REVIEW ARTICLE

Drugs 2004; 64 (1): 89-107
0012-6667/04/0001-0089/$34.00/0

Hypopigmentary Skin Disorders
Current Treatment Options and Future Directions

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Abstract

Alterations of skin and hair pigmentation are important features that have warranted treatment from ancient history on up to modern time. In some cultures, even today patients with vitiligo are regarded as social outcasts and are affected considerably both emotionally and physically. This article presents current options and future directions for the treatment of hypopigmentary disorders.

Whereas with congenital disorders, such as albinism and phenylketonuria, no causal therapy has been established up to now, several treatment options for acquired hypopigmentary disorders have been investigated. In particular, in vitiligo, one of the most prevalent hypopigmentary disorders, a number of treatment modalities have been employed in the past 30 years. However, most of them are only able to palliate, not cure, the disease. Depending on the distribution of the hypopigmented lesions (localised or generalised) and the state of the disease

(active or stable), several therapeutic options, for example phototherapy, surgical skin grafts, autologous melanocyte transplantation and immunomodulators, can be applied alone or in combination. For phototherapy, because of unfavourable results and adverse effects, ultraviolet (UV) A has been largely replaced by narrow-band UVB for repigmentation of generalised vitiligo. Although immunomodulators, such as corticosteroids, have been used both topically and systemically over the past 3 decades for the treatment of disseminated vitiligo, they are only suitable for the treatment of acrofacial and localised forms because of adverse effects. Hence, new immunomodulatory agents, such as calcineurin antagonists, have recently been introduced as new promising tools to treat acquired hypopigmentary disorders. However, all therapeutic approaches are hampered by the fact that the pathophysiology of hypopigmentary disorders is still poorly understood.

During early embryonic development, a subset of neural crest-derived cells migrate from the neural tube to the skin and pass into the melanoblast-melanocyte lineage, thereby giving rise to the pigmentary system.[1] Melanin is produced in melanosomes within melanocytes, which populate the basal layer of the epidermis and hair follicle, as well as mucous membranes, the brain (leptomeninges), the eye (retina and uvea) and the ear (cochlea, vestibulum). In humans, two major forms of melanin exist, eumelanin and pheomelanin, which are responsible for different skin colours (e.g. black, brown, yellow).

The starting point of melanin production is tyrosine, a non-essential amino acid, which is converted by tyrosinase to dopa and dopaquinone, and subsequently to melanin.[2] The level of tyrosinase activity directly correlates with the degree of melanisation.[3] Each melanocyte supplies its melanin-containing melanosomes to an average of 36 keratinocytes. The amount of supplied melanin determines the degree of cutaneous pigmentation.[4] Notably, the difference in pigmentation of black and white skin does not depend on the number of melanocytes, which is almost equal in both skin types, but on size, form, arrangement and melanin production of the cutaneous melanocytes. In black skin, melanocytes are larger, more dendritic and contain more melanosomes, which have a higher melanin content, than those in white skin.[5]

Hypopigmentary disorders may be the result of apoptosis as well as disorders of maturation leading to either a diminution of melanocytes in number and/or size (which could include up to a complete absence) or a reduction of melanin synthesis (see table I). Two inherited disorders may be used as examples to illustrate these scenarios: (i) in piebaldism, the lack of melanocytes in the unpigmented patches is due to a defect of migration of melanocytic stem cells during embryonal development from the neural crest to the skin;[5] and (ii) in albinism, the number of melanocytes is normal, however, melanin synthesis is inhibited by various degrees, depending on the genetic locus involved. More than ten different phenotypes are differentiated, ranging

Table I. Examples of congenital or acquired hypopigmentary disorders according to their cause

Type of disorder	Cause of hypopigmentary disorder	
	melanocytopenic disorders (diminution of melanocytes)	melanopenic disorders (reduction of melanin synthesis)
Congenital disorders	Piebaldism, Waardenburg syndrome, hypomelanosis ito	Albinism (oculocutaneous albinism [e.g. OCA 1A, OCA 1B, OCA 2, OCA 3], Hermansky-Pudlak syndrome, Chédiak-Higashi syndrome), phenylketonuria, nevus depigmentosus
Acquired disorders	Vitiligo, hypomelanosis guttata, halo-nevus, drug-induced leukoderma, chemical leukoderma	Post-inflammatory leukoderma/infectious diseases

from a partial reduction of eumelanin synthesis (oculocutaneous albinism [OCA] 2)[6] to a total absence of melanin in skin, hair and eyes (OCA 1). In the case of acquired hypopigmentary disorders, decreased melanin content of the lesions is in general due to a marked reduction or absence of melanocytes. The mechanism of destruction of melanocytes has been shown to be related to humoral and cellular autoimmune responses[7,8] as well as apoptosis induction.[9-11]

This review focuses on hypopigmentary disorders for which a causal therapy has been established or at least is in development. For the purposes of this article, only those therapeutic modalities that aim to cure the respective disorder (that is, adding melanocytes or increasing the production of melanin) are presented. Consequently, cosmetics, self-tanning externa, and non-melanin colouring agents such as canthaxanthine and β-carotinoids are not discussed. Special emphasis is given to the hypopigmentary disorder with the greatest clinical impact – vitiligo – for which a variety of therapeutic options exist.

1. Vitiligo

Vitiligo is one of the most prevalent hypopigmentary disorders. It is estimated to affect between 1% and 2% of the world population, although within different ethnic groups studies have demonstrated an incidence of 0.14–8.8%.[12] In almost 50% of patients, vitiligo starts before the age of 20 years. Depigmented patches may be present in a localised asymmetric form with a focal or segmental (dermatomal) distribution or in a generalised symmetric form with an acrofacial, disseminated or universal distribution.[12] The generalised form has a progressive course predominantly involving areas that are stressed mechanically, for example sites of trauma or sites affected by inflammation, including sunburns (figure 1a and b). The segmental type of vitiligo tends to be more limited and shows a more stable course.[12] In terms of progression and prognosis, vitiligo should therefore be classified into segmental and nonsegmental forms, the latter including the localised, acrofacial, generalised and universal types. The depigmentation corresponds to

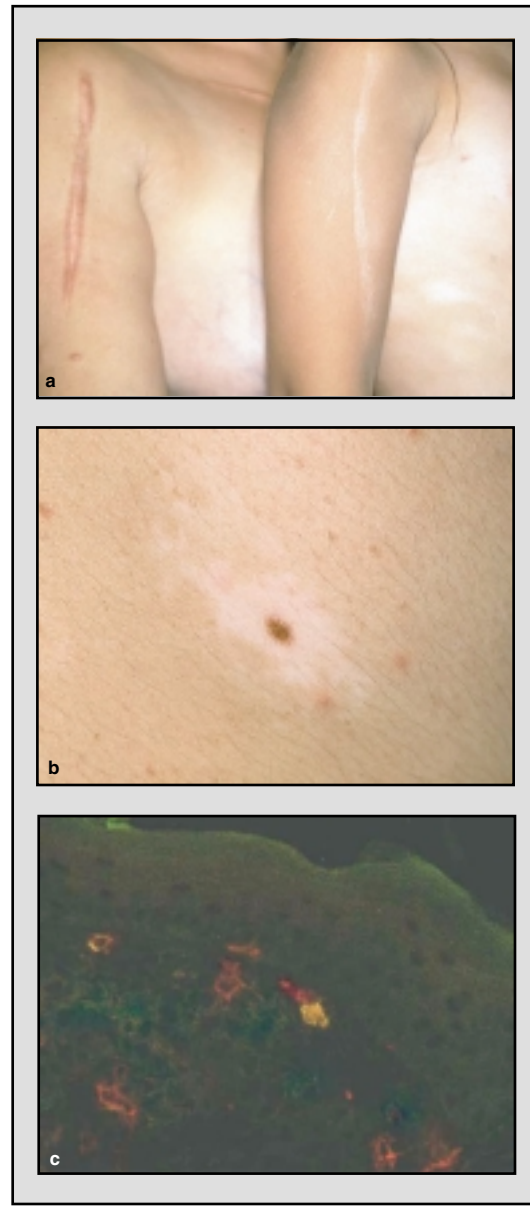

Fig. 1. Examples of acquired hypopigmentary disorders. (**a**) Trichromatic vitiligo induced by the isomorphic effect of scratching. A woman had keloids after an accident. Her 9-year-old daughter started manipulating the corresponding areas on her arm and subsequently developed vitiligo. (**b**) Halo nevus in a 16-year-old patient with generalised vitiligo. (**c**) Fluorescence microscopy to detect cytotoxic T cells (CD8 depicted as red areas) and T-cell receptors specific for the melanocyte differentiation antigen, MART-1 (26-35)/HLA-A2 multimers, depicted as green areas] in the margin of an active vitiligo lesion. **HLA** = human lymphocyte antigen; **MART** = the melanoma antigen recognised by T cells.

a loss of melanocytes in the epidermal compartment and the bulb/infundibulum of the hair follicle. Importantly, the outer root sheath of the hair follicle seems to be uninvolved in most patients and serves as a reservoir of (inactive and amelanotic) melanocytic stem cells.[13] Thus, repigmentation of vitiliginous patches with white hairs is difficult to achieve.

When considering therapy, the pathogenesis of the selective destruction of melanocytes is important. However, it is still not fully understood. Autoimmune mechanisms are supported by the association between vitiligo and autoimmune diseases as well as by the identification of autoantibody-dependent and direct cellular cytotoxicity.[7,8,14,15] Figure 1c depicts melanocyte-differentiation antigen-reactive cytotoxic T cells infiltrating the progressive border of a vitiligo patch. Recently, a possible involvement of keratinocyte- and lymphocyte-derived cytokines activating apoptosis of melanocytes has been assumed.[9-11] Neural mechanisms and viral infections were also postulated.[16,17] The involvement of autocytotoxic/metabolic mechanisms is supported by a defective calcium uptake and an overproduction of pterins in vitiliginous skin; both of the latter inhibit tyrosinase activity.[18,19] As there is accumulating evidence that pterins are synthesised during activation of cell-mediated immunity,[20] the distinction between autoimmune and autocytotoxic/metabolic pathogenesis is starting to recede. Table II presents some therapeutic approaches recommended for vitiligo, according to the disease state and distribution of lesions.

1.1 Phototherapy

1.1.1 Ultraviolet (UV) A Therapy

Over the past 30 years a major body of experience has been accumulated for ultraviolet (UV) A therapy combined with oral and topical photosensitisers, such as 8-methoxypsoralene (PUVA), phenylalanine (PAUVA) or khellin (KUVA).

Although PUVA therapy was the first treatment to be established as efficacious for vitiligo,[21,22] meta-analyses of observations obtained over the past decade showed the frequency of significant or complete repigmentation to be <10%; moreover, no success or even worsening was observed in almost 30% of patients.[23,24] One study showed that at least 1 year of continuous treatment is required with a mean number of 143 treatments, that is an average cumulative dose of between 451 and 729 Joules (J)/cm^2, resulting in 30–90% (moderate to extensive) repigmentation in 60.8% of the patients.[24] Cosmetic results may be unfavourable, with an increased contrast between repigmented areas and heavily pigmented normal skin. Symptoms in 38% of the patients recurred within 6 months of completion of PUVA therapy.[24] Further limitations of this time-consuming procedure include the requirement of eye protection and the occurrence of adverse effects such as nausea or fatigue.[24]

Several strategies have been investigated to improve the therapeutic efficacy of PUVA. To this end, the combination of PUVA with a potent topical corticosteroid proved to be three times more effective than either PUVA or the corticosteroid alone.[25] However, in this study, 29% of the patients withdrew from the evaluation, 17% because of insufficient repigmentation. An option to minimise systemic adverse effects is the use of topical PUVA,

Table II. Therapeutic approaches recommended for vitiligo according to the distribution of lesions and disease state

Disease state	Distribution of lesions	
	localised (focal, segmental)	generalised (acrofacialis, vulgaris, universal)
Active	Locally applied immunomodulatory agents (e.g. corticosteroids), crème-PUVA	UVB 311nm phototherapy, corticosteroid minipulse therapy
Stable	Surgical methods (punch grafts, blister grafts, split-thickness grafts, autologous melanocytes)	Combination of phototherapy (UVB 311nm) and surgical methods (punch grafts, blister grafts, split-thickness grafts, autologous melanocytes)

PUVA = photosensitiser plus ultraviolet A light; **UV** = ultraviolet.

which has been found to be effective in the treatment of localised vitiligo with good safety.[26,27] However, successfully treated patients mostly had darker skin types (skin phototype of III or higher [see table III]), thus having lower UV sensitivity than those with lighter skin pigmentation.[26,27] Moreover, phototoxic reactions on the vitiliginous areas, including blistering and perilesional hyperpigmentation, were frequently observed.[28]

With oral khellin as a photosensitiser, >70% repigmentation can be induced in 41% of patients. However, in one-quarter of the cohort, therapy had to be terminated because of abnormal liver function tests.[29] In an attempt to avoid this adverse effect, topical application of this photosensitiser was tested without success.[30] Thus, KUVA cannot be recommended for vitiligo therapy.

To date, results reported for oral phenylalanine 50–100 mg/kg in combination with UVA have been inconsistent. In an 8- and an 18-month treatment study, dense follicular repigmentation was seen in 26% and >60% repigmentation in 22% of the patients at the end of each study.[31,32] However, these promising results have not been observed by all investigators.[33,34] Hence, this form of therapy still needs further evaluation.

1.1.2 UVB

There is increasing evidence that UVB – either broad-band or narrow-band – is superior to UVA for inducing repigmentation of vitiliginous skin.[35-37] Köster and Wiskemann[35] reported that 75% repigmentation was achieved in 8 of 14 patients after 12 months of broad-band UVB therapy (average cumulative dose of 15.1 J/cm^2). However, 50% of the treated patients were classified with Fitzpatrick skin types IV–VI, and good clinical results were restricted to these patients. The reason for these differences in responses to phototherapy in patients with vitiligo has not been formally addressed yet.

Broad-band 280–320nm PUVB therapy seems to be equally effective as PUVA therapy, with moderate response after 10 weeks' therapy in 50% of the patients on the PUVB side and in 60% of the patients on the PUVA side.[38] Narrow-band 311nm UVB was slightly superior to topical PUVA, with repigmentation (not quantified) in 67% of the patients receiving 4 months' UVB therapy and 46% of the patients receiving PUVA therapy.[36] In a treatment study on children using 311nm UVB over 1 year (a cumulative dose of 91.3 J/cm^2), >75% repigmentation was achieved in 53% of patients.[39] Our own recent results confirm these observations.[40] In this study, narrow-band UVB was shown to be more effective in the treatment of Caucasian patients with vitiligo compared with broad-band UVB. Twenty-two percent of patients showed >75% repigmentation, whereas broad-band UVB had no effect.[40] A case example is presented in figure 2. The applied mean cumulative dose of narrow-band 311nm UVB was 68.6 J/cm^2; the mean single dose at the end of the 12-month therapy period was 1.0 ± 0.19 J/cm^2.

Table III. Characteristics of skin phototypes

Skin phototype	Characteristics
I	Always burns, never tans
II	Usually burns, sometimes tans
III	Sometimes burns, usually tans
IV	Never burns, always tans
V	Moderate constitutive pigmentation
VI	Marked constitutive pigmentation

The percentage of repigmentation reported in the study by Hartmann et al.[40] was less than that reported by Njoo et al.[39] and Scherschun et al.;[37] however, half of the patients in the studies reported by Njoo et al. and Scherschun et al. were of dark skin types, whereas the majority of patients treated in the study by Hartmann et al.[40] had Fitzpatrick skin types less than III. It should be noted that in all patients in the study by Hartmann et al., the active disease progression was stalled. Initial repigmentation was seen after 7–16 weeks (mean 11.8 weeks); best responses were achieved with lesions located on the arms, back, cheeks, buttocks and thighs. Patients presenting with a disseminated type of vitiligo experienced the best response, whereas patients with an acrofacial type or a localised form experienced only minimal or no benefit. Interestingly, in patients with vitiligo vulgaris, vitiliginous patches on the dorsum of hands and feet showed repigmentation, whereas in patients with acrofacial types

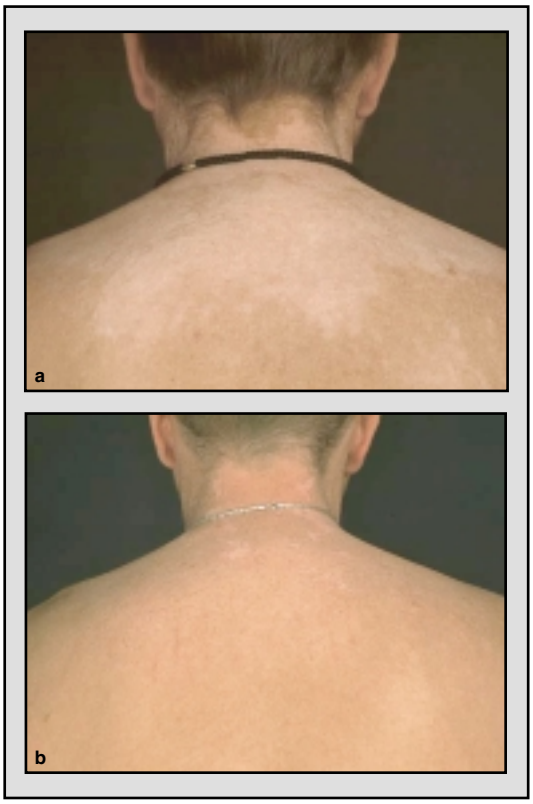

Fig. 2. A 36-year-old patient with progressive vitiligo (**a**) before and (**b**) after 6 months' therapy with narrow-band 311nm UVB.

of vitiligo no repigmentation could be seen in these regions.

In summary, compared with PUVA-therapy, repigmentation induced by UVB light occurs earlier with better cosmetic results and without systemic adverse effects. In addition, as shown by a dose-response model, long-term narrow-band UVB therapy should carry less risk for the induction of skin cancer than PUVA therapy.[41] However, further controlled studies are needed to confirm the efficacy and safety of narrow-band UVB.

1.1.3 Synergistic Drugs (Catalase, Calcipotriol)

The discovery that oxidative stress is likely to be involved in the pathophysiology of vitiligo opened new avenues for therapy. Accumulation of hydrogen peroxide is followed by oxidative degradation of catalase, an important radical scavenger.[18,19] As a result of its ability to remove hydrogen peroxide, pseudocatalase in combination with narrow-band UVB induced excellent repigmentation in 90% of patients. Notably, cosmetically relevant lesions on the face and the dorsum of the hands were successfully resolved.[42] However, these preliminary promising results were not confirmed in a large, controlled clinical trial.[43]

Calcipotriol (a vitamin D_3 analogue) combined with PUVA therapy represents an additional therapeutic strategy for vitiligo. The use of this therapeutic option in vitiligo was discovered following the occurrence of hyperpigmentation around psoriatic lesions when calcipotriol plus PUVA was applied to such lesions.[44] Recent clinical studies suggest that topical calcipotriol combined with sun exposure or PUVA potentiates repigmentation of vitiliginous skin.[45-48] Topical calcipotriol combined with psoralene and sunlight (PUVAsol) three times weekly for 1.5 years showed that there was 75% repigmentation in 76% of the patients receiving active treatment compared with 53% of the control group.[45] However, topical calcipotriol as monotherapy had no effect on vitiligo.[49] Furthermore, calcipotriol in combination with narrow-band UVB had no enhancing effect on repigmentation.[40] Moreover, calcipotriol slowed the process of repigmentation when combined with narrow-band UVB at a dose lower than 0.9 J/cm^2. To this end, both *in vitro* and clinical studies suggest that calcipotriol has photoprotective effects for low doses of UVB and exerts photosensitive effects only with higher doses of UVB.[50-52]

1.1.4 Excimer Laser

The use of xenon chloride XeCl excimer laser, generating UVB radiation at a wavelength of 308nm, has recently been reported to be more effective than narrow-band UVB (311–313nm) for the treatment of patients with localised psoriasis.[53] Since narrow-band UVB is effective in the treatment of vitiligo, whether this UVB laser also offers advantages for patients with leukoderma was investigated. Indeed, preliminary reports have shown that the excimer laser is very effective. In one patient with vitiligo on the elbows, nearly complete repigmentation was achieved after 6 months with UVB

excimer laser (the cumulative UVB dose was 70.8 J/cm2).[54] In patients with periorbital leukoderma, 50–75% improvement was noted after 4–5 weeks with an average cumulative dose of only 1.75 J/cm2.[55] In a recent study in 12 patients with vitiligo, six patients received 12 laser treatments three times a week, which resulted in repigmentation of most lesions.[56] The UVB excimer laser may represent a promising tool for the treatment of vitiligo, particularly in the acrofacial regions, which were hitherto resistant to all forms of conventional treatments or phototherapy.

A major advantage of XeCl excimer laser is the fact that only the vitiliginous lesions are treated, in contrast to phototherapy.[54] However, the exact physical effect of excimer laser radiation on vitiliginous skin has not been investigated. In the UV wavelength spectrum between 240 and 290nm, the major epidermal chromophores are DNA, amino acids and proteins.[57] Excimer laser radiation at a wavelength of 248nm was associated with DNA-damaging effects resulting in cytotoxicity and mutagenicity.[58,59] Therefore, the mutagenic long-time risk of 308nm excimer laser cannot be excluded.

1.2 Immunomodulators

Recent observations have supported the role of cell-mediated and humoral immune responses in the pathogenesis of vitiligo. Evidence for the importance of cell-mediated immunity in the pathogenesis of vitiligo has come from the field of melanoma research, indicating that CD8+ as well as CD4+ T cells play an important role in the destruction of melanocytes. Vitiligo-like lesions have been observed in patients with melanoma following treatment with interleukin (IL)-2 or interferon (IFN)-α (both of which induce T-helper [Th]-1-mediated cellular immunity).[60,61] Immunohistochemical studies revealed the presence of melanocyte differentiation antigen-specific CD8+ T cells in the perilesional sites of active vitiligo lesions (figure 1c).[8,62] On the other hand, uninvolved skin and, presumably also stable vitiliginous lesions, contained mainly CD4+ Th2 cells, associated with strong antibody responses.[9,63,64] On the basis of these findings, several immunomodulatory agents affecting Th1 and/or Th2 immune responses were employed for vitiligo therapy. Figure 3 summarises the assumed pathway of the effects of immunomodulators on vitiligo.

1.2.1 Corticosteroids

Corticosteroids are potent anti-inflammatory and immunosuppressive agents acting via the inhibition

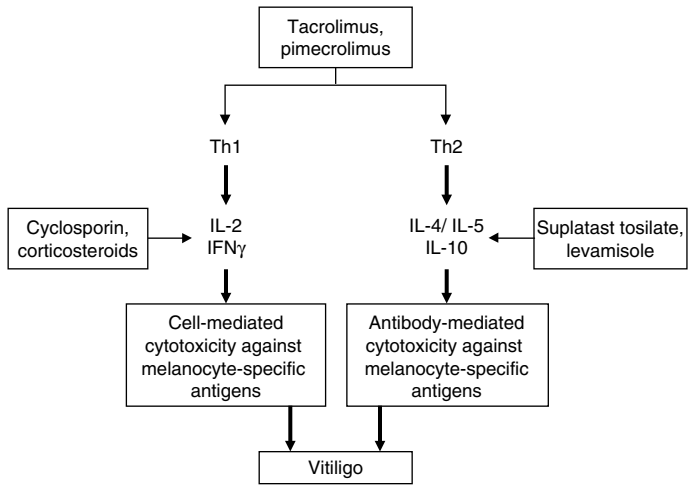

Fig. 3. Effects of immunomodulatory agents on T-helper (Th)-1 and Th2 cytokines, involved in pathogenesis of vitiligo. **IL** = interleukin; **IFN** = interferon.

of a variety of activation-induced gene products and induction of apoptosis of activated T cells.[65,66] The mechanism of action on vitiligo is supposed to be the suppression of direct or antibody-dependent cytotoxicity.[67,68] In a murine model of immune-mediated hypopigmentation, corticosteroids stopped the destruction of melanocytes, thereby allowing repigmentation.[69] Glucocorticoids have also been implicated in the modulation of Th1/Th2 cytokine production, presumably by suppressing type 1 cells and/or by switching Th cells from a Th1 to a Th2 phenotype.[66,70] Topical corticosteroids have been used to treat vitiligo since 1970 with varying results. The induction of marked or nearly complete repigmentation with potent corticosteroids (e.g. betamethasone valerate, triamcinolone) and very potent corticosteroids (e.g. clobetasol, fluticasone propionate) has ranged from 10% to 80%.[71-73] Notably, corticosteroids of low potency showed no therapeutic effect at all.[74]

Corticosteroid-induced repigmentation usually occurs within 1–4 months of treatment. It starts both from the hair follicles and the margins of the vitiligo patches.[71,72] Histological studies reveal that the repopulation of the repigmenting vitiliginous skin is with functional melanocytes.[71] The most favourable results with topical corticosteroids have been seen in children and in patients with dark skin types, lesions localised on the face and neck and lesions lasting <1 month, and when treatment has been applied during the active course of the disease.[72,73,75] The better responses seen in facial lesions may be attributed to the higher permeability of the epidermis in this region, especially in younger individuals, and the higher density of hair follicles. However, after 3–4 months of therapy, adverse effects occurred in the majority of patients. These included dermal atrophy, acneiforme papules, rosacea, teleangiectasia, ecchymoses and striae.[71,73] To minimise the incidence of these adverse effects, it is recommended to apply the corticosteroid once or twice daily for 6–8 weeks, followed by a treatment-free interval of several weeks.[72,73] Great caution should be exercised if corticosteroids have to be applied to the eyelids because of the risk of glaucoma.[72,76] Despite the clinical efficacy and the manageable adverse effects, the therapeutic value of topical corticosteroids is hampered by the limited duration of induced responses; relapses are frequently seen soon after the cessation of treatment.[74,77]

For generalised vitiligo, corticosteroids may be given systemically. For instance, prednisolone can be given continuously in dosages of 15–25 mg/day;[78,79] dexamethasone can be given either orally (i.e. 5–10mg on 2 consecutive days)[80-82] or as high-dose intravenous pulse therapy.[83] In general, systemic corticosteroids seem to be effective only in active disease. While the progression of the disease could be stopped in up to 89% of the patients, those with stable disease experienced no benefit from treatment.[79-81,83] Similarly to topical corticosteroids, initial repigmentation was seen from 2 weeks to 4 months after the start of therapy. However, no correlation could be made between clinical response with systemic corticosteroids and the duration of disease. Adverse effects were reported in up to 69% of the patients, including weight gain, acne, gastrointestinal distress, menstrual disturbance, insomnia and hypertrichosis.[78-81] Despite the fact that systemic corticosteroids have been shown to successfully amend active vitiligo, the serious systemic adverse effects reduce their clinical value; thus, they cannot be regarded as drugs of first choice.

1.2.2 Cyclosporin

Cyclosporin suppresses the activation of T cells and thereby T-cell-mediated melanocyte destruction via the inhibition of IL-2, an autocrine growth factor for T cells.[84] Oral cyclosporin has been successfully used in a variety of inflammatory and autoimmune diseases. Surprisingly, however, it exerts little or no effect on vitiligo.[85] Furthermore, adverse effects were common and included hypertension, renal dysfunction and gastrointestinal dysfunction. Topical cyclosporin preparations are not yet available.

1.2.3 Levamisole

Levamisole is an immunomodulatory agent suppressing Th2-type immune responses via inhibition of IL-4 and, thereby, shifting the immune balance

towards a Th1-type response (figure 3).[86] It was used hitherto in the treatment of leprosy and as adjuvant immunotherapy for colon and breast cancer as well as melanoma.[86,87] To date, its effect on vitiligo has only been tested in one study.[88] Thirty-six Indian patients with vitiligo, the majority (94%) presenting with active disease, received oral levamisole 150mg on 2 consecutive days every week for up to 48 months. Only 7% of the patients treated with levamisole alone showed >50% repigmentation. However, with levamisole given in combination with topical 0.1% fluocinolone acetonide or 0.05% clobetasole propionate, >50% repigmentation could be seen in 37% and 75% of the patients, respectively.[88] The first evidence of repigmentation was observed within 2–4 months. Adverse effects, such as mild nausea and occasional gastric pain, were minimal. Levamisole seems to be an effective (albeit only modestly) drug for limited and slow-spreading disease, with a good safety profile.

1.2.4 Suplatast Tosilate

Suplatast tosilate is an antiallergic agent that acts as a selective inhibitor of IL-4 and IL-5 synthesis *in vitro* (figure 3).[89] In a pilot study in ten patients with vitiligo, suplatast tosilate 300 mg/day stopped disease progression in seven patients and induced repigmentation in three patients.[90] There were no adverse effects associated with suplatast tosilate.

In corticosteroid-dependent patients with asthma (a disease in which Th2 cytokines are involved in the pathogenesis), the dose of corticosteroid was reduced when these patients were treated with suplatast tosilate.[91] Therefore, it is thought that patients with vitiligo may be effectively treated with a corticosteroid in combination with suplatast tosilate; such a regimen may mean that the dose of corticosteroid required to be therapeutic may be lower than that given in monotherapy. This has yet to be investigated.

1.2.5 Tacrolimus and Pimecrolimus

Multiple new immunomodulatory drugs are currently being investigated in several T-cell-mediated dermatological disorders. Topical immunosuppressive agents such as the macrolide lactone derivatives, tacrolimus and pimecrolimus were originally developed for the treatment of inflammatory skin disorders but hold promise as potent drugs for vitiligo therapy as well. Tacrolimus and pimecrolimus inhibit the transcription and release of T-cell cytokines, such as IL-2, IFNγ, IL-4 and IL-5 (figure 3).[92,93] They share many characteristics with cyclosporin in the mode of action, in that they interfere with phosphatase calcineurin. However, their immunosuppressive activity is 10- to 100-fold stronger than cyclosporin and they are suitable for topical application.[92,93] Local or systemic adverse effects are negligible.[92,93] Preliminary results suggest that tacrolimus may be an effective therapy for both localised and generalised vitiligo.[94,95] The twice daily application of 0.1% tacrolimus ointment for approximately 14 weeks led to repigmentation in all treated patients.[94] The degree of repigmentation was moderate to excellent in five of the six patients who received treatment. In a 2-month, double-blind, randomised trial of 0.1% tacrolimus versus 0.05% clobetasol (clobetasol propionate) in 20 children with vitiligo, the mean percentage of repigmentation was 41.3% for tacrolimus and 49.3% for clobetasol.[96] Further controlled trials are mandatory to delineate safety and efficacy.

1.3 Synthetic Melanotropic Peptides

α-Melanotropin (α-MSH) is a peptide hormone with structural homology to adrenocorticotropic hormone (ACTH). It stimulates dendricity of melanocytes and protects them from oxidative stress. Furthermore, it induces enhanced tyrosinase activity and, thus, synthesis melanin – preferentially eumelanin. α-MSH is expressed in the skin by different cell types, including melanocytes, and is reduced in both nonlesional and perilesional skin of patients with vitiligo.[97,98] Increased melanin expression and tanning was induced by subcutaneous administration of a natural and a synthetic analogue of α-MSH (melanotan) in normally pigmenting skin.[99,100] However, vitiliginous areas were unaffected when treated with an intradermal injection of α-MSH.[101] Recently, a sex steroid-thyroid hormone was reported to be a potent drug for the treatment of vitiligo as

a result of its stimulatory effect on melanocyte proliferation and melanin production via α-MSH.[102,103]

1.4 Surgical Therapy

The aim of surgical therapy for vitiligo is a homogeneously repigmented recipient area by transplantation of autologous melanocytes. Surgical techniques include autologous transplantation of minigrafts (punch grafts), transplantation of epidermal blisters induced by suction or kryosurgery (blister grafts), split-thickness grafts, cultured epidermal grafts and cellular grafts (non-cultured melanocytes alone or in combination with keratinocytes). A surgical approach should be considered, if:

- vitiligo lesions have existed for a long time, since the total destruction of melanocytes, including those in the hair bulb, is to be expected;
- <30% of the body surface area is involved;
- lesions have been stable for at least 1 year to minimise the risk of triggering vitiligo at the donor site or loss of pigmentation at the recipient site;
- no history of hypertrophic scars or keloid formation is known.

A predication of the possible clinical course can be obtained by the use of minigraft tests, that is implanting two or three punch biopsies of 2mm diameter in the lesions to be grafted. This makes it possible to evaluate the spread of pigmentation at the recipient site and the risk for triggering vitiligo at the donor site.[104,105]

1.4.1 Punch Grafts

Punch grafts are performed as full-thickness grafts with 1.5–3mm punch biopsies of 0.6–0.8mm thickness obtained from normal pigmented skin of either the medial aspect of the arm, the gluteal region or the thigh. Excess adipose tissue should be carefully trimmed before transplantation.[106] These grafts are implanted into perforations prepared at the recipient site by means of either a biopsy punch as well or a pulsed Erbium **:** Yttrium-Aluminum-Garnet (YAG)-laser.[107] Haemostasis at the recipient site should be achieved with saline-soaked cotton.[108] The recipient holes should be either equal in size or up to 0.5mm smaller in diameter than the grafts and, as melanocytes seem not to be able to migrate beyond 5mm, the grafts should be placed 4–8mm apart from each other.[108,109] After transplantation, the recipient site is covered with a paraffin-embedded gauze or with a transparent adhesive tape for 1–2 weeks.[110]

Pigment spread will occur gradually. The first signs of repigmentation of skin adjacent to the graft appears after between 2 and 6 weeks, and the maximum spread of repigmentation is reached within 6 months.[110] Repigmentation can be facilitated by exposing the treated area to sunlight or UVA.[108,109] Repigmentation of 90–100% has been achieved in 39%, 74% and 86% of patients, respectively, in three independent studies.[108-110] However, in 10% of the transplantations no spread of pigmentation may occur even after a positive minigraft.[107,108] The major advantage of this approach is that it can be easily performed without special surgical equipment, but the method is time consuming and complications are frequent, including spotted pigmentation, polka dot appearance (47.3% of patients), colour mismatch (34.3% of patients) and cobblestone appearance (between 27% and 32% of patients) of the recipient site.[111] Regrafting between the initial punch grafts may be required to achieve acceptable results.[108,111] The risk of a cobblestone appearance increases with the size of the punch biopsies[110,112] but can be reduced by trimming the adipose tissue from the grafts and ensuring that the recipient holes are about 1mm deeper than the thickness of the graft.[109] Initial hyperpigmentation is frequently observed, especially when PUVA or PUVAsol is administered after transplantation; however, it disappears gradually with time.[108,112]

1.4.2 Blister Grafts

This modality, first reported by Falabella,[113] relies on the transplantation of the epidermal site of artificially induced autologous blisters. Blisters can be induced from 3 hours up to 2 days before transplantation either with a vacuum pump, with liquid nitrogen applied for 3–5 seconds with three to six freeze-thaw cycles, or with sterilised syringes at a sustained negative pressure of approximately 200–400mm Hg for 30–180 minutes.[107,114,115] The

blister size can be up to 6cm in diameter,[106,116] and the mechanical split will be exactly at the dermoepidermal junction.

The medial aspect of the arm, the forearm, the thigh, the abdomen and the buttock are the preferred donor sites. The recipient site is prepared by dermabrasion, laser ablation (erbium : YAG- or ultrapulse carbon dioxide laser), or using a dermatome or by creating a blister.[107,116,117] The grafts are carefully removed from the donor site by cutting the top of the bullae with sharp scissors, are transferred using a glass slide, and are covered with an antibacterial ointment before being transplanted. The blister graft is secured with an adhesive tape or strip for 1 week. Donor and recipient sites usually heal within 8–10 days. Since the dermis is not injured, the donor site heals without scar formation.

Repigmentation occurs gradually within 3–4 weeks after transplantation, with the spread of pigmentation beyond the grafted area within 3–4 months. The success rate of epidermal grafting varies depending on the clinical appearance and the localisation of the vitiligo lesions. Excellent cosmetic results with >95% repigmentation have been seen in 50–88% of patients with segmental and localised vitiligo.[114,115,117] Vitiligo involving the face, especially the forehead, responded best; eyelids, lips, alae nasae, neck, axillae and skin over bony prominences are difficult to treat.[114] Repigmentation is enhanced by PUVA treatment, either before or after the grafting procedure.[114] Long-term observation of up to 5 years in accordingly treated patients has indicated that repigmentation obtained by this method is permanent.[114] The only adverse effect of this method is transient hyperpigmentations both at the donor and the recipient site.[117] The limitations of this technique relate to the fact that the grafts are very fragile, tear easily and are, therefore, difficult to handle, and the technique is also time consuming.[106] Furthermore, the maximal area of epidermal sheets that can be transplanted in one procedure is about 7×7 cm^2; hence, repeated procedures may be needed to treat large vitiliginous lesions.[114]

1.4.3 Split-Thickness Grafts

With split thickness skin grafts, areas of up to 190 cm^2 and/or multiple lesions may be covered at one time, giving immediate results.[111,118] A thin Tiersch graft of 0.1–0.2mm thickness is obtained by means of an electric dermatome. The recipient area can be conditioned as described for blister grafts (see section 1.4.2). The grafted sheets are placed close to each other with a slight overlap, fixed with adhesive strips or tape, and covered with a saline-moistened gauze or sterile adhesive foil. Transplanted areas have to be immobilised for 1 week to prevent local wrinkling and graft dislocation.[106] Although grafts always appear pink for the first few months, good to excellent results with >80% repigmentation have been reported in 50–80% of the transplanted areas.[118-120]

The clinical value of split-thickness grafting is restricted by the formation of miliae-like cysts at the recipient site, particularly on the face and neck, which may occur between 2 and 4 months after the procedure. This is likely to be caused because the transplanted epidermis occludes sweat glands. The cysts either disappear spontaneously or can be resolved by scratching. Partial loss of the grafts, haematoma formation, and thickening of the graft margins were described in 33% of patients.[111] Scarring of the donor site was reported in 7–16% of patients.[120] Thicker grafts with dermal fractions result in a stuck-on appearance.[106] Repigmentation rates were significantly higher with the split-thickness graft technique (90% repigmentation) compared with the suction blister techniques (25–65% repigmentation).[121]

1.4.4 Cultured Epidermal Grafts, Cultured Melanocytes and Melanocyte Suspensions

The major advantage of cultured grafts is the possibility of treating large areas with autologous cells obtained from small biopsies[122-126] and the possibility of cryostorage of the cells.[122]

The *in vitro* expansion of epidermal cells for therapeutic reasons was first introduced in 1975 by Rheinwald and Green[127] and was initially employed for the treatment of burn patients. After the separation of epidermis and dermis, the epidermis is

seeded in a medium that sustains cultivation of both keratinocytes and melanocytes. After 2–3 weeks, an epidermal sheet graft is obtained, which allows coverage of areas up to 240 cm^2.[128,129] The recipient sites are prepared according to the guidelines used for other transplantation techniques. Pigmentation of treated areas is usually observed 1 month after transplantation. Hyperpigmentation may occur during the first 3–6 months; however, it fades thereafter.[128,129] Satisfactory results are achieved within 3–12 months after grafting in 30–44% of patients.[128-130] However, acrofacial vitiligo, especially lesions on the dorsal aspects of the fingers, feet or joint areas, are hard to resolve. Disadvantages include the difficult handling of the fragile and delicate skin and the susceptibility to shear forces. The latter is well documented for cultured sheet grafts used in burn surgery.[131] Notably, melanocytes are depleted with serial cultivation and are preserved only in the early passages.[130] To overcome this limitation, the reconstruction of pigmented epidermis by integration of melanocytes in the basal layer *in vitro* was successfully introduced but is still in an experimental stage.[132,133]

Consequently, special emphasis was given to the expansion of isolated melanocytes. In 1987, Lerner et al.[134] were the first to transplant suspensions of autologous cultured melanocytes into a suction blister of depigmented skin. In the following years, different culture conditions and grafting devices were developed such as collagen or hyaluronic acid membranes, or syringes containing fibrinogen. A milestone was achieved when Olsson and Juhlin[135] in 1992 introduced a culture medium for melanocytes free from phorbol esters, pituitary extract and serum. After 3–8 weeks of culture, cell densities ranged from 5×10^6 to 50×10^6 melanocytes/100 cm^2. Thus, areas up to 500 cm^2 may be transplanted during one session.[123,124,134,136] Repigmentation occurs within 4–6 weeks. Initial hyperpigmentation is common, especially for lesions on hands and feet and in people with a darker skin type. However, within 6–8 months, transplanted areas acquire the same colour as the surrounding skin.[125] Optimal colour match and >95% repigmentation in approximately 40% of the treated areas was reported when seeding melanocytes in a density ranging from 7×10^6 cells/100 cm^2 to 15×10^6 cells/100 cm^2.[124,125] While areas on the trunk, face and dorsal aspect of the hands generally show a good to excellent result, fingers and elbows remain problem areas as for all other transplantation methods.[124,125]

2. Piebaldism

Piebaldism is a rare autosomal-dominant genetic disorder, characterised by congenital patches of white skin (leukoderma) and white hair. The most specific clinical feature is a white forelock (poliosis). The leukodermal patches may involve the ventral chest and the abdomen, the upper arms and lower legs. Unlike vitiligo, in piebaldism the depigmented symmetrical patches are present at birth and are static in shape and size. Occasionally, however, increased pigmentation at the margins and islands of hyperpigmentation within the leukodermal patches have been reported.[137] The heterogeneous distribution of pigmentation is caused by defective proliferation and migration of neural crest-derived melanoblasts during embryogenesis.

In human studies addressing piebaldism, several heterozygous point-mutations and deletions of the *c-kit* proto-oncogene were demonstrated, resulting in a graded series of piebald phenotypes.[138,139] This mutation results in a reduced KIT receptor protein expression. The KIT receptor (mast/stem cell growth factor receptor) is a member of the tyrosine kinase receptor family and is expressed on the surface of melanocytes. It is required for the normal development of dermal melanocytes.[140] Reduced expression results in an abnormal distribution of melanoblasts and decreased proliferation during embryologic development. The spontaneous appearance of pigmented islets within hypomelanotic macules as well as the presence of melanocytes in the depigmented areas has enhanced the interest in developing therapies for piebaldism. Oral and topical methoxalen plus UVA were successful in inducing new hyperpigmented spots within piebald lesions, however, the cosmetic effect was largely unsatisfactory.[137,141] Surgical approaches seem to be more

promising in repigmenting localised piebald lesions. Cultured epidermal autografts and split-thickness skin grafts, either alone or in combination with autologous minigrafts, as well as melanocyte suspensions proved to be successful. To this end, complete or nearly complete repigmentation was achieved in all accordingly treated patients. Notably, the piebald lesions treated involved a sizeable area of up to 1000 cm^2.[118,120,134,142]

3. Idiopathic Guttate Hypomelanosis

Idiopathic guttate hypomelanosis (IGH) was first described in 1951 as 'symmetric progressive leukopathy of the extremities' and is a common acquired pigmentary disorder with a high prevalence among patients with dermatological disorders.[143-145] It is characterised by multiple achromic or hypopigmented, irregularly shaped cutaneous macules of up to 8mm in diameter, primarily located on the legs and forearms. The number of lesions increases with age and spontaneous repigmentation has never been reported. The aetiology and pathogenesis are unknown, but familial aggregation indicates a genetic predisposition. Since sun-exposed areas are preferentially involved, actinic damage may also be a causal factor.[146] On the other hand, the detection of parietal cell antibodies in 30% of patients suggests an autoimmune mechanism.[147] Histological analysis demonstrated a decreased number of melanocytes, which are less dendritic and contain fewer melanosomes. These melanocytes also display a reduced tyrosinase activity. Thus, a marked reduction of melanin pigment of the keratinocytes was obvious.[146,148-150] Experimental grafting of skin from IGH lesions into normal epidermis induces centrifugal depigmentation of the skin surrounding the grafted area suggesting an active depigmentation process.[146]

Treatment of IGH is difficult. Intralesional triamcinolone 2 mg/mL, which was injected once monthly over 3 months, produced a certain degree of repigmentation in most patients, but a good response was seen in less than half of the patients.[146] Notably, an excellent response was achieved in 11 of 15 patients if triamcinolone was combined with autologous minigrafts.[146] Minigrafts alone in the aforementioned study did not modify the achromic defects. In contrast, autologous blister grafts seem to be a very effective treatment option.[146,151] In another study, IGH lesions were treated with liquid nitrogen for 10 seconds; this measure resulted in complete repigmentation within 6–8 weeks after treatment. Interestingly, the repigmented IGH lesions contained significantly more dopa-positive melanocytes than untreated lesions, probably as a result of the migration of melanocytes from adjacent normal skin.[149]

4. Post-Inflammatory Hypopigmentation

Post-inflammatory hypopigmentation is a frequent sequelae of various cutaneous inflammatory diseases. In addition, endocrinological, physical and chemical factors may cause this condition. Prolonged use of potent corticosteroids, chemical peels and surgical interventions such as dermabrasion, cryotherapy or laser ablation are among the most frequent causes. Post-inflammatory hypopigmentation usually appears in the form of hypochromatic or achromic maculae. Their configuration and distribution reflects the affected area of the respective trigger. Clinically, post-inflammatory hypomelanosis may be distinguished from other hypomelanotic dermatoses, such as vitiligo, by the presence of feathered margins. The underlying mechanisms developing hypo- or hypermelanosis are not well understood. Some authors proposed an 'inherited individual chromatic tendency' of melanocytes to respond to inflammation or trauma, with either decreased or increased melanin production.[152] Hence, the same trigger can lead to hyperpigmentation in one and hypopigmentation in another individual. However, severe inflammation or trauma leads to a variable degree of acquired hypopigmentation and depigmentation as a result of destruction of melanocytes.

Treatment of acquired hypopigmentation remains a challenge. Slightly hypochromatic macules such as those induced by inflammatory diseases (e.g. atopic dermatitis, pityriasis versicolor) are the result of altered melanin production and usually

disappear spontaneously within a few weeks or months. Achromic macules induced by severe inflammatory diseases (e.g. lupus erythematosus, scleroderma), severe trauma (e.g. second- and third-degree burn injury) and toxic chemicals (e.g. monobenzylether of hydroquinone, 4-tertiary butylcatechol, p-phenylendiamine, p-tertiary butylphenol) may be permanent because of irreversible destruction of melanocytes.[153] Sun exposure or exposure to UVA or UVB light as well as PUVA therapy may lead to repigmentation within months of therapy if melanocytes in the affected areas are still functional.[152,153] In achromic leukoderma with total loss of melanocytes, surgical treatments seem to be the only effective therapeutic options. Indeed, reported results of surgical treatments are excellent with satisfactory repigmentation in all of the transplanted patients soon after grafting.[142,151]

5. Future Directions – Gene Therapy and Tissue Engineering

Insights into the pathophysiology of hypopigmentary disorders and the emerging fields of gene therapy and tissue engineering are opening new therapeutic avenues. Specific genes are sequentially activated in melanocytes as they migrate from their embryonic origin. Further on in development, melanin content is regulated by a close interplay between melanocytes and keratinocytes. Factors released by keratinocytes control the proliferation and phenotypic behaviour of melanocytes (e.g. dendricity and melanisation), thereby controlling epidermal pigmentation.[154] A mutation in genes encoding these factors may disrupt this process, resulting in hypopigmentation. Most potent factors in this respect include basic fibroblast growth factor (FGF) and endothelin-1, which stimulate melanocyte proliferation and tyrosinase activity as well as hepatocyte growth factor/scatter factor or stem cell factor (SCF/KIT ligand).[155-158]

Basic FGF, endothelin-1 and SCF are primarily found in close proximity to highly pigmentary active melanocytes. They are actually released during photochemotherapy as well as during wound healing following dermabrasion or autologous grafting.[156] On the contrary, in vitiligo lesions a significantly lower expression of basic FGF and SCF was found, whereas that of IL-6 and tumour necrosis factor (TNF)-α was rather strong[159] (IL-1α, IL-6, TNFα and transforming growth factor-β are paracrine inhibitors of melanocyte proliferation and melanogenesis).[159] Therefore, it has been speculated that the substitution or induction of melanocyte stimulating growth factors, by local application, gene therapy or tissue engineering, may be a promising tool to treat hypopigmented skin disorders.

Gene therapy relies on the introduction of exogeneous DNA into a host cell to cause a therapeutic benefit. Both sustained expression or inhibition (e.g. by antisense technology) of proteins/growth factors in the target tissue can be called upon.[160] Keratinocytes are well suited as target cells for gene transfer, since their *in vitro* culture is well established.[127,161] Employing the epidermis as target tissue is also advantageous because of easy access and the ability to monitor the effect. Moreover, the potential to transduce epidermal stem cells minimises the number of treatments and, thereby, increases cost effectiveness.[162] There are two approaches of gene delivery, *ex vivo* and/or *in vivo*.

The *ex vivo* approach permits the introduction of genetic material directly into most cell types, which can be cultured. The transduction is performed by electrophoresis, lipofection, calcium phosphate or viral-mediated gene transfer. Subsequently, the cells are transplanted back into the donor. It offers the possibility to select and separate cells (e.g. stem cells) and to directly control transduction/transfection efficiency. However, the technical requirements limit this option to specialised centres. For *in vivo* gene transfer, the genes are delivered directly into the target tissue, which obviates the need for cell culture. However, direct gene transfer methods (e.g. lipofection, gene gun, receptor-mediated delivery vectors) are hampered by relatively low transfection efficacy.[163,164] A possible exception is the topical application of viral vectors or particle-mediated gene transfer in skin wounds.[165-167]

A number of clinical trials are currently evaluating gene therapeutic approaches for various inherit-

ed and acquired skin diseases.[164] An example is phenylketonuria: transfer of phenylalanine hydroxylase cDNA by a replication defective adenovirus leads to normalised phenylalanine serum levels and reversal of hypopigmentation.[168] Tissue-engineered skin is an attractive vehicle to deliver genetically modified epidermal cells. Skin constructs obtained by assembling keratinocytes alone or in combination with fibroblasts together with different extracellular matrix molecules were successfully used to cover burn injuries, acute and chronic wounds.[161,169,170] Recently, the introduction of active melanocytes into epidermal reconstructs has been reported.[132,133] Nevertheless, the construction of tissue-engineered pigmented skin is still in an experimental stage.

6. Conclusions

Hypopigmentary disorders, though not life threatening, nevertheless have a strong impact on the quality of life of affected individuals. A better understanding of the aetiology and pathogenesis at the molecular and cellular level is likely to improve already existing treatment options and may open new therapeutic avenues. This is important since none of the currently established therapies are able to produce satisfactory cosmetic results. Transplantation techniques using cultured melanocytes seem to be the most promising procedures to repigment large hypopigmented areas, and the possibility of cryopreserving the cells allows subsequent transplantation if needed. Moreover, *in vitro* cultured melanocytes provide the means to deliver genetic modifications designed to improve repigmentation.

Acknowledgements

The authors wish to express their appreciation to Henning Hamm, Würzburg, and Uta Hofmann, Düsseldorf, Germany, for fruitful discussions. Dr Becker was supported by the Deutsche Krebshilfe grant #10-1845-BeI), the Deutsche Forschungsgemeinschaft (grant #BE 139415-3) and the Wilhelm-Sander-Stifting (grant #2000.033.2).

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