NK cells (**J**) are shown among the total number of lymphocytes. Resident memory T cells (T_{rm}) were defined as $CD3^+CD8^+CD62L^-CD69^+$ cells among the total number of CD8 Cells (**F-G**). Regulatory T cells, defined as $CD3^+CD4^+CD25^+CD127^{low/-}$ cells, are displayed as a percentage of CD4 cells (**H**). Results are shown as individual dot plots with a line between visit 1 and visit 2 resembling the same patient. Differences between visit 1 and 2 were tested with the Wilcoxon signed rank test: no significant differences were found for any cell type.

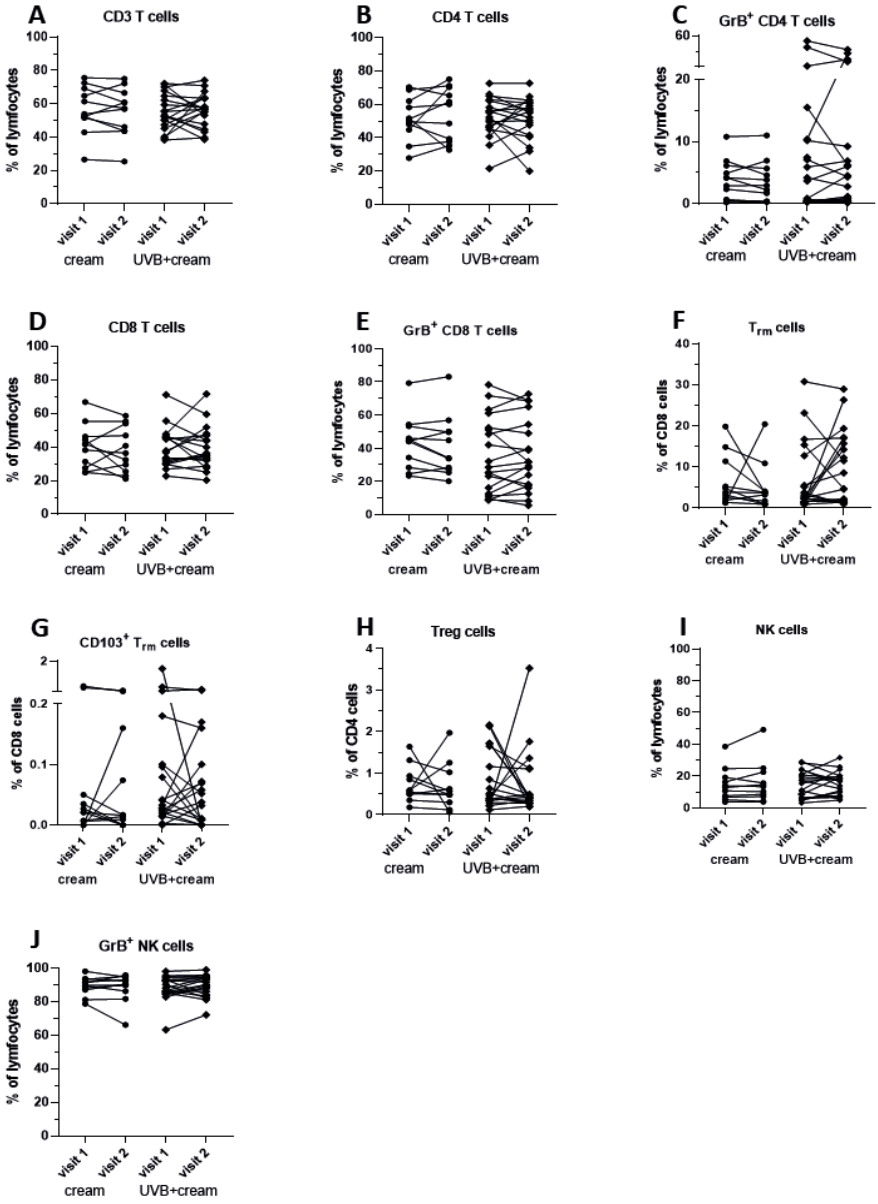


Figure 4. Change in cell percentages between visit 1 and 2 after treatment in skin. Lesional skin biopsies were taken from patients at visit 1 (baseline) and visit 2. Visit 2 took place after 3 months of treatment with either topical therapy alone (cream) or treatment with NB-UVB in combination with topical therapy (UVB+cream). The percentage of CD3 T cells (**A**), CD4 T cells (**B**), GrB^+CD4 T cells (**C**), CD8 T cells (**D**), GrB^+CD8 T cells (**E**), NK cells (**I**) and GrB^+NK cells (**J**) are shown among the total number of lymphocytes. Resident memory T cells (T_{rm}) were defined as $CD3^+CD8^+CD62L^-CD69^+$ cells among the total number of CD8 Cells (**F-G**). Regulatory T cells, defined as $CD3^+CD8^-CD25^+CD127^+$ cells, are displayed as a percentage of $CD8^-$ cells (**H**). Results are shown as individual dot plots with a line between visit 1 and visit 2 resembling the same patient. Differences between visit 1 and 2 were tested with the Wilcoxon signed rank test, significant as indicated; **P*<0.05 (p=0.044 in A) and ***P*≤0.005 (p=0.006 in B, p=0.005 in D).

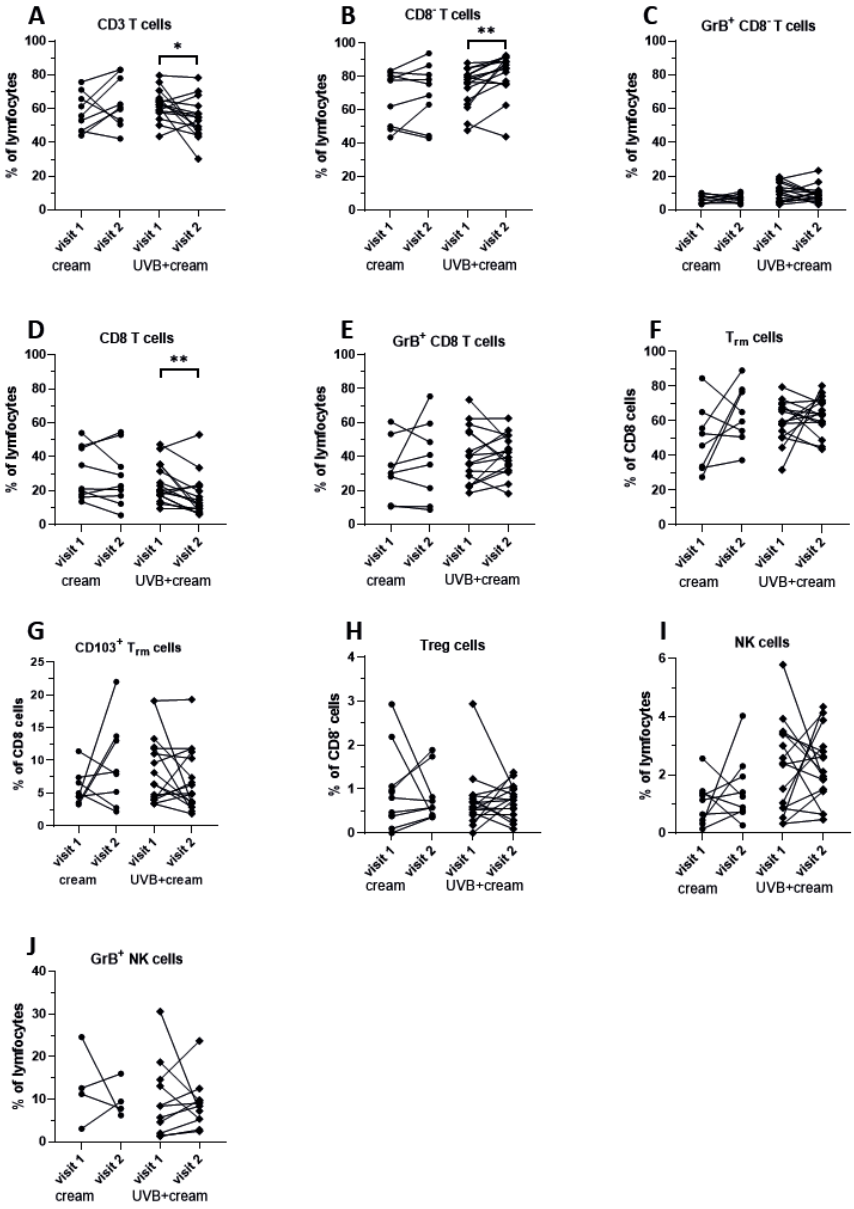


Figure 5. Correlation fold change of cell types with VES repigmentation in blood and skin. (**A**, **C**) The spearman coefficient r was calculated (x-axis) for the fold change of each cell type in relation to repigmentation and the corresponding P-value was transformed to –Log10 (y-axis). –Log10 P-values higher than 1.3 (dotted line) were considered statistically significant and correspond to p values <0.05. (**B**, **D**) Relations between repigmentation and the fold change of GrB^+CD8 cells (blood) and GrB^+CD8^- cells (skin) are shown. Black dots represent patients from the cream group and orange dots from the UVB+cream group. **(D)** Skin samples from 4 patients in total were excluded due to technical issues. The dotted line (at x=1) represents no change in cell percentage between V1 and V2.

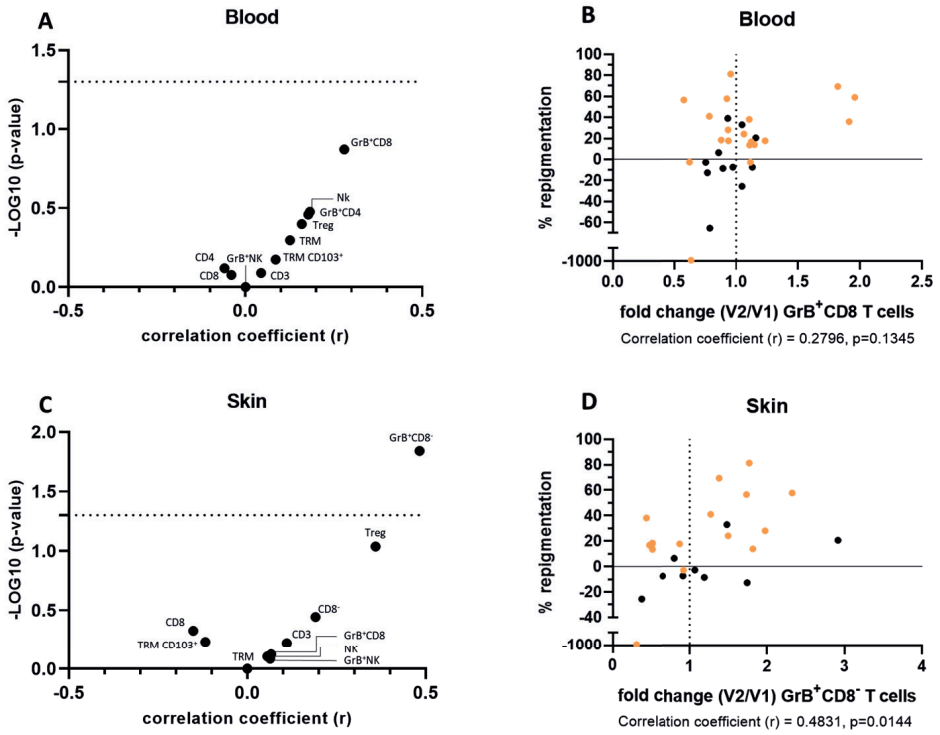
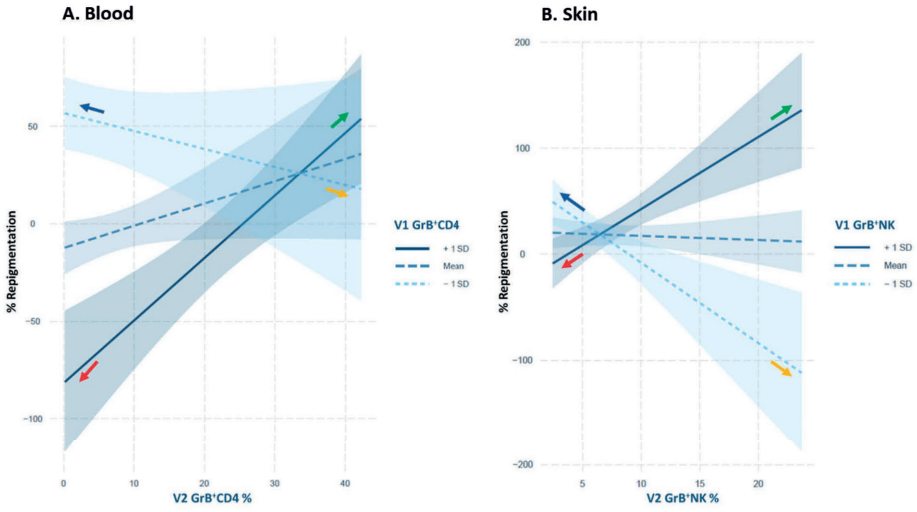


Figure 6. Visualization of significant relations between percentages of immune cell types at visit 1, visit 2 and repigmentation in blood and skin. (**A**) Significant interaction (p=0.001) is shown between GrB⁺CD4 cells percentages in blood at visit 1 (V1), visit 2 (V2) and the repigmentation % at visit 3 (V3, illustrated on the y-axis). The value of GrB⁺CD4 cells at V1 is expressed as above average (+ 1 standard deviation (SD), dark blue line), below average (-1 SD, light blue dotted line) and the average mean (blue dashed line). The value of GrB⁺CD4 cells at V2 is expressed as cell percentage (of lymphocytes) on the x-axis. When an above average value of GrB⁺CD4 cells is found at V1 and a higher cell percentage is found at V2 compared to V1, this results in more repigmentation (green arrow). A below average value of GrB⁺CD4 cells at V1 and lower cell percentage at V2 compared to V1, is also predictive for more repigmentation (blue arrow). An above average value of GrB⁺CD4 cells at V1 in combination with a low cell percentage at V2 is associated with further depigmentation (illustrated as negative repigmentation) (red arrow). Likewise, a below average value of GrB⁺CD4 cells at V1 and a higher percentage of these cells at V2 (compared to V1) also results in less repigmentation (yellow arrow). (**B**) Significant interaction (p=0.03) is shown between GrB⁺NK cells percentages in skin at V1, V2 and the repigmentation % at V3 (y-axis). The value of GrB⁺NK cells at V1 is expressed as above average (+ 1 SD, dark blue line), below average (-1 SD, light blue dotted line) and the average mean (blue dashed line). The value of GrB⁺NK cells at V2 is expressed as a cell percentage (of lymphocytes) on the x-axis. If an above average value of GrB⁺NK cells is found at V1 and a higher cell percentage is found at V2 (compared to V1), this results in more repigmentation (green arrow). A below average value of GrB⁺NK cells at V1 and lower cell percentage at V2, is also predictive for more repigmentation (blue arrow). An above average value of GrB⁺NK cells at V1 in combination with a low cell percentage at V2 is associated with less repigmentation (red arrow). Similarly, a below average value of GrB⁺NK cells at V1 and a higher percentage of these cells at V2 results in further depigmentation (yellow arrow)



DISCUSSION

This study provides important insights into the effects of standard of care therapy on local and systemic cellular immunity in vitiligo. We addressed the need for identifying biomarkers for early treatment response, by investigating T cell subsets (CD3, CD4, CD8, T_{RMs}, Tregs) and NK cells (in blood and skin) in relation to treatment response (repigmentation). We found that NB-UVB had a significant effect on the decrease of CD3 and CD8 T cells 3 months after therapy, and an increase of CD4 T cells in skin. In addition, our results showed that an early increase of GrB^+CD8^- T cells in skin was significantly correlated with a better treatment response. In blood, significant relations were seen of the same cell type (GrB^+CD4 T cells) between V1, V2 and repigmentation. Moreover, similar significant relations with repigmentation between GrB^+NK cells percentages at V1 and V2 were found in skin.

Remarkably, our results demonstrated no differences after 3 months of therapy in both the cream and UVB+cream group in blood. This finding is not unexpected for the cream group, as significant systemic absorption of topical calcineurin inhibitors and corticosteroids following normal use in vitiligo skin is minimal.[41–43] Contrary to our results, several studies have reported alterations in circulating blood cell types after NB-UVB therapy, indicating the systemic immunosuppressive effects of this therapy. [44–47] However Ekman et al.[48] report that based on their findings of UVB therapy in psoriasis, the effect of NB-UVB may be focused more on local rather than systemic inflammation. This is in line with our findings, showing a significant decreased number of T cells (CD3) and cytotoxic T cells (CD8) and increased number of T helper cells (CD4) in the skin after 3 months of NB-UVB therapy.

Furthermore, a trend was found towards a positive correlation between an increase in Tregs in the skin and a higher repigmentation response after 6 months. This can be explained by the possible increased effect of active suppression of the self-reactive T cells by the increased number of Tregs, finally resulting in repigmentation. In addition, we found that an increase of GrB^+CD8^- cells in skin after 3 months was significantly associated with a higher repigmentation after 6 months. This association of GrB^+CD8^- cells with repigmentation in our study was unexpected, as GrB is a serine protease traditionally secreted by NK cells and cytotoxic T cells and known for its proapoptotic role in autoimmune skin diseases. This left us with the question: to which phenotype of cells do these GrB^+CD4 cells belong and what was its function? Since our $CD8^-$ Tregs also were increased in skin, we hypothesized that (a part of) the Tregs could belong to this GrB^+CD4 population, as has been previously suggested by others.[49–51] Studies have shown that CD4 T cells are able to produce GrB.[52,53] In addition, GrB is highly upregulated in activated

T cells with a type 1 Treg (Tr1) phenotype.[54,55] These Tr1 cells can be classified as $CD4^{+}FoxP3^{-}$ Tregs that stimulate tolerance and have a suppressive capacity on inflammatory immune responses.[49] Activated GrB^{+}Tr1 cells can induce cytotoxicity against myeloid (dendritic) cells, leading to prevention of naïve T cell priming and suppression of effector T cells.[51,56] Another postulated explanation could be that these cells could perhaps play a role in immunosuppression by inducing apoptosis (through GrB) in pathogenic melanocyte specific T cells and thereby enhancing repigmentation. In summary, this finding may imply a special pathomechanism that needs further study.

The interaction between these same $GrB^{+}CD4$ cells in blood, before and 3 months after therapy, were found to have a significant prognostic role for repigmentation. Seemingly, the balance between the cell percentages at these two time points is of importance for an estimation of the treatment response after 6 months of therapy. Substantial differences in cell percentage after the first 3 months of therapy, is an indication for a poorer repigmentation, whereas minor changes seem to be an indication for a better repigmentation. In daily clinical practice, these changes in $GrB^{+}CD4$ percentage in blood could be taken into account to predict the treatment outcome after 6 months.

Likewise, GrB^{+}NK cells in skin have shown a similar relation with repigmentation. We found that an increase of these cells (starting from a below average GrB^{+}NK cell percentage at baseline), was associated with a negative repigmentation (meaning an increase of vitiligo lesions). This increase in GrB^{+}NK cells was not unexpected, as this indicates that these cells are actively exerting their cytotoxicity onto melanocytes, which is confirmed by others.[12,13,57] Interestingly, a unique finding was that a rise in these cells from an above average baseline value, seemed to be associated with a higher repigmentation percentage. Once more, this shows that the balance in GrB^{+}NK cell percentage between these two time points is essential, however a clear explanation for this rise with regards to a higher repigmentation percentage is lacking.

To our knowledge, our study is the first to evaluate these changes in cellular immunity before and during therapy in relation to repigmentation after 6 months of therapy. The current study was carried out prospectively and compliance to therapy was monitored accurately. However, a limitation of this study was the inability to assess the association between the cellular changes and treatment efficacy due to the nature of the study design and the limited number of patients. No distinction was made between the treatment groups, since this would have lowered the power of our analyses.

In summary, this study evaluated early changes in cellular immunity in relation to repigmentation after standard of care therapy, showing a positive relation between GrB^+CD8^- T cells in skin and therapeutic response. In addition, significant relations were found between repigmentation and GrB^+CD4 cells in blood and GrB^+NK cells in skin. Further studies, however, are needed to understand the exact pathomechanism behind these findings. For future implementations of an early on treatment biomarker, a systemic marker (from blood) would be convenient for the ease of use. In a follow-up study chemokines, cytokines and proteins involved in the vitiligo pathophysiology will be evaluated in blood, skin and blister fluid to gain more insight on potential biomarkers, which may be useful for future individualized therapeutic management of NSV patients.

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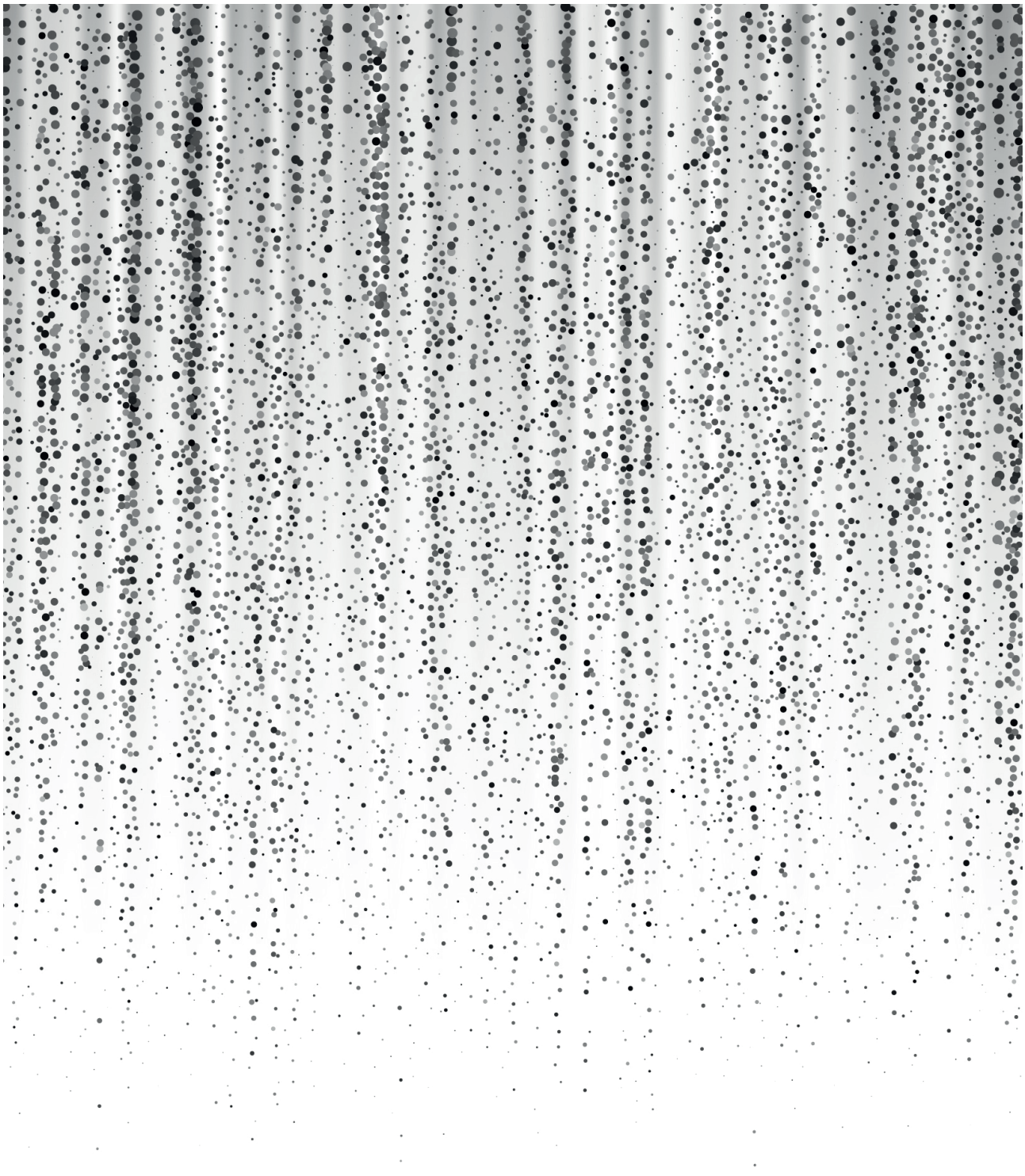
SUPPLEMENTS

Table S1. In- and exclusion criteria

Inclusion criteria:	
1.	Males and females aged ≥18
2.	Patients with non-segmental vitiligo commencing standard of care treatment, consisting of a potent topical corticosteroid, such as FP cream, alone or in combination with NB-UVB phototherapy for at least 6 months.
3.	Patients with active disease (new lesions and/or signs of disease progression) within the past 6 months prior to baseline.
4.	Patients able to communicate well with the investigator, to understand the requirements of the study, as well as understand and sign the written informed consent.
Exclusion criteria:	
1.	Patients that have received phototherapy or systemic immunosuppressive treatment during the last 6 weeks prior to baseline.
2.	Patients that have received topical anti-inflammatory treatment (topical corticosteroids, calcineurin inhibitors) during the last 4 weeks prior to baseline.
3.	Patients currently receiving treatments other than potent topical corticosteroids, such as FP 0.05% cream, calcineurin inhibitors (tacrolimus, pimecrolimus) or NB-UVB phototherapy for non-segmental vitiligo.
4.	Recurrent herpes simplex virus skin infections.
5.	Patients with a history of hypertrophic scars or keloids
6.	Patients with a history of hypersensitivity to (UVB or UVA) light and/or allergy to local anaesthesia.
7.	Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive hCG laboratory test (unless female is confirmed post-menopausal).
8.	Patients with haemophilia or other bleeding/clotting disorders
9.	Patients previously diagnosed with depression according to the DSM5 by a qualifying physician.
10.	Patients without lesions located at suitable areas for biopsies / suction blisters sampling (e.g. not on the face, genital areas, or other sensitive areas as judged by the investigator).

Table S2. Used antibodies

Antibody	Clone	Conjugate
Anti-human CD3	SK7	FITC
Anti-human CD4	SK3	BV750
Anti-human CD8	SK1	BV785
Anti-human CD69	FN50	BV605
Anti-human CD103	Ber-ACT8	BV711
Anti-human CD127	A019D5	PE
Anti-human CD62L	DREG-56	BV650
Anti-human CD56	HCD56	PE/Cy5
Anti-human CD25	BC96	BV510
Anti-human FoxP3	206D	BV421
Anti-human GrB	QA16A02	PE/Dazzle 594
Anti-human IFNg	4S.B3	BV570



CHAPTER 3

NB-UVB phototherapy response of different body regions in non-segmental vitiligo

Vidhya S. Narayan
Elnaz Alagha
Wouter Ouwerkerk
Sanne E. Uitentuis
Janny E. Lommerts
Samia Esmat
Rania M. Mogawer
Nanis Ragab
Sai Yee Chuah
Steven Thng
Albert Wolkerstorfer
Rosalie M. Luiten
Marcel W. Bekkenk

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ABSTRACT

Background

Narrow-Band Ultraviolet B (NB-UVB) is a widely used treatment for non-segmental vitiligo (NSV). The location of the vitiligo lesions is of influence on the degree of repigmentation. To date however, these differences in therapeutic response between specific anatomical areas of the body have not been fully characterized.

Objective

This study aimed to evaluate the clinical response to NB-UVB treatment in 19 different body regions.

Methods

A multicentre study of NSV patients treated with NB-UVB (alone or in combination with topical therapy and/or systemic steroids) was performed in hospitals in Egypt, Singapore and the Netherlands. Evaluation of the depigmented surface area of 19 different body regions was conducted with the Vitiligo Extent Score at baseline and after 6 months of therapy.

Results

A total of 101 NSV patients completed the study. The highest repigmentation was found in lesions on the lower facial region, followed by the upper facial region and lower trunk (>75% repigmentation in 58%, 48% and 46% of the patients, respectively). The lower face showed a significant better repigmentation than the upper face. The lowest repigmentation (<25% repigmentation) was observed at the feet (81%), followed by the hands (68%). Left versus right and dorsal versus ventral sides of the body demonstrated no significant differences. In addition, the torso and limbs showed similar repigmentation after treatment (median 47% and 42% respectively, *P=0.288*). We found that the body surface area can be classified into 4 composite body regions according to prognosis of repigmentation, i.e. face; torso&limbs; axillae; hands&feet, from highest to lowest median repigmentation (76%, 40%, 27%, 4%) respectively.

Conclusion

This study is the first to evaluate and compare 19 different body regions after NB-UVB therapy. These results may be of direct use to clinicians and vitiligo patients as it provides more detailed information on treatment prognosis per body region.

INTRODUCTION

Non-segmental vitiligo (NSV) is a chronic depigmenting autoimmune disorder caused by destruction of melanocytes, and has a significant negative effect on the quality of life, as many of the patients feel stressed and stigmatized by their condition.[1,2] Current therapy for NSV frequently consists of NB-UVB alone or in combination with topical therapy (such as calcineurin inhibitors and/or corticosteroids).[3,4] In cases of rapidly spreading vitiligo, systemic steroids are often added to this therapy to arrest progression of depigmentation.[3,5,6]

Patients can present variable repigmentation responses depending on the affected body region. Facial vitiligo is known to have a better response to treatment than vitiligo lesions on other body parts.[7] Moreover, acral areas, such as lesions on the hands or feet, are considered to be most resistant to therapy.[7] Until now, a few studies have investigated these differences in repigmentation between body regions after NB-UVB therapy.[8–10] However, in these studies the full body surface area (BSA) was roughly divided in 6 or less regions (e.g. face, neck, trunk, extremities, hands and feet). No distinction in location was made within these regions, such as left/right, front/back or upper/lower side of that body area. For example, the front side of the trunk could have a different response to treatment than the back side. Thus, potential differences within the abovementioned regions were not evaluable in these studies and could in fact be present. Additionally, more insight on this can be of use in clinical vitiligo trials, for example to determine the location of the target lesion. Moreover, this knowledge could be of great value to patients, since they often want to know the prognosis of their treatment. The burden that patients experience of vitiligo on various body regions differs.[11] Consequently, patients consider repigmentation of lesions on certain areas of the body to be more important than other areas, often leading to inquiries about the specific repigmentation of their vitiligo in a particular body region. This study will provide more insight regarding the repigmentation of lesions in various body regions after therapy.

The aim of this study was to evaluate the repigmentation after vitiligo therapy consisting of NB-UVB phototherapy (alone or in combination with topical therapy and/or systemic steroids) in several specific body regions as predefined by the Vitiligo Extent Score (VES).[12] Furthermore we aimed to assess the effect of demographic features such as sex, age, disease duration, disease activity and skin type on repigmentation after therapy.

PATIENTS AND METHODS

Patients

This international multicenter study was conducted in a cohort of NSV patients, aged 16 years or older, who received treatment with NB-UVB alone or in combination with topical therapy and/or systemic steroids (referred to as NB-UVB common therapy) for a minimum period of six months. Patients who received NB-UVB therapy in the month prior to starting therapy, were excluded. Eligible patients visiting the outpatient clinic were recruited in three different academic hospitals: in Egypt, Singapore and the Netherlands. We used data of other prospective studies (one published[13] and two unpublished) to include patients in our study. In addition, we included all consecutive patients with NSV who completed NB-UVB common therapy between January 2019 and January 2020 in the Amsterdam University Medical Center in the Netherlands. All patients provided written informed consent. Each center had received ethical approval from their Institutional Review Board. In addition, this study was not subject to the WMO (Medical Research Involving Human Subjects Act) as confirmed by the Medical Ethical Committee of the Amsterdam University Medical Center (EC number W20_317).

Treatment

All patients received NB-UVB treatment for at least 6 months, either in the hospital or at home. The equipment comprised of Waldmann UV-100 units with TL-01 lamps, irradiating light with a wavelength of 311 nm. The choice of treatment with a semi-circular whole body unit or with a partial body unit was dependant on the physicians advice in daily practice setting. The treatment schedule in all study centers was in line with the local standard of care therapy for NSV, namely irradiation three times a week on nonconsecutive days in combination with topical therapy on the non-irradiation days.[3] Patients were instructed to increase the intensity with 50mJ/cm2 each treatment, if no suberythema after irradiation occurred. Topical treatment consisted of a calcineurin inhibitor (tacrolimus 0.1% or pimecrolimus 1%) for lesions on the face and skinfolds and a corticosteroid (i.e. fluticasone propionate 0.005%) for lesions on the rest of the body. In cases of fast spreading vitiligo, an oral mini-pulse corticosteroid (such as dexamethasone 2-4mg, 2 consecutive days a week) was added to the treatment schedule.

Evaluation of treatment effects and data collection

The treatments response was expressed as a repigmentation percentage of the baseline lesional surface area. For patients receiving NB-UVB therapy with a partial body unit, only the treated regions were assessed. The overall repigmentation and repigmentation per specific body region were calculated by using the VES (https://

www.vitiligo-calculator.com).[12] The VES is a validated and feasible clinical scoring instrument that expresses the extent of the vitiligo lesions in percentage of the BSA.[12] It can be used to assess the vitiligo involvement of 19 separate areas of the body. Data were collected at two time points. At baseline the patient visited the physician before starting therapy and after 6 months of NB-UVB therapy (± 1 month window) the second visit took place. Photographs were taken of all vitiligo lesions at both time points. These photographs were used by the physicians to determine the VES scores at baseline and after 6 months of therapy. Using the VES on photographs has shown to give similar results compared to a live evaluation.[12] The repigmentation percentage was calculated by subtracting the 6 month VES score from the baseline VES score, followed by a division with the baseline VES score, multiplied by 100.

Statistical analysis

All statistical analyses were performed in SPSS software version 26.0 for Windows (IBM, Armonk, NY). Descriptive statistics were used for analyzing demographics and repigmentation percentages of the study population. Significance rates for unpaired data were determined by using the Mann-Whitney U (*U)* or Kruskal-Wallis H test for the treatment response in percentage repigmentation. To test statistical significant differences between body regions the Wilcoxon signed-rank test (*Wx*) was performed, as the data were paired and not normally distributed. For correlation and association analyses, the Spearman rank correlation coefficient test (r_s) was used. In addition, to determine whether demographic features were confounders of the treatment outcome, we performed a multiple linear regression analysis after a log transformation of the treatment outcome. Adjusted p-values are presented for post-hoc analyses according to Bonferroni. Statistical tests were two-sided and the level of significance was set at $P < 0.05$.

RESULTS

Patient characteristics

A total of 101 patients met the eligibility criteria and were included in the analyses. Ninety patients were included from other prospective studies (*n*=32 from Egypt, *n*=3 from Singapore, *n*=55 from the Netherlands) and 11 patients from the Netherlands were retrospectively included in this study. Patient and vitiligo characteristics are illustrated in Table 1. The average VES score at inclusion was 5.6% (median 2.9%, IQR 1.33-5.19) BSA. Concerning the distribution of the lesions, 93 patients (92%) showed involvement of more than one body region, and eight patients (8%) had lesions in only one region (e.g. in the face or axilla). The left and right hand were

the most common affected regions (in 78 and 76 patients respectively), followed by the upper and lower face (in 75 and 73 patients respectively). The waist and gluteal area were the least affected areas.

Table 1. Patient and vitiligo characteristics

Male : female ratio	35 : 66
Mean age ± SD (min-max) in years	40 ± 14 (16-76)
Mean age of onset ± SD (min-max) in years	30 ± 16 (2-66)
Disease duration median in years [IQR]	6 [3-16]
Disease activity N (%)	
Activity in past 6 months	88 (87)
Stable for 6 months or longer	13 (13)
Skin type N (%)	
I	1 (1)
II	35 (34.7)
III	25 (24.8)
IV	26 (25.7)
V	12 (11.9)
VI	2 (2)
Previous treatments N (%)	
Use of creams: yes	85 (84.2)
Use of NB-UVB phototherapy: yes	48 (47.5)
Skin transplantations: yes	9 (8.9)

SD, standard deviation; N, number of patients; IQR, interquartile range

Treatment and overall response

All patients received NB-UVB phototherapy for a minimum of 6 months. Ninety-four patients received whole body NB-UVB and eight patients were treated with partial NB-UVB for their localized vitiligo. Out of all 101 patients, 14 patients (14%) were treated with NB-UVB only, 73 patients (72%) received NB-UVB in combination with topical therapy and in 14 patients (14% with active, rapidly spreading vitiligo) dexamethasone was added to the treatment. There were no significant differences in total repigmentation (Kruskal-Wallis H 2.882, P=0.237) between these three treatment groups. Consequently, further analyses were performed on the total cohort of patients.

The overall treatment response (repigmentation) at 6 months varied between patients. Seventeen patients showed an excellent response (≥75% repigmentation), 24 patients a good response (between 50-74% repigmentation), 34 patients a moderate response (between 25-49% repigmentation) and 22 patients showed a poor response (between 0-24%). Four patients were unresponsive; their vitiligo

lesions increased during therapy. The overall affected BSA% of all patients however, did decrease from median 2.9 [IQR 1.3-5.2] to 1.5 [IQR 0.5-2.8].

Demographics correlated to treatment response

Female patients showed a significant better overall response to treatment than male patients (median 48% vs 33%, *U P*=0.029), although the distributions of the lesions over the various body regions and the repigmentation per body region did not significantly differ between sex (chi square tests, *P* >0.076). To assess whether age was of influence on the treatment response, we correlated this with the total repigmentation (r_s=0.085, *P*=0.398) and repigmentation per body region; no significant relations were found. In addition, no correlations were found between age of onset and disease duration with regard to the treatment responses. Furthermore, no differences in repigmentation were found between patients with active disease (new lesions in the past 6 months) and stable disease (*U P*=0.359). Finally, the skin type was assessed in relation to the treatment response, however this also did not show any significant correlation (r_s=0.100, *P*=0.320). Multiple linear regression analyses were performed demonstrating that sex, age, disease duration, disease activity and skin type were not confounding factors on the treatment outcome.

Comparison of treatment response between body regions

After analysing the treatment response per body region (Figure 1A), we found the highest repigmentation percentages in lesions located on the lower face, followed by the upper face and lower trunk (excellent repigmentation in 58%, 48% and 46% of the patients, respectively). On the contrary, the lowest repigmentation percentages (<25% repigmentation) were observed on the feet, followed by the right and left hand (81%, 68% and 65%, respectively). In Figure 1A, a full overview of the repigmentation percentages per body region is shown.

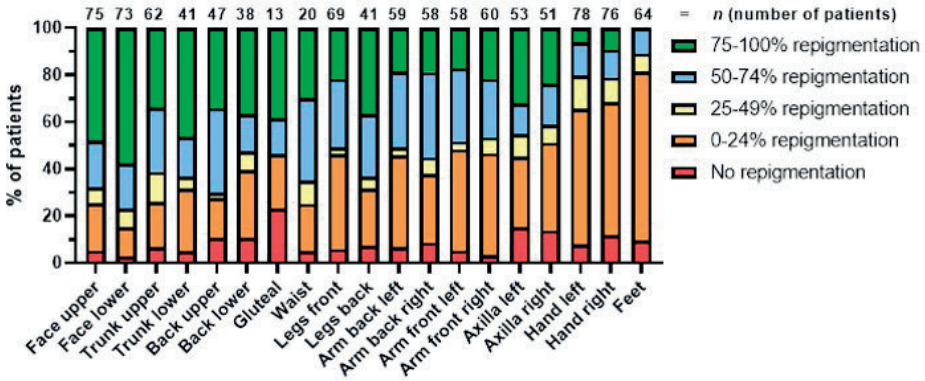
We also assessed potential differences in response of body regions within one area. The lower face showed a significant better repigmentation than the upper face (median 80% vs 64% respectively, *Wx n=66*, *P=0.008*) (Figure 1B). Other adjacent body regions such as, the upper and lower trunk, upper and lower back, waist and gluteal regions, front and back arms, front and back legs, and left and right axillae did not differ in treatment response (Figure 1B).

We defined 4 combined body regions based on repigmentation response, i.e. the face, torso&limbs, axillae and hands&feet (Figure 2). These combined body regions significantly differed in repigmentation response upon treatment (Friedman ANOVA test, *P*=0.000). Post-hoc analyses showed that the total face had a

significantly better repigmentation than the torso&limbs, axillae and hands&feet (Table 2). Subsequently, the hands&feet showed a significantly lower treatment response compared to the torso&limbs (*Wx P*=0.000). No significant differences in repigmentation were found between the torso&limbs and axillae, however the axillae did seem to show a trend towards lower repigmentation (Figure 1B).

Figure 1

A. Response to treatment by body region. The stacked bars show the repigmentation (divided into 5 groups) per body region. On the x-axis, the specific body regions are shown, and the y-axis represents the percentage of total number of patients (n) in which the lesions at this body region are present.



B. Response to treatment by body region with statistical analyses. The boxplots show the median repigmentation per body region. Statistical differences were tested with the Wilcoxon signed-rank test. ns, not significant; ** significant.

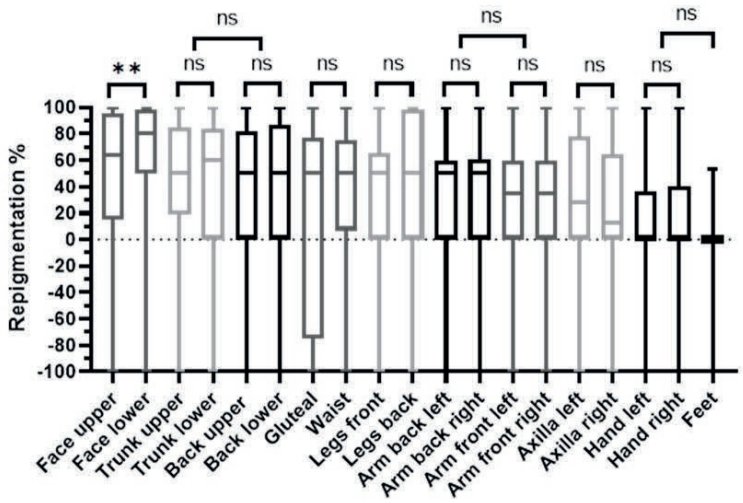


Figure 2. Overview of (combined) body regions with median repigmentation percentages. The body regions (as predefined by the Vitiligo Extent Score) are displayed with the median repigmentation percentage in the lower right corner (of each body region). Body regions with the same background colour, indicate a similar repigmentation response after therapy. Median repigmentation with the interquartile ranges (IQR) of these combined regions are shown on the right side of the figure.

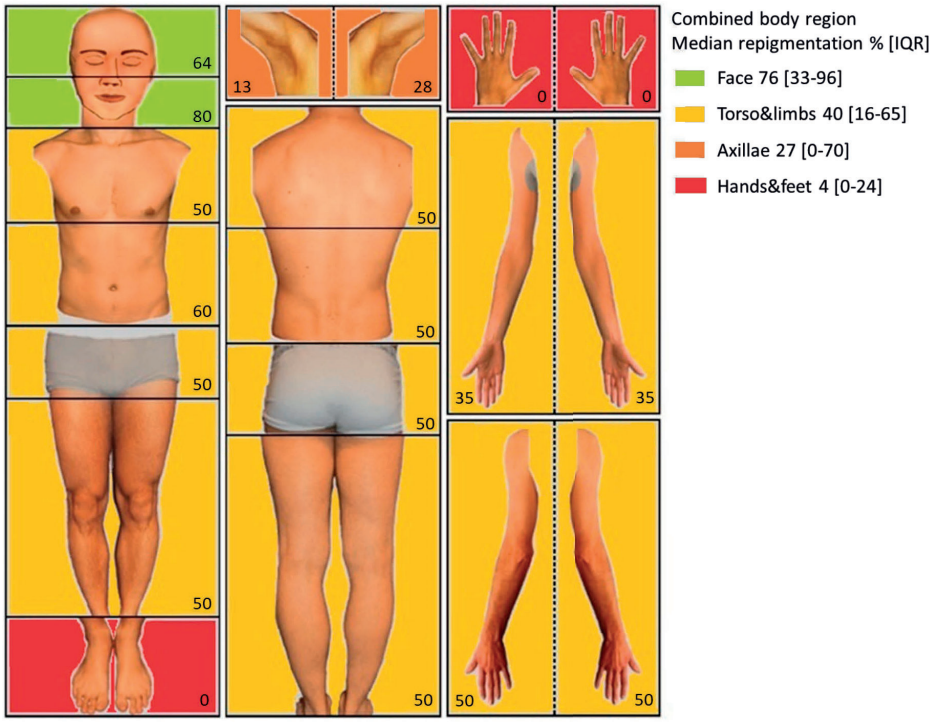


Table 2. Statistical differences in treatment response between combined body sites

Combined body region (median repigmentation %)	**Combined body region** (median repigmentation %)	***Wx* [*n*] *P*-value#**
Face (76)	Torso&limbs (40)	[74] 0.000 * (Face > Torso&limbs)
Face (76)	Axillae (27)	[49] 0.005 * (Face > Axillae total)
Face (76)	Hands&feet (4)	[69] 0.000 * (Face > Axillae total)
Torso&limbs (40)	Axillae (40)	[52] 0.999
Torso&limbs (4)	Hands&feet (4)	[81] 0.000 * (Torso&limbs > Hands&feet)
Axillae (27)	Hands&feet (4)	[47] 0.999

Wx, Wilcoxon signed-rank test; *statistically significant; #p-value after bonferroni correction; *n,*number of patients in which the comparison between the 2 body regions are made.

DISCUSSION

In this study, the clinical response to NB-UVB common therapy was evaluated and compared between 19 different body regions as predefined by the VES. We found that the best responses to treatment were seen in the face. On the contrary, the hands and feet showed the lowest repigmentation after 6 months of therapy. No significant differences in repigmentation were found between the torso, limbs and axillae. In addition, ventral versus dorsal and left versus right body regions were compared, demonstrating no differences in repigmentation after therapy. Furthermore, we correlated the treatment response to demographic features, showing that female patients had a significant better overall treatment response than male patients. Other characteristics such as age, disease duration, disease activity and skin type did not correlate with treatment response.

To our knowledge, this study is the first to evaluate and compare 19 different body regions after NB-UVB therapy using the VES, a validated measuring tool.[12] Our data demonstrate a varied response among the different anatomic body regions with facial lesions achieving the highest improvement and acral lesions achieving the least improvement, which is in line with previous studies.[8–10,14] Based on our findings, we concluded that the body surface area can be classified into 4 composite body regions (i.e. face; torso&limbs; axillae; hands&feet) with lesions in each of these body regions displaying a similar prognosis of repigmentation after treatment. Although the axillae did not significantly differ from the torso&limbs, trends were seen of a lower repigmentation in this body region after treatment (Figure 1B). These composite regions are largely in accordance with the body surface classification of the Vitiligo Area Scoring Index, supporting the distinct BSA division of this tool.[15]

Notably, a new finding of ours showed that the lower face significantly better responded to therapy than the upper face. The reason behind this difference is not completely clear, however might be related to the variation in hair follicle density between the lower and upper face. Hair follicles have been shown to be reservoirs for melanocytes.[16] The residual melanocytes that are located in the outer root sheath of the hair follicles are reactivated via the Wnt pathway after NB-UVB radiation, leading to migration to depigmented epidermis and (perifollicular) repigmentation.[17,18] This may also clarify why minimal hair-bearing sites (such as the hands and feet) have a lower response to therapy. However, this does not explain why body regions with a higher hair follicle density (i.e. limbs or axillae) are less responsive to therapy than the face. A possible explanation could be that the face is generally more exposed to UV (sun)light allowing an enhanced response to therapy.

In addition, the Wnt pathway is known to be physiologically inhibited in palms and soles[19], which could also be the case in other body regions. Other factors however, could play a role as well. Studies have identified crucial differences between distinct anatomical regions of the skin regarding local immune responses and histological differences.[20,21] Facial skin for instance is sebaceous gland rich, possibly serving as a reservoir for melanoblasts and non-melanogenic melanocytes.[22] During exposure to UV-radiation, these could differentiate, proliferate and migrate to the epidermis causing a better repigmentation compared to sebaceous gland poorer areas such as the limbs or axillae.[18] In addition, sebaceous gland rich regions may have a distinct non-inflammatory immune response which is different from sebaceous gland poor areas.[21] Summarizing, differences in repigmentation between body regions exist, however the exact mechanism of NB-UVB induced repigmentation and the anatomic variation in response to this, still remain to be fully elucidated.[18]

In our study, female patients had a better overall treatment response than male patients. This might be related to physiological differences between male and female skin, such as differences in hair growth, number of sebaceous glands or hormone metabolism.[23,24] However, behavioral factors (such as compliance to therapy) could play a role as well, as female patients seem to experience a greater quality of life impairment compared to male patients[25], although this was not recorded in our study. Our findings did not demonstrate any association between treatment response and age, disease duration, disease activity or skin type, as is confirmed by several other studies.[26–30]

The results of this study are not only of importance to clinicians for providing an estimate of repigmentation of specific body regions to patients, but also could be useful in clinical trials for determining the target lesion location. The strength of our study is that the follow-up data were recent up to January 2020, representing up-to-date results of modern-day vitiligo treatment with NB-UVB in three different countries. This study provides a thorough outline of the differences (and resemblances) between body region responses to vitiligo treatment in a daily practice setting. The precise compliance to therapy and total cumulative doses however, were not recorded due to practical reasons and thus not taken into consideration for the analyses. Another limitation is that it was unclear whether the genital area (part of the waist) was exposed to NB-UVB radiation and therefore perhaps inaccurately included into the analyses. Lastly, there was a high heterogeneity between the given treatments with regards to the equipment (whole body units versus partial body units), the treatment setting (home vs. outpatient) and the additional therapy (NB-UVB alone or in combination with topical therapy and/or systemic steroids) which may have had an influence on the repigmentation

differences. However, this heterogeneity does better reflect the reality in daily practice, possibly giving patients a better estimation of the expected repigmentation responses after therapy.

In conclusion, this study gives a clear overview of the detailed VES body regions response to NB-UVB treatment in a daily practice setting. Our findings demonstrate that these 19 body regions can be integrated into 4 composite body regions (face; torso&limbs; axillae; hands&feet) displaying a similar repigmentation within each composite region after therapy. Facial lesions achieved the highest repigmentation, even indicating a better response of the lower face compared to the upper face. Lesions on the hands&feet showed the lowest responses, followed by the axillae, torso&limbs. For daily clinical practice, the results of our study may be of direct added value to clinicians and vitiligo patients as it provides more detailed information on treatment prognosis per body region.

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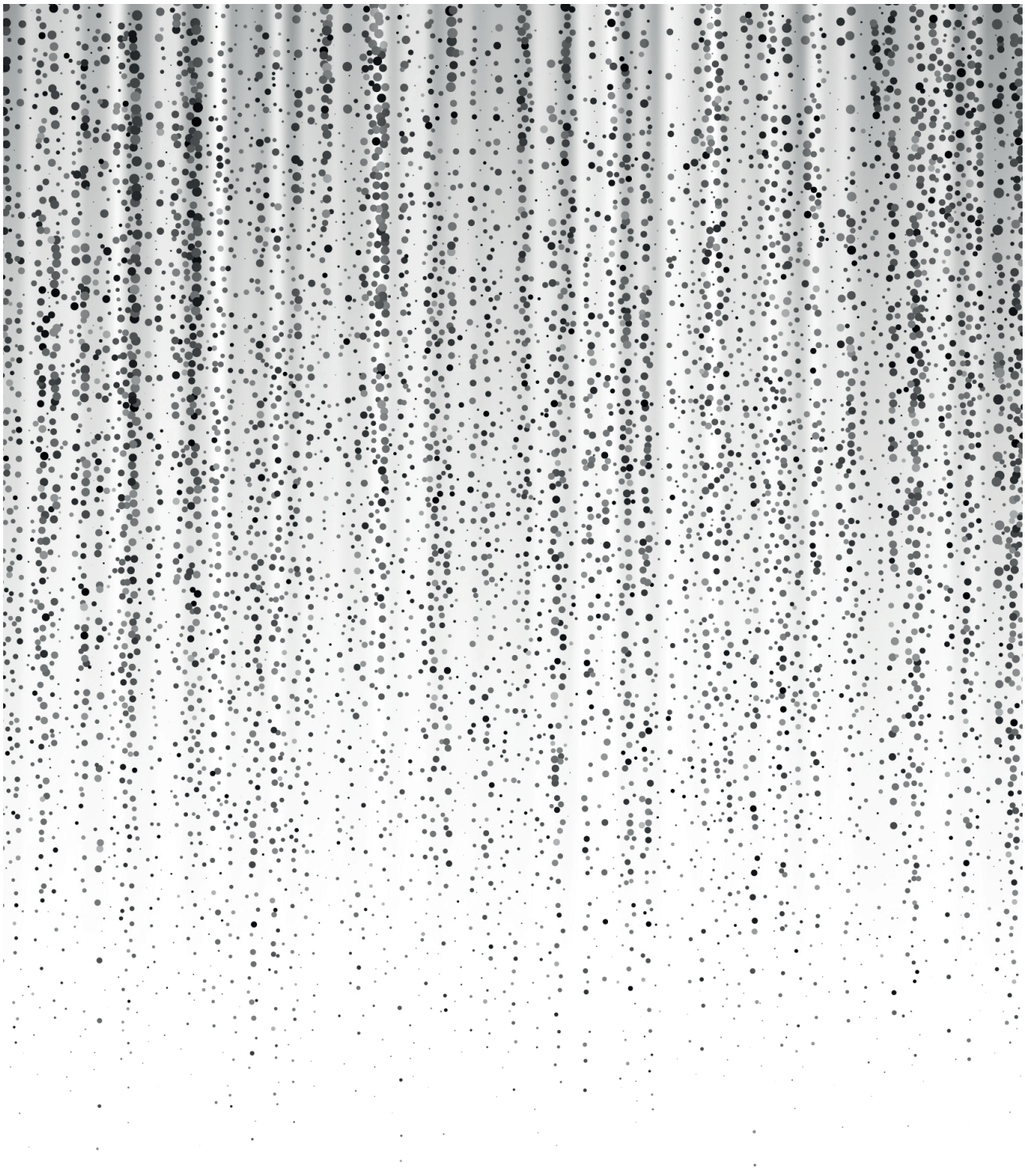

CHAPTER 4

Patient reported outcomes for intensified versus conventional NB-UVB treatment in non-segmental vitiligo

Sanne E. Uitentuis
Vidhya S. Narayan
Bas S. Wind
Marcel W. Bekkenk
Menno A. de Rie and
Albert Wolkerstorfer

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ABSTRACT

Introduction

Treatment of non-segmental vitiligo (NSV) remains a challenge. Efficacy of NB-UVB treatment may increase with more frequent use or in combination with topical agents. Currently, data on the most effective treatment regimen lacking. Our objective is to retrospectively compare NB-UVB treatment regimens for non-segmental vitiligo.

Methods

Patients with NSV treated with NB-UVB therapy were included in two time periods. Group I received NB-UVB therapy twice a week (conventional treatment) and group II received NB-UVB thrice a week, combined with topical agents (intensified treatment). Patients completed a questionnaire regarding the degree and onset of repigmentation, satisfaction and side effects.

Results

Repigmentation scores did not differ significantly between the two groups. Onset of repigmentation in the first three months seemed higher in group II, but this difference was not significant (23.4% vs 51.1%; p=0.11). In both groups the majority of the patients were moderately to very satisfied (group I: 70.2% group II: 73.3%). The occurrence of adverse effects was comparable.

Conclusions

This study indicates that conventional and intensified treatment for NSV seem to be comparable. The intensified treatment might be more effective to speed up the onset of repigmentation, but larger prospective studies are needed to objectify these findings.

INTRODUCTION

Non-segmental vitiligo (NSV) is an acquired chronic pigment disorder of the skin characterized by the development of patchy leukoderma, with a significant negative effect on the quality of life.[1–3] NSV is the most common depigmenting disorder affecting approximately 1% of the world's population, regardless of gender, ethnicity or skin type.[4] Current treatments include topical agents, phototherapy and surgical techniques which aim to improve melanocyte proliferation and stimulate repigmentation.[5] Topical therapy with corticosteroids or calcineurin inhibitors are suggested as first-line treatment.[6] The use of narrow band ultraviolet B (NB-UVB) phototherapy was first reported in 1997 by Westerhof et al.[7] and is now considered to be a growing modality for the treatment of generalized NSV.[5] NB-UVB is superior to psoralen and UVA (PUVA, another commonly used phototherapy regimen in vitiligo) due to its increased efficacy and decreased adverse effects.[8,9]

Currently, no consensus exists as to what the optimal treatment regimen and duration is of NB-UVB phototherapy, and practice varies widely.[10] At the Academic Medical Center (AMC), conventional treatment for NSV was NB-UVB alone twice a week, but this has changed in an intensified schedule with NB-UVB thrice a week in combination with topical treatment with fluticasone propionate and/or tacrolimus. This change was implemented in 2011 due to recent studies showing that the combination of topical therapy and NB-UVB is superior to monotherapy.[11,12] However, data on the most effective treatment frequency are lacking. Until now, the NB-UVB treatment regimens have never been compared directly. For the use of NB-UVB expert recommendations have been developed based on current literature by the Vitiligo Working Group Phototherapy Committee.[13] However, little evidence is available to guide clinicians on what is the most effective duration, dosage and treatment frequency of NB-UVB. Two studies have investigated the difference in effectiveness between twice and thrice weekly treatment regimens for vitiligo with the 308-nm excimer laser.[14,15] Both studies demonstrate that the final repigmentation does not depend on the treatment frequency but on the total number of treatments. However, the onset of repigmentation does occur faster with the three times weekly treatment. This could be the same for NB-UVB phototherapy but studies investigating this hypothesis are lacking.

For future improvement of vitiligo therapy it is important to assess, compare and evaluate the current treatment regimens, in order to determine what is most effective and satisfactory for the patient. Therefore, the aim of this study is to explore whether a combination of NB-UVB three times weekly and topical agents is superior to NB-UVB alone twice a week for the treatment of non-segmental vitiligo from patients' perspectives.

MATERIALS AND METHODS

Patients

This retrospective cohort study was performed at the Netherlands Institute for Pigment Disorders in Amsterdam. Inclusion criteria were: (i) diagnosis of non-segmental vitiligo; (ii) age 16 or older; (iii) received total body NB-UVB therapy for at least six months.

Patients were included in two different time periods: Group I finished their NB-UVB therapy between March 2008 and January 2009. Group II finished their therapy between February 2017 and October 2017. Questionnaires were sent to the patients within four months after the end of the NB-UVB therapy. In between these time periods the standard of treatment had changed (see treatment). Inclusion criteria were patients aged 16 years or older, diagnosed with NSV who received total body NB-UVB therapy at home prescribed for one year.

Patients were sent the questionnaire with a consent form and a return envelope. If a patient did not respond, he or she was reminded by phone with a maximum of three follow-up calls. In case a patient was not reachable by phone a second questionnaire was sent by post. This study was not subject to the WMO (Medical Research Involving Human Subjects Act) and approval was granted by the Medical Ethical Committee of the AMC.

Treatment

In both groups NB-UVB treatment was given at home. The equipment comprised of semi-circular Waldmann UV-100 units with TL-01 tubes. These tubes contain ultraviolet B lamps that irradiate light with a wavelength of 311 nanometer. Standardized cumulative doses were calculated by using the intensity measurements together with the treatment charts of patients. Nursing staff cared for guidance and explanation of the NB-UVB equipment and treatment. In group I irradiation took place twice a week on non-consecutive days. The patients were not advised to use an additional topical treatment except emollients. In group II irradiation was given thrice a week on non-consecutive days with topical therapy on the non-irradiation days, consisting of a calcineurin inhibitor (tacrolimus or pimecrolimus) for lesions on the face and skinfolds and a corticosteroid such as fluticasone propionate 0.005% for lesions on the rest of the body. Patients were advised to increase the intensity by 50 mJ/cm2 if no suberythema of the skin occurred directly after the NB-UVB therapy. When suberythema occurred, an equal dose was recommended for the next irradiation and when painful erythema or blisters were observed patients were instructed to omit the next treatment.

Questionnaire

A questionnaire comprising 21 questions was constructed (Supplementary Appendix I). Questions included patient characteristics, disease duration, The Vitiligo Disease Activity (VIDA) score for disease activity and previous treatments. Patients were requested to note the repigmentation percentage per treatment area (face, neck, trunk, arms, hands, legs and feet) and to state the onset of repigmentation. Additionally, patients were asked to rank their level of satisfaction regarding the result of the therapy and the appearance of the repigmentation. Seven questions were included about the NB-UVB regimen such as average times of irradiation per week and total amount of months of the treatment. Additionally, patients were asked whether they had used topical therapy and if so, which topical agent was used and the average application of the cream per week. Finally, questions were asked about adverse effects; temporary occurrence of suberythema, painful erythema and a burnt skin.

Statistical analysis

All statistical analyses were conducted using SPSS software version 24.0 for Windows (Armonk, NY, USA). Mean scores between both groups were compared using the T-test and Mann-Whitney-U test for normally and not-normally distributed numerical variables, respectively. For categorical variables Chi-square tests were performed to analyze differences between the groups. The Spearman's rank correlation test was used to define the association between the onset of repigmentation and patient satisfaction within the groups. Statistical tests were two-sided and the statistical level of significance was set at $P<0.05$.

RESULTS

Patient and treatment characteristics

A total of 109 eligible patients with non-segmental vitiligo aged 16 years or older were sent the questionnaire. The response rate was 47 (87%) in group I and 45 (82%) in group II. The patient characteristics are presented in Table 1. The two groups were comparable regarding age, duration of disease, previous treatments and skin type. The Vitiligo Disease Activity (VIDA) scores showed no significant differences in disease activity between the two groups ($p=0.46$).

Table 1. Patient Characteristics.

	Group I (conventional) N=47	Group II (intensified) N=45
Male : female ratio	1 : 1.8	1 : 1.1
Mean age ± SD (min-max)	43 ± 13 (17-68)	46 ± 14 (21 – 74)
Disease duration > 5 years	66%	56%
Skin type		
I	2%	4%
II	55%	64%
III	34%	11%
IV	4%	7%
V	4%	13%
VI	0%	0%

SD: standard deviation

Treatment characteristics and results are shown in Table 2. Patients in group II received significantly more irradiation per week. Of this group 96% had used additional topical agents during the irradiation period: 88% applied tacrolimus 0.1% for the face with a mean number of 3.3 times per week and 79% applied fluticasone propionate 0.005% with a mean application of 3.2 times per week. Other creams such as Pimecrolimus and Betamethasone were used by 9% of the patients.

Table 2. Characteristics and results of treatment.

	Group I (conventional)	Group II (intensified)	P-value
Mean number of treatments per week	2.3	3.0	<0.001*
Time investment per week in minutesmedian – [IQR]	20 – [10-40]	25 – [12-40]	0.72
Missed – more than 10 treatments	13%	27%	0.38
Suberythema – more than 20 times	40%	42%	0.27
Painful erythema occurrence – more than 10 times	8.5%	4.4%	0.79
Blistering	0%	4.4%	0.14

*significant

The majority of patients in both groups were treated more than nine months with the NB-UVB therapy (group I 68%, group II 83%). The median cumulative doses in group I and II were 62.3 (N=47) and 63.8 J/cm2 (N=22), respectively. In the intensified group half of the cumulative doses (23 patients) were unknown due to incomplete treatment charts of the patients.

Repigmentation and satisfaction

The median repigmentation within the two groups differed widely for different body sites. The median repigmentation for the face and neck were reported slightly

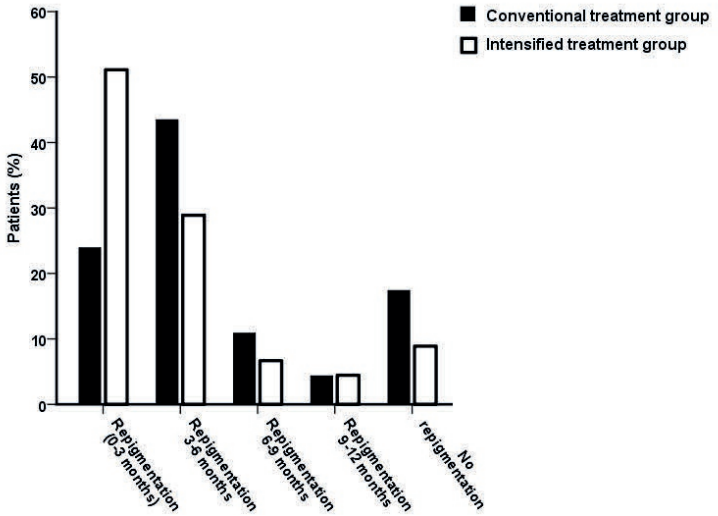
higher in group II, this difference was not significant. In both groups, patients reported that acral areas tended to repigment less than the face and neck (Table 3). The onset of repigmentation in the first three months seems higher in group II, but this difference is not significant (Group I 23.4%, group II 51.1%; p=0.11) (Fig. 1). The start of repigmentation in both groups was predominantly reported in the first six months (66% of the group I and 80% group II).

Table 3. Median repigmentation (%) at different body sites at the end of therapy.

	Group 1 (conventional) $_{\text{Median [IQR]}}$	N	Group II (intensified) $_{\text{Median [IQR]}}$	N	P-value
Face	60 [6 - 80]	28	75 [11 - 90]	40	0.20
Neck	40 [30 - 70]	19	50 [9 - 80]	25	0.79
Trunk	30 [10 - 55]	30	20 [6 - 50]	33	0.50
Arms	40 [10 - 60]	29	25 [6 - 60]	31	0.49
Hands	10 [0 - 30]	31	3 [0 - 30]	32	0.37
Legs	35 [6 - 58]	24	40 [6 - 70]	33	0.78
Feet	0 [0 - 15]	17	0 [0 - 5]	25	0.60

IQR: interquartile range

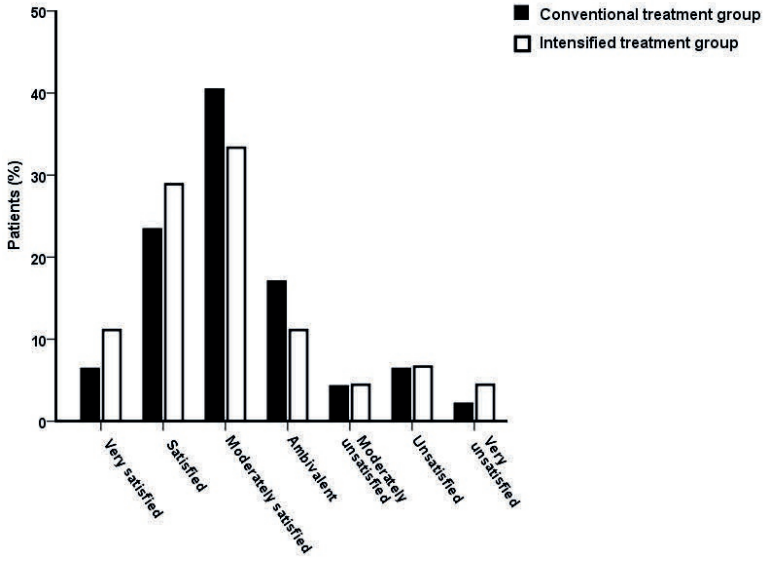
Figure 1. The onset of repigmentation per patient group. No significant differences were found between groups (Chi square test, p=0.11).



Additionally, a significant correlation between the onset of repigmentation and satisfaction of the result was found in group II (intensified); Spearman's rho 0.44, p=0.003. In group I (conventional) this correlation was not significant (Spearman's

rho 0.28; p=0.064). The majority of the patients were moderately to very satisfied in both groups (conventional group 70.2% versus intensified group 73.3%, p=0.91) (Fig. 2).
Furthermore, most of the patients would recommend their treatment to others (98% of the conventional group, 89% of the intensified group; p=0.08).

Figure 2. Satisfaction with result after one year NB-UVB therapy per patient group. No significant differences were found between groups (Chi square test, p=0.91).



DISCUSSION

This questionnaire study examined patient reported outcomes regarding repigmentation and satisfaction in non-segmental vitiligo patients after completing NB-UVB therapy. According to this retrospective study, the intensified treatment regimen with NB-UVB thrice a week in combination with topical agents does not seem to be superior to the conventional treatment regimen with NB-UVB twice a week.

Our NB-UVB protocol changed from twice to thrice a week (in combination with topical therapy), however no reports are available demonstrating the superiority of one above the other. Previous studies indicate that repigmentation is dependent on the total number of light treatments.[14,15] Earlier onset of repigmentation has been seen in the thrice weekly regimen, which seems to be comparable to our study. Similar

results have been observed in a study comparing twice and thrice weekly NB-UVB therapy for psoriatic patients.[16] Furthermore, systematic review studies comparing combination therapy (NB-UVB and topical calcineurin inhibitors) with monotherapy (NB-UBV) showed improved repigmentation for the combination therapy, especially in the face and neck.[17,18] However, we cannot confirm this in our study.

To our knowledge, our retrospective study is the first to compare different treatment regimens with each other from patients' perspectives. A strength of our study was that it concerns real life data from clinical practice. Another strength is that it had equal and comparable treatment groups regarding baseline patient and treatment characteristics, which was essential to make proper comparisons between the two different treatment regimens. In addition, the surveys were only sent to patients that had recently completed their NB-UVB therapy, which kept the recall bias as limited as possible.

However, there are some limitations to this study that need to be addressed. Firstly the study was performed in two different time periods with a 10 year interval, patients expectations might have changed over time. Since the prognosis and treatment regimens for vitiligo have not changed much in this period we expect this difference to be small. Furthermore this study was based on daily practice, additional therapy (such as topical agents and skin transplantation) during NB-UVB therapy were allowed in the conventional group. This could have had an influence on the outcomes and makes a proper comparison between our treatment regimens more difficult. Moreover, not all cumulative doses were known of the patients in the intensified group due to incomplete treatment charts. The observation that the final repigmentation per body site was comparable for both groups may be due to the similar cumulative doses in both groups. However this cannot be objectified due to the fact that not all cumulative doses could be determined. Another important limitation is the lack of validated outcome measures. However, it is remarkable that the percentage of repigmentation in the various body locations is compatible with the expected response pattern in both groups. Finally, disease extent and comorbidity were not assessed, although these factors might influence the patients' perspective on the therapeutic outcomes.

The design of our study was constructed in such a way that two and three times weekly NB-UVB therapy could not directly be compared with each other; we also added the topical treatment to our standard of care. A direct comparison between twice weekly and thrice weekly NB-UVB in vitiligo patients is still needed to determine what the optimal frequency is. It is possible that the additional topical treatment is responsible for the earlier repigmentation in the intensified group.

Thus, prospective studies are required, since it is difficult to critically investigate these regimens in retrospective study designs due to the possible variations in dosimetry, irradiation time and other outcome measures. A twice-weekly regimen might be more convenient regarding time and effort for patients. However, this could be accompanied by psychological distress experienced by patients due to slow responses, leading to reduced therapy compliance.[13]

In conclusion, this study shows that the twice-weekly and thrice-weekly regimen in combination with topical agents seem to be comparable regarding patient reported repigmentation, satisfaction and occurrence of short term adverse effects. Though, patients seem to notice an earlier onset of repigmentation in the intensified group, this difference is not significant. Finally, the start of repigmentation in the intensified group significantly correlates with the satisfaction after completing the NB-UVB therapy. The intensified treatment might be more effective to speed up the onset of repigmentation, but costs and burden should be taken into account. Larger prospective studies are needed to objectify these findings.

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We would like to express our gratitude to the volunteering patients who participated in this study.

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SUPPLEMENTARY APPENDIX I
THE QUESTIONNAIRE (IN DUTCH)

Vragenlijst voor patiënten met vitiligo die een UVB-kuur hebben gehad
Hieronder vindt u een aantal vragen over uw vitiligo en de behandeling met UVB.
Wilt u bij elke vraag het hokje aankruisen dat het meest op u van toepassing is?
Het is belangrijk dat u alle vragen beantwoordt en geen vragen overslaat.
Het invullen van deze vragenlijst kost ongeveer 5 minuten.

Voorletter(s) + achternaam : .....
Geboortedatum :...../...../.....
Geslacht : M / V

Voorgeschiedenis
1) Hoe lang heeft u vitiligo?

- ☐ Minder dan 1 jaar
- ☐ 1-5 jaar
- ☐ Langer dan 5 jaar

2) Heeft u voor deze laatste lichtbehandeling al eens eerder een behandeling voor vitiligo gehad? Zo ja, welke?
(Omcirkel uw antwoord, meerdere antwoorden mogelijk)

UVB-lichttherapie		ja	nee	weet niet
Zo ja,	Thuis	ja	nee	
	Ziekenhuis	ja	nee	
UVA (zonnebank)		ja	nee	
PUVA	lichttherapie	ja	nee	weet niet
Hormoonzalf/crème		ja	nee	weet niet
Huidtransplantatie		ja	nee	weet niet
Anders , namelijk:		.....		

3) Hoeveel jaar heeft u, de laatste UVB-kuur meegerekend, in totaal UVB-lichttherapie gehad?

- ☐ Minder dan 1 jaar
- ☐ 1 jaar
- ☐ 2 jaar
- ☐ 3 jaar
- ☐ Meer dan 3 jaar
- ☐ Anders:……..

4) Hoe gedroeg de vitiligo zich voordat u begon met de lichtbehandeling?

- ☐ Het breidde zich uit de laatste 6 weken voor start lichtbehandeling
- ☐ Het breidde zich uit de laatste 3 maanden voor lichtbehandeling
- ☐ Het breidde zich uit de laatste 6 maanden voor lichtbehandeling
- ☐ Het breidde zich uit de laatste 12 maanden voor lichtbehandeling
- ☐ Geen uitbreiding laatste 12 maanden voor lichtbehandeling
- ☐ Geen uitbreiding laatste 12 maanden en mijn huidskleur kwam spontaan terug in de witte plekken
- ☐ Ik kan het me niet meer herinneren

Vragen over uw laatste UVB-kuur

Wilt u bij elke vraag het hokje aankruisen dat het meest op u van toepassing is?

5) Waar vond de laatste lichtbehandeling plaats?

- ☐ Thuis
- ☐ SNIP

6) Hoe vaak belichtte u gemiddeld per week?

- ☐ 1x per week
- ☐ 2x per week
- ☐ 3x per week
- ☐ 4x per week
- ☐ 5x per week

7) Hoe lang heeft u belicht?

- ☐ Minder dan 3 maanden
- ☐ 3 tot 9 maanden
- ☐ 9-12 maanden
- ☐ Meer dan 12 maanden

8) Hoe vaak heeft u door omstandigheden een behandeling moeten missen?

- ☐ Nooit
- ☐ 1-5 keer
- ☐ 5-10 keer
- ☐ Meer dan 10 keer

9) Na hoeveel maanden merkte u dat de bruine kleur in de witte plekken terugkwam?

- ☐ Ongeveer 0-3 maanden
- ☐ Ongeveer 3-6 maanden
- ☐ Ongeveer 6-9 maanden
- ☐ Ongeveer 9-12 maanden
- ☐ Niet

10) Hoe vaak merkte u dat direct na de belichting de plekken tijdelijk (korter dan 12 uur) roze werden?

- ☐ Minder dan 10 keer
- ☐ 10-20 keer
- ☐ 20-40 keer
- ☐ Meer dan 40 keer

11) Bent u verbrand met pijnlijke roodheid tijdens de laatste periode lichtbehandeling?
(Indien 'ja', geef aan hoe vaak)

- ☐ nee
- ☐ ja:
  - ☐ minder dan 5 keer
  - ☐ 5-10 keer
  - ☐ meer dan 10 keer

12) Bent u verbrand tot blaren toe tijdens de laatste UVB-kuur?
(Indien 'ja', geef aan hoe vaak)

- ☐ Nee
- ☐ Ja
  - ☐ minder dan 5 keer
  - ☐ 5-10 keer
  - ☐ meer dan 10 keer

13) Als de plekken direct na de vorige belichting roze (minder dan 12 uur) werden,

- ☐ Sloeg ik hierna de volgende belichting over
- ☐ Ging ik bij de volgende belichting een stap terug
- ☐ Bleef ik gelijk bij de volgende belichting
- ☐ Ging ik bij de volgende belichting een stap hoger
- ☐ Niet van toepassing: de plekken werden nooit roze

14) Als de plekken na de vorige belichting rood en pijnlijk waren, …

- ☐ Sloeg ik hierna de volgende belichting over
- ☐ Ging ik bij de volgende belichting een stap terug
- ☐ Bleef ik gelijk bij de volgende belichting
- ☐ Ging ik bij de volgende belichting een stap hoger
- ☐ Niet van toepassing: de plekken werden nooit rood en pijnlijk

Vragen over gebruik van crèmes tijdens UVB-kuur

15) Heeft u gedurende de periode van de UVB-kuur ook crèmes gesmeerd voor de behandeling van vitiligo:
(Indien 'ja', geef aan welke (meerdere opties mogelijk) en hoe vaak)

- ☐ Nee
- ☐ Ja
  - ☐ Protopic
    - ☐ Gemiddeld 4 maal per week
    - ☐ Gemiddeld 3 maal per week
    - ☐ Gemiddeld 2 maal per week
    - ☐ Gemiddeld 1 maal per week
    - ☐ Zelden
  - ☐ Cutivate
    - ☐ Gemiddeld 4 maal per week
    - ☐ Gemiddeld 3 maal per week
    - ☐ Gemiddeld 2 maal per week
    - ☐ Gemiddeld 1 maal per week
    - ☐ Zelden
  - ☐ Overig, namelijk: ……
    - ☐ Gemiddeld 4 maal per week
    - ☐ Gemiddeld 3 maal per week
    - ☐ Gemiddeld 2 maal per week
    - ☐ Gemiddeld 1 maal per week
    - ☐ Zelden

Resultaten

16) Hoe tevreden bent u over het uiterlijk van de gerepigmenteerde huid na de behandeling?

- ☐ zeer tevreden
- ☐ tevreden
- ☐ tamelijk tevreden
- ☐ noch tevreden - noch ontevreden
- ☐ tamelijk ontevreden
- ☐ ontevreden
- ☐ zeer ontevreden

17) Bent u voldoende voorgelicht over de lichtbehandeling en het belichtingsschema?

- ☐ ja
- ☐ nee

Indien ‘nee’; wat hebt u gemist?
……

18) Hoe tevreden bent u over het resultaat van de behandeling?

- ☐ zeer tevreden
- ☐ tevreden
- ☐ tamelijk tevreden
- ☐ noch tevreden - noch ontevreden
- ☐ tamelijk ontevreden
- ☐ ontevreden
- ☐ zeer ontevreden

19) Hoe reageerde uw vitiligo op de laatste UVB kuur?
U kunt hier aangeven op welke plekken uw vitiligo aanwezig was en hoeveel bruine kleur in elke witte plek is teruggekomen (meerdere antwoorden mogelijk)

	Plaats	% verbetering
☐	Gelaat	……..%
☐	Hals	……..%
☐	Romp	……..%
☐	Armen	……..%
☐	Handen	……..%
☐	Benen	……..%
☐	Voeten	……..%
	_____	
☐	Totaal	……..%

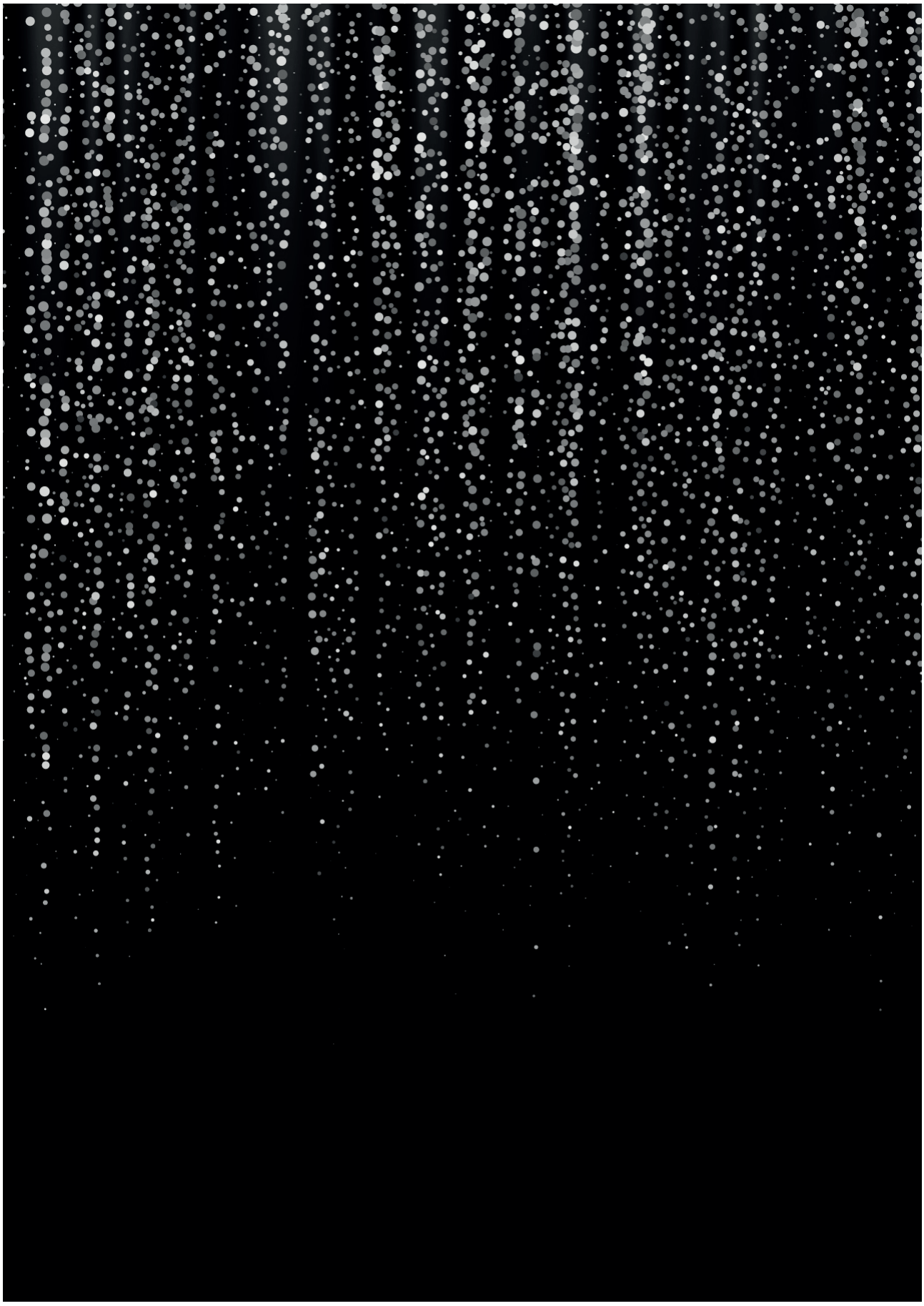
20) Zou u anderen de behandeling aanraden?

- ☐ Ja
- ☐ Nee

Waarom (niet)?
……
……
……

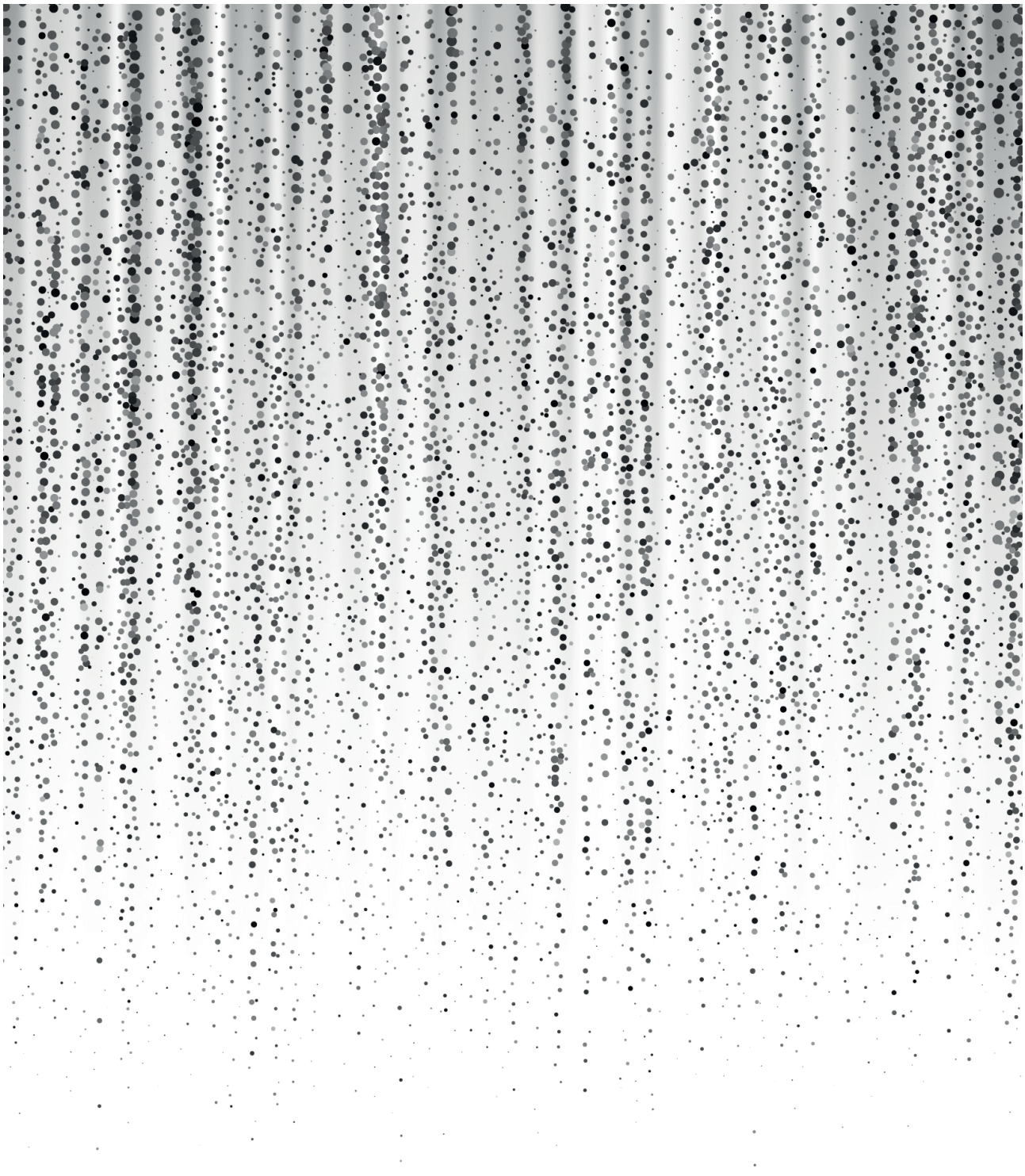
22) Hoeveel tijd was u in totaal per week kwijt met lichtbehandeling?
…… minuten …….. uren

Overige opmerkingen die u kwijt wil:
……
……



PART II

OPTIMIZATION OF SURGICAL TREATMENT



CHAPTER 5

Donor to recipient ratios in the surgical treatment of vitiligo and piebaldism: a systematic review

Vidhya S. Narayan
Lars L.C. van den Bol
Nanja van Geel
Marcel W. Bekkenk
Rosalie M. Luiten
Albert Wolkerstorfer

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ABSTRACT

Stabilized vitiligo resistant to conventional therapy (e.g. segmental vitiligo) and piebaldism lesions can be treated with autologous cellular grafting techniques, such as non-cultured cell suspension transplantation (NCST) and cultured melanocyte transplantation (CMT). These methods are preferred when treating larger surface areas due to the small amount of donor skin needed. However, the donor to recipient expansion ratios and outcomes reported in studies with cellular grafting vary widely and to date, no overview or guideline exists on the optimal ratio. The aim of our study was to obtain an overview of the various expansion ratios used in cellular grafting and to identify whether expansion ratios affect repigmentation and colour match. We performed a systematic literature search in MEDLINE and EMBASE, to review clinical studies that reported the expansion ratio and repigmentation after cellular grafting. We included 31 eligible clinical studies with 1591 patients in total. Our study provides an overview of various expansion ratios used in cellular grafting for vitiligo and piebaldism, which varied from 1:1 up to 1:100. We found expansion ratios between 1:1 and 1:10 for studies investigating NCST and from 1:20 to 1:100 in studies evaluating CMT. Pooled analyses of studies with the same expansion ratio and repigmentation thresholds, showed that when using the lowest (1:3) expansion ratio the proportion of lesions achieving >50% or >75% repigmentation after NCST was significantly better than when using the highest (1:10) expansion ratio (χ^2 P=0.000 and χ^2 P=0.006, respectively). Less than half of our included studies stated the colour match between different expansion ratios and results were variable. In conclusion, the results of our study indicate that higher expansion ratios lead to lower repigmentation percentages after NCST treatment. This should be taken into consideration while determining which expansion ratio to use for treating a patient.

INTRODUCTION

Vitiligo and piebaldism are skin disorders, in which large depigmented lesions can be present. These skin diseases can severely alter the physical appearance leading to social stigmatization and an impaired quality of life.[1,2] Several methods of autologous skin transplantation are available as treatment for repigmenting stable vitiligo and piebaldism lesions.[3,4] These surgical methods can roughly be divided into tissue grafting and cellular grafting.

The three major techniques of tissue grafting include punch grafting, epidermal blister grafting and split-thickness grafting.[5] Punch grafting is a simple and widely used technique, in which a donor skin area of 1cm^2 can approximately repigment a recipient depigmented skin area of 5cm^2 (donor to recipient expansion ratio is 1:5).[6] This ratio however, can vary due to the differences in pigment spread. Moreover, adverse effects such as a cobblestone appearance of the recipient site and scarring of the donor site are not uncommon.[7] The epidermal blister grafting and the split thickness grafting methods are found to have the highest repigmentation success rates[3,7], however the donor to recipient expansion ratios (DR expansion ratios) are approximately 1:1 for both techniques.[8–10] Due to these low expansion ratios, the tissue grafting techniques are poorly suited for treating large depigmented areas, requiring large donor areas to treat large recipient lesions.[11] In addition, repeated surgical operations are needed for treating large surface areas.

Cellular grafting techniques include non-cultured cell suspension transplantation (NCST) and cultured melanocyte transplantation (CMT). These methods are preferred when treating larger surface areas due to the small amount of donor skin needed.[11] However, DR expansion ratios and outcomes reported in studies with cellular grafting vary widely and to date, no overview or guideline exists on which ratio to use. Furthermore, little evidence is available on the correlation of expansion ratios with the repigmentation success rate.

We performed a systematic review to provide an overview of the various expansion ratios used during NCST and CMT. Furthermore we aimed to identify whether expansion ratios affect the repigmentation success rates and colour matching to the non-lesional surrounding skin.

METHODS

This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Guideline and was registered in the PROSPERO database (registration number: CRD42020176011).

Search strategy

We za systematic literature search in collaboration with a clinical librarian (M.M.) of the Amsterdam University Medical Center in EMBASE and MEDLINE from inception until 20th December 2019. Our search strategy contained main keywords and synonyms of vitiligo, piebaldism and cellular transplantation techniques (see Appendix S1 for entire literature search strategy). Original articles were obtained by excluding editorials, reviews and commentaries in the search. The same approach was used for excluding animal studies. Subsequently, the reference lists of identified relevant publications were screened manually for other relevant articles.

Study selection and eligibility criteria

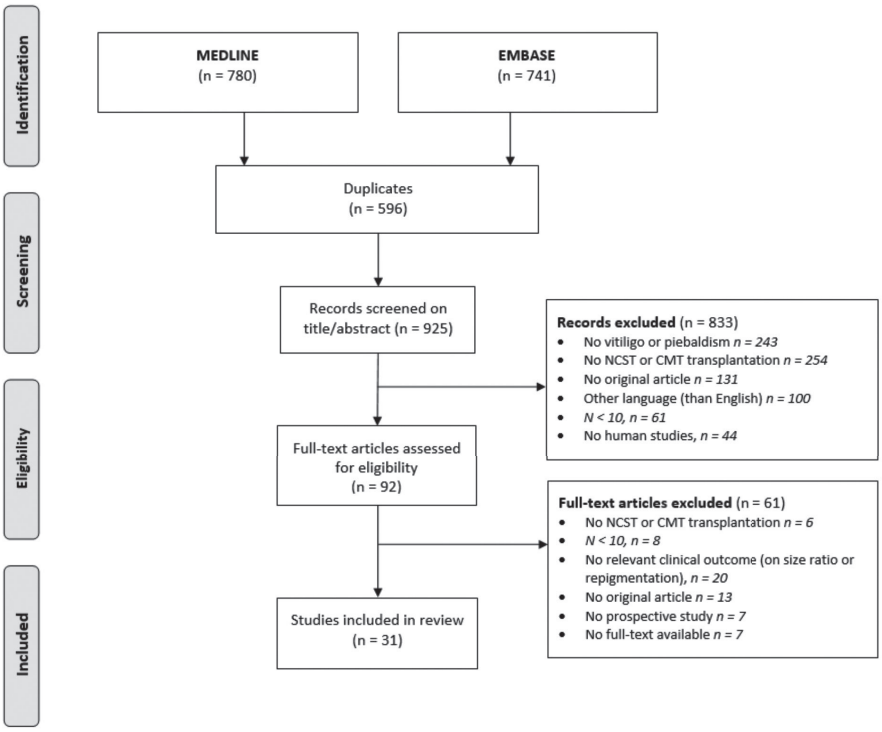
All articles retrieved by our search were screened independently by 2 reviewers (V.N. and L.B.) using the web-tool *Rayyan*[12], after removing duplicate findings. We performed a first selection by means of screening title and abstract, followed by full text screening based on the predefined eligibility criteria. Any discrepancies between the 2 reviewers were resolved through discussion with a third reviewer (A.W.). The inclusion criteria consisted of: (I) ≥10 vitiligo and/or piebaldism patients, (II) intervention study with cellular transplantation technique(s), (III) known donor and recipient sizes with quantitative repigmentation as outcome measure, (IV) prospective studies including (non)randomised clinical trials and case series and (V) studies written in English. Cellular transplantation techniques consisted of NCST and CMT. We did not exclude studies in which additional therapy (e.g. phototherapy) was given. The selection procedure for eligible studies and exclusion criteria are shown in the PRISMA flow diagram (Fig. 1).

Data extraction and analyses

The 2 reviewers (V.N. and L.B.) extracted the following information independently from each eligible study: author, publication year, study design, number of patients and/or lesions, patient characteristics, (sub)type of depigmentation, disease stability, preparation of recipient site and grafting type of donor site. In addition, for the study results we extracted information on transplantation technique, adjuvant treatment, DR expansion ratio, number of patients reaching repigmentation based on >90%, 75%, >70% >65% and >50%, colour match and follow-up duration.

Descriptive statistics and pooled analyses of studies with the same expansion ratio and repigmentation thresholds were carried out to meet our study aim. Chi-square (χ^2) tests were performed to analyse and compare categorical variables. Spearman's rank correlation (r_s) test was used to define the association between the DR expansion ratios and the repigmentation. Statistical analyses were executed using Statistical Package for Social Sciences (SPSS version 26.0 for Windows) and the statistical level of significance was set at $P < 0.05$.

Figure 1. Prisma flow diagram of selection procedure and included studies. The selection procedure for eligible studies and exclusion criteria are shown in this PRISMA flow diagram.



Quality assessment

We performed a risk of bias analysis to assess the quality of all included articles. We used the Cochrane Collaboration risk-of-bias tool for randomised trials (RoB 2)[13] to assess the bias (high, some concerns, low) of each included randomised controlled trial (RCT) regarding the (1) randomisation process, (2) deviations from the intended interventions, (3) missing data, (4) measurement of the outcome and (5) selection of reported result. For the quality assessment of the included case

series, we used the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Case Series.[14] For the included (non-randomised) clinical trials we used the JBI Critical Appraisal Checklist for Quasi-Experimental Studies.[15] Both checklists comprised of approximately 10 questions on quality appraisal, in which the overall appraisal was classified by 'include' for studies with a low or moderate bias and 'exclude' for studies with a high risk of bias (Appendix S3).

RESULTS

Study selection and characteristics

A total of 925 unique articles were initially identified by the database searches. Based on our eligibility criteria of title and abstract, 833 articles were excluded leaving 92 studies for full text screening of which 31 studies met our inclusion criteria. Our study selection process and reasons for exclusion are presented in the PRISMA flow diagram (Fig. 1). A total of 1591 patients and 3081 treated leukodermal lesions were investigated. Of the 31 articles, 11 were randomised controlled trials, 6 were (non-randomised) clinical trials and 14 were case series studies. All included studies were prospective in which vitiligo and/or piebaldism patients were treated with NCST (30 studies) and/or with CMT (4 studies). Twenty-eight of the 31 studies used the split-thickness skin grafting method to harvest skin from the donor site. Of the eligible articles, 29 studies treated vitiligo patients and 2 studies treated both vitiligo and piebaldism patients. In 26 studies different subtypes of vitiligo (i.e. non-segmental, segmental and focal vitiligo) were included (Table 1). Detailed patient characteristics can be found in Appendix S2, Supporting information. Nineteen studies mentioned one specific expansion ratio that was used (marked as 'precise DR expansion ratio' Table 1) and the other 12 studies reported a (wide) range of expansion ratios (marked as 'range of DR expansion ratios' in Table 1). Expansion ratios varied from 1:1 up to 1:100 in the included studies. The study characteristics, DR expansion ratios, results of repigmentation, colour match and treatments are summarised in Table 1.

Studies comparing DR expansion ratios in NCST

Two studies directly compared different expansion ratios and their repigmentation after NCST.[16,17] In the study of Tegta *et al.*[16] repigmentation after NCST was compared between a 1:3 expansion ratio group (n=10 patients) and a 1:5 expansion ratio group (n=10 patients). Three months after transplantation 5 patients in the 1:3 group had a > 75% repigmentation response, whereas no patients in the 1:5 group reached this response ($P < 0.05$).

Tawfik *et al.*[17] assessed repigmentation after NSCT in 42 non-segmental vitiligo (NSV) patients, randomly allocated to two groups: one group (*n*=21 patients) received a 1:3 expansion ratio in which a total of 50 lesions were treated. These lesions were subdivided into two groups: 25 lesions received additional NB-UVB therapy after surgery and the other 25 lesions did not. The other group (*n*=21 patients, 52 lesions) was treated with a 1:10 expansion ratio, of which half of the lesions received additional NB-UVB therapy. More patients showed >75% repigmentation in the 1:3 expansion ratio group (88% with NB-UVB and 80% without additional therapy) compared to the 1:10 expansion ratio group (12% with NB-UVB and 8% without post treatment). This repigmentation was significantly better in the 1:3 expansion ratio group compared to the 1:10 group (t-test P=0.000), however no significant differences were found between the subgroups. Both studies demonstrate great differences in repigmentation response between the lower and higher expansion ratio groups.

DR expansion ratio related to repigmentation and colour match in NCST

Given the heterogeneity of the studies (predominantly regarding outcomes, disease stability, follow-up and adjuvant treatment) it was not feasible to perform a meta-analysis. Instead, we performed pooled analyses on roughly comparable studies that stated a precise DR expansion ratio and used the same repigmentation outcome thresholds (i.e. 50%, 75% or 90% repigmentation). Studies that provided a form of additional treatment (such as NB-UVB, PUVASOL, excimer laser) were excluded from the pooled analysis since these could influence the outcomes.[18] Tables 2a, 2b and 2c show the number of lesions reaching >50%, >75% and >90% repigmentation respectively after NCST per DR expansion ratio used. The highest repigmentation rates are seen in the 1:3 expansion ratio group, whereas the lowest repigmentation percentages are found in the 1:10 expansion ratio group. These differences between expansion ratios were significant in the >50% (χ^2 P=0.000) and >75% (χ^2 P=0.006) repigmentation groups. Furthermore, a significant correlation was found in these groups between the expansion ratios and the repigmentation percentages (r_s = -0.228 P=0.000, r_s = -0.197 P=0.006, respectively). For the >90% repigmentation group (Table 2c), no significant association was found between expansion ratio and repigmentation (r_s = -0.109 P=0.08).

Table 1. Study characteristics and results

Author, year	Study design	*n* patients (lesions)	Type of depig-mentation	Stability disease (months)	Preparation recipient site	Treat-ment	Post-Treatment	D:R ratio	Repigmentation *n* (% of total)	Good colour match *n (% of total)*	Follow-up (months)
Precise DR expansion ratio											
Garg, 2019[41]	CS	10 (20)	8 NSV 2 SV	≥ 6	Er:YAG	NCST		1:3	> 75 %: 14 lesions (70%)	Unknown	6
Kachhawa, 2017[42]	CS	152 (437)	151 NSV 1 SV	≥ 12	Dermabrasion	NCST	PUVA	1:4	> 75 %: 179 lesions (41%)	Unknown	6
El-Zawahry, 2017[19]	CT	A. 10 (61) B. 21 (89)	NSV	≥ 12	A. CO_2 B. Cryo	NCST	UVB	1:5	A. > 75 %: 2 patients (20%) B. > 75 %: 9 patients (43%)	A. 6 patients (60%) B. unknown	18
Razmi, 2018[20]*	RCT	30 (42)	21 NSV 6 SV 3 FV#	≥ 12	Dermabrasion	NCST		1:5	> 75 %: 24 lesions (57%)	25 lesions (60%)	4
Tegta, 2006[16]	RCT	A. 10 B. 10	11 NSV 4 SV 5 FV	≥ 12	Blister or Dermabrasion	NCST		A. 1:3 B. 1:5	A. > 75 %: 5 patients (50%) B. > 75 %: 0 patients (0%)	A. 3 patients (30%) B. 1 patient (10%)	3
Tawfik, 2019[17]	RCT	42 1a. (25) 1b. (25) 2a. (26) 2b. (26)	NSV	≥ 12	Dermabrasion	NCST	1a. None 1b. NB-UVB 2a. None 2b. NB-UVB	1:3 1:10	1a. > 75 %: 20 lesions (80%) 1b. > 75 %: 22 lesions (88%) 2a. > 75 %: 2 lesions (8%) 2b. > 75 %: 3 lesions (12%)	1a. 25 lesions (100%) 1b. 25 lesions (100%) 2a. 17 lesions (65%) 2b. 17 lesions (65%)	6 6
Budania, 2012[21]*	RCT	21 (28)	8 NSV 10 SV 3 FV	≥ 12	Dermabrasion	NCST	sunlight exposure	1:10	> 75 %: 25 lesions (89%)	23 lesions (82%)	4
El-Zawahry, 2011[34]	CS	22	19 NSV 1 SV 2 FV#	≥ 12	Cryo	NCST	PUVA	1:10	> 50 %: 12 patients (55%)	Unknown	6-17
Huggins, 2012[22]	CS	23 (29)	19 NSV 2 SV 8 FV#	≥ 6	Dermabrasion	NCST		1:10	> 65 %: 14 lesions (48%)	18 lesions (62%)	3-6

Table 1. Continued.

Author, year	Study design	*n* patients (lesions)	Type of depig-mentation	Stability disease (months)	Preparation recipient site	Treat-ment	Post-Treatment	D:R ratio	Repigmentation *n* (% of total)	Good colour match *n (% of total)*	Follow-up (months)
Mulekar, 2006[43]	CS	25 A. 25 B. 25	17 NSV 8 SV#	≥ 6	Dermabrasion	NCST	A. None B. Oral beta-methasone	1:10	A. > 65 %: 8 patients (32%) B. > 65 %: 22 patients (88%)	Unknown	12
Mulekar, 2005[23]	CS	142	NSV	≥ 6	Dermabrasion	NCST		1:10	> 65 %: 95 patients (67%)	125 patients (88%)	12-72
Mulekar, 2003[44]	CS	175	114 NSV 43 SV 18 FV#	≥ 12	Dermabrasion	NCST		1:10	> 65 %: 129 patients (74%)	Unknown	12
Mutalik, 2017[45]	RCT	A. 25 B. 25	14 NSV 10 SV 26 FV	≥ 24	Dermabrasion	NCST	A. None B. Oral Cyclosporin	1:10	A. > 75 %: 7 patients (28%) B. > 75 %: 25 patients (100%)	Unknown	6
Pandya, 2005[33]	CS	A. 4 B. 23	25 NSV 2 SV	≥ 24	Dermabrasion	A.CMT B.NCST		A. unclear B. 1:10	A. > 65%: 2 patients (50%) B. > 65%: 16 patients (70%)	Unknown	6
Ramos, 2017[24]	CS	20 (24)	8 NSV 12 SV#	≥ 12	Dermabrasion	NCST	sunlight exposure	1:10	> 50 %: 15 patients (75%)	17 lesions (85%)	3-6
Thakur, 2019[25]	RCT	A. 10 B. 10	12 NSV 8 SV	A. 3-6 B. ≥ 12	Dermabrasion	NCST		1:10	A. > 75 %: 3 patients (30%) B. > 75 %: 6 patients (60%)	A. 8 patients (80%) B. 8 patients (80%)	6
Vazquez-Martinez, 2011[46]	CT	11	NSV SV FV+	≥ 12	Dermabrasion	NCST		1:10	Unclear	Unknown	12
Bao, 2015[31]	CT	83 A. (83) B. (83)	43 NSV 40 SV	≥ 12	CO_2	A.CMT B.NCST		A. 1:20 B. 1:5	A. > 50 %: 68 lesions (82%) B. > 50 %: 67 lesions (81%)	Nearly uniform in both methods	12
Verma, 2014[32]	RCT	A. 6 (50) B. 19 (50)	20 NSV 2 SV 3 FV	≥ 12	Dermabrasion	A.CMT B.NCST	A. Puvasol B. Puvasol	A. 1:100 B. 1:10	A. > 70 %: 26 lesions (52%) B. > 70 %: 31 lesions (62%)	Unknown	6

Table 1. Continued.

Author, year	Study design	*n* patients (lesions)	Type of depig-mentation	Stability disease (months)	Preparation recipient site	Treat-ment	Post-Treatment	D:R ratio	Repigmentation *n* (% of total)	Good colour match *n (% of total)*	Follow-up (months)
Range of DR expansion ratios											
van Geel, 2004[26]	RCT	A. 19 (22) B. 9 (11)	NSV	A. ≥ 12 B. < 12	CO_2	NCST	UVB or PUVA	1:1 - 1:4.5	A. > 75 %: 9 lesions (41%) B. > 75 %: 0 lesions (0%)	13 lesions (72%)	12
Lommerts, 2017[27]	RCT	10 A. (10) B. (10)	3 SV 7 P	≥ 12	A.CO_2.209 µm B.CO_2.144 µm	NCST	UVA	1:4-1:5	A. > 75 %: 5 lesions (50%) B. > 75 %: 4 lesions (40%)	A. 9 lesions (90%) B. 9 lesions (90%)	6
Khodadadi, 2010[28]	CS	10	6 NSV 4 FV	≥ 12	Intraepidermal injection	NCST		1:3-1:7	> 75 %: 4 patients (40%)	4 patients (40%)	6
Gill, 2019[47]	CS	50	40 NSV 4 SV 6 FV	≥ 12	Dermabrasion	NCST		1:3-1:10	> 70 %: 31 patients (62%)	Unclear	6
Gupta, 2019[36]	RCT	A. 15 (22) B. 17 (25)	18 NSV 6 SV 8 FV#	≥ 12	A. Er:YAG B. Dermabra-sion	NCST		1:3-1:10	A. > 50 %: 9 patients (60%) B. > 50 %: 10 patients (59%)	Unknown	6
Mulekar, 2004[35]	CS	64	49 SV 15 FV#	≥ 12	Dermabrasion	NCST		1:3-1:10	> 65 %: 55 patients (86%)	Unknown	60
Orouji, 2018[38]	CT	300 (1060)	231 NSV 10 SV 59 FV#	≥ 12	Intralesional injection	NCST		1:3-1:10	> 75 %: 109 patients (36%)	Unknown	6
Sahni, 2011[29]	CS	13 (19)	6 NSV 6 SV 1 FV#	≥ 12	Dermabrasion	NCST		1:3-1:10	> 75 %: 19 lesions (100%)	16 lesions (84%)	4
Olsson, 1998[37]	CS	23 (27)	17 NSV 3 SV 3 P#	≈ 89 mean	Dermabrasion	NCST		1:4-1:10	> 75 %: 23 lesions (85%)	Unknown	6-12

Table 1. Continued.

Author, year	Study design	*n* patients (lesions)	Type of depig-mentation	Stability disease (months)	Preparation recipient site	Treat-ment	Post-Treatment	D:R ratio	Repigmentation *n* (% of total)	Good colour match *n (% of total)*	Follow-up (months)
Parambath, 2019[48]	RCT	20 A. (20) B. (20)	13 NSV 7 SV#	≥ 12	Dermabrasion	NCST	A. None B. PRP	1:4-1:10	A. > 75 %: 11 lesions (55%) B. > 75 %: 16 lesions (80%)	Unknown	6
Ebadi, 2015[49]	CT	10 A. (9) B. (10)	NSV	≥ 12	Dermabrasion	NCST	A. None B. Excimer laser	1:5-1:10	A. > 65 %: 1 lesion (11%) B. > 65 %: 4 lesions (40%)	Unknown	3-4
Hong, 2011[30]	CT	A. 35 B. 67	12 NSV 90 FV	≥ 6	CO_2	CMT		A.<1:10 B.>1:10	A. > 50 %: 31 patients (89%) B. > 50 %: 57 patients (85%)	Unknown	6

Abbreviations: *n*, number of patients or lesions; DR ratio, donor-recipient size ratio; RCT, randomized controlled trial; CS, case series; CT, clinical trial; NSV, non-segmental vitiligo; SV, segmental vitiligo; FV, focal vitiligo; P, piebaldism; Er:YAG, erbium-doped yttrium aluminum garnet laser; CO2, CO2-laser; Cryo, cryoblebbing; NCST, autologous non-cultured cell suspension transplantation; CMT, autologous cultured melanocyte transplantation; PUVA, psoralen and ultraviolet A; UVB, ultraviolet-B phototherapy; NB-UVB, narrowband ultraviolet-B phototherapy; Puvasol, psoralen combined with sunlight exposure; PRP, platelet-rich plasma
*Only 1 (relevant) treatment arm is shown of this study
#Analysis of repigmentation was provided per vitiligo subtype in study
+Number of patients of each subtype unknown.

We identified 13 studies that specified the colour match of the recipient site to the non-lesional normally pigmented skin after treatment. In two studies directly comparing expansion ratios, a significantly better colour match was found in the lower expansion ratio group.[16,17] Other studies illustrated variable results of the colour matching in relation to the expansion ratios (Table 1).[19,20,29,21–28]

Table 2a. Percentage of NCST treated lesions with > 50% repigmentation in three DR expansion ratio groups

DR ratio	Author, year	Lesions > 50 %	Total lesions	Total percentage lesions with >50% repigmentation
1:3	Garg, 2019	18	20	
	Tegta, 2006	7	10	
	Tawfik, 2019	24	25	
	Total	**49**	**55**	49/55 = **89 % ***
1:5	Razmi, 2018	33	42	
	Tegta, 2006	1	10	
	Bao, 2015	67	83	
	Total	**101**	**135**	101/135 = **75 % ***
1:10	Tawfik, 2019	6	26	
	Budania, 2012	26	28	
	Mutalik, 2017	11	25	
	Ramos, 2017	15	20	
	Thakur, 2019	8	10	
	Total	**66**	**109**	66/109 = **61 % ***

*Chi square test P=0.000

Table 2b. Percentage of NCST treated lesions with > 75% repigmentation in three DR expansion ratio groups

DR ratio	Author, year	Lesions > 75 %	Total lesions	Total percentage lesions with >75% repigmentation
1:3	Garg, 2019	14	20	
	Tegta, 2006	5	10	
	Tawfik, 2019	20	25	
	Total	**39**	**55**	39/55 = **71 % ***
1:5	Razmi, 2018	24	42	
	Tegta, 2006	0	10	
	Total	**24**	**52**	24/52 = **46 % ***
1:10	Tawfik, 2019	2	26	
	Budania, 2012	25	28	
	Mutalik, 2017	7	25	
	Thakur, 2019	6	10	
	Total	**40**	**89**	40/89 = **45 % ***

*Chi square test P=0.006

Table 2c. Percentage of NCST treated lesions with > 90% repigmentation in three DR expansion ratio groups

DR ratio	Author, year	Lesions > 90 %	Total lesions	Total percentage lesions with >90% repigmentation
1:3	Tawfik, 2019	15	25	15/25 = **60 % ***
1:5	Razmi, 2018	13	42	
	Bao, 2015	44	83	
	Total	**57**	**125**	57/125 = **47 % ***
1:10	Tawfik, 2019	1	26	
	Budania, 2012	20	28	
	Pandya, 2005	12	23	
	Ramos, 2017	5	20	
	Thakur, 2019	4	10	
	Total	**40**	**107**	42/107 = **39 % ***

*Chi square test P=0.158

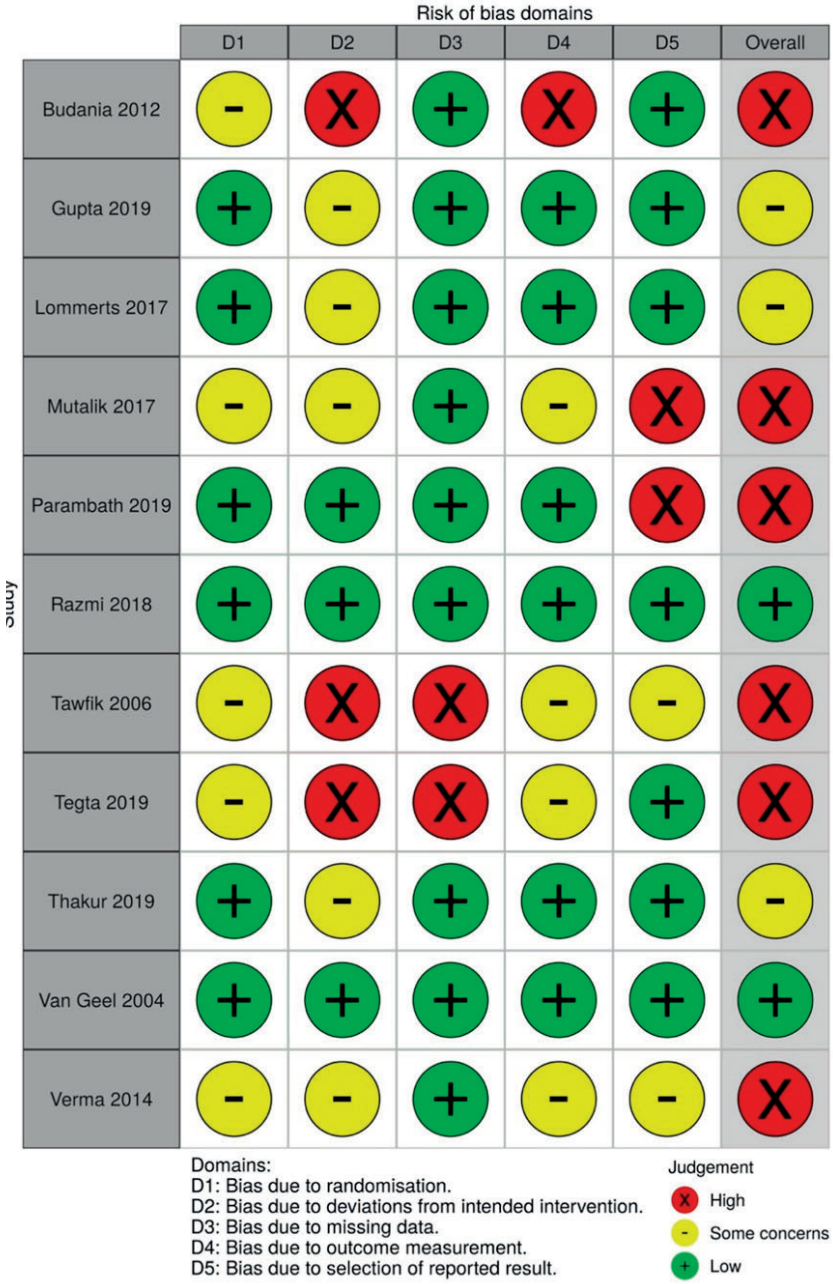
CMT

In four studies repigmentation after CMT was assessed in which the expansion ratios varied from 1:20 until 1:100. Hong *et al*.[30] divided 102 patients into two groups: 35 patients received CMT with a expansion ratio < 1:10 (mean 1:8) and 67 patients were treated with a expansion ratio > 1:10 (mean 1:27). The mean repigmentation was 77% in the low expansion ratio group and 78% for the high expansion ratio group (no significant difference between both groups, t-test P=0.958). Three studies compared CMT with NCST using different expansion ratios. In the study of Bao *et al*.[31] repigmentation in patients (*n*=83) receiving CMT with an expansion ratio of 1:20 (68 patients > 50% repigmentation) did not significantly differ from patients (*n*=83) receiving NCST with an expansion ratio of 1:5 (67 patients with >50% repigmentation), P=0.986. Verma *et al.*[32] compared repigmentation in 6 patients (50 lesions) after CMT with a 1:100 expansion ratio to 19 patients (50 lesions) after NCST with a 1:10 expansion ratio. Although >70% repigmentation was more frequently seen after NCST (62%) than after CMT (52%), the difference between these two groups was not significant (χ^2 P=0.058). In the case series of Pandya *et al*.[33] CMT was compared to NCST showing a higher >65% repigmentation response after NCST than CMT (70% vs 50%). The expansion ratio in the CMT group however, was not clearly stated in this study.

Quality assessment

The risk of bias assessment of the RCTs is shown in Figure 2. Six (6/11) studies showed an overall high risk due to biases in missing data, selection of reported results and deviations from intended interventions. Other studies had some concerns (3/11) or a low (2/11) risk of bias. The critical appraisal of 14 case series studies and 6 (non-randomised) clinical trials are summarised in Appendix S2, Supporting information. Four (4/20) studies showed an overall poor quality mainly due to the selective patient population and lack of reporting relevant information.

Figure 2. Risk of bias assessment of randomised controlled trials. The risk of bias assessment of all 12 included RCTs are shown in this 'traffic light' plot accompanied by the explanation of the 5 domains of bias and risk judgement.



DISCUSSION

This study provides a systematic overview of donor to recipient expansion ratios and its relation to repigmentation and colour match in cellular grafting of vitiligo and piebaldism. We reviewed 31 studies evaluating 1591 patients after NCST and/or CMT. We identified expansion ratios from 1:1 to 1:10 in studies investigating NCST and from 1:20 to 1:100 in studies evaluating CMT. Furthermore the results of our study indicate that lower expansion ratios lead to higher repigmentation percentages after NCST treatment.

In our pooled analyses of various studies performed in different countries and time periods, we found that studies with a 1:10 expansion ratio had a significant lower >50% and >75% repigmentation than the 1:3 or 1:5 expansion ratio groups after NCST. This relation was not significant for the >90% repigmentation (Table 2c), although a similar trend is seen as the >50% and 75% repigmentation groups. We found 2 RCTs comparing a lower expansion ratio to a higher expansion ratio, demonstrating substantially better outcomes for the lower expansion ratios.[16,17] These studies are in line with the findings of our pooled analyses. On the contrary, no significant differences in repigmentation were found between different expansion ratios after CMT treatment.[30] A possible explanation for this could be that in principle this technique yields a larger number of melanocytes for transplantation depending on the culture time. Remarkably, no significant differences were seen in studies comparing (different expansion ratios between) CMT and NSCT.[31–33] The cultured technique however, does have a few disadvantages since it is a time-consuming, expensive and complicated procedure, requiring advanced equipment, a sterile lab setup and trained personnel.

Less than half of our included articles state the colour match. Two studies demonstrate a better colour match, when using a lower expansion ratio[16,17], however the results of the other studies are variable.

Most studies included different vitiligo subtypes (*n*=26), however in only 12 studies an outcome analysis per subtype was provided (Table 1). Subsequently in seven[22,24,34–37] out of these 12 studies, segmental vitiligo (SV) and/or focal vitiligo (FV) demonstrated a higher repigmentation response after NCST treatment compared to NSV. Solely one study[38] showed the exact opposite. This suggests that a separate analysis per subtype is of importance, since various subtypes respond to treatment in a different manner due to differences in pathophysiology.[39] In NSV, even though stable for > 12 months, the persevering auto-immunity against melanocytes can have a negative impact on treatment outcome.

Furthermore, we found differences in preparation of the recipient site (i.e. dermabrasion, C02-laser, Er:YAG-laser, suction blister, cryoblebbing), what could have influenced the outcomes. However, Al-Hadidi et al. have reviewed these methods, stating that there is no evidence-based preference in terms of better outcomes.[40]

One of the limitations in our study was that only two databases were finally used for our search. In addition, we found a high heterogeneity between our included studies in terms of repigmentation measures, disease stability, vitiligo subtypes, follow-up duration, adjuvant therapy and quality. Moreover, many studies were lacking a control group. As a consequence, we were not able to perform a meta-analysis of the outcomes with regard to the expansion ratios. Instead, we pooled study results with the same expansion ratios and repigmentation threshold outcomes (excluding studies with post-surgical adjuvant therapies). These pooled studies were predominantly comparable in disease stability (>12 months) and follow-up duration (average 6 months). Another limitation is that little under half of all included studies stated a range of expansion ratios, making it somewhat difficult to draw conclusions from these studies.

Given these points, this review once again underlines the importance of establishing a consensus on (core) outcomes. Nevertheless, we attempted to integrate the outcomes of all relevant prospective studies as far as possible and to our knowledge this is the first systematic review to summarise different expansion ratios and their related outcomes reported in cellular grafting.

In conclusion, our study provides an overview of various donor to recipient expansion ratios used in cellular grafting for vitiligo and piebaldism. We found expansion ratios between 1:1 and 1:10 for studies investigating NCST and from 1:20 to 1:100 in studies evaluating CMT. Remarkably, no differences in outcomes were found in studies comparing NSCT with CMT. The results of our study indicate that higher expansion ratios lead to lower repigmentation percentages after NCST treatment. For clinical practice this should be taken into consideration, before deciding which DR expansion ratio to use.

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SUPPORTING INFORMATION

Appendix S1. Literature search

Search strategy per database

MEDLINE

("Skin Transplantation"[Mesh] OR "Melanocytes/transplantation"[Mesh] OR "Keratinocytes/transplantation"[Mesh] OR graft*[tiab] OR transplant*[tiab] OR autotransplant*[tiab] OR autograft*[tiab]) AND ("Hypopigmentation"[Mesh] OR hypopigment*[tiab] OR hypomelanos*[tiab] OR partial albinis*[tiab] OR cutaneous albinis*[tiab] OR piebald*[tiab] OR vitilig*[tiab] OR Leukoderm*[tiab] OR leucoderm*[tiab] OR depigment*[tiab]) NOT (animals[mh] NOT humans[mh]) NOT (letter[pt] OR comment[pt] OR editorial[pt])

EMBASE:

('skin transplantation'/exp/mj OR ('keratinocyte'/exp AND 'transplantation'/de) OR
('melanocyte'/exp AND 'transplantation'/de) OR graft*:ti,ab,kw OR transplant*:ti,ab,kw OR
autograft*:ti,ab,kw OR autotransplant*:ti,ab,kw) AND ('hypopigmentation'/mj OR 'depigmentation'/exp/mj OR 'albinism'/exp/mj OR 'piebaldism'/exp/mj OR hypopigment*:ti,ab,kw OR hypomelanos*:ti,ab,kw OR 'partial albinis*':ti,ab,kw OR 'cutaneous albinis*':ti,ab,kw OR piebald*:ti,ab,kw OR vitilig*:ti,ab,kw) NOT ([animals]/lim NOT [humans]/lim) NOT ('conference abstract'/it OR 'conference review'/it OR
'editorial'/it OR 'erratum'/it OR 'letter'/it OR 'note'/it)

Terms for search

Terms for vitiligo and piebaldism

Non-MESH: Vitiligo; Piebaldism; Depigmentation; Hypopigmentation; Leucoderma, hypomelanoses, partial albinism

MESH: Vitiligo (MESH); Hypopigmentation (MESH)

Terms for skin transplantation

Non-MESH: graft*, transplant*, autotransplant*, autograft*, skin transplantation, melanocyte transplantation, keratinocyte transplantation, graft*

MESH: Skin Transplantation (MESH); Melanocytes/transplantation (MESH); Keratinocytes/transplantation (MESH)

Appendix S2. Detailed patient and treatment characteristics table.

Author, year	Country	*N* Patients (lesions)	M/F	Age in years (range)	Type of depigmentation	Stability disease (months)	Treat-ment	Preparation recipient site	Harvesting of donor site
Precise DR expansion ratio									
Garg, 2019	India	10 (20)	8/2	21-30	8 NSV, 2 SV	≥ 6	NCST	Er:YAG-laser	STSG
Kachhawa, 2017	India	152 (437)	69/85[+]	11-50	151 NSV, 1 SV	≥ 12	NCST	Dermabrasion	Derma-braded skin
El-Zawahry, 2017	Egypt	1. 10 (61) 2. 21 (89)	9/25[+]	1. 22.4 mean 2. 24.1 mean	NSV	≥ 12	NCST	1. CO_2-laser 2. Cryoblebbing	STSG
Razmi, 2018	India	30 (42)	12/18	10-36	21 NSV, 6 SV, 3 FV	≥ 12	NCST	Dermabrasion	STSG
Tegta, 2006	India	20	10/10	10-54	11 NSV, 4 SV, 5 FV	≥ 12	NCST	Suction/ phototoxic/ nitrogen blister OR Dermabrasion	STSG
Tawfik, 2019	Egypt	42 (102)	13/29	13-48	NSV	≥ 12	NCST	Dermabrasion	STSG
Budania, 2012	India	21 (28)	7/14	12-27	8 NSV, 10 SV, 3 FV	≥ 12	NCST	Dermabrasion	STSG
El-Zawahry, 2011	Egypt	22	5/17	8-45	19 NSV, 1 SV, 2 FV	≥ 12	NCST	Cryoblebbing	STSG
Huggins, 2012	USA	23 (29)	12/11	18-60	19 NSV, 2 SV, 8 FV	≥ 6	NCST	Dermabrasion	STSG
Mulekar, 2006	Saudi-Arabia	25	7/18	5-45	17 NSV, 8 SV	≥ 6	NCST	Dermabrasion	STSG
Mulekar, 2005	India	142	88/85[+]	18-70	NSV	≥ 6	NCST	Dermabrasion	STSG
Mulekar, 2003	India	175	79/105[+]	12-70	114 NSV, 43 SV, 18 FV	≥ 12	NCST	Dermabrasion	STSG
Mutalik, 2017	India	50	18/32	12-68	14 NSV, 10 SV, 26 FV	≥ 24	NCST	Dermabrasion	STSG
Pandya, 2005	India	1. 4 2. 23	12/15	21-30	25 NSV, 2 SV	≥ 24	1.CMT 2.NCST	Dermabrasion	STSG
Ramos, 2017	Brazil	20 (24)	6/14	10-50	8 NSV, 12 SV	≥ 12	NCST	Dermabrasion	STSG
Thakur, 2019	India	1. 10 2. 10	11/9	> 18	12 NSV, 8 SV	1. 3-6 2. ≥ 12	NCST	Dermabrasion	STSG
Vazquez-Martinez, 2011	Mexico	11	5/6	35-48	NSV, SV, FV[*]	≥ 12	NCST	Dermabrasion	STSG
Bao, 2015	China	83 (1. 83) (2. 83)	38/45	25.2 mean	43 NSV, 40 SV	≥ 12	1.CMT 2.NCST	CO_2-laser	SBEG
Verma, 2014	India	1. 6 (50) 2. 19 (50)	13/12	Unknown	20 NSV, 2 SV, 3 FV	≥ 12	1.CMT 2.NCST	Dermabrasion	STSG

Appendix S2. Continued.

Author, year	Country	*N* Patients (lesions)	M/F	Age in years (range)	Type of depigmentation	Stability disease (months)	Treat-ment	Preparation recipient site	Harvesting of donor site
Range of DR expansion ratios									
van Geel, 2004	Belgium	1. 19 (22) 2. 9 (11)	14/14	15-65	NSV	1. ≥ 12 2. < 12	NCST	CO_2-laser	STSG
Lommerts, 2017	The Netherlands	10 (1. 10) (2. 10)	4/6	18-62	3 SV, 7 P	≥ 12	NCST	1. CO_2- 209 µm 2. CO_2 144 µm	STSG
Khodadadi, 2010	Iran	10	4/6	17-52	6 NSV, 4 FV	≥ 12	NCST	Intraepidermal injection	STSG
Gill, 2019	Iran	50	26/24	29.8 mean	40 NSV, 4 SV, 6 FV	≥ 12	NCST	Dermabrasion	STSG
Gupta, 2019	India	1. 15 (22) 2. 17 (25)	8/24	13-31	18 NSV, 6 SV, 8 FV	≥ 12	NCST	1. Er:YAG-laser 2.Dermabrasion	STSG
Mulekar, 2004	India & Saudi-Arabia	64	22/42	> 12	49 SV, 15 FV	≥ 12	NCST	Dermabrasion	STSG
Orouji, 2018	Iran	300 (1060)	111/189	12-71	231 NSV, 10 SV, 59 FV[§]	≥ 12	NCST	Intralesional injection	STSG
Sahni, 2011	India	13(19)	6/7	8-17	6 NSV, 6 SV, 1 FV	≥ 12	NCST	Dermabrasion	STSG
Olsson, 1998	Sweden	23 (27)	11/12	8-52	17 NSV, 3 SV, 3 P	≈ 88,9 mean	NCST	Dermabrasion	STSG
Parambath, 2019	India	20 (40)	8/13[+]	23.1 mean	13 NSV, 7 SV	≥ 12	NCST	Dermabrasion	STSG
Ebadi, 2015	Iran	10 (19)	4/6	21-48	NSV	≥ 12	NCST	Dermabrasion	STSG
Hong, 2011	China	102	45/57	9-55	12 NSV, 90 FV	≥ 6	CMT	CO_2-laser	SBEG

N, number of patients; M/F, male/female distribution; RCT, randomized controlled trial; CS, case series; CT, clinical trial; NSV, non-segmental vitiligo; SV, segmental vitiligo; FV, focal vitiligo; P, piebaldism; NCST, autologous non-cultured cell suspension transplantation; CMT, autologous cultured melanocyte transplantation; Er:YAG, erbium-doped yttrium aluminum garnet laser; STSG, split-thickness skin graft; SBEG, suction blister epidermal graft.

[+]Due to loss to follow-up, discrepancy between N total and M/F total.

[*] N unknown

Appendix S3. Critical Appraisal of Case Series and (non-randomised) Clinical trials

JBI Critical Appraisal Checklist for Case Series[14]

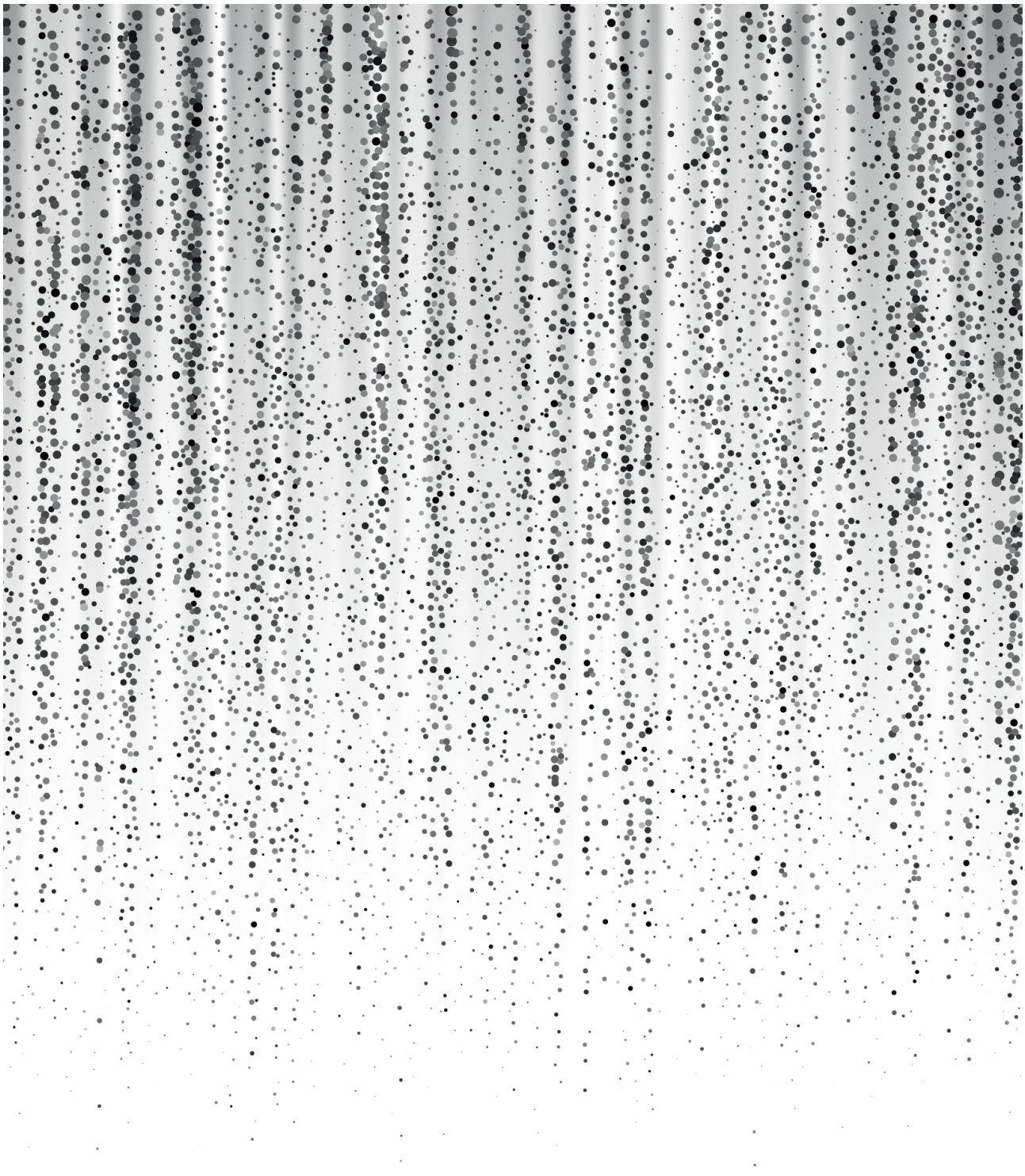
Author, year	1	2	3	4	5	6	7	8	9	10	Overall	Rationale
El-Zawahry *et al.* 2011	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NA	Include	-
Garg *et al.* 2019	Yes	UC	UC	No	Yes	Yes	No	Yes	No	NA	Exclude	Unclear reporting of many details such as recruitment of patients, measurement tool and outcome measure assessment.
Gill *et al.* 2019	Yes	Yes	UC	No	Yes	Yes	Yes	Yes	Yes	Yes	Include	-
Huggins *et al.* 2012	Yes	Yes	Yes	UC	Yes	Yes	Yes	Yes	Yes	Yes	Include	-
Kachhawa *et al.* 2017	Yes	Yes	No	UC	Yes	Yes	Yes	Yes	Yes	NA	Include	-
Khodadadi *et al.* 2010	Yes	Yes	UC	No	Yes	Yes	Yes	Yes	Yes	NA	Include	-
Mulekar *et al.* 2006	Yes	UC	UC	UC	Yes	Yes	Yes	Yes	Yes	NA	Exclude	Questionable study design and unclear reporting of instrument use for measuring outcome.
Mulekar *et al.* 2005	Yes	UC	UC	No	No	Yes	Yes	Yes	Yes	NA	Include	-
Mulekar *et al.* 2004	Yes	UC	No	UC	No	Yes	Yes	Yes	Yes	NA	Include	-
Mulekar *et al.* 2003	Yes	UC	UC	No	No	Yes	No	Yes	UC	NA	Exclude	Unclear reporting of many details such as recruitment of patients, clinical information, measurement tool and outcome measure assessment.
Olsson *et al.* 1998	No	No	No	No	Yes	Yes	UC	UC	Yes	NA	Exclude	Lack of reporting information such as inclusion criteria, measurement tool and outcome measurement assessment. Furthermore unclear reporting of relevant clinical information (disease stability).
Pandya *et al.* 2005	Yes	Yes	No	UC	Yes	UC	Yes	Yes	UC	NA	Include	-
Ramos *et al.* 2017	Yes	Yes	UC	No	Yes	Yes	Yes	Yes	Yes	NA	Include	-
Sahni *et al.* 2011	Yes	Yes	No	UC	Yes	Yes	Yes	Yes	Yes	NA	Include	-

Abbreviations: UC, unclear; NA, not applicable.

JBI Critical Appraisal Checklist for Quasi-Experimental Studies (non-randomized experimental studies)[15]

Author, year	1	2	3	4	5	6	7	8	9	Overall	Rationale
Bao *et al.* 2015	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Include	-
Ebadi *et al.* 2015	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Include	-
El-Zawahry *et al.* 2017	Yes	Yes	Yes	Yes	Yes	Yes	Yes	UC	Yes	Include	-
Hong *et al.* 2011	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Include	-
Orouji *et al.* 2018	Yes	NA	NA	No	Yes	Yes	NA	No	Yes	Include	-
Vazquez-Martinez *et al.* 2011	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Include	-

Abbreviations: UC, unclear; NA, not applicable.



CHAPTER 6A

Meek micrografting: a novel surgical technique for the treatment of depigmentation

Vidhya S. Narayan
Nicoline F. Post
Paul P.M. Van Zuijlen
Marcel W. Bekkenk
Albert Wolkerstorfer

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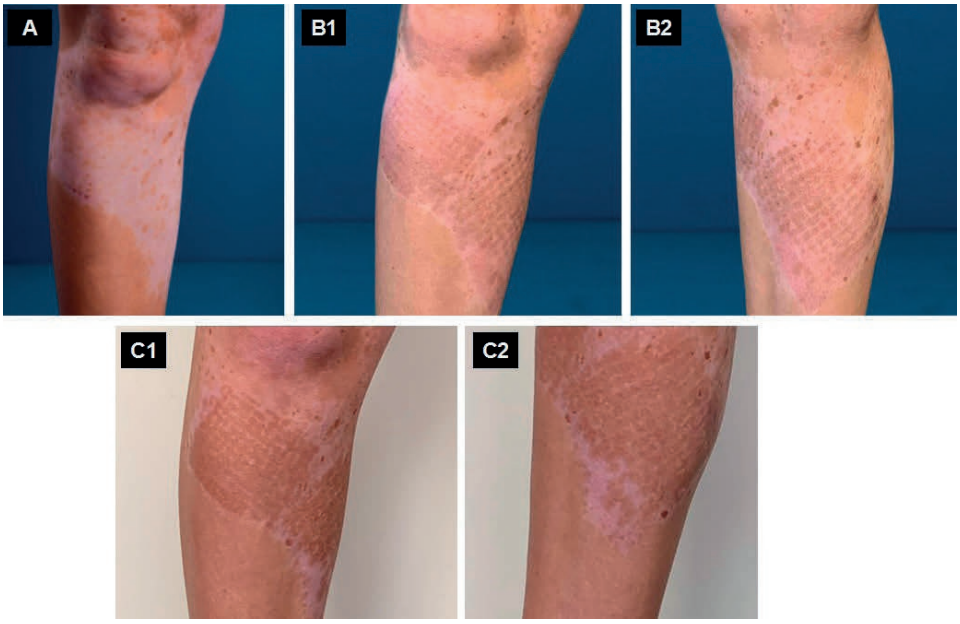
Dear Editor,

Depigmenting skin disorders, such as vitiligo and piebaldism, can severely alter the physical appearance leading to social stigmatization and an impaired quality of life.[1] To date, several surgical methods of autologous skin transplantation are available for treating stable depigmented lesions to achieve repigmentation.[2,3] Tissue grafting techniques, such as split-thickness and blister grafting, have the highest repigmentation rates[4], however they are poorly suited for treating larger lesions due to the low expansion rate of the donor skin (1:1 expansion ratio).[5] In addition, only a limited surface area can be treated per treatment session. On the contrary, larger surface areas (approximately 3-to-10-fold size of the donor skin) can be treated with the non-cultured epidermal cell suspension technique (NCES). This technique has been simplified over the years and frequently seems to result in a good and uniform repigmentation. Although NCES usually yields lower repigmentation success rates, when treating with a higher expansion ratio.[6] Furthermore, it is more time-consuming and requires chemical processing and trained personnel.[3] Summarising, the above-mentioned grafting techniques lack the capacity of easily treating large depigmented areas with high success rates. Thus, there is an unmet need for a simple, reliable and effective transplantation technique for large areas.

We herein present the first patient with stable depigmentations that was treated with the Meek micrografting technique using a 1:4 and 1:6 expansion ratio. This tissue grafting method was first presented in 1958 by C.P. Meek and currently the modification of this method is a milestone in the history of acute burns surgery.[7–9] However, this technique has never been utilized previously for the treatment of depigmented lesions. We used this method to treat a large surface area on the lower right leg of a 21-year-old female with piebaldism (Fig. 1A). During this procedure, we harvested a 200 μm split-thickness skin graft from non-lesional normally pigmented skin of the right hip using an electric dermatome (Humeca, the Netherlands). After that, the harvested skin was placed onto two square cork plates and cut into 196 small square pieces (measuring 3 x 3 mm) per cork plate (Fig. 2A) using the Meek Micromesher (Humeca, the Netherlands) (Fig. 2B). Subsequently, the micro skin grafts on the cork plates were transferred onto a prefolded expandable gauze (Fig. 2C) with aid of an adhesive spray. As result of the spray, the skin pieces adhered to the gauze (Fig. 2D) and the square cork was gently removed. The gauze, now containing the skin pieces, was manually unfolded allowing an up to 9-fold skin expansion (Fig. 2E). The recipient site was prepared with a CO_2 laser (Ultrapulse; Lumenis Ltd) ablating 209 μm of tissue, followed by fixation of the expanded gauzes on the laser-ablated site (Fig. 2F) with a self-

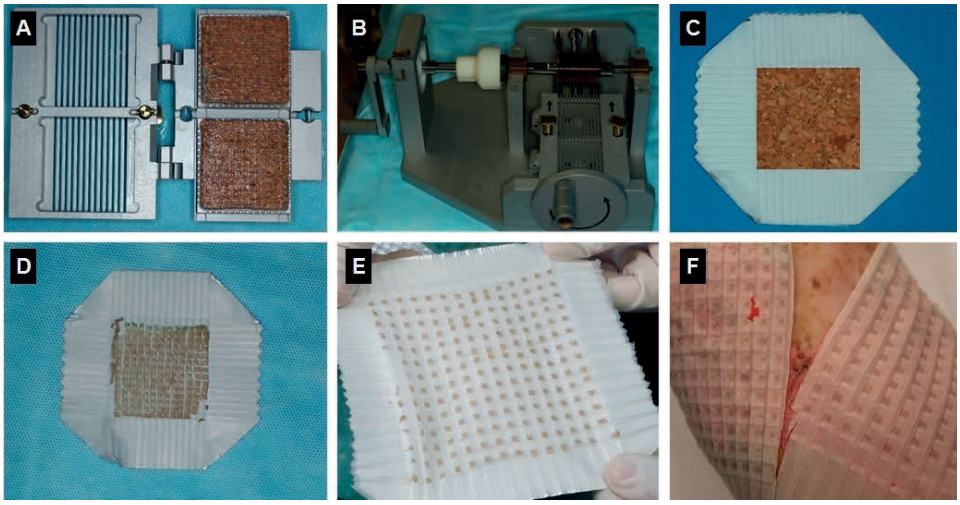
adhesive dressing (Medipore, 3M Health Care, Borken, Germany). Finally, the Meek gauzes and dressing were removed after one week. In our patient we used one gauze with a 1:4 expansion ratio and another gauze with a 1:6 expansion ration, both resulting in remarkably high repigmentation percentages after 3 (Fig. 1B) and 9 months (Fig. 1C). A side effect of this treatment, seems to be a 'grid-like' pattern of repigmentation in the first months after treatment, although this appearance did improve over time (Fig 1C). Adjuvant therapy (such as phototherapy) may have improved the speed and final repigmentation[10], however we did not combine any therapy after grafting for our patient. For future treatments of larger areas, higher expansion ratios (1:9) may be effective as well, given the similar repigmentation percentage between the 1:4 and 1:6 expansion ratio.

Figure 1. The lower right leg of a 21-year-old female with piebaldism before treatment (A). Same patient and same location 3 months after treatment with Meek micrografting: 1:4 ratio 87% repigmentation achieved (B1) and 1:6 ratio 76% repigmentation achieved (B2). Same site 9 months after treatment: 1:4 ratio 93% repigmentation achieved (C1) and 1:6 ratio 92% repigmentation achieved (C2). Digital surface measurement was done by using ImageJ software.



6A

Figure 2. Meek micrografting technique. The split-thickness skin graft was positioned (dermal side down) onto the square cork plates and placed in a cutting block (A), that moves along a crankshaft with cutting blades of the Meek Micromesher (B). After spraying with the adhesive spray, the cork (with the cut skin grafts) is placed onto the pleated prefolded Meek gauze (C), allowing the micro skin grafts to adhere to the gauze (D). The pleated gauze is pulled manually in all directions for expansion (E). Finally, the unfolded gauze, containing the skin grafts, is fixated to the recipient site (F).



In conclusion, we want to highlight the potential benefit of this novel technique for stable depigmented lesions, as it is a simple tissue grafting method that potentially reaches high expansion ratios and high repigmentation success rates. Thus, the Meek technique seems to be a promising novel technique for grafting large depigmented lesions.

ACKNOWLEDGEMENT

The patient in this manuscript has given written informed consent to the publication of her case details.

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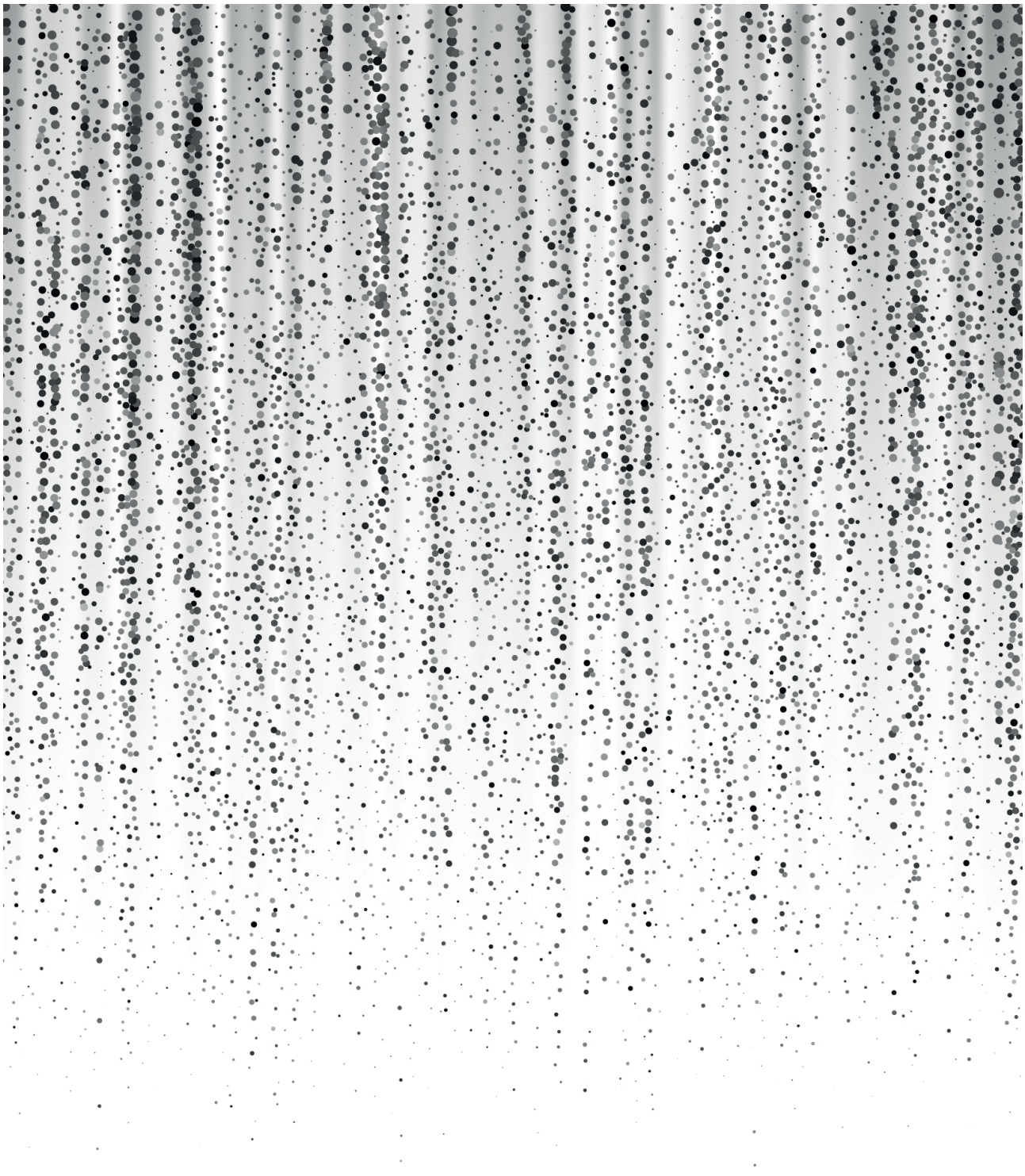

CHAPTER 6B

Meek micrografting, a novel surgical technique for the treatment of vitiligo and piebaldism: A case series

Nicoline F. Post*
Vidhya S. Narayan*
Marcel W. Bekkenk
Albert Wolkerstorfer

*Authors contributed equally.

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Dear Editor,

Several surgical techniques are available for the treatment of stable depigmentations caused by (segmental) vitiligo and piebaldism.[1,2] To date, autologous tissue grafting and cellular suspension methods are widely used for treating stable vitiligo and piebaldism[3]; however, limitations of these current techniques exist concerning the costs, size of transplanted skin, ease of use and repigmentation. There is an unmet need for treatment of large surface areas with a simple, reliable and effective transplantation technique. The Meek micrografting technique was first introduced in 1958 and is a milestone in the treatment of acute burns surgery,[4] however, this technique has only recently been introduced for treating depigmentations.[5]

The aim of this study was to evaluate the results of all vitiligo and piebaldism patients treated with the Meek micrografting technique, with regard to repigmentation, side-effects and patient- reported outcomes.

We retrospectively analysed the treatment response of all vitiligo and piebaldism patients who received Meek micrografting between September 2019 and December 2021. The donor skin was harvested from the hip region using an electric dermatome. The harvested skin was placed onto square cork plates and cut into small square pieces (3x3mm) using the Meek Micromesher (Humeca, the Netherlands). Then, the square skin pieces were transferred onto the prefolded expandable gauzes, which varied in expansion ratio (i.e. 1:3, 1:4, 1:6 or 1:9). Subsequently, the recipient site was prepared with a CO_2 laser, followed by fixation of the expanded gauzes on the laser-ablated site. A detailed description of the treatment procedure has recently been published.[5] The repigmentation after 6 weeks and 6 months of treatment was measured on UVA photographs using digital image analysis (ImageJ). In addition, patients filled in a questionnaire on the effectiveness, side-effects and their treatment experience.

A total of six patients received 11 treatments with different donor/recipient ratios of 1:3, 1:4, 1:6 and 1:9 (Table 1). Seventy-three percent achieved a ≥ 75% repigmentation and an overall median repigmentation of 64.9% (6 weeks follow-up) and 88.1% (6 months follow-up) (Figure 1). Remarkably, in 6 out of 11 treatments, a relatively high degree of repigmentation, was already achieved after 6 weeks (Table 1).

Table 1. Patient characteristics, treatment results and questionnaire outcomes

Patient number	Age/ sex	Skin type[a]	Diag-nosis	Expan-sion ratio (#corks)	Recipi-ent site	Median Repig. after 6 weeks (%)	Median Repig. after 6 months (%)	Thera-peutic history (median repig.% after 6 months)	QO: Meek compared to ther-apeutic history	QO: Onset of repig. (months)	QO: 75% repig. visible (months)	QO: Repig. % after 6 months	QO: Pain during and after treatment (VAS)	QO: Itch during and after treat-ment (VAS)	VNS
1	21/F	2	SV	1:4 (2)	Hip	67.9	88.0	PG (77.0) CS (79)	A lot better	1	3	100	7	6	No longer noticeable
				1:6 (1)	Leg	Not measured	81.0[b]								
				1:9 (2)	Hip	18.8	39.6								
2	29/M	2	SV	1:4 (2)	Chest	61.1	72.4	PG (75.9)	A lot better	Unknown	NA[c]	50	Unknown	0	A lot less noticeable
3	20/F	2	P	1:4 (1)	Leg	87.0	93.0	PG (9.5) CS (92)	Better	2	5	75	8	8	A lot less noticeable
				1:6 (1)	Leg	76.0	92.0								
				1:6 (2)	Leg	77.9	78.9								
4	17/F	2	SV	1:3 (1)	Hip	10.2	90.6	None	NA	4.5	6	75	9	8	A lot less noticeable
				1:6 (1)	Lower abdomen	19.3	95.2								
5	23/F	2	P	1:6 (1)	Leg	9.1	45.4	PG (77.7) CS (94)	Less	3.5	NA[c]	50	7	0	Slightly less no-ticeable
6	25/F	4	NSV	1:6 (1)	Leg	84.1	88.3	PG (85.8)	Better	2	4	75	6	0	A lot less noticeable

Abbreviations: #corks, number of cork plates (42 × 42 mm) used for harvesting the donor skin (1 cork plate equals 17.6 cm2 donor skin size); CS, cell suspension technique; F, female; M, male; NA, not applicable; NSV, nonsegmental vitiligo; P, piebaldism; PG, punch grafting technique; QO, questionnaire outcomes; Repig., repigmentation; SV, segmental vitiligo; VAS, visual analogue scale from 1 to 10; VNS, Vitiligo noticeability score.

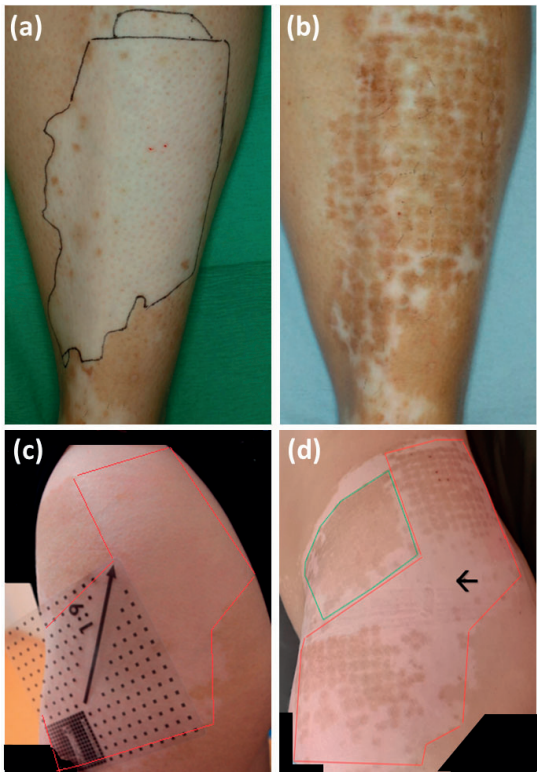
[a]Skin type according to Fitzpatrick skin scale.

[b]Measured after 1 year due to COVID-19 pandemic.

[c]Self-reported 75% repigmentation not achieved

Of the six patients, three patients previously received the cell suspension treatment and five patients had received treatments with the punch graft technique. Slight differences were found in median repigmentation after 6 months between the Meek technique (88%), the punch graft technique (77%) and the cell suspension technique (92%). Eighty-three percent of the patients would recommend this treatment to others. Moreover, patients reported to be very satisfied (17%), satisfied (50%) and neutral (33%) regarding the treatment results. In addition, 83% of the patients reported a Vitiligo Noticeability Score[6] of 4 or 5, and 67% reported a good to excellent colour match. No severe side effects occurred during or after the treatments. Commonly reported side effects were pain and itch (Table 1). Furthermore, hyperpigmentation was seen in two patients. In all patients a 'grid-like' pattern was visible, sometimes even 6 months after the treatment (Figure 1b, 1d).

Figure 1. Non-segmental vitiligo lesion on the left shin of patient 6, before (a) and at 6-month follow-up (b), with a 1:6 expansion ratio. A repigmentation rate of 88.3% was calculated by ImageJ. Segmental vitiligo lesion of the less hip of patient 1, before (c) and at 6-month follow-up (d). The area within the red lines was treated with a 1:9 expansion ratio, and 40% repigmentation is seen at a 6-month follow-up. The lines of the undergarment are marked by the arrow and show a band of depigmentation underneath after treatment. The area within the green lines (d) was treated with a 1:4 expansion ratio: 92% repigmentation is seen at a 1-year follow-up.



A disadvantage of this treatment is the initial high cost for purchasing the device. Moreover, we noticed that a slight fold or movement of the gauzes in the first week seemed to result in lower repigmentation (Figure 1d). Consequently, this leads to difficulties when treating joints or body folds. However, this issue has also been reported with the cell suspension technique.[7] Advantages are that expensive disposables or chemical processing are not required. Moreover, a small donor site can be used for large recipient sites. Once the machine is acquired, the costs for the corks, gauzes and maintenance are very low. Another, comparable automated micrografting technique is the fractional epidermal micrografting technique, which is based on multiple suction blisters.[8,9]

The main limitations of this study were the retrospective design and small sample size. Future studies are required to compare results with current transplantation techniques. *Summarizing,* our preliminary experience in the use of the Meek technique for treating depigmented macules suggests that it is a simple and safe transplantation technique that achieves relatively high repigmentation percentages and expansion ratios.

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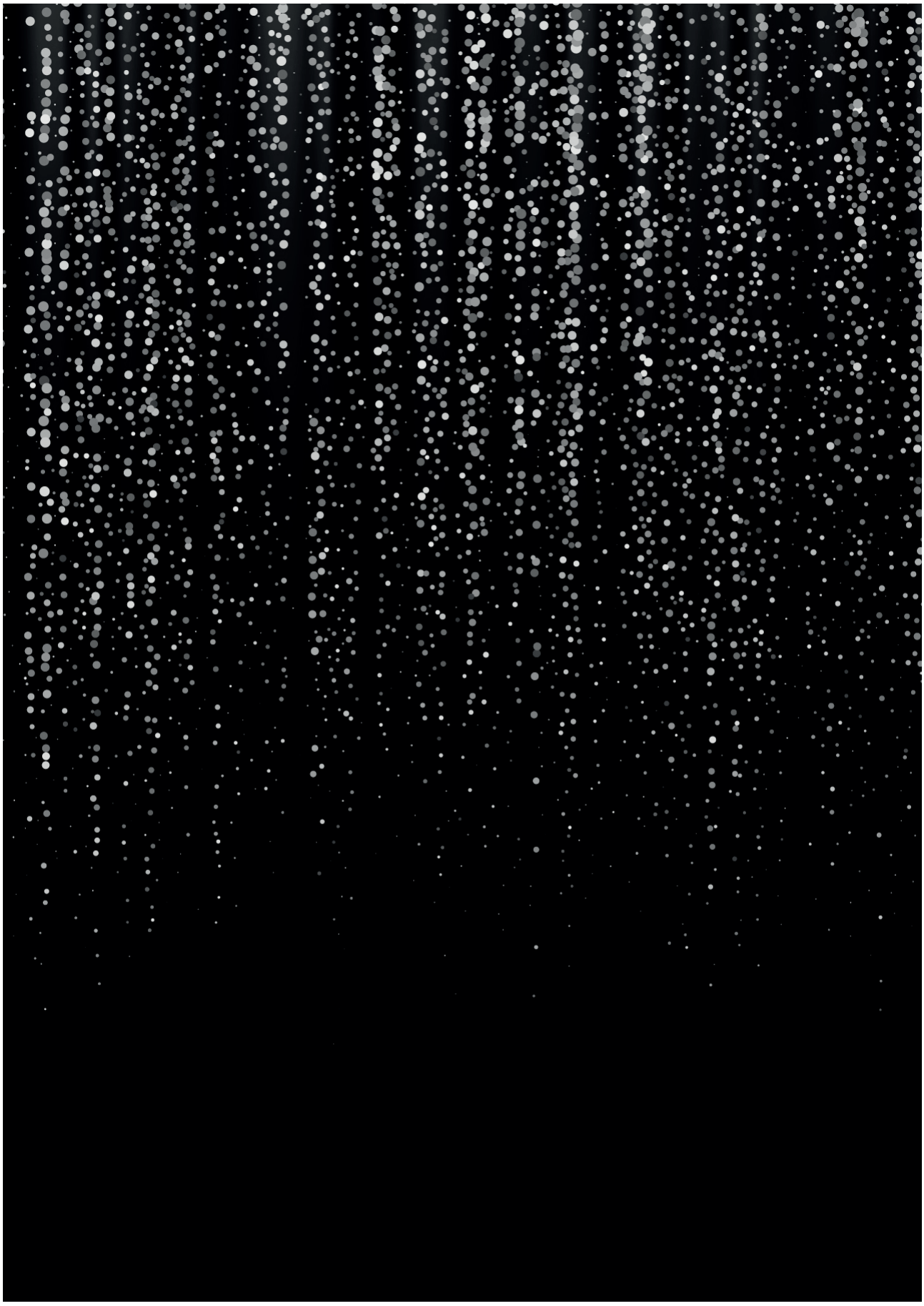
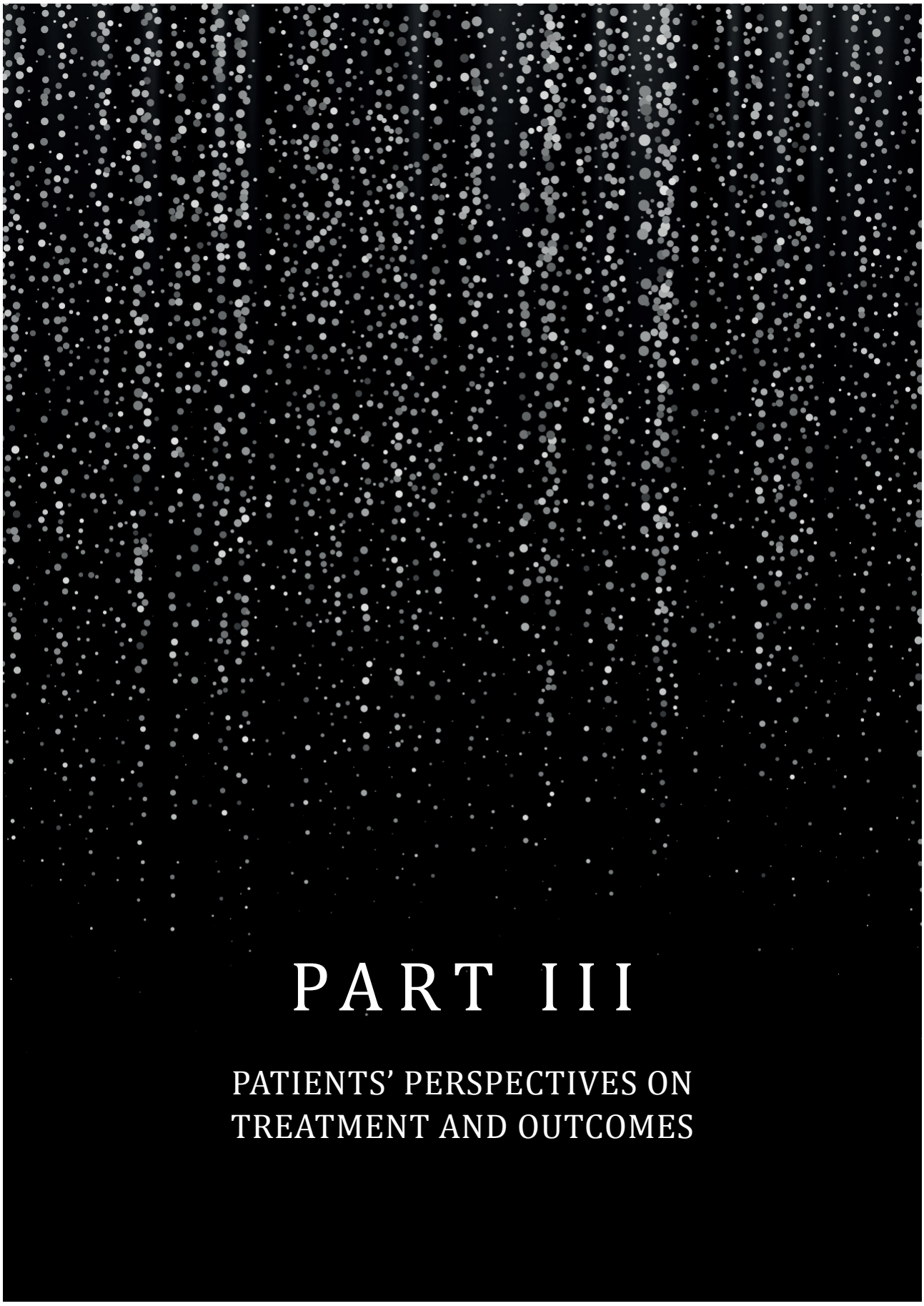
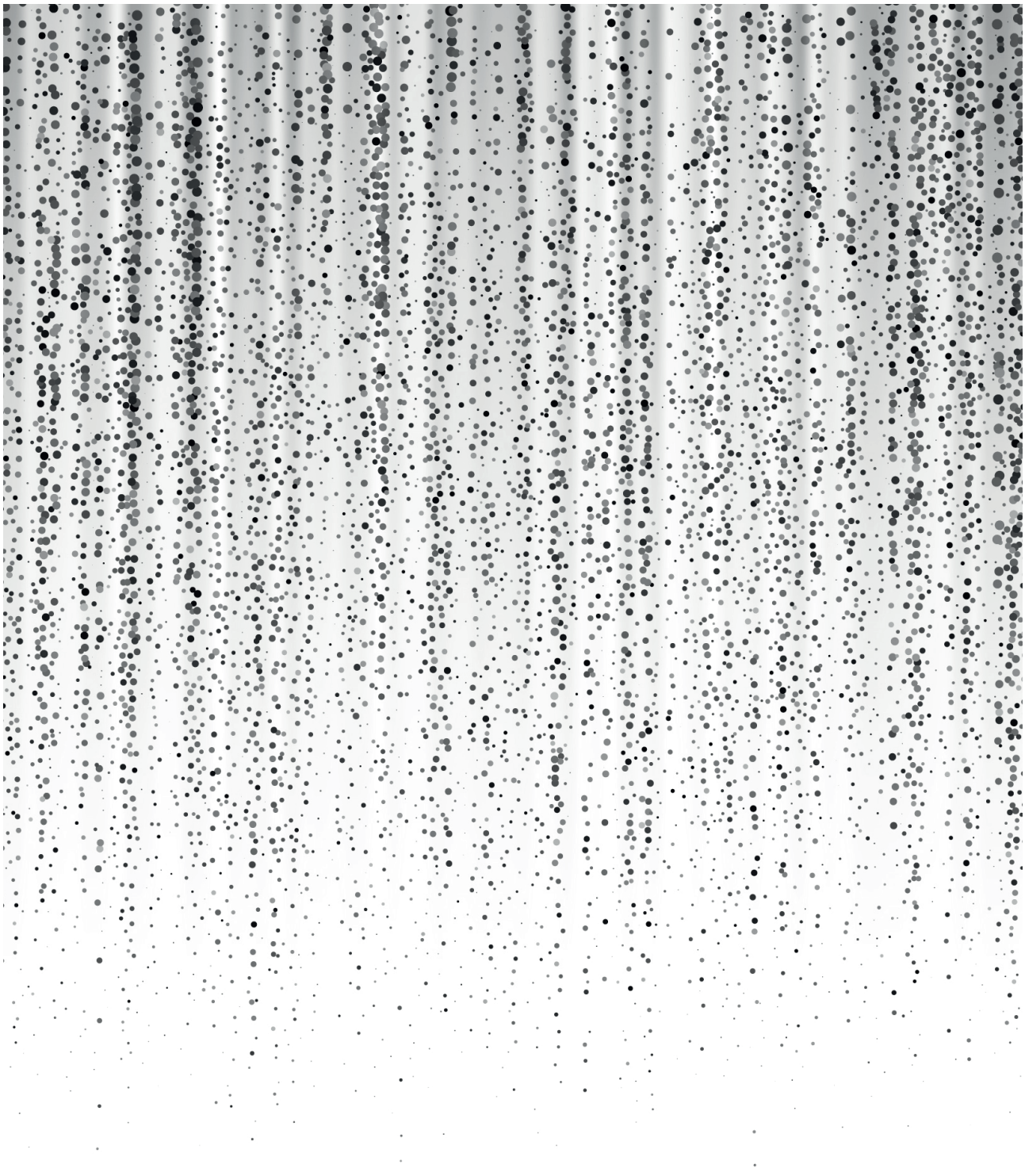




PART III

PATIENTS' PERSPECTIVES ON TREATMENT AND OUTCOMES



CHAPTER 7

What is a successful repigmentation in vitiligo from patients' view?

Vidhya S. Narayan
Sanne E. Uitentuis
Marcel W. Bekkenk
Albert Wolkerstorfer

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Dear Editor,

Based on an international e-Delphi consensus, repigmentation has been defined as a core outcome in vitiligo.[1] Many measurement instruments have been used to assess repigmentation[2], although to date the Vitiligo Extent Score and the Vitiligo Area Severity Index are the best validated.[3,4]

However, for interpretation of this outcome, little evidence is available on the thresholds of successful repigmentation. In previous Cochrane reviews on vitiligo, success was arbitrarily defined as more than 75% repigmentation.[5] But why was 75% chosen as the limit? In atopic eczema and psoriasis, the Psoriasis Area and Severity Index 75% and the Eczema Area and Severity Index 75% are commonly used to define successful treatment.[6,7] However, in vitiligo - where treatments generally yield less improvement - this analogy may not be true. Previous clinical studies defined successful repigmentation varying from 'any repigmentation' to a 100% repigmentation, and was usually defined by physicians.[2] Until now, little is known on patients' perspective regarding successful treatment in terms of repigmentation. In addition, these success percentages could differ depending on the location. Remarkably, Eleftheriadou et al. showed, in three focus groups, that an unanimous agreement was reached in all participating vitiligo patients that 80-100% repigmentation of a target lesion should be regarded as successful.[8]

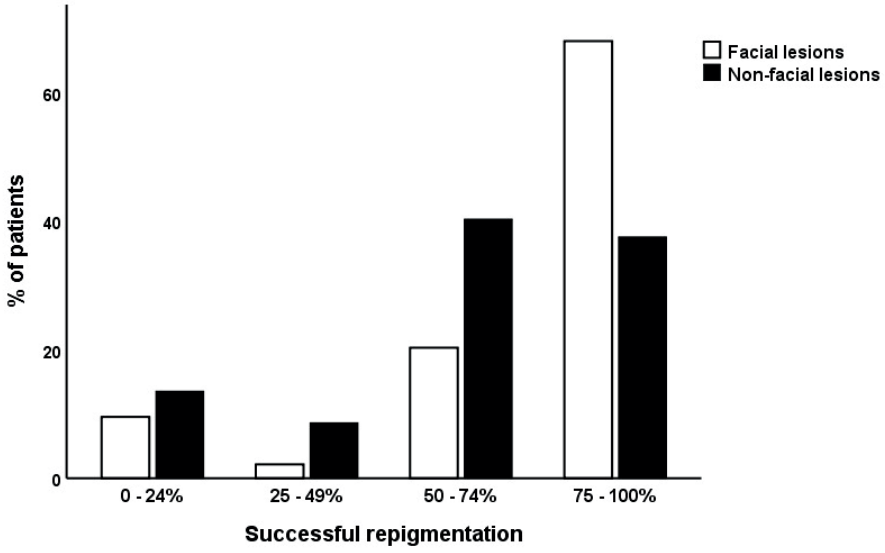
We aimed to evaluate the definition of successful repigmentation for facial and non-facial lesions from patients' perspective by carrying out a prospective cross-sectional questionnaire study.

All patients aged 16 years or older with non-segmental vitiligo who had consecutively visited our outpatient clinic at the Netherlands Institute for Pigment Disorders of the Amsterdam University Medical Center between April 2017 and January 2019 were asked to complete a secured online questionnaire (LimeSurvey version 2.6.7). Written informed consent was obtained from all patients. This survey consisted of questions regarding patient demographics, quality of life and successful repigmentation rates concerning the patient's own situation. Answers were based on a visual analogue scale ranging from 0% until 100%, with separate questions for facial and non-facial lesions (on the body). Only fully completed questionnaires were included in the analysis. This study was not subject to the Medical Research Involving Human Subjects Act, as confirmed by the Ethics Committee of the Amsterdam UMC (W17_349).

The overall response rate was 70%, (n=377; 60% fully completed, 8% not completed, 2% declined). The male : female ratio was 133 : 192, median age 40 years (range 16-77) and median disease duration was 7 years. Facial lesions were present in 86% of participants and non-facial lesions in 70%. Eighty-three per cent of patients had a light skin type (Fitzpatrick I-III) and 17% had a dark skin type (Fitzpatrick IV-VI) patients. The median of the Dermatology Life Quality Index total score was 2 [interquartile range 1-6].

Of the 325 patients that completed the questionnaire, 221 (68%) and 122 (37%) patients considered ≥75% repigmentation successful for their facial vitiligo and non-facial vitiligo lesion(s), respectively (Figure 1). The success threshold for facial lesions in patients with a dark skin type patients was significantly higher compared with patients with a light skin type (median 90% vs 80% respectively; $P_{\text{Mann-Whitney U-test}} = 0.018$). The same applied to non-facial lesions (dark skin type 70% vs light skin type 50%, $P_{\text{Mann-Whitney U-test}} = 0.035$). Twenty-two per cent of patients reported that 100% repigmentation should be regarded as successful for facial vitiligo.

Figure 1. Percentage repigmentation regarded as successful by patients for facial and non-facial lesions. The bar graph represents the percentages in repigmentation that vitiligo patients have reported to be regarded as successful for facial lesions (white bars) and for lesions on the body (black bars). The y-axis represents the percentage of the total number of patients.



Our results are largely in agreement with the study of Eleftheriadou et al. Remarkably, on in five patients only regards treatment successful if 100% of the facial lesions are repigmented. However, a considerable proportion also seem to be content with lower repigmentation rates, indicating a great variation in our population. In addition, for non-facial lesions, patients seem to be less critical and were satisfied with less repigmentation.

There are some limitations to this study. This questionnaire was constructed specifically for this study and was not validated. Moreover, as patients were included at a national referral centre, the patient population in this study may not fully reflect the larger community of people with vitiligo who do not seek care or are not referred to our centre. However, strengths of this study include the large cross-sectional group of patients and the specification of facial versus non-facial lesions. In addition, patients were able to complete the questionnaire at home without potential influences.

In conclusion, this study indicates a great variety among patients regarding their definitions of successful repigmentation. Furthermore, successful repigmentation seems to depend on the location of the lesions and on skin type. In line with a previous focus group study, we found that only a high percentage of repigmentation (≥75%) in the face is regarded as successful by the majority of the patients. For future research we recommend involving different ethnicities and more patients with a dark skin type, to determine successful repigmentation.

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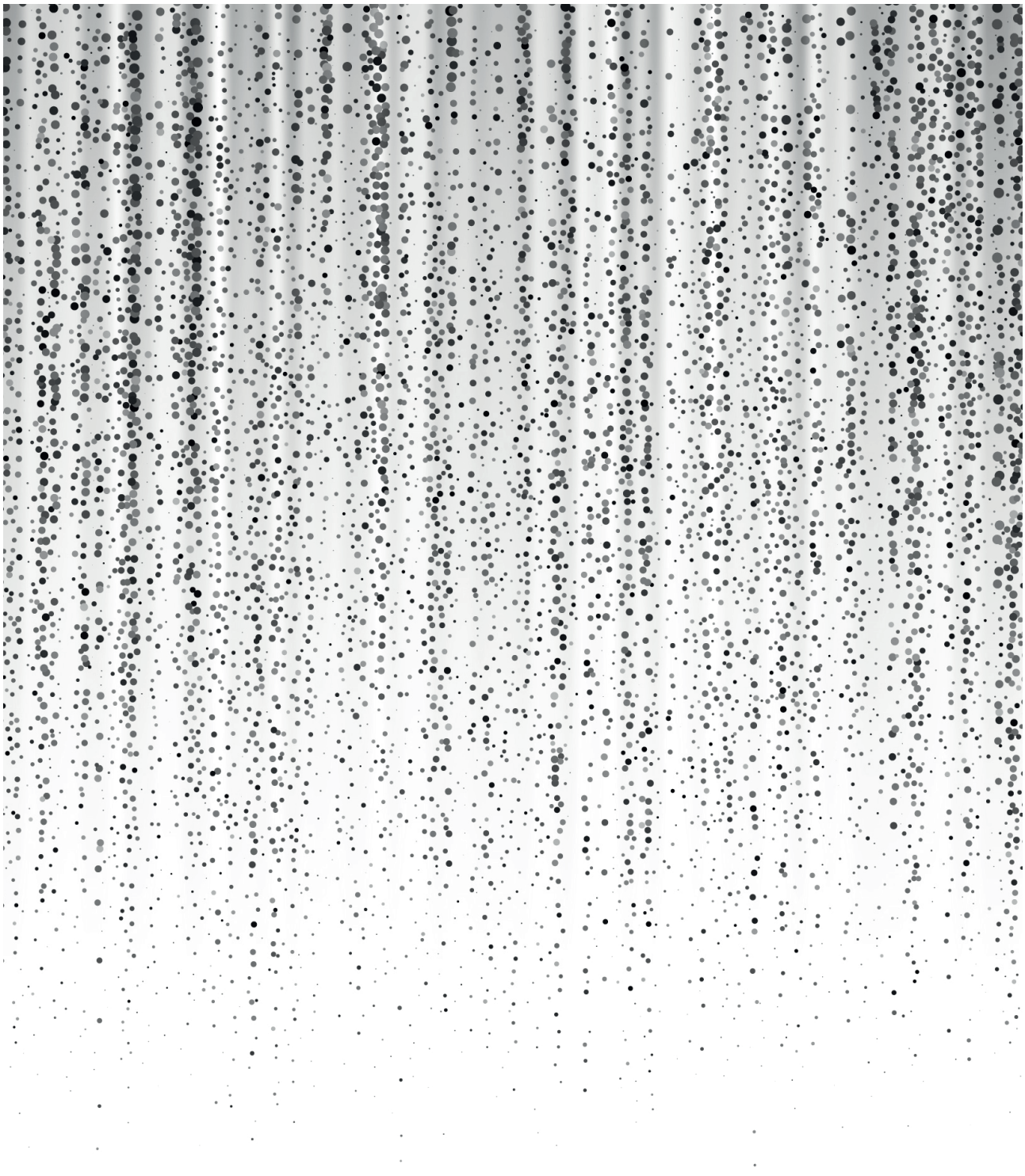

CHAPTER 8

Patients' perspective on current treatments and demand for novel treatments in vitiligo

Vidhya S. Narayan
Sanne E. Uitentuis
Rosalie M. Luiten
Marcel W. Bekkenk
Albert Wolkerstorfer

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ABSTRACT

Background

The treatment of non-segmental vitiligo (NSV) remains a challenge. Current treatments often achieve suboptimal clinical results. To improve these treatment results, several new therapies are being developed and investigated. There is, however, little evidence on the actual need for novel therapies.

Objective

To assess patients' perspective on current and novel therapies for vitiligo.

Methods

A prospective questionnaire study was conducted in a large cohort of vitiligo patients that consecutively visited the outpatient clinic of the Amsterdam University Medical Centre between April 2017 and January 2019. Patients were requested to fill in a digital questionnaire on patient characteristics, disease burden, quality of life, efficacy and satisfaction of current treatments and aspects regarding new treatments.

Results

A total of 325 vitiligo patients completed the questionnaire (60% response rate). Of the respondents, 94% believed that new and improved treatments are needed and 86% would be willing to participate in clinical trials investigating a new therapy. Sixty-nine percent would agree on taking weekly injections if it led to effective treatment results. Of the patients that had received therapy before, 49% reported that the current treatments were not effective and 50% was not satisfied with the current treatments. Sixty-seven percent of the patients experienced facial lesions as an extreme burden, whereas this was, 25%, 12% and 10% for lesions on the hands, trunk and feet, respectively. The emotional burden score was significantly higher in dark skin types compared to light skin types (respectively 8 vs 5, U $p < 0.05$).

Conclusion

There is a substantial need for new vitiligo therapies. A considerable number of patients in our study is dissatisfied with current treatments and is emotionally burdened by the disease. Moreover, the vast majority demands novel treatments and is willing to participate in clinical trials.

INTRODUCTION

Vitiligo is the most common depigmenting skin disease affecting approximately 1% of the world population, regardless of gender, ethnicity or skin type.[1] Although problems like itch and a history of sunburns are common, vitiligo generally does not lead to physical complaints.[2] However, the disease is strongly associated with a significant negative effect on the quality of life, as many of the patients feel stressed and stigmatized by their condition.[3,4]

To date, there is no curative treatment available for vitiligo. For many years now vitiligo therapy consists of topical agents, phototherapy and surgical techniques, which all aim to minimize disease progression and/or stimulate repigmentation.[5] So far, current treatment results vary between patients and a considerable number of patients does not sufficiently benefit from treatment.

To improve these suboptimal treatment results, several new therapies are being developed and investigated.[5–8] However, for the clinical implementation of these new treatments it is crucial to know the vitiligo patients' need for novel treatment modalities. What are these novel therapies worth to a patient? And how satisfied are patients in fact with the current treatments? More knowledge of these factors could be of great value to various stakeholders, such as physicians, policy-makers and pharmaceutical companies.

Furthermore, many studies have shown that vitiligo is associated with a substantial disease burden, using various measurement tools.[9–11] In addition, a few studies have related psychological complaints and quality of life to the location of the vitiligo lesions, showing a higher impact on exposed areas, such as the face.[12–14] However, various other studies indicate that other body regions have larger impact on the quality of life and/or disease burden. For example, Linthorst Homan et al. showed that vitiligo particularly on the chest had an impact on the health-related quality of life, whereas lesions on the hands and face did not.[2] Another study found that lesions on the genital area were associated with a high burden.[11] Florez-Pollack et al. illustrated that lesions on the hands were even more bothersome compared to the face and neck.[15] We hypothesize that the disease burden and its relation to the location of the various vitiligo lesions could be correlated to the demand for new therapies.

To address these issues, we performed a cross-sectional prospective survey. The aim of this study was to assess the patients' perspective on current and novel therapies for vitiligo. Furthermore, we aimed to compare demographic features,

such as gender, skin type and location of lesions (e.g. facial vs. non-facial) in relation to the primary outcomes on satisfaction of current treatments, disease burden and demand for new therapies.

PATIENTS AND METHODS

Patients

This prospective questionnaire study was conducted in a cohort of vitiligo patients at the Netherlands Institute for Pigment Disorders (NIPD) of the Amsterdam University Medical Centre (UMC). This institute is a centre of expertise for vitiligo and has a nation-wide patient population. All patients with non-segmental vitiligo (NSV), aged 16 years or older that had consecutively visited our outpatient clinic between April 2017 and January 2019 were approached. Patients with segmental, focal and universal vitiligo were not included, since these subtypes differ in management, treatment and prognosis and cannot be compared to NSV for this study purpose.

Patients of whom the email address was registered in the electronic patient system received an email with a secured online questionnaire carried out by LimeSurvey version 2.6.7. Patients of whom the email addresses were not registered, were not additionally contacted. If a patient did not respond, a reminder was sent after 1 month. Only fully completed responses were included in the final analysis. This study was not subject to the WMO (Medical Research Involving Human Subjects Act) as confirmed by the Medical Ethical Committee of the Amsterdam UMC (EC number W17_349).

Questionnaire

We developed an online questionnaire consisting of questions regarding; (i) demographics, (ii) disease burden, (iii) current treatment results, (iv) novel therapies and (v) quality of life (Supplementary Appendix I).
Demographic questions included age, gender, location of the vitiligo lesions and the Vitiligo Disease Activity (VIDA) score. Patients were requested to indicate the disease burden of their vitiligo lesions in relation to the location of these lesions. Furthermore, they were asked to score their emotional disease burden on a visual analogue scale from 0-10 (where 0 means no burden at all and 10 means an extreme burden). Patients were asked two questions in total about the effectiveness and satisfaction of current therapies. Three questions were included regarding novel therapies. First, patients were asked if they thought new and improved therapies for vitiligo are needed. Secondly, patients were requested to indicate whether they

would accept vitiligo therapy if it consisted of weekly injections. A third question was included about the willingness to participate in a clinical trial investigating a new vitiligo therapy, if patients would not receive any payments for participation and if this would be free of treatment costs. Finally, 10 questions of the Dermatology Life Quality Index (DLQI) were included to assess the quality of life.

Statistical analysis

Only fully completed responses were included in the final analysis. Descriptive statistics were used to analyse the demographics of the study population. To compare means and medians, respectively t-tests and Mann-Whitney U tests (U) were performed. Spearman rank correlation coefficient (r_s) was used for correlation analysis and Pearson Chi-square tests ($\chi 2$) were used to analyse categorical data. A p-value of <0.05 was considered statically significant. All statistical analyses were performed in SPSS version 25.0 (IBM, Armonk, NY).

RESULTS

Of the 920 patients with NSV, aged 16 or older that visited our outpatient-clinic, 542 patients had a functioning email address that was available to us. Questionnaires were sent to a total of 542 patients of whom 377 (70%) responded. Of these, 325 (60%) patients fully completed the questionnaire, 7 patients declined (1%) and 45 (9%) patients started the questionnaire, but did not complete it. Patient and vitiligo characteristics are presented in Table 1.

Primary survey outcomes

The primary survey outcomes are shown in Table 2. Of all patients, 94% indicated that new and improved treatment modalities are needed for vitiligo. Half of the patients were not or little satisfied with the current therapies and 49% indicated that the current treatments were not or little effective. The demand for new treatments did not significantly differ between males and females ($\chi 2$ p=0.305). No association was found between the location of vitiligo lesions and the demand for new therapies (Fisher's exact test p=0.558). Dark skin type patients (Fitzpatrick 4-6) did not have a higher need for new therapies than light skin type patients (Fitzpatrick 1-3) (Fisher's exact test p=0.217). However, darker patients were significantly more willing to receive a new effective treatment if it consisted of weekly injections ($\chi 2$ p=0.029). No relations were found for gender, location of lesions and skin type with regards to the willingness to participate in clinical trials.

Table 1. Patient and vitiligo characteristics

Male : female ratio	133 : 192
Mean age ± SD (min-max) in years	41 ± 14 (16-77)
Mean age of onset ± SD (min-max) in years	31 ± 16 (0-76)
Disease duration median in years [IQR]	7 [3-16]
Parameter	**N (%)**
Vitiligo disease activity (VIDA score)	
Activity in past 6 weeks	31 (10)
Activity in past 3 months	37 (11)
Activity in past 6 months	33 (10)
Activity in past year	104 (32)
Stable at least for 1 year	69 (21)
Stable for 1 year and spontaneous repigmentation	51 (16)
Skin type	
I	11 (3)
II	202 (62)
III	58 (18)
IV	20 (6)
V	25 (8)
VI	9 (3)
Previous treatments	
Has had treatment before for vitiligo	323 (99)
Use of creams	322 (99)
Use of NB-UVB light therapy	221 (68)
Skin transplantations	25 (8)

Table 2. Primary survey outcomes

Parameter	**N (%)**
Effectiveness of current treatments	
Not effective	56 (17)
Little effective	104 (32)
Effective	93 (29)
Very effective	55 (17)
Not applicable	17 (5)
Satisfaction regarding current treatments	
Not satisfied	63 (19)
Little satisfied	99 (31)
Satisfied	96 (30)
Very satisfied	45 (14)
Not applicable	22 (7)
Need for novel treatments: yes	305 (94)
Study participation: yes	281 (87)
New effective treatment if consisted of weekly injections: yes	224 (69)

Satisfaction with current treatments

The lesser satisfied patients were with current treatments, the more they indicated a need for new and improved therapies (r_s -0.18, p=0.002). The satisfaction is significantly correlated to the effectiveness from patients view of current treatments (r_s = 0.778, p=0,00). Patients who had received UVB therapy were significantly more satisfied with treatment than patients who had not received UVB therapy ($\chi 2$ p=0.001). The same applies to skin transplantation treatment ($\chi 2$ p=0.001). No relation was found between skin type and satisfaction with current therapies ($\chi 2$ p=0.19). Patients with facial vitiligo were significantly more satisfied with treatments than patients that did not have facial lesions ($\chi 2$ p=0.003). No difference in satisfaction was found between males and females ($\chi 2$ p=0.321).

Disease burden and Dermatology Life Quality Index

The median of the emotional disease burden score during lifetime was 5 [IQR 3-8] and over the past week was 3 [IQR 1-6] (Figure 1). The burden score over the past week was significantly lower than the score during lifetime (Wilcoxon signed rank test, p=0.000). Of all our patients, 28% had a DQLI total score higher than 5, which indicates a moderate to severe impaired quality of life (Figure 2). The emotional burden score of the past week was significantly correlated to the total scores of the DLQI (r_s = 0.607 p=0.000). The life time emotional burden score was significantly higher in darker skin types compared to lighter skin types (median 8 vs 5, U p=0.000). In addition, female patients had a significantly higher emotional burden score than male patients (t-test, p=0.002).

Figure 1. Emotional burden of vitiligo. The bar graph represents the emotional burden that vitiligo patients have experienced during their lifetime (black bars) and during the past week (white bars). On the x-axis, the numbers are shown for the degree of emotional burden (0 is no burden at all, 10 is an extreme burden). The y-axis represents the number of patients.

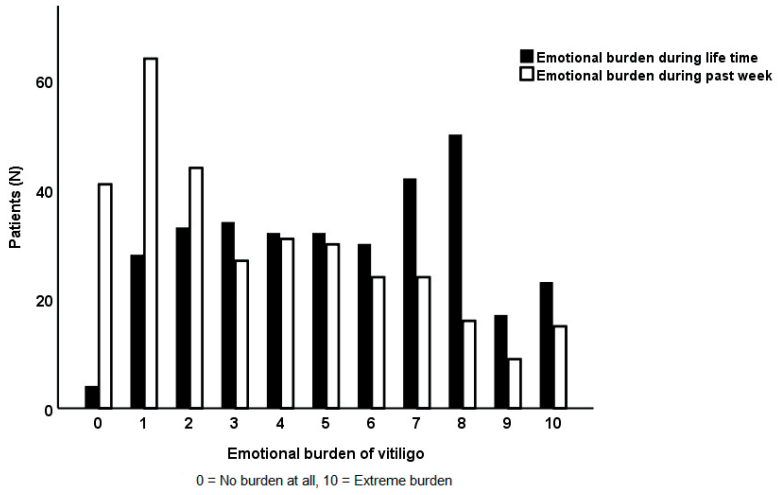
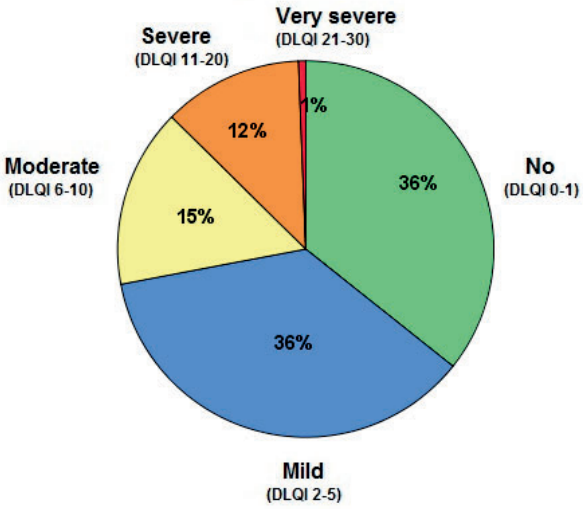
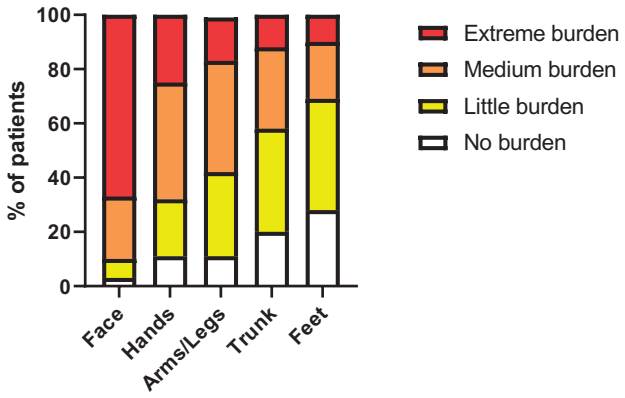


Figure 2. **Dermatology Quality Life Index total scores.** Dermatology Life Quality Index (DLQI) total scores distribution between the patients. Percentage of total number of patients (n = 325) shown. DLQI total scores of 0–1 represent no effect at all on patient's life, 2–5 represent a mild effect, 6–10 represent a moderate effect, 11–20 a severe effect and 21–30 a very severe effect on a patient's life.



In Figure 3 the burden of the disease is shown for each body region. Sixty-seven percent of the patients experienced their facial lesions as an extreme burden, whereas this was 25%, 12% and 10% for lesions on the hands, trunk and feet respectively. Patients that experienced facial lesions as an extreme burden were significantly more convinced that new and improved vitiligo treatments are needed ($\chi 2$ p=0.033). Furthermore, the emotional burden score of the past week had a significant relation with (i) the demand for novel treatment (r_s = 0.147 p=0.002), (ii) the willingness to participate in clinical trials (r_s = 0.131 p=0.018) and (iii) the acceptance of taking invasive therapy (r_s = 0.263 p=0.000). The same applies to the DLQI total scores with the demand for new treatment (r_s = 0.226 p=0.000), willingness to participate in clinical trials (r_s = 0.175 p=0.002) and acceptance of taking invasive therapy (r_s = 0.327 p=0.000). An overview of the statistical differences and correlations can be found in Supplementary Table S1.

Figure 3. Patient burden of disease per location. The stacked bar chart shows the patient burden of disease per body region. On the x-axis, the specific body regions are shown, and the y-axis represents the percentage of total number of patients (n = 325).



DISCUSSION

Our study clearly demonstrates the substantial demand of vitiligo patients for new therapies. Ninety-four percent of the vitiligo patients included in this study indicate that new and better treatments are needed for vitiligo. Consequently, a majority of these patients is willing to participate in clinical trials for improvement of vitiligo therapy. Moreover, approximately half of the patients felt that their current treatment was not effective and was not satisfied with it.

We analysed factors with potential influence on patient satisfaction. We found that patients who had received UVB therapy were significantly more satisfied than patients who had only received topical treatment, which is in line with previous studies.[16,17] Patients with facial vitiligo also seemed to be more satisfied with current treatments. This could be clarified by the fact that facial lesions often respond better to therapy than lesions on other body regions.[18]

Our results also show that patients that are little or not satisfied with current treatments, are more demanding for new treatments. It is however remarkable that even patients who were (very) satisfied with current treatments, still expressed the opinion that new treatments are needed for vitiligo.

Moreover, we found that the emotional burden was significantly higher in dark skin type patients (Fitzpatrick skin type 4-6). This is in accordance with our previous study which showed that vitiligo particularly has more impact on the quality of

life of patients with dark skin than patients with a light skin (Fitzpatrick skin type 1-3).[2] In addition, our results show a clear difference in experiencing burden of vitiligo lesions related to their location. The majority of the patients reported that they experienced their facial lesions as an extreme burden, followed by lesions on the hands in a quarter of the patients. This is not unexpected, since these body sites are usually exposed areas. On the contrary, lesions on the feet and on the trunk are experienced as little or no burden at all by the majority of the patients, probably since these usually involve covered areas. Thus, the degree of burden seems to be correlated to the visibility of the specific body region, which is in line with previous studies.[12–14,19] Furthermore, this study shows that a higher burden and impaired quality of life were significantly associated with a higher demand for new therapies, but also with a higher willingness to participate in clinical trials and a higher acceptance of invasive treatment for their vitiligo.

To our knowledge, our study is the first to analyse the demand for new therapies in a large cohort of vitiligo patients in the Netherlands. Similarly, the majority of vitiligo patients participating in a UK survey study reported the main priority of finding a cure or an effective lasting treatment.[20] As our collected data were recent up to October 2019, our study represents an up-to-date view of patients' perspective. The strength of our study is that all patients were approached consecutively from our outpatient department, thereby including patients from daily practice. Moreover, this survey is completed by a relatively large cohort of patients all over the Netherlands. However, there are some limitations to this study. Firstly, this questionnaire was constructed specifically for this study and was not validated. Secondly, the response rate was 70% but only 60% completed the full questionnaire, which may raise a bias, due to the lack of data available on the non-responding patients. Moreover, since patients were included at a national referral centre for pigment disorders, the population of vitiligo patients in this study may have a relative high disease burden and may not fully reflect the larger community of people with vitiligo who do not seek care or are not referred to our expert centre. In addition, 65% of our participants had skin types I and II, which is not fully representative for the global vitiligo population.

The results of this exploratory study underline the demand for improved and novel vitiligo therapies. A considerable fraction of patients in our study is dissatisfied with current treatments and emotionally burdened by the disease. In addition, the disease burden seems to be dependent on the visibility of the location of the vitiligo lesion. Moreover, the vast majority of all patients included demands novel treatments and is willing to participate in clinical trials.

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SUPPORTING INFORMATION

Table S1. Summary of statistical correlations

Parameter	Demographic feature	*P*-value
Need for novel treatments	- Gender (male/female) [x]	0.305
	- Location vitiligo lesions (facial/non-facial) [#]	0.558
	- Skin type (dark/light) [#]	0.217
Acceptance of receiving effective invasive therapy	- Gender [x]	0.023* (male > female)
	- Location vitiligo lesions [x]	0.415
	- Skin type [x]	0.029* (dark > light)
Willing to participate in clinical trials	- Gender	0.740
	- Location vitiligo lesions	0.530
	- Skin type	0.892
Satisfaction current treatments	- Gender [x]	0.321
	- Location vitiligo lesions [x]	0.003* (facial > non-facial)
	- Skin type [x]	0.189
	- Age	0.610 (r_s = -0.029)
Efficacy current treatments	- Gender [x]	0.623
	- Location vitiligo lesions [x]	0.024* (facial > non-facial)
	- Skin type [x]	0.286
	- Age	0.166 (r_s = -0.079)
Parameter	**Parameter**	***P*-value, correlation coefficient**
Satisfaction current treatments	- Use of creams	0.153, r_s = 0.082
	- Use of UVB-therapy	0.001, r_s = 0.210*
	- Skin-transplantation	0.003, r_s = 0.171*
Efficacy current treatments	- Use of creams	0.140, r_s = 0.084
	- Use of UVB-therapy	0.000, r_s = 0.257*
	- Skin-transplantation	0.001, r_s = 0.191*
DLQI total score	Emotional burden score (of the past week)	0.000, r_s = 0.607*
Emotional burden score (past week)	- Need for novel treatment	0.002, r_s = 0.147*
	- Willingness to participate trials	0.018, r_s = 0.131*
	- Acceptance of invasive therapy	0.000, r_s = 0.263*
DLQI total scores	- Need for novel treatment	0.000, r_s = 0.226*
	- Willingness to participate trials	0.002, r_s = 0.175*
	- Acceptance of invasive therapy	0.000, r_s = 0.327*

*statistically significant, [x] Chi-square test, [#] Fisher's exact test, r_s Spearman's rank-order correlation

Supplementary Appendix 1. Short survey vitiligo treatment

This survey consists of 24 (multiple)choice questions. We would like to know your opinion about current treatments and about the development of new therapies for vitiligo. In case you have any questions, you may address these to your attending physician.

Dear patient,

We would like to invite you to fill in this questionnaire regarding new treatments for vitiligo. This would take about 5 minutes.

Currently, medicines that are used for other auto-immune diseases, might have a favourable effect on vitiligo. For this reason, the Netherlands Institute for Pigment Disorders has started a questionnaire study to see if there is an actual need for new vitiligo therapies. With this research we also hope to gain more insight on the current situation of patients.
Your personal data will be analyzed confidentially and anonymously. This means that your treating physicians will not know your answers. The results of this study may be presented at medical congresses and published a scientific paper.

Thank you for participating.

Do you agree on completing this questionnaire?

I understand that filling in this questionnaire is voluntary
My answers will not have an influence on my regular treatment
My privacy will be respected at all times
I give my permission to participate in this research.

☐ Yes I agree ☐ No I do not agree

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1. What is your age? …….years

2. Are you male or female?: ☐ Male ☐ Female

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3. How old were you when your vitiligo started?

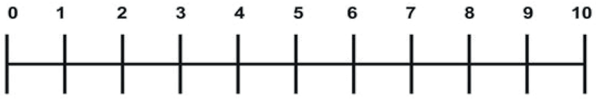
I was …… years old.

4. Where is your vitiligo located? (multiple answers possible)

☐ Face
☐ Hands
☐ Arms, legs
☐ Trunk
☐ Feet

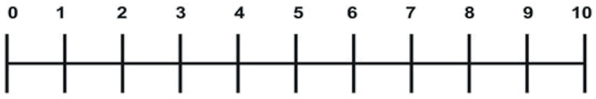
5. How much emotional burden did you experience from your vitiligo? Please indicate your emotional burden during your life time and over the past week on a scale from 0 to 10.

The emotional burden during my life time was (encircle your answer):



No burden Extreme burden

The emotional burden over the past week was (encircle your answer):



No burden Extreme burden

6. How much burden do you experience of the location of the vitiligo lesions?: If you do not have any vitiligo lesions on the body regions mentioned below, please indicate the burden you might experience if vitiligo lesions would occur on that region.

Face	☐ No burden	☐ Little burden	☐ Burden	☐ Extreme burden
Hands	☐ No burden	☐ Little burden	☐ Burden	☐ Extreme burden
Arms, legs	☐ No burden	☐ Little burden	☐ Burden	☐ Extreme burden
Trunk	☐ No burden	☐ Little burden	☐ Burden	☐ Extreme burden
Feet	☐ No burden	☐ Little burden	☐ Burden	☐ Extreme burden

7. Was your vitiligo active during the past period? So this means: have the white spots got bigger or have new white spots appeared?

☐ Active in the past 6 weeks
☐ Active in the past 3 months
☐ Active in the past 6 months
☐ Active in the past 1 year
☐ Stable for at least 1 year
☐ Stable for at least 1 year and spontaneous repigmentation

8. Which treatment(s) did you receive until now for your vitiligo? (multiple answers possible)

☐ Creams ☐ Phototherapy ☐ Skin transplantation ☐ Not applicable

9. How effective was the treatment for your vitiligo until now?

☐ Not effective ☐ Little effective ☐ Effective ☐ Very effective ☐ Not applicable

10. How satisfied are you with the treatment results until now?

☐ Not satisfied ☐ Little satisfied ☐ Satisfied ☐ Very satisfied ☐ Not applicable

11. Do you think new and improved therapies are needed for vitiligo?

☐ Yes ☐ No

12. Would you choose for a new and effective therapy for vitiligo if this

☐ Yes ☐ No

13. If a new treatment would be tested for vitiligo in a clinical trial, would you consider participating if this new treatment would be for free?

☐ Yes ☐ No

14. Do you want us to contact you if a new treatment is being investigated for vitiligo?

☐ Yes ☐ No

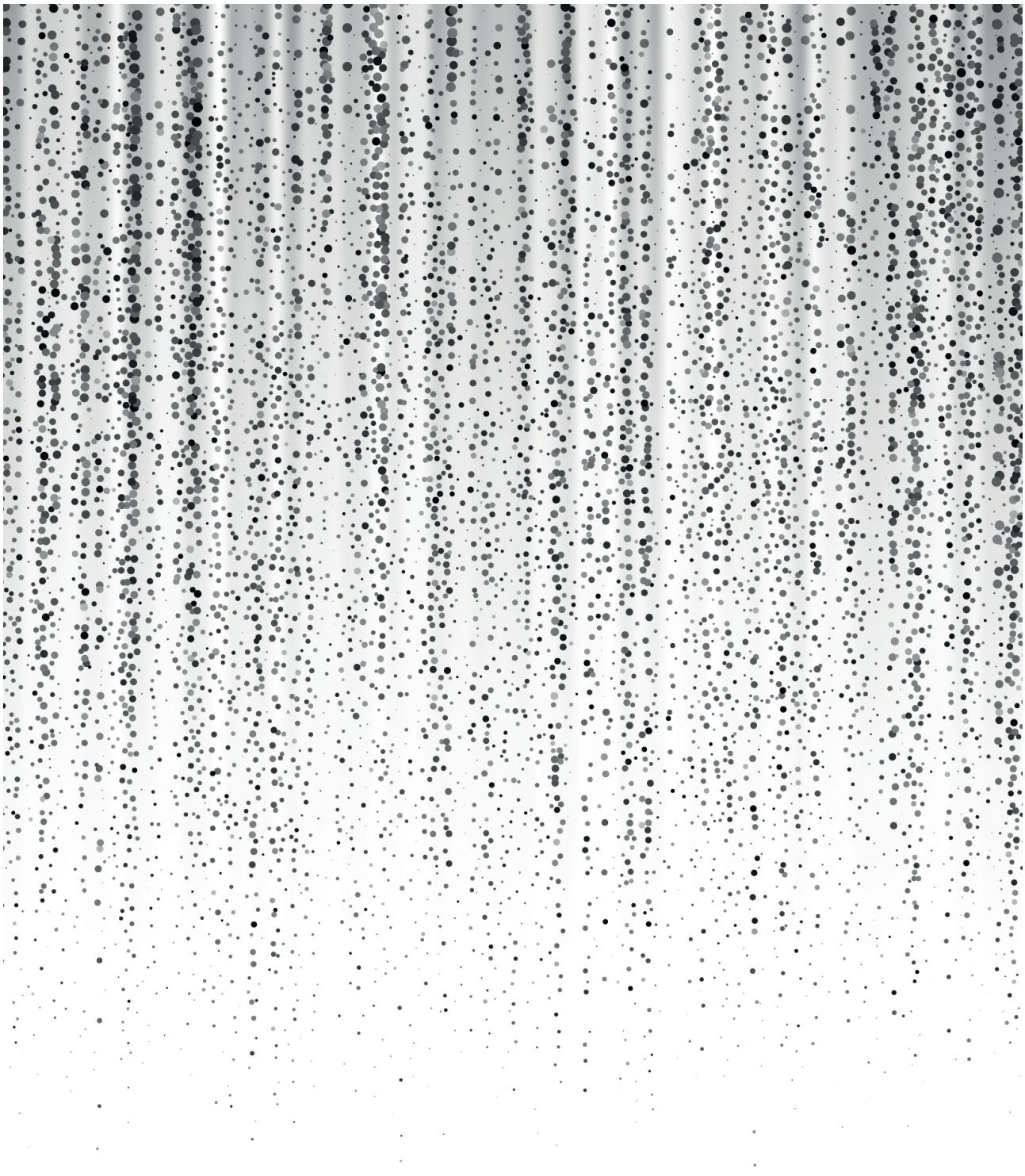
DERMATOLOGY LIFE QUALITY INDEX

The aim of this questionnaire is to measure how much your skin problem has affected your life OVER THE LAST WEEK. Please tick ☑ one box for each question.

1.	Over the last week, how **itchy**, **sore**, **painful** or **stinging** has your skin been?	Very much ☐ A lot ☐ A little ☐ Not at all ☐
2.	Over the last week, how **embarrassed** or **self-conscious** have you been because of your skin?	Very much ☐ A lot ☐ A little ☐ Not at all ☐
3.	Over the last week, how much has your skin interfered with you going **shopping** or looking after your **home** or **garden**?	Very much ☐ A lot ☐ A little ☐ Not at all ☐ Not relevant ☐
4.	Over the last week, how much has your skin influenced the **clothes** you wear?	Very much ☐ A lot ☐ A little ☐ Not at all ☐ Not relevant ☐
5.	Over the last week, how much has your skin affected any **social** or **leisure** activities?	Very much ☐ A lot ☐ A little ☐ Not at all ☐ Not relevant ☐
6.	Over the last week, how much has your skin made it difficult for you to do any **sport**?	Very much ☐ A lot ☐ A little ☐ Not at all ☐ Not relevant ☐

7.	Over the last week, has your skin prevented you from **working** or **studying**?	Yes	☐
		No	☐
		Not relevant	☐
	If "No", over the last week how much has your skin been a problem at **work** or **studying**?	A lot	☐
		A little	☐
		Not at all	☐
8.	Over the last week, how much has your skin created problems with your **partner** or any of your **close friends** or **relatives**?	Very much	☐
		A lot	☐
		A little	☐
		Not at all	☐
		Not relevant	☐
9.	Over the last week, how much has your skin caused any **sexual difficulties**?	Very much	☐
		A lot	☐
		A little	☐
		Not at all	☐
		Not relevant	☐
10.	Over the last week, how much of a problem has the **treatment** for your skin been, for example by making your home messy, or by taking up time?	Very much	☐
		A lot	☐
		A little	☐
		Not at all	☐
		Not relevant	☐

Please check you have answered EVERY question. Thank you.



CHAPTER 9

General discussion

GENERAL DISCUSSION

In this thesis 'shedding light on vitiligo' we aimed to study different facets of vitiligo with specific focus on current treatment outcomes in order to gain more knowledge about therapy responses and improve future clinical care for vitiligo. This final chapter reviews our main findings, places it in context of current literature and unveils future perspectives.

Evaluation of topical and phototherapy responses

In the first part of this thesis our objective was to assess the responses to current topical and narrow-band ultraviolet B (NB-UVB) phototherapy from cellular, clinical and patients' point of view.

Throughout the past several years, different immune cell types in blood and skin have been reported to be linked to the disease activity and treatment response in vitiligo.[1–3] Since there is an inevitable delay in repigmentation after therapy, the need for an early on treatment marker is substantial. In **chapter 2**, we provide important insights into the effects of topical and NB-UVB therapy on local and systemic cellular immunity of vitiligo in relation to repigmentation. We found that an early increase of Granzyme B $(GrB)^{+}CD8^{-}$ T cells in skin was significantly correlated with a better treatment response, which was an unexpected finding since GrB is known for its proapoptotic role in autoimmune skin diseases.[4] This led to the following question: to which phenotype of cells do these $GrB^{+}CD8^{-}$ cells belong and what was its function? Since our $CD8^{-}$ Tregs also were increased in skin, we hypothesized that (a part of) the Tregs could belong to this $GrB^{+}CD4$ population, as has been previously suggested by others.[5–7] The interaction between these same type $GrB^{+}CD4$ cells in blood, before and 3 months after therapy, were also found to have a significant prognostic role for repigmentation. Substantial differences in cell percentage after the first 3 months of therapy, was an indication for a poorer repigmentation, whereas minor changes seemed to be an indication for a better repigmentation. In daily clinical practice, these changes in $GrB^{+}CD4$ percentage in blood could be taken into account for providing an estimation of the treatment outcome after 6 months. A systemic marker (from blood) would be convenient for the ease of use as an early on treatment biomarker. Although to date, promising protein skin activity markers have been found such as chemokine ligand (CXCL) 9 and CXCL 10, that could be useful for an indication of disease activity[8] and perhaps also for therapeutic response, as demonstrated by Yang et al. after NB-UVB and topical treatment.[9] But, why is it so important to determine these (potential) biomarkers? Since repigmentation after vitiligo treatment is often incomplete and a delay in onset of repigmentation is seen after long-term therapeutic exposure,

an early on treatment biomarker could help to predict if continuation of therapy is beneficial or not. Based on these early treatment markers, unnecessary treatments and associated costs can be avoided. On the contrary, treatment markers related to a favourable response, could also motivate patients to continue therapy, even when there is no visible repigmentation initially. Moreover, a baseline biomarker or biomarker associated with repigmentation regardless of therapy, could also be of added value to patients, to help predict the chances on repigmentation. For future implementations of an early on treatment biomarker however, larger prospective studies are required to confirm these findings and to gain more insight on other potential cellular (circulating or local) biomarkers for treatment response.

Notably, during this trial but also during daily practice, we recognized that clinical treatment response (repigmentation) after therapy differs between body regions. These differences between specific anatomical areas of the body had not been fully characterized, which is why our aim was to assess the clinical response to NB-UVB in 19 different body regions **in chapter 3.** Our data demonstrated a varied response among the different anatomic body regions with facial lesions achieving the highest improvement and acral lesions achieving the least improvement, which is in line with previous studies.[10–13] Based on our findings, we concluded that the body surface area can be classified into 4 composite body regions according to prognosis of repigmentation after treatment, i.e. face; torso&limbs; axillae; hands&feet, from highest to lowest median repigmentation (76%, 40%, 27%, 4%) respectively. The reason behind this difference is not completely clear, however might be related to the variation in hair follicle density between different body regions. The residual melanocytes that are located in the outer root sheath of the hair follicles are reactivated via the Wnt pathway after NB-UVB radiation, leading to migration to depigmented epidermis and (perifollicular) repigmentation.[14,15] Although, this does not explain why body regions with a higher hair follicle density (i.e. limbs or axillae) are less responsive to therapy than the face. A possible explanation could be that the face is generally more exposed to UV (sun)light allowing an enhanced response to therapy. Another postulated clarification could be the distinct differences between anatomical regions of the skin with regards to local immune responses and histological differences.[16,17] In summary, differences in repigmentation between body regions exist, however the exact mechanism of NB-UVB induced repigmentation and the anatomic variation in response to this, still remain to be fully elucidated.[15] For daily clinical practice and for vitiligo research, the results of our study may be of direct added value to clinicians and vitiligo patients as it provides more detailed information on specific treatment prognosis per body region and is useful in clinical trials for determining the target lesion location. Based on these findings, patients' expectations could be managed, so

that they are well-informed and not disappointed with the treatment outcome. In addition, patients could decide to only treat lesions on body regions (for instance with a partial UVB body unit) that will presumably respond to treatment. Moreover, the burden that patients experience of vitiligo on various body regions differs, as confirmed in one of our studies.[18] Patients could also choose to treat a certain body region, based on the burden they experience from it, keeping in mind that treatment prognosis differs per body region.

To date, NB-UVB is considered to be the mainstay treatment modality for generalised (non-segmental) vitiligo due to its good efficacy and tolerance.[19] Although broadly used, previous literature reviews show no consensus on the frequency and dosing of NB-UVB therapy.[20,21] Expert recommendations are available, but NB-UVB treatment regimens still vary between hospitals and countries.[20,21] The majority of the hospitals have a thrice-weekly regimen, although some use the twice-weekly regimen. Others use a combination, for instance reducing the exposure frequency to twice a week once patients have achieved more than 75% repigmentation.[21] The efficacy of NB-UVB therapy might increase with the three- times weekly regimen in combination with topical treatment, although data on the most effective treatment scheme is lacking. In **chapter 4** we performed a retrospective study to explore whether a combination of topical agents and NB-UVB therapy three times weekly (intensified group) would be superior to NB-UVB alone two times weekly (conventional group), showing comparable results regarding repigmentation, satisfaction and occurrence of adverse effects from patients' view. However, the onset of repigmentation in the intensified group seemed to occur slightly earlier than the conventional group (51.1% vs 23.4% respectively; p=0.11), and this was positively correlated to satisfaction of treatment results in the intensified group. Thus, a three times weekly regimen could be more satisfactory and effective to speed up the treatment response. A twice-weekly regimen might be more efficient regarding time and effort for patients. Although, this could be accompanied by psychological distress experienced by patients as a result of delayed responses, leading to a reduced therapy compliance.[20] Since our study had its limitations regarding the retrospective design, variations in dosimetry, irradiation time and other outcomes, a direct comparison between twice weekly and thrice weekly NB-UVB in vitiligo patients is still required to determine what the optimal frequency is. Furthermore, concerns expressed by dermatologists on the higher weekly frequency and the number of exposures, is the carcinogenic potential of NB-UVB.[21] Currently, the risk of skin cancer after NB-UVB is not fully known yet. Several studies have investigated the risk of skin cancer after treatment with NB-UVB therapy.[22–25] No significant increased risk on the development of skin cancer was found after NB-UVB treatment. However, these studies were mostly retrospective and partially

based on psoriasis literature. Prospective multicentre studies of a large number of vitiligo patients with an adequate follow-up are required to make an accurate determination of the carcinogenic potential of NB-UVB therapy.[26] Notably, studies have shown that patients with vitiligo are found to have a decreased risk of both melanoma and nonmelanoma skin cancer, even though a substantial proportion of these patients were exposed to higher levels of UV radiation.[27,28]

Optimization of surgical treatment

Currently, several surgical techniques are available as treatment for repigmenting stable vitiligo and piebaldism lesions.[29,30] In essence, the donor to recipient expansion ratios (DR ratios) for tissue grafting techniques (i.e. punch grafting, epidermal blister grafting and split-thickness grafting) are set.[31] However, DR ratios and related outcomes reported in studies with cellular grafting vary widely and little evidence was available on the correlation between the DR ratios and repigmentation. Therefore, in **chapter 5,** we performed a systematic review to provide an overview of the various DR expansion ratios used during cellular grafting with non-cultured cell suspension transplantation (NCST) and cultured melanocyte transplantation (CMT). We found expansion ratios between 1:1 and 1:10 for studies investigating NCST and from 1:20 to 1:100 in studies evaluating CMT. The results of our pooled analyses indicated that higher expansion ratios lead to lower repigmentation percentages after NCST treatment. For clinical practice this should be taken into consideration, before deciding which DR expansion ratio to use. On the contrary, no significant differences in repigmentation were found between different expansion ratios after CMT treatment.[32] A possible explanation for this could be that in principle this technique yields a larger number of melanocytes for transplantation depending on the culture time. The cultured technique however, does have a few disadvantages since it is a time-consuming, expensive and complicated procedure, requiring advanced equipment, a sterile lab setup and trained personnel. Due to these disadvantages and strict regulations, this method is limitedly used.

As a consequence of the disadvantages of current surgical techniques and the lack of capacity of successfully treating large depigmented areas with high DR ratios, we sought for a novel transplantation technique to overcome these issues. In **chapter 6A** we shared our results of the first patient we treated with a novel tissue grafting method for depigmentations: the Meek micrografting technique.[33,34] Due to the successful results in our first patient, we continued to treat vitiligo and piebaldism patients with this Meek micrografting technique and evaluated the outcomes in a case-series study in **chapter 6B.** In this study, we assessed the results in 6 vitiligo and piebaldism patients, who received a total of 11 Meek micrografting treatments

with a 1:3 to 1:9 expansion ratio. We found an overall median repigmentation of 64.9% (6 weeks follow-up) and 88.1% (6 months follow-up). Since a relatively quick onset of repigmentation was seen after treatment, an early on treatment biomarker does not seem to be necessary for surgical treatments with the Meek technique. No major differences in repigmentation were found between the different body regions that were treated. Compared to previous treatments however, slight differences were found in median repigmentation after 6 months for the punch graft technique (77%, n=4) and the cell suspension technique (92%, n=3). Due to the limited number of treatments, larger studies are needed to assess the actual differences in repigmentation between these techniques, also taking the DR ratio differences into account. The main advantage of the Meek is that the loss of viable cells is minimal and that it is convenient to use for larger depigmented areas, while only a small area of donor skin is needed. The majority of the patients reported to be satisfied with this novel technique and would recommend this treatment to others. Although, common side effects, such as pain and itch during and shortly after the treatment were reported as well. Furthermore, a 'grid-like' pattern of repigmentation was visible (at the recipient site), which in some cases also persisted 6 months after treatment. Another disadvantage of this treatment is the initial high costs for purchasing the device. However, once the machine is acquired, the costs of the corks, gauzes and maintenance remain low. During this study, we observed that the median repigmentation differences of patients treated with the 1:4 and 1:6 DR ratio were negligible (88.0% vs 88.3%, respectively), although future prospective (randomized controlled) studies are required to better evaluate the repigmentation in relation to the different expansion ratios. In summary, our preliminary experience in the use of the Meek technique for treating depigmentations, suggests that it is a simple and safe transplantation technique that achieves relatively high repigmentation percentages and expansion ratios.

Patients' perspectives on treatment and outcomes

Over the past years, patient perspectives have become increasingly important. Consequently, the last part of my thesis focusses on patients' view on treatment and outcomes. Until now, little is known on patients' perspective regarding successful treatment in terms of repigmentation. In **chapter 7**, we conducted a prospective cross-sectional questionnaire study to evaluate the definition of successful repigmentation for facial and non-facial lesions from patients' perspective. Of the 325 patients that completed the survey, 221 (68%) and 122 (38%) patients considered ≥75% repigmentation successful for their facial vitiligo and non-facial vitiligo lesion(s) respectively. We however, did not distinguish the success thresholds between other body regions, which may differ as well based on the burden that patients experience from their vitiligo lesions. The success threshold

for lesions in dark skin type patients was significantly higher compared to light skin type patients. Remarkably, in our study 1 in 5 patients only regarded treatment successful if 100% of the facial lesions are repigmented. A considerable proportion also seems to be content with lower repigmentation rates, indicating a great variation in our population. Our results were largely in accordance with a previous focus group study of Eleftheriadou et al. stating that 80-100% repigmentation of a target lesion should be regarded as successful.[35] In conclusion, successful repigmentation seems to depend on the location of the lesions and on the skin types. However, for future research we recommend involving different ethnicities and more dark skin type patients to determine what a successful repigmentation is.

To date, patients often achieve suboptimal clinical results. To improve these results several new therapies are being developed and investigated.[36–38] Although, for the clinical implementation in daily practice, it is essential to understand the patients' need for these new treatment modalities. In **chapter 8** we clearly demonstrate the significant demand of vitiligo patients for new therapies. Of the 325 participating patients, 94% indicated that new and better treatments are needed for vitiligo. Moreover, approximately half of the patients felt that their current treatment was not effective and was not satisfied with it. A potential factor that could have been of influence on this dissatisfaction, is the therapeutic history. We found that patients who had received only topical treatment, were significantly less satisfied than patients who had received NB-UVB therapy, which is in line with previous studies.[39,40] This dissatisfaction could be less, if patients were better informed about their treatment prognosis per body region. In addition, an early on treatment biomarker may also help to manage patients' expectation and to finally improve their satisfaction on the outcomes of various treatments. Moreover, our analyses showed that patients with facial vitiligo, seemed to be more satisfied with current treatments. This could be clarified by the fact that facial lesions better respond to therapy than lesions on other body regions[10], as demonstrated as well in our previous study (chapter 3). Furthermore, our results show a clear difference in experiencing burden of vitiligo lesions related to their location. The degree of burden seems to be correlated to the visibility of the specific body region, which is also confirmed by other studies.[41–44] A higher burden and impaired quality of life, were significantly associated with a higher demand for new therapies, but also with a higher willingness to participate in clinical trials and a higher acceptance of invasive treatment for their vitiligo. Thus, the results of our exploratory study underline the need for improved and new therapies for vitiligo patients.

Future treatments

Since the interferon γ (IFN γ) signalling, mediated by the Janus kinase (JAK) – signal transducer and activator of transcription (STAT) pathway, plays a central role in the vitiligo pathogenesis[45], local inhibition of this signalling is a logical approach for developing new vitiligo treatments. Recently, large prospective randomized controlled trials (phase 2 and 3) have been conducted in Europe and the United States, investigating a novel topical treatment with ruxolitinib cream, an inhibitor of JAK 1 and 2.[46,47] Application of ruxolitinib cream as monotherapy was associated with considerable repigmentation of facial and non-facial vitiligo lesions after 24 and 52 weeks.[46,47] A ≥50% facial repigmentation was found in approximately 51% of the patients and a >50% total repigmentation was seen in approximately 22% of the patients after 6 months of treatment with ruxolitinib cream. In our study with the current standard of care therapy (NB-UVB phototherapy in combination with topical treatment and in some cases additionally oral mini pulse steroids) a >50% facial repigmentation was seen in 70% of the patients and >50% total repigmentation in 41% of the patients after 6 months. The current standard of care therapy seems to be more effective in terms of repigmentation, however is less convenient in the ease of use compared to topical therapy alone. The most common side effects of ruxolitinib cream were acne and pruritus of the application sites in approximately 6% and 5% of the patients, respectively.[47] To sum up, treatment with this cream seems to be promising for patients with vitiligo, however could perhaps be enhanced by combining with NB-UVB phototherapy.

In conclusion, this thesis *sheds light on* surgical and non-surgical treatments of vitiligo, immunological response biomarkers and patients' perspectives on vitiligo care. We found a positive relation between $GrB^{+}CD8^{-}$ T cells in skin and therapeutic response, which could be of use as an early on treatment biomarker, however further studies are needed to confirm our findings. In addition, we demonstrated that the body surface area can be divided into 4 composite body regions (face; torso&limbs; axillae; hands&feet) according to the degree of repigmentation after treatment. Furthermore, we compared a twice-weekly and thrice-weekly NB-UVB regimen in combination with topical therapy, showing comparable results regarding patient reported repigmentation, satisfaction and occurrence of adverse effects. We provided an overview of various expansion ratios used with non-cultured cell suspension transplantations, showing that higher expansion ratios lead to lower repigmentation. Moreover, we were the first to investigate a novel transplantation technique (Meek micrografting technique) for depigmentations, demonstrating it's simple and safe use with relatively high repigmentation percentages and expansion ratios. Finally, we found a great variety between patients regarding the definition of successful repigmentation and we underlined the demand for improved and novel

vitiligo therapies from patients' perspective.

This thesis addressed several aspects of vitiligo, providing an overview of current treatment results and a better understanding of patients' point of view on vitiligo care. Even despite the large variety in patients' perception of treatment success, discussing treatment prognosis per body region is important for managing the expectations of patients. Our recommendation for treating active vitiligo is in line with the current standard of care therapy (topical treatment in combination with NB-UVB phototherapy), however for stable vitiligo the Meek micrografting technique can safely and easily be used for treating depigmented areas. Currently, we have demonstrated early cellular changes during vitiligo standard of care therapy correlating to repigmentation, that require further studies to determine their use as potential treatment response biomarker. Future research will hopefully *shed* more *light on* these issues, ultimately contributing to improvement of vitiligo research and therapy.

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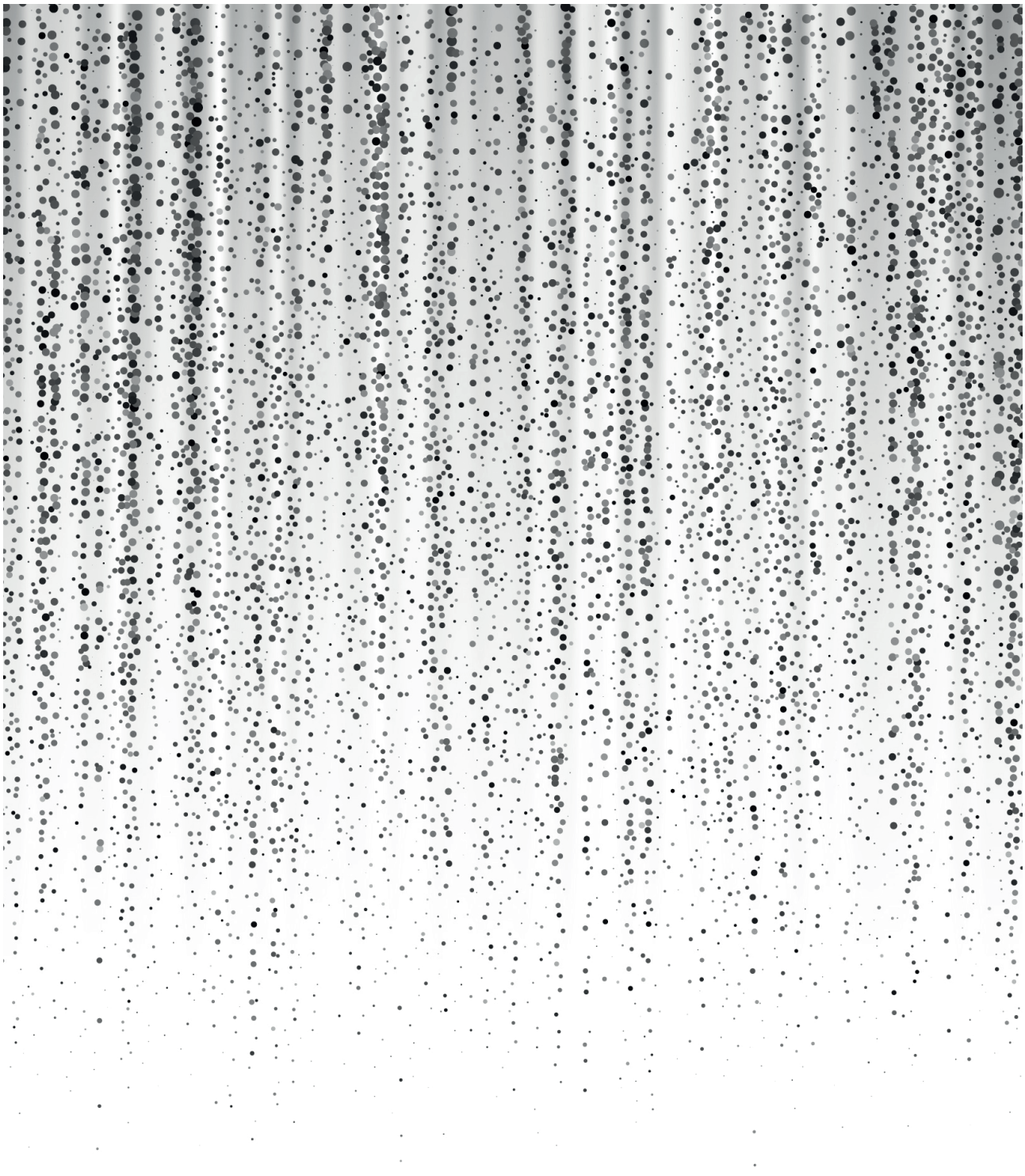


CHAPTER 10

Summary
Samenvatting

SUMMARY 'SHEDDING LIGHT ON VITILIGO'

Clinical, immunological and patients' perspectives

Vitiligo is the most common depigmenting skin disease affecting approximately 0.5-1% of the world population, regardless of sex, ethnicity or skin type. It is an auto-immune disease characterized by the development of white macules due to CD8 T cell mediated loss of melanocytes. The disease is strongly associated with an impaired quality of life, as many of the patients feel stressed and stigmatized by their condition. The overall aim of this thesis is to *shed light on* different clinical aspects of vitiligo to get a better understanding of the current state of affairs which may ultimately contribute to future improvement of vitiligo therapy and research.

Evaluation of topical and phototherapy responses

The treatment of non-segmental vitiligo (NSV) remains a challenge. Aside from a delay in visible repigmentation during therapy, it is uncertain whether a patient will ultimately respond to treatment. An early on treatment biomarker could help to predict the response to therapy. The aim of **chapter 2** was to evaluate the association between early changes within various immuno-cell types (T cell subsets and NK cells in blood and skin) and the clinical response to treatment. This prospective exploratory study was conducted in a cohort of NSV patients starting with standard of care treatment consisting of topical therapy alone or in combination with narrow band ultraviolet B (NB-UVB) phototherapy. Evaluation of the depigmented surface area was conducted with the Vitiligo Extent Score at baseline (V1), 3 months (V2) and 6 months (V3) after starting therapy. Tissue sampling of blood and lesional skin biopsies were performed at baseline and 3 months after therapy. Tissues were analysed for changes in T cell and NK cell populations as measured by CD3, CD4, Granzyme B^+ $(GrB)^+CD4$, CD8, GrB^+CD8, GrB^+CD8^-, T_{RM}, Treg, NK and GrB^+NK cells at V2 in relation to the repigmentation at V3. A total of 30 patients completed the study. Our results showed that an early increase of GrB^+CD8^- T cells in skin was significantly correlated to a higher repigmentation after 6 months of treatment. In blood, changes of this same sort of cell type (GrB^+CD4 T cells) between V1 and V2 showed significant relations with repigmentation at V3. Moreover, similar relations with repigmentation were found in skin between GrB^+NK cells percentages at V1 and V2. Other early changes in the analysed cell types did not show any significant relations with the treatment outcome. This study evaluated early changes in cellular immunity in relation to repigmentation after standard of care therapy. Both adaptive (GrB^+CD4 T cells) and innate (GrB^+NK cells) early detectable cellular immunological changes could play a role in predicting the treatment response after 6 months of therapy. For future implementations of an early on treatment biomarker, however, larger prospective

studies are required to confirm these findings and to gain more insights on other potential biomarkers.

NB-UVB is a widely used treatment for NSV. The location of the vitiligo lesions is of influence on the degree of repigmentation. To date however, these differences in therapeutic response between specific anatomical areas of the body have not been fully characterized. The objective of **chapter 3** was to evaluate the clinical response to NB-UVB treatment in 19 different body regions. A multicentre study of NSV patients treated with NB-UVB (alone or in combination with topical therapy and/or systemic steroids) was performed in hospitals in Egypt, Singapore and the Netherlands. Evaluation of the depigmented surface area of 19 different body regions was conducted with the Vitiligo Extent Score at baseline and after 6 months of therapy. A total of 101 NSV patients completed the study. The highest repigmentation was found in lesions on the lower facial region, followed by the upper facial region and lower trunk (>75% repigmentation in 58%, 48% and 46% of the patients, respectively). The lower face showed a significant better repigmentation than the upper face. The lowest repigmentation (<25% repigmentation) was observed at the feet (81%), followed by the hands (68%). Left versus right and dorsal versus ventral sides of the body demonstrated no significant differences. In addition, the torso and limbs showed similar repigmentation after treatment (median 47% and 42% respectively, *P=0.288*). We found that the body surface area can be classified into 4 composite body regions according to prognosis of repigmentation, i.e. face; torso&limbs; axillae; hands&feet, from highest to lowest median repigmentation (76%, 40%, 27%, 4%) respectively. In conclusion, this study was the first to evaluate and compare 19 different body regions after NB-UVB therapy. These results may be of direct use to clinicians and vitiligo patients as it provides more detailed information on treatment prognosis per body region.

Currently, data on the most effective treatment regimen with NB-UVB phototherapy is lacking. Efficacy of NB-UVB treatment may improve with more frequent use or in combination with topical agents. The objective of **chapter 4** was to retrospectively compare NB-UVB treatment regimens for NSV. Patients with NSV treated with NB-UVB therapy were included in two time periods. Group I received NB-UVB therapy twice a week (conventional treatment) and group II received NB-UVB thrice a week, combined with topical agents (intensified treatment). Patients completed a questionnaire regarding the degree and onset of repigmentation, satisfaction and side effects. Repigmentation scores did not differ significantly between the two groups. Onset of repigmentation in the first three months seemed higher in group II, but this difference was not significant (23.4% vs 51.1%; p=0.11). In both groups the majority of the patients were moderately to very satisfied (group I: 70.2%

group II: 73.3%). The occurrence of adverse effects was comparable. In conclusion, this study indicates that conventional and intensified treatment for NSV seem to be comparable regarding patient reported repigmentation, satisfaction and occurrence of adverse effects. The intensified treatment might be more effective to accelerate the onset of repigmentation, but larger prospective studies are needed to corroborate these findings.

Optimization of surgical treatment

Stabilized vitiligo resistant to conventional therapy (e.g. segmental vitiligo) and piebaldism lesions can be treated with autologous cellular grafting techniques, such as non-cultured cell suspension transplantation (NCST) and cultured melanocyte transplantation (CMT). These methods are preferred when treating larger surface areas due to the small amount of donor skin needed. However, the donor to recipient expansion ratios (DR ratios) and outcomes reported in studies with cellular grafting vary widely and to date, no overview or guideline exists on the optimal ratio. The aim of **chapter 5** was to obtain an overview of the various expansion ratios used in cellular grafting and to identify whether expansion ratios affect repigmentation and colour match. We performed a systematic literature search in MEDLINE and EMBASE, to review clinical studies that reported the expansion ratio and repigmentation after cellular grafting. We included 31 eligible clinical studies with 1591 patients in total. Our study provides an overview of various expansion ratios used in cellular grafting for vitiligo and piebaldism, which varied from 1:1 up to 1:100. We found expansion ratios between 1:1 and 1:10 for studies investigating NCST and from 1:20 to 1:100 in studies evaluating CMT. Pooled analyses of studies with the same expansion ratio and repigmentation thresholds, showed that when using the lowest (1:3) expansion ratio the proportion of lesions achieving >50% or >75% repigmentation after NCST was significantly better than when using the highest (1:10) expansion ratio (χ^2 P=0.000 and χ^2 P=0.006, respectively). Less than half of our included studies stated the colour match between different expansion ratios and results were variable. In conclusion, the results of our study indicate that higher expansion ratios lead to lower repigmentation percentages after NCST treatment. This should be taken into consideration when determining which expansion ratio to use for treating a patient.

To date, autologous tissue grafting and cellular suspension methods are widely used for treating stable vitiligo and piebaldism, however several limitations of these current techniques exist concerning the costs, size of transplanted skin, ease of use and repigmentation. There is an unmet need for treatment of large surface areas with a simple, reliable and effective transplantation technique. In **chapter 6A** we shared our results of the first patient we treated with a novel tissue

grafting method for depigmentations: the Meek micrografting technique. This tissue grafting method was first presented in 1958 by C.P. Meek and currently the modification of this method is a milestone in the history of acute burns surgery. During this procedure the donor skin is divided into many small pieces using the Meek Micromesher and then transferred onto a prefolded expandable gauze with aid of an adhesive spray. After that, the gauze is manually unfolded, allowing an up to 9-fold skin expansion. The expanded gauze is then placed onto the depigmented (laser-ablated) recipient site. For the treatment of our patient, we used one gauze with a 1:4 DR expansion ratio and another gauze with a 1:6 DR expansion ration, both resulting in remarkably high repigmentation rates after 3 and 9 months (1:4 ratio with a 87% and 93% repigmentation; 1:6 ratio with a 76% and 92% repigmentation, respectively). In conclusion, we wanted to underline the benefit of this novel technique for stable depigmented lesions, as it is a simple tissue grafting method that potentially reaches high expansion ratios and high repigmentation success rates.

Based on these successful results, we decided to continue treating vitiligo and piebaldism patients with this Meek micrografting technique and evaluated the outcomes in a case-series study in **chapter 6B.** The aim was to report the results of the Meek micrografting technique in vitiligo and piebaldism patients, with regard to repigmentation, side-effects and patient reported outcomes. We retrospectively analysed all vitiligo and piebaldism patients who received Meek micrografting between September 2019 and December 2021. Image analysis was used to calculate repigmentation after 6 weeks and 6 months of treatment. In addition, patients were requested to fill in a questionnaire on the effectiveness, side-effects and their treatment experience. A total of six patients received 11 treatments with a 1:3, 1:4, 1:6 and 1:9 expansion. Seventy-three percent of the treated lesions showed a ≥ 75% repigmentation. An overall median repigmentation of 64.9% (6 weeks follow-up) and 88.1% (6 months follow-up) were achieved. Eighty-three percent of the patients would recommend this treatment to others. Moreover, patients reported to be very satisfied (17%), satisfied (50%) and neutral (33%) regarding the treatment results. In addition, 83% of the patients reported that their vitiligo was a lot less up to no longer noticeable in the treated skin and 67% reported that the colour match was good to excellent. Reported side effects were pain, itch, transient hyperpigmentation and a 'grid-like' pattern. The main limitations of this study were the retrospective design and small sample size. Future prospective (randomized controlled) studies are required to better evaluate the repigmentation with different expansion ratios and to compare results with current transplantation techniques. *Summarizing,* our preliminary experience in the use of the Meek technique for treating depigmentations suggests that it is a simple and safe transplantation

technique that achieves relatively high repigmentation percentages and expansion ratios.

Patients' perspectives on treatment and outcomes

In vitiligo, repigmentation has been defined as a core outcome based on an international e-Delphi consensus. For interpretation of this outcome however, little evidence is available on the thresholds of successful repigmentation. In addition, little is known on patients' perspective regarding successful treatment in terms of repigmentation. In **chapter 7**, we aimed to evaluate the definition of successful repigmentation for facial and non-facial lesions from patients' perspective by carrying out a prospective cross-sectional questionnaire study. All patients with non-segmental vitiligo, aged 16 years or older that had consecutively visited our outpatient clinic at the Netherlands Institute for Pigment Disorders of the Amsterdam University Medical Centre between April 2017 and January 2019 were requested to complete a secured online questionnaire regarding patient demographics, quality of life and successful repigmentation concerning their own situation with a visual analogue scale ranging from 0% until 100%, separately for facial and non-facial lesions (on the body). The overall response rate was 70%, n=377 (60% fully completed, 8% not fully completed, 2% declined). Facial lesions were present in 86% and non-facial lesions in 70%. Eighty-three percent of the patients were light skin type (Fitzpatrick 1-3) and 17% were dark skin type (Fitzpatrick 4-6) patients. Of the 325 patients that completed the questionnaire, 221 (68%) and 122 (38%) patients considered ≥75% repigmentation successful for their facial vitiligo and non-facial vitiligo lesion(s) respectively. Remarkably 1 in 5 patients only regards treatment successful if 100% of the facial lesions are repigmented. The success threshold for lesions in dark skin type patients was significantly higher compared to light skin type patients. In conclusion, this study indicates a great variety between patients regarding the definition of successful repigmentation. Furthermore, successful repigmentation seems to depend on the location of the lesions and on the skin types.

Current treatments often achieve suboptimal clinical results. To improve these treatment results, several new therapies are being developed and investigated. There is, however, little evidence on the actual need for novel therapies. The objective of **chapter 8** was to assess patients' perspective on current and novel therapies for vitiligo. A prospective questionnaire study was conducted in a large cohort of vitiligo patients that consecutively visited the outpatient clinic of the Amsterdam University Medical Centre between April 2017 and January 2019. Patients were asked to fill in a digital questionnaire on patient characteristics, disease burden, quality of life, efficacy and satisfaction of current treatments and

aspects regarding new treatments. A total of 325 vitiligo patients completed the questionnaire (60% response rate). Of the respondents, 94% believed that new and improved treatments are needed and 86% would be willing to participate in clinical trials investigating a new therapy. Sixty-nine percent would agree on taking weekly injections if it led to effective treatment results. Of the patients who received prior treatment, 49% reported that the current treatments were not effective and 50% was not satisfied with the current treatments. Sixty-seven percent of the patients experienced facial lesions as an extreme burden, whereas this was, 25%, 12% and 10% for lesions on the hands, trunk and feet respectively. The emotional burden score was significantly higher in dark skin types compared to light skin types (respectively 8 vs 5, U $p < 0.05$). We concluded that there is a substantial need for new vitiligo therapies. A considerable number of patients in our study is dissatisfied with current treatments and is emotionally burdened by the disease. Moreover, the vast majority demands novel treatments and is willing to participate in clinical trials.

This thesis addressed several aspects of vitiligo, providing an overview of current treatment results and a better understanding of patients' point of view on vitiligo care. Even despite the large variety in patients' perception of treatment success, discussing treatment prognosis per body region is important for managing the expectations of patients. Our recommendation for treating active vitiligo is in line with the current standard of care therapy (topical treatment in combination with NB-UVB phototherapy), however for stable vitiligo the Meek micrografting technique can safely and easily be used for treating depigmented areas. Currently, we have demonstrated early cellular changes during vitiligo standard of care therapy correlating to repigmentation, that require further studies to determine their use as potential treatment response biomarker. Future research will hopefully *shed* more *light on* these issues, ultimately contributing to improvement of vitiligo research and therapy.

SAMENVATTING 'LICHT WERPEN OP VITILIGO'

Klinische, immunologische en patiënt perspectieven

Vitiligo is een van de meest voorkomende depigmenterende huidaandoeningen, die ongeveer 0,5-1% van de wereldbevolking treft, ongeacht geslacht, etniciteit of huidtype. Het is een auto-immuunziekte die wordt gekenmerkt door het ontstaan van witte plekken in de huid als gevolg van het verlies van melanocyten door auto-reactieve CD8 T cellen. De ziekte wordt sterk geassocieerd met een verminderde levenskwaliteit, aangezien veel patiënten stress ervaren en zich gestigmatiseerd voelen door hun aandoening. Het algemene doel van dit proefschrift is om licht te werpen op verschillende klinische aspecten van vitiligo om een beter inzicht te krijgen in de klinische, immunologische en patiënt perspectieven, wat uiteindelijk kan bijdragen tot toekomstige verbetering van vitiligo-behandeling en -onderzoek.

Evaluatie van topicale en lichttherapie behandelresultaten

De behandeling van non-segmentale vitiligo (NSV) blijft een uitdaging. De repigmentatie is tijdens behandeling vaak na enkele maanden vertraagd zichtbaar. Daarnaast is het onzeker of een patiënt uiteindelijk goed zal reageren op de behandeling. Een biomarker in een vroeg stadium tijdens de behandeling (vroege behandel-biomarker) zou kunnen helpen om de respons op behandeling te voorspellen. Het doel van **hoofdstuk 2** was om de associatie tussen veranderingen binnen verschillende immuuncelpopulaties (T-celsubtypen en NK-cellen in bloed en huid) en de klinische respons op behandeling te evalueren. Deze prospectieve exploratieve studie is uitgevoerd bij NSV-patiënten die een standaard vitiligo behandeling kregen bestaande uit alleen topicale therapie of in combinatie met narrow-band ultraviolet B (NB-UVB) lichttherapie. Evaluatie van het gedepigmenteerde huidoppervlak werd uitgevoerd met de Vitiligo Extent Score (VES) op baseline (V1, vòòr behandeling), 3 maanden (V2) en 6 maanden (V3) na aanvang van de behandeling. Op baseline en 3 maanden na behandeling werden weefselmonsters van bloed en laesionale huidbiopten afgenomen. De weefsels werden geanalyseerd op veranderingen in T-celsubtypen en NK cellen bestaande uit CD3, CD4, Granzyme B^+ $(GrB)^+CD4$, CD8, GrB^+CD8, GrB^+CD8^-, T_{RM}, Treg, NK en GrB^+NK-cellen bij V2 in relatie tot de repigmentatie bij V3. In totaal hebben 30 patiënten de studie voltooid. Uit onze resultaten bleek dat een vroege toename (dat wil zeggen na 3 maanden behandelingsduur) van GrB^+CD8^- T-cellen in de huid significant correleerde met betere repigmentatie na 6 maanden behandelingsduur. In het bloed vertoonde de interactie van hetzelfde soort celtype (GrB^+CD4 T-cellen) tussen V1 en V2 significante relaties met repigmentatie bij V3. Bovendien werden in de huid soortgelijke relaties met repigmentatie gevonden tussen GrB^+NK^-cel percentages bij V1 en V2. Andere veranderingen in de geanalyseerde celtypen

toonden geen significant verband met de repigmentatie van de behandeling. Deze studie evalueerde vroege veranderingen in cellulaire immuniteit in relatie tot repigmentatie na standaard vitiligo behandeling. Zowel adaptieve (GrB^+CD4 T-cellen) als aangeboren (GrB^+NK-cellen) vroegtijdig detecteerbare immuun veranderingen zouden een rol kunnen spelen bij het voorspellen van de behandelrespons na 6 maanden therapie. Voor toekomstige toepassingen van een vroege behandel-biomarker zijn echter grotere prospectieve studies nodig om onze bevindingen te bevestigen en meer inzicht te krijgen in andere potentiële biomarkers.

NB-UVB lichttherapie is een veel gebruikte behandeling voor NSV. De locatie van de vitiligolaesies op het lichaam is van invloed op de mate van repigmentatie. Tot op heden zijn deze verschillen in behandelrespons tussen specifieke anatomische gebieden van het lichaam echter niet volledig in kaart gebracht. Het doel van **hoofdstuk 3** was om de klinische respons op NB-UVB behandeling in 19 verschillende lichaamsregio's te evalueren. In ziekenhuizen in Egypte, Singapore en Nederland werd een multicentrisch onderzoek uitgevoerd onder NSV-patiënten die werden behandeld met NB-UVB (alleen of in combinatie met topicale therapie en/of systemische steroïden). Evaluatie van de gedepigmenteerde oppervlakte van 19 verschillende lichaamsregio's werd uitgevoerd met de Vitiligo Extent Score bij baseline en na 6 maanden behandeling. In totaal hebben 101 NSV-patiënten de studie voltooid. De laesies op het onderste gedeelte van het gezicht repigmenteerden het beste, gevolgd door de bovenste gezichtsregio en het onderste gedeelte van de romp (>75% repigmentatie bij respectievelijk 58%, 48% en 46% van de patiënten). Het onderste gedeelte van het gezicht vertoonde een significant betere repigmentatie dan het bovenste gedeelte van het gezicht. De laagste repigmentatie (<25% repigmentatie) werd waargenomen op de voeten (81%), gevolgd door de handen (68%). Links versus rechts en dorsale versus ventrale zijden van het lichaam vertoonden geen significante verschillen. Bovendien vertoonden de romp en de ledematen na de behandeling vergelijkbare repigmentatie percentages (mediaan respectievelijk 47% en 42%, P=0,288). Wij constateerden dat het lichaamsoppervlak kan worden ingedeeld in 4 samengestelde lichaamsregio's op basis van de prognose van repigmentatie, namelijk gezicht; torso&ledematen; oksels; handen&voeten, van hoogste naar laagste mediane repigmentatiegraad (respectievelijk 76%, 40%, 27%, 4%). Kortom, naar ons weten is dit de eerste studie die 19 verschillende lichaamsregio's na NB-UVB behandeling evalueerde en vergeleek. Onze resultaten zijn nuttig voor clinici en vitiligopatiënten aangezien het duidelijke en gedetailleerde informatie geeft over de behandelingsprognose per lichaamsregio.

Momenteel is niet bekend welk behandelschema het effectiefst is met NB-UVB lichttherapie. De effectiviteit van NB-UVB behandeling kan toenemen bij

frequentere belichting of in combinatie met topicale behandelingen. In **hoofdstuk 4** hebben wij retrospectief de NB-UVB behandelschema's vergeleken bij NSV patiënten. Patiënten met NSV die met NB-UVB therapie werden behandeld, waren in twee tijdsperioden (in 2008 en 2017) geïncludeerd. Groep I kreeg tweemaal per week NB-UVB therapie (conventionele behandeling) en groep II kreeg driemaal per week NB-UVB, gecombineerd met topicale therapie (geïntensiveerde behandeling). De patiënten vulden een vragenlijst in over het begin en de mate van repigmentatie, tevredenheid en bijwerkingen. De repigmentatie scores verschilden niet significant tussen de twee groepen. De aanvang van repigmentatie in de eerste drie maanden leek eerder te zijn in groep II, maar dit verschil was niet significant (23,4% versus 51,1%; p=0,11). In beide groepen was de meerderheid van de patiënten matig tot zeer tevreden (groep I: 70,2% groep II: 73,3%). Het optreden van bijwerkingen was vergelijkbaar. Op basis van deze studie lijken de conventionele en de geïntensiveerde behandeling voor NSV vergelijkbaar te zijn wat betreft de patiënt gerapporteerde repigmentatie, tevredenheid en bijwerkingenprofiel. Bij de geïntensiveerde behandeling zou de repigmentatie eerder zichtbaar kunnen zijn, maar er zijn grotere prospectieve studies nodig om deze bevindingen te bevestigen.

Optimalisatie van chirurgische behandeling

Gestabiliseerde vitiligo die therapie resistent is voor conventionele therapie (bv. segmentale vitiligo) en piebaldisme laesies kunnen worden behandeld met autologe cellulaire transplantatie technieken, zoals niet-gekweekte celsuspensie-transplantatie (NCST) en gekweekte melanocytentransplantatie (CMT). Deze methoden hebben de voorkeur bij de behandeling van grotere huidoppervlakken, vanwege de geringe hoeveelheid benodigde donorhuid. Studies met cellulaire transplantaties rapporteren vaak uiteenlopende expansieratio's die worden gebruikt voor de donorhuidoppervlakte ten opzichte van de acceptorhuidoppervlakte (DR-ratio's) en tot op heden bestaat er geen overzicht of richtlijn over de optimale ratio. Het doel van **hoofdstuk 5** was het verkrijgen van een overzicht van de verschillende DR expansieratio's die gebruikt worden bij cellulaire transplantatie en om daarbij na te gaan of die ratio's van invloed zijn op repigmentatie en kleurovereenkomst tussen de getransplanteerde huid en normale omliggende huid. We hebben een systematische literatuursearch uitgevoerd in MEDLINE en EMBASE om klinische studies te beoordelen die de DR ratio's en repigmentatie na cellulaire transplantaties rapporteerden. We hebben 31 klinische studies geïncludeerd met in totaal 1591 patiënten. Onze studie geeft een overzicht van de verschillende DR ratio's die bij cellulaire transplantatie voor vitiligo en piebaldisme werden gebruikt, variërend van 1:1 tot 1:100. Wij vonden DR ratio's tussen 1:1 en 1:10 voor studies die NCST onderzochten en van 1:20 tot 1:100 in studies die CMT evalueerden. Uit gepoolde analyses van de studies met dezelfde DR ratio en repigmentatie indelingen, bleek

dat bij gebruik van de laagste (1:3) DR ratio, de proportie van de laesies die >50% of >75% repigmentatie bereikten na NCST, significant beter was dan bij gebruik van de hoogste (1:10) DR ratio (respectievelijk χ^2 P=0,000 en χ^2 P=0,006). Minder dan de helft van onze geïncludeerde studies vermeldde de kleurovereenkomst na behandeling bij de verschillende expansieratio's en de resultaten waren variabel. Concluderend, wijzen de resultaten van onze studie erop dat hogere DR expansieratio's leiden tot lagere repigmentatiegraad na NCST-behandeling. Hiermee moet rekening worden gehouden bij de behandeling van patiënten.

Tot op heden worden autologe weefseltransplantaties en cellulaire suspensie-methoden veel gebruikt voor de behandeling van stabiele vitiligo en piebaldisme, maar deze huidige technieken hebben verschillende beperkingen wat betreft kosten, oppervlakte van de getransplanteerde huid, gebruiksgemak en repigmentatiegraad. Er is een evidente behoefte aan behandeling van grote oppervlakken met een eenvoudige, betrouwbare en effectieve transplantatietechniek. In **hoofdstuk 6A** deelden wij onze resultaten van de eerste patiënt die wij behandelden met een nieuwe weefseltransplantatiemethode voor depigmentaties: de Meek micrografting-techniek. Deze weefseltransplantatiemethode werd voor het eerst omschreven in 1958 door C.P. Meek en is momenteel een mijlpaal in de geschiedenis van de acute brandwondenchirurgie. Bij deze procedure wordt de donorhuid in vele kleine stukjes verdeeld met behulp van de Meek Micromesher en vervolgens met behulp van een kleefspray overgebracht op een voorgevouwen expandeerbaar gaasje. Daarna wordt het gaasje handmatig opengevouwen, waardoor de huid tot 9 keer kan uitzetten. Het geëxpandeerde gaasje wordt vervolgens op de gedepigmenteerde (met laser voorbehandelde) acceptorgebied geplaatst. Bij onze patiënt gebruikten wij een gaasje met een 1:4 DR expansieratio en een ander gaasje met een 1:6 DR expansie ratio, welke beide resulteerden in opmerkelijk hoge repigmentatie percentages na 3 en 9 maanden (1:4 ratio met respectievelijk 87% en 93% repigmentatie; 1:6 ratio met respectievelijk 76% en 92% repigmentatie). Wij wilden het voordeel van deze nieuwe techniek voor stabiele gedepigmenteerde laesies benadrukken, aangezien het een eenvoudige weefseltransplantatiemethode betreft die potentieel hoge DR expansieratio's en hoge repigmentatie percentages kan bereiken.

Naar aanleiding van deze succesvolle resultaten bij onze eerste patiënt, zijn wij vitiligo en piebaldisme patiënten blijven behandelen met deze Meek micrografting techniek en hebben we de resultaten geëvalueerd in een case-series studie in **hoofdstuk 6B**. Het doel was om de resultaten van de Meek micrografting techniek bij vitiligo en piebaldisme patiënten te rapporteren, met betrekking tot de repigmentatie, bijwerkingen en de door patiënt gerapporteerde uitkomsten.

We analyseerden retrospectief alle vitiligo en piebaldisme patiënten die tussen september 2019 en december 2021 behandeld zijn met de Meek micrografting techniek. Met behulp van beeldanalyse werd de repigmentatie 6 weken en 6 maanden na behandeling berekend. Daarnaast werden patiënten gevraagd een vragenlijst in te vullen over de effectiviteit, bijwerkingen en hun behandelervaring. In totaal kregen zes patiënten 11 behandelingen met een 1:3, 1:4, 1:6 en 1:9 DR expansieratio. Drieënzeventig procent van de behandelde laesies vertoonde een repigmentatie van ≥ 75%. Een totale mediane repigmentatie van 64,9% (6 weken follow-up) en 88,1% (6 maanden follow-up) werden bereikt. Drieëntachtig procent van de patiënten zou deze behandeling aan anderen aanbevelen. Bovendien gaven de patiënten aan zeer tevreden (17%), tevreden (50%) en neutraal (33%) te zijn over de resultaten van de behandeling. Daarnaast meldde 83% van de patiënten dat hun vitiligo veel minder tot niet meer zichtbaar was in de behandelde huid en 67% meldde dat de kleurovereenkomst met de niet-aangedane huid goed tot uitstekend was. Gerapporteerde bijwerkingen waren pijn, jeuk, voorbijgaande hyperpigmentatie en een 'rasterachtig' patroon. De belangrijkste beperkingen van deze studie waren de retrospectieve opzet en het gering aantal patiënten. Toekomstige prospectieve (gerandomiseerde gecontroleerde) studies zijn nodig om de repigmentatie met verschillende DR expansieratio's nader te evalueren en de resultaten te vergelijken met de huidige transplantatietechnieken. Samenvattend laat deze studie onze eerste ervaringen zien met de Meek-techniek voor de behandeling van depigmentaties. Het betreft een eenvoudige en veilige transplantatietechniek, die relatief hoge repigmentatie percentages en DR expansieratio's kan bereiken.

Patiëntperspectieven op behandeling en resultaten

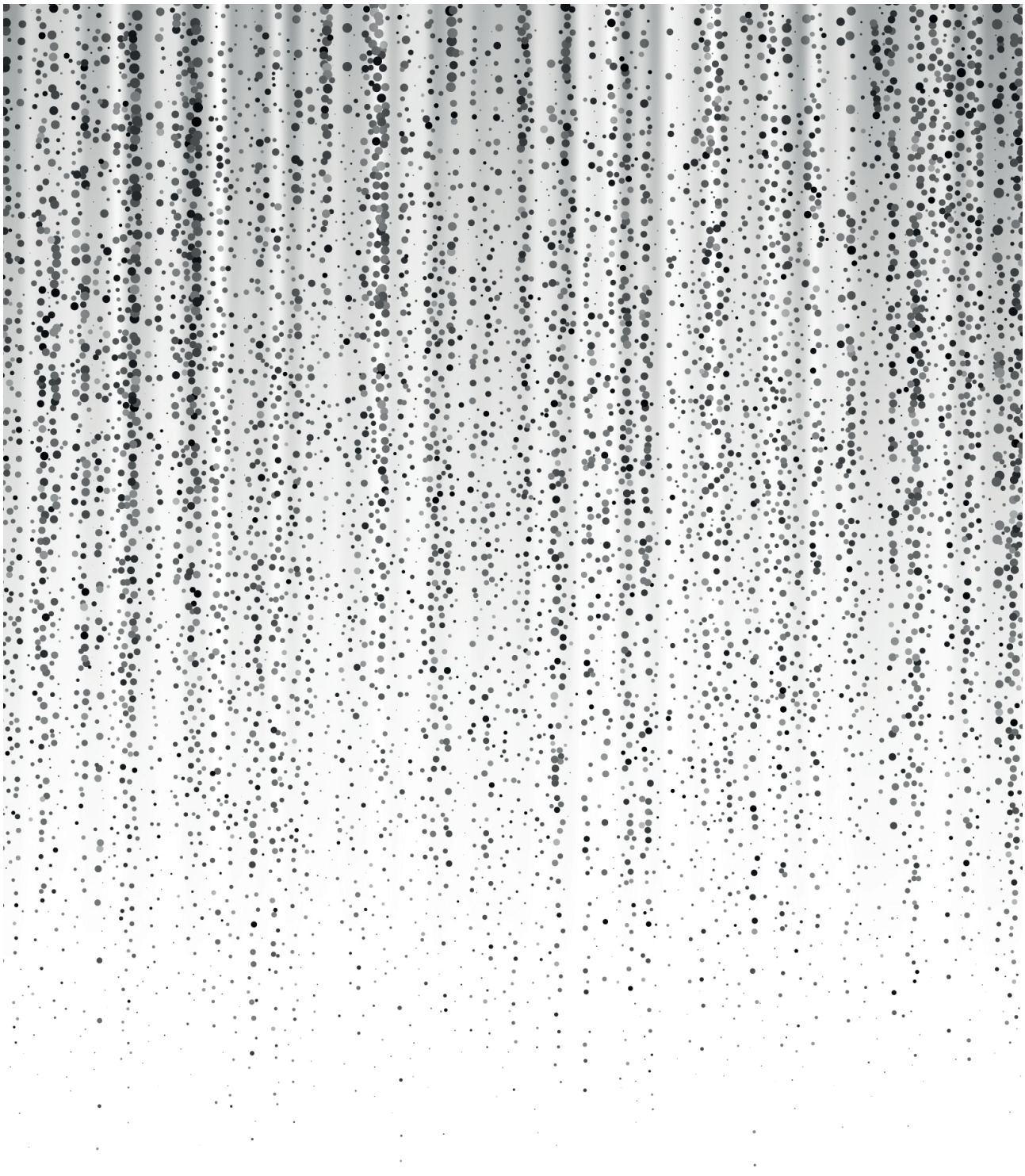
In een internationale e-Delphi-consensusstudie over vitiligo is repigmentatie gedefinieerd als een essentiële uitkomstmaat. Voor de interpretatie van deze uitkomst is echter weinig bewijs beschikbaar over de drempelwaarde voor succesvolle repigmentatie. Bovendien is er weinig bekend over welke mate van repigmentatie patiënten als een succesvolle behandelingsresultaat definiëren. In **hoofdstuk 7** hebben wij getracht de definitie van succesvolle repigmentatie voor faciale en non-faciale laesies vanuit het perspectief van de patiënten te evalueren, door een prospectieve cross-sectionele vragenlijststudie uit te voeren. Alle patiënten met non-segmentale vitiligo, van 16 jaar of ouder die tussen april 2017 en januari 2019 achtereenvolgens onze polikliniek van het Nederlands Instituut voor Pigmentstoornissen van het Amsterdam Universitair Medisch Centrum hadden bezocht, werden gevraagd een beveiligde online vragenlijst in te vullen over demografische gegevens, kwaliteit van leven en welke mate van repigmentatie zij op basis van hun eigen ervaringen een succesvolle behandeling vinden met

behulp van een visuele analoge schaal variërend van 0% tot 100%, afzonderlijk voor faciale en non-faciale laesies (op het lichaam). De totale respons op de vragenlijst bedroeg 70%, n=377 (60% volledig ingevuld, 8% niet volledig ingevuld, 2% geweigerd). Faciale laesies waren aanwezig in 86% en non-faciale laesies in 70%. Drieëntachtig procent van de patiënten had een lichte huidtype (Fitzpatrick 1-3) en 17% had een donkere huidtype (Fitzpatrick 4-6). Van de 325 patiënten die de vragenlijst invulden, achtten 221 (68%) en 122 (38%) patiënten, ≥75% repigmentatie succesvol voor respectievelijk hun faciale vitiligolaesies en hun non-faciale vitiligolaesies. Opmerkelijk is dat 1 op de 5 patiënten de behandeling pas geslaagd acht als 100% van de laesies in het gezicht gerepigmenteerd zijn. De succes drempelwaarde voor laesies bij patiënten van het donkere huidtype was significant hoger dan bij patiënten van het lichte huidtype. Concluderend, deze studie wijst op een grote variabiliteit in de mening van patiënten over de definitie van succesvolle repigmentatie. Bovendien lijkt het oordeel over succesvolle repigmentatie af te hangen van de locatie van de laesies en van het huidtype.

Met de huidige vitiligo behandelingen worden vaak suboptimale klinische resultaten bereikt. Om deze resultaten te verbeteren worden verschillende nieuwe therapieën ontwikkeld en onderzocht. Er is echter weinig zicht op de werkelijke behoefte aan nieuwe therapieën bij patienten. Het doel van **hoofdstuk 8** was om de mening van patiënten over de huidige behandelingen en hun behoefte naar nieuwe therapieën voor vitiligo te achterhalen. Een prospectieve vragenlijststudie werd uitgevoerd in een groot cohort van vitiligopatiënten die tussen april 2017 en januari 2019 achtereenvolgens de polikliniek van het Amsterdam Universitair Medisch Centrum bezochten. Patiënten werd gevraagd een digitale vragenlijst in te vullen over patiëntkenmerken, ziektelast, kwaliteit van leven, effectiviteit en tevredenheid over huidige behandelingen en aspecten met betrekking tot nieuwe behandelingen. In totaal vulden 325 vitiligo patiënten de vragenlijst in (60% respons). Van de respondenten was 94% van mening dat nieuwe en verbeterde behandelingen nodig zijn en 86% zou bereid zijn deel te nemen aan klinisch onderzoek naar een nieuwe therapie. Negenenzestig procent zou instemmen met wekelijkse injecties als dat tot effectieve behandelingsresultaten zou leiden. Van de patiënten die al eerder een behandeling hadden ondergaan, meldde 49% dat de huidige behandelingen niet effectief waren en 50% was niet tevreden met de huidige behandelingen. Zevenenzestig procent van de patiënten ervoer laesies in het gezicht als een extreme last, terwijl dit respectievelijk 25%, 12% en 10% was voor laesies aan de handen, romp en voeten. De emotionele belasting was significant hoger bij donkere huidtypes vergeleken met lichte huidtypes (respectievelijk 8 versus 5, p <0,05). Wij concludeerden dat er een aanzienlijke behoefte is aan nieuwe therapieën voor vitiligo. Een aanzienlijk aantal patiënten in onze studie was

ontevreden over de huidige behandelingen en emotioneel belast door de ziekte. Bovendien zou de overgrote meerderheid graag nieuwe behandelingen krijgen en is bereid deel te nemen aan klinische studies.

Deze thesis belicht verschillende aspecten van vitiligo, waarbij er een overzicht van de huidige behandelresultaten en een beter begrip van de mening van patiënten over vitiligo-zorg is gegeven. Het bespreken van de behandelprognose per lichaamsregio is belangrijk om de verwachtingen van patiënten te managen, ook al is er een grote variatie in wat als een succesvolle behandeling wordt beschouwd. Onze aanbeveling voor de behandeling van actieve vitiligo komt overeen met de huidige standaard behandeling (namelijk topicale behandeling in combinatie met NB-UVB lichttherapie). Voor stabiele vitiligo bevelen wij de Meek micrografting techniek aan, aangezien het veilig en gemakkelijk kan worden gebruikt voor de behandeling van gedepigmenteerde gebieden. Tevens hebben wij vroege cellulaire veranderingen aangetoond tijdens de standaardtherapie voor vitiligo die correleren met repigmentatie, alhoewel deze veranderingen verder moeten worden onderzocht om te bepalen of zij kunnen worden gebruikt als potentiële biomarker voor de respons op behandeling. Toekomstig onderzoek zal hopelijk meer licht werpen op deze kwesties en uiteindelijk bijdragen tot verbetering van vitiligo-onderzoek en -therapie.



APPENDICES &

List of contributing authors
Contribution of the authors per article
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About the author
Dankwoord

LIST OF CONTRIBUTING AUTHORS

Elnaz Alagha
Department of Dermatology, Netherlands Institute for Pigment Disorders, Amsterdam Infection and Immunity Institute, Amsterdam University Medical Centers, Location University of Amsterdam, Amsterdam, The Netherlands

Marcel W. Bekkenk
Department of Dermatology, Netherlands Institute for Pigment Disorders, Amsterdam Infection and Immunity Institute, Amsterdam University Medical Centers, Location University of Amsterdam, Amsterdam, The Netherlands

Lars L.C. van den Bol
Department of Dermatology, Netherlands Institute for Pigment Disorders, Amsterdam Infection and Immunity Institute, Amsterdam University Medical Centers, Location University of Amsterdam, Amsterdam, The Netherlands

Saskia Chielie
Department of Dermatology, Netherlands Institute for Pigment Disorders, Amsterdam Infection and Immunity Institute, Amsterdam University Medical Centers, Location University of Amsterdam, Amsterdam, The Netherlands

Sai Yee Chuah
Department of Dermatology, National Skin Centre, Singapore City, Singapore.

Samia Esmat
Department of Dermatology, Faculty of Medicine Cairo University, Cairo, Egypt.

Nanja van Geel
Department of Dermatology, Ghent University Hospital, Ghent, Belgium

Malika Hanser
Novartis Institutes for BioMedical Research, Basel, Switzerland

Rasmus B. Kjellerup
Novartis Institutes for BioMedical Research, Basel, Switzerland

Christian Loesche
Novartis Institutes for BioMedical Research, Basel, Switzerland

Janny E. Lommerts
Department of Dermatology, Medisch Centrum Jan van Goyen, Amsterdam, The Netherlands

Rosalie M. Luiten
Department of Dermatology, Netherlands Institute for Pigment Disorders, Amsterdam Infection and Immunity Institute, Amsterdam University Medical Centers, Location University of Amsterdam, Amsterdam, The Netherlands

Elizabeth McNamara
Novartis Institutes for BioMedical Research, Basel, Switzerland

Rania M. Mogawer
Department of Dermatology, Faculty of Medicine Cairo University, Cairo, Egypt.

Wouter Ouwerkerk
Department of Dermatology, Netherlands Institute for Pigment Disorders, Amsterdam Infection and Immunity Institute, Amsterdam University Medical Centers, Location University of Amsterdam, Amsterdam, The Netherlands.
National Heart Centre Singapore, Hospital Drive, Singapore City, Singapore.

Nicoline F. Post
Department of Dermatology, Netherlands Institute for Pigment Disorders, Amsterdam Infection and Immunity Institute, Amsterdam University Medical Centers, Location University of Amsterdam, Amsterdam, The Netherlands

Nanis Ragab
Department of Dermatology, Faculty of Medicine Cairo University, Cairo, Egypt.

Menno A. de Rie
Department of Dermatology, Amsterdam Infection and Immunity Institute, Amsterdam University Medical Centers, Location University of Amsterdam, Amsterdam, The Netherlands

Martin-Alexander Schneider
Novartis Institutes for BioMedical Research, Basel, Switzerland

Steven Thng
Department of Dermatology, National Skin Centre, Singapore City, Singapore.

Nathalie O.P. van Uden
Department of Dermatology, Netherlands Institute for Pigment Disorders, Amsterdam Infection and Immunity Institute, Amsterdam University Medical Centers, Location University of Amsterdam, Amsterdam, The Netherlands

Sanne E. Uitentuis
Department of Dermatology, Netherlands Institute for Pigment Disorders, Amsterdam Infection and Immunity Institute, Amsterdam University Medical Centers, Location University of Amsterdam, Amsterdam, The Netherlands

Bas S. Wind
Department of Dermatology, Bergman Clinics, Amsterdam, The Netherlands

Albert Wolkerstorfer
Department of Dermatology, Netherlands Institute for Pigment Disorders, Amsterdam Infection and Immunity Institute, Amsterdam University Medical Centers, Location University of Amsterdam, Amsterdam, The Netherlands

Paul P.M. Van Zuijlen
Department of Plastic, Reconstructive and Hand Surgery, Amsterdam University Medical Center, Amsterdam, The Netherlands.
Burn Center, Red Cross Hospital, Beverwijk, The Netherlands.

CONTRIBUTION OF THE AUTHORS PER ARTICLE

Early cellular and clinical responses to treatment in non-segmental vitiligo

V.S. Narayan: Contributions to conception and design, acquisition of data, analysis and interpretations of data, drafting the manuscript and revising it critically for important intellectual content and final approval of the version to be published.

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N.O.P. van Uden: Contributions to analysis and interpretations of data, drafting the manuscript and revising it critically for important intellectual content and final approval of the version to be published.

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C. Loesche: Contributions to conception and design.

M. Hanser: Contributions to analysis and interpretations of data.

E. McNamara: Contributions to conception and design, analysis and interpretations of data.

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S. Esmat: Contributions to acquisition of data, revising the article critically for important intellectual content and final approval of the version to be published.

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N. Ragab: Contributions to acquisition of data, revising the article critically for important intellectual content and final approval of the version to be published.

S.Y. Chuah: Contributions to acquisition of data, revising the article critically for important intellectual content and final approval of the version to be published.

S. Thng: Contributions to acquisition of data, revising the article critically for important intellectual content and final approval of the version to be published.

A. Wolkerstorfer: Contributions to conception and design, analysis and interpretations of data, drafting the article and revising it critically for important intellectual content and final approval of the version to be published.

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Patient reported outcomes for intensified versus conventional NB-UVB treatment in non-segmental vitiligo

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B.S. Wind: Contributions to acquisition of data and revising the article critically for important intellectual content and final approval of the version to be published.

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A. Wolkerstorfer: Contributions to conception and design, analysis and interpretations of data, revising the article critically for important intellectual content and final approval of the version to be published.

Donor to recipient ratios in the surgical treatment of vitiligo and piebaldism: a systematic review

V.S. Narayan: Contributions to conception and design, acquisition of data, analysis and interpretations of data, drafting the article and revising it critically for important intellectual content and final approval of the version to be published. Corresponding author.

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Meek micrografting: a novel surgical technique for the treatment of depigmentation

V.S. Narayan: Contributions to conception and design, acquisition of data, analysis and interpretations of data, drafting the article and revising it critically for important intellectual content and final approval of the version to be published. Corresponding author.

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Meek micrografting, a novel surgical technique for the treatment of vitiligo and piebaldism: A case series

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A. Wolkerstorfer: Contributions to conception and design, acquisition of data, analysis and interpretations of data, drafting the article and revising it critically for important intellectual content and final approval of the version to be published. Corresponding author.

What is a successful repigmentation in vitiligo from patients' view?

V.S. Narayan: Contributions to conception and design, acquisition of data, analysis and interpretations of data, drafting the article and revising it critically for important intellectual content and final approval of the version to be published. Corresponding author.

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A. Wolkerstorfer: Contributions to conception and design, acquisition of data, analysis and interpretations of data, drafting the article and revising it critically for important intellectual content and final approval of the version to be published. Corresponding author.

Patients' perspective on current treatments and demand for novel treatments in vitiligo

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LIST OF PUBLICATIONS

List of publications in this thesis:

- Uitentuis SE, Narayan VS, Wind BS, Bekkenk MW, de Rie MA, Wolkerstorfer A. Patient reported outcomes for intensified versus conventional NB-UVB treatment in non-segmental vitiligo. J Dermatolog Treat. 2019 Sep;30(6):594-597. doi: 10.1080/09546634.2018.1543851. Epub 2018 Nov 29. PMID: 30497304.
- Narayan VS, Uitentuis SE, Luiten RM, Bekkenk MW, Wolkerstorfer A. Patients' perspective on current treatments and demand for novel treatments in vitiligo. J Eur Acad Dermatol Venereol. 2021 Mar;35(3):744-748. doi: 10.1111/jdv.16927. Epub 2020 Oct 7. PMID: 32893377; PMCID: PMC7983948.
- Narayan VS, Uitentuis SE, Bekkenk MW, Wolkerstorfer A. What is successful repigmentation in vitiligo from the point of view of patients? Br J Dermatol. 2021 Jan;184(1):165-166. doi: 10.1111/bjd.19422. Epub 2020 Sep 1. PMID: 32726875; PMCID: PMC7818176.
- Narayan VS, van den Bol LLC, van Geel N, Bekkenk MW, Luiten RM, Wolkerstorfer A. Donor to recipient ratios in the surgical treatment of vitiligo and piebaldism: a systematic review. J Eur Acad Dermatol Venereol. 2021 May;35(5):1077-1086. doi: 10.1111/jdv.17108. Epub 2021 Feb 12. PMID: 33428279; PMCID: PMC8247963.
- Narayan VS, Post NF, Van Zuijlen PPM, Bekkenk MW, Wolkerstorfer A. Meek micrografting: a novel surgical technique for the treatment of depigmentation. J Eur Acad Dermatol Venereol. 2021 Nov;35(11):e798-e801. doi: 10.1111/jdv.17478. Epub 2021 Jul 12. PMID: 34166548.
- Narayan VS*, Post NF*, Bekkenk MW, Wolkerstorfer A. Meek micrografting, a novel surgical technique for the treatment of vitiligo and piebaldism: A case series. J Eur Acad Dermatol Venereol. 2022 Dec 22. doi: 10.1111/jdv.18829. Epub ahead of print. PMID: 36545938.
- Narayan VS, Alagha E, Ouwerkerk W, Uitentuis SE, Lommerts JE, Esmat S, Mogawer RM, Ragab N, Chuah SY, Thng S, Wolkerstorfer A, Luiten RM, Bekkenk MW. NB-UVB phototherapy response of different body regions in non-segmental vitiligo. J Eur Acad Dermatol Venereol. 2023 Feb 3. doi: 10.1111/jdv.18946. Epub ahead of print. PMID: 36734628.

List of other publications:

- Post NF, Ezekwe N, Narayan VS, Bekkenk MW, Van Geel N, Hamzavi I, Passeron T, Wolkerstorfer A. The use of lasers in vitiligo, an overview. J Eur Acad Dermatol Venereol. 2022 Jun;36(6):779-789. doi: 10.1111/jdv.18005. Epub 2022 Mar 1. PMID: 35176186; PMCID: PMC9314124.
- Willemsen M, Krebbers G, Tjin EPM, Willemsen KJ, Louis A, Konijn VAL, Narayan VS, Post NF, Bakker WJ, Melief CJM, Bekkenk MW, Luiten RM. IFN-γ-induced PD-L1 expression on human melanocytes is impaired in vitiligo. Exp Dermatol. 2022 Apr;31(4):556-566. doi: 10.1111/exd.14500. Epub 2021 Nov 17. PMID: 34758170.
- Willemsen M, Post NF, van Uden NOP, Narayan VS, Chielie S, Kemp EH, Bekkenk MW, Luiten RM. Immunophenotypic Analysis Reveals Differences in Circulating Immune Cells in the Peripheral Blood of Patients with Segmental and Nonsegmental Vitiligo. J Invest Dermatol. 2022 Mar;142(3 Pt B):876-883. e3. doi: 10.1016/j.jid.2021.05.022. Epub 2021 Jun 22. PMID: 34166674.

PhD PORTFOLIO

Name PhD student: Vidhya Shankar Narayan

PhD period: October 2018 – June 2023
Promotor: Prof. dr. R.M. Luiten
Co-promotores: dr. A. WolkerstorferProf. dr. M.W. Bekkenk

	Year	Workload ECTS
1. PhD training		
General courses and seminars		
The Amsterdam UMC World of Science	2018	0,7
Practical biostatistics, exam: 9	2019	1,4
Scientific writing in English	2019	1,5
Clinical and Research Data Management.	2019	1,0
Basic course immunology	2019	1,0
Flow Cytometry Introduction Course	2019	0,3
Electronic Basic Course Legislation and Organization for Clinical researcher (eBROK:'Basiscursus Regelgeving Klinisch Onderzoek')	2020	1,0
Systematic review	2020	0,7
Weekly scientific meeting of department	2018-2023	3,0
	Total	**10,6**
Presentations		
Annual meeting Dutch Society for Experimental Dermatology, Lunteren, the Netherlands *Poster presentation:* Patients' perspective on current and demand for novel treatment in vitiligo	2020	0,5
International Pigment Cell Conference, online booklet (due to COVID) *Selected as poster presentation:* 'Patients' perspective on current and demand for novel treatment in vitiligo'	2020	0,5
Selected as poster presentation: 'What is a successful repigmentation in vitiligo from patients' view?'	2020	0,5
Vitiligo International Symposium, digital (due to COVID) *Oral presentation:* Size ratios of donor and recipient areas in the surgical treatment of vitiligo: a systematic review	2021	0,5
Poster presentation: 'Patients' perspective on current and demand for novel treatment in vitiligo'	2021	0,5
Poster presentation: 'What is a successful repigmentation in vitiligo from patients' view?	2021	0,5
European Society for Pigment Cell Research, digital *Oral presentation:* NB-UVB therapy response of different body regions in non-segmental vitiligo	2022	0,5
European Society for Dermatological Research, Amsterdam *Poster presentation:* NB-UVB therapy response of different body regions in non-segmental vitiligo	2022	0,5

Continued.

	Year	Workload ECTS
1. PhD training		
Presentations		
Vitiligo International Symposium, Bangalore, India		
Oral presentation: NB-UVB therapy response of different body regions in non-segmental vitiligo	2022	0,5
	Total	**4,5**
(Inter)national conferences		
Annual meeting Dutch Society for Experimental Dermatology, Lunteren, Netherlands	2019	0,5
Annual meeting European Academy of Dermatology and Venereology, Madrid	2019	0,5
Annual meeting Dutch Society for Experimental Dermatology, Lunteren, Netherlands	2020	1,0
Vitiligo International Symposium, digital	2021	1,5
European Society for Pigment Cell Research, digital	2022	0,5
Vitiligo International Symposium, India	2022	1,25
2. Teaching		
Supervising master student for master thesis VUmc L.L.C. van den Bol	2019-2020	1.0
Supervising master student for master thesis AMC E. Alagha	2019-2020	1.0
Wetenschappelijke vergadering dermatologie Amsterdam UMC	2020	0.2
	Total	**2,2**
3. Parameters of esteem - Awards		
Travel grant, International Pigment Cell Conference, 2020, Tokyo, Japan		
Travel grant, Amsterdam Infection and Immunity Institute for Vitiligo International Symposium, India		

About the author

Vidhya Shankar Narayan was born on August 23rd 1992 in Veldhoven, Noord-Brabant, as second of two siblings to Shankar and Usha. She completed her primary and high school education in Valkenswaard. After successfully obtaining her gymnasium high school diploma, she moved to Amsterdam in 2010, to pursue her bachelor's and master's degrees in Medicine at the University of Amsterdam.

After her bachelor's degree, Vidhya arranged an additional internship at MS Ramaiah Hospital in Bangalore, India, for three months, gaining valuable experience in clinical practice. During her master's degree, Vidhya broadened her horizons by completing an internship in tropical medicine in Tanzania. After coming back, Vidhya developed a growing interest in dermatology which led her to pursue her final research internship at the Dermatology Department/Netherlands Institute for Pigment Disorders (SNIP).

Following her successful completion of her research internship, Vidhya was offered a Ph.D. position at the same department, working under the supervision of Prof. Dr. R.M. Luiten, Dr. A. Wolkerstorfer, and Prof. Dr. M.W. Bekkenk. Before starting her PhD., Vidhya worked as an MD (basisarts) at the dermatology department at Medisch Centrum Jan van Goyen and Brijder addiction care Spaarne hospital Hoofddorp to gain further clinical experience. In 2021, she moved to Berlin for six month to complete writing her PhD dissertation.

In July 2021, Vidhya began her residency program in dermatology at the Amsterdam University Medical Center, location AMC.

Dankwoord

Het zit erop! Basta! Finito! (Ph)Done! Ook ik kan nu zeggen dat ik ein-de-lijk klaar ben met mijn PhD! Laten we eerlijk wezen, de meesten van jullie zijn waarschijnlijk direct hiernaar toe doorgebladerd/gescrold om dit als eerste te lezen hè? Ik heb er daarom ook extra mijn best op gedaan. Mijn hele PhD traject en het afronden daarvan was natuurlijk nooit gelukt zonder de hulp van anderen. Ik wil daarom graag mijn promotor, copromotoren, medeauteurs, collega's, patiënten, vrienden en familie enorm bedanken. Jullie steun, feedback en aanmoediging waren onmisbaar. Daarnaast zou ik graag nog een aantal personen in het bijzonder willen bedanken.

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