Innovative Use of Pulsed Dye Laser and Liposomes in Dermatology

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Innovative Use of Pulsed Dye Laser and Liposomes in Dermatology

Innovatieve toepassingen van Pulsed Dye Laser en liposomen in de dermatologie

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CONTENTS

Innovative use of Pulsed Dye laser and Liposomes in Dermatology

Jaap de Leeuw

Chapter 1
Introduction

1.1 General considerations

An important issue in medicine is that the efficacy, the safety and the costs of medical treatment modalities are appropriately in concordance. Patient safety should be the dermatologist's number one priority, especially in the treatment of non-life threatening diseases such as acne, vitiligo and psoriasis. In dermatology, intra-patient studies (right-left studies) provide an ideal means to evaluate the efficacy of topical treatment modalities. Double-blind, crossover studies have proven their benefit in systemic treatment. In addition to the observations by physicians, the patient's quality of life studies became indispensable to determine the overall efficacy of the treatment. Assessing the costs of medical treatment is more complex. The costs of treatments are not only determined by the price of drugs, medical apparatus and duration of treatments, but also by the reduction of patient's working hours, travel costs, and, last but not least, the costs of treating side effects of medication. Physicians and patients are both increasingly aware of the risks of medical interventions.

Primum non nocere (nonmaleficence) is a Latin phrase, imputed to Hippocrates, means "First, not to harm". It reminds the physician and other health care providers that they must consider the possible harm that any medical intervention may inflict and that human acts with good intentions may have unwanted consequences. A closely related phrase is "Sometimes the cure is worse than the illness." The situation in which a subject has a complication of treatment that is more severe than the disease for which the treatment was been given must be avoided. The dictum *primum non nocere* is not necessarily the guiding principle in cancer therapy. The goal of cancer treatment is first to eradicate the cancer. Primum non nocere would therefore, theoretically, give way to *primum succurrere* - "first, hasten to help". This implies that successful treatment of skin cancer requires the detection and the diagnosis at an early stage, so that it can be cured with minimal surgical complications. Many patients all over the world suffer from psoriasis, acne, vitiligo, actinic keratosis and non-melanoma skin cancer (NMSC). Although seldom life threatening, these disorders of the skin are important considering the burden imposed on the quality of life of the sufferer, the costs of the treatment, the loss of productive time caused by treatments and the side effects of the treatments.

There is currently no cure for psoriasis, although a wide range of therapies are available, varying considerably in terms of efficacy and toxic effects.[1] The level

of compliance to treatments in patients with psoriasis is low, with reported non-compliance in 40% of the patients.[2,3] Factors such as efficacy[2,4] and the duration of the treatment can influence the decision of whether to continue with treatment.[3] Factors such as adverse effects, time to improvement and relapse influence the choice of treatment by patients with psoriasis. Most patients would be willing to try out different aspects of the treatment to achieve an improvement in their psoriasis with a minimum of adverse effects. In other words, patients may indicate that they would wait longer for a treatment to work if the chances of severe adverse effect such as skin cancer and liver damage were considerably reduced. Patients consider the long-term risks of skin cancer and liver damage to be the most important adverse effects and are prioritized above short-term risks of drug-induced hypertension or skin irritation.[5]

Vitiligo poses no threat to a patient's life, although the de-pigmented areas are susceptible to actinic damage. On the one hand, meticulous sun protection is imperative to prevent unfavorable consequences of sun damage such as phototoxic dermatitis and skin cancer. On the other hand, phototherapeutic approaches are most effective in the treatment of vitiligo.[6] Long-term treatments with UVB and PUVA induce accelerated photo-aging, cutaneous and systemic immunosuppression[7] and an increased risk of skin carcinoma.[8,9] The therapeutic use of immunosuppressive drugs is based on the hypothesis that vitiligo is an autoimmune disorder and their effectiveness supports it. Corticosteroids may be applied topically[10] or administered systemically.[11-13] Cyclophosphamide[14], 5-fluouracil[15] and levamisole[16] have been used to treat vitiligo by modifying the immune response with varying therapeutic efficacy. Severe side effects, which may occur with systemic immunosuppressive regimens, require careful analysis of the benefit–risk ratio. Potential serious side effects caused by long-term systemic steroids and immunosuppressive agents may not justify their use because vitiligo is a pigment disorder.[6] Recently, non-steroidal topical immunosuppressive therapy with calcineurin inhibitors (tacrolimus and pimecrolimus), has been introduced.[17-19] Their anticipated improvement in the risk–benefit ratio as compared with the ratio of steroids and other immunomodulators has not yet been established.[20] Acne vulgaris is a multi-causal disorder of the pilo-sebaceous glands. Four major factors are responsible for the development of acne lesions. These include abnormal desquamation of the follicular epithelium causing the obstruction of the pilo-sebaceous canal, the stimulation of sebaceous secretion by androgens at puberty, the presence and the growth of bacteria and the subsequent attraction of lymphocytes and leukocytes resulting in an inflammation. The inflammatory component of acne lesions is usually associated with the proliferation of Propionibacterium (P.) acnes in the follicular unit. Standard treatment today consists of peeling the skin and reducing P. acnes and inflammation by using

topical benzoylperoxide gel, azelaic acid cream, tretinoin cream, adapalene gel and topical antibiotics. Systemic antibiotics and anti-androgens (in female patients) are used when this approach is not effective. However, the efficacy of antibiotic treatment is progressively reduced by the increasing resistance of the bacteria to the used antibiotics. Side effects may also limit their use. When other treatments fail, the final remedy is oral isotretinoin, which is very effective, even in recalcitrant acne, but its use is even more limited by moderate to serious side effects. Photodynamic therapy (PDT) may be an alternative treatment for acne, but the lowest possible light dose should be used in order to minimize the side effects.[21]

There is a discrepancy between therapists on their goals of treatment. On the one hand, therapists may aim for a rapid and a sustained control of the disease process, irrespective of the adverse events. On the other hand, therapists may aim at an improvement in the quality of life with minimal side effects irrespective of the duration of improvement or a lack of complete control of the disease. Complete clearance of skin lesions is often not a realistic goal.[22] Generally, most patients will be willing to try out different aspects of the treatment to achieve an improvement in their skin disease with minimal adverse effects. Consultation with patients and evaluation of their goals are an important part of the therapeutic process. Potent treatments may provide more complete and prolonged clearance of widespread disease, but also have a higher risk of serious side effects, which must be considered when choosing a treatment strategy.[23]

The investigations described in this thesis were pursued in order to optimize the efficacy and the safety profiles of different therapeutic modalities for treating psoriasis, vitiligo, acne and non-melanoma skin cancer (NMSC).

1.2 Aims of the investigations described in this thesis were two-fold:

I. To optimize the risk-benefit ratio in the treatment of plaque type psoriasis by using Pulsed Dye Laser (PDL) therapy.
   a. An evaluation of the efficacy and the safety of PDL therapy.
   b. A comparison of the results of PDL therapy with those of Ultra-Violet B (UVB) treatment, which is the standard photo-therapy for psoriasis.
   c. An assessment of the putative synergism of the combined PDL- and UVB therapy.
   d. An investigation into the histopathological changes in psoriatic lesions at various time intervals after PDL therapy.

II. To evaluate the effect of enhanced penetration of drugs into the skin using liposomes as carriers for water- and fat soluble drugs.

a. An assessment of the efficacy and the side effects of Khellin encapsulated in liposomes in the treatment of vitiligo.

b. An assessment of the efficacy and the side effects of 5-aminolevulinic acid 0.5% (5-ALA) encapsulated in liposomes in the treatment of acne.

c. An evaluation of the quality of fluorescence detection of non-melanoma skin cancer at an early stage using 5-aminolevulinic acid 0.5% encapsulated in liposomes in combination with a specialized computerized detection and visualization system.

1.3 References

1. Fairhurst DA, Ashcroft DM, Griffiths CEM. Optimal management of severe plaque form of psoriasis. Am J Clin Dermatol 2005; 6: 283-294.
2. Richards HL, Fortune DG, Griffiths CEM. Adherence to treatment in patients with psoriasis. J Eur Acad Dermatol Venereol 2006; 20: 370-379.
3. Van de Kerkhof PC, De Hoop D, de Korte J, Cobelens SA, Kuipers MV. Patient compliance and disease management in the treatment of psoriasis in the Netherlands. Dermatology 2000; 200: 292-298.
4. Zaghoul SS, Goodfield MJ. Objective assessment of compliance with psoriasis treatment. Arch Dermatol 2004; 140: 408-414.
5. Seston EM, Ashcroft DM, Griffith*s* CEM. Balancing the benefits and risks of drug treatment. A stated-preference, discrete choice experiment with patients with psoriasis. Arch Dermatol. 2007; 143: 1175-1179.
6. Ortel B, Petronic-Rosic V, Calzavara- Pinton P. Phototherapeutic options of vitiligo. Dermatological Phototherapy and Photodiagnostic methods. Jean Krutmann et al. (Eds.), Springer 2009; chapter 7: 151-183.
7. Beissert S, Schwarz T. Mechanisms involved in ultraviolet light-induced immunosuppression. J Invest Dermatol Symp Proc 1999; 4: 61–64.
8. De Gruijl FR. Skin cancer and solar UV radiation. Eur J Cancer 1999; 35: 2003–2009.
9. Van der Leun JC. UV-carcinogenesis. Photochem Photobiol 1984; 39: 861–868.
10. Kumari J. Vitiligo treated with topical clobetasol propionate. Arch Dermatol 1984; 120: 631–635.
11. Kim SM, Lee HS, Hann SK. The efficacy of low-dose oral corticosteroids in the treatment of vitiligo patients. Int J Dermatol 1999; 38: 546–550.
12. Pasricha JS, Khaitan BK. Oral mini-pulse therapy with betamethasone in vitiligo patients having extensive or fast-spreading disease. Int J Dermatol 1993; 32: 753–757.
13. Radakovic-Fijan S, Fürnsinn-Friedl AM, Hönigsmann H, Tanew A. Oral dexamethasone pulse treatment for vitiligo. J Am Acad Dermatol 2001; 44: 814–817.
14. Gokhale BB, Parakh AP. Cyclophosphamide in vitiligo. Indian J Dermatol 1983; 28: 7–10.
15. Szekeres E, Morvay M. Repigmentation of vitiligo macules treated topically with Efudix cream. Dermatologica 1985; 171: 55–59.
16. Pasricha JS, Khera V. Effect of prolonged treatment with levamisole on vitiligo with limited and slow-spreading disease. Int J Dermatol 1994; 33: 584–587.

17. Coskun B, Saral Y, Turgut D. Topical 0.05% clobetasol propionate versus 1% pimecrolimus ointment in vitiligo. Eur J Dermatol 2005; 15: 88–91.
18. Kostovic K, Pasic A.. New treatment modalities for vitiligo: focus on topical immunomodulators. Drugs 2005; 65: 447–459.
19. Silverberg NB, Lin P, Travis L, Farley-Li J, Mancini AJ, Wagner AM, Chamlin SL, Paller AS. Tacrolimus ointment promotes repigmentation of vitiligo in children: a review of 57 cases. J Am Acad Dermatol 2004; 51: 760–766.
20. Hultsch T, Kapp A, Spergel J. Immunomodulation and safety of topical calcineurin inhibitors for the treatment of atopic dermatitis. Dermatology 2005; 211: 174–187.
21. Hörfelt C, Funk J. Frohm-Nilsson M, Wiegleb Edström D, Wennberg AM. Topical methyl aminolaevulinate photodynamic therapy for treatment of facial acne vulgaris: results of a randomized, controlled study. Br J Dermatol 2006; 155: 608-613.
22. Al-Suwaidan SN, Feldman SR. Clearance is not a realistic expectation of psoriasis treatment. J Am Acad Dermatol 2000; 42: 796-802.
23. Lebwohl M. A clinician's paradigm in the treatment of psoriasis. J Am Acad Dermatol 2005; 53: S59-69.

Chapter 2

Basic principles

2.1 *Sunlight and photobiology*

Without sunlight there would have been no organic life on our planet

Photobiology is the study of the effects of non-ionizing radiation on living systems and reflects all types of interaction of light with micro-organisms, plants, animals and human beings.[1] Sunlight provides the majority of the energy on earth. All living organisms require solar energy (electromagnetic radiation) in an assimilated and utilizable chemical form for the metabolic activities associated with growth and development.[2] Photosynthesis is the photochemical reaction whereby plants use chlorophyll to convert carbon dioxide and water into oxygen and energy-rich carbohydrates. These stored carbohydrates are ingested by herbivorous animals. Carnivorous animals in turn acquire photo-synthetically produced energy indirectly by feeding on plant-eating animals that have stored the ingested chemical energy in their tissues (food chain).[2] Although in The Holy Bible Genesis I is stated that God said "Let there be light and there was light, and God saw that it was good", sunlight in fact has a range of positive, but also negative biologic effects. (Table 1).[3] Two major positive physical properties of the sun are its light and its warmth. Light (visible radiation) provides the capacity to see, solar heat (infrared radiation) is responsible for the planet earth being inhabitable. Warmth is of great comfort to human beings and accounts for the positive values, such as "warm feelings". Worship of the sun as a religious symbol of strength, authority and majesty can be traced back to the beginnings of human history (Table 2). Photo-medicine involves diagnostic and therapeutic photo-biology.[1]

Electromagnetic radiation

Electromagnetic (EM) radiation is defined by the wavelength (λ) and the frequency (v). Wavelength is the distance between two corresponding points on each successive wave (Figure 1). Frequency is the number of waves that passes a point per second. The product of the wavelength and frequency λ x v was shown by Einstein to be constant and equal to the speed of light in a vacuum = 3 x 10^8 meters/second = 300,000 km per sec. Electromagnetic radiation is classified as a sequence of increasing wavelengths into cosmic rays, gamma rays, X-rays, ultraviolet light, visible light, infrared light, microwaves

and radio waves (Figure 2). Ultraviolet (UV) C of wavelengths between 200-280 nanometer (nm) does not reach the earth because it is absorbed by the ozone layer (thickness 3 mm) in the stratosphere.[2]

The UV radiation spectrum lies between the wavelengths of 100 nm and 400 nm according to the international agreement. The Commission Internationale de l'Eclairage (CIE) adopted the following general definitions[4]:

Ultraviolet C (UVC) = 200-280 nm
Ultraviolet B (UVB) = 280-315 nm
Ultraviolet A (UVA) = 315-400 nm, subdivided into UVA2 315-340 nm and UVA1 340-400 nm.

Alternative definitions in use for UV radiation are[4]:

Ultraviolet C (UVC) = 150-280 nm/100-290 nm
Ultraviolet B (UVB) = 280-320 nm/290-320 nm
Ultraviolet A (UVA) = 320-400 nm/320-410 nm

Longer wavelengths are divided into:

Visible light = 400-800 nm
Infrared light (heat) = 800-17000 nm
Microwaves and radio waves >17000 nm.

Wavelengths of shorter than about 200 nm propagate only in a vacuum and are not relevant in phototherapy. Very short wavelengths such as cosmic rays, gamma rays and X-rays penetrate deeply into the human tissues where they may cause extensive damage. This radiation is called ionizing radiation because it ionizes molecules due to its high energy. This ionizing radiation is useful in the treatment of malignant diseases when destruction is the goal of the therapy.[2] Optical penetration into the skin is defined by the combination of absorption and scattering. Both are higher at shorter wavelengths resulting in a gradual increase in the depth of penetration into the skin at longer wavelengths over a broad spectrum (290 nm to 1300 nm). Laser light with wavelengths above 1300 nm only penetrate superficially because of the high absorption coefficient of tissue water (Figure 3).

The concept that EM radiation consists of individual discrete packets of energy = quantum = photon was first proposed by Max Planck (1901: the quantum theory). The EM waves are described by the following three physical properties: the frequency ν, the wavelength λ and the photon energy E. Frequencies range from about a million billion Hertz (gamma rays) down to a few Hertz (radio waves). Wavelength is inversely proportional to the wave frequency so that gamma rays have very short wavelengths that are fractions of the size of atoms, whereas radio wavelengths are higher than 17000 nm. Photon energy is directly proportional to the wave frequency so that gamma rays have the highest energy and radio waves have very low energy. Planck related the energy of an individual packet to the frequency of the radiation by means of a constant, named the Planck's constant (h

= 6.63x10 $^{-34}$ Joule-second. [2] The energy (E) of a photon = hv, v = 1/λ => E = h/ λ. This means, that the energy of a photon is inversely proportional to the wavelength (Figure 1). In other words, the shorter the wavelength, the higher is the energy of the photon. The longer the wavelength the lower is the energy. The wavelength of the light waves (and other EM waves) is decreased when present in a medium (matter).

2.2 *Light and skin*

2.2.1 **Ultraviolet light B** (UVB: 280 nm to 320 nm)

Irradiation of the skin with UVB has become one of the most common therapies for several skin diseases such as psoriasis, eczema and vitiligo. This therapy can be given as broad-band (BBUVB, 280–320 nm) or narrow-band (NBUVB, 310-315 nm). In normal skin, UVB increases the synthesis of transforming growth factor (TGF)-α by keratinocytes, accelerates keratinocyte proliferation and increases the overall epidermal thickness.[5] Several cytokines thought to initiate or enhance psoriatic pathology are also increased after UVB irradiation of skin or cultured keratinocytes: Interferon (IFN)-γ[6], tumor necrosis factor (TNF)-α [7], interleukin (IL)-1[8] , IL-6[9] and IL-8.[10] Furthermore, UVB increases leukocyte infiltration[11] and activates neutrophil effector functions.[12] Therefore, it may be anticipated that the therapy of psoriatic skin with UVB would worsen hyperplasia and inflammation in the lesions. However, an entirely opposite effect was observed. The effects of UVB on normal skin are paradoxical in relation to its therapeutic effects. The effects on psoriatic skin include reduced epidermal thickness, a lowered keratinocyte proliferation, a decreased presence of reactive IL-6 in the lesions and the elimination of IFN-γ-induced proteins (HLA-DR and ICAM-1) from the psoriatic keratinocytes.[13,14] The explanation for this paradox was believed to be because of the consistent and the profound depletion of T lymphocytes from the psoriatic epidermis upon UVB therapy indicating that a systemic effect was a negligible factor.[14] Studies also showed that dermal lymphocytes were less affected by UVB therapy[14] because only 5-10% of the incidental radiation in the UVB spectrum penetrated the epidermis up to the basal layer. The remaining energy was scattered at the epidermal surface or was absorbed by keratinocyte proteins or other molecules.[15] Keratinocytes appeared to be relatively resistant to the cytotoxic effects of UVB. It was cytotoxic for keratinocytes at levels that were at least 10-fold higher than those for lymphocytes. The deletion of activated lymphocyte clones from psoriatic epidermis produced a sustained therapeutic improvement. [14] Thus, it was thought that UVB phototherapy was effective in chronic plaque psoriasis through local effects. Therapy with UVB also reduced contact hypersensitivity (CHS) to a number of antigens, [16,17] by targeting the epidermal compartment, inhibiting the

proliferation of keratinocytes and the migration of Langerhans cells (LC), abrogating antigen presentation and inducing apoptosis of activated skin-homing T cells.[14,18,19] Sensitization occurs when an antigen (Ag) is presented by dendritic cells to $CD4^+$ T lymphocytes carrying the appropriate T cell receptor (TCR) in association with the major histocompatibility complex (MHC) class II molecules. In addition, the interaction of co-stimulatory molecules such as B7 with CD28 is required. This suppression in the immune response is called local immunosuppression[20] because the sensitization and the UV exposure affect the same skin area. The systemic effect of UVB therapy on psoriasis was considered to be little and unlikely to be of clinical importance. This led to the conclusion that the systemic effect of UVB was too small to be a reason to alter the interpretation of the findings of within-subject comparative phototherapy studies.[21] Later on, it became apparent that the immunosuppression by UVB was more complex than that assumed earlier. UVB also exerted immunosuppressive effects that were independent of the direct cell cytotoxicity and depletion of the Langerhans cells at the site of exposure. [22] Higher UV doses also affect immune reactions induced at a distant, non-UV-exposed site. Accordingly, CHS could not be induced in mice which were exposed to high doses of UV radiation even if the contact allergen was applied at a non- irradiated site.[23] This type of suppression is called systemic immunosuppression, which is mediated via mechanisms other than the local immunosuppression. The question of how UV radiation can interfere with the induction of an immune response at a distant non-UV-exposed skin area remained unanswered for quite a long time. Today, it is clear that UV radiation stimulates keratinocytes to release soluble immunosuppressive mediators including IL-10, which enter the circulation and thereby can suppress the immune system in a systemic manner.[24] Studies in various UV-mediated tolerance models (local, systemic, high dose, low dose) showed different regulatory T cells (also called suppressor T cells) with unique phenotypes that were involved in these systems. Currently, best characterized are the regulatory T cells involved in the low dose suppression of CHS. UV-induced regulator cells transferring suppression belong to the $CD4^+CD25^+$ subtype,[25] they express CTLA-4,[24] bind the lectin dectin-2 [26] and in contrast to the classical $CD4^+CD25^+$ T cells release high amounts of IL-10 upon antigen-specific activation.[24] Therefore, several mechanisms are involved in UV-induced immunosuppression[20]:

- UV radiation suppresses the expression of MHC class II and co-stimulatory molecules (e.g. B7), which are expressed on antigen-presenting cells and which are crucial for the interaction with T cells. UV radiation also down-regulates the expression of CD80 and CD86 on human Langerhans cells and on blood-derived dendritic cells.[27,28]
- UV radiation stimulates keratinocytes to release soluble immunosuppressive

mediators such as IL-10 and TNF-α, which are responsible for the systemic immunosuppression.[25]

- UV radiation converts trans-urocanic acid (UCA) into cis-UCA, which also exerts immunosuppressive effects.[20] UVB induces reactive oxygen species which may contribute to impair the function of antigen- presenting cells after UV radiation.[29]

There is a certain proportion of human population whose immune response will be compromised by UV radiation. This indicates that there are UV-susceptible and UV-resistant individuals. The immune system does not only protect from infectious agents, but also from malignant cell transformation. At an early stage, transformed cells may be recognized as ‘‘foreign’’ and attacked by the immune system (tumor immunology). This may particularly apply in both non-melanoma skin cancer and malignant melanoma. There is striking evidence for a strong correlation between the risk of developing skin cancer and immunosuppression. Chronically immunosuppressed individuals such as organ transplant recipients run a significantly higher risk of developing skin cancer. This risk certainly increases with the cumulative UV load.[30] Currently available devices are designed to deliver wavelengths of between 310-315 nm (NBUVB) for treating certain dermatologic skin disorders. Erythemogenic doses below 300 nm produce significant clearing; however, these wavelengths also produce the highest erythema and burning. The wavelengths within the action spectrum, which produce the best therapeutic response at sub-erythemogenic doses of UV light therapy in psoriasis are between 310 and 315 nm.[31] The effects of UV light may result in two main categories of observable changes. The first category consists of acute changes, which include membrane damage, induction of cytoplasmic transcription factors, DNA damage and isomerization of urocanic acid. The second category consists of sub-acute changes, which include alteration of antigen-presenting cell populations and the modification of intracellular and intercellular signaling mechanisms. This overall effect creates a change in the environmental cytokine patterns in the epidermis and the dermis, which is more favorable for the development of a T helper (Th)-2 cell-like response as a result of UV effects on the skin.[32]

It is standard in NBUVB therapy to first obtain a minimal erythema dose for effectiveness while limiting the side effects of repetitive erythemogenic doses. The usual starting dose for NBUVB therapy ranges from 50% to 70% of the minimal erythema dose (MED). The MED is determined by a simple procedure that takes a total of 15 to 20 minutes. Delivery of the doses for the MED can be done at the time of the initial visit and the first full body or partial body dose of NBUVB therapy may be started 2 days later. The protocols may range from therapy of three to five times per week once the MED and the initial dose have been determined. Comparison trials

to determine the most effective methods for the delivery of NBUVB reported no real statistical difference, although there was a slight improvement with therapy of five times per week.[33] The more aggressive use of NBUVB therapy, using 70% to 90% of the MED was not statistically superior to a 50% of the MED.[34] Other important factors in the most effective use of NBUVB therapy included lubrication of the skin with a non-UVB absorbing lubricant such as mineral oil, which decreased the reflectance from the scales of the psoriatic plaques. Caution should be exercised not to use agents containing salicylic acid, which would absorb UVB.

Therapy with NBUVB is advanced by increasing the dose of each successive intervention by at least 10% of the MED up to 20% or even 25% of the MED and uses the clinical parameters such as the pinkness of the patient's skin or any complaint of burning to evaluate the degree of progress on a daily basis. Typically, 15 to 20 therapy sessions may be necessary to achieve an improvement of more than 50% in psoriasis. Some patients show no clearance with NBUVB because of the severity of their psoriasis or their intolerance to UV light therapy. Combination therapy, other modalities of UV light therapy or systemic treatments may be considered in such resistant cases.[31] A 308 nm excimer laser is a XeCl excimer laser, which emits a wavelength of 308 nm and has many physical properties of lasers: a monochromatic and coherent beam of light, selective treatment of the target, high penetration and the ability to deliver high fluence. It can directly irradiate lesional skin, thus sparing the surrounding normal skin from unnecessary radiation. Clinical data indicated that treatment of psoriasis vulgaris with a 308 nm excimer laser was effective after about 15 treatments.[35] Most treatments were scheduled twice a week. The initial dose is one to two folds of the MED and the subsequent doses are then increased by 20–30% on every other two to three treatments. Others used very high multiples of the MED, such as 4 and 6 times the MED and claimed initial long-term remissions in some patients.[36]

Adverse effects of UVB therapy may be acute or chronic.[37]

Acute adverse effects of UVB:

- Erythema and burning. NBUVB had a reduced incidence of burning episodes for therapeutically equivalent UV regimens as compared with BBUVB.
- Blisters occurred within the psoriatic lesions halfway through NBUVB therapy were reported, but usually resolved spontaneously in spite of continued treatment.[38]
- Pruritus was mainly associated with dryness of the skin. The use of regular emollients was recommended during the treatment and for about 4 weeks thereafter.

Chronic adverse effects of UVB:

- Photo-ageing is the result of cumulative DNA damage, whereby UVB

causes epidermal changes. Clinically it presents as wrinkling, dryness and coarseness of the skin, freckling, telangiectasia, yellowish and mottled pigmentation, loss of strength and comedones.
Histologically, there is elastosis in the upper dermis together with degenerated and slightly reduced collagen.

- Carcinogenesis. In a systemic review of the literature between 1980 and 1996 an increased risk of about 2% per year was estimated for non-melanoma skin cancers because of UVB therapy.[39]

2.2.2 Laser

2.2.2.1 *History*

The word LASER is an acronym for Light Amplification by the Stimulated Emission of Radiation. That radiation is in the form of photons of light, which are the end product of light amplification that is produced in turn by stimulated emission. The concept of stimulated light emission was initially introduced by Einstein in 1917. Einstein proposed that a photon of electromagnetic energy could stimulate the emission of another identical photon from atoms or molecules that are in an excited state.[40] The first laser was developed by Maiman in 1959 using a ruby crystal to produce red light with a wavelength of 694 nm.[41] In 1963, Goldman pioneered the use of lasers for cutaneous pathologies by promoting ruby laser treatment for a variety of cutaneous diseases.[42-45] The development of the Carbon dioxide (CO_2) and the Argon lasers soon followed and served as the focus of cutaneous laser research during the next 2 decades[42,46]. The CO_2 laser emitting infrared light at 10,600 nm was used for tissue vaporization and destruction of various epidermal and dermal lesions. Unfortunately, the continuous-wave (CW) CO_2 laser also had a high rate of hypertrophic scar formation and pigment alterations because of prolonged tissue exposure to laser energy resulting in excessive thermal injury to the skin.[42,47] The Argon laser produced blue-green light (wavelengths 488 and 514 nm) that was primarily used to treat benign vascular birthmarks. Although most port-wine stains and hemangiomas were effectively lightened, there was an unacceptably high rate of hypertrophic scar formation.[42,48,49] Cutaneous laser surgery was revolutionized in the 1980s with the introduction of the theory of selective photothermolysis by Anderson and Parrish (Table 3).[50]

2.2.2.2 *Stimulated emission, population inversion and light amplification* [51]

Every atom and molecule has a natural tendency to exist in its natural or resting state. The orbiting electrons of the atom or the molecule occupy a given natural position in this resting state. A photon of energy must be absorbed by the atom or the molecule for an

electron to move to a higher orbital level. Once an electron has moved to a higher orbital level, its natural tendency will be to return to its normal or resting state. In doing so, a given packet of energy will be released as a photon that will be characteristic for that atom or molecule. Such an emission of energy is called spontaneous emission. When many atoms or molecules undergo spontaneous decay, the emissions are out of phase with each other. However, if an electron in an excited state is stimulated by a photon of enough energy then that photon will cause orbital decay of the electron returning it to its resting state and result in the emission of a second photon identical to the incident photon. The net result is that two photons of the same wavelength (color) traveling in the same direction and in phase both spatially and temporally are released from the atom. This process is termed stimulated emission and is the first necessary element in the production of laser light. The majority of the particles are in the resting state in a normal population of atoms or molecules. Pumping enough energy into the system will raise the majority of atoms or molecules to their excited state. Such a change, called a population inversion, is necessary for the production of laser light. Once a population inversion has occurred and so long as the energy continues to be pumped into the system, the stimulated emission of in-phase photons will result on their impact on other excited-state electrons resulting once again in additional in-phase photons that, in turn will do the same i.e. produce more in-phase photons. When this chain-reaction “mass production” of in-phase photons occurs within the unique structure of the laser optical resonator, the process is magnified further resulting in light amplification (Figure 4).

2.2.2.3 *Laser Design* [51-52]

The design of every laser is basically the same. A laser uses a power source, a lasing medium, and a chamber to stimulate the emission of photons (Figure 4). The chamber (also called the optical cavity, laser cavity, or resonator) has a fully reflecting mirror at one end and a partially reflecting mirror at the opposite end. The partially reflecting mirror has an opening with a shutter. The chamber contains a medium, which can be solid (ruby- and Nd:YAG-lasers), liquid (dye lasers), or gas or a mixture of gases (Argon- and CO_2 lasers). The medium is called an active medium when energized into its excited state by a power source. The active medium can be achieved through a variety of different sources including electricity, radio-frequency enhancement, light, chemical reaction and mechanical power. The external power source excites the atoms in the lasing medium. As unstable atoms release their photons, they travel through the laser chamber, are reflected by the mirrors at both the ends of the chamber and collide with other excited atoms in the lasing medium. This triggers a cascade of reaction resulting in numerous photons. Photons of the same wavelength, energy and phase are released at the same moment by opening the shutter. The laser light continues to be amplified as long as the population inversion to an excited state continues. Laser light has several unique properties that

distinguish it from other light sources. These key properties (monochromaticity, coherence, collimation and high power) are the basis for the therapeutic applications of laser energy. On the one hand, non-laser light sources such as intense pulsed light devices emit light of many different wavelengths modulated by a cutoff filter. On the other hand, laser light is monochromatic, i.e. all of the light emitted by a laser is of a single, discrete wavelength determined by the laser medium. The monochromatic nature of laser light is a critical property for the application of laser technology in clinical practice because cutaneous chromophores selectively absorb light of different wavelengths. The specific wavelength of laser light also affects the distance it can penetrate into the tissue. The depth of the penetration of laser light generally increases with the increasing wavelength within the spectrum of visible light. The depth of the target chromophore as well as the specific wavelength absorbed by that chromophore must be taken into account when one chooses a laser for clinical use. Another unique property of laser light is that the light is coherent, i.e. the waves of light are in phase with each other in both time and space. The coherent nature of laser light is due to the process of stimulated emission. The light emitted from a laser is in the same direction and in the same phase. Collimation refers to the parallel nature of the waves emitted by a laser.[42] A laser creates a collimated beam by reflecting the light in a chamber between two mirrors that allows the exit of parallel waves only. The tendency toward divergence is low because the waves of light are parallel to each other. The amplification process within the laser cavity produces a high power density. Energy and power both quantify the amount of light emitted from a laser. The unit of energy is the joule (Joule = name of English physicist, 1818-1889, 1 joule = 0.239 calorie, 1 calorie = amount of heat required to warm 1 gram of water to 1 degree) and represents work. Fluence refers to the energy density of a laser beam measured in joules per square centimeter. Irradiance, the power density of a laser beam, is the power of the laser beam divided by the area of the laser beam (spot size = $\pi \times r^2$) and is expressed as watts per square centimeter (W/cm^2). Peak power is inversely proportional to the pulse width or W = J/sec: 1 joule in 1 second = 1 Watt, 1 joule in 1msec = 1000 Watts, 1 joule in 1 µsec = 1,000,000 Watts. Fluence and irradiance are directly proportional to each other: Fluence (J/cm^2) = irradiance x exposure time = W/cm^2 x sec. The effect of laser light on human skin can be affected by power, time and spot size. If the power or time is doubled, fluence increases by a factor of 2. If the spot size is doubled the fluence decreases by a factor of 4. Decreasing the spot size by a factor of 2, results in an increases the fluence by a factor of 4.

2.2.2.4 *Laser-tissue interactions*

Light may interact with tissues in four key ways: transmission, reflection, scattering and absorption. Transmission refers to the passage of light through a tissue without having any effect on that tissue or on the properties of the light. Reflection refers to the repelling of the

light from the surface of the tissue without an entry into the tissue. Approximately 4% to 7% of the light is reflected from the surface of the skin.[50] The amount of the light reflected increases with the increasing angle of incidence with the least reflection occurring when the laser beam is directed perpendicularly to the tissue. Scattering of the light occurs after it has entered the tissue. Scattering is due to the heterogeneous structure of tissues, with variations in the particle size and the index of refraction between different parts of the tissue determining the amount of scatter. Scattering spreads out the beam of light within the tissue resulting in the irradiation of a larger area than anticipated. Scattering also limits the depth of penetration because it can occur forwards as well as backwards. Most scattered light is because of the interaction with dermal collagen in the skin. Generally, the amount of scattering of laser light is inversely proportional to the wavelength of the laser. Longer wavelengths thus penetrate the tissue more deeply. An exception to this rule is the laser light beyond the mid-infrared region in the electromagnetic spectrum.[53] Laser light with wavelengths of more than 1300 nm only penetrates superficially because of the high absorption coefficient of the tissue water. Laser light absorption by specific tissue targets is the fundamental goal of clinical lasers. According to the Grothus-Draper law, light must be absorbed by the tissue to produce any effect in that tissue. The absorption of the photons of laser light is responsible for its effects on the tissue. The components of the tissue that absorb the photons preferentially depend on the wavelength. These light-absorbing tissue components are known as chromophores. Frequently targeted chromophores in the skin include melanin, hemoglobin, water and exogenous tattoo inks (Figure 3). Absorption of energy by a chromophore results in the conversion of that energy into thermal energy. A very short pulse of intense laser light will cause an explosive expansion in the tissue called a photomechanical (or photo acoustic) reaction. A longer pulse of less intense laser light causes rapid heating and denaturation in the tissue called photo-thermal effect. Lower intensities applied for longer times may result in photochemical tissue reactions. The theory of selective photothermolysis refers to laser energy absorption by a target chromophore without any significant thermal damage to the surrounding tissue. The laser must produce a beam of light with a wavelength preferentially absorbed by the chromophore in the lesion to achieve selective photothermolysis. Equally important is the pulse duration of the laser beam that must be shorter than the thermal relaxation time of the chromophore to prevent the spread of thermal energy beyond the targeted chromophore. The thermal relaxation time (TRT) is defined as the time required by the chromophore to cool down to half of its peak temperature after laser irradiation. The TRT is proportional to the square of the size (d = diameter) of the chromophore and inversely proportional to the thermal diffusivity of the tissue ($k = 1.3 \times 10^{-3}$ cm^2/sec). Therefore, smaller objects cool down faster than larger ones. For example, 0.5- to 1.0-μm melanosomes have a TRT of approximately 1 μsec, whereas 100-μm capillaries have a thermal relaxation time of approximately 10 msec. If the pulse width is larger than

the thermal relaxation time, non-specific thermal damage occurs because of the heat diffusion into the surrounding tissue. Finally, the energy (fluence) delivered to the target must be high enough to destroy the chromophore within the pulse duration. Based on the theory of selective photothermolysis, the wavelength, the pulse duration and the fluence of a laser can be tailored to provide selective damage to the lesions without non-specific thermal damage to the surrounding tissues.

Most cutaneous lasers produce a beam with a Gaussian profile in which the intensity peaks at the center of the beam and attenuates at the periphery. Clinically, this results in the necessity of treating tissue with some overlap of the laser beam to deliver energy to the tissue in a more uniform manner. Other modes of operation for lasers result in a doughnut-shaped or target-shaped distribution of energy delivery with the intensity of the laser light at the edge of the beam being higher than at the center or fluctuating across the diameter of the beam.[54] These modes tend to approach a more constant intensity profile, thus overlapping needs to be kept to a minimum to prevent overheating of the tissue at the periphery of the laser beam. The spot size of a laser is equivalent to the laser beam cross section. The spot size directly affects the fluence and the irradiance of a laser beam as mentioned above. The spot size is also clinically important because of the scattering of the laser light in the skin. A small spot size allows more scattering both sideways and backwards than a larger spot size. This results in a more rapid reduction of the energy fluence in the tissue than with a larger spot size. A large spot size of 7- to 10-mm is needed for the maximum penetration of laser light into mid-dermal or deeper targets. Increasing the depth of the penetration levels off with spot sizes of 10- to 12-mm.

Lasers may differ in the manner in which their light is emitted or delivered. Some lasers, notably those with gaseous active media are capable of the continuous discharge of light also called continuous wave (CW). The power output of such lasers does not vary with time and is usually relatively low when compared with other forms of delivery. Such continuous waves can be broken up by an optical or a mechanical shutter that simply breaks up the delivery profile of the CW beam, but does not alter the power output of the beam per se. Such delivery, although confusing, is called pulsed delivery (of the CW beam). Lasers not capable of CW delivery (notably, solid lasers) are able to build up a “head” of power before releasing their “shock wave” or pulse of relatively high power. The pulses delivered by such lasers are usually quite short and are followed by a lag phase before another pulse can be generated. The duration and the power of the pulse, as well as the duration of the lag phase differ for the various pulsed lasers. When the chromophore such as oxy-hemoglobin in the blood vessels (target) is located deeper in the skin, then unintended overlying targets such as epidermal melanin may be damaged. The selectivity of the laser for its intended target can also be improved by surface cooling. Cooling is also used to reduce the pain during laser therapy.

2.2.2.5 *Pulsed Dye Laser (PDL)* [42]

The basis of the laser therapy of vascular lesions is the destruction of abnormal vessels by the absorption of laser energy without damaging the surrounding tissues. The principles of selective photothermolysis are defined as the targeting of an exact dose of intense photo-thermal energy at a specific wavelength to a specific structure, a chromophore, to achieve a selective destruction of the target. The PDL conforms to the principles of selective photothermolysis. These are: 1) The pulse duration is less than the TRT of the target. The pulse duration of 0.45 milliseconds (ms) is shorter than the TRT (1 millisecond) of small- to medium- sized blood vessels; 2) The wavelengths 585 nm and 595 nm reach and are preferentially absorbed by oxy-hemoglobin in the desired target tissues (blood vessels in the superficial dermis) (Figure 5); 3) The proper fluence reaches a damaging temperature in the target tissue without damaging the surrounding tissue structures. The PDL therapy is performed with fluences ranging from 3 to 10 J/cm^2 and a spot size of 2- to 10-mm with no more than 10% pulse overlap to minimize the risk of extensive thermal injury. It is well documented that hemoglobin peaks at 420 nm, 540 nm and 577 nm. The longer wavelengths penetrate the tissue deeper than the shorter wavelengths (Figure 6). Initially, the wavelength chosen for pulsed dye lasers was 577 nm. However, wavelengths from 585 to 595 nm provide a deeper penetration with an insignificant difference in the absorption by hemoglobin. As the laser light passes through the epidermis to the target vessels, it is absorbed by the hemoglobin and converted into heat within the abnormal vessel walls. Normal sized vessels are also heated, but because they are much smaller, they conduct heat away very rapidly and do not reach the temperature that is high enough to cause coagulation. The heating of the vessel walls causes coagulation or destruction of the vessel. The Cynosure laser system is different from other flash lamp pulsed dye lasers because of the different beam profiles characteristic of the individual manufacturer. The Cynosure beam profile resembles a “top hat” with sharper edges. Other systems have a beam profile that is not as clearly defined and with fuzzy edges. The homogeneous beam profile of the Cynosure laser eliminates the need to overlap the delivered pulses.

Adverse effects of PDL treatment:

- Purpura always occurs, may become darker 24-48 hours after treatment and lasts approximately 10 days.
- Hypo-pigmentation is represented by a decreased amount of pigment in the treated area as compared with the untreated, surrounding tissue. It is more commonly seen in dark skinned patients, but may also occur after sun exposure prior to laser treatment.
- Hyper-pigmentation is represented by an increased amount of pigment in the treated area as compared with the untreated, surrounding tissue. It may be caused by extravasation of erythrocytes through the laser-damaged vessels,

but it is usually caused by the activation of melanocytes in de epidermis. Pigmentation changes are usually transient and last for an average of 6 months.

- Small minute spots of depression may occur post-laser therapy, if laser fluences were too high or if there was a significant amount of overlapping during the therapy. In most cases, punctate depressions will fill in naturally during the healing phase.
- Textural surface changes have occasionally been noted in patients who have failed to comply with the post-therapy skin care instructions. Surface changes usually involve small areas of excoriation. Hypertrophic scarring has never been reported.
- Blisters and crusts may be an indication of sun exposure or a too high fluence for the skin type. The blistered area should be treated with antibiotic ointment twice daily.

Air cooling devices or cryospray systems are highly recommended to be used in conjunction with PDL therapy to minimize pain during the procedure and to prevent the activation of melanocytes or any damage to the melanocytes in the epidermis.

2.2.3 *Light absorption* [2]

The absorption of light is a specific and a unique event because each type of molecule is capable of absorbing radiation only in specific wavelength ranges. A molecule that has absorbed light enters an “excited” state, which exists for only a fraction of a second. Following photon absorption, molecules leave the ground state and are raised to a higher energy level known as the singlet excited state $^{*}S^{1}$. If an excited $^{*}S^{1}$ molecule does not undergo chemical change to form a photoproduct, it may emit the energy as light in a process referred to as fluorescence or it may shed its excess energy as heat (decay). An excited $^{*}S^{1}$ molecule may also form a longer-lived triplet state $^{*}T^{1}$ by inter-system crossing. An $^{*}T^{1}$excited molecule may shed its excess energy as light. This process is called phosphorescence or heat (decay) (Figure 6). Heat can be used medically in laser surgery and diathermy. The quantum theory makes clear how light absorption works. The entire energy of a photon is absorbed by a molecule or it is not absorbed at all and the absorption of one photon involves only one molecule. The energy of the photon is taken up by the molecule and the photon ceases to exist as a result of absorption. Molecules exist only in states of specific energy. The energy possessed by a molecule cannot be varied continuously, but only in specific jumps depending on the specific orbital distributions of the electrons in the molecule, which conform to the laws of quantum mechanics. Each of the allowed electronic states or levels has a specific energy.

The energy difference (ΔE) between the ground state and the excited state is the energy required to raise the molecule from the ground state to that excited level. The energy of a photon of wavelength λ is h/ λ. The light of specific wavelengths only which correspond to the energy differences between the ground state and the excited states of a given molecule will be absorbed by that molecule. The energy level of the excited state is inversely proportional to the absorbed wavelength as ΔE = h/ λ. Each absorption process corresponds to a certain ΔE. The minimum amount of energy (hv1) necessary to raise a molecule to an electronically excited state is the amount required for the lowest excited state corresponding to the longest wavelength of light absorbed by the molecule. More energy (hv2) and consequently a shorter wavelength of light are required to excite a molecule to a higher excited state. More wavelengths may be absorbed by a molecule according to the number of the orbital distributions of the electrons in the molecule. These wavelengths are read in a spectrometer as the absorption spectrum of a molecule. Actually, such simple line spectra are obtained only for atoms. When atoms are covalently joined together into molecules, new motions such as rotation and vibration occur. These add additional energy to the electronic states resulting in many more absorptions. The absorption spectrum of a molecule is made up of numerous lines representing the absorption probability at each wavelength. The lines are spaced so closely together that they cannot be distinguished and only the contour of their heights is recorded. The wavelengths that have the highest probability of absorption are called the absorption maxima of the absorption spectrum. The absorption maxima may be used as identifying characteristics of a compound. The absorption spectrum of a chemical compound is related to the molecular structure of that compound and the color of a compound is determined by the wavelengths that are absorbed (Figure 7).

2.2.4 *Topical photodynamic therapy (PDT)*

Heme-containing enzymes are essential for energy metabolism. Protoporphyrin (Pp) IX is the immediate precursor of heme in its biosynthetic pathway and thus every nucleated cell in the body must have at least a minimum capacity to synthesize PpIX. PpIX does not enter the cells from the extra-cellular fluid via the plasma membrane, but is synthesized within the mitochondria of the cells. 5-Aminolaevulinic acid (ALA) is a naturally occurring delta amino acid that is converted into PpIX. The synthesis of heme is regulated by a feedback mechanism in which free heme inhibits the synthesis of 5-ALA catalyzed by 5-ALA synthetase. This process is regulated so closely that photo-sensitizing concentrations of PpIX never accumulate under normal conditions. However, an excess of exogenous 5-ALA bypasses this negative feedback control mechanism resulting in

a transient intracellular accumulation of PpIX.[55] Photo-sensitization can be achieved either by administering exogenous photo-sensitizing molecules (porphyrins, chorins, and phthalocyanines) or by taking advantage of the endogenous pathways via the topical application of precursors such as 5-aminolaevulinic acid (5-ALA) or its derivatives.[56]

The molecule 5-ALA itself is not a photo-sensitizer. [57] Topically applied, 5-ALA and its ester derivatives use the intrinsic cellular heme biosynthetic pathway to produce photo-active porphyrins via a series of enzymatic reactions between the mitochondria and the cytosol. Following activation of PpIX with light of the appropriate wavelength, reactive oxygen species (ROS), particularly singlet oxygen are generated by transfer of the energy to the molecular oxygen present in the tissue. These ROS modify either continuous cellular functions, damage other components of the target cell including the mitochondria, the endoplasmic reticulum and the plasma membrane [58,59] or induce cell death by necrosis or apoptosis[60,61] depending on their amount and their location within the target tissue. Supplementing this direct assault are the indirect pathways of cellular destruction such as the recruitment of inflammatory cells, increased immune response and vascular compromise.[62] Singlet oxygen can also destroy the photo-sensitizing agent itself and prevent further action, a process referred to as photo-bleaching. Following PDT, after photo-bleaching, the synthesis of PpIX continues and reappearance of PpIX is subsequently observed.

The accumulation of PpIX following administration of 5-ALA is more pronounced in rapidly proliferating cells such as pre-malignant and malignant cells as compared with the surrounding normal tissue.[63-65] This phenomenon is due to the decreased activity of ferrochelatase in the neoplastic cells. The latter enzyme catalyses the insertion of iron into PpIX finally forming non-photodynamically active heme. Along with the deficiencies in the metabolic pathway of heme, other factors including the increased 5-ALA uptake, the limited availability of iron, the cell cycle, the proliferation activity, the mitochondrial density, the increased temperature and the lower pH values were suggested to alter the biosynthesis of heme in the neoplastic cells.[64,66] The increased permeability of many cutaneous neoplasms because of a damaged skin barrier will further enhance the activity when 5-ALA is applied topically. [67] Two products for topical PDT of actinic keratoses (AK) have received marketing authorization. In the USA, the 5-ALA hydrochloride 20% (Levulan® Kerastick®, DUSA Pharmaceuticals Inc., Wilmington, MA, USA) has been approved for the photodynamic therapy of AK in combination with blue light (417 nm) for 1000 sec (16 min and 40 sec) at 10 J/cm^2, after 14–18 hours incubation with light protection.[68,69] Shorter incubation times (1-3 hours) in combination with blue light (405 nm) were reported in different studies.[70,71] In Europe the methyl ester of 5-ALA methyl 5-aminolaevulinate (MAL) 16% in a cream formulation (Metvix® , Galderma, Photocure ASA Oslo, Norway), has been approved for the therapy of AK and basal cell carcinoma (BCC) in combination with

red light. MAL showed a more selective accumulation in AK and BCC and being more lipophilic than 5-ALA led to higher porphyrin synthesis than 5-ALA.[64,65] MAL is applied after removal of crusts for 3 hours under occlusion. Both ALA and MAL led to the production of PpIX, which showed a large peak in absorption spectra at 409 nm (Soret-band) with much smaller peaks at 506 nm, 532 nm, 580 nm and 635 nm (Q-bands) (Figure 8). While blue light such as that emitted by the Blu-U® (DUSA Pharmaceuticals, Wilmington, MA) or Omnilux Blue™ (Photo Therapeutics Inc., Carlsbad, CA) takes advantage of the highest absorption spike at 417 nm, it is limited by the depth of penetration to about 1.5- to 2-mm. Red light (>600 nm) requires higher energy levels to achieve the same effect (because of the lower PpIX light absorption at longer wavelengths), but has the advantage of being able to penetrate deeper.[68]
The effectiveness of PDT depends on (1) the photo-sensitizer used, its ability to selectively penetrate the diseased tissue and the duration of application; (2) the activating light source, its ability to penetrate into the desired target and the duration of exposure; and (3) the type of target cells and their oxygenation status. To be effective, the damage resulting from PDT must surpass cellular repair mechanisms, a feature referred to as the minimum photodynamic dose.
The goal of PDT is the selective destruction of targeted abnormal cells while preserving the normal structures. The initial step to PDT is the photo-sensitization of the abnormal cells.[72] The key cellular principle is the preferential accumulation of photo-sensitizers in tumor cells causing a cascade effect of radical oxygen species once illuminated. Many photo-sensitizers are lipophilic and accumulate in membrane structures damaging both the plasma membrane as well as the mitochondrial structures.[73] The net result is cell death by apoptosis and necrosis. It is important to note that although PDT results in singlet oxygen species, these reactive radicals are short lived with a radius of action of only 0.01 μm and therefore, have very low mutagenic potential for DNA damage.[74] At the tissue level, PDT was found to have an effect on both the tumor and its vascular supply. It is postulated that water-soluble sensitizers have a higher affinity for the vascular system, whereas the lipophilic molecules have a direct effect on the tumor itself.[55]
Conventional light sources are divided into four sub-categories (the first three covering the entire visible spectrum):[68]

- Incandescent lamps are essentially conventional light bulbs
- High-pressure arc lamps
- Low-pressure arc lamps
- Light emitting diodes (LED) are small semiconductors with a narrow wavelength band of 20–50 nm

Lasers by definition are monochromatic light sources. Diode lasers (632 nm and 670 nm) and pulsed dye lasers (585 nm and 595 nm) have been used in PDT. Given that lasers emit coherent light, they can be focused with high precision even on very small target

areas with sharp boundaries.[75]

It was reported in a comparative trial that light of shorter wavelengths is less effective in the treatment of Bowen's disease at a theoretically equivalent dose. [76] Therefore, only the use of red light was recommended for PDT of skin tumors.[81] Non-melanoma skin cancer of up to a thickness of 2- to 3-mm can be treated with red light; thicker lesions require multiple treatments or tissue preparation (de-bulking) prior to PDT.[78,79] Laser or incoherent light sources may be used for a successful ALA/MAL-PDT. Pulsed laser light sources matching one of the Q-bands at 585 nm were reported to have similar results as compared with those using an incoherent light source in the treatment of AK.[80] Although not ideally matching the porphyrin absorption spectrum, the use of a long-pulsed dye laser at 595 nm (V-beam, Candela Corporation, Wayland, MA, USA) was also reported to be effective for treating AK.[81] In contrast to lasers, light sources with wide illumination fields enable the simultaneous irradiation of larger areas. Here, incoherent light sources such as lamps (PDT 1200L, Waldmann Medizintechnik, Villingen- Schwenningen, Germany) or light emitting diodes (LEDs) (Aktilite, Galderma, France; Omnilux PDT, Phototherapeutics, Altrincham, UK), which match the absorption maxima of the ALA- or MAL-induced porphyrins are preferred. [80,82-84] A broad-spectrum red light (580–700 nm) dose of 100–150 J/cm^2 (100–200 mW/cm^2) is chosen for treating malignant tumors. The values are significantly lower (37–80 J/cm^2) for the more narrow emission spectra of the LED systems (bandwidth approximately 30 nm). The light intensity should not exceed 200 mW/cm^2 to prevent hyper-thermic effects.[80,82] A light dose of 10–40 J/cm^2 and a light intensity of 50–70 mW/cm^2 (broad-spectrum red light) is recommended for inflammatory dermatoses. During irradiation, both the patient and clinic staff should be wearing protective goggles in order to avoid the risk of eye damage.[85] Since proliferating, relatively iron-deficient tumor cells of epithelial origin are highly sensitized by ALA or MAL, tissue damage is mostly restricted to the sensitized cells almost omitting the surrounding tissue, especially cells of the mesenchymal origin such as fibroblasts resulting in an excellent cosmesis. [86] The same applies for the cells in inflammatory dermatoses. It was reported that fractionated laser light (on-off) was more effective than continuous illumination at the same power density and using the same photo-sensitizers. [87,88] A fractionated illumination of 20 + 80 J/cm^2 separated by a 2-hour dark interval after a single application of 20% ALA in treatment of superficial BCC, Bowen's disease and AK lesions achieved high complete response rates after a 2-year follow-up period.[88] It was suggested by Braathen et al[89] and Christansen et al[90] that shorter incubation times may be sufficient for a successful treatment.

Until recently, clinically approved indications were restricted to AK, superficial and nodular BCC and Bowen's disease. The range of indications has been expanding continuously. Nowadays, PDT is also used for the treatment of non-malignant disorders such as acne

vulgaris, verrucae vulgaris, leishmaniasis and skin ageing.[91] Various guidelines on PDT have been published, but no complete consensus has been reached as yet .[92,93]
Adverse effects of PDT [94]:

- Pain of varying intensity is experienced by most patients undergoing PDT. The pain is described as a burning, stinging or a prickling sensation in the treatment area during illumination. The mechanism behind the PDT-induced pain is probably a consequence of nerve stimulation or damage by reactive oxygen species generated during illumination. The subsequent inflammatory reaction may also contribute to the long-term pain sensation. Measures such as topical anesthetic to reduce the pain during PDT have no effect.[95-97] Unilateral peripheral nerve block appeared to be very efficient in reducing MAL-PDT induced pain and did not affect clinical outcome.[98] Subcutaneous infiltration anesthesia was significantly more effective in reducing 5-ALA PDT-related pain than oral analgesics.[99] Administration of oral analgesics may be indicated, especially if the irradiation field is extended. A significant reduction in pain score during PDT treatment was reported when cold air analgesia [100] and cooling by fans or spraying water on the lesion were used. It was reported that the pain during illumination depended on the location, the size and the histological type of the lesion. Large lesions on the face and the scalp were most painful.[101] Localized erythema and edema in the treated area are usually seen after PDT, followed by a dry necrosis restricted to the treated area over the next days after tumor treatment.[69]
- Post-treatment photo-toxicity up to 48 hours.
- Contact allergy to MAL (approximately 1 % risk).[102]

2.2.5 *Fluorescence detection (FD)*

After application, 5-ALA is taken up actively at higher concentrations in tumor cells than in normal cells leading to a concentration ratio of PpIX. This ratio can be used in PDT to selectively destroy the diseased tissue and in fluorescence detection to visualize the diseased tissue (Figure 9).[103] PpIX absorbs light entering into the excited state. The molecule leaves the excited state via 3 different pathways. The first 2 options are the return to the ground state by either transforming the stored light into heat or by emitting the energy as light, the so-called fluorescence. The fluorescence is shifted to the longer wavelength (600-700 nm) as compared with the excitation light (400 nm). The third pathway is the cross-over to the intermediate state of the photo-sensitizer and the subsequent generation of ROS for PDT. The fluorescence quantum yield is the percentage of molecules that convert the absorbed light energy into fluorescence. The fluorescence quantum of PpIX is only 15% so

that the molecules must be excited with light at 400 nm, which coincides with their maximum absorption. Light emitting diodes are spectrally centered at 405 nm and have a spectral half-width of 20 nm. The LED light is transformed by PpIX into red fluorescence. The fluorescence is detected using a digital camera. A special optical filter in the front of the camera blocks the unwanted blue light imaging the red PpIX fluorescence only. A normal color image of the skin area under investigation is possible for diagnostic use without this filter. Blue light penetrates the skin to a depth of 1- to 2-mm. Excitation using deeper penetrating wavelengths such as green- or red light (Q- bands) is theoretically possible, but these bands are 10- to 20-fold smaller than the Soret-band (blue light).[103] The light intensity is kept at values of lower than 1 mW/cm^2 to prevent any PDT effect.

The traditional fluorescence aided detection of malignant and pre-malignant skin lesions is a three- step process as follows:

1. Application of a photo-sensitizer such as 5-ALA 20% in moisturizing cream, methyl ester of 5-ALA 16% (Metvix) or 5-ALA 20% in propylene glycol and alcohol (Kerastick – USA) under occlusion.
2. Blue light (417 nm) delivered to the tumor and the immediate vicinity
3. Fluorescence detection at wavelengths above 600 nm.

An incoherent light source is sufficient for exciting the porphyrins in the skin lesions of interest after topical application of ALA. The fluorescence is excited by a flash lamp emitting light in the spectral range of 380 nm to 450 nm at a repetition rate of about 10 Hz. The area of interest is immediately exposed to the excitation flash and the fluorescence of the lesion is monitored by a CCD-camera triggered by the flash lamp. This pulsed system enables a dermatologic fluorescence diagnosis in an ambient light situation (Dyaderm, Biocam GmbH, Regensburg, Germany). The camera is connected to a personal computer and provides the normal RGB-image and the fluorescence images at the same time. These images are displayed in real-time on a PC screen. Moreover, the fluorescence images are processed by analyses routines, which help to estimate the size of each lesion. Additionally, the resulting fluorescent area is false color-coded and superposed on the normal RGB-image to show the exact position and the size of the lesion in the clinical image of the skin.[103] Calibration using the surrounding tissue allows one to enhance the contrast of the lesion with respect to the background fluorescence in a single image. The fluorescence intensity is thus given as a ratio between the lesion and the reference. The non-homogeneous irradiation of the investigated skin by the light source can also be corrected for by using a quantitative read-out. Additional parameters such as the age, the skin type, the pigmentation and the inflammation that influence the measured fluorescence intensity can thus be considered and corrected for by employing various algorithms. Multiplication of the resulting fluorescence image with

the specific adjustable parameters enables one to make optimum use of the dynamic range of the CCD-camera in order to obtain the highest possible contrast. The false color presentation then makes it easier for the investigator to evaluate the distribution of the fluorescence intensity. In addition, the false color-coded fluorescence image can be superposed on the clinical image. Thus, the fluorescence contrast and the white light images of the lesion are combined in a single image. The fluorescence detection system is limited by the penetration of the used chromophore and the penetration depth of the excitation light.[104] The image processing described above enabled the detection of the suspected areas, which showed a fluorescence ratio of > 2.0 for a neoplastic transformation as compared with the surrounding peri-lesional tissue. Fluorescence detection may also be used to estimate the efficacy of PDT. The digital image processing allows quantitative and user-independent analyses of the detected fluorescence intensities, which significantly enhances the fluorescence contrast between the tumor and the surrounding tissue.[103]

2.3 Liposomes

Liposomes are microscopic vesicles formed from phospholipids as biological membranes. A fundamental feature of cell membranes is the organization of lipids into bi-layers providing permeability barriers between the exterior and the interior compartments. A large group of biological membrane lipids that spontaneously form bi-layers in water are the phospholipids. A class of phospholipids commonly used to construct liposomes for drug delivery is phosphatidylcholine (lecithin).[105] The ability of phospholipids to form a bi-layer structure is because of their amphipathic character resulting from the presence of a polar or hydrophilic (water-attracting) head-group region and a non-polar or lipophilic (water-repellent) tail. The hydrophilic head groups orientate toward the aqueous phase and the lipophilic tails orientate to each other in the presence of water.[105] Therefore, liposomes contain a lipophilic compartment within the bi-layer membranes and hydrophilic compartments between the membranes. Under the right conditions, water-soluble substances can be stored into the water phase and lipophilic substances into the lipid phase.[106] In general, phospholipids spontaneously form large multi-lamellar liposomes and special process conditions and post-process steps are required to produce appropriately sized (uni-) lamellar liposomes. Liposomes may be small, uni-lamellar vesicles (SUVs 25–50 nm in diameter), large, uni-lamellar vesicles (LUVs 50–500 nm in diameter) or large multi-lamellar vesicles (LMVs 500–10,000 nm in diameter). SUVs are less suitable for drug delivery because they lack stability and their volume is too small for entrapping drugs. The penetration of liposomes through the stratum corneum decreases with increasing diameters. Therefore, the preferred structures for drug delivery are LUVs that are 50–500 nm in diameter.[105] Next to the preferred sizes, an essential

characteristic of liposomes for penetration through the stratum corneum is their state in a liquid crystal phase. The lipid bi-layer passes from a gel into a liquid crystal phase at a critical phase transition temperature (cptT). At the cptT, the head groups become fully hydrated and the lipid chains become freely mobile in the membrane. The cptT is determined by the length and the saturation of the paired lipid chains.[107] (Lipids such as phosphatidylcholine, form bi-layers at cptT below room temperature. This liquid crystal state is essential for liposomes to interact simultaneously with the lipid and the aqueous compartments of the stratum corneum, and for delivering the entrapped drugs into the skin.[108] The similarity in the lipid composition of liposomes and the membranes of the intercellular lamellae and the keratinocytes enables the liposomes and the drugs encapsulated into them to penetrate into the epidermal barrier to a higher extent than that with other forms of application.[109] This may result in an increased absorption, but a decreased clearance of drugs from the epidermis thereby promoting a prolonged sustained release of drugs in the epidermis and a lower absorption of drugs into the blood circulation. Therefore, liposomes may not only act as drug transporters, but also as drug localizers.[110] Fluorescent liposomes applied to the skin were found inside the epidermal keratinocytes.[111] Liposomes fuse with the outer cell membrane and deliver their contents into the cytoplasm via endocytosis.[112] It is possible to introduce even large molecules into the cytoplasm of keratinocytes by using liposomes as intracellular transporters after which they may prove their efficacy.[113] Moreover, 'empty' liposomes (without encapsulated drugs) hydrate the skin simply by providing lipids to the stratum corneum.[114] This hydration capacity may be of benefit in the treatment of xerosis cutis and atopic dermatitis.[115]

2.4 Summary of laser basics relevant in this study:[116]

a. Thickness of the compartments of the human skin:

- Stratum corneum 10 µm = 0.01 mm
- Epidermis 100 µm = 0.1 mm
- Papillary dermis 200 µm = 0.2 mm
- Reticular dermis 2.800 µm = 2.8 mm
- Total dermis = 3.0 mm.

b. The main chromophores in the skin are:

- Water present in all the tissues, absorption wavelengths above 1.300 nm
- Oxy-hemoglobin in the blood vessels absorption wavelengths 400 nm to 600 nm
- Melanin in the epidermis, the dermis and the hair follicles preferentially absorbs wavelengths of between 600 nm and 1.300 nm.

c. Selective Photothermolysis

Thermal injury is restricted to a given target without damaging the surrounding tissues, if:

- The wavelength of the laser light corresponds to the absorption peak of the target
- The pulse duration is shorter than the thermal relaxation time of the target
- The energy delivered during exposure is sufficient to destroy the target
- The effective penetration depth of the laser light encompasses the entire target. The effective skin penetration of the PDL 585 nm is 1.2 mm.

d. Thermo-kinetic selectivity (TKS)

- Large targets of the same chromophore retain heat longer than the smaller ones
- High energy required to damage a large target will spare a smaller structure if the energy is infused for a period that exceeds the required time for the smaller structure to release its absorbed heat. For example: The TRT has to be between the TRT of the epidermis (3-10 msec) and the TRT of the hair follicle (40-100 msec) to heat the melanin in the hair follicles sufficiently to damage the hair follicle, but without damaging the epidermis by heating the epidermal melanin. Thus, the pulse durations of between 10 and 50 msec are sufficient to destroy hair follicles while minimizing the undesired epidermal injury.

e. Extended Theory of Selective Photothermolysis and Thermal Damage Time (TDT). Chromophores such as melanin in the hair follicles and oxy-hemoglobin in the larger blood vessels may be used as a source of heat, which may destroy the target by conduction to the outer (non-chromophore containing) structures. The pulse width has to be long enough to avoid damage to the chromophore, and must be long enough to heat and damage the surrounding structures (the bulge of the hair follicle, the wall of blood vessels). This is called Thermal Damage Time (TDT).

f. Absorption of energy by a chromophore results in the conversion of that energy into thermal energy

- A very short pulse of intense laser light will cause an explosive expansion of the tissue and is referred to as photomechanical (or photo-acoustic) reaction
- A longer pulse of less intense laser light causes rapid heating and denaturation of the tissue and is referred to as photo-thermal effect
- Lower intensities applied for longer times may result in photochemical tissue reactions.

References

1. Ferguson J, Dover JS. Photodermatology. 1e ed.Manson publishing Ltd 2006; 1: 5.
2. Harber LC, Bickers DR Photosensitivity Diseases: principles of diagnosis and treatment. Philadelphia: Saunders Company 1981; chapter 1-3.
3. Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. Am J Clin Nutr 2004; 79: 362-371.
4. Taylor DK, Anstey AV, Coleman AJ, Diffey BL, Farr PM, Ferguson J, Ibbotson S, Langmack K, Lloyd JJ, MCCann P, Martin CJ, Menagé H Du P, Moseley H, Murphy G, Pye SD, Rhodes LE, Rogers S. Guidelines for dosimetry and calibration in ultraviolet radiation therapy: a report of a British Photodermatology Group workshop. Br J Dermatol 2002; 146: 755–763.
5. Murphy GM, Quinn DG, Camp RDR, Hawk JLM, Greaves MW. In-vivo studies of the action spectrum and time course for release of transforming growth factor- α by ultraviolet irradiation in man. Br J Dermatol 1991; 125: 566-568.
6. Livden, J K , Bjerke JR, Degre M, Matte M. The effect of Goeckerman therapy on interferon in serum and suction blister fluid from patients with psoriasis. Br J Dermatol 1986; 114: 217-225.
7. Köck A, Schwarz T, Kirnbauer R, Urbanski A , Perry P, Ansel JC, Luger TA. Human keratinocytes are a source of tumor necrosis factor alpha: evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light .J Exp Med 1990; 172: 1609-1614.
8. Konnikov N, Pincus SH, Dinarello CA. Elevate d plasma interleukin-1 levels in humans following ultraviolet light therapy for psoriasis . J Invest Dermatol 1989; 92: 235-239.
9. Kirnbauer R, Köck A, Neuner P, Forster E, Krutmann J, Urbanski A, Schauer E, Ansel JC, Schwarz T, Luger TA. Regulation of epidermal cell interleukin-6 production by UV light and corticosteroids. J Invest Dermatol 1991; 96: 484-489 .
10. Kondo S, Kono T, Sauder DN, McKenzie RC. IL-8 gene expression and production in human keratinocytes and their modulation by UVB. J Invest Dermatol 1993; 101: 690-694.
11. Soter NA. Acute effects of ultraviolet radiation on the skin . Sem. Dermatol 1990; 9: 11-15.
12. Savage JE, Theron AJ, Anderson R. Activation of neutrophil membrane-associated oxidative metabolism by ultraviolet radiation. J. Invest Dermatol

1993 ; 101: 532-536.
13. Grossman RM, Krueger J, Yourish D, Granelli-Piperno A, Murphy DP, May LT, Kupper TS, Sehgal PB, Gottlieb AB. Interleukin-6 (IL-6) is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Nat Acad Sci USA. 1989; 86: 6367-6371.
14 Krueger JG, Wolfe JT, Nabeya RT, Vallat VP, Gilledeau P, Heftler NS, Austin LM, Gottlieb AB. Successful ultraviolet B treatment of psoriasis is accompanied by a reversal of keratinocyte pathology and by selective depletion of intraepidermal T cells. J Exp Med 1995; 182: 2057-2068
15. Anderson RR, Parrish JA. The optics of human skin . J Invest. Dermatol 1981; 77: 13-19..
16. Tokura Y. Mechanisms of local, low-dose UVB induced immunosuppression in contact hypersensitivity. J Dermatol 1992; 19: 923-931.
17. Mommaas AM, Mulder AA, Vermeer M, Boom B, Tseng C, Taylor J, Streilein J. Ultrastructural studies bearing on the mechanism of UVB-impaired induction of contact hypersensitivity to DNCB in man. Exp. Immunol.1993; 9: 487-493 .
18. Hamakawa M, Sugihara A, Okamoto H, Horio T. Ultraviolet B radiation suppresses Langerhans cell migration in the dermis by down-regulation of alpha-4 integrin. Photodermatol Photoimmunol Photomed 2006; 22: 116-123.
19. Ozawa M, Ferenczi K, Kikuchi T et al. 312-nanometer ultraviolet B light (narrow-band UVB) induces apoptosis of T cells within psoriatic lesions. J Exp Med 1999; 189: 711-718.
20. Schwarz T. Mechanism of UV-induced immunosuppression. Keio J Med 2005; 54: 165-171.
21. Dawe RS, Cameron H, Yule S, Man I, Ibbotson SH, Ferguson J. UV-B Phototherapy clears psoriasis through local effects. Arch Dermatol 2002; 138:1071-1076.
22. Aberer W, Schuler G, Stingl G, Hönigsmann H, Wolff K. Ultraviolet light depletes surface markers of Langerhans cells. J Invest Dermatol 1981; 76: 202–210.
23. Noonan FP, De Fabo EC, Kripke ML. Suppression of contact hypersensitivity by ultraviolet radiation: an experimental model. Springer Semin Immunopathol 1981; 4: 293–304.
24. Schwarz A, Beissert S, Grosse-Heitmeyer K, Gunzer M, Bluestone JA, Grabbe S, Schwarz T: Evidence for functional relevance of CTLA-4 in ultraviolet-radiation-induced tolerance. J Immunol 2000; 165: 1824–1831
25. Schwarz A, Maeda A, Wild MK, Kernebeck K, Gross N, Aragane Y, Beissert

S, Vestweber D, Schwarz T. Ultraviolet radiation-induced regulatory T cells not only inhibit the induction but can suppress the effector phase of contact hypersensitivity. J Immunol 2004; 172: 1036–1043.

26. Aragane Y, Maeda A, Schwarz A, Tezuka T, Ariizumi K, Schwarz T. Involvement of dectin-2 in ultraviolet radiation-induced tolerance. J Immunol 2003; 171: 3801–3807
27. Weiss JM, Renkl AC, Denfeld RW, de Roche R, Spitzlei M, Schopf E, Simon JC. Low-dose UVB radiation perturbs the functional expression of B7.1 and B7.2 co-stimulatory molecules on human Langerhans cells. Eur J Immunol 1995; 25: 2858–2862.
28. Young JW, Baggers J, Soergel SA. High-dose UV-B radiation alters human dendritic cell costimulatory activity but does not allow dendritic cells to tolerize T lymphocytes to alloantigen in vitro. Blood 1993; 81: 2987–2997
29. Caceres-Dittmar G, Ariizumi K, Xu S, Tapia FJ, Bergstresser PR, Takashima A. Hydrogen peroxide mediates UV-induced impairment of antigen presentation in a murine epidermal-derived dendritic cell line. Photochem Photobiol 1995; 62: 176–.183
30. Euvrard S, Kanitakis J, Pouteil-Noble C, Claudy A, Touraine JL. Skin cancers in organ transplant recipients. Ann Transplant 1997; 2: 28–32.
31. Zanolli M. Phototherapy treatment of psoriasis today. J Am Acad Dermatol 2003; 49: 78-86.
32. Duthie MS, Kimber I, Norval M. The effects of ultraviolet radiation on the human immune system. Br J Dermatol 1999; 140: 995-1009.
33. Dawes RS, Wainwright NJ, Cameron H, Ferguson J. Narrowband ultraviolet B phototherapy for chronic plaque psoriasis: three times or five times weekly treatment? Br J Dermatol 1998; 138: 833-839.
34. Hofer A, Fink-Puches R, Kerl H, Wolf P. Comparison of phototherapy with near vs. far erythemogenic doses of narrow band ultraviolet B in patients with psoriasis. Br J Dermatol 1998; 138: 96-100.
35 He YL, Zhang XY, Dong J, Xu JZ, Wang J. Clinical efficacy of a 308 nm excimer laser for treatment of psoriasis vulgaris. Photodermatol Photoimmunol Photomed 2007; 23: 238–241.
36 Feldman SR, Mellen BG, Housman TS, Fitzpatrick RE, Geronemus RG. Efficacy of the 308 nm excimer laser for treatment of psoriasis: results of a multicenter study. J Am Acad Dermatol 2002; 46: 900-906.
37. Laube S, George SA Adverse effects with PUVA and UVB phototherapy. J Dermatol Treat 2001; 12, 101–105.
38. George SA, Ferguson J. Lesional blistering following narrow-band (TL-01) UVB phototherapy for psoriasis: a report of four cases. Br J Dermatol 1992;

127: 445–446.
39. Pasker-de Jong PCM, Wielink G, van der Valk PGM, van der Wilt GJ. Treatment with UV-B for psoriasis and non-melanoma skin cancer. Arch Dermatol 1999; 135: 834–840.
40. Einstein A. Zur Quantentheorie der Strahlung. Physikalische Zeitschrift 1917; 18: 121-128.
41. Maiman T. Stimulated optical radiation in ruby. Nature 1960; 187: 493-494.
42. Tanzi EL, Lupton JR, Alster TS, Lasers in dermatology: Four decades of progress**.** J Am Acad Dermatol 2003; 49: 1-31.
43. Goldman L, Blaney DJ, Kindel DJ, Kindel DJ Jr, Franke EK. Effect of the laser beam on the skin: preliminary report. J Invest Dermatol 1963; 40: 121-122.
44. Goldman L, Blaney DJ, Kindel DJ, Kindel DJ Jr, Franke EK. Pathology of the effect of the laser beam on the skin. Nature 1963; 197: 912-914.
45. Goldman L, Rockwell RJ, Meyer R, Otten R. Investigative studies with the laser in the treatment of basal cell epitheliomas. South Med J 1968; 61: 735-742.
46. Arndt KA, Noe JM. Lasers in dermatology. Arch Dermatol 1982; 118: 293-295.
47. Lanzafame RJ, Naim JO, Rogers DW, Hinshaw JR. Comparisons of continuous-wave, chop wave, and super-pulsed laser wounds. Lasers Surg Med 1988; 8: 119-124.
48. Noe JM, Barsky SH, Geer DE, Rosen S. Port wine stains and the response of argon laser therapy: successful treatment and the predictive role of color, age, and biopsy. Plast Reconstr Surg 1980; 65: 130-139.
49. Apfelberg DB, Maser MR, Lash H, Rivers JL. Progress report on the extended clinical use of the argon laser for cutaneous lesions. Lasers Surg Med 1980; 1: 71-83.
50. Anderson RR, Parrish JA. Selective photothermolysis: precise microsurgery by selective absorption of pulsed radiation. Science 1983; 220: 524-527.
51. Ratz JL. Laser Physics. Clinics in Dermatol 1995; 13: 11-20.
52. Carroll L, Humphreys TR. Laser-tissue interactions. Clinics in Dermatology 2006; 24, 2–7.
53. Massey R, Marrero G, Goel-Bansal M, Gmyrek R, Katz BE. Lasers in dermatology: a review. Lasers Dermatol 2001; 67: 477- 484.
54. Reinisch L. Laser physics and tissue interactions. Otolaryngol Clin North Am 1996; 29: 893-914.
55. Kennedy JC, Pottier RH, Pross DC. Photodynamic therapy with endogenous

protoporphyrin IX: basic principles and present clinical experience. J Photochem Photobiol B Biol 1990; 6: 143–148.
56. Ceburkov O, Gollnick H. Photodynamic therapy in dermatology. Eur J Dermatol 2000; 10: 568–576.
57. Fink-Puches R, Hofer A, Smolle J, Kerl H, Wolf P. Primary clinical response and long-term follow-up of solar keratoses treated with topically applied 5-aminolevulinic acid and irradiation by different wave bands of light. J Photochem Photobiol B Biol 1997; 41: 145–151.
58. Barr H, Kendall C, Reyes-Goddard J, Stone N. Clinical aspects of photodynamic therapy. Sci Prog 2002; 85: 131–150.
59. Hilf R, Mitochondria are targets of photodynamic therapy. J Bioenerg Biomembr 2007; 39: 85–89.
60. Szeimies RM, Karrer S, Abels C, Landthaler M, Elmets CA. Photodynamic therapy in dermatology. In: Dermatological phototherapy and photodiagnostic methods. Krutmann J, Hönigsmann H, Elmets CA, Bergstresser PR, eds. Berlin: Springer 2001; 209–247.
61. Lam M, Oleinick N, Nieminen AL. Photodynamic therapy-induced apoptosis in epidermoid carcinoma cells. Reactive oxygen species and mitochondrial inner membrane permeabilization. J Biol Chem 2001; 276: 47379–47386.
62. Korbelik M. PDT-associated host response and its role in the therapy outcome, Lasers Surg Med 2006; 38: 500–508.
63. Szeimies RM, Sassy T, Landthaler M. Penetration potency of topical applied delta-aminolevulinic acid for photodynamic therapy of basal cell carcinoma. Photochem Photobiol 1994; 59: 73–76.
64. Gaullier JM, Berg K, Peng Q et al. Use of 5-aminolevulinic acid esters to improve photodynamic therapy on cells in culture. Cancer Res 1997; 57: 1481–1486.
65. Uehlinger P, Zellweger M, Wagnieres G et al. 5-Aminolevulinic acid and its derivatives: physical chemical properties and protoporphyrin IX formation in cultured cells. J Photochem Photobiol B 2000; 54: 72–80.
66. Wolf P. Photodynamic therapy in dermatology: state of the art. J Eur Acad Dermatol Venereol 2001; 15: 508–509.
67. Lopez RFV, Lange N, Guy RG, Bentley MVLB. Photodynamic therapy of skin cancer: controlled drug delivery of 5-ALA and its esters. Adv Drug Del Rev 2004; 56: 77– 94.
68. Kormeili T, Yamauchi PS, Lowe NJ. Topical photodynamic therapy in clinical dermatology, review article. Br J Dermatol 2004; 150: 1061–1069.
69. Babilas P, Karrer S, Sidoroff A , Landthaler M, Szeimies RM. Photodynamic therapy in dermatology – an update. Photodermatol Photoimmunol

Photomed 2005; 21: 142–149.
70. Smith S, Piacquadio D, Morhenn V, Atkin D, Fitzpatrick R. Short incubation PDT versus 5-FU in treating actinic keratoses. J. Drugs Dermatol. 2003; 2: 629–635.
71. Touma D, Yaar M, Whitehead S, Konnikov N, Gilchrest BA. A trial of short incubation, broad-area photodynamic therapy for facial actinic keratoses and diffuse photodamage. Arch. Dermatol. 2004;140: 33–34.
72. Dougherty TJ, Kaufmann JE, Goldfarb A. Photoradiation therapy for the treatment of malignant tumors. Cancer Res 1978; 38: 2628–2635.
73. Taylor EL, Brown SB. The advantages of aminolevulinic acid photodynamic therapy in dermatology. J Dermatol Treat 2002; 13 (Suppl. 1): 3–11.
74. Fuchs J, Weber S, Kaufmann R. Genotoxic potential of porphyrin type photosensitizers with particular emphasis on 5-aminolevulinic acid: implications for clinical photodynamic therapy. Free Radic Biol Med 2000; 28: 537–548.
75. Brancaleon L, Moseley H. Lasers and non-laser light sources for photodynamic therapy. Lasers Med Sci
2002; 17: 173–186.
76. Szeimies RM, Abels C, Fritsch C et al. Wavelength dependency of photodynamic effects after sensitization with 5-aminolevulinic acid in vitro and in vivo. J Invest Dermatol 1995; 105: 672–677.
77. Morton CA, Whitehurst C, Moore JV, MacKie RM. Comparison of red and green light in the treatment of Bowen's disease by photodynamic therapy. Br J Dermatol 2000; 143: 767–772.
78. Haller JC, Cairnduff F, Slack G, et al. Routine double treatments of superficial basal cell carcinomas using aminolaevulinic acid-based photodynamic therapy. Br J Dermatol 2000; 143: 1270–1274.
79. Thissen MRTM, Schroeter CA, Neumann HAM. Photodynamic therapy with delta- aminolaevulinic acid for nodular basal cell carcinomas using a prior debulking technique. Br J Dermatol 2000; 142: 338–339.
80. Clark C, Bryden A, Dawe R, Moseley H, Ferguson J, Ibbotson SH. Topical 5-aminolaevulinic acid photodynamic therapy for cutaneous lesions: outcome and comparison of light sources. Photodermatol Photoimmunol Photomed 2003; 19: 134–141.
81. Alexiades-Armenakas MR, Geronemus RG. Laser-mediated photodynamic therapy of actinic keratoses. Arch Dermatol 2003; 139: 1313–1320.
82. Brown SB. The role of light in the treatment of non-melanoma skin cancer using methyl aminolevulinate. J Dermatol Treat 2003; 14 (Suppl. 3): 11–14.

83. Varma S, Wilson H, Kurwa HA et al. Bowen's disease, solar keratoses and superficial basal cell carcinomas treated by photodynamic therapy using a large-field incoherent light source. Br J Dermatol 2001; 144: 567–574.
84. Yang CH, Lee JC, Chen CH, Hui CY, Hong HS, Kuo HW. Photodynamic therapy for bowenoid papulosis using a novel incoherent light-emitting diode device. Br J Dermatol 2003; 149: 1297–1299.
85. Morton CA. Methyl aminolevulinate (Metvix) photodynamic therapy – practical pearls. J Dermatol Treat 2003; 14 (Suppl. 3): 23–26.
86. Morton CA, Brown SB, Collins S et al. Guidelines for topical photodynamic therapy: report of a workshop of the British Photodermatology Group. Br J Dermatol 2002; 146: 552–567.
87. Müller S, Walt H, Dobler-Girdziunaite D, Fiedler D, Haller U. Enhanced photodynamic effects using fractionated laser light. J Photochem Photobiol B 1998; 42: 67-70.
88. De Haas ERM, de Vijlder HC, Sterenborg HJCM, Neumann HAM, Robinson DJ. Fractionated aminolevulinic acid-photodynamic therapy provides additional evidence for the use of PDT for non-melanoma skin cancer. J Eur Acad Dermatol 2008; 22: 426-430.
89. Braathen LR, Paredes BE, Saksela O, Fritsch C, Gardlo K , Morken T , Frølich KW , Warloe T, Solér AM, Ros AM. Short incubation with methyl aminolevulinate for photodynamic therapy of actinic keratoses. J Eur Acad Dermatol Venereol 2009; 23: 550–555.
90. Christiansen K, Bjerring P, Troilius A. 5-ALA for photodynamic photorejuvenation-optimization of treatment regime based on normal-skin fluorescence measurements. Lasers in Surg and Med 2007; 39: 302-310.
91. Klein A, Babilas P, Karre S, Landthaler M, Szeimies RM. Photodynamic Therapy in dermatology- an update 2008. J Dtsch Dermatol Ges 2008; 6: 839-846.
92. Stockfleth E, Kerl H. Guideline Subcommittee of the European dermatology forum, guidelines for the management of actinic keratoses. Eur. J. Dermatol. 2006; 16: 599–606.
93. De Berker D, McGregor JM, Hughes BR. British association of dermatologists therapy guidelines and audit subcommittee, guidelines for the management of actinic keratoses. Brit. J. Dermatol. 2007; 156: 222–230.
94. Wiegell SR, Skiveren J, Philipsen PA, Wulff HC. Pain during photodynamic therapy is associated with protoporphyrin IX fluorescence and fluence rate. B J Dermatol 2008; 158: 727-733.
95. Holmes MV, Dawe RS, Ferguson J, Ibbotson SH. A randomized, double blind, placebo-controlled study of the efficacy of tetracaine gel (Ametop) for

pain relief during topical photodynamic therapy. Br J Dermatol 2004; 150: 337–340.

96. Skiveren J, Haedersdal M, Philipsen P A, Wiegell SR, Wulff HC. Morphine gel 0.3% does not relieve pain during topical photodynamic therapy: a randomized, double-blind, placebo-controlled study. Acta Derm Venereol (Stockh) 2006; 86: 409–411.
97. Langan SM, Collins P. Randomized, double-blind, placebo-controlled prospective study of the efficacy of topical anaesthesia with a eutetic mixture of lignocaine 2.5% and prilocaine 2.5% for topical 5-aminolaevulinic acid–photodynamic therapy for extensive scalp actinic keratoses. Br J Dermatol 2006; 154:146–149.
98. Paoli J, Halldin C, Ericson MB, Wennberg AM, Nerve blocks provide effective pain relief during topical photodynamic therapy for extensive facial actinic keratoses. Clin. Exp. Dermatol. 2008; 33: 559–564.
99. Borelli C, Herzinger T, Merk K, Berking C, Kunte C, Plewig G, Degitz K. Effect of subcutaneous infiltration anesthesia on pain in photodynamic therapy: a controlled open pilot trial, Dermatol. Surg. 2007; 33: 314–318.
100. Pagliaro J, Elliott T, Bulsara M, King C, Vinciulli C. Cold air analgesia in photodynamic therapy of basal cell carcinomas and Bowen's disease: an effective addition to treatment: a pilot study. Dermatol Surg 2004; 30: 63–66.
101. Grapengiesser S, Ericson M, Gudmundsson F et al. Pain caused by photodynamic therapy of skin cancer. Clin Exp Dermatol 2002; 27: 493–497.
102. Harries MJ, Street G, Gilmour E, Rhodes LE, Beck MH. Allergic contact dermatitis to methyl aminolevulinate (Metvix®) cream used in photodynamic therapy. Photodermatol Photoimmunol Photomed 2007; 23: 35-36.
103. Bäumler W, Abels C, Sziemies RM. Fluorescence Diagnosis and Photodynamic Therapy in Dermatology. Med Laser Appl 2003; 18: 47–56.
104. Szeimies RM, Landthaler M. Photodynamic therapy and fluorescence diagnosis of skin cancers. Rec Res Cancer Res 2002; 160: 240–245.
105. Hope MJ, Kitson CN. Liposomes: a perspective for dermatologists. Dermatol Clin 1993; 11: 143–154.
106. Egbaria K, Weiner N. Liposomes as a drug delivery system. Adv Drug Del Rev 1990; 5: 287– 300.
107. Cullis PR, Hope MJ. Physical properties and functional roles of lipids in membranes. In: Vance DE, Vance JE, eds. Biochemistry of Lipids and Membranes. Menlo Park: Benjamin/Cummings 1985: 25–72.
108. Gabrijelcic V, Sentjurc M, Kristl J. Evaluation of liposomes as drug carriers

into the skin by one-dimensional EPR imaging. Int J Pharm 1990; 62: 75–79.
109. Plessis J, Egbaria K, Weiner N. Influence of formulation factors on the deposition of liposomal components into the different strata of the skin. J Soc Cosmet Chem 1992; 43: 93–100.
110. Schmid MH, Korting HC. Therapeutic progress with topical liposome drugs for skin disease. Adv Drug Del Rev 1996; 18: 335–342.
111. Yarosh D, Bucana C, Cox P, Alas L, Kibitel J, Kripke M. Localization of liposomes containing a DNA repair enzyme in murine skin. J Invest Dermatol 1994; 103: 461–468.
112. Ostro M. Liposomes. Sci Am 1987; 256: 102–111.
113. Yarosh D, Klein J. The role of liposomal delivery in cutaneous DNA repair. Adv Drug Delivery Rev 1996; 18: 325–333.
114. Artmann C, Roding J, Ghyczy M. Influence of various liposome preparations on skin humidity. Parfum Kosm 1990; 90: 326.
115. Neugebauer D. Liposomen: Neues therapeutisches princip bei ekzemen und psoriasis. Apotheker J 1993; 6: 20–26.
116. Rosio TJ. Basic Laser Physics. In: Roenigk & Roenigk's Dermatologic Surgery: Principles and Practice, ed Roenigk RK, Roenigk HH, sec ed 1996; 53: 947-976.

Positive effects of sunlight	*Negative effects of sunlight*
Photosynthesis	Induction of skin cancer
Ability of vision	Cataract of the eye lens
Warmth	Sunburn
Vitamin D production => prevention of : - rickets and osteoporosis - multiple sclerosis, DM Type I, , hypertension rheumatoid arthritis - carcinoma (prostate, mamma, ovary, colon - non-Hodgkin's lymphoma => improvement of: - mental depression - schizophrenia	Ageing of the skin (helio-dermatosis): - wrinkles - hyper-pigmentation - hypo-pigmentation - hyperkeratosis - actinic keratosis
Inhibition of virulent viruses and bacteria	Induction or exacerbation of - discoid or systemic LE - rosacea - porphyria
Improvement (therapy) of skin diseases: - tuberculosis - acne - psoriasis - eczema - vitiligo	Photo-dermatosis - polymorphous light eruption - solar urticaria - photo-allergic skin reactions - photo-toxic skin reactions - phytophoto-dermatitis

Table 1. Light and shadow sides of sunlight (modified Ferguson 1).

Epoch	*Country*	*Worship*
Second millennium B.C. Thirteenth century B.C.	Egypt	Ra the sun god Amenhotep III earthly messenger and mortal link conducting solar power to the earthlings
Tenth century B.C.	Greece	Zeus (all-powerful deity) His son Apollo (sun god and father of medicine) Aesculapius (mortal son of Apollo) became a famous physician and his staff, intertwined by a serpent became the symbol of medical profession
Sixth century B.C	Persia	Teaching of Zoroaster: Ahura Mazda (god of wisdom) Mithras (deity of truth and light = sun god), asserted the victory of good over evil, of light over darkness Day of rest = Sunday
Second century B.C.	Pergamum, (Asia minor)	Utopia of Aristonicus “Commonwealth of the sun” Sun as symbol for justice and equality by shedding light and warmth upon all people
Fifteenth century	North America	Tonatiuh (powerful sun god) whose face forms the center of the Aztec calendar
1643-1715	France	Louis XIV King of France selected the sun as his symbol of authority and required that his subjects regard him as a sun god
Nineteenth century	Europe	Tanned skin = poverty (working in the fields) Pale skin = wealth (no need to work in the fields)
Twentieth century	Western world	Tanned skin = wealth (no need to work in factories, plenty of recreation time) Pale skin = troublesome life
Twenty-first century	Whole world	Sunburn is stupid Solar energy replacing fossil energy

Table 2. Historical overview of human’s attitude to sunlight (modified Harber).

Ancient Egyptians 8-methoxypsoralen (8-MOP)+ sunlight: treatment of vitiligo
1901 Max Planck introduced the quantum theory and the concept of photons
Early nineties Finsen lamp: tuberculosis of the skin
1917 Einstein stimulation of light emission LASER
1925 Goeckermann crude coal tar + ultraviolet light-B (UVB): psoriasis
1960 Maimann Ruby laser: pigmentation disorders
1964 Goldman first publication on laser surgery
1970 Broad-band UVB: psoriasis
1974 Psoralen (8-methoxpsoralen) + Ultraviolet light A (PUVA) systemic treatment, followed by topical treatment (bath PUVA): psoriasis
1975 Argon laser: port-wine stain
1983 Anderson and Parrish Selective Photothermolysis: Pulsed Dye Laser fort port-wine stain
1985 Narrow-band UVB: psoriasis and eczema
CO_2-laser and Erbiumlaser for skin ablation
1989 Retinoin-PUVA: psoriasis
1996 Pulsed Dye Laser: psoriasis
2000 Photodynamic therapy (PDT) with 5-aminolevulinic acid (5-ALA) and blue light (USA) or PDT with methylaminolaevulinate and red light (Europe) for actinic keratosis
2009 Acne treatment with 5-ALA 0.5% liposomale spray and IPL, vitiligo treatment with khellin 0.005% liposomes and UVAB, Fluorescence Detection with 5-ALA 0.5% liposomale spray and blue light.

Table 3. History of light in Dermatology.

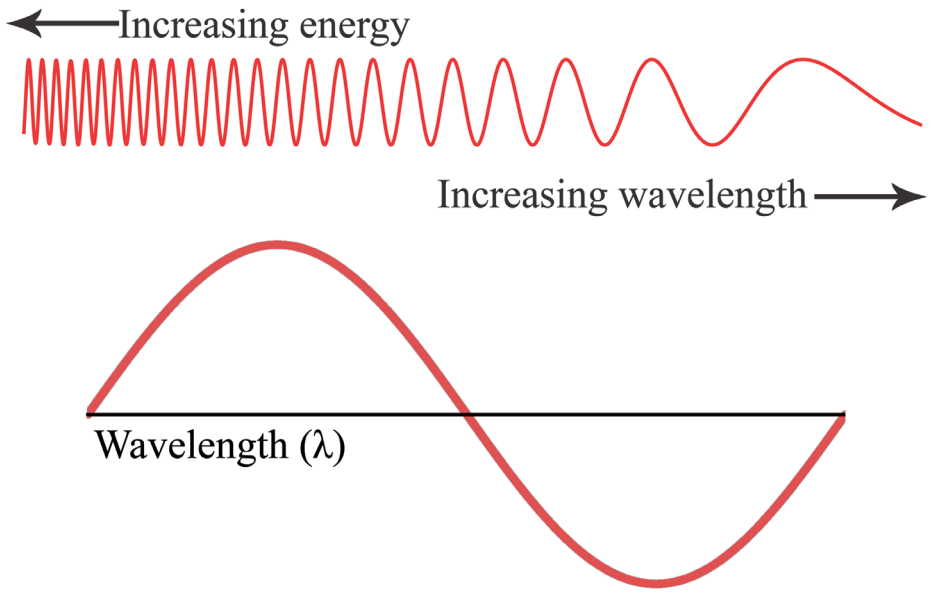

Figure 1. Wavelength is the distance between two corresponding points on each successive wave. Frequency (v) is the number of waves that passes a point per second. Einstein: λ x v is equal to the speed of light (300.000 km per sec). The energy of a photon is inversely proportional to the wavelength.

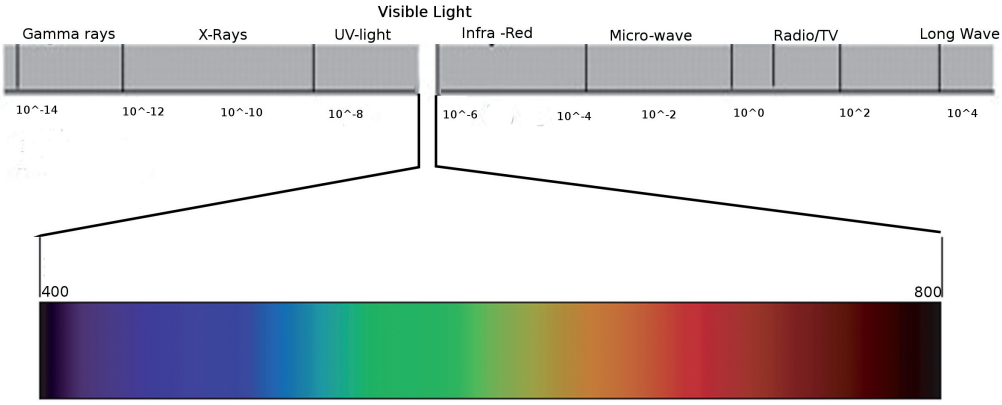

Figure 2. The electromagnetic spectrum.

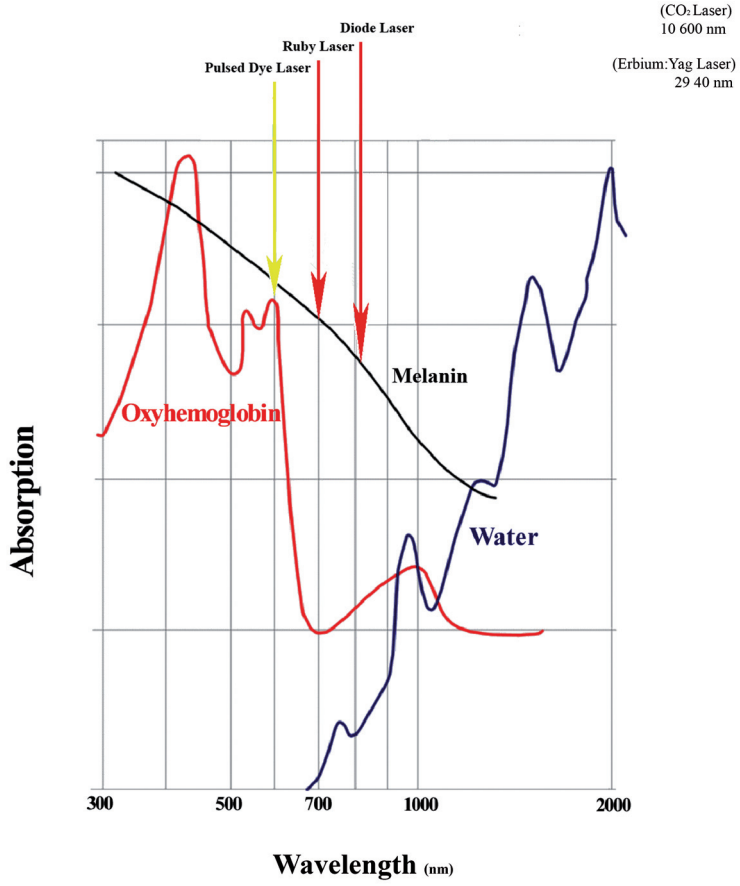

Figure 3. Absorption curves of melanin, oxy-hemoglobin and water.

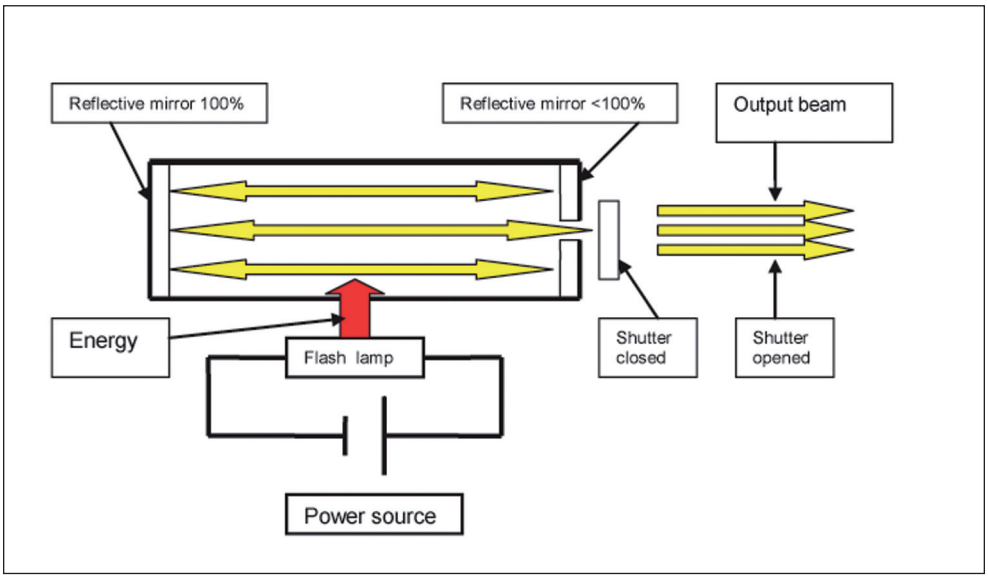

Figure 4. Design of the Pulsed Dye Laser.

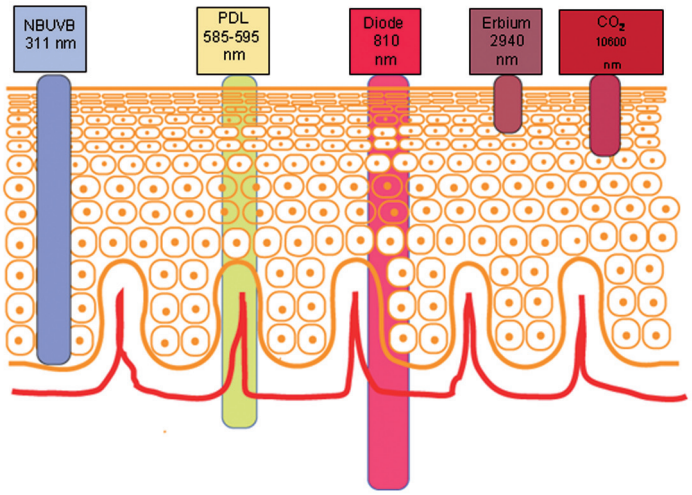

Figure 5. Penetration depth of lasers depends on the wavelength:

NBUVB light penetrates only into the epidermis

PDL light penetrates into the superficial dermis

Diode light penetrates into the mid-dermis

Erbium and CO_2 light is absorbed by water

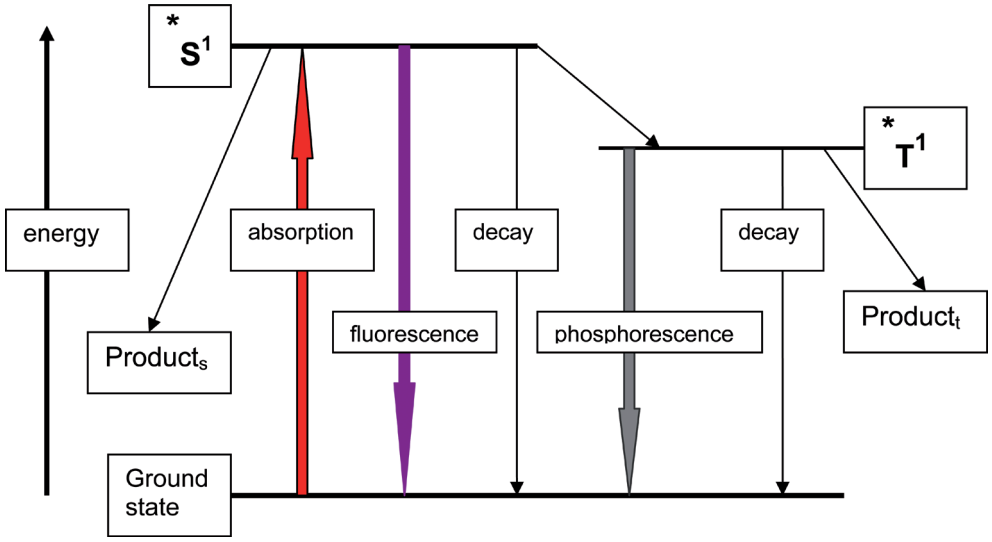

Figure 6. Photon absorption raises molecules to the singlet excited state $^{*}S^{1}$, energy dissipation include fluorescence, phosphorescence, triplet $^{*}T^{1}$ state formation and formation of photoproducts.

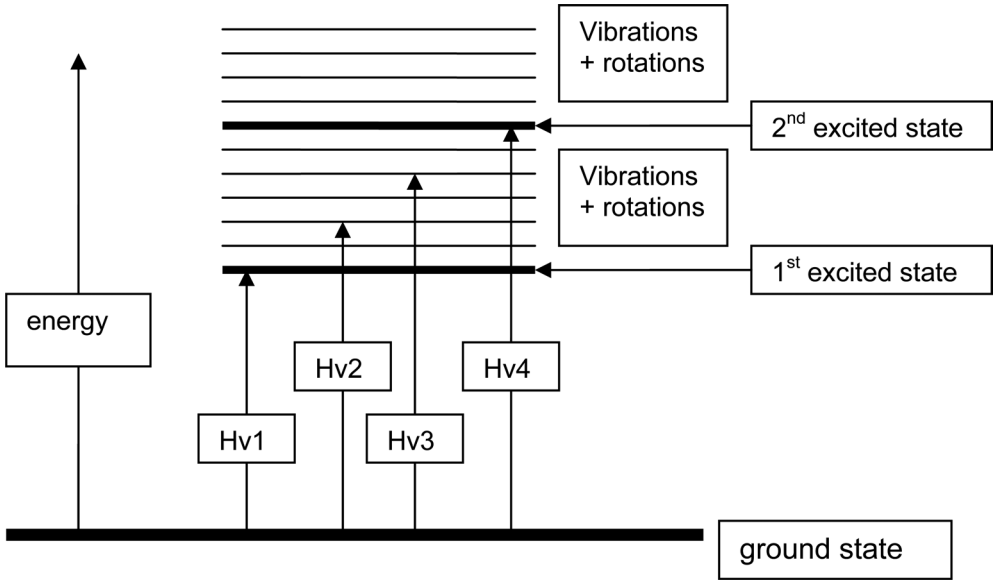

Figure 7. Absorption spectrum of a molecule.

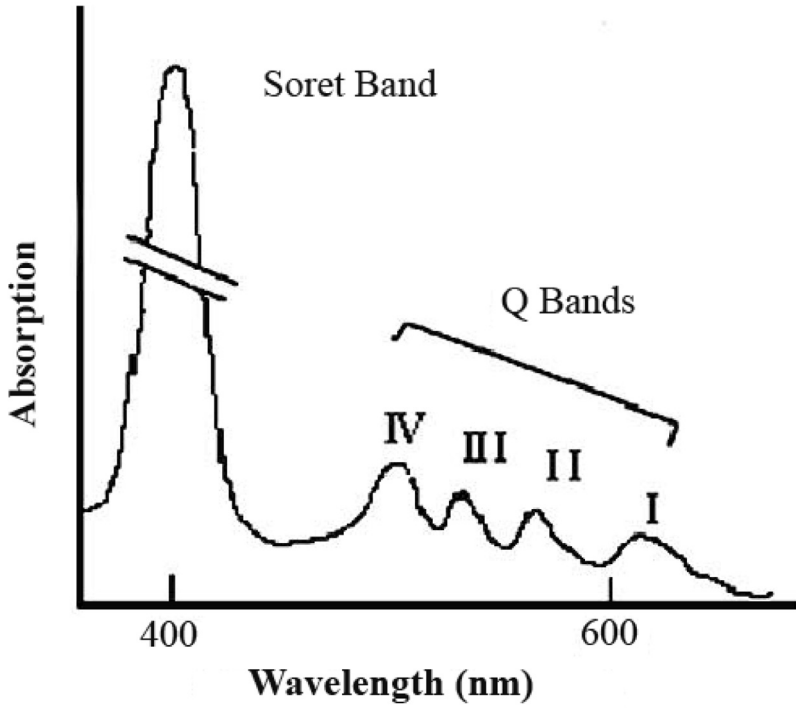

Figure 8. Protoporphyrin IX absorption spectrum.

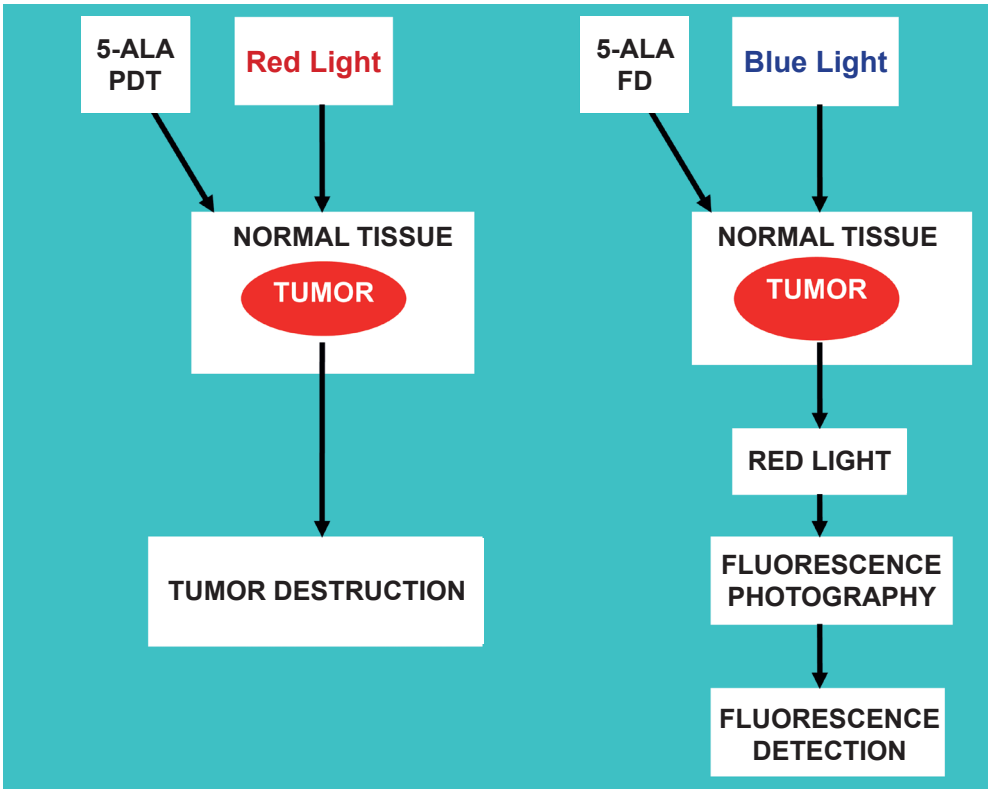

Figure 9. Photodynamic therapy (PDT) and fluorescence detection (FD).

Chapter 3

3.1 Psoriasis

3.1.1 *History, epidemiology and clinical aspects*

The term psoriasis is derived from the Greek word psora meaning scale and was first used by the Greek physician Galen to describe a scaly skin condition (129-99 B.C.). This affliction included probably true psoriasis, but also skin diseases such as eczema, scabies and leprosy. The confusion between psoriasis and leprosy continued for many centuries. People suffering from psoriasis were isolated from their community as lepers, the church declared them dead and in the 14th century Philip the Fair even ordered them to be burnt at the stake. Ferdinant von Hebra definitely separated the clinical features of psoriasis from those of leprosy in 1841.[1] In 1908, Robert Willan specifically described psoriasis as a recognizable entity.

Psoriasis is a chronic inflammatory and proliferative skin disease that affects approximately 2% of the world's population.[2,3] Different prevalence of psoriasis was reported for different countries, geographical regions and ethnic groups. Its prevalence ranges from 0% in Samoa to 4.6% in the USA, but is only 0.7% in African Americans. The estimated prevalence in Europe is 2-3%, and 0.7% in India, Nigeria and East Africa. Psoriasis is very rare in American Indians, Eskimos, Greenland Inuit Eskimos and Australian aboriginals. The risk of incurring psoriasis is about 20% if one parent has psoriasis, and is about 75% if both parents are affected. If one monozygotic twin suffers from psoriasis, the probability is more than 55% that the other will be affected too.[4] No significant gender differences have been reported. In psoriasis two distinct patient cohorts have been recognized. Type I is characterized by a peak age of onset of psoriasis at between 16 and 21 year and type II onset occurs at about the age of 55 years. Type I patients are more likely to show widespread and recurrent disease as compared with late onset patients. They also have a history with affected parents (44% in type I and 0% in type II), and have a higher frequency of HLA Cw6 as compared with late onset patients (85% vs 14%) and for HLA DR7 (70% vs 30%).[2,5] Although only rarely life-threatening, psoriasis is disabling, incurable and the cause of significant morbidity and of a poor quality of life of the patient.[6] Plaque-type psoriasis (Figure 10) is the most common form of the disease (occurring in more than 80% of the cases), which is characterized by the presence of symmetrical, well-demarcated, raised and erythematous scaly plaques.[7] These plaques are commonly situated on the scalp and the extensor surfaces of the limbs. However, any skin site may be affected. Psoriasis usually runs a remitting course, its clinical behavior

varying between patients and also in the affected patient at different times. Active disease is distinguished by plaques increasing in size and number, whereas the most acute form, pustular psoriasis, is characterized by localized or widespread areas of superficial cutaneous postulation.[7]

Other types of psoriasis are as follows:

- Psoriasis guttata, a common form of psoriasis in children, associated with preceding upper respiratory infection and raised anti-streptolysin titer indicating the relevance of streptococcal infections. The lesions vary from 2-mm to 1-cm in diameter and are round or oval. They are distributed more or less evenly over the body, particularly on the trunk and the proximal part of the limbs.[8]
- Psoriasis inversa (flexural psoriasis) characterized by red, sharply demarcated thin plaques in body folds such as the axillae, sub-mammary region, groins and the inter-gluteal cleft (often a central fissure is seen).
- Psoriasis palmoplantaris (Figure 11) exhibits red symmetric, sharply bordered plaques with very adherent yellow-white scale and frequently with painful fissures. In psoriasis pustulosa of the palms and the soles, the scaly erythematous plaques are admixed with sterile pustules and yellow-brown macules.
- Psoriasis of the nails affects about 30-50% of the psoriasis patients. The numbers are even higher in those with psoriasis arthritis. The most typical sign is pitting when the nail matrix is involved. Psoriatic changes in the nail bed result in the “oil spot” phenomenon. Sub-ungual hyperkeratosis and distal onycholysis are due to the parakeratosis of the nail bed.
- Psoriatic arthritis occurs in 5-30% of the patients with cutaneous psoriasis.
- Psoriatic erythroderma represents the generalized form of the disease that affects the whole body.

Although generally associated with cutaneous morbidity only, extensive skin involvement may be accompanied by systemic disturbances in the thermo-regulation, the fluid balance and the protein metabolism.[9]

Psoriasis follows a precarious course with regard to the age of onset, the morphology, the distribution, the exacerbations and the remissions as well as the response to treatment. Exacerbations may be triggered by stress[10-12], alcohol consumption & smoking,[13] physical trauma (Koebner’s phenomenon),[4] medications such as β-blockers, lithium, chloroquine, non-steroidal anti-inflammatory agents, tetracyclines, interferons, imiquimod[14] and infections. [8,11,14]

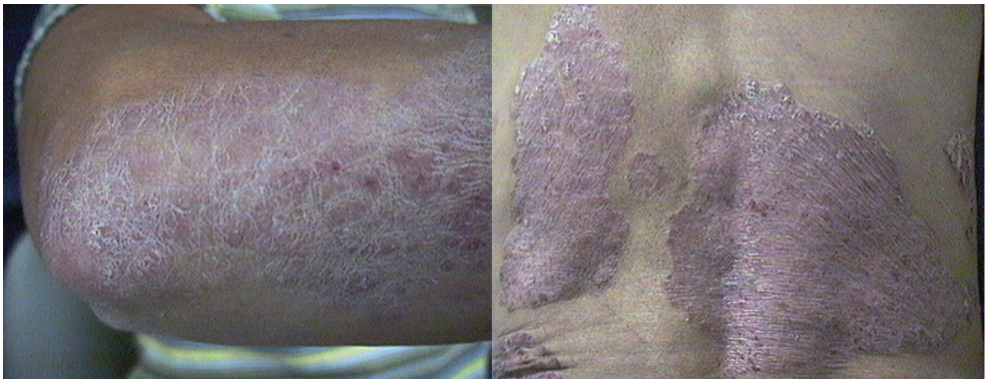

Figure 10. Plaque type psoriasis

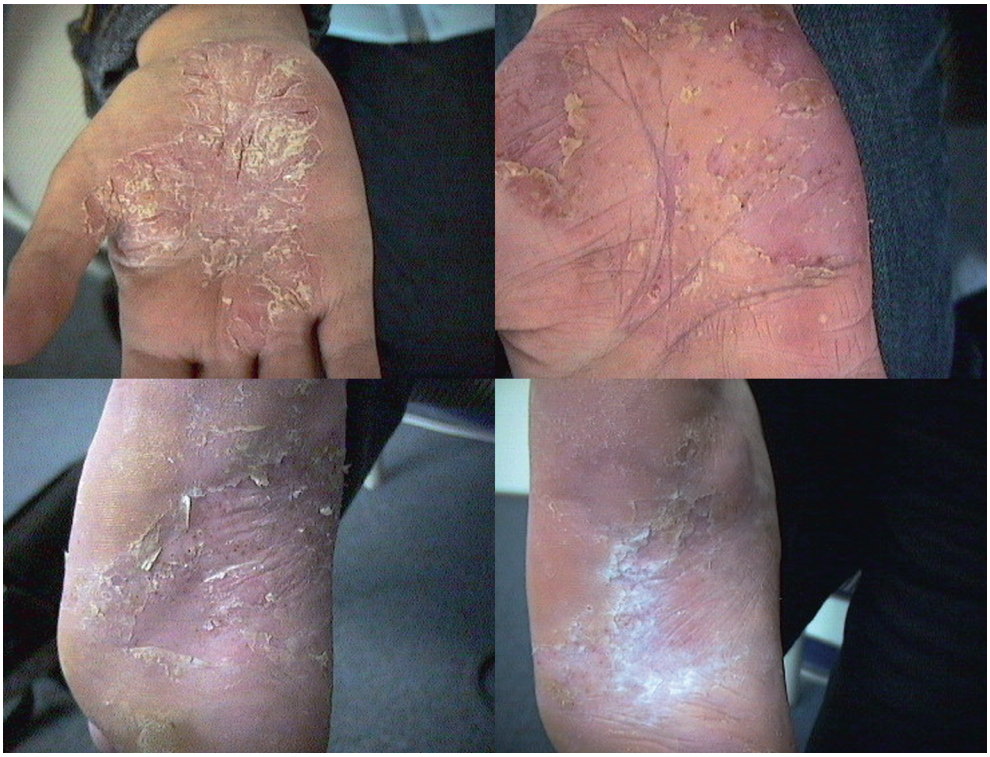

Figure 11. Psoriasis palmoplantaris

3.1.2 *Histopathology*

Psoriasis is histologically characterized by hyperproliferation of the epidermal keratinocytes, hyperkeratosis and infiltration of immunocytes accompanied by angiogenesis resulting in a typical thickening and scaling of the erythematous skin. The mitotic activity of basal keratinocytes is increased by a factor of 50 in the psoriatic skin resulting in an increased number of keratinocytes, the thickening of the spinous layer (acanthosis) and the elongation of the epidermal rete. The keratinocytes migrate from the basal layer to the cornified layer in about 4 days instead of the normal 28 days. (Figure 12).[4] There is an aberrant expression of cytokeratins 6, 16 and 17 that are associated with the hyperproliferation in the psoriatic epidermis.[15] The increased recruitment of keratinocytes in the psoriatic epidermis is visualized by staining for the Ki67 nuclear antigen, which is a representative marker for the growth fraction of the keratinocytes.[16]. The differentiation of keratinocytes is highly altered in psoriasis and parallels the 'regenerative maturation', an alternative cell differentiation program that occurs transiently during wound repair. The granular layer of the epidermis where the terminal differentiation begins is highly reduced or totally absent in psoriatic lesions. Consequently, the stratum corneum forms from incompletely differentiated keratinocytes that aberrantly retain a cell nucleus (parakeratosis). Scaling and thickening of the cornified layer (hyperkeratosis) is caused by the failure of psoriatic corneocytes (terminally differentiated keratinocytes) to stack normally. They secrete extracellular lipids and adhere to one another (Figure 12).[17] Other histological features of psoriasis include the presence of neutrophils within small foci in the stratum corneum and significant mononuclear infiltrates in the epidermis, which are detectable with immunostaining. In addition, there is a marked infiltration of mononuclear leukocytes such as T cells and dendritic cells (DCs) into the dermis. Blood vessels in the papillary dermal region are elongated and show hyperplasia. Marked dilation of these vessels causes the visible redness of psoriatic skin lesions. Many lymphocytes, monocytes and neutrophils clearly adhere to the endothelial cells that acquire characteristics of high endothelial venules, which are usually found in lymph nodes.[18] Leukocytes may enter the skin parenchyma by transmigration through the reactive vessels, but resident skin leukocytes may also proliferate resulting in the dense infiltrates observed in the psoriatic lesions.[17]

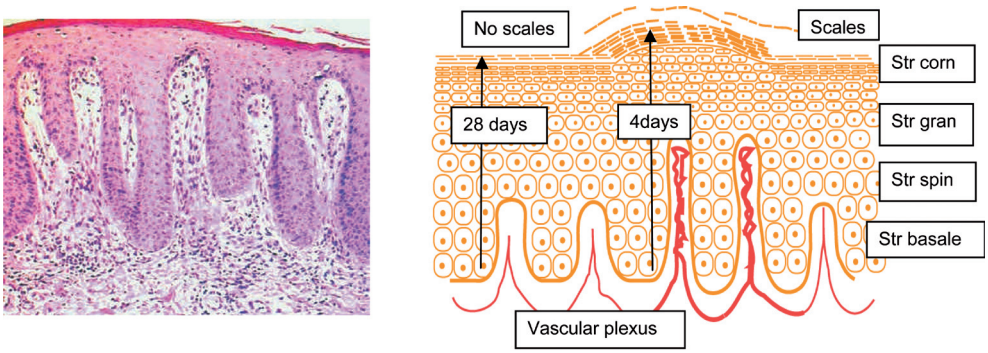

Figure 12. Histopathology of psoriasis
Keratinocytes require 28 days un the normal skin, but only 4 days in the psoriatic skin to migrate from the stratum basale (str. bas.) to the stratum corneum (str. corn) resulting in:

- Hyperkeratosis and scaling
- Incompletely differentiated keratinocytes that aberrantly retain a cell nucleus in the str. corn
- Reduction or absence of the stratum granulare (str. gran)
- Thickening of the stratum spinosum (str. spin)
- Infiltration of immunocytes and granulocytes into the epidermis and the dermis
- Dilation, elongation and hypertrophy of vacular plexus resulting in sharply demarcated, elevated, red, scaly patches.

3.1.3 *Pathogenesis*

3.1.3.1 *Genetics.*

The pathogenesis of psoriasis is not completely elucidated. The development of psoriatic lesions depends on multiple interactions between the susceptibility genes and the environmental factors.[19] Molecular genetic studies indicate that there are multiple susceptibility loci (PSORS1-8) present throughout the human genome. A number of genetic loci have been identified by genome wide linkage scans and two loci have been replicated: PSOR1 on chromosome 6, within the MHC, including HLA-Cw6, HLA-B13 and HLA-Bw57 and PSOR2 on chromosome 17q.[20,21]

3.1.3.2 *Innate and adaptive immune response*

As a primary defense organ, the skin forms a major barrier for the external elements and is thus permanently exposed to microbial, chemical and physical insults. Therefore, the skin has the capacity to generate both the innate as well as the adaptive immune responses.[22] The innate immune response mediates the initial protection against infections and the adaptive immune response develops more slowly and mediates the later, even more effective, defense against infections. The innate response

is antigen non-specific, lacks immunological memory and involves the actions of keratinocytes, DCs, macrophages, natural killer (NK) cells and cytokines. The cell-mediated adaptive immune response is antigen specific, has the ability to recognize and respond to many different microbes, provides enhanced responses to recurrent or persistent infections and prevents injurious immune responses against host cells and tissues and involves the action of antigen-presenting cells (APCs), macrophages, DCs and T-lymphocytes.[22] Innate and adaptive immune responses do not operate as separate or parallel systems, but represent a single highly integrated biological system designed to respond to infectious insults in a complementary fashion.

It is critical that a proper balance between the innate and the adaptive responses be maintained because on the one hand, septic shock may result from a cutaneous bacterial infection if the innate response is inadequate, whereas on the other hand, an exaggerated innate response may result in a chronic autoimmune inflammatory-type response.[23]

3.1.3.3 *Toll-like receptors*

Toll-like receptors (TLRs) are phylogenetically well conserved type I transmembrane proteins representing the major pattern recognition receptors of the innate immune system for various pathogen-associated molecular patterns (PAMPs).[24] Toll-like receptor signaling leads to the activation of the nuclear factor kappa B (NF-κB) through the adaptor protein MyD88-dependent pathway and induces a battery of immune adjuvant effects, which are principally mediated by pro-inflammatory cytokines. To date, 11 TLR family members together with their agonists have been identified. TLR3 binds double-stranded RNA synthesized by viruses, TLR4 recognizes bacterial lipopolysaccharides, TLR5 accepts bacterial flagellin and TLR9 is activated by bacterial and viral DNA. TLR7 and TLR8 are triggered by imiquimod and their innate antiviral and anti-tumor effects are because of the induction of type I interferon (IFN). Type I IFNs also shape the adaptive arm of the immune response by driving TH1 responses. In human peripheral blood, the principal producer of type I IFNs in response to imiquimod is represented by plasmacytoid dendritic cell precursors (PDCs), a novel subset of lymphoid-related cells selectively expressing high levels of TLR7 and TLR9. PDCs are the key players in innate antiviral immunity because of their unique ability to produce large amounts of type I IFN. In addition, PDCs differentiate into DCs with the ability to stimulate T cell–mediated adaptive immunity upon viral stimulation. TLR-mediated activation of innate immunity is involved not only in the host defense against pathogens, but also in immune disorders.[24] Activation of innate immunity is a crucial step in the development of antigen-specific acquired immunity.[25] Psoriasis is a chronic and relapsing T cell–mediated autoimmune disease, in which type 1 cytokine secretion by T cells induces keratinocyte hyperproliferation

in genetically predisposed individuals. In recent years, the observation that psoriatic plaques are resistant to microbial infection despite a compromised skin barrier has led to growing interest in the role of the innate immune system in psoriasis. It was shown that exacerbation of chronic psoriasis plaque may follow a repeated topical application of the TLR7 agonist imiquimod. A TLR7-mediated effect was indicated by the massive induction of lesional type I IFN after imiquimod therapy. Keratinocytes, as non-professional APCs possess a remarkable production repertoire of pro-inflammatory cytokines and constitutively express TLR1, -2, -3, -5 and -9, but not TLR7. Among professional APCs, TLR7 is principally expressed by PDCs, which in addition to their unique ability to produce large amounts of type I IFN in response to viral infection, also represent the principal producer of type I IFN in response to imiquimod. PDCs classically described in blood, secondary lymphoid organs, bone marrow, and thymus have also been described in inflammatory skin lesions and large numbers of PDCs have been identified in the dermal infiltrate in psoriasis. PDCs may represent key cellular mediators of innate immune responses driving the pathogenic events leading to psoriasis.[24] TLR9 is the only known receptor to detect immunostimulatory DNA such as bacterial DNA.[26] Activation of TLR9 leads to the activation of IFN-regulatory factor 5 (IRF5), tumor necrosis factor receptor-associated factor 6 (TRAF6), and NF-κB and the rapid production of inflammatory cytokines and chemokines including interleukin (IL)-8/*CXCL8*, tumor necrosis factor (TNF)-α, IL-6, IL-12, and type I IFNs.[27] IL-8 stimulates proliferation of keratinocytes and is also a chemoattractant for neutrophils.[28] Keratinocytes express functional TLRs and play an important role in initiating and fine-tuning innate and adaptive immune responses in the skin via secretion of cytokines and chemokines in addition to killing microbes by producing antimicrobial peptides (β-defensins).[29] Human β-defensin-2 (HBD-2) is induced as a part of the inflammatory response in the skin, which in psoriasis, is a part of the regenerative maturation process involving hyperproliferation and induction of marker genes such as those encoding elafin and cytokeratins 6, 16 and 17. It was reported that HBD-2 and other skin β-defensins have cytokine-like properties in addition to their antimicrobial activity. The central role of these proteins in the innate immune system of the skin indicated that β -defensin genes may be candidate genes for psoriasis susceptibility.[30]

3.1.3.4 *Apoptosis*

Apoptosis is the general name for physiologic cellular suicide caused by activation of an energy-dependent suicide program.[31-33] This process can be triggered by various stimuli including cytokines, hormones, viruses and toxic insults. Changes at the cell surface include the externalization of phosphatidylserine and other alterations that promote recognition by the phagocytes. Intracellular changes include the degradation

of the chromosomal DNA into the high molecular weight oligonucleosomal fragments and the cleavage of a specific subset of cellular polypeptides. This cleavage is now known to be accomplished by a specialized family of cysteine-dependant aspartate-directed proteases called caspases, which has been referred to as “executioner” proteins because of their roles in apoptosis. Some caspases are also required in the immune system for the maturation of cytokines. Failure of apoptosis is one of the main factors in the development of tumors and autoimmune diseases.[34,35] Therapeutic modalities such as UVB phototherapy and PUVA for psoriasis were reported to induce apoptosis of lesional T lymphocytes.[36,37] Keratinocytes undergo terminal differentiation and finally cornification during their normal migration to the epithelial surface. This differentiation process is morphologically characterized first by cellular enlargement followed by shrinkage of the cytoplasm and nucleus within the upper part of the spinous layer. Their chromatin is finally degraded and the remaining nuclear structure dissolved within the stratum granulosum. The molecular mechanism of this process, called anoikosis resembles the apoptotic pathway. UVB-phototherapy induced keratinocyte apoptosis with an increase in caspase-3 activity in time after irradiation, reaching a maximum at 12 hours, after which it declined slightly but still remained at a high level.[38] Caspase-3 is one of the key executioners of apoptosis as it is either partly or totally responsible for the proteolytic cleavage of many key proteins leading to the characteristic “DNA ladder” seen in the apoptotic cells.[34] Induction of apoptosis by PDL was not observed in an experimental study using hamster skin.[39]

3.1.3.5 *Immune cell kinetics*

Antigen-reactive T cells enter the skin at sites that harbors antigen during the course of a normal cutaneous immune reaction. Subsequently, the antigen is eliminated by an immune mechanism appropriate to the eliciting antigen (Ag). The initial step of every specific immune reaction to an Ag is the recognition and the uptake of the Ag by professional APCs such as the DCs or the macrophages. The DCs are a rather unique cell type in that they may function in both innate and adaptive immunity. The second step is the migration of the cells to the T cell areas of local draining lymph nodes, the processing of the Ag and the presentation of selected Ag fragments (peptides) on the surface of the DCs to naïve T cells.[40] The third step is the migration of mature T cells to the skin and the various roles played by the cytokines released from these T cells and other cells.[41] In normal, non-inflamed skin, immature Langerhans cells (LCs) are present as a three-dimensional network in the suprabasal layers where they can monitor the environment as sentinel cells on-guard to detect foreign intruders. The immature LC is well suited for this task as it can phagocytose and then process antigen, and begins an efficient migratory pathway

towards the draining lymph node to initiate an immune response as a fully mature dentritic APC.[23] Upon antigenic stimulation by the APCs, the naïve $CD4^+$ T cells are activated, expand and differentiate into three different effector subsets termed T helper 1(Th1) , T helper 2 (Th2) and T helper 17 (Th17), which are characterized by the production of distinct cytokines and effector functions.[42] Th1 cells produce interferon-γ (IFN-γ), and lymphotoxin (LT) and can mobilize the cellular arm of the immune system to combat intracellular pathogens. Th2 cells secrete IL-4, IL-13 and IL-25, which are essential for the generation of appropriate classes of antibodies and for the elimination of extracellular phatogens.[43] Th17 cells play an important role in the host's defense against extracellular pathogens, which are not efficiently cleared by Th1-type and Th2-type response.[42] While these subsets have specific effector functions in clearing infections, unregulated expansion of $CD4^+$ Th effector T cells causes immunopathology. Excessive Th1 responses are associated with various autoimmune and inflammatory disorders, whereas enhanced Th2 cytokine production is involved in atopic diseases including allergies and asthma. The cutaneous immune reaction is overstrained in psoriasis.[18,40] The Th17 cytokines IL-17, IL-21 and IL-22 are highly pro-inflammatory and Th17 cells with specificity for self-antigens lead to severe autoimmunity. [42] IL-17 expression has been detected in the target tissue in human autoimmune disease such as rheumatoid arthritis and psoriatic lesions.[44] IL-12 and more recently, IL-23 have both been implicated in the pathogenesis of psoriasis. IL-12 plays an important role in the cell-mediated immune response, which is responsible for the defense against certain intracellular bacterial and parasitic infections. IL-12 is composed of a covalently linked heavy chain (IL-12p40) and a light chain (IL-12p35). The receptor comprises two transmembrane subunits, IL-12Rß1 and IL-12Rß2. [45] IL-12 is produced by the DCs cells and the macrophages. It induces differentiation of CD4 naïve T cells to Th1 cells and activates NK cells. [46] These Th1 cells and activated NK cells produce interferon IFN-γ and other type 1 cytokines such as IL-2 and TNF-ß. IFN-γ plays a critical role in the pathogenesis of psoriasis by facilitating T cell infiltration into the epidermis and induce keratinocyte proliferation.[47] IL-23 is a more recently described cytokine that is structurally closely related to IL-12. Both cytokines are heterodimers that share the common subunit IL-12p40 (IL-12p40 + IL-23p19 = IL-23; IL-12p40 + IL-12p35 = IL-12). Furthermore, the receptor for IL- 23 shares the common subunit, IL-12Rß1, to which the common IL-12p40 subunit binds. The dominant role of IL-23 involves the stimulation of a subset of $CD4^+$ T cells (sometimes called IL-17 T cells) to produce IL-17.[48] IL-17 induces the production of pro-inflammatory cytokines predominately by the endothelial cells and the macrophages. It is believed that IL-17 and IFN-γ synergize to increase the production of pro-inflammatory cytokines by the keratinocytes, which is important for the development of inflammation in the skin seen in psoriasis.[49] IL-12 and IL-23 are

important in the pathogenesis of psoriasis. However, IL-12 and IL-23 deficiencies in human beings and knockout mice indicate a potential risk for increased susceptibility to infection, particularly with Mycobacterium and Salmonella species in patients treated with IL-12p40 targeting agents.[50]

3.1.3.6 *Vascular changes*

The first microscopically visible events in developing psoriatic plaques are the superficial peri-vascular infiltration of lymphocytes and monocytic cells and the dilation of the blood vessels in the dermal papillae.[51] T cells and macrophages appear in the dermal infiltrates of psoriatic lesions before the development of significant epidermal changes.[52] The migration of lymphocytes into the site of inflammation begins with the adhesion of the lymphocytes to the dermal papillary vascular endothelium via cell-cell interactions.[7,41] The passage of leukocytes from the blood vessels into the tissue occurs in five steps. Endothelial cells play a decisive role in these steps. In the first step (rolling), leukocytes roll along the blood vessel wall. Rolling reduces the flow velocity of the leukocytes and is mediated by the interaction between P- and E-selectin expressed by the endothelial cells and the selectin ligands expressed by the leukocytes. E-selectin is the endothelial ligand for CLA on memory T cells, which is responsible for skin-homing of memory T cells. E-selectin is normally not expressed on the endothelial cells of the cutaneous microvessels, but it is up-regulated during cutaneous inflammation facilitating the accumulation of T cells in the psoriatic skin.[53,54] Endothelial cells express both P- and E-selectin in the uninvolved skin, but their expression is highly enhanced in the psoriatic skin. In the second step of skin infiltration, the immune cells rolling along the blood vessel wall recognize chemokines presented by the endothelial cells and are activated (triggering). The third step of skin infiltration is characterized by the formation of tight adhesions between the endothelial cells and the immune cells (adhesion). This is achieved by integrins expressed on the immune cells and their ligands expressed on the endothelial cells. Chemokines induce the integrin-dependent adhesion of the immune cells to the endothelial cells and cause the rapid arrest of the immune cells. Lymphocyte function antigen-1 (LFA-1, CD11a／CD18) is an important integrin for skin homing. It binds to ICAM-1(CD54) and ICAM-2 (CD102) expressed by the endothelial cells. The passage of the immune cells through the endothelial wall is called diapedesis (fourth step) and occurs via pores formed between the endothelial cells. Endothelial cells in psoriatic lesions express a number of leukocyte-related adhesion proteins (CD31, CD34 and addressin) that are normally associated with high endothelial venules in lymph nodes by the action of T cell triggered vascular endothelial growth factor (VEGF). These venules have fenestrations allowing naïve cells entry into chronic lesions of psoriasis.[18] This is the last step is the migration into

the target tissue. The number of T cells in non-lesional psoriatic skin is higher than that in the skin from healthy participants.[55] In psoriatic skin lesions, CD8+ T cells primarily home into the epidermis, whereas CD4+ cells are mainly present in the dermis.[56] The reasons for these different anatomical homing patterns may be caused by the varied expression of chemokine receptors such as CXCR3 and integrins such as CD103 on CD4+ and CD8+ T cells. $CD45RO^{+}$ T cells constitute 36.6% of the proliferating dermal cells in involved skin.[57] Once keratinocytes synthesize ICAM-1 it is possible for LFA-1 positive T cells to migrate into the inflamed epidermis through LFA-1/ICAM-1 interaction. T cells may be able to migrate into the epidermis by adhesive interactions between αεβ7 integrin, which is expressed on the epidermal T1 cells and E-cadherin synthesized by the keratinocytes. Cytokines from intradermal T cells can trigger keratinocytes to synthesize and release IL-8, the main chemotactic signal for the recruitment of neutrophils into the epidermis from the vascular stores. Once arrived in the epidermal regions, the activated T cells release Th1 cytokines including IFN-γ, IL-2 and TNF-α. IL-2 is associated with psoriasis and is a known T-cell growth stimulator.[58] TNF-α is a potent inducer of ICAM-I, CD40 and major histocompatibility complex (MHC) class II proteins on the epidermal keratinocytes and stimulates angiogenesis.[18] Persistence of mature LCs, which produce abundant IL-12 provides the background for direct stimulation of Th1 cell subsets in the lesional psoriatic skin.[18] In normal skin, the superficial microvasculature is composed of capillary loops, which arise from the terminal arterioles in the upper horizontal dermal vascular plexus, pass up into the dermal papilla and arch back to connect with post-capillary venules in the horizontal plexus.[59] Non-lesional skin has short lengths of microvessels in the superficial, papillary dermis, whereas lesional psoriatic skin is characterized by dilated and elongated superficial capillaries passing into the dermal papillae, representing exaggerated tortuosity, coiling of the apical segment of the capillary loop, neo-angiogenesis and high endothelial venule (HEV) formation.[60-62] HEV formation may be important for the extravasation and trafficking of the activated T cells in the psoriatic lesions.[62] Vascular defects in psoriasis are confined to the superficial capillary loops. Factors secreted by the epidermis such as the VEGF govern the vascular changes in order to support the increased nutritional requirement during the disease activity.[63,64] If the hypothesis that the reduced resistance of the expanded capillary bed is responsible for the elevated blood flow in psoriatic plaques is true, then elimination of this capillary component should normalize blood flow. The diameter measurements of psoriatic capillaries vary from 6- to 17-micrometer.[61,65,66] An average basal flux of 35.15 ± 40.38 AU was recorded in port wine stain skin using laser Doppler flowmetry.[67] This was less than the basal flux values recorded in psoriatic plaque skin (269.79 ± 232.37).[68] These results indicated that the capillary expansion by itself is not sufficient to generate a substantially elevated blood flow

and further supports the hypothesis that other factors such as an increased number of resistant vessels and/or chronic structural widening of pre-existing arterioles may contribute in the grossly elevated blood flow in psoriatic plaque skin.

During the last decade, the lymphatic system has been the subject of increasing interest with the discovery of specific factors among which are the lymphatic growth factors VEGFC and VEGF-D.[69] Their tyrosine kinase receptor VEGFR3 found in venous and lymphatic endothelial cells during embryogenesis becomes restricted to lymphatic cells early in the postnatal period except for the fenestrated blood vessels of some organs such as endocrine glands.[70] The activation of VEGFR3 by VEGF-C or VEGF-D leads to the proliferation of the lymphatic endothelial cells while targeted deletion of VEGF-C in mice impairs the development of small lymphatic vessels.[69] Lymphatic vessels are expanded in psoriasis, but little is known on the mechanism of their development in the lesions and the potential alterations in the non-involved skin of patients with psoriasis. Vascular volume and the expression of ICAM-1 and E-selectin were reported to be increased in the non-lesional psoriatic skin.[71, 72] These observations indicated the presence of a pre-psoriatic phenotype in the distant non-lesional skin, but little is known on their potential significance in the patho-mechanisms in psoriasis.[73] The expression of the different isoforms of VEGF-A, VEGFR2, VEGF-C, VEGFR3 and prox-1 are higher in lesional psoriatic skin than in non-lesional skin.[73] There is a four-fold increase in the endothelium of the superficial microvasculature in lesional psoriatic skin, but not in the deeper vasculature indicating that microvascular expansion is restricted to the upper plexus.[74] Current evidence such as the rapid response of psoriasis to cyclosporine, which inhibits T cell activation and cytokine release indicates that psoriasis is primarily a T cell-dependent disease.[75] However, the prominent increase in the dermal microvasculature in the lesional skin indicates that psoriasis is also angiogenesis-dependent.[76-78]

3.1.4 *Treatment of psoriasis, adverse effects and Pulsed Dye Laser (PDL)*

Traditional therapies for psoriasis have focused on the inhibition of epidermal proliferation and/or suppression of inflammation. Treatments include monotherapy or combinations of topical vitamin D analogues, tazarotene, corticosteroids, anthralin tar and salicylic acid, phototherapy (broad-band and narrow-band UVB and Psoralen-UVA) and systemic treatments with retinoids, methotrexate (MTX), cyclosporine CsA), fumaric acid esters and more recently biologicals. Limiting factors are that none of these agents is effective in all patients. Each treatment may also have adverse effects and the effects of the treatment generally decrease over time.[79,80] On the one hand, topical therapy is less effective as compared with photo-(chemo) therapy and systemic treatments, but on the other hand, adverse effects of topical treatments are generally less severe than those of photo-(chemo) therapy and systemic treatments

(Table 4). Combinations of treatments associated with increased toxicity are bone marrow suppressants (e.g., methotrexate, hydroxyurea and 6-thioguanine) and drugs that may increase the risk of skin cancer (e.g. cyclosporine and PUVA).
The treatment with the most optimum efficacy/adverse effect profile for the particular patient should be chosen because of the diverse presentations of psoriasis, the patient's individual needs and other coexistent factors.
The alterations in the capillaries have been neglected as a possible therapeutic target, although it was reported that in a new psoriasis lesion one of the earliest observable changes is an increase in the dermal papillary vasculature.[76-77,78,81] The clinical improvement during the treatment is preceded by micro-vascular improvement.[82] This indicated, that the expanded superficial micro-vascular bed in psoriasis skin is an essential component for maintaining clinical lesions.[82] These observations have led to the hypothesis, that in psoriasis selective destruction of the dilated papillary vessels by selective photothermolysis may reduce the transmigration of inflammatory cells resulting in the clearing of the psoriasis plaques (Figures 13 and 14).[83] Selective photothermolysis, destroying only the dilated papillary vessels, but sparing the normal sized vessels and without causing damage to the epidermis became a reality after the introduction of the PDL machine.[84,85] Partial and total clearance of psoriasis after PDL treatment was reported in several studies.[86-91] Efficacy and safety aspects of PDL treatment of plaque type psoriasis were studied in this thesis.

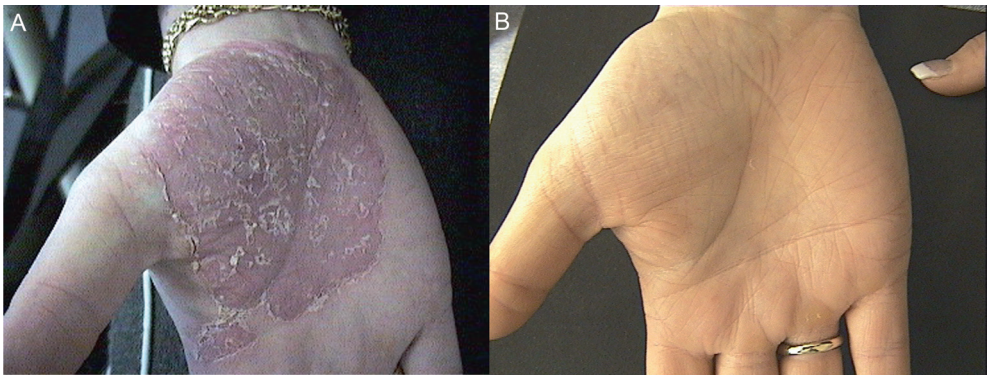

Figure 13. A. Psoriasis palmaris, woman 22 years old, duration of psoriasis 8 years.
B. PD-laser after 5 treatments with concomitant calcipotriol ointment, stable with 1 PDL treatment per 3 months.

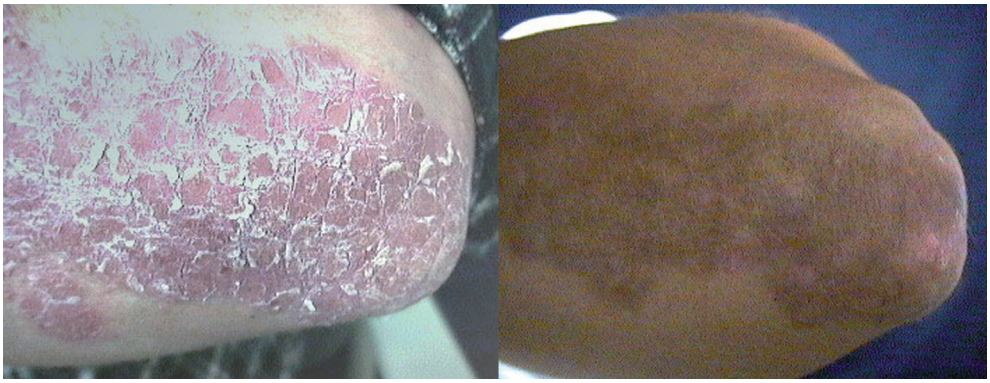

Figure 14. Psoriasis PDL treatment before and after pictures.
Side effect: hyperpigmentation

Topical therapy	*Common adverse effects*
Salicylic acid	Risk of salicylate toxicity with application to more than 20% body surface area
Vitamin D3 derivatives Calcipotriol Calcitriol	Skin irritation; risk of hypercalciuria and hypercalcemia at more than 100 gram a week
Vitamin A derivative Tazarotene	Tazarotene is a pregnancy category X drug Not registered in the Netherlands
Topical corticosteroids	Skin atrophy, striae distensae, teleangiectases, purpura, acne, skin infections, Cushing's syndrome, hypothalamic-pituitary-adrenal axis suppression

Tars	Skin irritation, folliculitis, phototoxic reactions, staining of skin and clothes, odor, carcinogenicity following tar application in psoriatics remains unconfirmed, renal dysfunction during large body surface treatment
Dithranol (anthralin)	Skin irritation, burning, erythema, staining of skin and clothes
Phototherapy	*Common adverse effects*
Narrow-band UVB	Erythema and burning from overexposure, premature aging of the skin, carcinogenic risk
PUVA	Phototoxic reactions, lentigines, induction of photo-aging, increased risk of squamous cell carcinoma and malignant melanoma, ingestion of oral psoralen may produce nausea, oral psoralen is a pregnancy category X drug
Systemic therapy (conventional)	*Common adverse effects*
Fumaric acid esters	Flushing, nausea, vomiting, stomachache, diarrhea, depression, edema in the legs, anemia, leucopenia, thrombocytopenia, hepato- and renal toxicity, contra-indicated during pregnancy and lactation (pregnancy category X drug)
Methotrexate	Hepato-toxicity, gastrointestinal symptons, malaise, headache, reactivation of phototoxic reactions, ulcerative stomatitis, myelosuppression, anemia, pneumonitis, pulmonary fibrosis, induction of lymphomas, teratogenicity, methotrexate is a pregnancy category X drug.
Cyclosporin	Renal toxicity, hypertension, gastrointestinal symptons, flu-like symptoms, hypertrichosis, gingival hypertrophy, increase in cutaneous malignancies, cyclosporine is a pregnancy category C drug
Acitretin	Teratogenicity, hepato-toxicity, hyperostosis, hyperlipidemia, mucocutaneous adverse effects, such as dry skin, cheilitis sicca, dermatitis and alopecia, arthralgias and myalgias, pseudo-tumor cerebri, acitretin is a pregnancy category X drug and should not be used during breast-feeding

Systemic therapy (biologicals)	*Common adverse effects*
Infliximab	Upper respiratory tract infections, acute infusion reactions with fever, chills, chest pain, hypotension, nausea and dyspnea, anaphylactoid reactions, reactivation of latent tuberculosis, six-fold greater risk of lymphomas, increase of liver enzymes, de-myelinating diseases, cardiac insufficiency, leucopenia, thrombocytopenia, pancytopenia, lupus-like syndrome
Etanercept	Transient injection-site reactions with itching, pain, erythema and swelling, infections of the upper respiratory tract, bronchitis, skin infections, lupus-like syndrome and positive ANA, lymphoma, de-myelinating disorders of the central nervous nervous system, congestive heart failure
Efalizumab	Removed from the market by the EMEA because of progressive multi-focal leucoencephalopathy
Adalimumab	Pain at the injection site, infections, malignancy, development of anti-nuclear antibodies
Alefacept	T-lymphocyte depletion. Not approved by the EMEA in Europe

Table 4. Overview of anti-psoriatic therapies and their adverse effects.
Category C: drugs in which risk cannot be ruled out during pregnancy and lactation because of inadequate data, two-thirds of all drugs fall in this category. Category X: drugs that are absolutely contra-indicated during pregnancy. EMEA = European Medicines Agency.

3.1.5 References

1. Glickman FS. Lepra, psora, psoriasis. J Am Acad Dermatol 1986; 14: 863-866.
2. Christophers E. Psoriasis-epidemiology and clinical spectrum. Clin Exp Dermatol 2001; 26: 314-320.
3. Roenigh HH, Maibach HI. Psoriasis third edition, 1998, Marcel Dekker, inc. pp 112-113, 543-557.
4. Schon MP, Boehncke WH. Psoriasis. N Engl J Med 2005; 352: 1899-1912
5. Ferrandiz C, Pujol RM, Garcia-Patos V, Bordas X, Smandia JA. Psoriasis of early and late onset: A clinical and epidemiologic study from Spain. J Am Acad Dermatol 2002; 46: 867-873.
6. Finlay AY, Khan GK, Luscombe D, Salek MS. Validation of sickness impact profile and psoriasis disability index in psoriasis. Br J Dermatol 1990; 123: 751-756.
7. Lebwohl M. Psoriasis. The lancet 2003; 361: 1197-1204
8. McFadden JP, Baker BS, Powles AV, Fry L. Psoriasis and streptococci: the natural selection of psoriasis revisited. Br J Dermatol 2009; 160: 929-937.
9. Creamer D, Sullivan D, Bicknell R, Barker J. Angiogenesis in psoriasis. Angiogenesis 2002; 5: 231-236.
10. Farber EM, Nickoloff BJ, Recht B, Prahi JE. Stress, symmetry and psoriasis: possible role of neuropeptides. J Am Acad Dermatol 1986; 14:305-311.
11. Naldi L, Peli L, Parazzini F, France Carrel C. Family history of psoriasis, stressful life events, and recent infectious disease are risk factors for a first episode of acute guttate psoriasis: results of a case-control study. J Am Acad Dematol 2001; 44: 433-438
12. Schmid-Ott G, Jaeger B, Boehm T, Langer K, Stephan M, Raap U, Werfel T. Immunological effects of stress in psoriasis. Br J Dermatol 2009; 160: 782-785.
13. Poikolainen K, Reunala T,Karvonen J. Smoking, alcohol and life events related to psoriasis among women. Br J Dermatol 1994; 130: 473-477.
14. Fry L, Baker BS. Triggering psoriasis: the role of infections and medications. Clin Dermatol 2007; 25: 606–615.
15. Leigh IM, Navsaria H, Purkis PE, McKay IA, Bowden PE, Riddle PN. Keratins (Kl6 and Kl7) as markers of keratinocyte hyperproliferation in psoriasis in vivo and in vitro. Br J Dermatol 1995; 133: 501-511.
16. Ando M, Kawashima T, Kobayashi H, Ohkawara A. Immunohistological detection of proliferating cells in normal and psoriatic epidermis using Ki-67 monoclonal antibody. J Dermatol Sci 1990; 1: 441-446.
17. Lowes MA, Bowcock AM, Krueger JG. Pathogenesis and therapy of psoriasis.

Nature 2007; 445: 866-873.
18. Krueger JG. The immunologic basis for the treatment of psoriasis with new biologic agents. J Am Acad Dermatol 2002; 46: 1-23.
19. Jullien D, Barker JN. Genetics of psoriasis. J Eur Acad Dermatol Venereol 2006; 20 (Suppl. 2): 42–51.
20. Barker JNWN. Genetic aspects of psoriasis. Clin Exp Dermatol. 2001; 26: 321-325.
21. Bowcock AM, Barker JN. Genetics of psoriasis: The potential impact on new therapies. J Am Acad Dermatol 2003; 49: S51-56.
22. Schwarz T. Skin immunity. Br J Dermatol 2003; 149 (Suppl. 66): 2-4.
23 Nickoloff BJ. Cutaneous dendritic cells in the crossfire between innate and adaptive immunity. J Dermatol Sci 2002; 29: 159-165.
24 Gilliet M, Conrad C, Geiges M, Cozzio A, Thürlimann W, Burg G, Nestle FO, Dummer R Psoriasis triggered by Toll-like receptor 7 agonist Imiquimod in the presence of dermal plasmacytoid dendritic cell precursors. Arch Dermatol. 2004; 140: 1490-1495.
25 Arancibia SA, Beltran CJ, Aguirre IM, Silva P, Peralta AL, Malinarich F, Hermoso MA. Toll-like Receptors are Key Participants in Innate Immune Responses. Biol Res 2007; 40: 97-112.
26 Pivarcsi A. Toll-Like Receptor 9 independent suppression of skin Inflammation by oligonucleotides. J Invest Dermatol 2007; 127: 746-748.
27 Vollmer J. TLR9 in health and disease. Int Rev Immunol 2006; 25:155–181.
28 Terui T, Ozawa M, Tagami H. Role of neutrophils in induction of acute inflammation in T-cell mediated immune dermatosis, psoriasis: a neutrophil-associated inflammation-boosting loop. Exp Dermatol 2000; 9: 1-10.
29. Nagy I, Pivarcsi A, Koreck A, Szell M, Urhan E, Kemeny L. Distinct strains of propionibacterium acnes induce selective human beta-defensin-2 and interleukin-8 expression in human keratinocytes through toll-like receptors. J Invet Dermatol 2005; 124: 931-938.
30. Hollox EJ, Huffmeier U, Zeeuwen PLJM, Palla R, Lascorz J, Rodijk-Olthuis D, van de Kerkhof PCM, Traupe H, de Jongh G, den Heijer M, Reis A, Armour JAL, Schalkwijk J. Psoriasis is associated with increased β -defensin genomic copy number. Nature Genetics 2008; 40: 23-25.
31. Cohen JJ. Apoptosis. Immunol today. 1993; 14: 126-130.
32. Raskin CA. Apoptosis and cutaneous biology. J Am Acad Dermatol. 1997; 36: 885–896.
33. Haake AR, Polakowska RR. Cell death by apoptosis in epidermal biology. J Invest Dermatol 1993; 101: 107-112.

34. Earnshaw WC, Martins LM, Kaufmann SH. Mammalian caspases: structure, activation, substrates, and functions during apoptosis. Annu Rev Biochem 1999; 68: 383-424.
35. Thornberry NA, Lazebnik Y. Caspases: enemies within. Science 1998; 281: 1312-1316.
36. Krueger JG, Wolfe JT, Nabeya RT, Vallat VP, Gilledeau P, Heftler NS, Austin LM, Gottlieb AB. Successful ultraviolet B treatment of psoriasis is accompanied by a reversal of keratinocyte pathology and by selective depletion of intraepidermal T cells. J Exp Med 1995; 182: 2057-2068.
37. Coven TR, Walters IB, Cardinale I, Krueger JG. PUVA-induced lymphocyte apoptosis: Mechanism of action in psoriasis.. Photodermatol Photoimmunol Photomed 1999: 15: 22-27.
38. Mass P, Hoffmann K, Gambichler T, Altmeyer P, Mannherz HG. Premature keratinocyte death and expression of marker proteins of apoptosis in human skin after UVB exposure. Arch Dermatol Res 2003; 295: 71-79.
39. Babilas P, Shafirstein G, Baumler W, Baier J, Landthaler M, Szeimies RM, Abels C. Selective Photothermolysis of blood vessels following Flashlamp-Pumped Pulsed Dye Laser irradiation: In vivo results and mathematical modelling are in agreement. J Invest Dermatol 2005; 125: 343 –352.
40. Sabat R, Philipp S, Höflich C, Kreutzer S, Wallace E, Asadullah K, Volk HD, Sterry W, Wolk K
Immunopathogenesis of psoriasis. Exp Dermatol 2007, 16, 779–798.
41 Mehlis SL, Gordon KB. The immunology of psoriasis and biologic immunotherapy. J Am Acad, Dermatol 2003; 49: S44-50.
42. Bettelli E, Korn T, Kuchroo VK. Th17: the third member of the effector T cell trilogy Current Opion Immunol 2007; 19: 652-657.
43. Mosmann TR, Coffman RL. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. Ann Rev Immunol 1989; 7: 145-173.
44. Steinman L. A brief history of T(H)17, the first major revision in the T(H) 1/T(H)2 hypothesis of T cell-mediated tissue damage. Nat Med 2007; 13: 139-145.
45. Hunter CA. New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. Nat Rev Immunol 2005; 5: 521-531.
46. Robertson MJ, Ritz J. Interleukin 12: basic biology and potential applications in cancer treatment. Oncologist 1996; 1: 88-97.
47. Hong K, Chu A, Ludviksson BR, Berg EL, Ehrhardt RO. IL-12, independently of IFN-gamma, plays a crucial role in the pathogenesis of a murine psoriasis-like skin disorder. J Immunol 1999; 162: 7480-7491
48. Aggarwal S, Ghilardi N, Xie MH, de Sauvage FJ, Gurney AL. Interleukin-23

promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. J Biol Chem 2003; 278: 1910-1914.
49. Teunissen MB, Koomen CW, de Waal Malefyt R, Wierenga EA, Bos JD. Interleukin-17 and interferon-gamma synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. J Invest Dermatol 1998; 111: 645-649.
50. Torti DC, Feldman SR. Interleukin-12, interleukin-23, and psoriasis: Current prospects. J Am Acad Dermatol 2007; 57: 1059-1068.
51. Ragaz A, Ackerman A B. Evolution, maturation and regression of lesions of psoriasis. New observations and correlation of clinical and histologic findings. Am J Dermatopathol 1979: 1: 199–214.
52. Bjerke JK, Krough HK, Matre R. Characterization of mononuclear cell infiltrates in psoriatic lesions. J invest Dermatol 1978; 71: 340-343.
53. Groves RW, Allen MH, Barker JNWN, Haskard DO, MacDonald DM. Endothelial leucocyte adhesion molecule-1 (ELAM-1) expression in cutaneous inflammation. Br J Dermatol 1991; 124: 117-23.
54. Picker LJ, Kishimoto TK, Wayne Smith C, Warnock RA, Butcher EC. ELAM-1 is an adhesion molecule for skin-homing T cells. Nature 1991; 349: 796-799.
55. Lowes MA, Chamian F, Abello MV et al. Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). Proc Natl Acad Sci USA 2005: 102: 19057–19062.
56. Bos JD, Hagenaars C, Das PK, Krieg SR, Voorn WJ, Kapsenberg ML. Predominance of 'memory' T cells (CD4+, CDw29+) over 'naive' T cells (CD4+, CD45R+) in both normal and diseased human skin. Arch Dermatol Res 1989: 281: 24–30.
57. Morganroth GS, Chan LS, Weinstein GD, Voorhees JJ, Cooper KD. Proliferating cells in psoriatic dermis are compromised primarily of T cells, endothelial cells, and factor XIIIa+ perivascular dendritic cells. J Invest Dermatol 1991; 96(3): 333-340.
58. Lee RE, Gaspari AA, Lotze MT, Chang AE, Rosenberg SA. Interleukin 2 and psoriasis. Arch Dermatol 1988; 124: 1811-1815.
59. Yen A, Braverman IM. Ultrastructure of the human microcirculation: the horizontal plexus of the papillary dermis. J Invest Dermatol 1976; 66: 131-142.
60. Braverman IM, Yen A. Ultrastructure of the capillary loops in the dermal papillae of psoriasis. J Invest Dermatol 1977; 68: 53-60.
61. Bull RH, Bates DO, Mortimer PS. Intravital video-capillaroscopy for the study of the circulation in psoriasis. Br J Dermatol 1992; 126: 436-445.
62. Guenther LC, Ortonne JP. Pathophysiology of psoriasis: science behind

therapy. J Cutan Med Surg 2002; 6: 2-7.
63. Detmar M, Brown LF, Claffey KP et al. Overexpression of vascular permeability factor/vascular endothelial growth factor and its receptors in psoriasis. J Exp Med 1994; 180: 1141-1146.
64. Detmar M, Yeo KT, Nagy JA. et al. Keratinocyte-derived vascular permeability factor (vascular endothelial growth factor) is a potent mitogen for dermal microvascular endothelial cells. J. Invest Dermatol 1995; 105: 44-50.
65. Mordovstev VN, Albanova VI. Morphology of skin microvasculature in Psoriasis. Am J Dermatopathol 1989; 11 :33-42
66. Bravermann IM. The cutaneous microcirculation: ultrastructure and microanatomical organisation. Microcirculation 1997; 4: 329-340.
67. Jernbeck J, Malm M. Calcitonin gene-related peptide increases the blood flow of port wine stains and improves continues wave dye laser treatment. Plast Reconstr Surg 1993; 91: 245-251.
68. Hern S, Stanton AWB, Mellor RH, Harland CC, Levick JR, Mortimer PS. Blood flow in psoriatic plaques before and after selective treatment of the superficial capillaries. Br J Dermatol 2005; 152: 60-65.
69. Karkkainen M, Haiko P, Sainio K et al. Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins. Nat Immunol 2004; 5: 74–80.
70. Partanen TA, Arola J, Saaristo A et al. VEGF-C and VEGF-D expression in neuroendocrine cells and their receptor, VEGFR-3, in fenestrated blood vessels in human tissues. FASEB J 2000; 14: 2087–2096.
71. Barton SP, Abdullah MS, Marks R. Quantification of microvascular changes in the skin in patients with psoriasis. Br J Dermatol 1992; 126: 569–574.
72. De Boer OJ, Wakelkamp IMJ, Pals ST et al. Increased expression of adhesion receptors in both lesional and non-lesional psoriatic skin. Arch Dermatol Res 1994; 286: 304–311.
73. Henno A, Blacher S, Lambert C, Colige A, Seidel , Noël A, Lapière, de la Brassinne M, Usgens BV. Altered expression of angiogenesis and lymphangiogenesis markers in the uninvolved skin of plaque-type psoriasis. Br J Dermatol 2009; 160: 581-590.
74. Creamer D, Allen MH, Sousa A, Poston R, Barker JNWN. Localisation of endothelial proliferation and microvascular expansion in active psoriasis. Br J Dermatol 1997; 136: 859-865.
75. Ellis CN, Fradin MS, Messana JM, Brown MD, Siegel MT, Hartley AH et al. Cyclosporine for plaque-type psoriasis. results of a multidose, double-blind trial. N Engl J med 1991; 324: 277-284.
76 Pinkus H, Mehregan AH. The primary histologic lesion of seborrhoeic dermatitis

and psoriasis. J Invest Dermatol 1966; 46:109-116.
77 Goodfield M, Macdonald Hull S, Holland D et al. Investigations of the “active” edge of plaque psoriasis: Vascular proliferation precedes changes in epidermal keratin. Br J Dermatol 1994; 131: 808-813.
78. Barker JNWN. Pathophysiology of psoriasis. Lancet 1991; 338: 227-230.
79. Lebwohl M. A clinician’s paradigm in the treatment of psoriasis. J Am Acad Dermatol 2005; 53: s59-69.
80. Macdonald Hull S, Goodfield M, Wood EJ, Cunliffe WJ. Active and inactive edges of psoriatic plaques: identification by tracing and investigation by laser-Doppler flowmetry and immunocytochemical techniques. J Invest Dermatol 1989; 92: 782-785.
81. Heng MCY, Allen SC, Haberfelde G, Song MK. Electronmicroscopic and immunohistochemical studies of the sequence of events in psoriatic plaque formation following tape-stripping. Br J Dermatol 1991; 125: 548-556.
82. Hacker S, Rasmussen J. The effect of flash lamp-pulsed dye laser on psoriasis. Arch Dermatol 1992; 128: 853-855.
83. Anderson RR, Parrish JA. Microvasculature can be selectively damaged using dye lasers: a basic theory and experimental evidence in human skin. Lasers Surg Med 1981; 1: 263-276.
84. Anderson RR, Parrish JA. Selective photothermolysis: precise microsurgery by selective absorption of pulsed radiation. Science 1983; 220: 524-527.
85. Ros AM, Garden JM, Bakus AD, Hedblad MA. Psoriasis response to the pulsed dye laser. Lasers Surg Med 1996; 19: 331-335.
86. Katugampola GA, Rees AM, Lanigan SW. Laser treatment of psoriasis. Br J Dermatol 1995; 133: 909-913.
87. Zelickson BD, Mehregan DA, Wendelschfer-Crabb G et al. Clinical and histologic evaluation of psoriatic plaques treated with a flashlamp pulsed dye laser. J Am Acad Dermatol 1996; 35: 64-68.
88. Bjerring P, Zachariae H, Søgaard H. The flashlamp-pumped Dye Laser and dermabrasion in Psoriasis- Further studies on the reversed Köbner phenomenon. Acta Derm Venereol (Stockh) 1997; 77: 59-61.
89. Hern S., Allen M.H., Sousa A.R., Harland C.C., Barker J.N.W.N., Levick J.R., Mortimer P.S. Immunohistochemical evaluation of psoriatic plaques following selective photothermolysis of the superficial capillaries. Br J Dermatol 2001; 145: 45-53.
90. Erceg A, Bovenschen HJ, Kerkhof van der PCM, Seyger MMB. Efficacy of the pulsed dye laser in the treatment of localized recalcitrant plaque type psoriasis: a comparative study. Br J Dermatol 2006; 155; 110-114.
91. Norobio R, Kurokawa M, Kabayasi K, Morita A. Evaluation of the clinical and

immunohistological efficacy of the 585-nm pulsed dye laser in the treatment of psoriasis. J Eur Acad Dermatol 2009; 23: 420-424.

3.2 Vitiligo

3.2.1 *Epidemiology and clinical aspects*

Vitiligo is an acquired disease characterized by scattered, sharply demarcated white macules. These de-pigmented macules often appear symmetrically, usually on the face (Figure 15), the nape, the axillae, the elbows, the hands (Figure 16), the knees and the genitals. Vitiligo usually occurs in a localized or generalized pattern, but rarely in a dermatomic area. Epidermal and mucosal variants occur. The course of vitiligo is usually one of slow progression, but it may exacerbate rapidly or stabilize. Vitiligo may also re-pigment spontaneously, but only partially.[1] Histopathological and histochemical examinations showed a reduction in the number and the function of melanocytes in vitiligo lesions.[2] Usually, the diagnosis is clinically obvious and often skin biopsies for the histopathological evaluation is unnecessary. The prevalence of vitiligo in the general population is 1% to 2%, without race or gender predilection and with an onset peak at an age of between 10 and 30 years. A genetic component is likely because there is a positive family history in about 30% of the patients.[3,4] Vitiligo is commonly considered to be only a cosmetic problem, but the implications of vitiligo are beyond the limits of a cosmetic disease. The impact on the quality of life of the patient is comparable with that in psoriasis on the emotional and socially functioning scales.[5-8] In addition, many patients experience phototoxic reactions in the de-pigmented macules after exposure to sunlight in some patients in spite of the use of high sun protection factor agents.

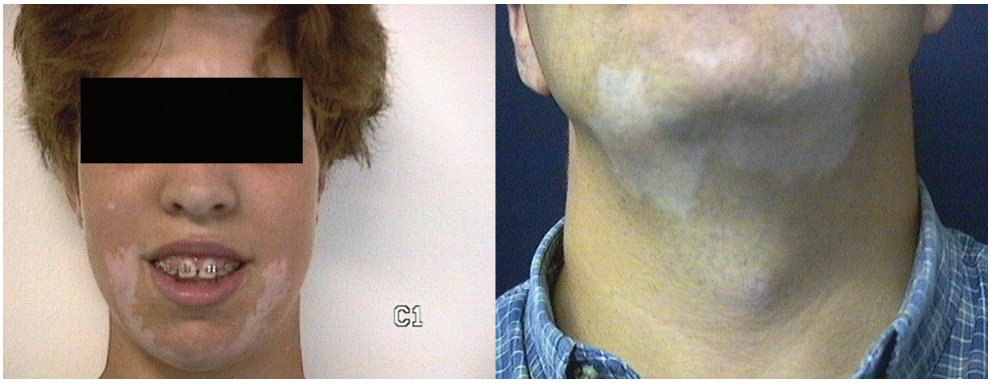

Figure15. Facial vitiligo

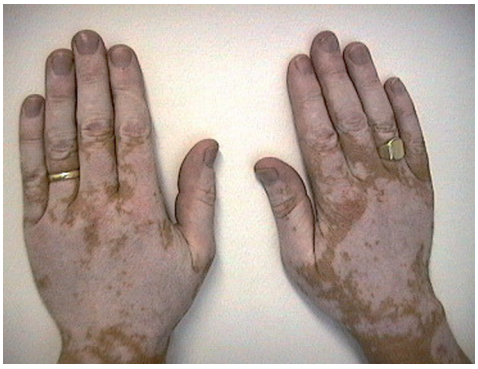

Figure 16. Vitiligo of the hands

3.2.2 *Pathogenesis*

The exact etiology of vitiligo remains obscure to date, but many factors including infections, stress, neural abnormalities, melatonin receptor dysfunction, impaired melanocyte migration and genetic susceptibility have been implicated in the development of the disease.[9,10] The cause of vitiligo is not yet fully understood. However, it is clear that there is a lack of functional melanocytes in the affected skin. There are several hypotheses on the pathogenesis of vitiligo, but none of them fully explain the disease. Four hypotheses are particularly important.[11] These are as follows:

1). The autoimmune hypothesis is based on the observation that several autoimmune diseases such as autoimmune thyroid disease and type I diabetes mellitus often accompany vitiligo. Moreover, vitiligo patients may have elevated serum levels of antibodies against melanocytic antigens such as tyrosinase and tyrosinase-related proteins 1 and 2. Several studies have reported a role for both cellular and humoral immunity in the pathogenesis of vitiligo.[12-14]

2). The neural hypothesis assumes that the altered reactions of melanocytes towards neuropeptides, catecholamines and their metabolites are responsible for the destruction of melanocyte. A close contact between melanocytes and nerve endings that is rarely seen in normal skin is observed in the de-pigmented skin. In addition, degenerated and regenerated autonomous nerve fibers and thickened basement membranes of Schwann cells are present at the center and at the periphery of the de-pigmented skin lesions. Nerve endings at such sites show aberrations in the expression of nerve growth factor (NGF) and neuropeptides.[11]

3). The self-destruct hypothesis states that melanocytes destroy themselves because of the defects in the protective mechanisms that are responsible for eliminating toxic melanin precursors. These defects lead to the accumulation of indole derivatives and free radicals, which are melanotoxic.

4). The biochemical hypothesis argues that the destruction of melanocytes

destruction is because of the accumulation of toxic metabolites of melanogenesis, the breakdown of free-radical defense and an excess of hydrogen peroxide.[15] The biochemical hypothesis assumes an over-synthesis of hydrobiopterin, a cofactor of tyrosine hydroxylase, resulting in an increased synthesis of catecholamine. This also results in an increase in the reactive oxygen species (ROS) toxic for melanocytes. Moreover, reduced levels of catalase in combination with higher concentrations of hydrogen peroxide were found in affected and unaffected skin of vitiligo patients.[16] Ultimately, all of these different factors may act independently or together to yield the same effect, namely the disappearance of melanocytes from the skin and this was proposed in the convergence theory.[10] For example, autoimmunity may arise as a secondary phenomenon following the self-destruction of pigment cells and this may then amplify the damage to the melanocytes. Different pathogenic mechanisms may account for the various clinical types of vitiligo. The neural theory is usually related to the segmental vitiligo, whereas the autoimmune hypothesis is thought to be involved in the generalized (non-segmental) form of the disorder. Indeed, non-segmental vitiligo is characterized by an association with autoimmune diseases and with unstable results after autologous melanocyte grafting.[17] Vitiligo is often associated with other autoimmune conditions such as autoimmune thyroid disease, autoimmune poly-endocrine syndrome types 1 and 2, pernicious anemia, type 1 diabetes mellitus, Addison's disease, alopecia areata, systemic lupus erythematosus, rheumatoid arthritis, psoriasis and myasthenia gravis.[9] Furthermore, these same autoimmune diseases occurred at an increased frequency in the first-degree relatives of the patients.[18,19]

Histological examination of skin biopsies from vitiligo patients showed that inflammatory cells were most prominent at the periphery of the vitiligo lesions.[20] The dermal and the epidermal infiltrate consisted of cytotoxic and helper T cells that were closely associated with the areas of melanocyte depletion. Expression of the MHC class II antigen HLA-DR indicated that many of the infiltrating T cells were activated and a significant number of T cells at the edges of the vitiligo lesions also showed high levels of the expression of CLA antigen, which is typical for skin-homing T cells.[20,21]

In addition, there was evidence for the expression of IL-2 and IFN-γ receptors on the lymphocytic infiltrate.[22] Although these findings do not distinguish between whether the immune infiltrate is the result or the cause of the disease, the results indicate the involvement of local immune reactivity in melanocyte destruction.[9]

There is evidence that both cellular and humoral immunity can act together in vitiligo pathogenesis. Firstly, both T and B cells were found to be simultaneously increased in patients with recent onset vitiligo.[23] Secondly, both autoantibodies and T lymphocytes reduced the number of pigment cells in an in vitro experiment

in a dose-dependent manner.[24] Re-pigmentation in vitiligo patients treated with immunosuppressive agents such as corticosteroids, ultraviolet (UV) radiation with psoralen (PUVA), and cytotoxic drugs like 5-fluorouracil indirectly supports the idea that an autoimmune-mediated process is involved in vitiligo pathogenesis. [25-27]
UV radiation can induce the expression of anti-inflammatory cytokines, modulate the expression of ICAM-1, interleukin (IL) receptors (R) 1 and 2 and induce apoptosis of skin-infiltrating T lymphocytes.[28-30] Furthermore, HLA-DR antigens, Fc and C3b receptors are decreased on Langerhans cells (LCs) after UV treatment which may adversely affect the role of LCs. Following PUVA therapy, a reduction in the number of LCs was noted in vitiligo patients and a decrease in the expression of a vitiligo-associated melanocytic antigen was observed, which could have inhibited the antibody-dependent cell-mediated cytotoxicity (ADCC) against pigment cells.[31,32]
Tacrolimus exerts an immunosuppressive effect on T cells by blocking the action of the cytokine gene-activating cofactor calcineurin. Topical application of tacrolimus to vitiligo lesions resulted in successful re-pigmentation.[33,34] This treatment again indicated the role of immune reactions in vitiligo pathogenesis.
The final result of all the pathogenic mechanisms involved during the de-pigmenting process in vitiligo is melanocyte destruction and an absence of pigmentation.[35] However, the melanocytes in the hair follicles usually remain unaffected. During the process of re-pigmentation, these melanocytes proliferate and mature to an active state as they migrate into the epidermis.[36]

3.2.3 *Treatment*

Therapeutic approaches are aimed at reversing the progressive loss of melanocytes and reconstituting normal skin coloration, but none is uniformly effective and adverse effects may occur. Most common treatments are topical corticosteroids, tacrolimus, systemic and topical psoralen ultraviolet-A light (PUVA), narrow- and broad-band ultraviolet-B (NB-UVB and BB-UVB, respectively) and topical khellin with UVA (KUVA).[1,37,38] NB-UVB was reported to be effective in the treatment of vitiligo with an overall re-pigmentation of 75% in 53% of the patients and a stabilization of the disease in 80% of the patients.[39] Khellin (ami visnaga), a furanochromone with a chemical structure resembling that of the psoralen family (Figure 17), is activated by UVA and UVB.[40] Khellin has been used to treat vitiligo, because it has phototherapeutic properties that are similar to those of the psoralens, but it has substantially lower phototoxic and mutagenic effects.[40,41] The current status on the position of khellin for treating vitiligo was recently reported and it was concluded that the research into this area remains inconclusive and at times contradictory.[38] In a study comparing khellin or placebo under the influence of natural sunlight, 12 of the 30 patients with vitiligo in the khellin group showed more than 50% re-pigmentation compared with none in the

placebo group.[42] A left versus right study in 72 patients comparing khellin with UVA versus UVA alone concluded that re-pigmentation was because of the UVA and not the khellin.[43] There was no difference between the khellin and the placebo groups in a left versus right study comparing khellin plus sunlight with vehicle plus sunlight in 41 vitiligo patients.[44] However, in a later study the same authors compared a khellin gel and UVA with UVA alone and reported that both groups responded, but khellin plus UVA was superior to UVA alone ($P < 0.01$).[45] A study in 33 patients compared topical khellin plus UVA with PUVA and reported that khellin plus UVA may induce re-pigmentation that was comparable with that induced by systemic PUVA, but required a longer period.[46]

The selection of the vehicle in which khellin is incorporated is highly important for the adequate availability of khellin in the skin because of its very low solubility in water.[45] Khellin encapsulated in phosphatidylcholine liposomes facilitates the availability of khellin in the epidermis and in the hair follicles.[47,48]

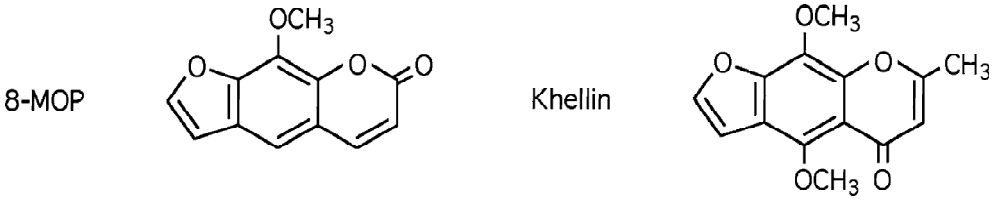

Figure 17. Molecular structure formula of khellin and psoralen

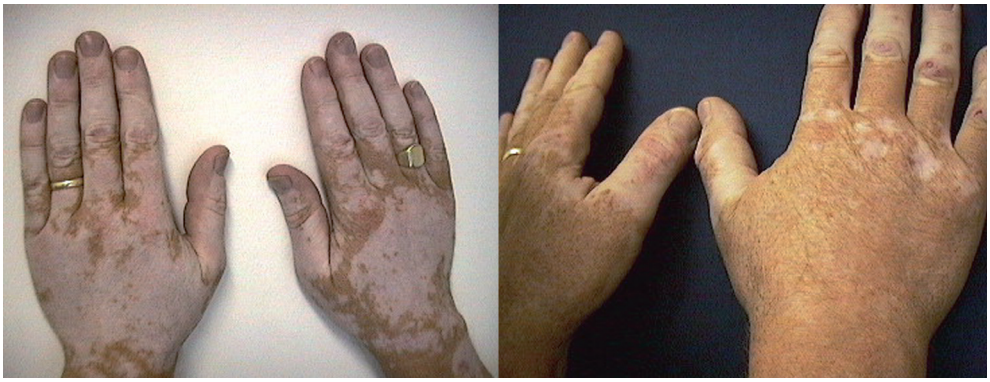

Figure 18. Before and after Khellin-Liposomal- Ultraviolet (KLUV) treatment.

3.2.4 *Surgical treatment*

Surgical treatment is limited to vitiligo maculae, refractory to the aforementioned treatments. An important condition for surgical therapy is the stability of the diseas.[35] In stable vitiligo (such as segmental unilateral vitiligo), the outcome of transplantation therapy is usually excellent. In some cases, transplantation may indeed be the only effective treatment.[49] Unfortunately, to date there is no reliable test to predict the activity and the outcome of melanocyte transplantation treatment in patients with vitiligo. Vitiligo can be active in one skin area and inactive or even in regression in another area at the same time and fluctuations in time are often seen.[49] Thus, it may be difficult to draw conclusions from patient's anamnesis and from performing a few test grafts prior to more extensive treatments as an approach to predict stability. While there is no consensus on definite parameters for stability, various recommendations indicate a period of disease inactivity ranging from 6 months to two years.[50] Njoo et al recommended a 6-point scale on which the disease activity by history was scored by means of the "vitiligo disease activity score (VIDAscore)" before and after 12 months of therapy. This is a scoring system of the patient's own opinion of the present disease activity within the time periods indicated as follows: active in the past 6 weeks (score +4); active in the past 3 months (score +3); active in the past 6 months (score +2); active in the past year (score +1); stable for at least 1 year (score 0); and stable for at least 1 year with spontaneous re-pigmentation (score –1). The term "active" is defined as the expansion of the existing lesions or the appearance of new lesions. "Stable" refers to the condition when these symptoms are not present.[51]

Methods of surgical modalities for vitiligo include both tissue grafts and cellular grafts. Both these techniques have their own advantages and disadvantages (Table 5) according to the recently published guidelines.[50] Tissue grafts comprises punch grafting, suction blister epidermal grafting and split-thickness grafting. Cellular grafts represent methods, which require specialized trained personnel and appropriate laboratory facilities and are expensive.[50,52] In vitiligo surgery the most frequently reported complication is the lack of take or the survival of grafts and the absence of re-pigmentation. De-pigmentation of grafts may also occur (often as a result of vitiligo instability) in spite of a good take of the graft. Another problem may be the quality of the preparation of the melanocytes before transplantation. In vitro cultured autologous skin cells have been chemically and mechanically prepared and/or cultured. For cell preparation it is important to use a medium containing a minimum of foreign proteins and if they have to be used, they should be from the safest possible source. These precautionary measures are taken in order to reduce the risk of transmitting slow viruses, prions and similar agents to the recipient site.[49] Liquid nitrogen, sandpaper, suction devices, topical methoxsalen followed by PUVA, CO_2 laser and Erbium-YAG

lasers were used for preparing the recipient site.[53,54] The disadvantage of preparing the recipient site with liquid nitrogen and topical methoxsalen followed by PUVA is that it is difficult to control the final result. Today, lasers are a good means to ablate the skin in a controlled fashion. It was reported that the Erbium-YAG laser-assisted ablation resulted in more pigment spread around the graft than areas prepared with topical methoxsalen followed by PUVA.[54]

The first investigation on vitiligo described in this thesis involved the evaluation of the efficacy and the safety of the treatment of vitiligo with khellin encapsulated in liposomes in combination with UV light therapy (KLUV). The second investigation involved a surgical approach for transplanting of suction blister roof epidermal grafts and Erbium laser ablation for re-pigmentation of refractory and stable vitiligo patches. The third study involved additional surgery for refractory vitiligo patches, particularly on the 'emotionally charged' body sites such as the face, the head, the neck and the hands after 1 year of KLUV treatment.

Surgical methods	Advantages	Disadvantages
Punch grafting	Easiest and least expensive	Only suitable for small areas Scars at donor and recipient sites (cobblestone effect)
Suction blister epidermal grafting	Excellent cosmetic results Minimum chances of scarring at donor and recipient sites	Large areas require several treatments
Split-thickness grafting	Treating a relatively large area in a short period of time	Scares at donor site Color mismatch Milia Peri-graft de-pigmentation halo Stuck-on appearance of recipient site
Transplantation of autologous epidermal cell suspension	Fairly large area can be treated with a donor-to-recipient expansion ratio ranging from 5 to 10 fold	Requires a well-equipped laboratory and trained personnel
Transplantation of cultured autologous melanocytes	Treating a relatively large area in a short period of time	Safety concerns on the use of cultured autografts. This method is expensive and requires a tissue culture laboratory setup
Autologous cultured epithelial grafts	Treating large areas	Requires both specialized equipment and personnel and is expensive

Table 5. Advantages and disadvantages of surgical methods in the treatment of vitiligo.

3.2.5 *References*

1. Kovacs S. Continuing medical education: vitiligo. J Am Acad Dermatol 1998; 38: 647-666.
2. LePoole IC, Das PK. Microscopic changes in vitiligo. Clin Dermatol 1997; 15: 863–873.
3. Handa S, Kaur I. Vitiligo: clinical findings in 1436 patients. J Dermatol 1999; 26: 653–657.
4. Halder RM. Childhood vitiligo. Clin Dermatol 1997; 15: 899–906.
5. Kent G, al-Abadie M. Factors affecting responses on Dermatology Life Quality Index items among vitiligo sufferers. Clin Exp Dermatol 1996; 21: 330-333.
6. Parsad D, Pandhi R, Dogra S, Kanwar AJ, Kumar B. Dermatology Life Quality Index score in vitiligo and its impact on the treatment outcome. Br J Dermatol 2003; 148: 373-374.
7. Sampogna F, Picardi A, Chren MM et al. Association between poorer quality of life and psychiatric morbidity in patients with different dermatological conditions. Psychosom Med 2004; 66: 620-624.
8. Ongenae K, van Geel N, De Schepper S, Naeyaert JM. Effect of vitiligo on self-reported health-related quality of life. Br J Dermatol 2005; 152: 1165-1172.
9. Rezaei N, Gavalas NG, Weetman AP, Kemp EH. Autoimmunity as an aetiological factor in vitiligo. J Eur Acad Dermatol Venereol 2007; 21, 865–876.
10. Le Poole IC, Das PK, van den Wijngaard RMJG, Bos JD, Westerhof W. Review of the etiopathomechanism of vitiligo: a convergence theory. Exp Dermatol 1993; 2: 145–153.
11. Forschner T, Buchholtz S, Stockfleth E. Current state of vitiligo therapy – evidence-based analysis of the literature J Dtsch Dermatol Ges 2007; 5: 467–476.
12. Ochi Y, DeGroot LJ. Vitiligo in Graves' disease. Ann Intern Med 1969; 71: 935–940.
13. Naughton GK, Eisinger M, Bystryn JC. Antibodies to normal human melanocytes in vitiligo. *J* Exp Med 1983; 158: 246–251.
14. Ogg GS, Dunbar PR, Romero P, Chen JL, Cerundolo V. High frequency of skin-homing melanocyte-specific cytotoxic T lymphocytes in autoimmune vitiligo. J Exp Med 1998; 188: 1203–1208.
15. Pawelek J, Korner A, Bergstrom A, Bologna J. New regulation of melanin biosynthesis and autodestruction of melanoma cells. Nature 1980; 286: 617–619.

16. Schallreuter KU, Wood JM, Berger J. Low catalase levels in the epidermis of patients with vitiligo. J Invest Dermatol 1991; 97: 1081–1085.
17. Taeib A. Intrinsic and extrinsic pathomechanisms in vitiligo. Pigment Cell Res 2000; 13 (Suppl. 8): 41–47.
18. Alkhateeb A, Fain PR, Thody A, Bennet DC, Spritz RA. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their relatives. Pigment Cell Res 2003; 16: 208–214.
19. Laberge G, Mailloux CM, Gowan K, Holland P, Bennet DC, Fain PR, Spritz RA. Early onset and increased risk of other autoimmune diseases in familial generalised vitiligo. Pigment Cell Res 2005; 18: 300–305.
20. Al Badri AMT, Todd PM, Garioch JJ, Gudgeon JE, Stewart DG, Goudle RB. An immunohistological study of cutaneous lymphocytes in vitiligo. J Pathol 1993; 170: 149–155.
21. Van den Wijngaard R, Wankowicz-Kalinska A, Le Poole C, Tigges B, Westerhof W, Das P. Local immune response in skin of generalised vitiligo patients. Destruction of melanocytes is associated with the prominent presence of CLA+ T cells at the perilesional site. Lab Invest 2000; 80: 1299–1309.
22. Abdel Naser MB, Kruger-Krasagakes S, Krasagakis K, Gollnick H, Abdel Fattah A, Orfanos CE. Further evidence for involvement of both cell mediated and humoral immunity in generalised vitiligo. Pigment Cell R*es* 1994; **7**: 1–8.
23. Hann SK, Park YK, Chung KY, Sungbin IM, Won JH. Peripheral blood lymphocyte imbalance in Koreans with active vitiligo. Int J Dermatol 1993; 32: 286–289.
24. Wankowicz-Kalinska A, van den Wijngaard RM, Tigges BJ, Westerhof W et al. Immunopolarization of CD4+ and CD8+ cells to Type-1-like is associated with melanocyte loss in human vitiligo. Lab Invest 2003; 83: 683–695.
25. Parrish JA, Fitzpatrick TB, Shea C, Pathak MA. Photochemotherapy of vitiligo: use of orally administered psoralens and a high intensity long wave ultraviolet light system. Arch Dermatol 1976; 112: 1531–1534.
26. Kumari J. Vitiligo treated with topical clobetasol propionate. Arch Dermatol 1984; 120: 631–635.
27. Tsuji T, Hamada T. Topically administered fluorouracil in vitiligo. Arch Dermatol 1983; 119: 722–777.
28. Krutmann J, Morita A. Mechanisms of ultraviolet (UV) B and UVA phototherapy. J Invest Dermatol Symp Proc 1999; 4: 70–72.
29. Duthie MS, Kimber I, Norval M. The effects of ultraviolet radiation on the immune system. Br J Dermatol 1999; 140: 995–1009.

30. Halder RM, Young CM. New and emerging therapies for vitiligo. Dermatol Clin 2000; 18: 79–89.
31. Kao CH, Yu HS. Comparison of the effect of 8-Methoxypsoralen (8-MOP) plus UVA (PUVA) on human melanocytes in vitiligo vulgaris and in vitro. J Invest Dermatol 1992; 98: 734–740.
32. Viac J, Groujon C, Misery L, Staniek V, Faure M, Schmitt D, Claudy A. Effect of UVB 311 nm irradiation on normal human skin. Photodermatol Photoimmunol Photomed 1997; 13: 103–108.
33. Tanghetti EA. Tacrolimus ointment 0.1% produces repigmentation in patients with vitiligo: results of a prospective patient series. Cutis 2003; 71: 158–162.
34. Lepe V, Moncada B, Castanedo-Cazares JP, Torres-Alvarez MB et al. A double-blind randomised trial of 0.1% tacrolimus vs 0.05% clobetasol for the treatment of childhood vitiligo. Arch Dermatol 2003; 139: 581–585
35. Falabella R. Surgical treatment of vitiligo: why, when and how. J Eur Acad Dermatol Venereol 2003; 17: 518–520.
36. Cui J, Shen L, Wang G. Role of hair follicles in the repigmentation of vitiligo. J Invest Dermatol 1991; 97: 410-416.
37. Antoniou G, Katsambas A. Guidelines for the treatment of vitiligo. Drugs 1992; 43: 490-498.
38. Gawkrodger DJ, Ormerod AD, Shaw L, Mauri-Sole I, Whitton ME, Watts MJ, Anstey AV, Ingham J, K. Young. Guideline for the diagnosis and management of vitiligo. Br J Dermatol 2008; 159: 1051-1076.
39. Njoo MD, Bos JD, Westerhof W. Treatment of generalized vitiligo in children with narrow-band (TL-01) UVB radiation therapy. J Am Acad Dermatol 2000; 42: 245-253.
40. Morliere P, Honigsmann H, Averbeck D, Dardalhon M, Hüppe G, Ortel B, Santus R, Dubertet L. Phototherapeutic, Photobiologic and Photosensitizing properties of khellin. J Invest Dermatol 1988; 90: 720-724.
41. Ortel B, Tanew A, Honigsmann H. Treatment of vitiligo with khellin and ultraviolet A. J Am Acad Dermatol 1988; 18: 693-701.
42. Abdel-Fattah A, Aboul-Enein MN, Wassel GM, El-Menshawi BS. An approach to treatment of vitiligo by khellin. Dermatologica 1982; 165: 136–40.
43. Procaccini EM, Riccio G, Montfrecola G. Ineffectiveness of topical khellin in photochemotherapy of vitiligo. J Dermatol Treatment 1995; 6: 117–120.
44. Orecchia G, Perfetti L. Photochemotherapy with topical khellin and sunlight in vitiligo. Dermatology 1992; 184: 120-123.
45. Orecchia G, Sangalli ME, Gazzaniga A., Giordano F. Topical photochemotherapy of vitiligo with a new khellin formulation: preliminary

results. J Dermatol Treatment 1998; 9: 65-69.
46. Valkova S, Trashlieva M, Christova P. Treatment of vitiligo with local khellin and UVA: comparison with systemic PUVA. Clin Exp Dermatol 2004; 29:180-184.
47. Lieb LM, Ramachandran C, Egbaria K, Weiner N. Topical delivery enhancement with multilamellar liposomes into pilosebaceous units. J. Invest Dermatol 1992; 99:108-113.
48. Hoffman RM. Topical liposome targeting of dyes, melanins, genes, and proteins selectively to hair follicles. J Drug Target 1995: 67-74.
49. Olsson MJ. What are the needs for transplantation treatment in vitiligo, and how good is it? Arch Dermatol 2004; 140: 1273-1274
50. Parsad D, Gupta S. Standard guidelines of care for vitiligo surgery. Indian J Dermatol Venereol Leprol 2008; 74: s37-45.
51. Njoo MD, Das PK, Bos JD, Westerhof W. Association of the Kobner phenomenon with disease activity and therapeutic responsiveness in vitiligo vulgaris. Arch Dermatol 1999; 135: 407-413.
52. Falabella R. Surgical therapies for vitiligo. Clin Dermatol 1997; 15: 927-939.
53. Oh CK, Cha JH, Lim JY, Jo JH, Kim SJ, Jang HS, Kwon KS. Treatment of vitiligo with suction epidermal grafting by the use of an ultrapulse CO2 laser with a computerized pattern generator. Dermatol Surg, 2001; 27(6): 565-568.
54. Pai GS, Vinod V, Joshi A. Efficacy of erbium YAG laser-assisted autologous epidermal grafting in vitiligo. J Eur Acad Dermatol Venereol, 2002. 16: 604-606.

3.3 Acne vulgaris

3.3.1 *Epidemiology and clinical aspects.*

Acne vulgaris is one of the most common disorders of the skin with 70–96% of the population suffering from it at some point in their life and may have a considerable impact on the quality of life.[1,2] For many patients, acne has the characteristics conform the definition of chronic disease, psychological sequels that do not always correlate with the clinician's assessment of the severity at one point in time.[3] These characteristics are a prolonged course, a pattern of recurrence or relapse, manifestation as acute outbreaks or slow onset, and a psychological and social impact that affects the sufferer's quality of life.[4] Acne vulgaris is clinically characterized by

non-inflammatory comedonal lesions, inflammatory acne papules, pustules, nodules or cystic lesions.[5]

The histopathological picture of acne depends on the stage of genesis. Early lesions are seen as microcomedones, which show mildly distended follicles and narrowed follicular openings. The follicular distention increases and passes into a compact cystic structure containing an eosinophilic, keratinaceous debris, hair, and bacteria in closed comedones. Open comedones have broad follicular openings with a peri-vascular mononuclear cell infiltrate surrounding the distended follicle. The sebaceous glands become atrophic. The follicular contents may rupture into the dermis following the follicular distension and induce an inflammatory response. In the acute phase of inflammation neutrohils first appear creating a pustule. As the lesions mature, foreign-body granulomatous inflammation engulfs the follicle and end-stage scarring often results.[6]

Clinical classification of acne: [5]

1. Neonatal acne is characterized by multiple small, erythematous, closed comedones on the nose, the forehead and the cheeks of a neonate with an onset frequently at between 0 and 6 weeks of age. Nearly 1 in 5 neonates has mild acne. Neonates have open comedones, inflammatory papules, or pustules less often. Neonatal acne lesions predominantly resolve spontaneously within 1 to 3 months and don not need treatment.
2. Infantile acne that is seen later in infancy has an onset beginning in months 3 to 6 and is classified as infantile acne. It is less common than neonatal acne and is characterized by more numerous inflammatory papules and pustules with an occasional presentation of nodular acne. Scarring from infantile acne is a risk. Patients with infantile acne may run an increased risk of developing severe acne vulgaris during the teenage years.
3. Acne vulgaris
    - Teenager acne is often related to the hormonal changes before and during puberty. Teenaged boys are more often affected than teenaged girls, with some estimates indicating that 100% of the teenaged boys are more or less affected by acne. After peaking during the teenage years, the prevalence of acne progressively decreases.
    - Adult acne affects approximately 8% of the adults aged 25 to 34 years and only 3% of the adults aged 35 to 44 years. Young adult acne may be a continuation of teenage acne or start de novo. Most teenaged boys with acne may anticipate a clearing by the age of 20 to 25 years. In contrast, women may continue to have acne well into

their adult life, up to and even beyond the age 40 years. Acne may persist into adult years in as many as 50% of the individuals.[7] Eighty-five percent of the women experienced a premenstrual flare of their acne.

- Acne papulopustulosa is characterized by non-inflammatory comedones, inflammatory papules, pustules with occasional presence of nodules and cicatrices (Figure 19A)
- Acne conglobata is a severe, eruptive nodulocystic variant of acne without systemic manifestations (Figure 19B).[6]
- Acne fulminans is the most severe form of cystic acne and is characterized by the abrupt onset of nodular and suppurative acne in association with varying systemic manifestations. Both forms of cystic acne are characterized by the formation of deep inflammatory lesions that often cause scarring. [6]
- Acne cosmetica is most commonly found in women between the ages of 20 to 40 years and is associated with the use of cosmetics containing comedogenic substances. It is characterized by persistent, low-grade closed comedones that resolve slowly once the causing product is no longer used.[6]
- Pomade acne is a variation of acne cosmetica seen almost exclusively in AfricanAmericans and Europeans. It is associated with the use of greases and oils applied on the scalp and the face. The lesions are closely packed closed comedones found only at the site of pomade application.[5]

Physical scars or persistent hyper-pigmentation are both not uncommon sequels of acne and are usually expensive and difficult to treat effectively. Adverse psychological effects including anxiety, depression and social withdrawal have all been reported in individuals with acne and acne scars.[8,9] The effects of acne can persist for many years, even in individuals who had self-limited adolescent acne.[10] Other micro-organisms are involved in acne-like diseases such as rosacea and Gram-negative folliculitis. Acneiform lesions may be also be induced by medicaments such as corticosteroids, iodides, bromides, lithium, isoniazid and others (drug-induced acne). An abrupt, monomorphous eruption of inflammatory papules distinguishes drug-induced acne from the heterogeneous morphology of acne vulgaris. Acne mechanica occurs secondary to the repeated mechanical and frictional obstruction of the pilosebaceous outlet resulting in comedo formation. Linear and geometric distribution of comedones and papulopustels is sometimes accompanied by hyper-pigmentation and lichenification indicating acne mechanica.[6]

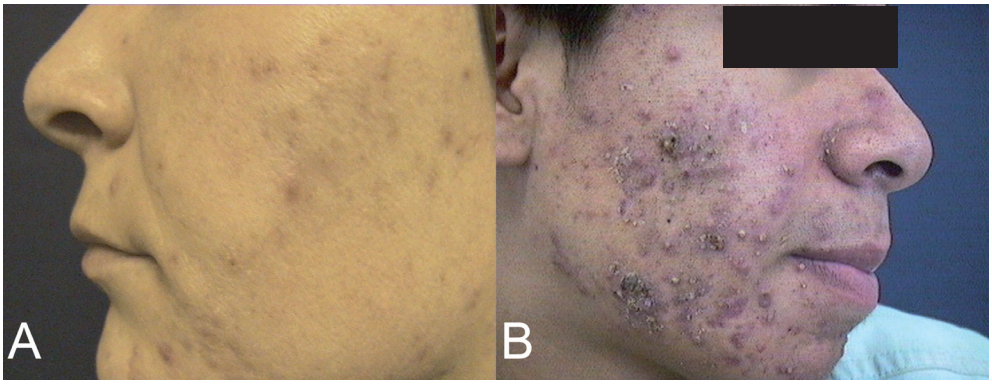

Figure 19. A. acne papulopustulosa B. acne conglobata

3.3.2 *Acne pathogenesis*

The formation of an acne lesion begins with the microcomedo. This lesion, which is not yet clinically visible forms when excess sebum collects in the follicle and abnormal epithelial desquamation occurs along with proliferation of Propionibacterium (P.) acnes. The microcomedo is the precursor of all acne lesions such as comedones and papules/pustules.[11] Four major factors are responsible for the development of acne lesions.[10]

1. Sebum production by the sebaceous gland
2. Follicular colonization by P. acnes
3. Alteration in the keratinization
4. Release of inflammatory mediators into the skin

Ad 1. Sebaceous lipids are regulated by peroxisome proliferator-activated receptors (PPARs) and sterol response element binding proteins.[12,13] PPARs are members of the nuclear hormone receptor family that alter sebaceous lipid production in concert with retinoid X receptors to regulate epidermal growth, differentiation and lipid metabolism.[12] Sterol response element binding proteins mediate the increase in sebaceous lipid formation induced by insulin-like growth factor-1.[13] The sebaceous gland acts as an independent endocrine organ in response to changes in androgens and hormones.[14] This aspect of sebaceous gland function is primarily influenced by corticotrophin-releasing hormone and corticotrophin receptors. Corticotrophin-releasing hormone levels change in response to stress and its role in regulating sebaceous gland function is a likely link in the brain-skin connection that is thought to explain the relationship between stress and skin disorders with an inflammatory component such as acne.[15-17] In addition, the sebaceous gland has both direct and indirect antibacterial activities. Palmitoleic acid isomer, a lipid in sebum, has innate antimicrobial activity against Gram-positive bacteria and is up-regulated by activation of TLR-2 by skin bacteria.[18, 19]

Ad 2. Interaction between P. acnes and TLR-2 triggers an inflammatory response, which induces the synthesis of TNF-α and IL-1.[20-22] These inflammatory cytokines amplify the signaling pathways that activate the activator protein (AP)-1 transcription factor.[23] Activation of AP-1 induces matrix metalloproteinase (MMP) genes whose products degrade and alter the dermal matrix.[23] Retinoids are known to inhibit AP-1.[24] Retinoids may induce monocytes to develop into $CD209^+$ macrophages that phagocytose P. acnes bacteria.[25] These data further support the anti-inflammatory activity of currently available treatments such as topical retinoids in acne.

Ad 3. Immune changes and inflammatory responses occur before hyperproliferation of keratinocytes, with a pattern similar to a type IV delayed hypersensitivity response.[26] Oxidized squalene can stimulate hyperproliferative behavior of keratinocytes indicating that this lipid may be responsible for comedo formation.[27] It has been hypothesized that lipoperoxides exert a proinflammatory effect on the pilosebaceous duct.[28,29] Lipoperoxides produce leukotriene B4, which is a powerful chemoattractant that can recruit neutrophils, macrophages and stimulate the production of proinflammatory cytokines.[26, 28,30] Matrix metalloproteinases (MMPs), which include collagenases, gelatinases, stromelysins, and matrilysins originate in keratinocytes and sebocytes and have a prominent role in both inflammatory matrix remodeling and proliferative skin disorders.[31] Oral isotretinoin can reduce the concentrations of MMPs in sebum paralleling the clinical improvement.[30]

Ad 4. The immune response is led by $CD4^+$ lymphocytes and macrophages.[26] Subsequent productions of cytokines activate local endothelial cells, up-regulating inflammatory vascular markers such as E-selectin, VCAM-1, ICAM-1 and human leukocyte antigen-DR (HLA-DR) in the vasculature around the pilosebaceous follicle[26] and the subsequent attraction of lymphocytes and leukocytes resulting in an inflammation. Acne is a disease that involves the innate and adaptive immune system and inflammatory events. Therefore, treatment that targets both immune system activation and inflammatory pathways is desirable. Many of the agents currently used to treat acne have effects on cellular receptors, inflammatory mediators, and other molecular targets.[10]

3.3.3 *Treatment and side effects*

Standard treatment of acne consists of peeling the skin and reducing P. acnes and inflammation by using topical benzoylperoxide gel, azelaic acid cream, tretinoin cream, adapalene gel and topical antibiotics. Systemic antibiotics and anti-androgens (in female patients) are used when this approach is not efficacious. Antibiotics were the first effective treatment for acne. However, the efficacy of antibiotic treatment is progressively reduced by increasing resistance of the bacteria to the used antibiotics. Adverse effects may also limit their use (Table 6). When other treatments fail, the final

remedy is oral isotretinoin, which is very effective, but its use is even more limited by moderate to serious adverse effects (Table 6). Patients with acne are often treated with multiple antibiotics and their flora is exposed to a significant selective pressure for resistance development. It has been observed that patients with acne treated with antibiotics had 2.15 times higher risk of developing an upper respiratory tract infection than patients with acne who were not treated with antibiotics.[32] In addition, there have been an increasing number of reports of infections caused by P. acnes and include arthritis, endocarditis and endophthalmitis.[33-35] Even an increased risk of (fatal) breast cancer by cyclins was reported.[36] Current guidelines and publications emphasize the need to limit antibiotic use, both frequency and duration, and to add the non-antibiotic antimicrobial agent benzoylperoxide (BPO) gel when long-term antibiotic use is necessary because BPO is a highly efficient bactericidal agent that minimizes the development of resistance at sites of application.[37-40] Strategies have been developed to limit antibiotic resistance in acne management:[10]

- Since acne is a multi-factorial disease, multiple classes of drugs are used in the clinical setting. Combination therapy is now recommended as the first-line choice.
- Combination of a topical retinoid such as tretinoin and adapalene with an antimicrobial (oral or topical) agent because of the complementary modes of action that have been observed clinically.
- Oral antibiotics must be discontinued when there is no further notable improvement and should ideally be used for no longer than 3 months.
- Replacing oral and topical antibiotics by a topical BPO-containing base because BPO reduces the possibility of antibiotic resistance of P. acnes and rapidly reduces the number of sensitive and resistant strains of P. acnes at the site of application.[40]
- The use of topical retinoids for maintenance therapy with added BPO for an antimicrobial effect if needed. Maintenance therapy is an effective strategy to minimize the risk of relapse.[41]
- Avoiding of the use of antibiotics for maintenance therapy.

This approach may have several problems in the clinical setting:

- Discontinuation of antibiotic treatment may often lead to an exacerbation of acne in spite of the replacement of topical BPO and the continuation of topical retinoids. In such cases, most of the patients are willing to continue the antibiotic treatment for longer than 3 months for good reason.
- Patients may have aversion to the use topical BPO because of the white discoloration of clothes.
- Adverse effects such as burning, itching, redness, dry skin, scaling, cracks, crusting, blistering and allergic contact dermatitis of BPO may limit its use.

- Concerns on the long-term carcinogenesis effect of BPO.[42]
- Adverse effects such as burning, itching, dry skin, redness, desquamation, contact allergy, crustation, hypo- and hyper-pigmentation and photo-sensitization are also possible during the use of tretinoin and to a lesser extent also during the use of adapalene.

The increasing antibiotic resistance of P. acnes and the growing awareness on the adverse effects of topical and systemic drugs in the treatment of acne vulgaris by physicians and patients motivated the search for new efficacious and safe treatment modalities such as light-based therapies.

Light-based treatments have three primary therapeutic targets.[43-46] These are as follows:

1. Reduction of P. acnes levels
2. Disruption of sebaceous gland function and subsequently reduction of sebum production
3. Reduction of inflammation via action on inflammatory cytokines

Acne often improves after exposure to sunlight or artificial light sources.[43] As part of its normal metabolism, P. acnes produces light-sensitive porphyrin compounds such as uroporphyrin, coproporphyrin III and protoporphyrin IX (PpIX). PpIX absorbs visible light at several wavelengths of between 400 and 700 nm. Absorption of light excites the PpIX, causing formation of singlet oxygen and ROS resulting in phototoxic reactions and destruction of bacteria[47] and damage to the sebaceous glands.[48] The activation of PpIX is visualized by the phenomenon of PpIX-fluorescence with fluorescence photography (Figures 19 and 20). The theoretically most effective wavelength for photo-activation of PpIX is 415 nm. Red light (635 nm) is less absorbed by photo-activating porphyrins, but penetrates more deeply into the tissue and may also have anti-inflammatory effects by influencing the release of cytokines from macrophages.[49] Initially, light-based systems for treating acne emitted blue light, [50] red light or a combination of blue- and red light. [47] However, clinical improvement was limited and multiple sessions were required. Photodynamic therapy (PDT) after application of photo-sensitizers such as 5-ALA or MAL has the potential to damage the sebaceous gland by the action of free oxygen radicals because of the higher availability of PpIX. The initial disruption of the sebaceous glands accompanied by the temporary release of pro-inflammatory mediators may help to explain why acne can flare after initiation of therapy. The subsequent reduction of the sebaceous gland function eliminates or reduces sebum production for prolonged periods.[51] Therefore, PDT with 5-ALA or MAL was introduced to achieve more effectiveness with fewer treatment sessions.[52] Studies on the efficacy and the tolerability of PDT differ in the used light source, the treatment schedules, the parameters and the concentration of

5-ALA.[53-59] In those studies, adverse effects such as pain, erythema, crusts (Figure 3) and post-treatment hyper-pigmentation were encountered highlighting the need for optimizing PDT for the treatment of acne. Split-face studies using 5-ALA and IPL reported that the combination of 5-ALA and IPL was more effective than IPL alone.[52,53,55]

Liposomes are used in the treatment of hair follicle- and sebaceous gland-associated disorders because of their potential to carry lipophilic drugs and hydrophilic drugs such as 5-ALA into the pilosebaceous structures.[60-63] Promising results were obtained with liposomal encapsulated drugs in the treatment of acne.[64-67] Christiansen et al performed a study on the fluorescence distribution patterns of normal skin after topical application of 20% 5-ALA in a moisturizing cream and after application of low concentrations of 5-ALA (0.5% and 1%) encapsulated in liposomes. The 5-ALA concentration could be lowered by a factor of 40 by changing the vehicle for delivering the 5-ALA from a moisturizing cream to liposome encapsulation while inducing the same level of fluorescence in the skin. The need for occlusion was eliminated at the same time. The low post-treatment fluorescence also significantly reduced the risk of post-treatment phototoxicity.[68] It was not substantiated that follicular obstruction was reduced by PDT. The combination of a topical retinoid and an antimicrobial agent is currently the preferred approach for almost all patients with acne.[10] The rational for this combination is that it attacks 3 of the 4 major pathogenic factors of acne: abnormal desquamation, P. acnes colonization and inflammation. Retinoids are anticomedogenic, comedolytic, and have some anti-inflammatory effects, whereas antibiotics have anti-inflammatory and antimicrobial effects and BPO is antimicrobial with some keratolytic effects. The combination of topical retinoids and PDT promises an attack on all 4 major pathogenic factors of acne including the normalization of keratinization, destruction of P. acnes, reduction of sebum excretion and reduction of inflammation. The efficacy of photodynamic therapy (PDT) of acne vulgaris using 5-ALA 0.5% liposomal spray and Intense Pulsed Light (IPL) in combination with topical keratolytic agents (Li-PDT-PC) was investigated in the study described in this thesis.

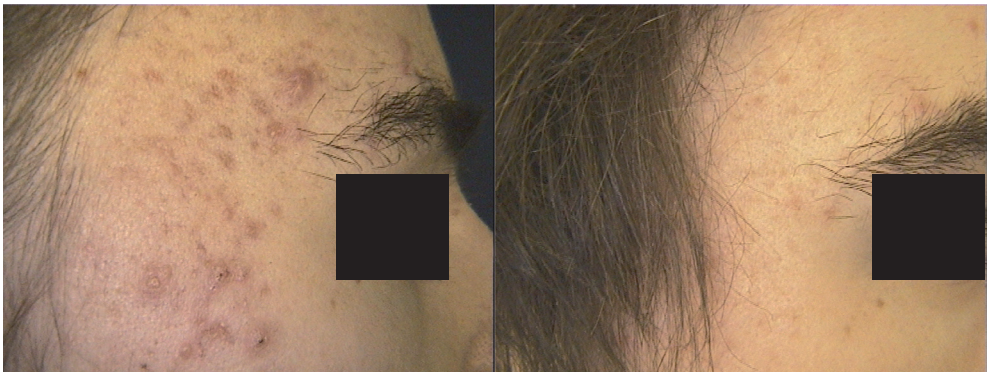

Figure 20. Before and after PDT treatment using 5-ALA 0.5% liposomal spray and Intense Pulsed Light in combination with topical keratolytic agents (Li-PDT-PC).

	Topical drugs				
	salicylic acid	benzoylperoxide	azelaic acid	tretinoin, adapalene,	erythromycin, clindamycin
Skin and mucosae	Irritation, dermatitis, contact allergic eczema	Burning, itching, erythema, dry skin, scaling, cracks, crusting, blistering, allergic contact dermatitis	Burning, itching, erythema, desquamation, contact allergy, hypo-pigmentation, photo-sensitization	Burning, itching, dry skin, erythema, desquamation, contact allergy, crustation, hypo- and hyper-pigmentation, photo-sensitization	Burning, itching, dry skin, erythema, desquamation, contact allergy, gram-negative folliculitis
Gastro-intestinal	Intoxication following application on large skin areas				Clindamycin: Belly ache, diarrhea, colitis
Blood	Intoxication following application on large skin areas				
Kidneys					
Neurological and psychological					

	Topical drugs				
	salicylic acid	benzoylperoxide	azelaic acid	tretinoin, adapalene,	erythromycin, clindamycin
Pregnancy and lactation	Treatment of large areas and under occlusion contra-indicated in pregnancy. Lactation no problem	No problem	No date available	Small quantities probably no problem. Treatment of large areas and under occlusion contra-indicated in pregnancy and lactation	No date available
Carcino-genicity		Concern about carcinogenesis in the long-term[42]			
Others		White staining of clothes			

		Systemic drugs		
	PDT 5-ALA 10% to 20% cream	tetracycline, doxycycline, minocycline	erythromycin	isotretinoin
Skin and mucosae	Pain, stinging, itching erythema, oozing, crustae and pustules. Phototoxicity 48 hours after treatment	Urticaria, angio-edema, phototoxical- and photoallergic reactions, Sweet's syndrome	Pruritus, urticaria, exanthema, toxic epidermal necrosis, Stevens-Johnson-syndrome	Xerosis cutis, pruritus, dermatitis, exanthema, epistaxis, conjunctivitis-, blepharitis- and cheilitis sicca, alopecia, paronychia, nail dystrofia, hyperhidrosis, hirsutism, vasculitis
Gastro-intestinal		Nausea, vomiting, diarrhea, glossitis, hairy tongue, stomatitis, pseudo-membranous colitis, prutitus ani, hepar dysfunction	Anorexia, nausea, vomiting, cramps, diarrhea, pseudo-membranous colitis, hepatitis, pancreatitis	Nausea, gastro-intestinal bleeding, intestinal inflammation, hepar dysfunction, hepatitis, pancreatitis, malaise.
Blood		Anemia, thrombocytopenia, neutropenia. Doxycycline: induction of hypoglycemia		Anemia thrombocytopenia, neutropenia, hyperglycemia, elevated cholesterol- and triglyceride levels, low HDL, thrombosis
Kidneys		renal dysfunction		Glomerulonephritis

		Systemic drugs		
Neurological and psychological		Benign intracranial hypertension	Impaired hearing	Headache, benign intracranial hypertension, photophobia, blurred vision, disturbance dark adaptation, cataract, impaired hearing, convulsions, somnolence, mental depression, psychosis, suicide.
Pregnacy and laction	Pregnancy category C[7]	Contra-indicated during pregnancy and lactation Unreliability of oral contraceptive devices	No problem	Absolutely contra-indicated during pregnancy and lactation
Carcinogenicity		Increased risk of incident and fatal breast cancer[36]		
Others		From minocin also reported: -lupus pneumonia -hypersensitivity -pneumonia -progressive respiratory failure		Myalgia and artralgia exostose, premature closure of epiphysis, arthritis

Table 6. Adverse effects and concerns of current acne treatment.

3.3.4 References

1. Leyden JJ. Therapy for acne vulgaris. N Engl J Med 1997; 336:1156–1162.
2. Del Roso J. Acne in the adolescence patient: Interrelationship of psychological impact and therapeutic options. Today Ther Trends 2001; 19: 473-484.
3. Niemeier V, Kupfer J, Demmelbauer-Ebner M, Stangier U, Effendy I, Gieler U. Coping with acne vulgaris: evaluation of the chronic skin disorder questionnaire in patients with acne. Dermatology 1998; 196: 108-115.
4. O'Halloran J, Miller GC, Britt H. Defining chronic conditions for primary care with ICPC-2. Fam Pract 2004; 21: 381-386.
5. White GM. Recent findings in the epidemiologic evidence, classification, and subtypes of acne vulgaris J Am Acad Dermatol 1998; 39: S34-37.
6. Zaenglein AL, Thiboutot DM. Acne vulgaris. Dermatology, volume one.

(Editors: Bologna JL, Jorizzo JL, Rapini RP). Mosby 2003; 38: 531-544.
7. Collier CN, Harper JC, Cantrell WC, Wang W, Foster KW, Elewski BFl. The prevalence of acne in adults 20 years and older. J Am Acad Dermatol 2008; 58: 56-59.
8. James WD. Clinical practice: acne. N Engl J Med 2005; 352: 1463-1472.
9. Kellett SC, Gawkrodger DJ. The psychological and emotional impact of acne and the effect of treatment with isotretinoin. Br J Dermatol 1999;140:273-282.
10. Thiboutot D, Gollnick H, Bettoli V, Dréno B, Kang S,. Leyden JJ, Shalita AR, Torres Lozada . New insights into the management of acne: An update from the Global Alliance to Improve Outcomes in Acne Group. J Am Acad Dermatol 2009; 60: S1-50.
11. Cunliffe WJ, Holland DB, Clark SM, Stables GI. Comedogenesis: some etiological, clinical and therapeutic strategies. Dermatology 2003; 206: 11-16.
12. Smith TM, Cong Z, Gilliland KL, Clawson GA, Thiboutot DM. Insulin-like growth factor-1 induces lipid production in human SEB-1 sebocytes via sterol response element-binding protein-1. J Invest Dermatol 2006; 126: 1226-1232.
13. Trivedi NR, Cong Z, Nelson AM, Albert AJ, Rosamilia LL, Sivarajah S et al. Peroxisome proliferator-activated receptors increase human sebum production. J Invest Dermatol 2006; 126: 2002-2009.
14. Zouboulis CC, Baron JM, Bohm M, Kippenberger S, Kurzen H, Reichrath J, Thielitz A. Frontiers in sebaceous gland biology and pathology. Exp Dermatol 2008;17: 542-551.
15. Zouboulis CC, Bohm M. Neuroendocrine regulation of sebocytes - a pathogenetic link between stress and acne. Exp Dermatol 2004; 13(Suppl 4): 31-35.
16. Ziegler CG, Krug AW, Zouboulis CC, Bornstein SR. Corticotropin releasing hormone and its function in the skin. Horm Metab Res 2007; 39: 106-109.
17 Slominski AT, Botchkarev V, Choudhry M, Fazal N, Fechner K, Furkert J et al. Cutaneous expression of CRH and CRH-R: is there a ‘‘skin stress response system?’’ Ann N Y Acad Sci 1999; 885: 287-311.
18. Wille JJ, Kydonieus A. Palmitoleic acid isomer (C16:1delta6) in human skin sebum is effective against gram-positive bacteria. Skin Pharmacol Appl Skin Physiol 2003; 16: 176-187.
19. Georgel P, Crozat K, Lauth X, Makrantonaki E, Seltmann H, Sovath S et al. A toll-like receptor 2 - responsive lipid effector pathway protects mammals against skin infections with gram-positive bacteria. Infect Immun 2005; 73:

4512-4521

20 .Kim J, Ochoa MT, Krutzik SR, Takeuchi O, Uematsu S, Legaspi AJ et al. Activation of toll-like receptor-2 in acne triggers inflammatory cytokine responses. J Immunol 2002; 169: 1535-1541.

21 Kapetanovic R, Cavaillon JM. Early events in innate immunity in the recognition of microbial pathogens. Expert Biol Ther 2007; 7: 907-918.

22. Jugeau S, Tenaud I, Knol AC, Jarrousse V, Quereux G, Khammari A, Dreno B. Induction of toll-like receptors by Propionibacterium acnes. Br J Dermatol 2005; 153: 1105-1113.

23. Kang S, Cho S, Chung JH, Hammerberg C, Fisher GJ, Voorhees JJ. Inflammation and extracellular matrix degradation mediated by activated transcription factors nuclear factor- kappaB and activator protein-1 in inflammatory acne lesions in vivo. Am J Pathol 2005; 166: 1691-1699.

24. Czernielewski J, Michel S, Bouclier M, Baker M, Hensby JC. Adapalene biochemistry and the evolution of a new topical retinoid for treatment of acne. J Eur Acad Dermatol Venereol 2001; 15(Suppl): 5-12.

25. Liu PT, Phan J, Tang D, Kanchanapoomi M, Hall B, Krutzik SR KimJ. $CD209^{+}$ macrophages mediate host defense against Propionibacterium acnes. J Immunol 2008; 180:4919-4923.

26. Jeremy AH, Holland DB, Roberts SG, Thomson KF, Cunliffe WJ. Inflammatory events are involved in acne lesion initiation. J Invest Dermatol 2003; 121: 20-27.

27 Ottaviani M, Alestas T, Flori E, Mastrofrancesco A, Zouboulis CC, Picardo M. Peroxidated squalene induces the production of inflammatory mediators in HaCaT keratinocytes: a pos sible role in acne vulgaris. J Invest Dermatol 2006; 126: 2430-2437.

28. Zouboulis CC. Leukotrien-antagonisten bie atopischen Erkrankungen und Akne. Akt Dermatol 2003; 29: 419-425.

29. Zouboulis CC, Saborowski A, Boschnakow A. Zileuton, an oral 5-lipoxygenase inhibitor.directly reduces sebum production Dermatology 2005; 210: 36-38.

30. Alestas T, Ganceviciene R, Fimmel S, Muller-Decker K, Zouboulis CC. Enzymes involved in.the biosynthesis of leukotriene B4 and prostaglandin E2 are active in sebaceous glands. J Mol Med 2006; 84: 75-87.

31 Papakonstantinou E, Aletras AJ, Glass E, Tsogas P, Dionyssopoulos A, Adjaye J et al. Matrixmetalloproteinases of epithelial origin in facial sebum of patients with acne and their regulation by isotretinoin. J Invest Dermatol 2005; 125: 673-684.

32. Margolis DJ, Bowe WP, Hoffstad O, Berlin JA. Antibiotic treatment of acne may be associated with upper respiratory tract infections. Arch Dermatol

2005; 141: 1132-1136.
33. Levy PY, Fenollar F, Stein A, Borrione F, Cohen E, Lebail B, Raoult D. Propionibacterium acnes postoperative shoulder arthritis: an emerging clinical entity. Clin Infect Dis 2008; 46:1884-1886.
34. Delahaye F, Fol S, Celard M, Vandenesch F, Beaune J, Bozio A, de Gevigney G. Propionibacterium acnes infective endocarditis: study of 11 cases and review of literature [French]. Arch Mal Coeur Vaiss 2005; 98: 1212-1218.
35. Bagyalakshmi R, Madhavan HN, Therese KL. Development and application of multiplex polymerase chain reaction for the etiological diagnosis of infectious endophthalmitis. Postgrad Med 2006; 52: 179-182.
36. Velicer CM, Heckbert SR, Lampe JW, Potter JD, Robertson CA, Taplin SH. Antibiotic use in relation to the risk of breast cancer. JAMA 2004; 291: 827-835.
37. Dréno B, Bettoli V, Ochsendorf F, Perez-Lopez M, Mobacken H, Degreef H. European recommendations on the use of oral antibiotics for acne. Eur J Dermatol 2004; 14: 391-399.
38. Del Rosso JQ, Leyden JJ. Status report on antibiotic resistance: implications for the dermatologist. Dermatol Clin 2007; 25: 127-132.
39. Eady EA, Bojar RA, Jones CE, Cove JH, Holland KT, Cunliffe WJ. The effects of acne treatment with a combination of benzoyl peroxide and erythromycin on skin carriage of erythromycin-resistant propionibacteria. Br J Dermatol 1996; 134: 107-113.
40. Eady EA, Farmery MR, Ross JI, Cove JH, Cunliffe WJ. Effects of benzoyl peroxide and erythromycin alone and in combination against antibiotic-sensitive and -resistant skin bacteria from acne patients. Br J Dermatol 1994; 131: 331-336.
41. Thiboutot DM, Shalita AR, Yamauchi PS, Dawson C, Kerrouche N, Arsonnaud S, Kang S. Adapalene gel, 0.1%, as maintenance therapy for acne vulgaris: a randomized, controlled, investigator-blind follow-up of a recent combination study. Arch Dermatol 2006; 142: 597-602.
42. O'Connell JF, Klein-Szanto AJ, DiGiovanni DM, Fries JW, Slaga TJ. Enhanced malignant progression of mouse skin tumours by the free-radical generator benzoylperoxide. Cancer Res 1986; 46 (6): 2863-2865.
43. Cunliffe WJ, Goulden V. Phototherapy and acne vulgaris. Br J Dermatol 2000; 42, issue 5: 855-856. Taub AF. Procedural treatments for acne vulgaris. Dermatol Surg 2007; 33: 1005-1026.
45. Shnitkind E, Yaping E, Geen S, Shalita AR, Lee WL. Antiinflammatory properties of narrow-band blue light. J Drugs Dermatol 2006; 5: 605-610.
46. Mariwalla K, Rohrer TE. Use of lasers and light-based therapies for treatment

of acne vulgaris. Lasers Surg Med 2005; 37: 333-342.
47. Papageorgiou P, Katsambas A, Chui A. Phototherapy with blue (415 nm) and red (660) nm light in the treatment of acne vulgaris. Br J Dermatol 2000; 142: 973-978.
48. Divaris DX, Kenedy JC, Poittier RH. Phototoxic damage to sebaceous glands and hair follicles of mice after systemic administration of 5-aminilevulinic acid correlates with localized protoporphyrin IX fluorescence. Am J Pathol 1990; 136: 891-897.
49 Young S, Bolton P, Dyson M, Harvey W, Diamantopoulos C. Macrophage responsiveness to light therapy. Lasers Surg Med 1989; 9: 497-505.
50. Tzung TY, Kuan-Hsing W, Huang ML. Blue light phototherapy in the treatment of acne. Photodermatol Photoimmunol Photomed 2004; 20: 266-269.
51. Hongcharu W, Taylor CR, Chang Y, Aghasi D, Suthamjariya K, Anderson RR. Topical ALA-photodynamic therapy for the treatment of acne vulgaris. J Invest Dermatol 2000; 115: 183-192.
52. Rojanamatin J, Choawawanich P. Treatment of inflammatory facial acne vulgaris with intense pulsed light and short contact of topical 5-aminolevuleinic acid: a pilot study. Dermatol Surg 2006; 32: 991-997.
53. Gold MH. A multi-center study of photodynamic therapy in the treatment of moderate to severe inflammatory acne vulgaris with topical 20% 5-aminolevulinic acid and a new intense pulsed light source. J Am Acad Dermatol 2004; 50 (s1): 14.
54. Pollock B, Turner D, Stringer MR, Bojar RA, Goulden V, Stables GI, Cunliffe WJ. Topical aminolaevulinic acid-photodynamic therapy for the treatment of acne vulgaris: a study of clinical efficacy and mechanism of action. Br J Dermatol 2004; 151: 616-622.
55 Santos MA, Belo VG, Santos G. Effectiveness of photodynamic therapy with topical aminolevulinic acid and intense pulsed light versus intense pulsed light alone in the treatment of acne vulgaris: comparative study. Dermatol Surg 2005; 31: 910-915.
56. Wiegell SR, Wulf HC. Photodynamic therapy of acne vulgaris using 5-aminolevulinic acid versus methyl aminolevulinate. J Am Acad Dermatol 2006; 54: 647-651.
57. Wiegell SR, Wulf HC. Photodynamic therapy of acne vulgaris using methylaminolaevulinate: a blinded, randomized, controlled trial. Br J Dermatol 2006; 154: 969-976.
58. Hörfelt C, Funk J. Frohm-Nilsson M, Wiegleb Edström D, Wennberg AM. Topical methyl aminolaevulinate photodynamic therapy for treatment of facial acne vulgaris: results of a randomized, controlled study. Br J Dermatol

2006; 155: 608-613. 59.
59. Hörfelt C, Stenquist B, Larkö O, Faergemann J, Wennberg AM. Photodynamic therapy for acne vulgaris: a pilot study of the dose-response and mechanism of action. Acta Derm Venereol 2007; 87: 325-329.
60. Hope MJ, Kitson C.N. Liposomes: A Perspective for Dermatologists. Dermatol Clin 1993; 11: 143-154.
61. Lieb LM, Ramachandran C, Egbaria K, Weiner N. Topical delivery enhancement with multilamellar liposomes pilosebaceous units. J. Invest Dermatol 1992; 99: 108-113.
62. Bernard E, Bubois JL, Wepeirre J. Importance of sebaceous glands in cutaneous penetration of an antiandrogen: target effect of liposomes. J Pharm Sci 1997; 86: 573-578.
63. De Leeuw J, de Vijlder HC, Bjerring P, Neumann HAM. Liposomes in Dermatology Today, review article. J Eur Acad Dermatol Venereol 2009; 23: 505-516.
64. Schäfer-Korting M, Korting HC, Ponce-Pöschl E. Liposomal tretinoin for uncomplicated acne vulgaris. Clin Invest 1994; 72: 1086-1091.
65. Patel VB, Misra AN, Marfatia YS. Topical liposomal gel of tretinoin for the treatment of acne: research and clinical implications. Pharm Dev Technol 2000; 5: 455-464.
66. Patel VB, Misra AN, Marfatia YS. Preparation and comparative clinical evaluation of liposomal gel of benzoylperoxide for acne. Drug Dev Ind Pharm 2001; 27: 863-870.
67. Fluhr JW, Barsom O, Gehring W, Gloor M. Antibacterial efficacy of benzoylperoxide in phospholipids liposomes. A vehicle-controlled, comparative study in patients with papulopustular acne. Dermatology 1999; 198: 273-277.
68. Christiansen K, Bjerring P, Troilius A. 5-ALA for photodynamic photorejuvenation-optimization of treatment regime based on normal-skin fluorescence measurements. Lasers Surg Med 2007; 39: 302-310.

Non-melanoma skin cancer

3 4.1 *Epidemiology*

Non-melanoma skin cancer (NMSC) is the most common cancer in Caucasians and its incidence is of epidemic proportions worldwide.[1-5] Although, the term NMSC covers all cutaneous cancers excluding melanomas, it is normally used to refer to two major types of skin cancers namely basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). These two types of skin cancer account for more than 95% of all NMSC.[5] BCCs arise de novo, which means there are no known precursor lesions.[5] SCCs may arise from actinic keratoses (AKs). Actinic keratoses have been often considered to be pre-malignant precursor lesions by some authors[6-8], whereas according to other authors, AKs are true epithelial neoplasmata from the beginning.[3,9] From this standpoint of view, the evolution from AK to SCC represents progression rather than transformation, and therefore, AK should be recognized as incipient SCC.[10] It has been estimated that 6% to 10% of AKs develop into invasive SCC.[11,12] This highlights the value of AK as a marker for SCC.[3] Thus, if these high-risk patients with AKs are monitored closely, invasive tumors that develop could be treated at an early stage when cure rates are high, morbidity low, mortality non-existent and costs of treatment are limited.[3] The average annual increase of NMSC in the Caucasians in Europe, the United States, Canada and Australia has been 3% to 8% since the 1960s.[4] Today, the incidence of NMSC is higher than all other cancers combined and NMSC is among the five most costly cancers reported in the USA.[13] There are many reasons for the worldwide increase in the incidence of NMSC.

In the Netherlands, the total number of skin cancer patients calculated over a period of 15 years is expected to increase from 20,654 cases in 2000 to 37,342 cases in 2015 (an increase of 81%).[14] The number of cases of BCC is expected to increase by 78%. The highest increase is expected in those aged 15-64 years (males, 66% increase; females, 94% increase), especially at sites other than the head and the neck. An overall increase of 80% in SCC is expected. An increase of 79% is expected in older males and females and an increase of 93% is expected in middle-aged females aged 35–64 years.[14]

There are many reasons for the worldwide increase in the incidence of NMSC. Etiological factors that underlie the development of skin cancer are of endogenous origin as well as of exogenous origin.[4] Major endogenous factors are the age, the genetic pre-disposition such as the skin type (especially skin types I, II and III) and hereditary diseases such as xeroderma pigmentsum. The key environmental risk factor for NMSC is the ultraviolet (UV) radiation from exposure to sunlight, artificial tanning lamps,[15-18] iatrogenic exposure to psoralen & ultraviolet A (PUVA) and ultraviolet B (UVB) modalities often used for the treating psoriasis and vitiligo.[19,20]

Those with occupational or recreational outdoors exposure such as commercial fisherman, construction workers, park rangers, farmers, aircraft pilots, yachtsmen, golfers, skiers, mountaineers and sun-addicts have a higher incidence rate of AKs than those who are indoors.[21-24] Other exogenous factors include carcinogens such as arsenic, pesticides, tar, certain industrial oils, dyes, solvents, ionizing radiation, human papilloma viral infections.[25] and immunosuppression, especially in organ transplant recipients.[26] Tobacco smoking has also been recently linked to SCC.[27] In 2002, more than 1 million cases of NMSC were diagnosed in the United States.[28] More than 20% of the metastatic diseases and deaths in NMSC are from SCC. Therefore, the ability of a physician to distinguish pre-cancerous lesions with a high degree of accuracy is very important and will decrease the morbidity and mortality from SCC.[29] The differential diagnosis of NMSC includes many benign and (superficial) malignant lesions (Table 4).[12,29] The diagnostic accuracy or the positive predictive value (PPV) of the clinical diagnosis of AKs made by specialized physicians appeared to be only 74% after histological evaluation.[29] An increasing problem is posed by a particular cancerous area with multiple (pre)malignant lesions diffusely spread over UV-exposed skin. Today, physicians often need to take multiple biopsies from clinically suspected malignant lesions, which can be a significant burden for the patient.[30]

3.4.2 *Clinical features NMSCs*

- Actinic keratosis: solitary or multiple, red, scaly papules or plaques, 2- to10-mm in diameter, occurring in sun-exposed sites, may bleed and become hypertrophic.[29]
- Squamous cell carcinoma in situ: solitary or multiple, sharply demarcated, scaling, or hyperkeratotic macule, papule or plaque, often pink or red in color, erosions and crusts may be present.
- Differentiated squamous cell carcinoma: red, yellow or skin-colored, indurated plaque, papule or nodule, with adherent scaling or hyperkeratosis, erosion, ulceration, crust in the center and a firm, hyperkeratotic border.
- Undifferentiated squamous cell carcinoma: red, fleshy, granulating, easily bleeding, erosive, crusting papules, nodules papillomatous vegetations and ulceration with a necrotic base.
- Superficial basal cell carcinoma: red, shiny or slightly scaly, thin plaques, often with a fine pearly border.
- Superficial multi-centric basal cell carcinoma appears as multiple, thin, pink or red plaques with fine teleangiectasia.
- Nodular basal cell carcinoma: translucent or pearly papule or nodule, sometimes with black, brown or blue hyper-pigmentation.
- Morphea-like basal cell carcinoma: whitish, sclerotic patch with ill-defined

borders and sometimes with pearly colored papules at the periphery.

- Cystic basal cell carcinoma may present as firm, translucent papules and noduli occasionally with a depressed (umbilicated) center.

Differential diagnosis of actinic keratosis	
Benign	Malignant
Kerarosis (seborrheic, stucco, arsenical, lichenoid) Porokeratosis Keratoacanthoma Lentigo solaris Discoid lupus erythematosus Psoriasis	Bowen's disease Basal cell carcinoma Squamous cell carcinoma

3.4.3 *Dermatoscopy*

Dermatoscopy is a noninvasive method that allows the in vivo evaluation of colors and microstructures of the epidermis, the dermoepidermal junction, and the papillary dermis not visible to the naked eye.

Dermatoscopic characteristics of NMSC:

Actinic keratosis:

- Uniform pink or tan-colored background.
- Prominent keratin (white or yellow scale).[31]

Morbus Bowen:

- Irregular 'glomerular vessels' are characteristic.
- Scaly surface.[32]

Basal cell carcinoma:

- Arborizing vessels
- Grey-blue ovoid nests
- Maple leaf-like areas
- Spoke wheel areas
- Ulceration
- Small brown globules and/or homogeneous pigmentation.[32,33,.34]

Dermatoscopic characteristics of benign skin disorders, which may show positive fluorescence after application of 5-ALA:

Sebaceous hyperplasia:

- Solitary or aggregated white or yellow nodules
- The nodules are surrounded by groups of orderly winding, scarcely branching

vessels extending towards the center, but never crossing it: “crown vessels”.[34,35,36]

Poriasis:

- Vessels are tiny red dots in a homogenous pattern
- Light pink background
- Non-adherent scaling (in flakes).[32]

Common wart:

- Papilliform structure
- Thrombosed capillaries.

3.4.4 *Fluorescence detection of NMSCs*

Following topical application of 5-ALA, PpIX is induced selectively in epithelial tumor cells with high metabolism. The tumor becomes visible upon irradiation with light and can be delineated from the surrounding tissue.[37] This method is called fluorescence diagnosis (FD). The contrast of the acquired fluorescence images can be significantly enhanced by using a highly light sensitive charged couple device (CCD) camera system together with a specially designed digital imaging (Dyaderm system, Biocam GmbH, Regensburg, Germany).[30,37,38]

The clinical differential diagnosis between AK, (superficial) SCC, (superficial) BCC and certain benign skin disorders may be difficult as already mentioned. Dermatoscopy is very helpful, but is time-consuming for examining large areas of the skin. The advantage of FD is that it indicates the “points of most interest“ for determining the optimal site for dermatoscopic examination or for taking a biopsy. This results in a considerable reduction of procedure time. FD is also a helpful tool for evaluating the efficacy of PDT or for marking the boundaries of the tumor for pre-operative planning. Although promising, the results up till now were limited because of technological constraints in terms of the application of 5-ALA, the illumination and the observation of fluorescence.

A new FD system with a purpose-made digital camera and software in combination with the application of 5-ALA encapsulated in liposomes was evaluated in the studies described in this thesis in order to improve the detection of early NMSCs (clinically visible as well as non-visible lesions) on the face, the neck, the chest, the back and the hands of patients treated with UV and outdoor workers.

3.4.5 References

1. Marks R. An overview of skin cancers. Incidence and causation. Cancer 1995; 75: 607-612.
2. English DR, Armstrong BK, Kricker A, Fleming C. Sunlight and cancer. Cancer Causes Control 1997; 8: 271-283.
3. Salasche SJ. Epidemiology of actinic keratoses and squamous cell carcinoma. J Am Acad Dermatol 2000; 42: S4-7.
4. Diepgen TL, Mahler V. The epidemiology of skin cancer. Br J Dermatol 2002; 146, Suppl 61: 1-6.
5. Trakatelli M, Ulrich C, del Marmol V, Euvrard S, Stockfleth E, Abeni D. Epidemiology of nonmelanoma skin cancer (NMSC) in Europe: accurate and comparable data are needed for effective public health monitoring and interventions. Br J Dermatol 2007; 156, S 3: 1-7.
6. Marks R, Rennie G. Malignant transformation of solar keratoses to squamous cell carcinoma. Lancet 1988: 795-797.
7. Jorizzo JL, Carney PS, Ko WT, Robins P, Weinkle SH, Werschler WP. Treatment options in the management of actinic keratosis. Cutis 2004; 74 (S 6): 9-17.
8. Epstein E. Quantifying actinic keratosis: assessing the evidence. Am J Clin Dermatol 2004; 5: 141-144. x
9. Ackerman AB, Mones JM. Solar (actinic) keratosis is squamous cell carcinoma. Brit J Dermatol 2006; 155: 9-22.
10. Oppel T, Korting HC. Actinic keratosis: the key event in the evolution from photoaged skin to squamous cell carcinoma. Skin Pharmacol Physiol 2004; 17: 67-76.
11. Dodson JM, DeSpain J, Hewett JE, Clark DP. Malignant potential of actinic keratoses and the controversy over treatment: a patient-oriented perspective. Arch Dermatol 1991; 127: 1029-1031.
12. Rossi R, Mori M, Lotti T. Actinic keratosis. Int J Dermatol 2007; 46: 895-904.
13. Housman TS, Feldman SR, Williford PM, Fleisher AB, Goldman ND, Acostamadiedo JM, Chen GD. Skin cancer is among the most costly of all cancers to treat for Medicare population. J Am Acad Dermatol 2003; 48: 425-429.
14. De Vries E, van de Poll-Franse LV, W.J. Louwman WJ, de Gruijl FR, Coebergh JWW.
    Predictions of skin cancer incidence in the Netherlands up to 2015. Br J Dermatol 2005; 152: 481–488.
15. Armstrong BK, Kricker A. The epidemiology of UV induced skin cancer. J

Photochem, Photobiol B: Biol 2001 ; 63 :8– 18.
16. 16. Young AR. Tanning devices-fast track to skin cancer? Pigment Cell Res 2004; 17: 2–9.
17. MacKie RM. Long-term health risk to the skin of ultraviolet radiation. Prog Biophys Mol Biol, 2006; 92: 92–96.
18. The International Agency for Research on Cancer Working Group on Artificial Ultraviolet Light and Skin Cancer. The association of use of sun beds with cutaneous malignant melanoma and other skin cancers: A systemic review. Int J Cancer 2006; 120: 1116–1122.
19. Nijsten TEC, Stern RS. The increased risk of skin cancer is persistent after discontinuation of psoralen+ultraviolet A: A cohort study. J Invest Dermatol 2003; 121: 252–258.
20. Lim JL, Stern RS. High levels of ultraviolet B exposure increase the risk of non-melanoma skin cancer in psoralen and ultraviolet A-treated patients. J Invest Dermatol 2005; 124: 505–513.
21. Vitasa BC, Taylor HR, Strickland PT, Rosenthal F, West S et al. Association of non-melanoma skin cancer and actinic keratosis with cumulative solar ultraviolet exposure in Maryland watermen. Cancer 1990; 65: 2811–2817.
22. Buja A, Lange JH, Perissinotto E, Rausa G, Grigoletto F, Canova C, Mastrangelo G. Cancer incidence among male military and civil pilots and flight attendants: An analysis on published data. Toxicol Ind Health 2005; 21: 273– 282.
23. Moehrle M. Outdoor sports and skin cancer. Clin Dermatol 2008; 26: 12–15.
24. Schwartz RA, Bridges TM, Butani AK, Ehrlich A. Actinic keratosis: An occupational and environmental disorder. J Eur Acad Dermatol Venereol 2008: 22: 606-615.
25. Masini C, Fuchs PG, Gabrielli F, Stark S et al. Evidence for the association of human papilloma virus infection and cutaneous squamous cell carcinoma in immunocompetent individuals. Arch Dermatol 2003; 139: 890–894.
26. Euvrard S, Kanitakis J, Claudy A. Medical progress: Skin cancers in organ transplant recipients. N Engl J Med 2003; 348: 1681-1691.
27. De Hertog SAE, Wensveen CAH, Bastiaens MT, Kielich CJ, Leiden skin cancer study. Relation between smoking and skin cancer. J Clin Oncol 2001; 19: 231-238.
28. Jemal A, Thomas A, Murray T, Thun M. Cancer statistics, 2002. CA Cancer J Clin 2002; 52: 23-47.
29. Venna SS, Lee D, Stadecker MJ, Rogers GS. Clinical recognition of actinic keratoses in a high-risk population. Arch Dermatol 2005; 141: 507-509

30. Smits T, Kleinpenning MM, Blokx WAM, Kerkhof PCM, van Erp PEJ, Gerritsen MP. Fluorescence diagnosis in keratinocytic intraepidermal neoplasias. J Am Acad Dermatol 2007; 57: 824-831.
31. Peris K, Micantonio T, Piccolo D, Fargnoli MC. Dermoscopic features of actinic keratosis. J Dtsch Dermatol Ges 2007; 5: 970–976.
32. Pan Y, Chamberlain AJ, Bailey M, Chong AH, Haskett M, Kelly JW. Dermatoscopy aids in the diagnosis of the solitary red scaly patch or plaque - features distinguishing superficial basal cell carcinoma, intraepidermal carcinoma, and psoriasis. J Am Acad Dermatol 2008; 59: 268-274.
33. Scalvenzi M, Lembo S, Francia MG, Anna Balato A . Dermoscopic patterns of superficial basal cell carcinoma. Int J Dermatol 2008; 47: 1015–1018.
34. Argenziano G, Zalaudek I, Corona R, Sera F, Cicale L, Petrillo G, Ruocco E, Hofmann-Wellenhof R, Soyer HP. Vascular Structures in Skin Tumors A Dermoscopy Study. Arch Dermatol. 2004; 140: 1485-1489.
35. Bryden AM, Dawe RS, Fleming C. Dermatoscopic features of benign sebaceous proliferation. Clin Exp Dermatol 2004, 29, 676–677.
36. Zaballos P, Ara M, Puig S, Malvehy J. Dermoscopy of Sebaceous Hyperplasia. Arch Dermat 2005; 141: 808.
37. Bäumler W, Abels C, Szeimies RM. Fluorescence diagnosis and photodynamic therapy in dermatology. Med Laser Appl 2003; 18: 47-56.
38. Fauteck JD, Ackermann G, Birkel M, Breuer M, Moor ACE, Ebeling A, Ortland C. Fluorescence characteristics and pharmacokinetic properties of a novel self-adhesive 5-ALA patch for photodynamic therapy of actinic keratoses. Arch of Dermatol Res 2008; 300: 53-60.

Chapter 4

Published as

Treatment of chronic plaque type psoriasis with the MultiCare pulsed dye laser

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Summary

In psoriasis, the earliest changes before the occurrence of inflammation and epidermal hyperproliferation are in the dermal papillary vasculature. Previous investigations have demonstrated a reduction of psoriasis after selective photothermolysis of the dermal vasculature with the pulsed dye laser. This laser was used to treat 74 patients with chronic plaque type psoriasis unresponsive to conventional topical therapies. After laser treatment, improvement appeared to be good to very good in 73% of patients.

Introduction

Psoriasis is histologically characterized by epidermal keratinocyte hyperproliferation, an intradermal and intraepidermal inflammatory infiltrate composed mainly of T cells, accumulation of neutrophil granulocytes, increased numbers of dendritic cells and mast cells, and expansion of the superficial dermal vasculature. Traditional therapies for psoriasis have generally focused on inhibition of epidermal proliferation and /or inflammation. The abnormities in the capillary vasculature have therapeutically been neglected, although it has been reported that in a new psoriasis lesion the earliest observable changes are in the dermal papillary vasculature.[1,2,3] These changes include increased vascular dilatation and proliferation, increased tortuosity of capillaries, swelling of endothelial cells, and widening of the gaps between endothelial cells.[2,3] In psoriasis skin capillary loops display the morphological characteristics of venous capillaries, including bridged fenestrations of the endothelial cell layer, facilitating the exchange between the vessel and the interstitium.[4] In addition,T-helper cells adhere to dermal papilla capillary endothelium in psoriatic but not in normal kin.[5] These studies have led to the hypothesis that selective destruction by photothermolysis of the dilated papillary vessels leads to elimination of the extravasation of mediators of

inflammation in the interstitium resulting in clearing of psoriasis plaques.[6]

The Pulsed Dye laser (PDL) is very suitable for selective photothermolysis of dermal vessels.[7] This machine has been proven to be effective in the treatment of teleangiectasia and port wine nevi. [8,9,10] Previous studies have demonstrated partial and total clearance of psoriasis with the PLD.[6,11,12,13]

Katugampola et al treated 8 patients with chronic plaque type psoriasis. An improvement of more than 50% was shown in 5 patients at week 10 after the last of 3 treatment sessions. A complete remission of the treated plaques was seen in 1 patient. [6]

Ros et al observed a clinical improvement after 1 to 3 treatment sessions in 6 of 10 patients. Pathohistologic findings of biopsies from treated skin of 3 patients revealed no epidermal damage. [11]

In an elegant study performed by Zelickson et al, a psoriasis plaque was divided into 2 halves, one half was treated with PDL, the other half was not treated. The authors demonstrated a remission of psoriasis after 2 treatments, while the non-treated half did not show any improvement at all. After 13 months an expansion of psoriasis was seen from the non-treated half into the treated half of the plaque. The remission time of psoriasis plaques treated by PDL appeared to be considerable longer than the remission time of psoriasis plaques treated by triamcinolon cream. Confocal microscopy demonstrated normalisation of diameter and tortuosity of papillary vessels in psoriasis skin after PDL treatment.[12]

Bjerring et al compared the results of PDL treatment in 11 patients with chronic plaque type psoriasis with the results of simultaneous treatment with dermabrasion in 6 of the 11 patients. Complete remission was seen in 3 patients after PDL and in 5 patients after dermabrasion. Partial remission was observed in 6 patients treated by PDL and in 1 patient treated by dermabrasion. The PDL treatment appeared to be less damaging than dermabrasion.[13]

The purpose of this open prospective study was to evaluate the effectiveness and the safety of pulsed dye laser treatment in 74 patients with plaque type psoriasis.

Patients and methods

Selection of the patients

The study group comprised 74 patients with stable plaque type psoriasis or psoriasis pustulosa circumscripta on the hands and feet, which was resistant to previous topical treatments (calcipotriol, corticosteroids, tar, broadband UVB,and topical psoralen-UVA). Patients with photointolerance (toxic or allergic), on medication with phototoxic or photoallergic drugs, who were pregnant, or with pre-existent or

manifest cutaneous malignancy were excluded.
All included patients provided written informed consent to participate in the study.
Four patients did not show up, 1 patient because of intercurrent mamma carcinoma and the other 3 patients without any definite reason.

Methods

Laser treatment: Patients were treated with PDL equipment (MultiCare, VLS Cynosure, Chelmsford, Mass, USA) that had adjustable settings for wavelengths from 585 to 600 nm; pulse durations of 450 and 1500 microseconds; and 3-, 5-, 7-, and 10-mm spot diameters. Depending on the spot size, fluences up to 20-J/cm^2 are possible. In this study a wavelength of 585 nm and a pulse duration of 0.450 msec was used. Treatment fluences ranged from 6 to 8 J/cm^2 for 5-mm spot size, 5 to 6 J/cm^2 with a 7-mm spot diameter. The patients were treated once every 4 to 6 weeks with an overlap of ± 20% of the spot size, treating the entire lesion with a margin of about 5 mm in the surrounding skin. During the treatment the skin was cooled with cold air (Cryo 5, Zimmer Elektromedizin GmbH, Ulm, Germany). Patients were requested to report any adverse events immediately.
In each patient the initial PDL treatment was carried out with a spot diameter of 5 mm and a fluence of 6.0 J/cm^2. At following visits the fluence was increased with 0.5 to 1.0 J/cm^2 in case the psoriasis lesions showed a lower improvement than 50%, up till a maximum fluence of 8.0 J/cm^2. Psoriasis lesions with thick infiltration and/or abundant scales were treated with a spot diameter of 7mm and initial fluence of 5.0 J/cm^2, and the fluence was raised on indication up till a maximum of 7.0 J/cm^2.
The PDL treatment was terminated in those cases, which did not show an improvement of more than 30 % after 5 treatments.

Comedication

To promote the penetration of the laser light into the skin, scaly psoriatic lesions of the patients were initially treated with calcipotriol ointment between laser treatments. However, soon we noticed that there was more hyperpigmentation in patients after they had received a combination of calcipotriol ointment and laser therapy than after laser therapy alone. Replacement of calcipotriol ointment by salicylic acid 5% to 10% in petrolatum ointment resulted in sufficient keratolysis and less hyperpigmentation. The patients were also instructed to discontinue any other medication.

Evaluation of PDL treatment

Treatment efficacy was evaluated by blinded comparison of photographs (Fotofinder, TeachSreen Software GmbH, Bad Birnbach, Germany) of the lesions taken in each patient at inclusion time and every control visit before PDL treatment. Changes of

lesions were assessed concerning erythema, desquamation and size by experienced dermatologists. The results were recorded as follows (Table 1):

Results

Of the 74 treated, 54 patients (73%) achieved greater than 70% improvement after an improvement average of 2.5 treatments, 35 of the patients (47%) achieved a clearance between 91% and 100%, 19 of the patients (26%) an improvement between 71% and 90%, and 11 of the patients (15%) an improvement between 31% and 70% clearance (Table 2). All 9 patients (12%) exhibiting 30% and less clearance were deemed treatment failures for purposes of the study. Improvement of lesions and outcomes were well correlated to the number of treatments received (Figure 1). Of those receiving four or more treatments, only one (6%) had improvement less than 70%.

Of those exhibiting greater than 70% improvement in their lesions, the duration of remission averaged 14.7 months following the final treatment (Figure 2). Those with 90-100% improvement exhibited the longest duration of remission, up to 36 months with an average of 17.2 months. Those with between 70 and 90% improvement exhibited up to 24 moths of remission with an average of 10.8 months. The degree of improvement was well correlated with duration of remission.

Side effects of the PDL treatment were post laser treatment purpura in all the patients with spontaneous resolution in 1 to 2 weeks, non-severe pain during and shortly after the laser treatment (local anesthesia was seldom necessary), transient hypopigmentation in 3% of the patients, transient hyperpigmentation in 27% of the patients, and transient crustae in 3% of the patients. Scarring was not observed. There were no dropouts because of adverse events associated with PDL treatment.

Summery of the results: 73% of the patients suffering from recalcitrant plaque type psoriasis who were treated with the pulsed dye laser showed an improvement of 71% to complete clearance after 1 – 5 treatment sessions (Figures 3 – 6). The mean time of psoriasis remission appeared to be 14 months. Serious side effects were not observed.

Discussion

The pathogenesis of psoriasis is not yet entirely known, although present evidence indicates that psoriasis is primarily a T cell–dependent immunological disease.[14] Problems may often be encountered in the course of treatment of psoriasis. Topical treatment using calcipotriol, corticosteroids, tar and ultraviolet light (UV) is not always effective and side effects of topical anti-psoriasis agents may limit their use. In those cases that topical treatment does not work sufficiently, the next step is to change to systemic treatments with fumarates, retinoids, methotrexate or cyclosporine. Although

these systemic agents are more efficacious than the topical agents, they may have even more serious side effects. Every expansion of the therapeutic armamentarium with methods minimizing side effects is welcome.

Laser light with a wavelength of 585 nm passes through the epidermis without damaging epidermal structures, because the epidermis contains no chromophores for 585 nm light.[11] Lack of epidermal damage prevents the Köbner phenomenon to occur.[13] Only abundant melanin in sun tanned skin and in skin type 4 to 6 absorbs 585 nm light, which may result in hyper-and hypopigmentation. The thermal relaxation time (TRT) of blood vessels in the skin vary from 0.2 msec for vessels with a diameter of 10 micrometer up till 3 msec for larger vessels with diameter of 40 micrometer.[15] Thus, the pulse duration of the pulsed dye laser (0.450 msec) is longer than the TRT of the small vessels and therefore long enough to avoid damage to the small vessels. The pulse duration of 0.450 msec is short enough to eliminate the dilated vessels with diameters of about 20 micrometer in the stratum papillare of psoriatic skin. [15] Zelickson et al did not observe a significant difference in the efficacy of PDL light with a pulse duration of 0.450 msec compared with a pulse width of 1.5 msec. In this study best clinical results were achieved using the 5-mm spot diameter, which is in accordance with previous studies. Penetration of laser light increases with increasing spot diameter making a spot diameter of 7-mm more suitable for more infiltrated, thick psoriasis lesions.

The 7-mm spot diameter has also the advantage of shorter treatment duration. PDL treatment using the 10 mm spot diameter appeared to be less effective than treatment using 5-mm and 7-mm spot sizes. This is probably due to the lower energy per mm^3 on the level of the papillary vessels in the skin column provided by the 10-mm spot size compared with the energy in the 5-mm and 7-mm skin column, because the 10-mm spot size light penetrates deeper into the skin than the 5-mm and 7-mm spot size light.

The fluence of the PDL has to be high enough for photothermolysis of the dilated vessels in psoriatic skin. For 5-mm spot size fluences of 6 to 8 J/cm^2 are appropriate, and in recalcitrant cases even 9 J/cm^2. A spot diameter of 7-mm requires fluences of 5 to 7J/cm^2. If the fluence is chosen to low, no purpura will be seen. Therefore, the appearance of purpura during PDL treatment indicates the appropriate fluence. Cooling the skin during treatment using an air-cooled device reduces discomfort and should be a standard procedure. No analgesia is required and treatments are well tolerated. The treatment of a psoriasis lesion sized 100 cm^2 takes about 10 minutes Therefore, the treatment of wide spread psoriasis is to time-consuming Additional daily application of salicylic acid 5% in petrolatum removes much of the scaling associated with psoriatic plaques, thereby improving the amount of energy delivered

to the dermal vasculature. The application of salicylic acid ointment was stopped 2 days before each PDL treatment, because of its photo-protective capacity. Calcipotriol ointment has also keratolytic capacity, but it causes more post laser hyperpigmentation than salicylic acid ointment does.

Conclusion:

Seventy-three percent of patients with plaque type psoriasis resistant to traditional topical treatments achieved between 70% and 100% remission in the treated lesions. A mean duration of remission appeared to be 14 months. PDL treatment is indicated for patients with persistent plaque type psoriasis, not responding to usual treatments (particularly on "non-covered body" sites), plaque type psoriasis complicated by side effects of medication, psoriasis palmoplantaris, psoriasis inversa, patients not willing to use topical corticosteroids or systemic treatment, and patients at risk for skin cancer provocation by UVB therapy or immunosuppression.

References

1. Pinkus H, Mehregan AH. The primary histologic lesion of seborrhoeic dermatitis and psoriasis. J Invest Dermatol 1966; 46: 109–116.
2. Macdonald D, Hull S, Goodfield M, et al. Active and inactive edges of psoriatic plaques: identification by tracings and investigation by laser-Doppler flowmetry and immunocytochemical techniques. J Invest Dermatol 1989; 92: 782–785.
3. Heng MCY, Allen SG, Haberfelde GH, Song MK. Electronmicroscopic and immunohistochemical studies of the sequence of events in psoriatic plaque formation following tape-stripping. Br J Dermatol 1991; 125: 548–556.
4. Braverman IM, Yen A. Ultrastructure of the capillary loops in the dermal papillae of psoriasis. J Invest Dermatol 1977; 68: 53–60.
5. Barker JNWN. Pathophysiology of psoriasis. Lancet 1991; 338: 227-230.
6. Katugampola GA, Rees AM, Lanigan SW. Laser treatment of psoriasis. Br J Dermatol 1995; 133: 909–913.
7. Anderson RR, Parrish JA. Selective photothermolysis: precise microsurgery by selective absorption of pulsed radiation. Science 1983; 220: 524–527.
8. Tan OT, Sherwood K, Gilchrest BA. Treatment of children with port-wine stains using the flashlamp-pumped pulsed dye laser. N Engl J Med 1989; 320: 416-421.
9. Tan OT, Morrison P, Kuban AK. 585 nm For the treatment of port-wine stains. Plast Reconstr Surg 1990; 86:1112- 1117.
10. Lowe NJ, Behr KL, Fitzpatrick R, Goldman M, Ruiz-Esparza J.Flashlamp pumped dye laser for rosacea-associated teleangiectasia and erythema. J

Dermatol Surg Oncol 1991;17: 522-525.
11. Ros AM, Garden JM, Bakus AD, Hedblad MA. Psoriasis response to the pulsed dye laser. Lasers Surg Med 1996; 19: 331-335.
12. Zelickson BD, Mehregan DA, Wendelschfer-Crabb, Ruppman D, Cook A, O'Connell P, et al. Clinical and histologic evaluation of psoriatic plaques treated with a flashlamp pulsed dye laser. J Am Acad Dermatol 1996; 35: 64-68.
13. Bjerring P, Zachariae H, Søgaard H. The flashlamp-pumped dye laser and dermabrasion in psoriasis - further studies on the reversed Ko¨ bner´s phenomenon. Acta Derm Venereol 1997; 77:59-61.
14. Baker BS, Fry L. The immunology of psoriasis. Br J Dermatol 1992;126:1-9.
15. Garden JM, Tan OT, Kerschmann R, Boll J, Furumoto H, Anderson RR. Effect of dye laser pulse duration on selective cutaneous vascular injury. J Invest Dermatol 1986; 87:653-657.

91% to 100% change	complete remission	Score 4
71% to 90% change	good improvement	Score 3
31% to 70% change	moderate improvement	Score 2
11% to 30% change	some improvement	Score 1
0% to 10% change	no improvement	Score 0

Table 1.

Number of treatments	score 4	score 3	score 2	score 1	score 0	Total
1	9.46%	1.51%	2.70%	1.35%	2.70%	29.73%
2	13.51%	6.76%	6.76%	4.05%	2.70%	33.78%
3	6.76%	4.05%	4.05%	0.00%	1.35%	16.22%
4	12.16%	1.35%	1.35%	0.00%	0.00%	5.41%
5	5.41%	0.00%	0.00%	0.00%	0.00%	5.41%
Total	47.3%	25.68%	14.86%	5.41%	6.76%	100.00%

Table 2.

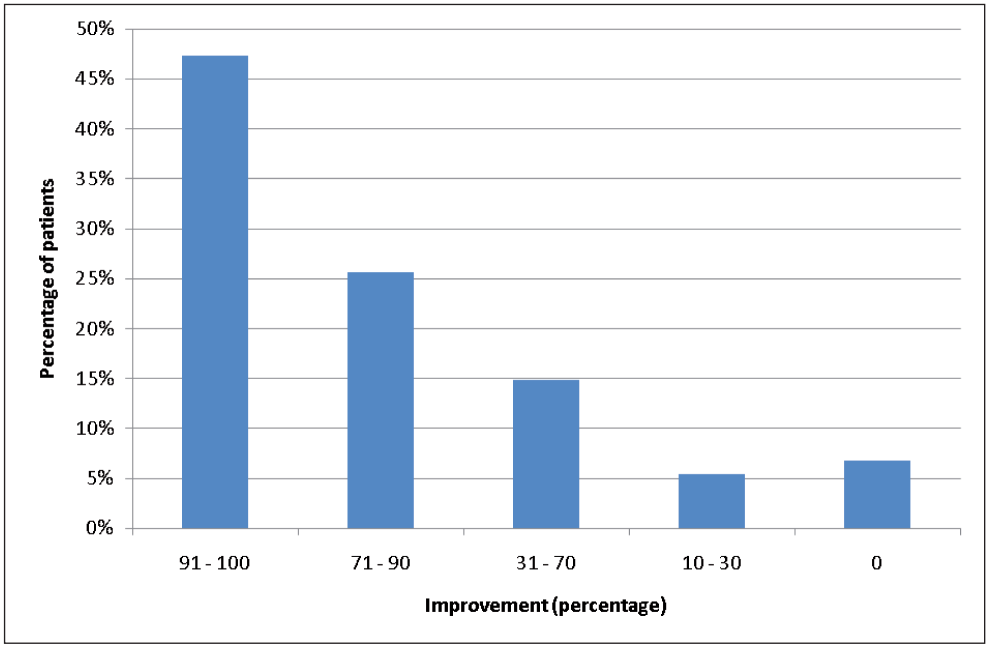

Figure 1. Results after 1 to 5 PDL treatments

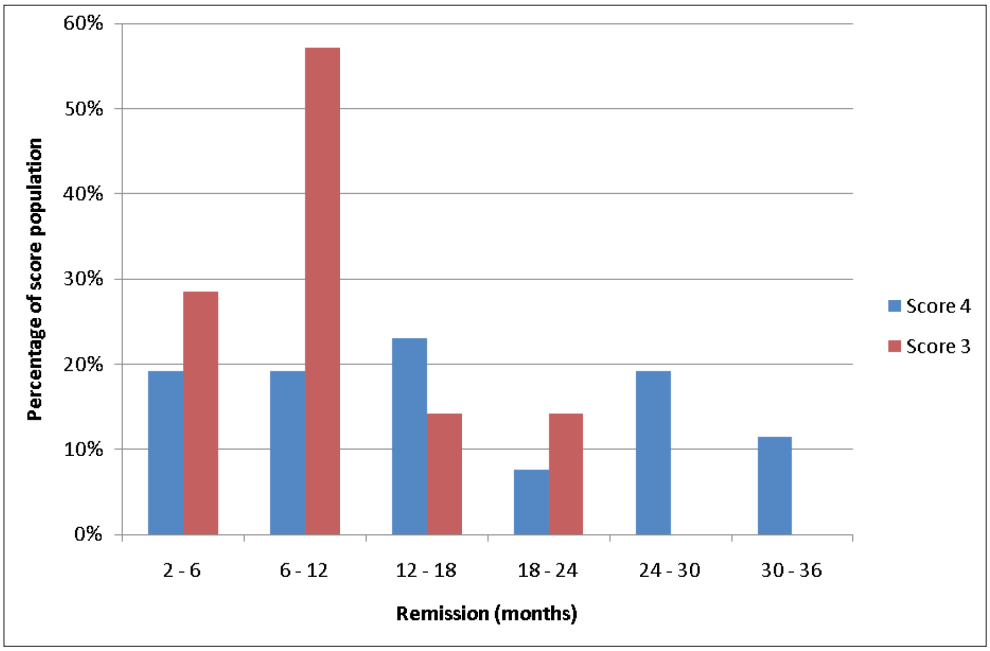

Figure 2. Remission periods in relation with treatment scores

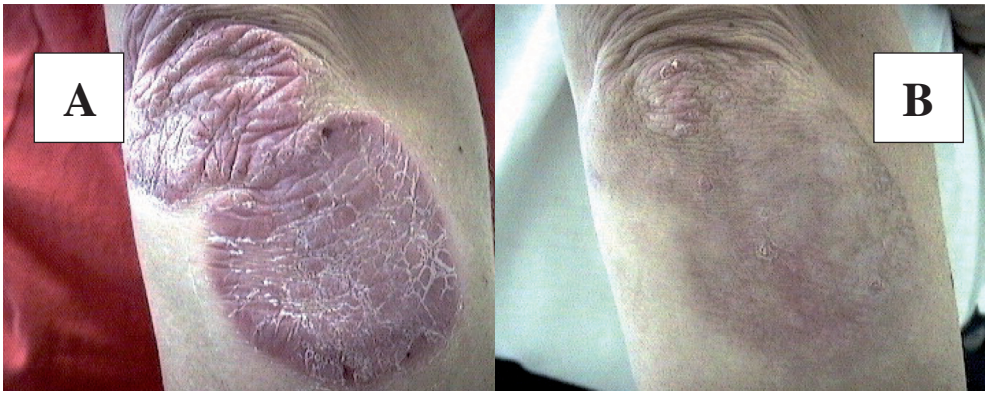

Figure 3. Score 4: improvement 95% after 2 PDL treatments

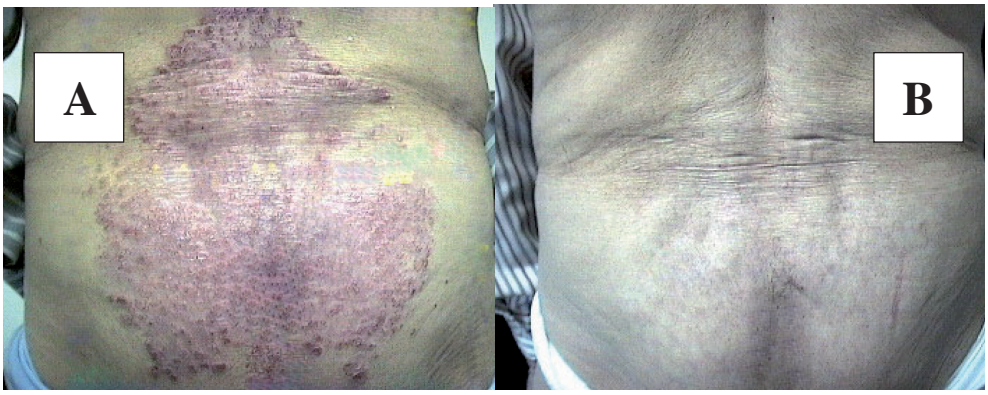

Figure 4. Score 4: improvement 100 % after 5 PDL treatments

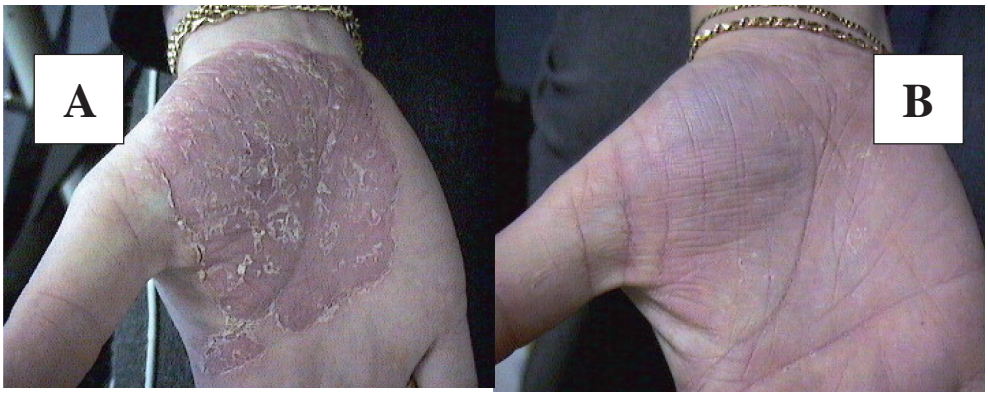

Figure 5. Score 4: improvement 95% after 2 PDL reatments

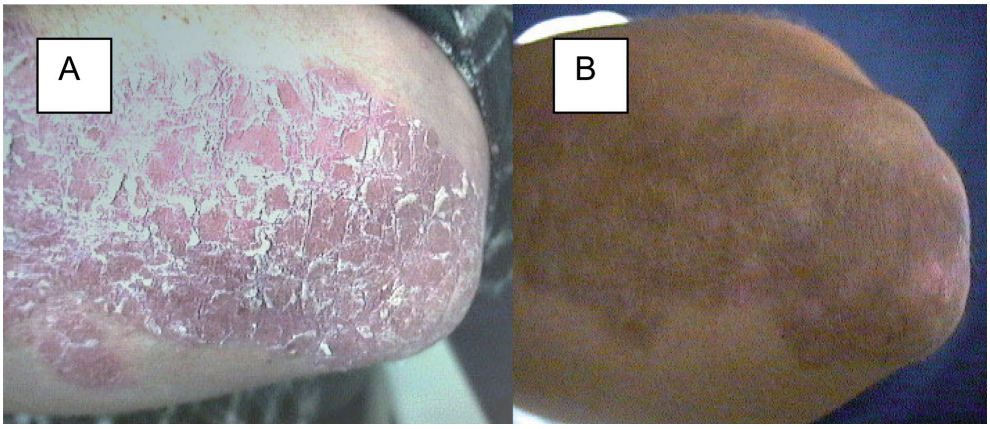

Figure 6. Score 3: improvement 71- 90 % after 2 PDL treatments
Side effect hyperpimentation

Chapter 5

Published as

Concomitant treatment of psoriasis of hands and feet with pulsed dye laser and topical calcipotriol, salicylic acid, or both: A prospective open study in 41 patients

Jaap de Leeuw, Bhupendra Tank, Peter J. Bjerring,
Suzanne Koetsveld, Martino Neumann.

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Abstract

Background: Psoriasis of the hands and feet is a chronic disease which is often resistant to the usual topical therapies. It has considerable morbidity and seriously affects the quality of life of patients.

Objective: We sought to prospectively evaluate the efficacy and safety of pulsed dye laser (PDL) treatment of psoriasis of the hands and feet.

Methods: In all, 41 patients with therapy-resistant psoriasis of the hands and feet were treated once every 4 to 6 weeks with PDL at 585-nm wavelength, 450 microsecond pulse duration, 7-mm spot diameter, and 5- to 6.5 J/cm2 fluence. Calcipotriol ointment and salicylic acid 5% to 10% ointment were used as keratolytic agents. Treatment efficacy was evaluated by blinded comparison of photographs of the lesions taken before and after PDL treatment in each patient.

Results: A good to very good improvement in the lesions was observed in 76% of the patients after treatment. An average duration of remission was 11 months. Side effects were transient purpura, moderate discomfort during the treatment, transient hyperpigmentation or hypopigmentation, and incidental transient crustae.

Limitations: This was an open prospective study with a limited number of patients who were concomitantly treated with calcipotriol and salicylic acid ointment. Patients with photointolerance, on medication with phototoxic or photoallergic drugs, and with widespread psoriasis were excluded.

Conclusions: Concomitant treatment with PDL and topical calcipotriol, salicylic acid,

or both was a satisfactory modality for treating psoriasis of the hands and feet. There was a subjective improvement in the symptoms and quality of life in all patients.

Introduction

Psoriasis is a chronic disease causing significant morbidity and poor quality of life in many patients.[1,2] In particular, psoriasis of the hands and feet is a distressing and disfiguring disease, often resistant to treatment, especially in case of pustulosis palmoplantaris. Topical and systemic treatments are not always adequately effective and their use may be limited by side effects. Therefore, clinicians have sought alternative methods and have turned to lasers for treating psoriasis.[3] Traditional therapies for psoriasis have focused on the inhibition of epidermal proliferation, inflammation, or both. The earliest changes noted in a new psoriatic lesion are in the capillaries.[4-7] These studies have led to the idea that selective destruction of the dilated papillary vessels by selective photothermolysis eliminates the extravasation of inflammatory mediators into the interstitium resulting in clearing of psoriatic plaques.[8]

The theory of selective photothermolysis stipulates that selective target destruction can be achieved by selection of a wavelength optimally absorbed by the chosen target, a pulse duration of less than the inherent thermal relaxation time of the target, and a fluence that is sufficient to cause nonrepairable injury.[9]

Selective photothermolysis of cutaneous blood vessels can be achieved with the flashlamp-pumped pulsed dye laser (PDL).[10-12] Previous studies have demonstrated partial and total clearance of psoriasis by selective photothermolysis of dermal vessels with PDL.[13-17] The aim of this open prospective study was to evaluate the efficacy and safety of PDL treatment in patients with psoriasis manuum et pedis. Changes in the disease-related symptoms and the quality of life after treatment were also assessed.

Methods

Patients

The study group comprised 41 patients (23 female, 18 male) aged 11 to 70 years (mean: 46.4 years) with clinical and histologic features of plaque-type psoriasis and psoriasis pustulosa on the hands and feet, which was resistant to previous topical treatments (calcipotriol, corticosteroids, tar, broadband UVB, topical psoralen-UVA). Patients with photointolerance (toxic or allergic), on medication with phototoxic or photoallergic drugs, who were pregnant, or with pre-existent or manifest cutaneous malignancy were excluded. And institutional review board approved this study. All included patients provided written informed consent to participate in this open prospective study.

Laser treatment

Patients were treated with PDL equipment (MultiCare, VLS Cynosure, Chelmsford, Mass) that had adjustable settings for wavelengths from 585 to 600 nm; pulse durations of 450 and 1500 microseconds; and 3-, 5-, 7-, and 10-mm spot diameters. Each patient was treated with the PDL at a 585-nm wavelength, 450-microsecond pulse duration, 7-mm spot size, and 5- to 6.5-J/cm2 fluence. The patients were treated once every 4 to 6 weeks with an overlap of 20% of the spot size, treating the entire lesion with a margin of about 5 mm in the surrounding skin. During the treatment the skin was cooled with cold air (Cryo 5, Zimmer Elektromedizin GmbH, Ulm, Germany). Patients were requested to report any adverse events immediately.

Comedication

To promote the penetration of the laser light into the skin, scaly psoriatic lesions of the patients were initially treated with calcipotriol ointment between laser treatments. However, soon we noticed that there was more hyperpigmentation in patients after they had received a combination of calcipotriol ointment and laser therapy than after laser therapy alone. Replacement of calcipotriol ointment by salicylic acid 5% to 10% in petrolatum ointment resulted in sufficient keratolysis and less hyperpigmentation. The patients were also requested to discontinue any other medication.

Evaluation of PDL treatment

Treatment efficacy was evaluated by blinded comparison of photographs (Fotofinder, TeachSreen Software GmbH, Bad Birnbach, Germany) of the lesions taken before and after PDL treatment in each patient. Experienced dermatologists subjectively assessed the photographs. Improvement was scored as follows: no improvement, 0% to 10% clearance; poor improvement, 11% to 30% clearance; moderate improvement, 31% to 70% clearance; good improvement, 71% to 90% clearance; and very good improvement, 91% to 100% clearance.

PDL treatment was discontinued after complete clearance of the psoriatic lesions or after 5 treatment sessions if the improvement was not better than 30%.

The follow-up in each patient was continued for up to 36 months to determine the duration of remission once the psoriatic lesions had cleared completely. During the follow-up period the patients were permitted to use calcipotriol, salicylic acid ointment, or both.

Results

In all, 41 patients with psoriasis manuum et pedis participated in this open prospective study. The results of the treatment are summarized in Table I. Briefly, 31 (76%) of the 41 patients who were treated achieved more than 71% clearance

after an average of 4.2 treatment sessions (Table II). A representative example of the photographs taken before and after 4 treatment sessions from these 31 patients are shown in Figs 1 and 2. The remaining 10 (24%) patients who showed less than 70% improvement were considered as treatment failures for the purposes of this study. Representative examples of the photographs from these 10 patients taken before and after 5 treatment sessions are shown in Fig 3. The duration of disease remission was also assessed in all 31 patients who showed an improvement of more than 71% during a follow-up period of 36 months. The results of remission are summarized in Table II. The duration of remission averaged 10.7 months after the final treatment in patients who showed an improvement of more than 71% in their lesions. An average duration of remission of 12 months was noted in patients with 91% to 100% improvement in their lesions. Side effects of the PDL treatment were postlaser treatment purpura in all the patients with spontaneous resolution in 1 to 2 weeks, nonsevere pain during and shortly after the laser treatment (local anesthesia was seldom necessary), transient hypopigmentation in 3% of the patients, transient hyperpigmentation in 27% of the patients, and transient crustae in 3% of the patients. There were no dropouts because of adverse events associated with PDL treatment. All the patients verbally commented on the subjective improvement in their quality of life and a reduction in the symptoms of their disease at each visit.

Discussion

The exact cause of psoriasis has not yet been elucidated.[18] Psoriasis is histologically characterized by epidermal keratinocyte hyperproliferation, intradermal and intraepidermal inflammatory infiltrate composed mainly of T cells, and vascular dilation and proliferation in the papillary dermis.[3] The question still remains whether it is the epidermal proliferation that triggers inflammation and vascular changes or whether it is the reverse. The changes in the capillary vasculature of psoriasis have been therapeutically neglected, although it was reported that these were the earliest observed changes both in a new psoriatic lesion and at the margin of an extending lesion.[1-3] Laser light with a wavelength of 585-nm penetrates the epidermis without causing epidermal damage because the keratinocytes have no chromophore absorbing this wavelength whereby Koebner´s phenomenon is prevented from occurring.[11] The thermal relaxation time of the capillary vessels is around 200 microseconds for the smallest vessels with a diameter of 10-µm. The diameter of the dilated psoriatic vessels is about 20-µm. The pulse duration of the PDL (450 microseconds) is short enough to destroy the psoriatic vessels, but long enough to spare the normal capillary vessels.[16] The fluence must be high enough to destroy the psoriatic vessels. The destruction of the vessels is visible as purpura, so that the appearance of purpura is an indication that the fluence has been correctly chosen.

This postlaser purpura disappears 1 to 2 weeks after treatment.

It appeared that a 5-mm spot diameter required a fluence of 6 to 8 J/cm2 to be effective and a 7-mm spot diameter required a fluence of 5 to 6.5 J/cm2. The results of both those treatments were more or less equal.[14] In this open prospective study, we chose treatment with 7-mm spot diameter and fluence of 5 to 6.5 J/cm2. The PDL treatment was generally well tolerated by all patients. Although there was some discomfort, local anesthesia was not required in most of the patients. The pain may be reduced by pretreatment with lidocain-prilocain cream under occlusion, oral paracetamol tablet, or both. This is especially useful in patients with psoriasis of the palms and soles. Hypopigmentation was most common in patients with tanned skin. Hyperpigmentation may be because of sunlight exposure after PDL treatment. Therefore, patients were requested to avoid exposure to (artificial) sunlight 6 weeks before and 6 weeks after PDL treatment. Hyperpigmentation is also often seen after the combination of calcipotriol ointment and PDL treatment. To remove the scales from the psoriatic lesions, salicylic acid ointment does well without augmenting the risk of post-PDL treatment hyperpigmentation.

Cooling of the skin during PDL treatment is obligatory to reduce the pain and the risk for pigment changes. Koo and Lebwohl[19] examined the duration of remission reported with many therapies currently used for psoriasis. The remissions varied from 1 month to 1 year depending on the therapeutic modality. In many clinical studies, ‘‘remission’’ and ‘‘relapse’’ are not actively defined. Not all the investigators tend use the same definitions even when these terms are defined. The least stringent definition of relapse involves a recurrence of psoriasis to 50% of the baseline. The strictest definition involves any recurrence of psoriasis after clearing.[20] We defined a relapse as recurrence of psoriasis to 30% or more of the baseline. During remission, patients were allowed to use emollients or calcipotriol ointment. The mean duration of remission in our study in 31 patients with improvement of 71% to 100% was 10.7 months after the final treatment, whereas the mean duration of remission in the 22 patients with 91% to 100% improvement was 12 months.

Almost identical clinical results were recently reported by Feldman et al, 21 who treated 30 patients with psoriasis using the excimer laser (308-nm, 10 mJ pulse, 3.2-cm^2 spot) (PhotoMedex XTRAC, Montgomeryville, Pa) twice weekly, starting at 2 to 4 minimum erythema dose, increasing it if there was no response after the first treatment. An improvement of 75% was achieved in 72% of patients after an average of 6.2 treatment sessions. Common adverse events were erythema, blistering, hyperpigmentation, and erosions. At 12 months, 24% of the patients showing improvement were remission free. In our study, patients were treated once every 4 to 6 weeks with PDL. After an average of 4.2 treatment sessions, more than 71% clearance was achieved in 76% of the patients. In these patients, the mean duration

of remission was 10.7 months. Crustea were noted in only 3% of the patients and no blistering was observed in any of the patients. Furthermore, the wavelength of 308-nm (excimer laser) is potentially carcinogenic in the long term, whereas the wavelength of 585-nm (PDL) is noncarcinogenic. These differences argue in favor of treating patients with psoriasis using PDL. Good candidates for PDL treatment appeared to be patients with limited plaque-type psoriasis resistant to other topical treatments; patients in whom other treatments caused adverse events; and patients with disseminated psoriasis in important areas such as the hands, arms, legs, and genital area where prolonged treatment is difficult to sustain.[14]

In conclusion, the results of this open prospective study showed that concomitant calcipotriol, salicylic acid ointment, or both and PDL treatment was effective. However, there were limitations to the study reported here. Briefly, the limited number of included patients and the concomitant treatment restrict any definite recommendations concerning its routine use for treating therapy-resistant psoriasis of the hands and feet. This can only be established in large well-designed clinical studies.

References

1. Roenigk HH, Maibach HI. Psoriasis. 3rd ed. New York: Marcel Dekker Inc; 1998. pp. 112-3, 543-557.
2. Finlay AY, Khan GK, Lascombe D, Salek MS. Validation of sickness impact profile and psoriasis disability index in psoriasis. Br J Dermatol 1990;123:751-756.
3. Tournas JA, Lowe NJ, Yamauchi PS. Laser and novel light source treatments for psoriasis. Lasers Surg Med 2004; 35: 165-173.
4. Barker JNWN. Pathophysiology of psoriasis. Lancet 1991; 338: 227-230.
5. Pinkus H, Mehregan AH. The primary histologic lesion of seborrhoic dermatitis and psoriasis. J Invest Dermatol 1966; 46:109-116.
6. Macdonald D, Hull S, Goodfield M, Wood EJ, Cunliffe W. Active and inactive edges of psoriatic plaques: identification by tracings and investigation by laser-Doppler flowmetry and immunocytochemical techniques. J Invest Dermatol 1989; 92: 782-785.
7. Heng MCY, Allen SG, Haberfelde GH, Song MK. Electronmicroscopic and immunohistochemical studies of the sequence of events in psoriatic plaque formation following tape-stripping. Br J Dermatol 1991; 125: 548-56.
8. Yen A, Braverman IM. Ultrastructure of the human microcirculation: the horizontal plexus of the papillary dermis. J Invest Dermatol 1976; 66: 131-142.
9. Anderson RR, Parrish JA. Selective photothermolysis: precise microsurgery by selective absorption of pulsed radiation. Science 1983; 220: 524-527.

10. Tan OT, Sherwood K, Gilchrest BA. Treatment of children with port-wine stains using the flashlamp- pumped pulsed dye laser. N Engl J Med 1989; 320: 416-421.
11. Tan OT, Morrison P, Kuban AK. 585 nm For the treatment of port-wine stains. Plast Reconstr Surg 1990; 86: 1112-1117.
12. Lowe NJ, Behr KL, Fitzpatrick R, Goldman M, Ruiz-Esparza J. Flashlamp pumped dye laser for rosacea-associated teleangiectasia and erythema. J Dermatol Surg Oncol 1991; 17: 522-525.
13. Katugampola GA, Rees AM, Lanigan SW. Laser treatment of psoriasis. Br J Dermatol 1995; 133: 909-913.
14. Ros AM, Garden JM, Bakus AD, Hedblad MA. Psoriasis response to the pulsed dye laser. Lasers Surg Med 1996; 19: 331-335.
15. Zelickson BD, Mehregan DA, Wendelschfer-Crabb, Ruppman D, Cook A, O'Connell P, et al. Clinical and histologic evaluation of psoriatic plaques treated with a flashlamp pulsed dye laser. J Am Acad Dermatol 1996; 35: 64-68.
16. Bjerring P, Zachariae H, Søgaard H. The flashlamp-pumped dye laser and dermabrasion in psoriasis-further studies on the reversed Köbner's phenomeon. Acta Derm Venereol 1997; 77: 59-61.
17. De Leeuw J, Neugebauer WD. Behandeling van chronische plaque psoriasis met de MultiCare pulsed dye laser. NTvDV 2001; 11:3-7.
18. Baker BS, Fry L. The immunology of psoriasis. Br J Dermatol, 1992; 126:1-9.
19. Koo J, Lebwohl M. Duration of remission of psoriasis therapies. J Am Acad Dermatol 1999; 41: 51-9.
20. Garden JM, Tan OT, Kerschmann R, Boll J, Furumoto H, Anderson RR, et al. Effect of dye laser pulse duration on selective cutaneous vascular injury. J Invest Dermatol 1986; 87: 653-7.
21. Feldman SR, Mellen BG, Housman TS, Fitzpatrick RE, Geronemus RG, Friedman PM, et al. Efficacy of the 308-nm excimer laser for treatment of psoriasis: results of a multi-center study. J Am Acad Dermatol 2002; 46: 900-6.

Improvement	No of patients	Patients %
0%-10%	3	7
11%-30%	1	2
31%-70%	6	15
71%-90%	9	22
91%-100%	22	54
Total	41	100

Table I. Improvement in scores in the 41 patients after pulsed dye laser treatment. Improvement of less than 71% was regarded as pulsed dye laser treatment failure.

Follow-up in 31 patients after successful pulsed dye laser treatment			
Improvement	No of patients	Mean No. of PDL treatment sessions	Mean duration of remission, mo
71%-100%	31 (76%)	4.2	10.7
91%-100%	22 (54%)	4.9	12

Table II. All patients were followed up for 36 months. Improvement of 71% to 100% and 91% to 100% in the patients with the corresponding mean number of PDL treatment sessions and the mean duration of remission are shown.

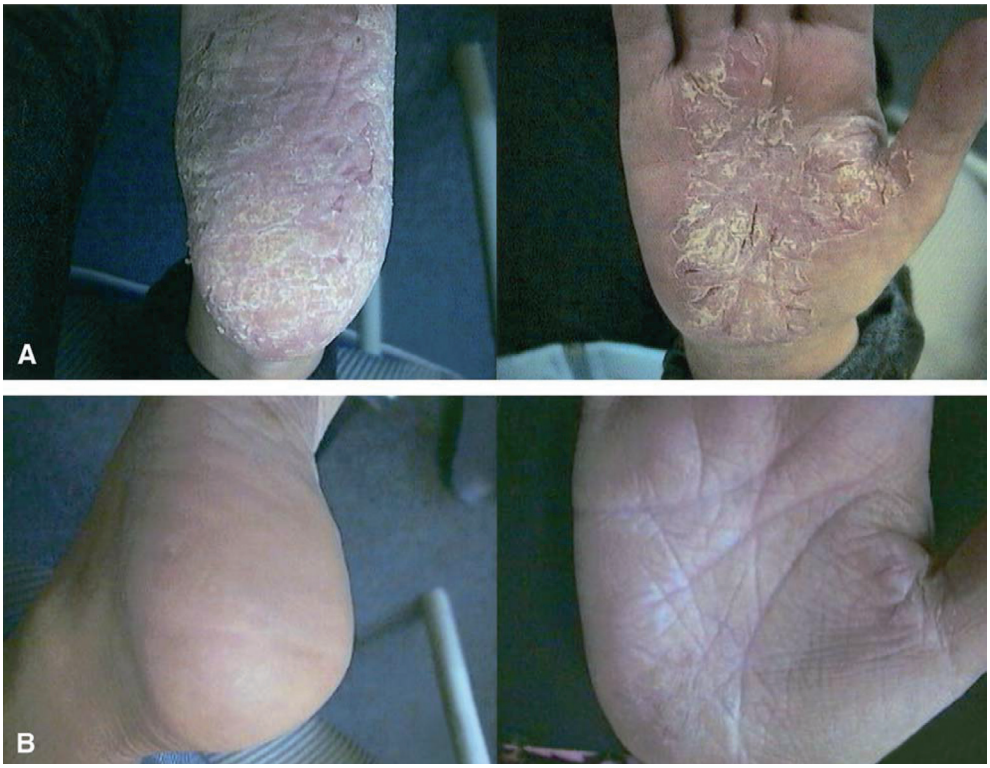

Fig 1. A, Girl aged 14 years with duration of psoriatic lesions for 6 years before pulsed dye laser (PDL) treatment. B, Lesions improved by 95% in same patient after 4 PDL sessions. Lesions were in remission with one treatment each 6 months.

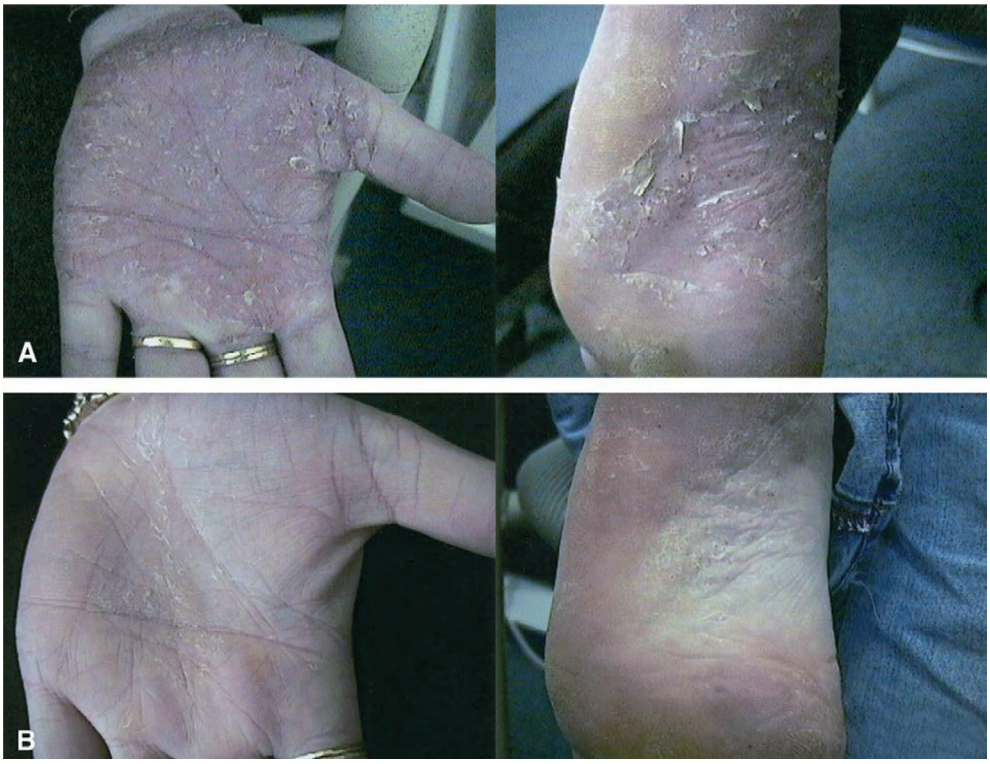

Fig 2. A, Woman aged 22 years with duration of psoriatic lesions for 8 years before pulsed dye laser (PDL) treatment. B, Improvement in hand and foot lesions in same patient of 90% and 70%, respectively, after 4 PDL sessions. Lesions were still in remission after 36 months.

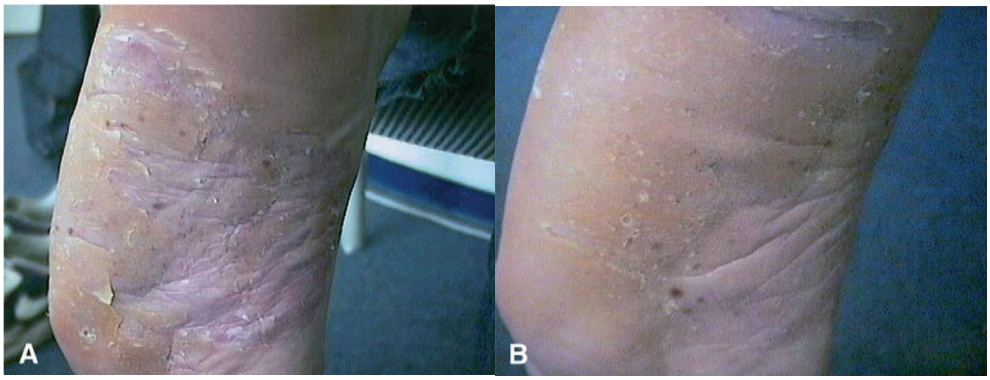

Fig 3. A, Woman aged 44 years with duration of psoriatic lesions for 18 years before pulsed dye laser (PDL) treatment.
B, There was only 50% improvement in lesions of same patient after 5 PDL sessions.

Chapter 6

Published as

A Comparative Study on the Efficacy of Treatment with 585-nm Pulsed Dye Laser and Ultraviolet B-TL01 in Plaque Type Psoriasis

Jaap de Leeuw, Rosanne G. van Lingen, Hilde Both, Bhupendra Tank, Tamar Nijsten, H.A.Martino Neumann.

Dermatologic Surgery 2009; 35: 80-91.

Abstract

Background: Narrow-band Ultraviolet-B and Pulsed Dye Laser both affect psoriasis, but via different pathways.

Objective: To compare the results of Pulsed Dye Laser with Ultraviolet-B therapy and to look for synergism of both therapies in patients with plaque type psoriasis.

Methods: In each eligible individual four similar target plaques were selected and halves of these plaques were treated either by Pulsed Dye Laser, Ultraviolet-B, combination of Pulsed Dye Laser and Ultraviolet-B, or not treated. Results were recorded single-blind using the Physician's Global Assessment-score at study enrolment and at week 13. Non-parametric, paired statistical tests were used to test for differences within and between therapies.The results were also analysed after dichotomisation of the changes in the Physician's Global Assessment-score into responsive and non-responsive to treatment.

Results: A significant improvement of the psoriasis lesions was noted at Week 13 ($p<.001$) with each therapy. No significant differences were noted between the therapies. Synergism of Pulsed Dye Laser and Ultraviolet-B was not observed.

Conclusions: Pulsed Dye Laser is safe for treating plaque type psoriasis, but its efficacy is limited to a sub-group of patients. Combining Pulsed Dye Laser with Ultraviolet-B has no additional benefit.

Introduction

Epidermal hyperproliferation with epidermal thickening, parakeratosis and hyperkeratosis, an intra-dermal and intra-epidermal inflammatory infiltrate composed mainly of T cells, accumulation of neutrophil granulocytes, increased numbers of dendritic cells and mast cells, and expansion of the superficial dermal vasculature histologically characterize psoriasis.[1] In normal skin, the superficial microvasculature is composed of capillary loops that arise from terminal arterioles in the upper horizontal dermal vascular plexus, pass up into the dermal papillae and arch back to connect with the postcapillary venules in the horizontal plexus.[2] Non-lesional skin has short lengths of microvessels in the superficial, papillary dermis, whereas dilated and elongated superficial capillaries passing into the dermal papillae, representing exaggerated tortuosity and coiling of the apical segment of the capillary loop, characterize lesional psoriatic skin.[3,4] The endothelium of the superficial microvasculature in lesional skin is four times thicker than in normal skin but not in the deeper vasculature, indicating that microvascular expansion is restricted to the upper plexus.[5] Although, present evidence indicates that psoriasis is primarily a T cell-dependent disease, the prominent increase in the dermal micro-vasculature in lesional skin indicates that psoriasis is also angiogenesis-dependent.[6-8] Most traditional therapies for psoriasis have focused on the inhibition of epidermal proliferation and suppression of inflammation. Limiting factors are that none of these treatments is effective in all patients, each may have serious side effects, and the efficacy generally decreases over time.[9,10] The alterations in the capillaries have been neglected as a possible therapeutic target, although it has been reported that, in a new psoriasis lesion, one of the earliest observable changes is an increase in the dermal papillary vasculature.[6-8,11,12] During treatment, microvascular improvement precedes clinical improvement.[13] This indicates that the expanded superficial microvascular bed in psoriasis skin is an essential component for maintaining clinical lesions.[13] These observations have led to the hypothesis that, in psoriasis, selective destruction of the dilated papillary vessels by selective photothermolysis may reduce the transmigration of inflammatory cells resulting in clearing of psoriasis plaques.[14] Selective photothermolysis, destroying only the dilated papillary vessels, sparing the normal- sized vessels and without causing damage to the epidermis, became an effective procedure once the Pulsed Dye Laser (PDL) was introduced.[15,16] Partial and total clearance of psoriasis after PDL treatment was reported in several studies.[14, 17-22] Narrow-band ultraviolet-B light therapy (UVB), which is highly efficacious against psoriasis, [23-28] appeared to be superior to broad-band UVB therapy [28,29] and nearly as effective as psoralen ultraviolet-A (PUVA) therapy, and because there are no photosensitizer-related adverse reactions and a possible lower long-term cancer risk, it can be considered to be the standard light therapy.[30] UVB inhibits the proliferation of keratinocytes and induces immunosuppression.[31-34] UVB targets primarily structures in the epidermis, whereas PDL light passes through the epidermis and targets primarily

the superficial vessels in the dermis, indicating that UVB and PDL clear psoriasis via different pathways. The aim of this study was to evaluate the efficacy and the safety of PDL treatment in plaque type psoriasis, to compare the results of PDL treatment with the results of the UVB treatment and with those of the PDL+UVB treatment, and to look for a synergistic effect of PDL+UVB treatment on psoriasis.

Materials and Methods

Subjects

Patients were recruited from the outpatient dermatology clinic of the Erasmus MC, University Medical Center, Rotterdam. Adult patients with stable plaque psoriasis with at least four psoriasis plaques were considered eligible. Patients who were intolerant to light (toxic or allergic), were using drugs with photo toxic or photoallergic potency, were younger than 18 years, were pregnant or had preexistent or manifest skin malignancy were excluded. A wash-out period of 2 weeks was indicated for topical drugs and of 4 weeks for photo (chemo) - therapy and systemic drugs, except for emollients. All included patients provided written informed consent to participate in the study. The Medical Ethical Committee of the Erasmus MC, University Medical Center, Rotterdam approved this study. The study was started in October 2004 and ended in March 2006.

Materials

PDL treatment was performed using the V Star PDL (Cynosure Inc., Chelmsford, MA, USA). The active medium is a rhodamine dye that emits yellow light at a wavelength of 585-nm. During treatment, the skin was cooled with cold air from the Zimmer 6 air cooler (Zimmer Elktromedizin, Neu-Ulm, Germany).[35] PDL treatment parameters were wavelength 585-nm, pulse duration 0.450 ms, spot diameter 7-mm, spots overlapping ± 20% and fluences between 5.5- and 6.5 J/cm².

Ultraviolet 311-nm treatment was performed with the UVB-TL01 S (wavelength 311-nm) handheld device (Cosmedico Medizintechnik GmbH, Villingen-Schwenningen, Germany). Mean irradiance 19.4 mW/cm². During treatment the aperture of the appliance (5.0 cm x 10.0 cm) was held against the skin, the implication being, that the distance between the device and the skin was constant in every patient. Minimal erythema dose (MED) was determined by placing a metal cover with 6 test squares (each 1 cm²) on the MultiCare device. The squares allowed the penetration of 100%, 83%, 65%, 49%, 34% and 17% of UVB-TL01 irradiance of the device (19.4 mW/cm²). After 24 hours the test squares were examined. The square showing just observable erythema was considered as MED. Mean MED was 0.86 J/cm² (range 0.34-1.2 J/cm²).

Treatment schedule

In each patient, four plaques were randomly allocated, divided into two halves, and treated as shown in Figure 1. This paired approach enabled us to assess the effect of UVB, PDL and UVB+PDL at Week 13 and to compare the effect of UVB and PDL with control sides in plaque b and in plaque c. The sides of plaques a and b that were not treated with UVB were protected with metal foil during exposure. Plaque d was treated with the combination of UVB and PDL (PDL+UVB) on both sides, and the effect was compared with that of UVB (plaque b) and PDL (plaque c). Therefore, four plaques were available for blind observation: two plaques treated on both sides (plaque a, plaque d) and two plaques treated on one side (plaque b, plaque c). In some patients, the psoriatic lesions were too small to divide into two halves. In these cases, two separate, comparable, nearby plaques were selected as substitutes. The four target plaques were selected, as much as possible, on symmetrical sites of the right arm, the left arm, the right leg and the left leg. A clock wise rotation schedule of the various above-mentioned treatments, of plaque a through plaque d, was used in which, in every new included patient, the schedule started on a different body site. The remaining plaques in patients who presented with more than the four study plaques were treated as follows: half of the body with UVB-TL01, the other half with PDL, following a rotation model according to the sequence of inclusion.

Patients were treated with PDL every 3 weeks for 10 weeks, in total four treatments (Table 1). During laser treatment, the patient's skin was cooled with cool air to reduce the pain and to prevent damage to the melanocytes due to overheating.

UVB treatment parameters were wavelength 311 nm, start dose 70% of the MED, dose increments each treatment 20% of previous treatment until erythema threshold or clearing of the lesion was reached, three weekly treatments for 11 weeks (in total 30-33 treatments) (Table1).[36,37] Mean initial dose was 0.62 J/cm² (range 0.24-0.86 J/cm²), cumulative dose 84 J/cm² (range 40-120 J/cm²).

Concomitant topical treatment with salicylic acid 5% in petrolatum was permitted for all plaques to reduce scattering of the laser beam by scales but was discontinued 2 days prior to PDL treatment.

Clinical assessment

Specialized dermatological nurses recorded clinical results single-blind using the Physician's Global Assessment (PGA) score[38] (Table 2) at Week 13. This moment was chosen 2 weeks after cessation of the UVB treatment and 3 weeks after the last PDL treatment (ensuring that the post-laser purpura had totally disappeared) (Table 1). Although two PGA scores were recorded for the PDL+UVB treated lesions (plaque d) to maintain blind assessment, we restricted the analysis to the right side of the treated plaques.

Definition of response to treatment

An improvement of 3 points or more from baseline on the PGA score (maximum score 5) or a total clearance (PGA score of 0) in plaque-sides selected for this study was defined as response to treatment.

Statistical analyses

The Friedman's test was used to test for statistically significant differences in the change of PGA scores of the UVB-, PDL- and PDL+UVB-treated plaques at Week 13. We also assessed the difference in the degree of PGA score change after each of these therapies in different plaques within the same patient. For UVB and PDL, change in PGA score was compared with the control side of the same plaque at week 13. For each of these paired statistical tests, the Wilcoxon signed rank test was used. Good clinical response (change of 3 levels of PGA score or clear at week 13) between the three therapy regimens was compared using the McNemar's test.

Results

Study population

In this single-centre, single-blind, prospective, paired randomized controlled study 27 patients (17 men and 10 women), aged 20 to 65 years (mean 44.4 years), with skin types I to IV, and stable plaque psoriasis with at least four psoriasis plaques were included ((in total 108 plaques). The mean duration of psoriasis was 14 years (range 4 to 35 years). The distribution of the baseline PGA scores in each of the treatment groups was comparable (Figure 2)

Direct Paired Comparison Between Baseline and Week 13

The PGA scores after treatment with UVB, PDL, and PDL + UVB were significantly reduced at Week 13 ($p < 0.0001$) (Table 3 and Fig 2). For each therapy, in about 20% of plaques no improvement was achieved, more than 60% showed improvement of two levels in the PGA score and less than 20% showed an improvement of three or more levels in the PGA score (Figure 3). No statistical differences were observed in treatment induced PGA score changes between these three therapies (Table 3). A comparison between PGA scores of the UVB- or PDL- treated halves and the control halves of the psoriasis plaques showed no significant differences ($p=0.47$ and 0.42, respectively). In plaque a, a clearly visual difference between UVB treatment and PDL treatment was seen in 6 patients in favour of UVB and in 4 patients in favour of PDL (Figure 4).

Analyses of PGA-scores in the Sub-group of Plaques that Responded to Treatment.

Good clinical response (improvement of ≥ 3 points in the PGA score from baseline or clearing of psoriasis) was achieved in seven (25.9%) UVB-treated, nine (33.3%)

PDL-treated, five (18.5%) PDL+UVB-treated and two (7.4%) non-treated (NT) halves of the 27 psoriasis plaques.

Total clearance (PGA score of 0) was achieved in the UVB-treated sides of five (18.5%) plaques, in the PDL-treated sides of five (18.5%) plaques, in the PDL+UVB-treated side of two (7.4%) plaques, and in a non-treated side of one (3.7%) plaque. In one patient, total clearance was obtained after PDL mono-therapy and UVB mono-therapy. Total clearance was achieved after PDL treatment in four plaques and after UVB treatment also in four plaques.

Side effects of treatment

Regarding side effects of the PDL therapy, all patients reported transient purpura (occasionally accompanied by crusts) during 1 to 2 weeks, 20 (74%) reported moderate discomfort (which is considerably reduced by cooling the skin during treatment), and 20 (74%) reported evident, but transient hyperpigmentation after therapy. Although hypopigmentation and atrophic scarring were reported in some studies,[16,18] these side effects were neither reported in a recent study[22] nor observed in our study. Regarding side effects of the UVB treatment, nine (33%) patients reported pain, 15 (55%) reported photo-dermatitis, and 20 (74%) reported some hyperpigmentation. Regarding side effects of PDL+UVB therapy, all patients reported purpura, 17 (63%) reported pain, 13 (48%) reported photo-dermatitis, and 19 (70%) reported hyperpigmentation.

Discussion

Recent breakthroughs in the treatment of psoriasis have led to better understanding of the pathogenesis of this disease.[34,39,40] Although major advances in the development and the use of targeted biologicals for controlling psoriasis have been made, the need to develop more safe and cost-effective cures remains. Potential targets for future drugs are cytokines involved in T-cell activation and T-cell trafficking.[40] Inhibition of immunocyte trafficking by selective photothermolysis of the elongated and dilated papillary blood vessels by PDL was demonstrated to be efficacious in plaque type psoriasis.[13,16-22] The PDL was developed on the concept of selective absorption of a brief radiation pulse that generates and confines heat to oxy-haemoglobin, resulting in the destruction of the dilated papillary blood vessels, while minimizing damage to the normal-sized papillary blood vessels and to the surrounding structures .[14, 15, 20]

Two wavelengths, 585- and 595-nm, are currently used for treating skin disorders, which are accompanied by vascular dilatation. Controversy exists as to which wavelength induces greater photo-thermal damage to the blood vessels and subsequent resolution of the particular skin disorder.[41,42] Before laser irradiation oxy-haemoglobin absorbs 4.8 times more light with a wavelength of 585-nm than

light with a wavelength of 595-nm. However, during laser irradiation the absorption increases more for 595-nm than for 585-nm, yielding a 585- to 595-nm ratio of 1.2. This is due to bathochromic (red) shift in blood-absorption and conversion of oxy-haemoglobin to met-haemoglobin, which reduces the 585-nm light absorption and increases that of 595-nm compared with native oxy-haemoglobin, both caused by heating of the blood during irradiation. The remaining small disparity between 585-nm and 595-nm can easily be corrected by augmenting the fluence. Therefore, similar results may be obtained with the wavelengths of 585-nm and 595-nm. The purpura level for both wavelengths is a good indicator of the proper vascular damage that has been achieved.[43]

According to the principles of photothermolysis the optimal pulse-duration to treat dilated capillary vessels in a psoriasis plaque is considered to be in the range of 0.450- to 1.5- msec (less or equal to the Thermal Relaxation Time of the vessels),[18] which is short enough to damage the dilated psoriasis vessels and long enough to spare the normal-sized capillary blood vessels.

The optimal spot diameters for destroying psoriasis vessels are 5-and 7-mm. The 7-mm spot diameter has the advantage that the number of pulses required to treat the clinical area is 35% lower than when treating with the 5-mm spot diameter, reducing the time of treatment, and that the penetration of the laser light is slightly deeper.[44]

The beam profile of the PDL machine has a Gaussian-like distribution of energy, resulting in considerable more light energy in the centre of the beam compared with the border.45 In order to obtain a uniform pattern of applied energy, overlapping of the spots by about 20% is imperative. From this point of view it is likely that without overlapping, the inhibition of the immunocyte trafficking in psoriasis is sub-optimal, resulting in decreased clinical responses.

In order to have a correct simultaneous comparison between the results of UVB and PDL, the endpoint of the study was chosen to be just after ± 30 treatments with UVB, which is a standard number of treatments, and 3 weeks after 4 treatments with PDL. An universal evidence-based standardized protocol concerning time-intervals for PDL therapy is not yet available.

The PGA scoring system was chosen instead of the PASI scoring system, because in the observation of individual plaques the percentage of the total surface area involvement is not an issue, the PGA scoring is far less time-consuming than the PASI scoring, and also because the blinded observers (research nurses) were better trained in scoring the PGA than the PASI. In addition it has been shown, that PGA and PASI are highly correlated and share a high overall reliability[38].

In the statistical data analyses of all included patients PDL and UVB, as well as the combination therapy PDL+UVB resulted in a significant improvement ($p < 0.0001$ for all 3 treatment modalities) in the PGA scores at T13 compared with T0 (Figure

2, Table 3). However, there were no statistically significant differences between the three treatment modalities mutually (Table 3). After dichotomising the changes in PGA scores into responsive and non-responsive to treatment, PDL scored better than UVB and UVB better than PDL+UVB, but according to the McNemar's test these differences were not statistically significant. This indicates that in terms of response, PDL was statistically as effective as UVB treatment, but there was no synergistic effect of combining PDL treatment with UVB treatment in the sub-group of patients who responded to treatment. A speculative explanation for this lack of synergism may be that the inhibition of vessel-active cytokines produced by keratinocytes and immunocytes by UVB-treatment normalises the diameters of the papillary blood vessels, whereby these vessels no longer form a target for PDL. A total clearance of psoriasis after both UVB and PDL was seen in only 1 patient. Complete clearance after UVB, but not after PDL was achieved in 4 patients and after PDL, but not after UVB also in 4 patients, indicating that patients who do not respond to UVB may respond to PDL and vice versa.

The non-treated sides of the study plaques also showed an unexpectedly good response. The differences between the UVB-treated and the non-treated sides, and between the PDL-treated and the non-treated sides were not significant ($p = 0.47$ and $P = 0.42$, respectively). A putative explanation for this phenomenon is that the non-treated sides could have benefited from the UVB- and the PDL treatment of the neighbouring sides, although in a previous study it was suggested that there was no carry-over effect between the side treated with PDL and the non-treated side.[18] In contrast, the differences between the improvements reflected in the PGA scores of the NT sides seen in the Figures 5, 6, and 7 indicate an inverse relationship between improvement and the distance between PDL treated sides and NT sides, indicating an effect of PDL in a limited area around the irradiated site. This observation is supported by Babilas and colleagues who demonstrated tissue damage and thrombus formation in and also outside the irradiated area of hamster skin 24 hours after PDL treatment.[46] Although the carry-over effect in PDL warrants further study, our findings indicate that overlapping spot sizes may not be necessary in the PDL treatment of psoriasis. This implies multiple benefits such as a reduction in the duration of treatment, less discomfort to the patients, and lower costs. Other possible mechanisms by which the non-treated sides could have benefited are via a systemic effect of the treatment of the remaining plaques with UVB and PDL, and/or a non-expended good effect of the permitted concomitant treatment with salicylic acid ointment. A spontaneous remission was not likely, because the majority of the patients (19) were treated during the winter.

Taibjee and colleaques[47] conducted a trial on the treatment of localized plaque psoriasis in which the efficacy of the excimer laser was compared with the efficacy

of the PDL laser (wavelength 595-nm, spot diameter 7-mm, pulse width 1.5 msec and fluence 10 J/cm²). They reported a complete clearance in 41% of the patients in the excimer group after 17 treatments and in 27% of the patients in the PDL group, requiring 3.2 treatments. Many patients in that study found PDL to be a very convenient treatment modality because of monthly hospital visits. Side effects caused by the excimer were blistering in 68% and hyperpigmentation in 41% of the patients versus 0% blistering and 9% hyperpigmentation caused by the PDL.[47] Those results are in good agreement with the results presented here.

During the laser treatment, cooling of the skin is imperative in order to reduce the pain, to protect the melanocytes in the epidermis from damage, and to prevent activation of epidermal effector T cells via pericapillary heat diffusion after treatment, which will tend to promote the persistence of clinical lesions.[20]

Better results of PDL treatments may be obtained by increasing the number of sessions, reducing the time intervals between treatments, increasing the fluence and the pulse width to respectively 10 J/cm² and 1.5 msec (Taibjee and colleagues)[47], applying mineral oil immediately prior to PDL treatment and by concomitant treatment with calcipotriol ointment.[48]

Conclusions and limitations

All tested treatment modalities appeared to be efficacious in the treatment of plaque type psoriasis. There were no statistically significant differences between the efficacy of PDL treatment and UVB treatment. A combination of PDL and UVB treatment had no additional value because of lack of synergism. Surprisingly, no statistically significant differences were seen between UVB treatment and PDL treatment on the one hand and the non-treated sides on the other hand. There is no clear explanation for this, but an unexpected carry-over effect (although a previous study suggested otherwise)[18] from the neighbouring PDL- and UVB-treated sides is supported by the observation of an inverse relationship between improvement of NT sides on the one hand and the distance between the PDL treated sides and the NT sides on the other hand. Another possibility is a systemic effect of the treatment of remaining plaques. After all, it seems likely that dividing of plaques is not the best approach to study the efficacy of topical therapies, but if pursued, then the treatment has to be strictly restricted to study plaques, without treatment of other remaining plaques. In the present study, patients with a complete clearance of the target lesion appeared to be divided into either PDL responders or UVB responders, indicating that patients who do not respond to UVB may respond to PDL and vice versa. Treatment with PDL resulted only in mild and transient side effects and therefore appeared to be safe. PDL treatment is certainly not the panacea for every psoriasis patient and should not be used in widespread psoriasis or in patients who respond adequately to simple

topically applied treatments. PDL treatment should be reserved for patients with persistent plaque type psoriasis, not responding to usual treatments (particularly on “non-covered body” sites), plaque type psoriasis complicated by side effects of medication, psoriasis palmoplantaris, psoriasis inversa, patients not willing to use topical corticosteroids or systemic treatment and patients at risk for skin cancer caused by actinic damage or immunosuppression. Most patients in our study preferred PDL therapy above UVB (20 versus 7 patients) because of the less frequent treatment sessions, the subjective improvement in the quality of life, and the lack of reports on carcinogenesis due to PDL treatment, whereas long-term side effects including carcinogenesis caused by excimer UVB and narrow-band UVB remain uncertain. With regard to the patient’s preference and the efficacy, the PDL treatment should be considered as a serious option in the treatment of plaque type psoriasis.

References

1. Creamer D, Sullivan D., Bicknell R, Barker J. Angiogenesis in psoriasis. Angiogenesis 5: 231-236, 2002.
2. Yen A, Braverman IM. Ultrastructure of the human microcirculation: the horizontal plexus of the papillary dermis. J Invest Dermatol 1976; 66: 131-42.
3. Braverman IM, Yen A. Ultrastructure of the capillary loops in the dermal papillae of psoriasis. J Invest Dermatol 1977; 69: 53-60.
4. Bull RH, Bates DO, Mortimer PS. Intravital video-capillaroscopy for the study of the microvascular expansion in active plaque psoriasis. Br J Dermatol 126: 436-45, 1992.
5. Creamer D, Allen MH, Sousa A et al. Localisation of endothelial proliferation and microvascular expansion in active psoriasis. Br J Dermatol 136: 859-65, 1997.
6. Pinkus H, Mehregan AH. The primary histologic lesion of seborrhoeic dermatitis and psoriasis. J Invest Dermatol 46:109-16, 1966.
7. Goodfield M, Macdonald Hull S, Holland D et al. Investigations of the “active” edge of plaque psoriasis: Vascular proliferation precedes changes in epidermal keratin. Br J Dermatol 131: 808-13, 1994.
8. Barker JNWN. Pathophysiology of psoriasis. Lancet 1991; 338:227-30.
9. Lebwohl M. A clinician’s paradigm in the treatment of psoriasis. J Am Acad Dermatol 2005;53:s59-69.
10. Macdonald D, Hull S, Goodfield M, Wood EJ, Cunliffe W. Active and inactive edges of psoriatic plaques: identification by tracings and investigation by laser-Doppler flowmetry and immunocytochemical techniques. J Invest Dermatol 1989; 92: 782-5.
11. Heng MCY, Allen SG, Haberfelde GH, Song MK. Electronmicroscopic and immunohistochemical studies of the sequence of events in psoriatic plaque

formation following tape-stripping. Br J Dermatol 1991; 125:548-56.
12. Yen A, Braverman IM. Ultrastructure of the human microcirculation: the horizontal plexus of the papillary dermis. J Invest Dermatol 1976; 66: 131-42.
13. Hacker S, Rasmussen J. The effect of flash lamp-pulsed dye laser on psoriasis. Arch Dermatol 1992; 128: 853-5.
14. Anderson RR, Parrish JA. Microvasculature can be selectively damaged using dye lasers: a basic theory and experimental evidence in human skin. Lasers Surg Med 1981; 1: 263-76.
15. Anderson RR, Parrish JA. Selective photothermolysis: precise microsurgery by selective absorption of pulsed radiation. Science 1983; 220: 524-527.
16. Ros AM, Garden JM, Bakus AD et al. Psoriasis response to the pulsed dye laser. Lasers Surg Med 1996; 19: 331-35.
17. Katugampola GA, Rees AM, Lanigan SW. Laser treatment of psoriasis. Br J Dermatol 1995; 133: 909-13.
18. Zelickson BD, Mehregan DA, Wendelschfer-Crabb et al. Clinical and histologic evaluation of psoriatic plaques treated with a flashlamp pulsed dye laser. J Am Acad Dermatol 1996; 35: 64-8.
19. Bjerring P, Zachariae H, Søgaard H. The flashlamp-pumped Dye Laser and dermabrasion in Psoriasis- Further studies on the reversed Köbner phenomenon. Acta Derm Venereol (Stockh) 1997; 77: 59-61.
20. Hern S., Allen M.H., Sousa A.R., Harland C.C., Barker J.N.W.N., Levick J.R., Mortimer P.S. Immunohistochemical evaluation of psoriatic plaques following selective photothermolysis of the superficial capillaries. Br J Dermatol 2001; 145: 45-53.
21. Hern S, Stanton AWB, Mellor RH, Harland CC, Levick JR, Motimer PS. In vivo quantification of the structural abnormalities in psoriatic microvessels before and after pulsed dye laser treatment. Br J Dermatol 2005; 152; 505-511.
22. Erceg A, Bovenschen HJ, Kerkhof van der PCM, Seyger MMB. Efficacy of the pulsed dye laser in the treatment of localized recalcitrant plaque type psoriasis: a comparative study. Br J Dermatol 2006; 155; 110-114.
23. Parrish JA, Jaenicke KF. Action spectrum for phototherapy of psoriasis. J Invest Dermatol 1981; 76(5): 359-62.
24. Weelden H van, Faille HB de la, Young E, Leun JC van der. A new development in UVB phototherapy for psoriasis. Br J Dermatol 1988; 119: 11-19.
25. Picot E, Picot-Debeze MC, Meunier L et al. Narrow-band UVB phototherapy (Philips TL-01) in psoriasis. Ann Dermatol Venereol 1992; 119: 639-42.
26. Green C, Ferguson J, Lakshmipathi T, Johnson BE. 311 nm UVB phototherapy-an effective treatment for psoriasis. Br J Dermatol 1988;119: 691-696.
27. Ferguson J. The use of narrowband UV-B (Tube Lamp) in the management of

skin disease. Arch Dermatol 1999; 135: 589-90.
28. Walter IB, Burack LH, Coven TR, Gilleaudeau P, Krueger JG. Suberythematogenic narrow-band UVB is markedly more effective than conventional UVB treatment of psoriasis vulgaris. J Am Acad Dermatol 1999; 40: 893-900.
29. Coven TR, Burack LH, Gilleandeau R, Keogh M, Ozawa M, Krueger JG. Narrowband UV-B produces superior clinical and histopathological resolution of moderate-to-severe psoriasis in patients compared with broadband UVB. Arch Dermatol 1997; 133: 1514-22.
30. Tanew A, Radakovic-Fijan S, Schemper M, Höningsmann H. Narrowband UVB phototherapy vs photochemotherapy in the treatment of chronic plaque type psoriasis. A paired comparison study. Arch Dermatol 1999; 135: 519-524.
31. Aubin F. Mechanisms involved in ultraviolet light-induced immunosuppression. Eur J Dermatol 2003; 13: 515-523.
32. Ozawa M, Ferenczi K, Kikuchi T et al. 312-nanometer Ultraviolet B Light (Narrow-Band UVB) induces apoptosis of T cells within psoriatic lesions. J Exp Med 1999: Vol 189 (4): 711-18.
33. Hofer A, Fink-Puches R, Kerl H, Wolf P. Comparison of phototherapy with near vs. far erythemogenic doses of narrow-band ultraviolet B in patients with psoriasis. Br J Dermatol 1998; 138: 96-100.
34. Lebwohl M. Psoriasis. Lancet 2003; 361:1197-1204.
35. Tan OT, Morrison P, Kurban AK. 585 nm for the treatment of port-wine stains. Plast Reconst Surg 1990; 86: 1112-1117.
36. Dawe RS, Wainwright NJ, Cameron H, Ferguson J. Narrow-band (TL-01) ultraviolet B phototherapy for chronic plaque psoriasis: three times or five times weekly treatment? Br J Dermatol 1998; 138: 833-9.
37. Wainwright NJ, Dawe RS, Ferguson J. Narrowband ultraviolet B (TL-01) phototherapy for psoriasis: which incremental regimen? Br J Dermatol 1998; 139: 410-4.
38. Langley RG, Ellis CN.Evaluating psoriasis with Psoriasis Area and Severity Index, Psoriasis Global Assessment, and Latice System Physician's Global Assessment. J Am Acad Dermatol 2004;vol 51, nr 4:563-569.
39. Krueger G, Ellis CN. Psoriasis-recent advances in understanding its pathogenesis and treatment. J Am Acad Dermatol 2005;53: s94-100.
40. Nickoloff B.J., Stevens S.R. What have we learned in dermatology from the biologic therapies? J Am Acad Dermatol 2006;54, nr 3: s143-151.
41. Kimel S, Svaasand LO, Hammer-Wilson MJ, Stuart Nelson J. Influence of Wavelength on Response to Laser Photothermolysis of Blood Vessels: Implications for Port Wine Stain Laser Therapy. Lasers in Surg Medicine; 2003, 33; 288 – 295.

42. Van Gemert MJ, Smithies DJ, Verkruysse W, Milner TE, Nelson JS. Wavelengths for port wine stain laser treatment: Influence of vessel radius and skin anatomy. Phys Med Biol 1997; 42: 41-
43. Pikkula BM, Chang DW, Stuart Nelson J, Anvari B. Comparison of 585 and 595 nm Laser-induced Vascular Response of Normal In Vivo Human Skin. Lasers in Surgery and Medicine 2005; 36:117-123.
44. Goldman MP, Fitzpatrick RE. Cutaneous Laser Surgery. Mosby Inc, second edition 1999, 26.
45. Goldman MP, Fitzpatrick RE. Cutaneous Laser Surgery. Mosby Inc, second edition 1999, 25.
46. Babilas P, Shafirstein G, Bäumler W, Baier J, Landthaler M, Szeimies RM, Abels C. Selective photothermolysis of blood vessels following flashlamp-pumped pulsed dye laser irradiation: in vivo results and mathematical modelling are in agreement. J Invest Dermatol 2005; 125: 343-352.
47. Taibjee SM, Cheung ST, Laube S, Lanigan SW. Controlled study of excimer and pulsed dye lasers in the treatment of psoriasis. Br J Dermatol 2005; 153:960-966.
48. Leeuw de J, Tank B, Bjerring .J, Koetsveld S, Neumann HAM. Concomitant treatment of psoriasis of hands and feet with pulsed dye laser and topical calcipotriol, salicylic acid, or both prospective open study in 41 patients. J Am Acad Dermatol 2006; 54, nr 2: 266-271.

Week	0	1	2	3	4	5	6	7	8	9	10	11	12	13
PDL		X			X			X			X			
UVB		3X	3X	3X	3X	3X	3X	3X	3X	3X	3X	3X		
Score	X													X

Table 1: Schedule of actions

0 = clear:	No evidence of erythema, but there may be residual discolourations (e.g. hyperpigmentation and pigmented macules), no evidence for scaling and induration (no plaque elevation above normal skin levels)
1 = minimal	Considering all involved areas, the overall clinical picture shows plaques with slight erythema with discrete induration (possible trace elevation above normal skin) and occasional fine scaling over <5% of lesions
2 = mild	Considering all involved areas, the overall clinical picture shows plaques with red colouration, slight but definite induration (slight but definite elevation of plaques above normal skin) and a fine thin scaling
3 = moderate	Considering all involved areas, the overall clinical picture shows plaques with definite red colouration, moderate induration (moderate elevation from normal skin to plaques with rounded or sloped edges), and coarse scaling
4 = severe	Considering all involved areas, the overall clinical picture shows plaques with very bright red colouration, marked induration (marked elevation with hard sharp edges to plaques), and a coarse, thick scaling
5 = very severe	Considering all involved areas, the overall clinical picture shows plaques with dusky to extreme deep red colouration, very marked induration (with very hard sharp edges between normal skin and plaques) and very severe, tenacious, coarse, thick scaling

Table 2: Physician's Global Assessment-score

Within therapies[+]	In favour of T13	In favour of T0	Equal effect at T13	p-value
UVB T13 vs UVB T0	20	0	7	<0.0001
PDL T13 vs PDL T0	20	1	6	<0.0001
PDL+UVB T13 vs PDL+UVB T0	21	1	5	<0.0001
Between therapies[#]	In favour of 1st therapy	In favour of 2nd therapy	Equal effect of therapies	p-value
UVB vs PDL	9	12	6	0.52
UVB vs PDL + UVB	10	9	8	0.82
PDL vs PDL + UVB	9	7	11	0.63
Compared with control[†]	In favour of 1st therapy	In favour of 2nd therapy	Equal effect of therapies	p-value
UVB vs control	10	7	10	0.47
PDL vs control	8	5	14	0.42

Abbreviations: UVB = ultraviolet-B; PDL = pulsed dye laser, vs = versus.

* Wilcoxon signed ranks test

\+ Comparison of the PGA-scores of the same psoriasis plaque between baseline (T0) and at week 13

\# Comparison of degree of change in PGA-scores of psoriasis plaques between different therapies at week 13 within the same patient.

† Comparison of degree of change in PGA-scores of the halves of treated and non-treated psoriasis plaques at week 13.

Table 3. Comparison[*] of within and between therapies at Week 13 (T13) of the study.

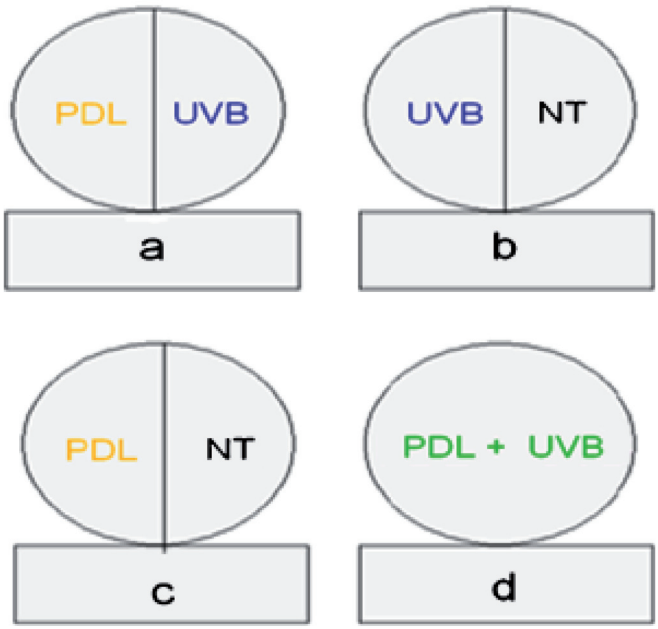

Figure 1: Treatment schedule of 4 plaques in each patient.
PDL = Pulsed Dye Laser, UVB = Ultraviolet-B TL01,
PDL+UVB = combination therapy, NT = no treatment

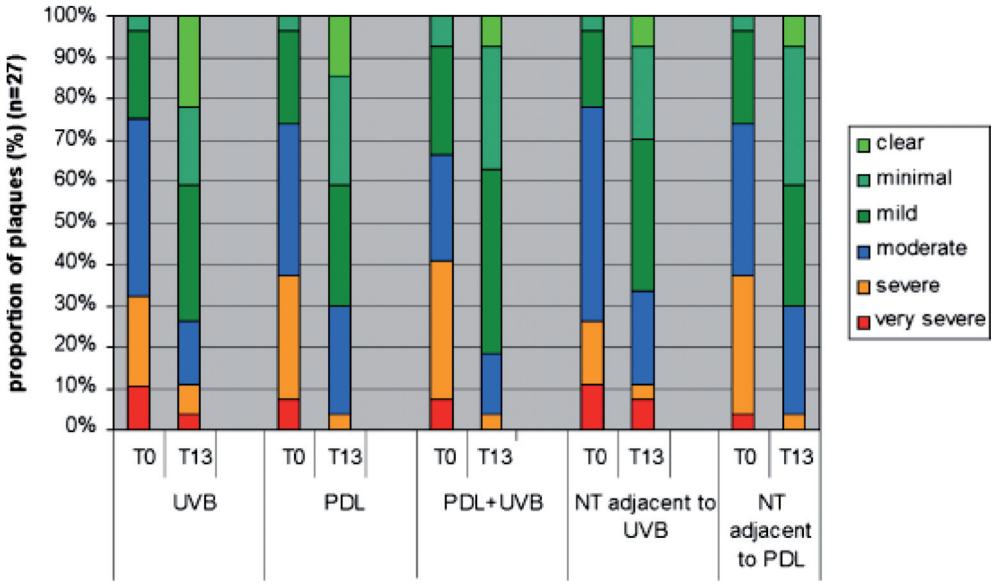

Figure 2: For each therapy, the distribution of Physician's Global Assessment scores at baseline (T0) and after 13 weeks (T13) of treatment.

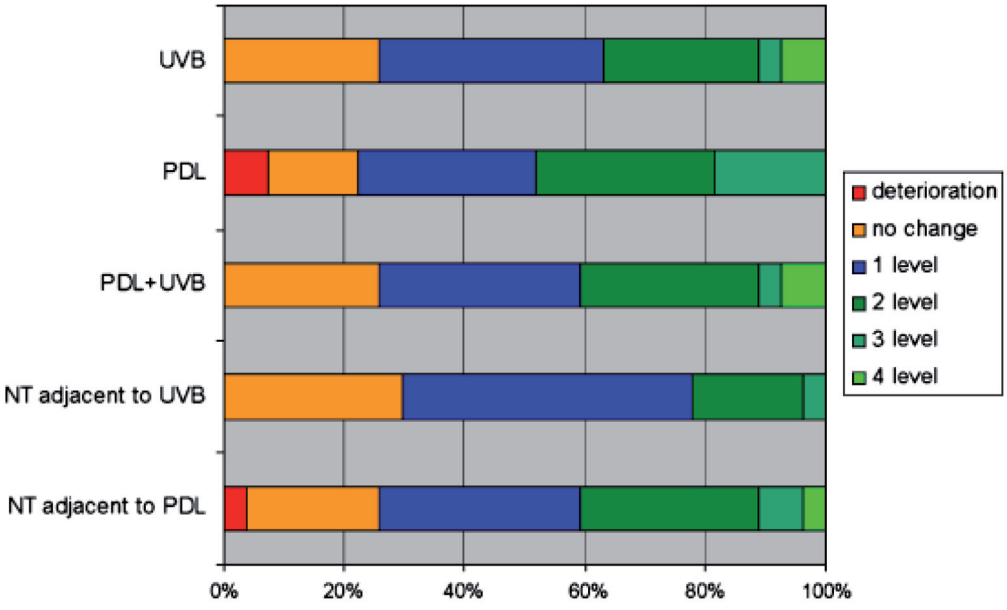

Figure 3. For each therapy, the distribution of the degree of change in Physician's Global Assessment score at week 13. Proportion of plaques (%) (n=27).

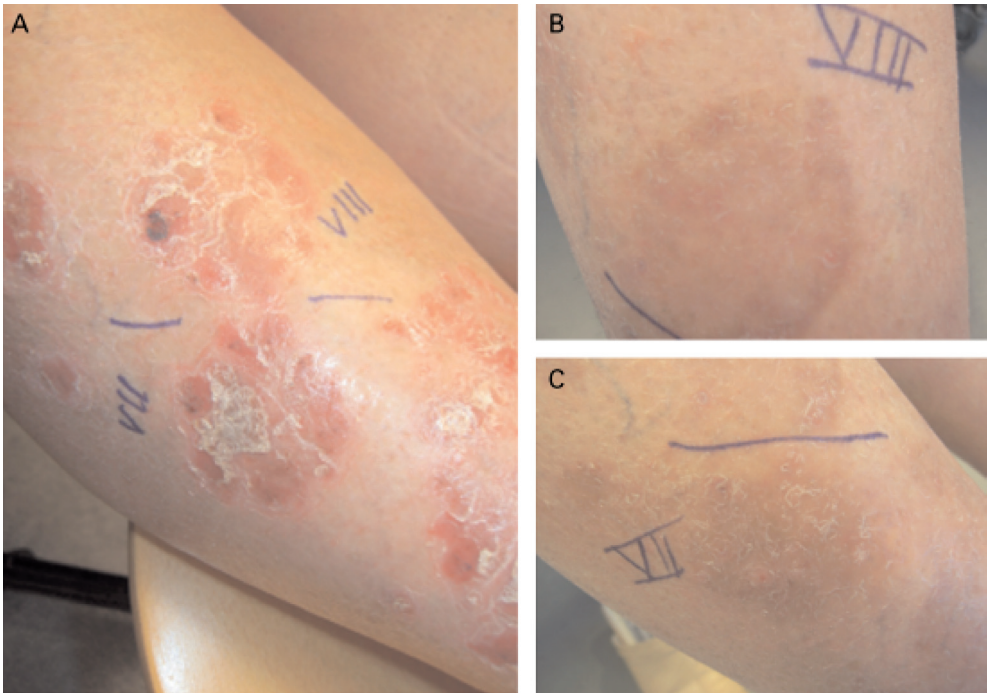

Figure 4: Two confluent plaques right lower leg. A. Pre-treatment. B. Post-treatment PDL side. C. Post-treatment UVB side. VII = UVB, VIII = PDL

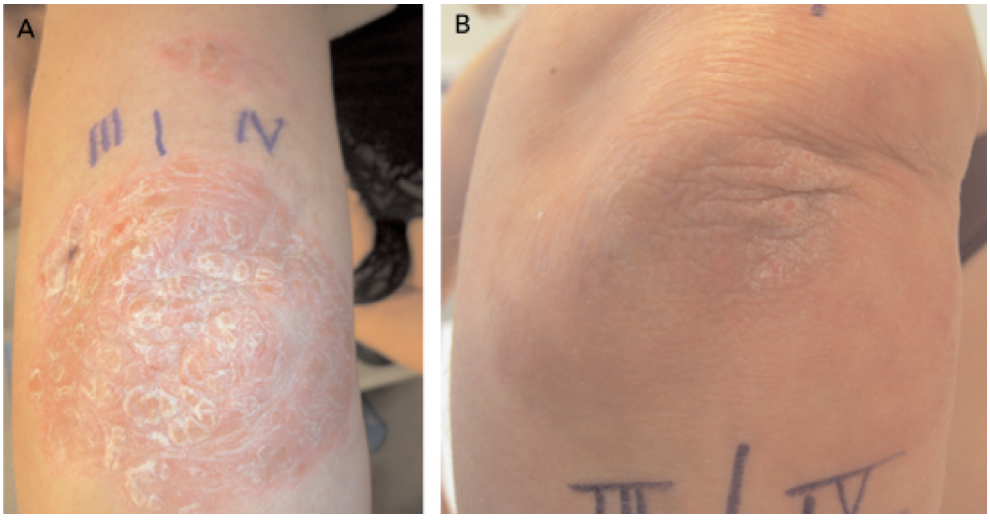

Figure 5: One plaque divided into two halves on the left elbow. A. Pre-treatment. B. Post-treatment. III = PDL, IV = NT. Considerable improvement of NT side adjacent to PDL side.

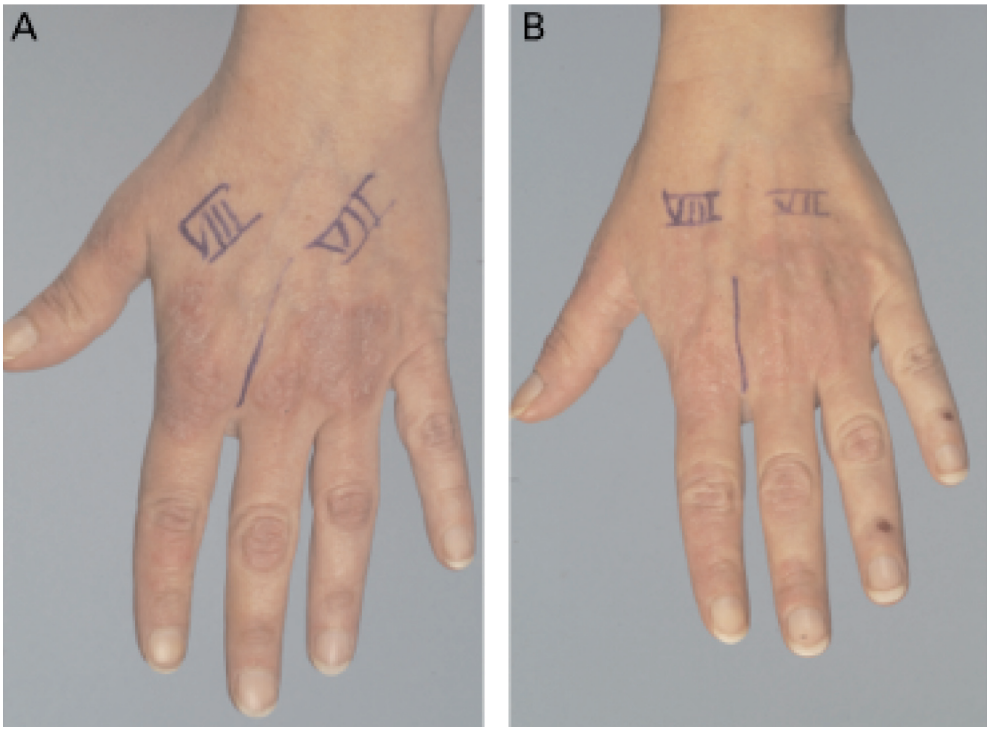

Figure 6: Two almost confluent plaques on the dorsum of the left hand. A. Pre-treatment. B. Post-treatment. VII = PDL, VIII = NT. Some improvement of NT side near PDL side.

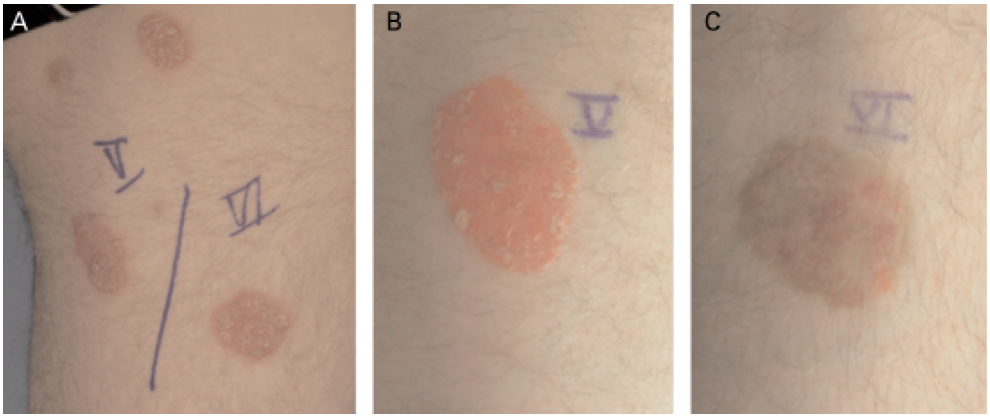

Figure 7: Two small plaques separated from each other by non-psoriatic skin on the right upper leg. A. Pre-treatment. B. Post-treatment of the NT side. C. Post-treatment of the PDL side. V = NT, VI = PDL. No improvement of NT side separated from PDL side by non-psoriatic skin.

Chapter 7

Cellular and molecular effects of pulsed dye laser and local narrow-band UVB therapy in psoriasis

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Abstract

Background: Pulsed dye laser (PDL) therapy is effective in clearing psoriasis plaques, but the mechanism of action is only partially understood. Local Narrow-band ultraviolet B (NB-UVB), which has a better defined mode of action is an effective standard treatment for psoriasis.

Objectives: To evaluate the cellular and the molecular effects of PDL and compare them with those of local NB-UVB in order to gain further insight into their mechanisms of action in psoriasis.

Patients and Methods: Nineteen patients with stable plaque-type psoriasis were treated either with PDL or NB-UVB. Lesional punch biopsies were obtained from all the patients before treatment. Additional biopsies were obtained at 3 hours and 24 hours after PDL treatment in 5 of these patients. In 14 patients additional biopsies were taken after 7- and 13 weeks of treatment. Samples were histopathologically examined for the level of dermal T cell infiltrate, and the expression of epidermal β-defensin 2, immune cell-derived tumor necrosis factor (TNF)-α, endothelial E-selectin, VEGFR2 and VEGFR3, and the expression of Interleukin (IL)-23 before and after treatment.

Results: The expression of VEGFR2, VEGFR3 and E-selectin was decreased in clinically high responders within 24 hours after PDL treatment. The expression of IL-23, TNF-α mRNA and E-selectin protein were significantly reduced after 2 PDL treatments, whereas the expression of epidermal markers and dermal T cell infiltrates had normalized after 4 PDL treatments. The expression of epidermal activation markers and E-selectin were significantly reduced after 13 weeks of NB-UVB treatment.

Conclusions: The expression of epidermal activation markers and the dermal T cell infiltrates were decreased after both the treatments. The decreased expression of VEGFR2 and VGFR3 followed by the down-regulation of TNF-α and IL-23p19 may be contributory factors in the efficacy of PDL in stable plaque-type psoriasis.

Introduction

The dilation and proliferation of the dermal papillary microvasculature is one of the early changes seen in a new psoriatic plaque.[1] The increased dermal microvasculature facilitates the trafficking of leukocytes from the circulation into the skin and therefore plays an important role in maintaining inflammation in psoriasis.[2] Interference with leukocyte trafficking via a selective destruction of the dilated capillaries may be an effective therapeutic intervention in psoriasis. Selective targeting of blood vessels may be achieved with the flash-lamp pumped pulsed dye laser (PDL).[3] The PDL technique is based on the selective absorption of short pulses of 585 nm light by oxy-hemoglobin inducing photothermolysis of capillaries leaving the other nearby structures in the skin undamaged.[4]

Several studies reported that psoriatic plaques were partly or completely cleared by PDL treatment.[5-8] In a comparative study, PDL showed a significantly higher efficacy than a class II topically applied corticosteroid.[8] PDL was also effective in clearing recalcitrant psoriatic plaques,[9] whereas palmoplantar psoriasis responded well to treatment with PDL alone or in combination with topical calcipotriol or salicylic acid.[10]

NB-UVB therapy is a standard treatment modality for psoriasis. The mechanism of action of NB-UVB has been investigated more thoroughly than that of PDL. It is known that NB-UVB targets the epidermal compartment inhibiting the proliferation of keratinocytes, abrogating antigen presentation, migration of Langerhans cells[11] and inducing apoptosis of activated skin-homing T cells.[12,13] Prolonged exposure to NB-UVB and a high cumulative dose may result in premature ageing of the skin and lead to a higher risk of skin cancer. However, recent follow-up studies in patients receiving NB-UVB for psoriasis did not report any increase in the incidence of skin cancer as compared with controls. [14,15] Long-term side effects of PDL treatment have not yet been reported.

No significant differences in the clinical efficacy between PDL and NB-UVB were reported in a single-blind, prospective, paired randomized controlled study,[16] indicating that PDL is a valid treatment modality for psoriasis. However, to date there is only a poor insight into the cellular and the molecular mechanisms that are affected by PDL explaining its efficacy in psoriasis. The aim of the present study was to investigate the effects of PDL treatment on the cellular and the molecular markers of disease activity in psoriasis using immunohistochemical and quantitative real time (RT)-PCR techniques and lesional skin biopsies taken before, during and at the

end of the treatment and compare them with those of NB-UVB treatment.

Materials and Methods

Patients and biopsies

Nineteen patients with stable plaque type psoriasis were enrolled into the study after they had provided written informed consent. The study was approved by the Medical Ethical Committee, Erasmus MC, University Medical Center, Rotterdam (approval nr: MEC-2004-154). Patients intolerant to light (toxic or allergic), using drugs with phototoxic or photo-allergic potency, younger than 18 years, were pregnant, or with pre-existing or manifest skin malignancy were excluded. A wash-out period of 2 weeks was indicated for topical drugs and of 4 weeks for photo (chemo) therapy and systemic drugs. Emollients were allowed.

The most representative psoriasis plaque was selected for a punch biopsy before treatment (baseline) in each patient. In 5 patients (2 women and 3 men aged 45 to 59 years) additional 3-mm punch biopsies were taken at 3 hours and 24 hours after the first PDL treatment for examining the short-term effects. The psoriasis plaques were randomly selected and treated either with PDL or with NB-UVB or left untreated in the remaining 14 patients (7 women and 7 men aged 20 to 65 years). Two 3-mm punch biopsies, one from a PDL-treated site and one from a NB-UVB-treated site were taken at week 7 (after 2 treatments of PDL and 15 treatments with NB-UVB) and at week 13 (after 4 treatments of PDL and 30 to 33 treatments of NB-UVB) from each of these14 patients. The punch biopsies were snap-frozen in Tissue-Tek OCT (Miles, Elkhart, IN, USA) and stored at -80°C until further processing.

Treatments

The V Star PDL (Cynosure Inc., Chelmsford, MA), emitting yellow light at a wavelength of 585 nm was used for PDL treatment. The skin was air-cooled using a Zimmer 6 air cooler (Zimmer Elktromedizin, Neu-Ulm, Germany) during the treatment. The PDL treatment parameters were: pulse duration 0.50 ms, spot diameter 7-mm, spots overlapping ± 20%, and fluences between 5.5 and 6.5 J/cm^2. Patients were treated with the PDL every 3 weeks for 10 weeks, a total four treatments. The UVB-TL01 S (wavelength 311 nm) handheld device (Cosmedico Medizintechnik GmbH, Villingen-Schwenningen, Germany) was used for the NB-UVB treatment. Mean UV-output was 19.4 mWatt/cm^2. The devices were calibrated with the Optometer P 9710 (Gigahertz Optik, Puchheim, Germany) before the treatment. The aperture of the appliance (5.0 × 10.0 cm) was held against the skin assuring that the distance between the device

and the skin was constant in each patient during the treatment. Minimal erythema dose (MED) was determined in each patient prior to the study. Mean MED NB-UVB was 0.86 J/cm^2 (range 0.34–1.2 J/cm^2). NB-UVB treatment (3 times per week) parameters were: a start dose of 70% of the MED, dose increments of 20% of the previous treatment until persistent erythema appeared or clearing of the lesion was achieved. A total 30–33 treatments were given during 11 weeks. Mean initial dose was 0.62 J/cm^2 (range 0.24–0.86 J/cm^2), mean cumulative dose 84 J/cm^2 (range 40–120 J/cm^2).

Plaques were randomly chosen and treated with PDL, NB-UVB or only with emollient in each patient. Concomitant topical treatment with salicylic acid 5% in petrolatum was permitted for all plaques in between the treatments sessions to reduce reflectance of the PDL and the NB-UVB beam by scales, but was discontinued 2 days before the PDL treatment and directly before the NB-UVB treatment. Clinical efficacy was assessed as described previously (16).

Immunohistochemistry

Cryostat sections (6 µm) were cut from each punch biopsy, mounted on glass slides, fixed in acetone and stained for markers of psoriasis using antibodies shown in Table 1. Endogenous peroxidase activity was neutralized using 4-chloro-1-naphtol. The slides were washed in phosphate-buffered saline (PBS) containing 0.05% Tween 20 and 0.5% bovine serum albumin (BSA) and incubated with the appropriate dilution of the primary antibodies (Table 1) for 1 hour at room temperature. They were subsequently incubated with biotinylated secondary antibodies (rabbit-anti-mouse IgG (Dako, 1:400) or donkey-anti-rabbit IgG (Amersham Biosciences, 1:800)) for 30 minutes and with horseradish peroxidase (HRP)-linked streptavidin (Dako, High Wycombe, UK) for 1 hour at room temperature. Any non-specific staining was prevented by adding unlabeled (normal) secondary antibody and normal human AB serum. HRP activity was visualized with amino-9-ethylcarbazole (Sigma-Aldrich, St. Louis, MO, USA) as a chromogen, resulting in a bright red staining. Sections were counter-stained with hematoxylin and mounted in glycerin-gelatin (Dako).

A representative region was selected in each glass slide and photographed at 100x magnification using an AxioCam MRc5 camera (Zeiss, Goettingen, Germany). The acquired images were analyzed by computer-assisted image analysis using the WCIF VisionJ (http://rsb.info.nih.gov/ij) software. The area occupied by the stained tissue was expressed as a fraction of the total area of interest (i.e. epidermis or dermis). For statistical analysis of the quantitative data, the Wilcoxon Signed Ranks test and the Kruskal-Wallis test were used for unpaired and paired sets of data, respectively.

RNA extraction and RT-PCR

The total RNA extraction was as follows. Twenty 10 μm cryostat sections were cut under RNAase-free conditions from each punch biopsy, directly placed in RNA lysis buffer (Sigma) followed by mRNA extraction. RNA was transcribed into cDNA and RT-PCR was performed using newly designed primers and probes. ABL1 was used as a housekeeping control gene. Primer and probe sequences are listed in Table 2.

Results

Clinical assessment

The Physician's Global Assessment (PGA) score was used to establish clinical improvement. The PGA score is a 5 point scoring system, where score 0 denotes symptom-free state, whereas scores 1-5 represent increasing severity. Definition of each score, as well as the clinical outcomes of the study have been reported in more detail elsewhere (16). The results of the present study showed that the mean clinical improvement (mean reduction in the PGA score) at week 13 was 46% for the PDL-treated plaques and 52% for the NB-UVB-treated plaques indicating that the clinical efficacy of both treatments were comparable. Six high responders (defined as a PGA reduction > 50%) were selected from the 14 patients in order to evaluate the effects of both treatments. Clinical improvement as assessed by the PGA score in the whole group and in the 6 high responders during both the treatments is shown in Figure 1.

Early effects of the PDL

Early changes (within 24 h) in psoriasis plaques after PDL treatment were investigated in order to elucidate its mechanism of action. No changes were observed in mRNA expression levels of markers of the activated epidermal psoriasis phenotype such as β-defensin 2 (HBD2) and keratin 17 (KRT17), TNF-α and vascular endothelial growth factor (VEGF)-A in biopsies taken 3- and 24 hours after PDL treatment (results not shown). The expression of the anti-apoptotic, heat-regulated molecule Bcl 2 also remained unaltered at the same time points (results not shown). The expression of the endothelial molecules E-selectin and VEGFR2 were significantly decreased in the biopsies taken 3 hours after the PDL treatment (Figures 2a & b). The expression of E-selectin was still reduced, whereas the expression of VEGFR2 returned to baseline indicating that the reduction of VEGFR2 was transient (Figures 2a & b) in the biopsies taken at 24 hours. Interestingly, the expression of the mRNA of the lymphatic marker VEGFR3 showed an alteration that was similar to that of VEGFR2 (Figure 2c). The expression of IL-23p19 mRNA was significantly reduced at 3 hours and remained reduced, although not significantly at 24 hours after the PDL treatment (Figure 2d).

The PDL treatment affected the expression of VEGFR2 and VEGFR3 mRNA as early as 3 hours, whereas E-selectin expression was significantly reduced 24 hours after treatment. The expression of IL-23p19 mRNA was also affected by PDL treatment, although not significant at 24 hours.

Long-term alterations at the mRNA level

Selected psoriasis plaques were treated either with PDL or with NB-UVB in 14 patients. The PDL treatment was carried out every 3 weeks for 10 weeks (a total four treatments), whereas NB-UVB treatment was three times weekly with increasing doses. The mRNA expression of keratinocyte-, immune cell- and endothelial cell-associated activation markers was assessed by quantitative RT-PCR in punch biopsies taken before, at week 7 and at week 13 of therapy in the group of clinical responders in order to determine the course of the molecular changes during the treatment. The expression of keratin 17 and β-defensin 2 decreased significantly in the PDL-treated punch biopsies corresponding with the clinical improvement. There were no significant alterations in the expression of keratin 17 and β-defensin 2 after the NB-UVB treatment in responders (Figures 3 a-d).

The expression of TNF-α mRNA was decreased significantly in the biopsies after the PDL treatment, but not after the NB-UVB treatment (Figures 3e & f). The expression of IL-23p19 mRNA was suppressed in the punch biopsies at week 7 of the PDL treatment, but returned to the baseline levels after 13 weeks, whereas there were no significant alterations in the expression of IL-23p19 mRNA in the clinical responders after the NB-UVB treatment (Figures 3g & h).

The expression of VEGFR2 mRNA was up-regulated during both the treatments indicating an activated angiogenesis (Figures 3i & j). In addition, a reduced expression of the anti-apoptotic molecule Bcl 2 was observed in the biopsies after the PDL treatment (results not shown).

In the 6 clinical responders, the PDL treatment resulted in the decreased expression of TNF-α and IL-23p19 mRNA at 7 weeks, and the decreased expression of β-defensin 2 (HBD2), keratin 17 and Bcl 2 at 13 weeks, whereas the expression of the angiogenic molecule VEGFR2 was induced. The expression of mRNA of these markers in the punch biopsies varied considerably between the patients treated with the NB-UVB.

Immunohistochemical alterations

Punch biopsies from all the 14 patients were analyzed by immunohistochemistry. The expression of transglutaminase K (TGK), an early differentiation marker with strong expression in psoriasis was assessed in order to follow any epidermal alterations. The level of T lymphocyte infiltrates (CD3) in the dermis was determined to assess

the inflammatory components of the disease activity. Changes in the contents of the blood vessels were followed by staining for the von Willebrand's factor (vWF), whereas endothelial activation was assessed by the expression of E-selectin.

Global improvement of the psoriatic pathology as assessed in the hematoxylin and eosin (H&E)-stained sections was observed in about 50% of the patients corresponding with the clinical improvement in psoriasis. No significant changes were observed in the PDL-treated plaques, whereas the expression of TGK and E-selectin were significantly decreased in the NB-UVB-treated plaques (Figure 4) after 13 weeks of treatment in the group of 14 patients (responders and non-responders).

We analyzed punch biopsies from 6 clinically high responders (defined as a PGA reduction > 50%) for epidermal and dermal markers of psoriasis in order to evaluate the effects of both treatments. The expression of epidermal TGK and the dermal T cell infiltrates were markedly reduced by both treatments (Figures 5a-d) in these 6 patients after 13 weeks. The expression of vWF was significantly decreased in the NB-UVB-treated plaques; a marked, but not significant decrease was also noted after PDL treatment (Figures 5e & f). The expression of the endothelial activation marker E-selectin showed a significant decrease in the PDL-treated punch biopsies after 7 weeks, but this change did not reach significance at week 13. The expression of E-selectin showed a gradual persistent decrease in the NB-UVB-treated lesional punch biopsies (Figures 5g & h).

Discussion

The results of this study showed that the clinical improvement of psoriasis after the PDL treatment was accompanied by alterations in certain classical markers of disease activity. The observed effects at the mRNA level were comparable in responders to the PDL- and the NB-UVB treatment. The relatively low clinical efficacy (52% improvement versus minimally 60% reported for total body irradiation) of NB-UVB in this study may be explained by the fact that local NB-UVB treatment was used instead of total body irradiation. It is assumed that total body NB-UVB irradiation may also exert a systemic effect.[17]

A hallmark of psoriatic skin is the remarkable transformation in the local microvascular system characterized by the dilation and the tortuosity of capillaries, increased permeability, and high endothelial venule formation, which is usually observed in lymph nodes.[18] Active angiogenesis in psoriatic lesions evidenced by the up-regulation of VEGF and VEGFR2 is accompanied by endothelial cell activation as observed by the up-regulation of ICAM-1, vascular cell adhesion molecule-1 (VCAM-1) and E-selectin. During this process, vascular endothelial growth factor (VEGF) signaling on endothelial cells represents the major rate-limiting step.[18] VEGF acts by engaging with its tyrosine kinase receptors VEGFR1 and VEGFR2 in endothelial

cells. Although VEGF binds to both receptors, it appears that most of its biological functions are mediated via VEGFR2.[18,19]

Our observation that the early effects of the PDL treatment involve the decreased expression of the endothelial molecules VEGFR2 and E-selectin corresponds with its proposed primary target. However, an additional early effect was also noted on VEGFR3 (FLT4), a lymph-endothelial marker.[20] Lymphatics are expanded in psoriasis and the expression of VEGFR3 is increased in both the involved as well as the uninvolved psoriatic skin.[19] The decreased expression of VEGFR3 early after PDL treatment may contribute to its efficacy in psoriasis.

Interestingly, the expression of VEGFR2 was up-regulated at later time points indicating re-activation of angiogenesis. This active angiogenesis may counteract the anti-psoriatic effect of the PDL treatment and may explain the insufficient clinical response in some patients. Another possibility may be that the main source of VEGF in psoriatic lesions, the activated keratinocyte, is not targeted by the PDL treatment. Therefore, activated lesional keratinocytes are less inhibited and may continue to stimulate the lesional microvasculature. The up-regulation of VEGFR2 may explain the enhanced efficacy of the PDL treatment when combined with calcipotriol ointment, which targets activated keratinocytes.[10]

TNF-α and IL-23 mainly produced by the inflammatory dendritic cells (DC) in the dermis are critical cytokines in the pathogenesis of psoriasis.[21, 22] Their importance is underlined by the fact that biologics targeting TNF-α and IL12/IL23p40 are effective in treating psoriasis.[23] Furthermore, both the cytokines are down-regulated during the treatment with other effective modalities.[24] The results of the present study showed that after 7 weeks of the PDL treatment, TNF-α and IL-23 mRNA were down-regulated in psoriatic skin, whereas IL-23 was already observed to be down-regulated within 3 hours after the PDL treatment. In combination with the observed decreased number of dermal CD3+ T cells, it may indicate that the vascular damage induced by the PDL treatment may also affect the peri-vascular immune cells.

Markers of keratinocyte activation and of the psoriasis phenotype were only significantly reduced at the end of the series of the PDL and the NB-UVB treatment sessions. Keratin 17 expression is high in the psoriatic epidermis correlating with the clinical severity.[25] Keratin 17 is considered to be a candidate auto-antigen in psoriasis.[26] Human β-defensin 2 (HBD2) is a molecule with antimicrobial activity expressed by epidermal keratinocytes under inflammatory conditions such as psoriasis.[27] Patients with psoriasis have higher genomic copy numbers of the gene encoding β-defensin 2[28] and have higher serum β-defensin 2 levels than healthy controls. Serum β-defensin 2 levels were shown to correlate positively with the disease severity.[29] Since the PDL treatment does not target the epidermis primarily, it is conceivable that the PDL-associated decrease in the expression of epidermal

keratin 17 and HBD2 is secondary to the dermal blood vessel damage and to the subsequent reduction in the inflammatory infiltrate and their mediators.

Our finding that the PDL treatment is effective in approximately 50% (a subgroup) of patients has also been reported by others.[30] In our study, the group of PDL-responders could not be distinguished from the non-responders in terms of specific baseline expression patterns (results not shown) or immunohistochemical alterations induced by the treatment. Further studies are essential for identifying the prognostic markers of PDL-responsiveness in psoriasis.

In conclusion, PDL and local NB-UVB treatment are clinically equally effective in stable plaque-type psoriasis. At the end of the treatment period, both the treatments resulted in the decreased expression of epidermal markers of keratinocyte activation as well as decreased dermal T cell infiltrates. This indicated that alterations in the expression of markers of psoriasis activity do not clearly disclose the clinical treatment modality used and may just reflect the clinical improvement in the disease. Furthermore, we observed early effects of the PDL treatment on VEGFR2, VEGFR3 and the down-regulation of TNF-α and IL-23p19. These are previously unrecognized factors for the efficacy of the PDL treatment in psoriasis.

References

1. Pinkus H, Mehregan AH. The primary histologic lesion of seborrheic dermatitis and psoriasis. J Invest Dermatol 1966; 46(1):109-116.
2. Heng MCY AS, Haberfelde GH, Song MK. Electronmicroscopic and immunohistochemical studies of the sequence of events in psoriatic plaque formation following tape-stripping. Br J Dermatol 1991; 125:548-556.
3. Hacker SM, Rasmussen JE. The effect of flash lamp-pulsed dye laser on psoriasis. Arch Dermatol 1992; 128(6):853-855.
4. Herd RM, Dover JS, Arndt KA. Basic laser principles. Dermatol Clin 1997; 15(3):355-372.
5. Katugampola GA, Rees AM, Lanigan SW. Laser treatment of psoriasis. Br J Dermatol 1995; 133(6):909-913.
6. Ros AM, Garden JM, Bakus AD, Hedblad MA. Psoriasis response to the pulsed dye laser. Lasers Surg Med 1996; 19(3):331-335.
7. Hern S, Allen MH, Sousa AR, Harland CC, Barker JN, Levick JR, Mortimer PS. Immunohistochemical evaluation of psoriatic plaques following selective photothermolysis of the superficial capillaries. Br J Dermatol 2001; 145(1):45-53.
8. Zelickson BD, Mehregan DA, Wendelschfer-Crabb G, Ruppman D, Cook A, O'Connell P, Kennedy WR. Clinical and histologic evaluation of psoriatic plaques treated with a flashlamp pulsed dye laser. J Am Acad Dermatol

1996; 35(1):64-68.
9. Erceg A, Bovenschen HJ, van de Kerkhof PC, Seyger MM. Efficacy of the pulsed dye laser in the treatment of localized recalcitrant plaque psoriasis: a comparative study. Br J Dermatol 2006; 155(1):110-114.
10. de Leeuw J, Tank B, Bjerring PJ, Koetsveld S, Neumann M. Concomitant treatment of psoriasis of the hands and feet with pulsed dye laser and topical calcipotriol, salicylic acid, or both: a prospective open study in 41 patients. J Am Acad Dermatol 2006; 54(2):266-271.
11. Hamakawa M, Sugihara A, Okamoto H, Horio T. Ultraviolet B radiation suppresses Langerhans cell migration in the dermis by down-regulation of alpha4 integrin. Photodermatology Photoimmunology & Photomedicine 2006; 22:116-123.
12. Krueger JG, Wolfe JT, Nabeya RT, Vallat VP, Gilleaudeau P, Heftler NS, Austin LM, Gottlieb AB. Successful ultraviolet B treatment of psoriasis is accompanied by a reversal of keratinocyte pathology and by selective depletion of intraepidermal T cells. J Exp Med 1995; 182(6):2057-2068.
13. Ozawa M, Ferenczi K, Kikuchi T, Cardinale I, Austin LM, Coven TR, Burack LH, Krueger JG. 312-nanometer ultraviolet B light (narrow-band UVB) induces apoptosis of T cells within psoriatic lesions. J Exp Med 1999; 189(4):711-718.
14. Weischer M, Blum A, Eberhard F, Rocken M, Berneburg M. No evidence for increased skin cancer risk in psoriasis patients treated with broadband or narrowband UVB phototherapy: a first retrospective study. Acta Derm Venereol 2004; 84(5):370-374.
15. Hearn R, Kerr A, Rahim K, Ferguson J, Dawe R. Incidence of skin cancers in 3867 patients treated with narrow-band ultraviolet B phototherapy. Br J Dermatol 2008; 159:931-935.
16. De Leeuw J, Van Lingen RG, Both H, Tank B, Nijsten T, Martino Neumann HA. A comparative study on the efficacy of treatment with 585 nm pulsed dye laser and ultraviolet B-TL01 in plaque type psoriasis. Dermatol Surg 2009; 35(1):80-91.
17. Schwarz T. Mechanisms of UV-induced immunosuppression. Keio J Med 2005; 54(4):165-171.
18. Costa C, Incio J, Soares R. Angiogenesis and chronic inflammation: cause or consequence? Angiogenesis 2007; 10(3):149-166.
19. Henno A, Blacher S, Lambert C, Colige A, Seidel L, Noel A, Lapiere C, de la Brassinne M, Nusgens BV. Altered expression of angiogenesis and lymphangiogenesis markers in the uninvolved skin of plaque-type psoriasis. Br J Dermatol 2009; 160(3):581-590.

20. Iljin K, Karkkainen MJ, Lawrence EC, Kimak MA, Uutela M, Taipale J, Pajusola K, Alhonen L, Halmekyto M, Finegold DN, Ferrell RE, Alitalo K. VEGFR3 gene structure, regulatory region, and sequence polymorphisms. Faseb J 2001; 15(6):1028-1036.
21. Zaba LC, Cardinale I, Gilleaudeau P, Sullivan-Whalen M, Suarez-Farinas M, Fuentes-Duculan J, Novitskaya I, Khatcherian A, Bluth MJ, Lowes MA, Krueger JG. Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. J Exp Med 2007; 204(13):3183-3194.
22. Lowes MA, Chamian F, Abello MV, Fuentes-Duculan J, Lin SL, Nussbaum R, Novitskaya I, Carbonaro H, Cardinale I, Kikuchi T, Gilleaudeau P, Sullivan-Whalen M, Wittkowski KM, Papp K, Garovoy M, Dummer W, Steinman RM, Krueger JG. Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). Proc Natl Acad Sci U S A 2005; 102(52):19057-19062.
23. Krueger GG, Langley RG, Leonardi C, Yeilding N, Guzzo C, Wang Y, Dooley LT, Lebwohl M. A human interleukin-12/23 monoclonal antibody for the treatment of psoriasis. N Engl J Med 2007; 356(6):580-592.
24. Zaba LC, Krueger JG, Lowes MA. Resident and “inflammatory” dendritic cells in human skin. J Invest Dermatol 2009; 129(2):302-308.
25. de Jong EM, van Vlijmen IM, van Erp PE, Ramaekers FC, Troyanovski SM, van de Kerkhof PC. Keratin 17: a useful marker in anti-psoriatic therapies. Arch Dermatol Res 1991; 283(7):480-482.
26. Bonnekoh B, Bockelmann R. Keratin 17/interferon-gamma autoimmune loop as a vicious circle driving psoriasis pathogenesis. J Am Acad Dermatol 2007; 56(1):162; author reply 162-164.
27. Harder J, Schroder JM. Psoriatic scales: a promising source for the isolation of human skin-derived antimicrobial proteins. Journal of leukocyte biology 2005; 77(4):476-486.
28. Hollox EJ, Huffmeier U, Zeeuwen PL, Palla R, Lascorz J, Rodijk-Olthuis D, van de Kerkhof PC, Traupe H, de Jongh G, den Heijer M, Reis A, Armour JA, Schalkwijk J. Psoriasis is associated with increased beta-defensin genomic copy number. Nat Genet 2008; 40(1):23-25.
29. Jansen PA, Rodijk-Olthuis D, Hollox EJ, Kamsteeg M, Tjabringa GS, de Jongh GJ, van Vlijmen-Willems IM, Bergboer JG, van Rossum MM, de Jong EM, den Heijer M, Evers AW, Bergers M, Armour JA, Zeeuwen PL, Schalkwijk J. Beta-defensin-2 protein is a serum biomarker for disease activity in psoriasis and reaches biologically relevant concentrations in lesional skin. PLoS ONE 2009; 4(3):e4725.

30. Taibjee SM, Cheung ST, Laube S, Lanigan SW. Controlled study of excimer and pulsed dye lasers in the treatment of psoriasis. Br J Dermatol 2005; 153(5):960-966.

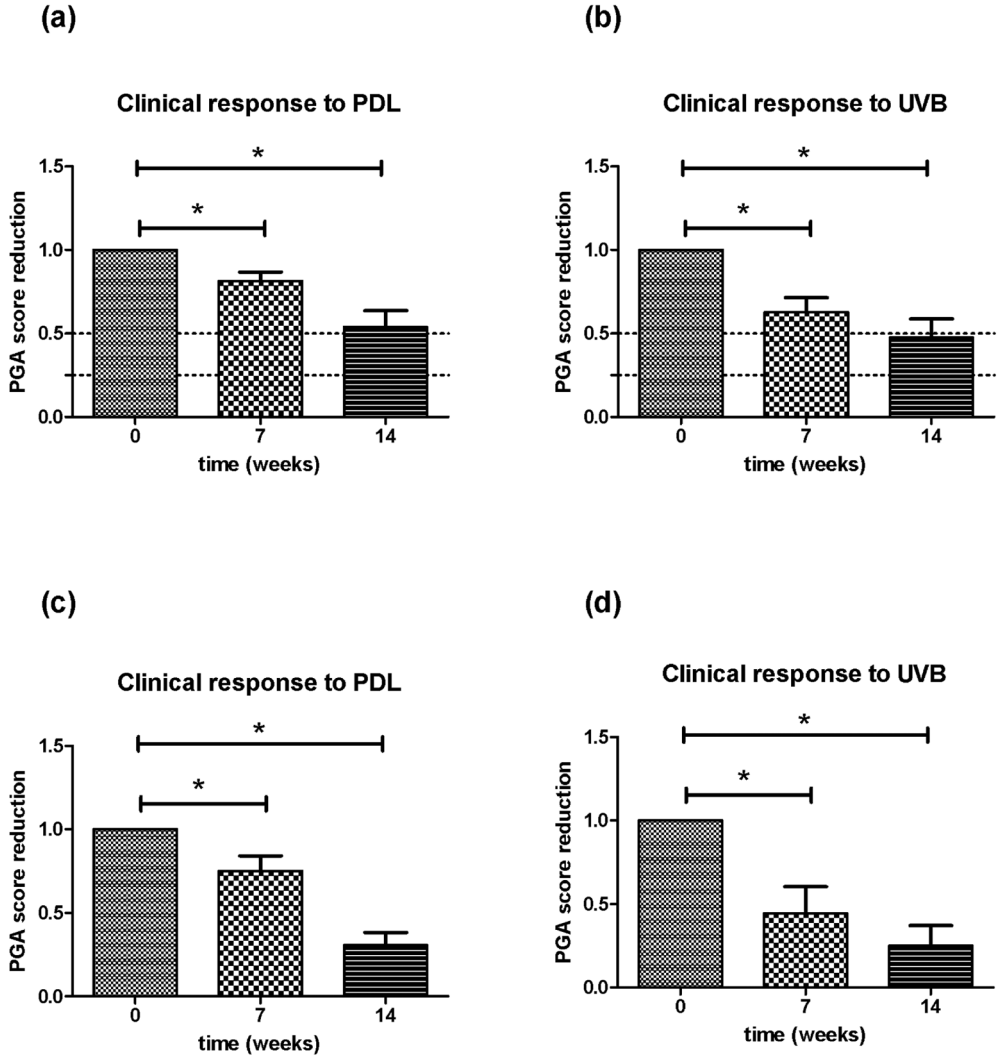

Figure 1. Clinical improvement in the psoriatic lesions during the PDL and the NB-UVB treatment from baseline to week 13. In each patient, stable psoriatic plaques were randomly selected and treated with PDL or NB-UVB. Clinical improvement was evaluated using the Physician's Global Assessment (PGA) score. The average clinical improvement in the 14 patients (1a & b). The average clinical improvement in 6 patients with high clinical response (1c & d). Error bars represent the standard error of the mean (SEM).

Figure 2.

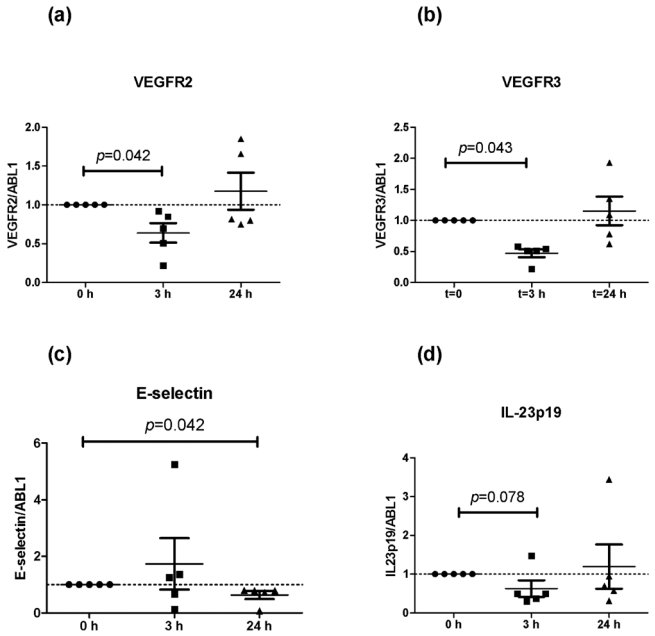

Figure 2. The effects of the PDL treatment on the expression of the vascular endothelial growth factor receptors, E-selectin and IL-23p19 in psoriatic skin. The expression of VEGFR2 (a), VEGFR3 (b), E-selectin (c) and IL-23p19 (d) was determined by RT-PCR in punch biopsies taken from psoriatic lesions before, 3- and 24 hours after the PDL treatment in 5 patients with psoriasis. The Figures depict the expression in the individual patients relative to the baseline. At each time point, the mean +/- SEM are marked. *p*-values are shown as calculated with the Wilcoxon signed ranks test.

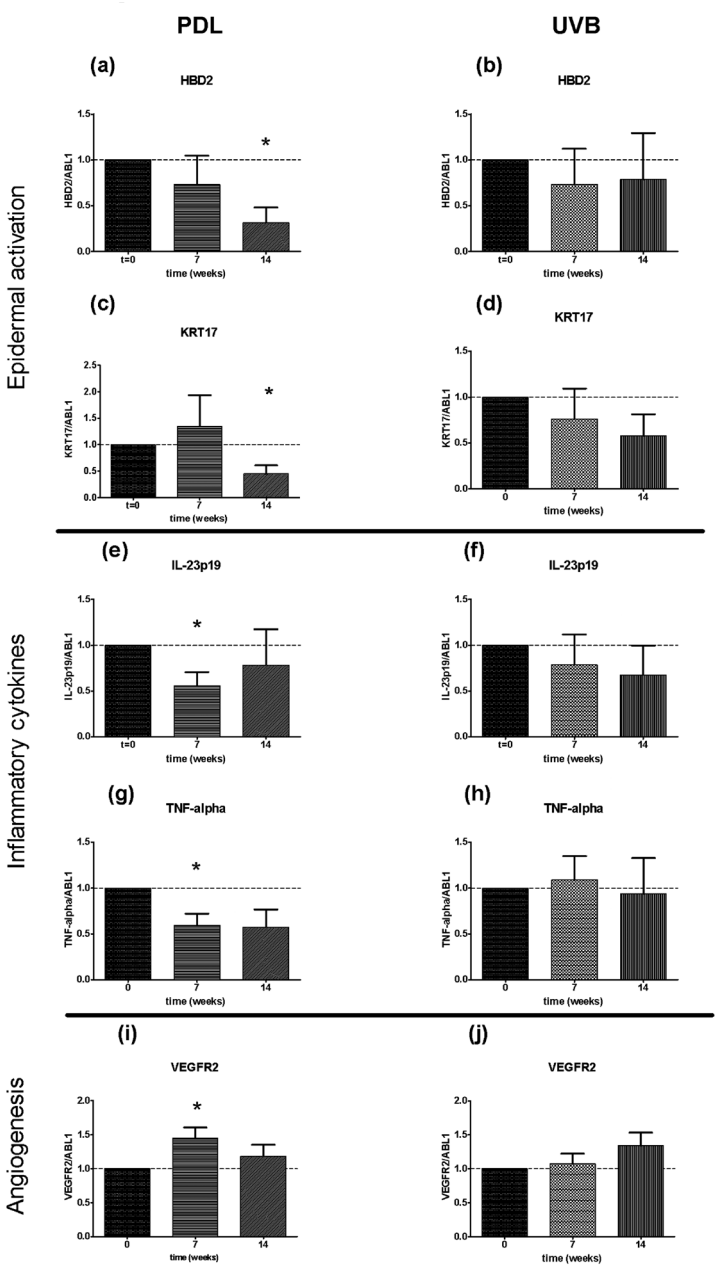

Figure 3. The effects of the PDL and the NB-UVB on the expression of the psoriasis markers in lesional psoriatic skin. The expression of mRNA of β-defensin 2 (a & b), keratin 17 (c & d), IL-23p19 (e & f), TNF-α (g & h) and VEGFR2 (i & j) was determined by the quantitative RT-PCR in punch biopsies from 6 patients. ABL1 was used as a housekeeping control gene. Bars show average expression levels relative to the baseline, error bars represent SEM. *p*-values are shown as calculated with the Wilcoxon signed ranks test.

Figure 4.

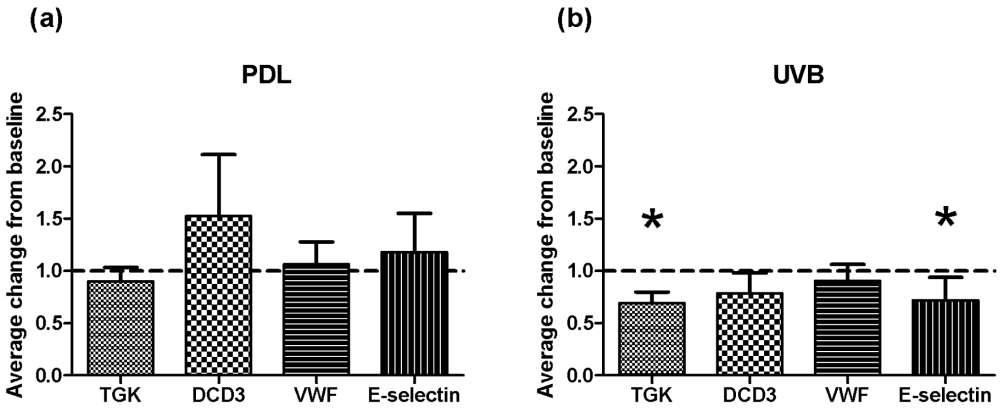

Figure 4. Immunohistochemical staining. Punch biopsies from 14 patients with psoriasis were taken before and 13 weeks after the start of the treatment. Immunohistochemical staining of the cryostat sections for epidermal transglutaminase K (TGK), CD3, von Willebrand's factor (vWF) and E-selectin (E-sel) was performed. The level of staining was quantified using digital image analysis. The level of staining for CD3 was quantified separately in the dermis (DCD3). Bars show the level of staining at 13 weeks relative to the baseline. Error bars represent the SEM.

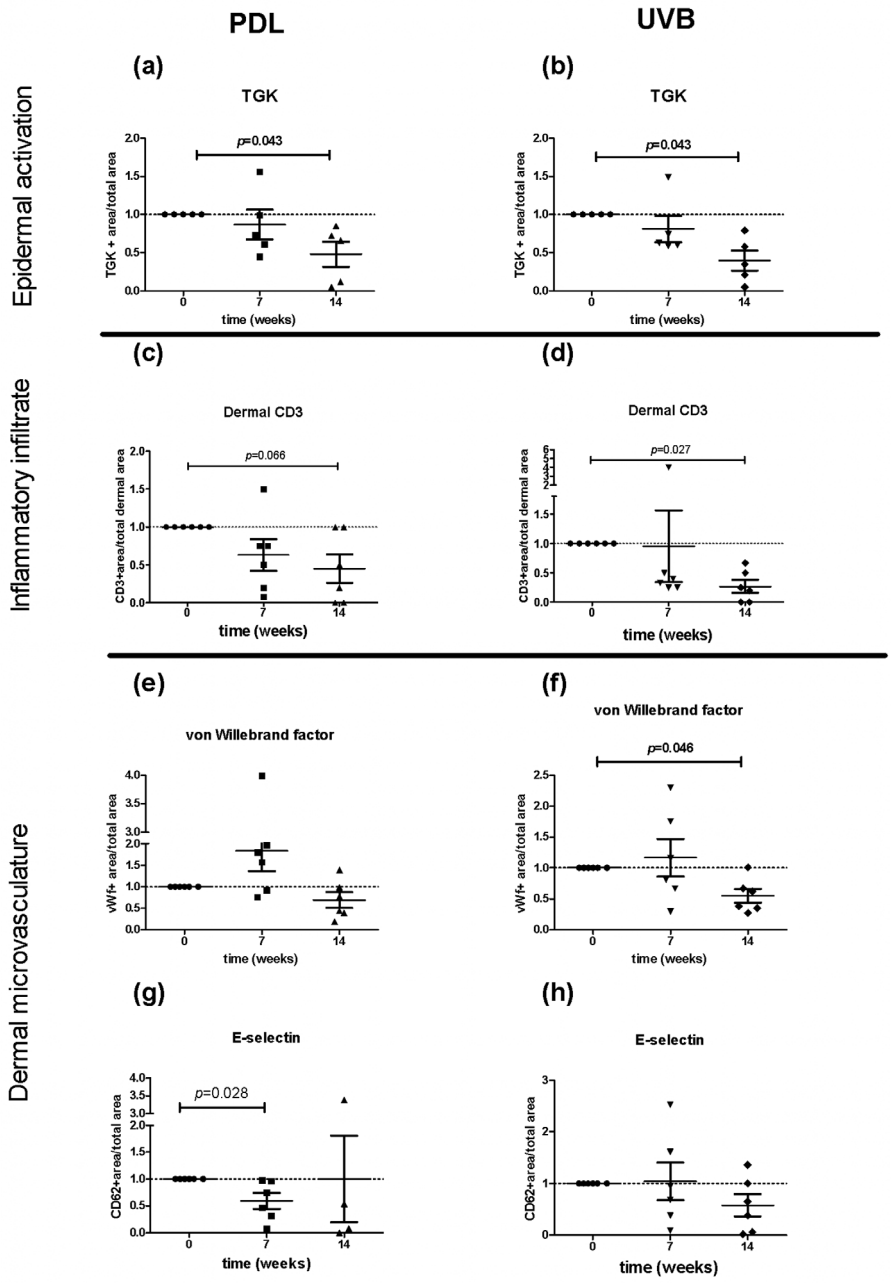

Figure 5. Immunohistochemical staining in punch biopsies from clinical responders. Cryostat sections of punch biopsies from 6 clinical responders taken before the start of treatment and 7 and 13 weeks later were stained using antibodies against transglutaminase K (a & b), CD3 (c & d), von Willebrand's factor (e & f) and E-selectin (g & h). The level of staining was quantified using digital image analysis. Figures depict the level of the expression in individual patients relative to the baseline. At each time point, the mean +/- SEM are marked. *p*-values are shown as calculated with the Wilcoxon signed ranks test.

Table 1. Antibodies used in the study

Antibody	Target molecule	Supplier	Species	Titer
anti-TGK	Transglutaminase K	Biomedical Technologies Inc.	Mouse	1:200
anti-CD3	CD3, a cell surface molecule on T lymphocytes	Dako	Rabbit	1:100
anti-vWF	von Willebrand's factor, a coagulation factor synthesized by endothelial cells	Abcam	Rabbit	1:25000
anti-E-selectin	E-selectin (CD62E), an adhesion molecule on endothelial cells	R&D systems Inc .	Mouse	1:20
anti-ICAM-1	Intercellular adhesion molecule 1 (CD54), expressed on endothelial cells and keratinocytes	R&D systems Inc.	Mouse	1:50

Table 2. Primers and probes for quantitative real-time PCR.

	Forward primer	Reverse primer	Probe [1]
RT17	TTGAGGAGCTGCAGAACAAG	AGTCATCAGCAGCCAGACG	76
HBD2	TCAGCCATGAGGGTCTTGTA	GGATCGCCTATACCACCAAA	35
TNF-α	CAGCCTCTTCTCCTTCCTGAT	GCCAGAGGGCTGATTAGAGA	29
IL-23p19	GTTCCCCATATCCAGTGTGG	TCCTTTGCAAGCAGAACTGA	76
E-selectin	ACCAGCCCAGGTTGAATG	GGTTGGACAAGGCTGTGC	86
VEGF-A	TGCCCGCTGCTGTCTAAT	TCTCCGCTCTGAGCAAGG	1
VEGFR2	GCTCAAGACAGGAAGACCAAG	GGTGCCACACGCTCTAGG	27
VEGFR3	CAAGAAAGCGGCTTCAGGTA	GCAGAGAAGAAAATGCTGACG	8
Bcl2	CAACACGCAGAGAATGTAAAGC	GGTAGGAGCTGTGGCGACT	45
ABL1	TGGAGATAACACTCTAAGCA TAACTAAAGGT	GATGTAGTTGCTTGGGACCCA	CCATT TTTGG TTTGG GCTTC ACACC ATT

[1] Probe numbers of the Exiqon probe library system (Exiqon, Vedbaek, Denmark).

Chapter 8

Published as

Liposomes in Dermatology Today

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Abstract
Liposomes are vesicles consisting of spherical phospholipid bi-layers with specific properties making them useful for topical application of drugs. Liposome research has expanded considerably over the last 30 years and nowadays, it is possible to construct a wide range of liposomes varying in size, phospholipids composition and surface characteristics to suit the specific application for which they are intended. In dermatology, the topical application of liposomes has proven to be of therapeutic value. Liposomes can be used as carriers for hydrophilic as well as lipophilic therapeutic agents because of their amphipathic character. They may improve stabilization of instable drugs by encapsulating them and serve as penetration enhancers facilitating the transport of compounds that otherwise cannot penetrate the skin. Liposomes help in reducing skin irritation by sustaining the release of drugs and by hydration of the epidermis. They also have the potential to target drugs into the pilosebaceous structures and hence they have an additional advantage for treatment of hair follicle-associated disorders. Clinical data indicate that 5-ALA encapsulated in liposomes improves the quality of Fluorescence Diagnosis by ALA-induced Porphyrins (FD) and optimizes the results of Photodynamic Therapy (PDT).

Introduction
The skin is an organ, which is directly accessible for topical application of drugs. However, this does not imply that drugs incorporated in conventional transport media such as creams and ointments are delivered at the optimal concentration to the right

target structure in the skin. Optimum topical delivery of drugs into the skin requires penetration of the vehicle through the stratum corneum, liberation of the drug from the vehicle, absorption of the drug through the different layers of the skin, adsorption of the drug to the tissue structure(s) involved in the pathological process and permeability of the drug through the cell membrane in case of intended intracellular action. Safety aspects of topical drug delivery include the reduction of the risk of local and systemic side-effects by using appropriate drug concentrations, optimal vehicles and valid treatment schedules.[1] Liposomes were already described in 1965 by Bangham et al., who noticed the spontaneous aggregation of phospholipids into vesicles after addition of water to phospholipids.[2] These vesicles, called liposomes, were initially used as a model for membrane system studies.[3] Since 1970, liposomes have received considerable attention as a system for delivering drugs to the target tissue (drug targeting).[3] The delivery of liposomal agents seemed to be superior to their conventional counterparts in certain fields (e.g. reducing tumour burden in squamous cell lung carcinoma).[4] The active or passive targeting by liposomes offered new approaches in the therapy of neoplasms (e.g. Kaposi's sarcoma, leukaemias and myelomas).[4] Liposomes may be effective in the therapy of micrometastases when liposomes are taken up by surrounding tissue from the blood vessels during angiogenesis.[4] In spite of promising prospects, the systemic application of liposomal drugs has been limited to just a few indications, but the topical application of liposomal preparations has attracted increasing attention in dermatology.[3,5]

Liposomes are microscopic vesicles formed from phospholipids as biological membranes. A fundamental feature of cell membranes is the organization of lipids into bi-layers, providing permeability barriers between exterior and interior compartments. A large group of biological membrane lipids that spontaneously form bi-layers in water are the phospholipids. A class of phospholipids commonly used to construct liposomes for drug delivery is phosphatidylcholine (lecithin; Fig. 1).[5] The ability of phospholipids to form a bi-layer structure is because of their amphipathic character resulting from the presence of a polar or hydrophilic (water-attracting) head-group region and a non-polar, lipophilic (water-repellent) tail. The hydrophilic head groups orientate toward the aqueous phase and the lipophilic tails orientate to each other in the presence of water (Fig. 1).[5] Therefore, liposomes contain a lipophilic compartment within the bi-layer membranes and hydrophilic compartments between the membranes. Under the right conditions, water-soluble substances can be stored into the water phase and lipophilic substances into the lipid phase (Fig. 1).[6] In general, phospholipids spontaneously form large multilamellar liposomes and special process conditions and post-process steps are required to produce appropriately sized (uni)lamellar liposomes. Liposomes may be small, unilamellar vesicles (SUVs 25–50 nm in diameter), large, unilamellar vesicles (LUVs 50–500

nm in diameter) or large multilamellar vesicles (LMVs 500–10 000 nm in diameter). SUVs are less suitable for drug delivery because they lack stability and their volume is too small for entrapping drugs. The penetration of liposomes through the stratum corneum decreases with increasing diameters. Therefore, the preferred structures for drug delivery are LUVs that are 50–500 nm in diameter (Fig. 1).[5] Next to the preferred sizes, an essential characteristic of liposomes for penetration through the stratum corneum is their state in a liquid crystal phase. The lipid bi-layer passes from a gel into a liquid crystal phase at a critical phase transition temperature (cptT). At the cptT, the head groups become fully hydrated and the lipid chains become freely mobile in the membrane. The cptT is determined by the length and the saturation of the paired lipid chains.[7]

Lipids, such as phosphatidylcholine, form bi-layers at cptT below room temperature. This liquid crystal state is essential for liposomes to interact simultaneously with the lipid and the aqueous compartments of the stratum corneum, and for delivering entrapped drugs into the skin.[8] Cell membranes contain many more classes of lipid than phosphatidylcholine, and the next, quantitatively important phospholipid is phosphatidylethanolamine. This lipid, in isolation, does not form a bilayer but adopts a structure known as the hexagonal (H_{11}) phase. The ability of lipids to adopt structures other than the bilayer configuration upon hydration is known as lipid polymorphism. A generalized shape property can be ascribed to lipid molecules, which reflects the phase structure they prefer in excess water. The bilayer phase lipids exhibit a cylindrical geometry, whereas H_{11} phase lipids can be considered cone shaped in that the acyl chains subtend a larger cross-sectional area than the polar head-group region. The reverse cone shape is adopted by detergens-type lipids which form micelles.[5] Evidence is accumulating that inverted H_{11} structures act as intermediates in membrane fusion and that LUVs made from lipid mixtures containing unsaturated phosphatidylethanolamine can fuse and mix with the skin lipids to loosen their structure providing a penetration enhancing effect.[5,9] Standard nanocarriers encompassed pure phosphatidylcholine vesicles (non-rigid), phosphatidylcholine vesicles with cholesterol (membrane stabilized liposomes), and two rigid vesicles of dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylcholine with cholesterol. The ultradeformable (flexible) vesicles include phosphatidylcholine with sodium cholate, phosphatidylcholine with Span 80 and phosphatidylcholine with Tween 80. All types of liposomes improve deposition into and permeation through the epidermis of encapsulated drugs compared with a saturated aqueous control. The ultradeformable vesicles are better than the standard liposomes with respect only to transepidermal drug flux.[9] The majority of the conventional liposome preparation techniques involve the application of volatile organic solvents or detergents. These solvents may influence the stability of vesicles, leading to leakage of drugs from

the liposomes. They may also exert toxicity towards molecules dissolved in the aqueous phase of the liposomes and towards membranes of cells. Because of these disadvantages, removal of the solvents and the detergents by gel filtration or dialysis are required.[10] Recently, a new method for the production of liposomes without the use of solvents and detergents has been described. This method involves the hydration of liposome components in an aqueous medium followed by the heating of these components in the presence of glycerol.[11]

The skin acts as a two-way barrier, controlling the inward and outward passage of water and electrolytes. This barrier is situated in the stratum corneum and consists of the cornified material of the terminal differentiated keratinocytes, proteins and intercellular lipids. Filaggrin is a crucial protein in the formation of the protective stratum corneum and has been indicated to play a role in determining the degree of dryness of human skin.[12] Filaggrin aggregates the keratin filament cytoskeleton, causing the collapse of the granular cells into flattened squames. This collapsed cytoskeleton, together with its attachment proteins and membrane components, undergoes extensive cross-linking to form the cornified cell envelope. The main function of the cornified envelope is to form the outermost barrier layer of the skin, which prevents water loss and keeps out allergens and infectious agents.[12] Intercellular lipids are produced by ovoid lamellar bodies, which are provided by the Golgi area in the keratinocytes in the stratum spinosum. In the ovoid lamellar bodies, lipids are arranged in bi-layer discs, which represent flattened unilamellar liposomes. In the stratum granulosum, the ovoid lamellar bodies migrate to the cell periphery, fuse with the cell membranes and discharge their contents into the intercellular spaces. After extrusion, the discs become arranged parallel to the cell membranes and then fuse to form uninterrupted sheets of water-retaining lipid bi-layers between the corneocytes.[13,14] Thus, the stratum corneum contains hydrophilic and lipophilic compartments, which act as a buffer to retard both water loss and absorption of water.[15] These lipid-rich structures might also influence the desquamation process in the stratum corneum.[16,17] Three mechanisms of the penetration of liposomes into the skin have been described:

1. Via lateral diffusion of liposomes in the stratum corneum. The molecular structure of liposomes is similar to that of endogenous skin lipids. Lipid exchange between human membranes is a common physiological phenomenon. The exchange occurs via molecular diffusion from one membrane to the other.[5] The rate of phospholipid exchange depends upon the relative sizes of the hydrophobic portions of the molecule. In general, phospholipids with short acyl chains (< 16 carbons long) exchange rapidly (minutes to hours), whereas longchain phospholipids move slowly between membranes into the stratum corneum (many hours to days).[18,19]

2. Via a trans-epidermal osmotic gradient and hydration force through which liposomes are sucked into the epidermis. In accordance with this putative mechanism is the observation that an effective penetration of liposomes into the skin requires a dry skin surface. Under occlusion (maximal hydration), no penetration of liposomes occurs.[20,21]

3. Via the pilosebaceous units.[22–24]

The similarity of lipid composition of liposomes and membranes of intercellular lamellae and keratinocytes enables the liposomes to penetrate into the epidermal barrier to a greater extent as compared with other application forms and so do the compounds encapsulated into these liposomes.[25] This may result in an increase of drug absorption into the epidermis and decrease of drug clearance from the epidermis, promoting longer sustained release of drugs in the epidermis and lower absorption of drugs into the blood. This is why liposomes may act not only as drug transporters, but also as drug localizers.[3] Fluorescent liposomes applied to the skin have been found inside the keratinocytes of the epidermis.[26] Liposomes fuse with the outer cell membrane and deliver their contents to the cytoplasm via endocytosis (Fig. 2).[27] It is possible to introduce even large molecules into the cytoplasm of keratinocytes by using liposomes as intracellular transport system after which these molecules may unfold their efficacy.[28]

Mezei and Gulaskharam were the first to report that triamcinolone acetonide in liposomes, applied topically to rabbit skin resulted in a higher concentration of triamcinolone acetonide in the epidermis and lower triamcinolone acetonide concentrations in the blood and urinary excretion as compared with triamcinolone acetonide applied in a gel formulation.[29,30] A similar effect was also shown in the human skin using liposome encapsulated tertracaine [31] and hydrocortisone.[32,33]

Moreover, 'empty' liposomes (without encapsulated drugs) hydrate the skin simply by providing lipids to the stratum corneum.[34] This hydration capacity may be of benefit in the treatment of xerosis cutis and atopic dermatitis.[35]

Indications for liposomes as drug carriers in dermatology

Acne

Acne is one of the most common disorders of the skin and has a considerable impact on the quality of life of adolescents. Standard treatment today consists of peeling the skin, eliminating the corynebacterium acnes and reducing inflammation by using topical benzoyl peroxide (BPO), tretinoin, adapalene and antibiotics. If this approach is not efficacious, systemic antibiotics and antiandrogens are the next step. However, the efficacy of antibiotic treatment is progressively reduced by increasing resistance of bacteria to antibiotics, and side-effects may also limit their

use. In case of failure of treatment the final remedy is oral isotretinoin, which is very effective, even in recalcitrant acne, but its use is limited by moderate to serious side-effects. The efficacy and safety profiles of certain antiacne drugs may be improved by encapsulating them into liposomes.

The superiority of liposome-encapsulated 1% clindamycinsolution vs. 1% clindamycin-solution in the treatment of acne was demonstrated in 73 patients. No side-effects were reported.[36]

Topical tretinoin is an anti-acne agent especially for reducing the size and the number of comedones.

Its use is limited by skin irritation, with erythema, scaling, burning sensation and increased susceptibility to sunlight at the sites of application. In a doubleblind study, the efficacy and the local tolerability of liposomal tretinoin 0.01% were compared with those of a commercial gel preparation with either 0.025% or 0.05% in 20 patients with uncomplicated acne vulgaris. The efficacy of liposomal tretinoin appeared to be equipotent to the reference gels, but liposomal tretinoin was superior with respect to skin irritancy.[37]

A comparative double-blind study in 30 patients demonstrated a 1.5-fold enhancement of drug efficacy and a marked decrease in all side-effects associated with tretinoin therapy for liposomal tretinoin gel compared with conventional tretinoin gels after 3 months of treatment.[38]

BPO is an effective topical agent in the treatment of acne and acts by inhibition of the propionibacterium acnes in the pilosebaceous units. Although resistance to BPO is not evident, local irritation with burning and erythema, and also bleaching of dyed clothing may limit patient compliance. A comparative doubleblind study of liposomal BPO vs. conventional BPO in 30 patients demonstrated a significant improvement in the therapeutic response (about 2-fold) after 3 months of treatment with liposomal BPO as compared with a conventional BPO gel, with marked reduction in adverse symptoms and bleaching of clothing.[39] In another study, a significantly higher antibacterial effect of a BPO liposome formula was observed as compared with commercial BPO formulations.[40]

Photodynamic therapy (PDT) is widely used to treat acne. However, there are only a few studies in the literature regarding treatment efficacy and treatment parameters.[41–44] Hyper-pigmentation was more often seen at higher doses of light, and pain was experienced more often by the patients when higher doses were used. The authors concluded that PDT could be an alternative treatment for acne and that the lowest possible light dose should be used for minimal side-effects.[43] Considering, the information provided by these publications, the need for optimizing PDT for treatment of acne is justified, especially concerning discomfort and side-effects during and after treatment. Christiansen et al. performed a study on the fluorescence

distribution patterns of normal skin after topical application of 20% 5-aminolevulinic acid (5-ALA) in a moisturizing cream and after application of low concentrations ALA (0.5% and 1%) encapsulated in liposomes. Changing the 5-ALA vehicle from a moisturizing cream to liposome encapsulation, the 5-ALA concentration could be lowered by a factor of 40, and still induced the same skin fluorescence and at the same time eliminated the need for occlusion.[45,46] The low post-treatment fluorescence also significantly reduced the risk of post-treatment pototoxicity.[45] In a preliminary study 16 patients with moderate to severe acne were treated with 0.5% ALA in a liposomal spray (each 10 min for 1 h) and subsequent illumination with an intense-pulse laser (IPL; 400–720 nm) 3×3.5–3×4.5 J/cm^2, every 4–6 weeks, and concomitant treatment with tretinoin 0.02% cream at night. Topical or systemic antibiotics were not used. The clinical results appeared to be promising without any side-effects (de Leeuw, unpublished observation; Fig. 3).

Ichthyosis vulgaris, xerosis cutis, and atopic skin

Recently, mutations in the filaggrin gene (R501X and 2282del4) were identified in patients with ichthyosis vulgaris and were shown to be an important predisposing factor for atopic dermatitis. These mutations, which are found in 10% of those of European origin, may lead to (in)-complete absence of filaggrin expression. [12] This results in a disturbance of the outermost barrier layer of the skin, which normally prevents water loss and keeps out allergens and infectious agents.[47,48] In atopic skin, more nonextruded lamellar bodies remain undelivered within the uppermost stratum granulosum cells, resulting in reduced delivery of pro barrier polar lipids in the intercellular space. This affects water permeability, leading to increased trans-epidermal water loss.[16,49] Healthy skin has the ability to regulate disturbed barrier function by increased lipid synthesis. This ability is reduced in atopic patients because of the (in)-complete absence of filaggrin expression and the altered extruding mechanism of lamellar bodies. Environmental influences such as central heating systems, increased washing, increased use of detergents, environmental pollutants, and many other influences on the epithelial barrier may increase the susceptibility of the skin leading to exacerbation of atopic dermatitis.[48] Increased lamellar body secretion was demonstrated with coincidental clinical improvement after wet-wrap dressing treatment of atopic eczema.[50] Similar effects on the restoration of the skin barrier were described after application of liposomes on the skin. Due to the similarity between the structure of the liposomes and the lipid layers of the stratum corneum, the liposomes bind to the keratin layer of the stratum corneum and form a thin occlusive film, which reduces trans-epidermal water loss giving the patient immediate relief from the discomfort associated with dry skin.[51] Additionally, the water content of liposomes promotes further hydration of the skin.[18] The wet-wrap

treatment is very time-consuming, needs training and is not well tolerated by every patient. Application of liposomal spray is a fast, easy to use and requires no training. Moreover, a doubleblind study showed that the patient's acceptance for liposomes containing preparations was significantly higher than that for commercially available moisturizing preparations.[52]

Atopic dermatitis, irritant and allergic contact dermatitis

Topically applied corticosteroid therapy is the first choice of treatment for atopic dermatitis and irritant and allergic contact dermatitis in addition to restoration of the damaged skin barrier and recognizing and elimination of a causative agent. Liposomes promote the restoration of the skin barrier resulting in a reduction of penetration of irritant- and allergenic substances and subsequently in a reduction of inflammation.[18,35] Furthermore, pharmacokinetic evaluation of triamcinolone acetonide and hydrocortisone showed higher drug levels in the skin and lower systemic availability following application of liposomal corticosteroids as compared with conventional preparations.[29,30,32,33] Higher and sustained concentrations of a corticosteroid in the skin promise higher efficacy, less side-effects from absorption of corticosteroids into the blood and lower frequency of application.[3,53]

Psoriasis

Recent breakthroughs in the treatment of psoriasis have led to improved understanding of the pathogenesis of this disease.[54,55] Although major advances in the development and the use of targeted biologicals for controlling psoriasis have been made, the need to develop safe, cost-effective and disease-effective cures remains.

Vitamin D_3 analogues such as calcipotriol, calcitriol and tacalcitol have antipsoriasis capacities due to suppression of inflammation and hyper-proliferation and promotion of epidermal differentiation. Calcitriol was tested at concentrations of 5, 15 and 20 µg/g in liposomes and in petrolatum in a mouse tail test.

The highest increase in the orthokeratosis indices was observed for calcitriol 5 µg/g in liposomes as compared with 15 and 20 µg/g in liposomes and 5, 15 and 20 µg/g in petrolatum. Similar results were also observed with tacalcitol in liposomes. The authors suggested that by using a liposomal preparation, the vitamin D_3 concentration can be reduced as compared with that of currently available commercial preparations without sacrificing therapeutic effect and meanwhile reducing local side-effects such as skin irritation and systemic side-effects.[56] Dithranol generally used as short contact therapy is safe and efficacious in the treatment of plaque type psoriasis. However, its use is inconvenient and troublesome because of its side-effects such as skin irritation, burning sensation, staining and necrotizing effect on normal as well as the diseased skin. The entrapment of dithranol in liposomal vesicles promotes its bioavailability

in the epidermis, which makes it possible to reduce the dose and in turn, the dose-dependent side-effects.[57] In a double-blind study, the superiority of 0.5% dithranol encapsulated in a liposomal gel as compared with a conventional cream containing 1.15% dithranol, 1.15% salicylic acid and 5.3% coal tar was reported.[58] Cyclosporine A (CyA), a potent immunosuppressive drug is highly efficacious in the treatment of psoriasis and other skin disorders. However, systemic administration of CyA is associated with serious side-effects, especially nephrotoxicity. Topical delivery of CyA is hindered by its physicochemical properties and the barrier property of statum corneum. CyA liposomal vesicles containing 10% and 20% ethanol showed statistically enhanced deposition of CyA into the stratum corneum, as compared with vesicles prepared without ethanol. These results can be considered as a step forward for the topical delivery of CyA using liposomes as a tool for the treatment of inflammatory skin diseases like psoriasis.[59]

Systemic methotrexate can be used for controlling recalcitrant psoriasis. However, the systematic use may provoke a number of side-effects, notably hepatotoxic effects. In order to overcome these side-effects, clinical studies have been pursued with topical application of methotrexate. A major problem in topical application of methotrexate is that the drug is hydro-soluble and is mostly in the dissociated form, thereby limiting its capacity for passive diffusion. However, by applying methotrexate in a liposomal preparation, 50% of the administered dose was found in the skin indicating that liposomes may be valuable in the topical application of methotrexate in the treatment of psoriasis.[60,61] It was confirmed that psoriasis can be treated by topical application of liposomalentrapped methotrexate-paraffin wax in a small clinical trial. This preparation was applied once a day on the psoriatic lesions, while the psoriasis plaques in the control group of patients were treated with free methotrexate and empty liposomes in paraffin wax. After 2 weeks of treatment, all plaques treated with liposomal methotrexate showed total clearance, whereas those receiving free methotrexate did not improve.[62]

Vitiligo

Vitiligo is an acquired de-pigmentation disorder affecting about 1% of the population. It may have an important negative impact on the quality of life, particularly of those with pigmented skin.

Autoimmunity, destroying the melanocytes in the epidermis, is the most accepted pathogenic mechanism, although other mechanisms have also been proposed. The inactive melanocytes in the outer root sheath of the hair follicles are not affected. Phosphatidylcholine liposomes are able to target molecules like khellin into the hair follicles.[22,23] After khellin is activated by ultraviolet A (UVA) and UVB, the inactive melanocytes proliferate and mature to an active state as they migrate into the

epidermis.[63] The results of systemic khellin in combination with UVA are comparable with the rates reported from psoralen photochemotherapy (PUVA).[64] The major advantage of khellin is that it does not induce photo-toxic skin erythema and does not induce detectable DNA mutations in contrast to PUVA, but side-effects such as liver dysfunction have been reported.[64] Topical photochemotherapy of vitiligo with khellin has also been reported to be efficacious.[65] The efficacy and the safety of topical treatment with 0.005% khellin encapsulated in L-phenylalanine stabilized phosphatidylcholine liposomes in combination with UVA/UVB light (KPLUV) therapy was demonstrated in 74 patients with vitiligo in a retrospective open trial.[66]

Actinic keratoses and skin cancer

Skin cancer is the most common cancer in white people. [67] Basal cell carcinoma and squamous cell carcinoma are the most frequently occurring skin cancers.[68] Squamous cell carcinoma has the potency to metastasize. Actinic keratosis is believed to be premalignant. The change of progression to invasive squamous cell carcinoma is about 8%, and many patients with squamous cell carcinoma have multiple actinic keratoses.[69] Skin cancer incidence rates have steadily increased over the last decades and are expected to keep on rising.[70] Exposure to solar UV radiation is the main environmental factor linked to the formation of skin cancers.[70] Sun-addicts are particularly at risk as are patients treated for skin disorders with UVB and PUVA. Other contributing factors are the ageing of the population and the increasing consumption of immunosuppressive drugs by organ transplant recipients and by individuals on treatment for autoimmune disorders, malignancies and skin disorders. Wavelengths especially in the UVB (280- to 320-nm) range damage DNA in the cells of human skin. Un-repaired UV-induced lesions such as cyclobutane pyrimidine dimers (CPD) in the DNA may lead to mutations involved in skin tumorgenesis.[71]

The bacteriophage T4 produces a DNA repair enzyme (T4 endonuclease V), which is able to substitute the UV-damaged enzyme complex in humans to initiate excision repair.[72] It has been shown in preclinical studies that T4 endonuclease V encapsulated in liposomes (T4N5 liposomes) cross the stratum corneum and were present in the epidermis 1 hour after application of a lotion containing T4N5 liposomes to mouse skin.[73] T4N5 liposomes, leading to a reinforcement of DNA repair, were observed in the cytoplasm and in the nuclei of keratinocytes in mouse and human skin. [26,74] T4N5 liposomes are antimutagenic not only because they remove CPD, but also because the re-synthesis step is less subject to error.[73] In addition, T4N5 liposomes have the ability to suppress the upregulation of tumour necrosis factor-α (TNF-α) and interleukin-10 (IL-10) after UVB irradiation of the skin. TNF-α is responsible for inflammatory and immunosuppressive responses by the immune system. IL-10 is also induced by UVB and shifts the cellular immune response from an active Th1

mode to a suppressive Th2 mode. It was reported that T4N5 liposomes, applied after UV exposure penetrate human skin and deliver T4 endonuclease into keratinocytes and epidermal Langerhans cells in 15 volunteers with preceding skin cancers.[75]

In the same study, a significant reduction of TNF-α and IL-10 gene transcription, a reduction of IL-10 protein and also an increase in DNA repair was demonstrated in biopsies taken 6 h after the T4N5 liposome application.[75] A reduction (45%) of UV-induced skin cancer in mice by topical application of T4N5 liposomes to the skin was already demonstrated in 1995.[76] Patients with xeroderma pigmentosa have an autosomal-recessive genetic defect in the pathway that repairs suninduced damage to DNA.[77]

As a result, the rates of (pre) malignancies of the skin are much higher than in the general population. This defect in DNA repair has been overcome in cell culture by the intracellular delivery of the bacterial DNA incision repair enzyme T4 endonuclease V. Daily applications of T4N5 liposome lotion for 1 years to sun-damage skin of 30 xeroderma pigmentosa patients (with a history of skin cancer or actinic keratosis) lowered the rate of new actinic keratoses and basal cell carcinomas significantly.[78] In summary, T4N5 liposomes protect the skin against UV-induced carcinogenesis by promoting the DNA repair and by inhibiting immunosuppression.

PDT, employing 5-ALA, was initially used in the treatment of actinic keratoses and later on also in the treatment of superficial basal cell carcinomas and morbus Bowen.[79–83] The mechanism of ALA- PDT is based on the selective accumulation of 5-ALA in malignant cells (and also in non-malignant cells with increased metabolism) as compared with normal adjacent tissue, and transformation of 5-ALA into the photosensitizer protoporphyrin IX (PpIX). PpIX induces phototoxicity and tissue destruction upon absorption of light of an appropriate wavelength and dose.[84] The hydrophilic nature of the ALA molecule limits the penetration through the skin as well as the cell membranes. Hence, ALAinduced PpIX formation is often restricted to superficial layers.[85] In order to improve the efficacy of 5-ALA, particularly in thicker lesions, a number of options have been investigated. Higher PpIX levels have been obtained in cell cultures by using the lipophilic methylester of 5-ALA methylaminolevulinate (MAL) compared with 5-ALA.[86] Higher PpIX levels have also been demonstrated using penetration enhancers, iron chelators, iontophoresis, and also after curettage prior to 5-ALA treatment.[87] To optimize the topical delivery system, the in vitro delivery performance of liposomes containing 5-ALA in the epidermis and the dermis without stratum corneum was studied. A higher skin retention and a lower skin permeation of 5-ALA was demonstrated as compared with 5-ALA in aqueous solution.[87] Another study assessed the tissue distribution and the kinetics of porphyrin synthesis after topical application of 5-ALA encapsulated in liposomes

to tumour bearing mice. In contrast to the kinetics of porphyrins formed from free 5-ALA, tumour and overlying skin produced maximal amount of porphyrins 24 hour after liposomal application.[85] The results of these studies indicated that 5-ALA in liposomes may be adequate for the 5-ALA PDT treatment of skin cancer because the liposomes deliver 5-ALA to the target skin layers (viable epidermis and dermis).[87]

Early tracing of (pre) malignancies is important for reducing the extensiveness of subsequent medical interventions, resulting in less patient discomfort, higher cure rates and reducing the costs of more extensive interventions and increased chances of side-effects. Actinic keratoses may be clinically detected as red and rough papules and plaques by inspection and palpation. However, diagnostic approaches have been unsatisfactory to date and there is a great demand for non-invasive clinical methods.[88]

Fluorescence diagnosis by ALA-induced porphyrins (FD), a technique based on PDT, was developed to distinguish various types of tissues and cells on the basis of differences in fluorescence. Recently, laserinduced fluorescence spectroscopy at 410-nm excitation has been shown to be effective as a non-invasive method for objective evaluation of non-melanoma skin cancers.[89] The depth of penetration of 5-ALA into the skin is a major limitation in the diagnosis, especially in hypertrophic and hyperkeratotic actinic keratosis lesions, and in psoriasis.[90–92] The mechanical barrier by the stratum corneum is responsible for the diffusion rate of ALA and/or penetration of light.92 Liposomes provide an enhanced passage of 5-ALA through the stratum corneum, resulting in a more precise targeting of drugs into the diseased cells. Liposomeencapsulated 5-ALA transformation into PpIX is also higher in actinic keratosis and basal cell carcinoma cells as compared with the cells in the normal adjacent skin. This is why liposomes may contribute toward improved diagnostic and therapeutic results. This property of liposomes as a targeting method is not available in other topical applications.[45]

Thrombophlebitis

Superficial vein thrombosis is generally considered to be relatively harmless. However, deep venous thrombosis and pulmonary embolism following superficial vein thrombosis have been reported.[93]

Therefore, the importance of treatment with low molecular weight heparin (LMWH) in addition to compression therapy has increased, mainly because of the antithrombotic and antiphlogistic actions of heparin.[94] Topical liposomal heparin spray-gel appeared to be equally efficacious in reduction of pain, erythema and thrombus size as compared with subcutaneous injections of LMWH.[94]

Subcutaneous injections and applications of topical LMWH in ointment, cream and gels are painful (rubbing into the skin at the site of the thrombophlebitis) in contrast with liposomal heparin spray-gel. In a double-blind study, 124 patients with superficial

vein thrombosis were treated. One half of the patients were treated with liposomal heparin spray and the other half of the patients with placebo liposomal spray. All the patients were also treated with ambulant compression therapy. Remarkably, better efficacy was observed in the patients treated with liposomal heparin spray as compared with those treated with the placebo liposomal spray. All patients underwent Doppler scanning of the deep venous
system. Thrombus propagation was not encountered in any case during the three weeks observation period.[95]

Hair removal

Unwanted facial and body hair can represent a severe cosmetic disturbance with social and psychological implications often strong enough to motivate patients, especially women, to seek dermatologic treatment. The goal of laser hair removal is to damage stem cells in the bulge of the follicle or to replace the hair follicle at the level of the dermis with connective tissue through thermal injury. The primary mechanism of laser hair removal is selective photothermolysis whereby follicular melanin is the target chromophore for destruction by light energy. Laser hair removal of white, blond and grey hair is often unsuccessful due to the lack of melanin in the hair follicle. Melanin-encapsulated liposomes have demonstrated to deliver melanin selectively to the hair follicle and the hair shaft. (Hoffman 1998) Melanin-encapsulated liposomes (Lipoxome®) are used for staining hair follicles of people with blond, white or grey hair.96,97 Histological studies have confirmed the presence of new deposits of melanin in the hair follicle after application of melanin-encapsulated liposomes. Pre-treatment with melanin liposomal spray improved the results of 800-nm diode laser therapy in 38 hirsute women and 2 men with light blond, white and grey hair.98 Lipoxome® spray was applied 6–8 times a day, during 14 days prior to laser treatments with an 800-nm diode laser (treatment intervals 6–8 weeks). At 6 months after the last laser treatment, 90% of patients experienced a permanent hair reduction of over 75% within 10 treatments. A correlation was found between the quantity of Lipoxome® applied to the area in the 14-day pretreatment period and the extent of laser hair removal (correlation of 0.785 at a significant level of 99.9%) In the control group, 20 hirsute women also having light blond, white and grey hair, were only treated with the 800-nm diode laser. At the last control visit, 6 months after the last laser treatment, these patients hardly showed any notable hair reduction. Side-effects were seen in both groups: transient discomfort, erythema, perifollicular oedema, crusting and transient hyperand hypopigmentation.[98] Pretreatment with Lipoxome® spray improved also the results of long pulsed Nd:Yag laser treatment for pigmented underarm hair. At 6 months after three laser treatment sessions, the percentage of hair reduction was 73% with the combined therapy and 43% with laser

treatment alone.[99]

Topical anaesthesia for intravenous cannulation and superficial surgery

In contrast with EMLA® cream, there is no need for occlusion after application of tetracaine 5% or lidocaine 4% in liposomal cream. Adequate anaesthesia is already obtained after 30 min, vasoconstriction does not occur and there is no association with methemoglobinemia.[100–105]

Skin rejuvenation

It became apparent that PDT of (pre) malignant disorders rejuvenated the adjacent skin in the irradiated area, which was clinically seen as a reduction of wrinkles, solar lentigines, scars and large skin pores.[106,107] In double-blind, split-face studies, a significantly higher clinical improvement was demonstrated on the facial halves receiving topical 5-ALA and IPL as compared with the facial halves receiving only IPL.[108,109] The concentrations of 5-ALA and MAL used in PDT, respectively, 20% and 16%, were originally chosen for the photodynamic treatment of precancerous and superficial malignant skin disorders, at which the side-effects were acceptable for the beneficial clinical efficacy. However, in cosmetic treatments, the safety and the lack of discomfort are of primary importance, side-effects such as pain, oedema, erythema, desquamation and phototoxicity during 24–48 hour after 3 hour of 20% 5-ALA and 16% MAL treatments are not acceptable.[45] This is why attempts have been made to reduce discomfort and at the same time maintain or augment efficacy of PDT by reducing incubation time to 1 hour[84,107] and even to 30 min[110] and/or reducing 5-ALA concentration to 5%.[79] Since these studies demonstrated that 0.5- to 1-hour incubation times of 20% 5-ALA cream under occlusion was sufficient to obtain a skin rejuvenation effect, comparable clinical results are to be expected from 1 to 2-hour application of 5-ALA 0.5% liposomal solution without occlusion.

Instead of PDT or in addition to PDT, tretinoin can be used to treat acne, for skin rejuvenation, to treat sun-damaged skin and to prevent epithelial (pre)malignancies because of its ability to regulate sebum production, collagen synthesis and epithelial cell growth and differentiation.[111,112] It has been demonstrated that the hydration of the epidermis and the storage of tretinoin in the epidermis is strongly promoted by incorporation of tretinoin in negatively charged liposomes resulting in more efficacy and less irritation of the skin as compared with tretinoin in a conventional cream.[113]

Conclusions

Liposome research has expanded considerably over the last 30 years, and nowadays, it is possible to construct a wide range of liposomes varying in size, phospholipids composition and surface characteristics to suit the specific application

for which they are intended.[114] Liposomal formulations should have high entrapment efficiencies, narrow size distributions, long-term stabilities and ideal release properties. Consequently, the production method requires the potential to produce liposomes using a wide range of ingredients of molecules (e.g. lipids/phospholipids) that promote liposome stability. In addition, liposomes should be free of toxic solvents and detergents. There are numerous lab-scale and a few large-scale techniques for liposome preparation giving rise to vesicles of different sizes and consisting of one or more bi-layers. However, most of these techniques are not suitable for the encapsulation of sensitive substances because of their exposure to mechanical stresses, harmful chemicals (e.g. volatile organic solvents and detergents) or low/high values of pH during the preparation. Conventional and novel preparation techniques have been introduced, each with its own advantages and possible limitations. Topical application of liposomal preparations is rational, based on clinical data and also from a pharmacologic point of view because of their advantages concerning drug delivery and restoration of skin barrier, including:

1. The similarity of lipid composition of liposomes and membranes in the epidermis enables the liposomes to penetrate into the epidermal barrier to a higher extent as compared with other application forms and so do the compounds encapsulated into the liposomes. This may result in an increased drug absorption into the epidermis and a decreased clearance of drug from the epidermis resulting in a much longer sustained drug release and reduction of drug absorption into the blood. This is why liposomes may act as drug transporters as well as drug targeters.[3] This may lead to increased effectiveness, reduction in side-effects and a higher compliance of patients to treatment.[3] Liposomes have also the potential to target drugs into the pilosebaceous structures and hence they can be employed for treatment of hair follicle- and sebaceous gland-associated disorders.[22–24] So, liposomes may serve as penetration enhancers facilitating the transport of compounds through the epidermis. The use of conventional penetration enhancers (e.g. dimethylsulphoxide or propylene glycol) leads on the one hand, to an improved transport rate through the epidermal barrier but, on the other hand, to more unwanted side-effects because of an increased systemic drug level[115] In addition, irritant- and allergic reactions to penetration enhancers have been reported leading to the conclusion that addition of penetration enhancers does not always mean an advantage in topical drug administration.[3,115] Side-effects from liposomes are not to be expected because liposomes are similar to epidermal lipids, biodegradable and non-toxic.[116]
2. Liposomes can be used as carriers for hydrophilic as well as lipophilic drugs because of their amphipathic character.[4,5] Compounds like methotrexate and

cyclosporin which cannot penetrate the epidermis may be usable for topical application when encapsulated in penetrating liposomes.[59,60,117] Liposomes may also improve stabilization of instable drugs by encapsulating them.[116–118]

3. 'Empty' liposomes (without encapsulated drugs) hydrate the skin simply by contributing lipids to the stratum corneum.[18,19,35,51] Additionally, the water content of liposomes promotes further hydration of the skin. In contrast with the 'wet-wrap' method, application of liposomal spray is a fast, easy procedure without any need of training.[51,52] Liposomes will help to reduce skin irritation to drugs (e.g. tretinoin) by hydration of the epidermis.[37,38,118,119]
4. Promising results have been obtained with liposomal encapsulated drugs in the treatment of acne,[36–40] xerosis,[51,52] atopic dermatitis,[53] psoriasis,[57,58] vitiligo,[66] superficial vein thrombosis[94,95] and hair removal.[96–99]
5. T4N5 containing liposomes protect the skin from UVinduced carcinogenesis by promoting the DNA repair and by inhibiting immunosuppression.[26,72–77] T4N5 liposomes protect against the development of basal cell carcinoma and actinic keratoses in patients with xeroderma pimentosa.[78,120]
6. PDT is widely used to treat actinic keratoses and superficial basal cell carcinoma. In addition, PDT is increasingly used to treat acne and for skin-rejuvenation. Side-effects limit patient's acceptance, especially in case of non-malignant skin disorders. Changing the 5-ALA vehicle from a moisturizing cream to liposome encapsulation, the 5-ALA concentration can be lowered by a factor of 40 and still induce the same skin fluorescence and at the same time eliminate the need for occlusion.[45]
7. Liposomes provide the opportunity for a more precise targeting of drugs into diseased cells and may contribute to optimize the quality of fluorescence diagnosis. This property of liposomes as a targeting method is not available in other topical applications.[45] It may be evident, that this review does not provide a complete detailed overview on the production and the usage of liposomal preparations in medicine. Based on what is already known, our view is that liposomal preparations have rightly earned a place in topical dermatological treatment and that future studies may show the benefits of these preparations for other indications.

It may be evident, that this review does not provide a complete detailed overview on the production and the usage of liposomal preparations in medicine. Based on what is already known, our view is that liposomal preparations have rightly earned a place in topical dermatological treatment and that future studies may show the benefits of these preparations for other indications.

References

1 Braun-Falco O, Plewig G, Wolff HH, Burgdorf WHC. Dermatology, 2nd edn. Springer-Verlag, New York, 2000; chapter 69: 1719–1721.

2 Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol 1965; 13: 238–252.

3 Schmid MH, Korting HC. Therapeutic progress with topical liposome drugs for skin disease. Adv Drug Del Rev 1996; 18: 335–342.

4 Allen TM. Long-circulating (sterically stabilized) liposomes for targeted drug delivery. Trends Pharmacol Sci 1994; 15: 215–220.

5 Hope MJ, Kitson CN. Liposomes: a perspective for dermatologists. Dermatol Clin 1993; 11: 143–154.

6 Egbaria K, Weiner N. Liposomes as a drug delivery system. Adv Drug Del Rev 1990; 5: 287–300.

7 Cullis PR, Hope MJ. Physical properties and functional roles of lipids in membranes. In: Vance DE, Vance JE, eds. Biochemistry of Lipids and Membranes. Benjamin, Cummings Publishing Co., Inc., Menlo Park, CA, 1985: 25–72.

8 Gabrijelcic V, Sentjurc M, Kristl J. Evaluation of liposomes as drug carriers into the skin by one-dimensional EPR imaging. Int J Pharm 1990; 62: 75–79.

9 El Maghraby GM, Barry BW, Williams AC. Liposomes and skin: from drug delivery to model membranes. Eur J Pharm Sci 2008; 34: 203–222.

10 Mozafari MR. Liposomes: an overview of manufacturing techniques. Cell Mol Biol Lett 2005; 10: 711–719.

11 Mozafari MR, Reed CJ, Rostron C. Development of non-toxic liposomal formulations for gene and drug delivery to the lung. Technol Health Care 2002; 10: 342–344.

12 McGrath JA, Uitto J. The filaggrin story. novel insights into skin barrier function and disease. Trends Mol Med 2008; 14: 20–27.

13 Elias PM. Epidermal lipids, membranes and keratinization. Int J Dermatol 1981; 20: 1–19.

14 Schürer NY, Plewig G, Elias PM. Stratum corneum lipid function. Dermatologica 1991; 183: 77–94.

15 Imokawa G, Akasaki S, Minematsu Y, Kawai M. Importance of intercellular lipids in water-retention properties of the stratum corneum: induction and recovery study of surfactant dry skin. Arch Dermatol Res 1989; 281: 45–51.

16 Fartach M. Epidermal barrier in disorders of the skin. Micr Res Techn 1997; 38: 361–371.

17 Potts RO, Francoeur ML. The influence of stratum corneum morphology on water permeability. J Invest Dermatol 1991; 96: 495–499.

18 Artmann C, Roding J, Ghyczy M, Prazel HG. Liposomes from soya phospholipids as percutaneous drug carriers. Arzneim Forsch 1990; 40: 1365–1368.
19 Junginger HE, Hofland HE, Bouwstra JA. Liposomes and niosomes: Interactions with human skin. Cosmetics Toiletries 1991; 106: 45.
20 Cevc G. Transfersomes, liposomes and other lipid suspensions on the skin, permeation enhancement, vesicle penetration, and transdermal drug delivery. Crit Rev Ther Drug Carrier Syst 1996; 13: 257–388.
21 Cevc G, Blume G. Lipid vesicles penetrate into intact skin owing to the transepidermal osmotic gradients and hydration force. Biochim Biophys Acta 1992; 1104: 226–232.
22 Lieb L, Ramachandran C, Egbaria K, Weiner N. Topical delivery enhancement with multilamellar liposomes into pilosebaceous units. In vitro evaluation using fluorescent techniques with hamster ear model. J Invest Dermat 1992; 99: 108–113.
23 Hoffman RM. Topical liposome targeting of dyes, melanins, genes, and proteins selectively to hair follicles. J Drug Target 1998; 5: 67–74.
24 Bernard E, Bubois JL, Wepeirre J. Importance of sebaceous glands in cutaneous penetration of an antiandrogen: target effect of liposomes. J Pharm Sci 1997; 86: 573–578.
25 Plessis J, Egbaria K, Weiner N. Influence of formulation factors on the deposition of liposomal components into the different strata of the skin. J Soc Cosmet Chem 1992; 43: 93–100.
26 Yarosh D, Bucana C, Cox P, Alas L, Kibitel J, Kripke M. Localization of liposomes containing a DNA repair enzyme in murine skin. J Invest Dermatol 1994; 103: 461–468.
27 Ostro M. Liposomes. Sci Am 1987; 256: 102–111.
28 Yarosh D, Klein J. The role of liposomal delivery in cutaneous DNA repair. Adv Drug Delivery Rev 1996; 18: 325–333.
29 Mezei M, Gulasekharam V. Liposomes – a selective drug delivery system for the topical route of administration: lotion dosage form. Life Sci 1980; 26: 1473–1477.
30 Mezei M, Gulasekharam V. Liposomes – a selective drug delivery system for the topical route of administration: gel dosage form. J Pharm Pharmacol 1982; 34: 473–474.
31 Gesztes A, Mezei M. Topical anaesthesia of the skin by liposomeencapsulated tetracaine. Anesth Analg 1988; 67: 1079–1081.
32 Wohlrab W, Lasch J. Penetration kinetics of liposomal hydrocortisone in human skin. Dermatologica 1987; 174: 18–22.
33 Wohlrab W, Lasch J. The effect of liposomal incorporation of topically applied

hydrocortisone on its serum concentration and urinary excretion. Dermatol Monatschr 1989; 175: 348–352.
34 Artmann C, Roding J, Ghyczy M. Influence of various liposome preparations on skin humidity. Parfum Kosm 1990; 90: 326.
35 Neugebauer D. Liposomen: Neues therapeutisches princip bei ekzemen und psoriasis. Apotheker J 1993; 6: 20–26.
36 Honzak L, Sentjurc M. Development of liposome encapsulated clindamycin for treatment of acne vulgaris. Eur J Physiol 2000; 440 (Suppl.): 44–45.
37 Schäfer-Korting M, Korting HC, Ponce-Pöschl E. Liposomal tretinoin for uncomplicated acne vulgaris. Clin Invest 1994; 72: 1086–1091.
38 Patel VB, Misra AN, Marfatia YS. Topical liposomal gel of tretinoin for the treatment of acne: research and clinical implications. Pharm Dev Technol 2000; 5: 455–464.
39 Patel VB, Misra AN, Marfatia YS. Preparation and comparative clinical evaluation of liposomal gel of benzoylperoxide for acne. Drug Dev Ind Pharm 2001; 27: 863–870.
40 Fluhr JW, Barsom O, Gehring W, Gloor M. Antibacterial efficacy of benzoylperoxide in phopholipid liposomes. A vehicle-controlled, comparative study in patients with papulopustular acne. Dermatology 1999; 198: 273–277.
41 Gold MH, Bradshaw VL, Boring MM, Bridges TM, Biron JA, Carter LN. The use of a novel intense pulsed light and heat source and ALA-PDT in the treatment of moderate to severe inflammatory acne vulgaris. J Drugs Dermatol 2004; 3: 15–19.
42 Santos MA, Belo VG, Santos G. Effectiveness of photodynamic therapy with topical 5-aminolevulinic acid and intense pulsed light versus intense pulsed light alone in the treatment of acne vulgaris: comparative study. Dermatol Surg 2005; 31: 910–915.
43 Hörfelt C, Stenquist B, Larkö O, Faergemann J, Wennberg AM. Photodynamic therapy for acne vulgaris: a pilot study of the dose–response and mechanism of action. *Acta Derm Venereol* 2007; 87: 325–329.
44 Wiegell SR, Wulf HC. Photodynamic therapy of acne vulgaris using 5-aminolevulinic acid versus methyl aminolevulinate. J Am Acad Dermatol 2006; 54: 647–651.
45 Christiansen K, Bjerring P, Troilius A. 5-ALA for photodynamic photorejuvenation-optimization of treatment regime based on normal-skin fluorescence measurements. Lasers Surg Med 2007; 39: 302–310.
46 Klein A, Babilas P, Karrer S, Landthaler M, Szeimies RM. Photodynamic therapy in dermatology – an update 2008. J Dtsch Dermatol *Ges* 2008; 6:

1–7.
47 Irvine AD, McLean WHI. Breaking the (un) sound barrier: Filaggrin is a major gene for atopic dermatitis. J Invest Dermatol 2006; 126: 1200–1202.
48 Palmer CNA, Irvine AD, Terron-Kwiatkowski A, Zhao Y. Common loss-of-function variants of the epidermal barrier protein filaggrin are major predisposing factor for atopic dermatitis. Nat Genet 2006; 38: 441–446.
49 Fartasch M, Bassukas ID, Diepgen TL. Disturbed extruding mechanism of lamellar bodies in dry non-eczematous skin of atopics. Br J Dermatol 1992; 127: 221–227.
50 Lee JH, Lee SJ, Kim DS, Bang D. The effect of wet-wrap dressing on epidermal barrier in patients with atopic dermatitis. J Eur Acad Dermatol Venereol 2007; 21: 1360–1368.
51 Lautenschlager H. Liposomes in dermatological preparations. Part 1 Cosmet Toilet*ries* 1990; 105: 89–96.
52 Sveinsson SJ, Olafsson JH, Valgardsson VS. The effect of liposomal moisturizing cream on dry skin: a preliminary double-blind study. J Dermatol Treatment 1993; 4: 187–189.
53 Korting HC, Zienicke H, Schafer-Korting M, Braun-Falco O. Liposome encapsulation improves efficacy of betamethasone dipropionate in atopic eczema but not in psoriasis vulgaris. Eur J Clin Pharmacol, 1990; 39: 349–351.
54 Lebwohl M. Psoriasis. Lance*t* 2003; 361: 1197–1204.
55 Krueger G, Ellis CN. Psoriasis-recent advances in understanding its pathogenesis and treatment. J Am Acad Dermatol 2005; 53: s94–s100.
56 Körbel JN, Sebök B, Kerényl M, Mahrle G. Enhancement of the antiparakeratotic potency of calitriol and tacalcitol in liposomal preparations in the mouse tail test. Skin Pharmacol Appl Skin Physiol 2001; 14: 291–295.
57 Agarwal R, Katare OP, Vyas SP. Preparation and in vitro evaluation of liposomal/niosomal delivery systems for antipsoriatic drug dithranol. Int J Pharmac 2001; 228: 43–52.
58 Saraswat A, Agarwal R, Katare OP, Kaur I, Kumar B. A randomized, double-blind, vehicle-controlled study of a novel liposomal dithranol formulation in psoriasis. J Dermatol Treat 2007; 18: 40–45.
59 Verma DD, Fahr A. Synergistic penetration enhancement effect of ethanol and phospholipids on the topical delivery of cyclosporin A. J Control Release 2004; 97: 55–66.
60 Trotta M, Peira E, Carlotti ME, Gallarate M. Deformable liposomes for dermal administration of methotrexate. Int J Pharmaceutics 2004; 270: 119–125.
61 Patel HM. Dermatologic ointment. UK Patent 1984 GB 2; 143: 433A.

62 Moghimi SM, Patel HM. Current progress and future prospects of liposomes in dermal drug delivery. J Microencaps 1993; 10: 155–162.
63 Cui J, Shen L, Wang G. Role of hair follicles in the repigmentation of vitiligo. J Invest Dermatol 1991; 97: 410–416.
64 Ortel B, Tanew A, Honingsman H. treatment of vitiligo with khellin and ultraviolet *A. J Am Acad Dermatol* 1988; 18: 693–701.
65 Orechia G, Sangalli ME, Gazzaniga A, Giordano F. Topical photochemotherapy of vitiligo with a new khellin formulation: preliminary results. J Dermatol Treatm 1998; 9: 65–69.
66 De Leeuw J, van der Beek N, Maierhofer G, Neugebauer WD. A case study to evaluate the treatment of vitiligo with khellin encapsulated in 1- phenylalanin stabilized phophatidylcholine liposomes in combination with ultraviolet light therapy. Eur J Dermat 2003; 13: 474–477.
67 Kraemer K. Sunlight and skin cancer. Proc Natl Acad Sci USA 1997; 94: 11–14.
68 Dahl E, Aberg M, Rausing A, Rausing EL. Basal cell carcinoma. Cancer 1992; 70: 104–108.
69 Glogau R. The risk of progression to invasive disease. J Am Acad Dermatol 2000; 42: s23–s24.
70 De Vries E, van de Poll-Franse LV, Louwman WJ, de Gruijl FR, Coebergh JWW. Predictions of skin cancer incidence in the Netherlands up to 2015. B J Dermatol 2005; 152: 481–488.
71 Ananthaswamy HN, Pierceall WE. Molecular mechanism of ultraviolet radiation carcinogenesis. Photochem Photobiol 1990; 52: 19–136.
72 Tanaka K, Sekiguchi M, Okada Y. Restoration of ultraviolet-induced unscheduled DNA synthesis of xeroderma pigmentation cells by the concomitant treatment with bacteriophage T4 endonuclease V and HVJ (Sendai virus) Proc Natl Acad Sci USA 1975; 72: 4071–4075.
73 Yarosh D. Liposome-encapsulated enzymes for DNA repair. In: Braun-Falco O, Korting H, Maibach H, eds. Liposome Dermatics. Springer-Verlag, Berlin, 1992: 258–268.
74 Yarosh D, Tsimis J, Yee V. Enhancement of DNA repair of UV damage in mouse and human skin by liposomes containing a DNA repair enzyme. J Soc Cosmet Chem 1990; 41: 85–92.
75 Wolf P, Maier H, Müllegger R. Topical treatment with liposomes containing T4 endonuclease V protects human skin in vivo from ultraviolet-induced upregulation of interleukin-10 and tumor necrosis factor-α. J Invest Dermatol 2000; 114: 149–156.
76 Bito T, Ueda M, Nagano T, Fujii S, Ichihashi M. Reduction of ultravioletinduced

skin cancer in mice by topical application of DNA excision repair enzymes. Photodermatol Photoimmunol Photomed 1995; 11: 9–13.
77 Cleaver J. Defective repair replication of DNA in xeroderma pigmentosum. Nature 1968; 218: 652–656.
78 Yarosh D, Klein J, O'Connor A, Hawk J, Rafal E, Wolf P. Effect of topically applied T4 endonuclease V in liposomes on skin cancer in xeroderma pigmentosum: a randomised study. *Lancet* 2001; 357: 926–929.
79 Lowe NJ, Lowe PL. A pilot study to determine the efficacy of ALA-PDT photorejuvenation for the treatment of facial aging. J Cos Laser Ther 2005; 7: 159–162.
80 Kim HS, Yoo JY, Cho KH, Kwon OS, Moon SE. Topical photodynamic therapy using intense pulsed light for treatment of actinic keratosis: Clinical and histopathologic evaluation. Dermatol Surg 2005; 31: 33–36.
81 Vinciullo C, Elliott T, Francis D et al. Photodynamic therapy with topical methylaminolaevulinate for 'difficult-to-treat' basal cell carcinoma. Br J Dermatol 2005; 152: 765–772.
82 Marmur ES, Schmults CD, Goldberg DJ. A review of laser and photodynamic therapy for the treatment of nonmelanoma skin cancer. Dermatol Surg 2004; 30: 264–271.
83 Britton JE, Goulden V, Stables G, Stringer M, Sheehan-Dare R. Investigation of the use of the pulsed dye laser in the treatment of Bowen's disease using 5-aminolaevulinic acid phototherapy. Br J Dermatol 2005; 153: 780–784.
84 De Rosa FS, Bentley MVLB. Photodynamic therapy of skin cancers. sensitizers, clinical studies and future. Pharm Res 2000; 17: 1447–1455.
85 Casas A, Batlle A. Aminolevulinic acid derivates and liposome delivery as strategies for improving 5-aminolevulinic acid-mediated photodynamic therapy. Curr Med Chem 2006; 13: 1157–1168.
86 Kloek J, Akkermans W, van Henegouwen GMJB. Derivates of 5-aminolevulinic acid for photodynamic therapy: enzymatic conversion into protoporphyrin. Photochem Photobiol 1998; 67: 150–154.
87 Pierre MBR, Tedesco AC, Marchetti JM, Bentley MVLB. Stratum corneum lipids liposomes for the topical delivery of 5-aminolevulinic acid in photodynamic therapy of skin cancer: preparation and in vitro permeation study. BMC Dermatol 2001; 1: 1–5.
88 Oppel T, Korting HC. Actinic keratosen: the key event in the evolution from photoaged skin to squamous cell carcinoma. Skin Pharmacol Physiol 2004; 17: 67–76.
89 Panjehpour M, Julius CE, Phan MN, Vo-Dihn T, Overholt S. Laserinduced fluorescence spectroscopy for in vivo diagnosis of non-melanoma skin cancers.

Lasers Surg Med 2002; 31: 367–373.
90 Aghassi D, Anderson RR, Gonzalez S. Confocal laser microscopic imaging of actinic keratosis in vivo: a preliminary report. J Am Acad Dermatol 2000; 43: 42–48.
91 Smits T, Kleinpenning MM, Blokx WAM, van der Kerkhof PCM, van Erp PEJ, Gerritsen MJP. Fluorescence diagnosis in keratinocytic intraepidermal neoplasias. J Am Acad Dermatol 2007; 57: 824–831.
92 Kleinpenning MM, Smits T, Ewalds E, van Erp PEJ, van de Kerkhof PCM, Gerritsen MJP. Heterogeneity of fluorescence in psoriasis after application of 5-aminolaevulinic acid: an immunohistochemical study. Br J Dermatol 2006; 155: 539–545.
93 Gorty S, Patton-Adkins J, Dalanno M. Superficial venous thrombosis of the lower extremities. Analysis of risk factors, and recurrence and role of anticoagulation. Vasc Med 2004; 9: 1–6.
94 Katzenschlager R, Hirschl M, Minar E, Ugurlouglu A. Liposomal heparinspray gel in comparison with subcutaneous low molecular weight heparin in patients with superficial venous thrombosis. A randomized, controlled, open multicentre study. J Kardiol 2003; 10: 375–378.
95 Katzenschlager R, Ugurlouglu A, Sipos G, Bihari I. Efficacy and tolerability of liposomal heparin spray gel as an add-on treatment in the management of superficial venous thrombosis. Angiology 2007; 58 (Suppl. 1): 27s–35s.
96 Sand M, Bechara FG, Sand D, Altmeyer P, Hoffmann K. A randomized, controlled, double-blind study evaluating melanin-encapsulated liposomes as a chromophore for laser hair removal of blond, white, and grey hair. Ann Plast Surg 2007; 58: 551–554.
97 Tierney E, Goldberg DJ. Laser hair removal pearls. J Cosm Laser Ther 2008; 10: 17–23.
98 De Leeuw J, van der Beek N, Neugebauer D. Permanent hair removal of white, grey and light blond hair after laser treatment combined with melanin encapsulated liposomes. URL: www.lipoxome.com (medical studies 2002).
99 Chattinakorn K. A prospective study analyzing the effects of pretreatment topical melanin in liposome compared with long pulsed Nd: Yag laser alone for hair removal. J Am Acad Dermatol 2007; 56 (Suppl. 2): AB203.
100 Taddio A, Kaur Soin H, Schuh S, Koren G, Scolni D. Liposomal lidocaine to improve procedural success rates and reduce procedural pain among children: a randomized controlled trial. CMAJ 2005; 172: 1691–1695.
101 Hallen B, Calsson P, Uppfeldt A. Clinical study of lignocaine-prilocaine cream to relieve pain of venepuncture. Br J Anaesth 1985; 57: 326–328.
102 Sims C. Thickly and thinly applied lignocaine-prilocaine cream prior to

venepuncture in children. Anaesth Intens Care 1991; 19: 343–345.
103 Eichenfield LF, Funk A, Fallon-Friedlander S, Cunningham BB. A clinical study to evaluate the efficacy of ELA-Max (4% liposomal lidocaine) as compared with eutectic mixture of local anesthetics cream for pain reduction of venepuncture in children. Pediatrics 2002; 109: 1093–1099.
104 Koh JL, Harrison D, Myers R, Dembinski R, Turner H, McGraw T. A randomized, double-blind comparison study of EMLA and ELA–Max for topical anesthesia in children undergoing intravenous insertion. Pediatr Anaesth 2004; 14: 977–982.
105 Fisher R, Hung O, Mezei M, Stewart R. Topical anaesthesia of intact skin: liposome-encapsulated tetracaine vs EMLA. Br J Anaesth 1998; 81: 972–973.
106 Gold MH. Photodynamic therapy with lasers and intense pulsed light. Facial Plast Surg Clin N Am 2007; 15: 145–160.
107 Biter PH. Noninvasive rejuvenation of photodamaged skin using serial, full-face intense pulsed light treatments. *Dermatol Surg* 2000; 26: 835–842.
108 Alster TS, Tanzi EL, Welsh CW. Photorejuvenation of facial skin with 20% 5-aminolevulinic acid and intense pulsed light. J Drug Dermatol 2005; 4: 35–38.
109 Gold MH, Bradshaw VL, Boring MM. Split-face comparison of photodynamic therapy with 5-aminolevulinic acid and intense pulsed light versus intense pulsed light alone for photodamage. Dermat Surg 2006; 32: 795–801.
110 Touma D, Yaar M, Whitehead S. A trial of short incubation, broad-area photodynamic therapy for facial actinic keratoses and diffuse photodamage. Arch Dermatol 2004; 140: 33–40.
111 Olsen EA, Katz HI, Levine N. Tretinoin emollient cream: a new therapy for photodamaged skin. J Am Acad Dermatol 1992; 26: 215–224.
112 Lippman SM, Meyskens FL. Results of the use of vitamin A and retinoids in cutaneous malignancies. Pharmac Ther 1989; 40: 107–122.
113 Sinico C, Manconi M, Peppi M, Lai F, Valenti D, Fadda AM. Liposomes as carriers for dermal delivery of tretinoin: in vitro evaluation of drug permeation and vesicle–skin interaction. J Controlled Rel 2005; 103: 123–136.
114 Banerjee R. Liposomes: Applications in Medicine. J Biomater Appl 2001; 16: 3–21.
115 Barry BW. Penetration enhancers. In: Shroot B, Schäffer H, eds. Skin Pharmacokinetics. Karger, Basel, 1987: 121–127.
116 Dreher F, Walde P, Luist PL, Elsner P. Human skin irritation studies of a lecithin microemulsion gel and of lecithin liposomes. Skin Pharmacol 1996; 9: 124–129.

117 Patel H. Liposomes as a controlled-release system. Biochem Soc Trans 1985; 13: 513–516.
118 Date AA, Naik B, Nagarsenker MS. Novel drug delivery systems: potential in improving topical delivery of antiacne agents. Skin Pharmacol Physiol 2006; 19: 2–16.
119 Brisaert M, Gabriëls M, Matthijs V, Plaizier–Vercammen J. Liposomes with tretinoin: a physical and chemical evaluation. J Pharm Biomed Anal 2001; 26: 909–917.
120 Yarosh D, Klein J, Kibitel J. Enzyme therapy of xeroderma pigmentosum: safety and efficacy of T4N5 liposome lotion containing a prokaryotic DNA repair enzyme. Photodermatol Photoimmunol Photomed 1996; 12: 122–130.

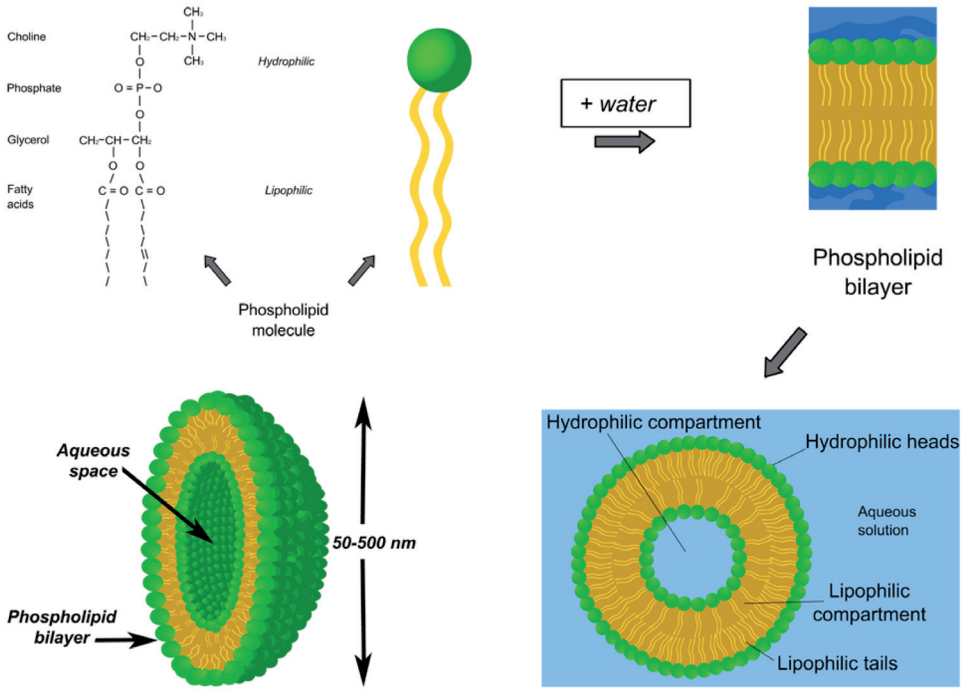

Figure 1. Top left structural formula of the phophatidylcholine molecule. In the presence of water phopholipid bilayers are formed, which create vesicles, enclosing an aqueous core. Lipid soluble substances can be stored in the outer lipid phase (yellow ring) and water soluble substances in the inner aqeous phase (blue centre).

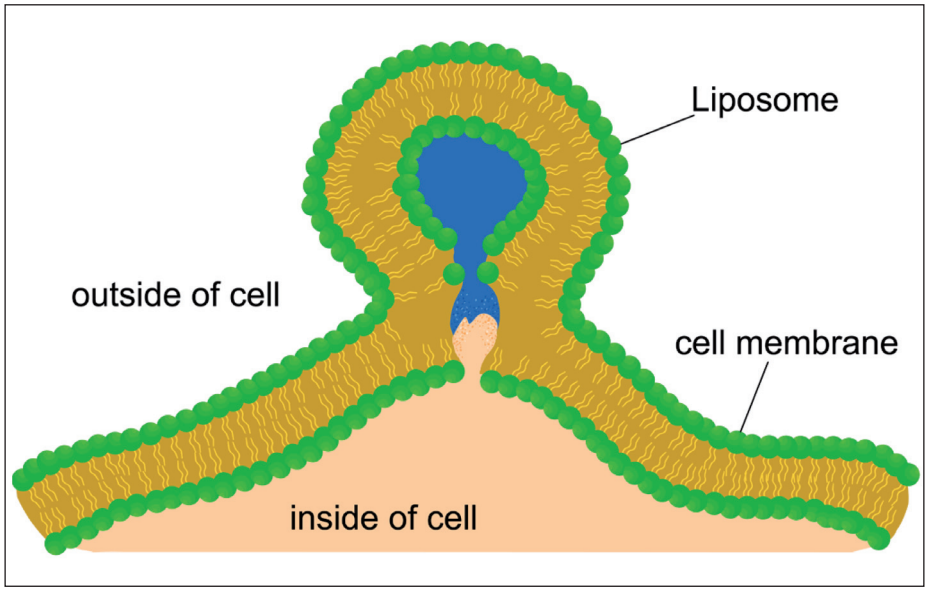

Figure 2. Liposomes fuse with the outer cell membrane and deliver their contents to the cytoplasm of cells via endocytosis.

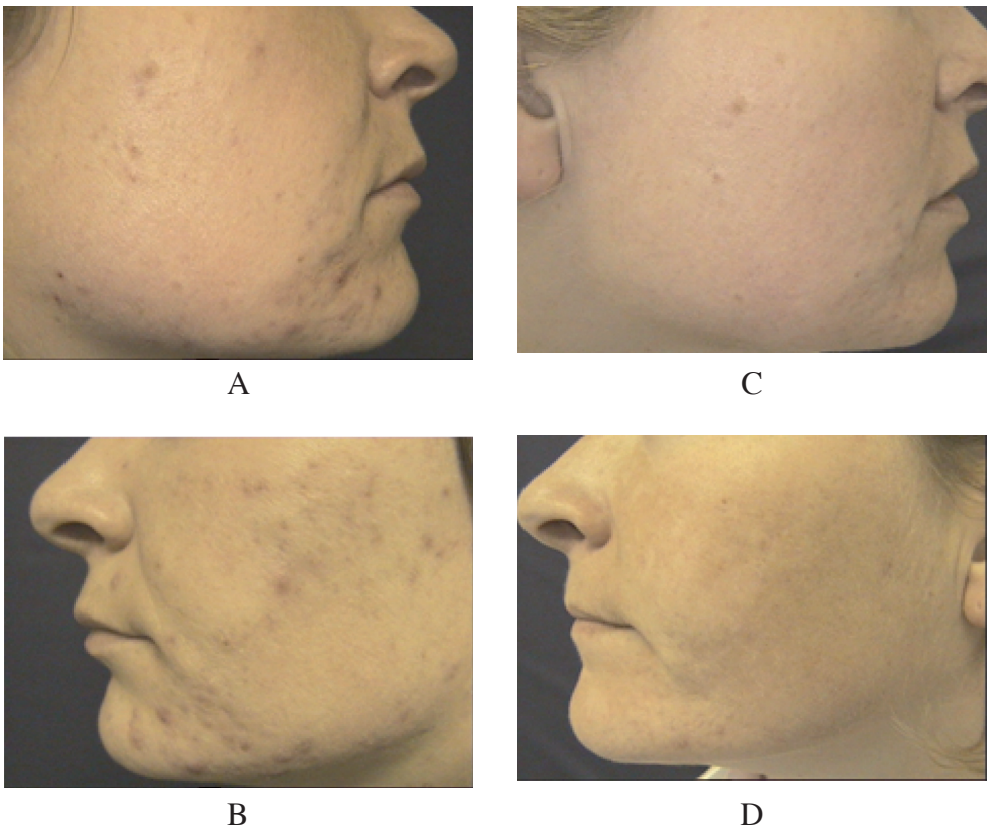

Figure 3. Female patient: a. and b. before treatment, c. and d. after 7 treatments with 0.5% ALA in a liposomal spray and subsequent illumination with an IPL (400 nm to 720 nm) every 4 to 6 weeks, concomitant treatment with tretinoin cream. Topical or systemic abtibiotics were not used.

Chapter 9

An earlier version of this chapter has been published as

A case study to evaluate the treatment of vitiligo with khellin encapsulated in L-phenylalanin stabilized phosphatidylcholine liposomes in combination with ultraviolet light therapy

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European Journal of Dermatology 2003; 13: 474-477.

Abstract

Vitiligo destroys the melanocytes in the epidermis; the inactive melanocytes in the outer root sheaths are not affected. Phosphatidylcholine liposomes are able to target molecules contained in them into the hair follicles. Khellin is activated by UVA and previous studies have shown that a combination of khellin and UVA (KUVA) can be effective in the treatment of vitiligo. The aim of this study was to determine in an open trial the efficacy and safety of treatment with khellin encapsulated in L-phenylalanine stabilized phosphatidylcholine liposomes in combination with UVA/UVB light therapy(KPLUV) in74 subjects with vitiligo. After a mean treatment period of 12 months (range 10-14 months) 72% of the treated locations had a repigmentation response of 50% to 100%. Repigmentation of 75-100% was achieved on the face in 63%, the back in 59%, the arms in 58%, the trunk in 57%, the legs in 56% and on the hands in 4% of the patients.Side effects were not seen with KPLUV. The patients in the control group, only treated with UVA/B - light, hardly showed any repigmentation (Figure 2). This indicates that the exposure of the skin to UV light alone is not responsible for the results of KPLUV.

Introduction

Vitiligo, an acquired depigmention disorder affecting about 1% of the population,[1, 2] may be a psychosocial disaster, particular in people with pigmented skin, especially in those countries where there is confusion of vitiligo with leprosy in the public mind.[3] Vitiligo is histologically characterized by the absence of melanocytes in the epidermis.

Inactive melanocytes in the outer root sheaths of the hair follicles are not affected. During the process of repigmentation, these inactive melanocytes proliferate and mature to an active condition as they migrate into the epidermis.[4]

Therapeutic approaches are aimed at reversing the progressive loss of melanocytes and reconstituting normal skin coloration, but none is uniformly effective and side effects may occur. Most common treatments are topical corticosteroids, systemic and topical PUVA, broad- and narrowband UVB, systemic and topical L-phenylalanine with UVA (PAUVA)[5-9], systemic and topical khellin with UVA (KUVA).[10-14] Surgical treatment is limited to small and stable vitiligo maculae.[1]

Khellin, a furanochromone with a chemical structure closely resembling that of the psoralen family, is activated by UVA (365 nm).[10]

Liposomes are small vesicles of membrane lipids. They can be used as microscopic carrier capsules for selective delivery of drugs into a specific disease site. Phosphatidylcholine-based liposomes have a high potential for selectively targeting molecules contained in them into the hair follicles.[15, 16, 17] Encapsulating khellin in phosphatidylcholine liposomes may enhance the penetration of khellin into the area of the inactive melanocytes in the hair follicle in order to provide optimal availability of khellin to stimulate the inactive melanocytes. L-phenylalanine is added to the formula in order to promote the stability of the solution. L-phenylalanine was also chosen because of its reputation to be effective, both orally and topically, together with UVA therapy in the treatment of vitiligo.[8, 9] Schallreuter has suggested that oxidative stress plays a role in the pathomechanism of vitiligo[18] and because L-phenylalanine has anti-oxidant properties this may be the way it works.[19] The Arimed B light source has a UV spectrum approximating that of sunlight (approximately UVB 5% and UVA 95%). It can be argued that such a simulated sunlight source represents a more cautious approach to home therapy than conventional broad spectrum UVB therapy.[20] The advantage of home therapy is that the high social and economic costs of hospital visits are reduced. The disadvantage of home therapy is that medical supervision is less frequent.

In this open, retrospective study the efficacy of topical treatment of vitiligo with khellin encapsulated in L-phenylalanine stabilized phosphatidylcholine liposomes in combination with ultraviolet light therapy (KPLUV) as home phototherapy is determined.

Subjects and methods

65 patients (50 females and 15 males) with vitiligo vulgaris and 9 patients (5 females and 4 males) with vitiligo universalis were included in the study. The average age was 43 years (range 12-75 years). Mean duration of vitiligo was 15 years (range 4-60 years). All patients had been treated before with topically applied

corticosteroids, 48 patients also with PUVA and 18 patients with broadband-UVB, without notable repigmentation. Exclusion criteria were phenylketonuria, pregnancy and breastfeeding, impaired hepatic and renal functions, malignant skin diseases, history of exposure to arsenates or ionizing radiation and photo-induced diseases. The patients were instructed to apply twice a day, in the morning and half an hour prior to the UVA therapy, a spray containing khellin in a concentration of 0.005% encapsulated in phosphatidylcholine liposomes, stabilized with L-phenylalanine 0.1%. The light source (Arimed B Cosmedico, Stuttgart, Germany) has a spectrum from 295 to 400 nm and peak emissions at 317 and 356 nm. The phototreatment schedule started with one minute daily 5 times a week raising the exposure time by one minute every week until the erythematic dose was reached. The treatment was continued with the suberythema dose. The exposure time was never more than l5 minutes. The light source was given to the patients for treatment at home as a panel and not as a booth in order to save space, so that patients with vitiligo on the frontal and dorsal parts of the body underwent two exposures. As soon as a marked improvement was achieved, the treatment schedule was reduced from 5 times a week to 3 times a week.

This study is a case-study and not a placebo-controlled, randomized study. The control group consisting of 30 patients used the same UVA/B light source (Arimed B Cosmedico), with the same photo treatment schedule, but not in combination with the application of the khellin and phenylalanine containing liposomal solution. The liposomal vehicle of the spray has an absorption spectrum from 190- to 210-nm. This is out of the range of the emission spectrum of the used UVA/B light source, excluding the need to use the vehicle in the control group. Photographs (Fotofinder, TeachScreen Software GmbH, Germany) of the subjects were taken at the beginning of the therapy and than once every two months for one year. The extent of repigmentation was recorded by visual comparison of the successive photographs, making use of planimetry.

The before-and-after evaluation was performed by 2 experienced clinicians, who did not know whether the patients were treated with UVA/B light therapy in combination with topical liposomal solutions (study group) or with UVA/B as monotherapy (control group).

Results

After a mean treatment period of 12 months (range 10 to 14 months) 72% of treated locations had a repigmentation response of 50% to 100%. The repigmentation was not equal for different parts of the body. Repigmentation of more than 50% was achieved on the face in 79%, the trunk in 73%, the arms in 76%, the legs in 70%, and on the hands in 65%. Repigmentation of 75%-100% was achieved in the face

in 63%, the arms in 58%, the trunk in 73%, the legs in 56% and the hands in 4% (Figure1). Surprisingly eight out of the nine patients (88%) with vitiligo universalis showed a repigmentation of more than 75%. The patients in the control group treated with only Uvlight (Arimed B), without the use of the khellin spray, hardly showed any repigmentation (Figure 2).

Mean cummulative annual Joules doses of UVA 679 J/cm^2 and of UVB 25.9 J/cm^2 (table 1).

Discussion

The treatment of vitiligo can be frustrating to patients and doctors. A standard modality that cures every patient does not yet exist.[1, 2] Topical corticosteroids, topical- and systemic PUVA, broad- and narrow-band UVB light therapy are the most common methods, although they are not always effective and side effects may limit the therapeutic options.[5-9] Narrow-band UVB is effective in the treatment of vitiligo. An overall repigmentation of 75% in 53% of the patients and a stabilization of the disease in 80% has been seen.[21] But not all investigators came to the same conclusion.[22] Surgical treatment is limited to small and stable vitiligo maculae.[1] The results of systemic khellin in combination with UVA[11-14] are comparable to the rates reported from psoralen phototherapy.[1] The major advantage of khellin is that it does not induce phototoxic skin erythema and it does not induce detectable DNA mutations in contrast to PUVA.[11,12] Ortel et al. studied the effect of oral khellin in combination with UVA light in 26 patients. A repigmentation of more than 70% was achieved in 41 % of the patients who had received 100 to 200 treatments. A mild elevation of liver transaminases was observed in 28% of the patients on oral treatment. Of three patients who were treated with topical khellin and UVA, two patients had a repigmentation of only 30%.[12] In our study with topically administered khellin in liposomes, a repigmentation between 50% and 100% was seen in 72% of the patients. Hofer et al. performed a study to assess the effectiveness and short-term and long-term safety of oral khellin plus UVA light therapy (KUVA) in patients with vitiligo. Of 17 patients, 41 % had a response of more than 70% repigmentation after a mean of 194 treatments. Short term side effects were episodes of nausea in 29% of the patients, elevated liver enzymes in 7% and gastritis in 7% of the patients. Long-term side effects have not been seen, no skin cancer or actinic damage of vitiliginous skin was found in any patient. Their data indicate that KUVA seems to be safe as well as effective for vitiligo, provided treatment is administered long enough.[13] Orecchia et al. concluded from their study that topically applied khellin is effective in the treatment of vitiligo, but that the results are vehicle dependant.[14] A potential concern is the hepatotoxic side effect of khellin. The low active ingredient concentration of 0.005% khellin in the topically applied spray would tend to exclude

this risk. Vitiligo destroys only the active melanocytes in the epidermis; inactive melanocytes in the outer root sheath of the hair are not affected. During the process of repigmentation, these inactive melanocytes proliferate and migrate from the outer root sheath to the epidermis, meanwhile maturing from the inactive phase to an active state.[4]

The ideas behind the treatment of vitiligo with khellin in L-phenylalanine stabilized liposomes as described in this article are based on:

- Enhancing the penetration of khellin by carrier liposomes into the hair follicles in order to provide optimal availability of khellin in the area of the inactive, nonaffected melanocytes.
- Stimulation of the melanogenesis by using topical khellin and UVA light.
- Avoidance of side effects by using only topical- and not systemic khellin treatment.
- L-phenylalanine was added to the formula because of its stabilizing effect on the solution, and its anti-oxidant properties.
- The safe light source and the fact that topically applied khellin has a very low phototoxic effect makes this approach suitable for home therapy.
- Home therapy is cost reducing and comfortable for the patients.

This study demonstrates that KPLUV is effective (Figures 3A+B and 4A+B) and safe in the treatment of vitiligo.

An open study has many shortcomings and further investigation is needed to determine the optimal combination of the ingredients in the spray and the optimal application frequencies.

References

1. Kovacs SO. Vitiligo. J Am Acad Dermatol 1998; 38: 647-666.
2. Westerhof W, Njoo MD, Schallreuter KU. Vitiligo. Hautarzt 1997; 48: 677-693.
3. Fitzpatrick TB, Eisen AZ, Wolff K. Dermatology in general medicine. New York: Mc Graw-Hill, 1993:923-924.
4. Cui J, Shen L, Wang G. Role of hair follicles in the repigmentation of vitiligo. J Invest Dermatol 1991; 97: 410-416.
5. Antoniou G, Katsambas A. Guidelines for the treatment of vitiligo. Drugs 1992; 43: 49049-8.
6. Antoniou C, Schulpis H, Michas T. Vitiligo therapy with oral and topical Phenylalanine with UVA exposure. Int J Derm 1989; 28: 545-547.
7. Anstey A, Hawk JLM. PUVA treatment for vitiligo. Br J Dermatol 1994: 131 (suppl 44): 18.

8. Camacho F, Mazuecos J. Treatment of vitiligo with oral and topical phenylalanine: 6 years of experience. Arch Dermatol 1999; 135: 216-217.
9. Cormane RH, Siddiqui AH, Westerhof W. Phenylalanine and UVA light for the treatment of vitiligo. Arch Dermatol Res 1985; 277:126-130.
10. Vedaldi D, Caffieri S, Dalla'acqua F. Khellin, a naturally occuring (urochrome, used for the photochemotherapy of skin diseases: Mechanism of action. Il Farmaco 1987; 43: 333-346.
11. Morliere P, Honigsmann H, Averbeck D. Phototherapeutic, Photobiologic and Photosensitizing properties of khellin. J Invest Derm 1988; 90: 720-724.
12. Ortel B, Tanew A, Honigsmann H. Treatment of vitiligo with khellin and ultraviolet A. J Am Acad Dermatol 1988; 18: 693-701.
13. Hofer A, Karl H, Wolf P. Long-term results in the treatment of vitiligo with oral khellin plus UVA. Eur J Dermatol 2001,1 1: 225-229.
14. Orecchia G, Sangalli ME, Gazzaniga A., Giordano F. Topical photochemotherapy of vitiligo with a new khellin formulation: preliminary results. J Der Treatm 1998; 9: 65-69.
15. Hoffman RM. Topical liposome targeting of dyes, melanins, genes and proteins selectively to hair follicles. J of Drug Targeting 1997; 3: 67-74.
16. Hope MI, Kitson C. Liposomes. A perspective for Dermatolo gists. Derm Ther Jan 1993; 2: 17-21.
17. Mezei M. Biodisposition of liposome encapsulated active ingredients applied on the skin. Liposome- Dermatitis. Braun Falco, Korting Maibach Springer Verlag 1992; 206-214.
18. Schallreuter KU. Pseudocatalase and the depigmentation disorder vitiligo. Retinoids 1998; 14: 57-59
19. Maskos Z, Rush JD, Koppenol WH. The hydroxylation of phenylalanine and tyrosine: A comparison with salicylate and tryptophan. Arch Biochem Biophys 1992; 296: 521-529.
20. Sarkany RPE, Anstey A, Diffey BL. Home phototherapy: report on a workshop of the British Photodermatology Group, December 1996. Br J Derm 1999; 140: 195-199.
21. Njoo MD, Bos JD, Westerhof W. Treatment of generalized vitiligo in children with narrow-band (TL-01) UVB radiation therapy. J Am Acad Dermatol 2000; 42: 245-253.
22. Meas M, van Weelden H, van Vloten WA. Vitiligo: epidemiologie en behandeling, met name met UVB (311 nm). Ned Tijdschr Dermatol Venerel 1999; 9: 30-33.

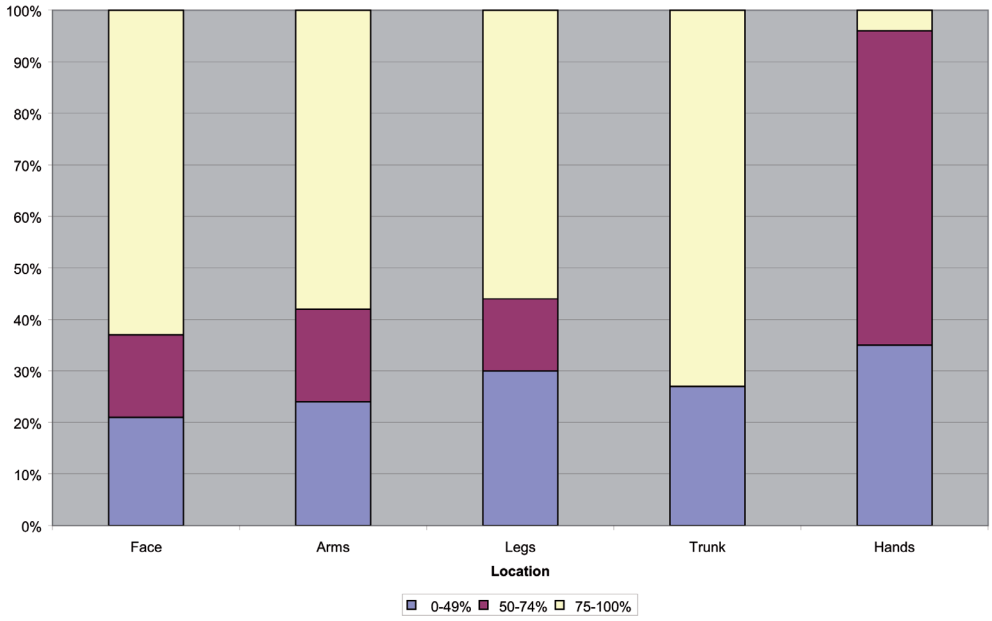


Figure 1. KPLUV group: % of repigmentation of vitiligo spot sizes for different parts of the body.

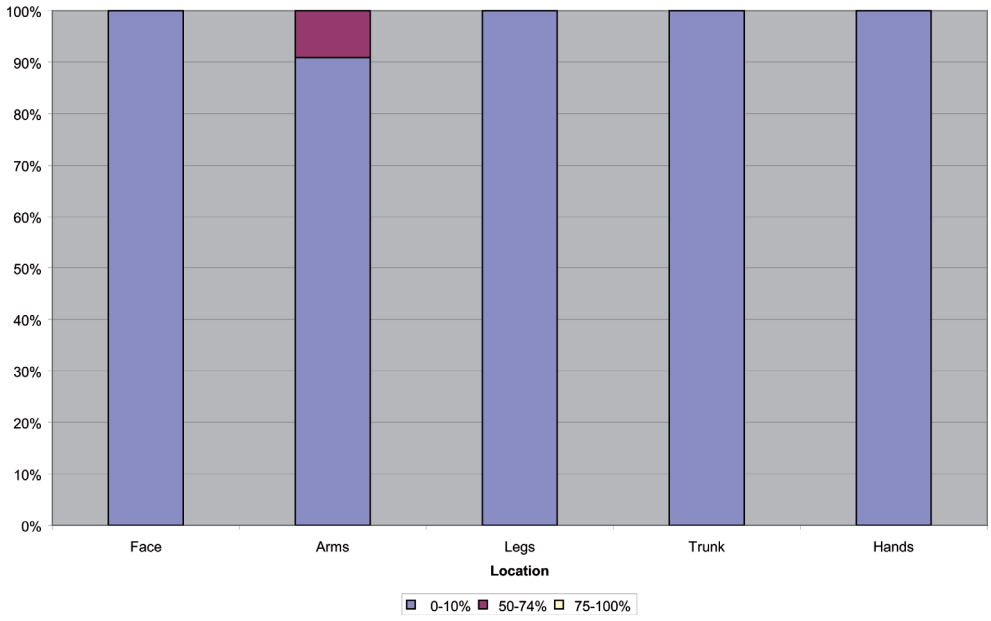


Figure 2. Control group: % of repigmentation of vitiligo spot sizes for different parts of the body: almost no repigmentation

	UVA J/cm^2	UVB J/cm^2
Mean	679	25.9
Maximum	1680	61,7
Minimum	180	7,2

Table 1. Cummulative annual Joules doses of UVA and UVB.

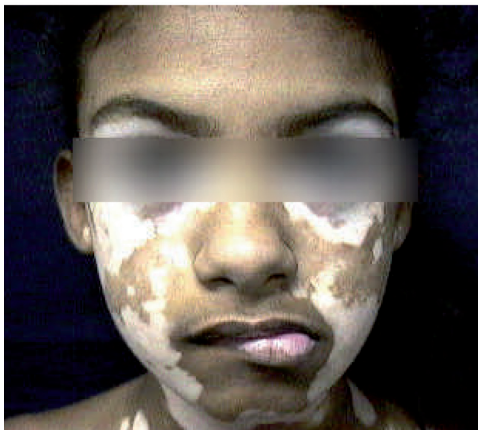


Figure 3A. 14 year-old female patient before treatment

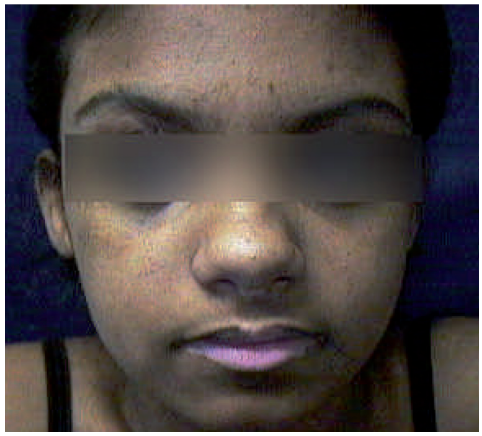


Figure 3B. 14 year-old female patient after treatment

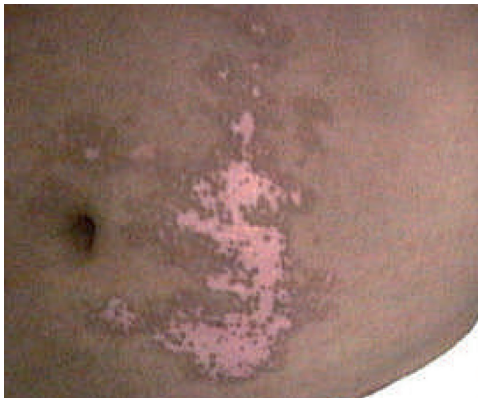


Figure 4A. 41 year-old male patient before treatment.

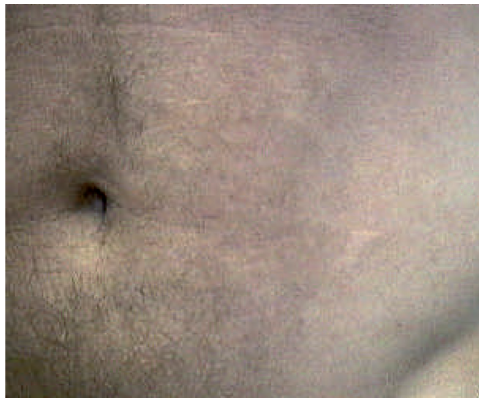


Figure 4B. 41 year-old male patient after treatment.

Chapter 10

Epidermal blister grafts for vitiligo

Y. J. Assen, J. de Leeuw, P. Bjerring, H.A.M. Neumann.

Submitted for publication

Abstract
Vitiligo is commonly encountered in dermatology. A variety of surgical and non-surgical treatment modalities have been described. Surgical techniques such as the epidermal blister graft transplantation may be effective in re-pigmenting the lesions in stable or refractory vitiligo. Our objective was to re-pigment vitiligo lesions using an easy technique without scarring. It can replace techniques that do cause scarring.

Introduction
Vitiligo is a common disease characterized by areas of de-pigmentation. It is widely accepted that the functional melanocytes disappear.[1] Although vitiligo is sometimes regarded as a cosmetic problem, the impact on the quality of life is considerable.[2-4] A variety of surgical and non-surgical treatment modalities have been described to treat vitiligo. Surgical interventions are based on transplanting functional melanocytes to the de-pigmented areas.[5] Surgical techniques are generally reserved for stable and refractory vitiligo. 0ne of the promising surgical techniques, besides minigrafts using punch biopsies, split-thickness grafts and transplantation of non-cultured cell suspension or cultured melanocytes, is blister roof transplantation in which epidermal blister grafts are transplanted onto the prepared de-pigmented areas.[5] We used this technique in patients with a clinical diagnose of vitiligo with a stable disease. This technique was observed to be effective, safe and easy to use and caused no scarring.

Technique
The blister roofs are the donor source of melanocytes in epidermal blister graft transplantation. The transplantation is performed in three phases. In the first phase

the blisters are created with a suction device, connected to a rectangular plastic block. This block divides the negative pressure over 28 holes each with a diameter of 5-millimetre (mm) and is attached to a skin area with normal pigmentation, preferably the patient's upper arm (Figures 1 and 2). It takes about one to one and a half hours to obtain manageable blisters using a vacuum of 300-mm of mercury. The second phase consists of creating superficial erosions with diameters of 4-mm to 5-mm in the recipient area by ablation with an Erbium-YAG laser with a fluence of 4.8 J/cm^2 (Figure 3). In the third phase, the blister roofs are removed with a pair of scissors and smooth forceps and transferred to the erosions on the recipient area (Figures 4 and 5). Smooth forceps are used because the blister roofs tend to stick in the ridges, interfering with the removal from the serrated forceps.

The transplants in the recipient area are protected with silicone sheeting, which is covered with standard gauze and fixed with an adhesive bandage (fixomull stretch) to prevent it from shifting. It is important that the graft remains stationary during the first few days. The bandage is carefully removed after a week and replaced by a new bandage for another week. Additional therapy such as phototherapy can be started two weeks after the transplantation.

The donor site re-pigments spontaneously. This is confirmed by the histological examination of a biopsy of the suction blister. The melanocytes are equally distributed between the roof and the bottom of the blister (Figure 6).

Discussion

We discuss our views on the choices we made within the options of this technique. We comment on the position of this technique in the range of surgical techniques available for vitiligo.

Choice of parameters

Various techniques have been used for blister roof transplantation. Previous studies reported that transplantation of epidermal blister grafts in vitiligo was effective.[1,5,6] Liquid nitrogen and suction devices were used for preparing the donor site.[7] Application of liquid nitrogen is not only painful, but may easily damage the melanocytes. The medial side of the upper arm was chosen as the donor site because it is a relatively inconspicuous area in case of a temporary discoloration and it is easily accessible. It enables patients to move freely during the creation of the blisters with the block on their arm and the suction device on a cord over their shoulder.

A larger area as compared with the sum of the individual graft surface can be re-pigmented as a result of the post-operative peripheral spreading of the pigment. A blister roof with a diameter of 5-mm corresponds to a surface area of 0.2 cm^2. Generally, the peripheral pigment spread around a transplanted graft is 1-mm to

2-mm resulting in an expansion rate of the surface area by a factor 2 to 3 In the case where grafts are used with a diameter of 10-mm corresponding to a surface area of 0.79 cm^2, the peripheral spreading of pigment of 1-mm to 2-mm increases the expansion rate of the surface area by a factor 1.4 to 1.9.[8] Therefore, 5-mm grafts provide optimum tissue and also have the advantage that they are easier to handle than the 10-mm grafts. The erosions caused by the laser ablation in the de-pigmented areas are about 2-mm apart from each other because of the expanding of pigment into the periphery. The Erbium laser ablation is stopped at the moment pin-point hemorrhages appear indicating that the ablation has reached the papillary dermis. Laser ablation may be painful and often requires topical anesthesia with lidocaine 1%-2%. Adrenalin is not used to prevent the masking of the pin-point hemorrhages. A variety of techniques, besides the Erbium-YAG laser are used for preparing the recipient site. Generally used techniques are liquid nitrogen, sandpaper, suction devices, diamond fraise, topical methoxsalen followed by PUVA and CO_2 lasers.[6,9] The disadvantages of these techniques are the difficulty to control the final result and the chances of scarring. Today, lasers are a good method to dermabrade the skin in a controlled fashion. In our opinion the Erbium laser is superior to the CO_2 laser because it causes less thermal damage in the recipient area than the CO_2 laser.

Comparison with other surgical techniques

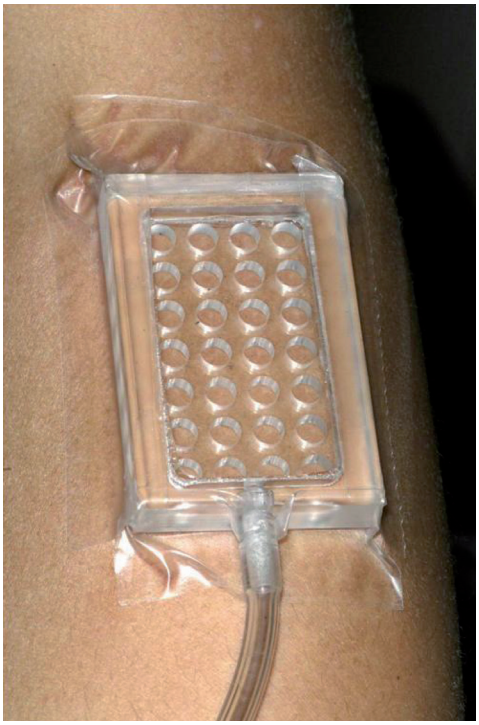
Besides blister roof transplantation, other known surgical techniques are the Thiersch split-thickness skin grafts, the cell suspension technique and the punch grafts. Thiersch grafts are known to cause scarring at both the donor- and the recipient site.[10] Moreover, a cosmetic treatment should have no or only a limited chance of aesthetic complications. In the above mentioned epidermal blister transplantation technique scarring has not yet been encountered. A temporary hyper- or hypopigmentation may occur at the donor site.

The cell suspension technique seemed to result in a good re-pigmentation rate, but the donor skin was harvested with a dermatome, so that there was a chance of scarring at the donor site. It also requires a laboratory that can process the cell suspension. It is important to prepare cell suspension in medium containing a minimum of foreign proteins and adequate precautionary measures must be taken to reduce the risk (even theoretical) of transmitting slow viruses, prions, and other pathogens to the recipient site.[11] Punch grafting is a simple technique and that is probably the reason for its wide use. It is know that punch grafts are effective in re-pigmentation, but they often cause a cobblestone effect.[12] According to the literature both epidermal blister grafts and punch grafts have the same re-pigmentation rate.[9]. However, punch grafts result in more scar formation and thus justify treating vitiligo with the blister roof transplantation. It is our experience that epidermal blister grafts

leave no scarring either at the donor- or the recipient sites as compared with other surgical techniques. However, more studies are necessary to exactly establish its rightful place in the treatment of vitiligo.

References

1. Taieb A, Picardo M. Vitiligo. N Engl J Med 2009; 360: 160-9
2. Kent G, al-Abadie M. Factors affecting responses on Dermatology Life Quality Index items among vitiligo sufferers. Clin Exp Dermatol1996; 21: 330-333.
3. Sampogna F, Picardi A, Chren MM et al. Association between poorer quality of life and psychiatric morbidity in patients with different dermatological conditions. Psychosom Med, 2004; 66: 620-624.
4. Ongenae K, Van Geel N, De Schepper S, Naeyaert JM. Effect of vitiligo on self-reported health-related quality of life. Br J Dermatol 2005; 152: 1165-1172.
5. Parsad D, Gupta S. Standard guidelines of care for vitiligo surgery. Indian J Dermatol Venereol Leprol 2008; 74: s37-45.
6. Oh CK, Cha JH, Lim JY et al.Treatment of vitiligo with suction epidermal grafting by the use of an ultrapulse CO2 laser with a computerized pattern generator. Dermatol Surg 2001; 27: 565-568.
7. Hann SK, Im S, Bong HW, Park YK. Treatment of stable vitiligo with autologous epidermal grafting and PUVA. J Am Acad Dermatol 1995: 32: 943-8.
8. Awad SS. Chinese Cupping: A Simple Method to Obtain Epithelial Grafts for the Management of Resistant Localized Vitiligo. Dermatol Surg 2008; 34: 1186-1193
9. Pai GS, Vinod V, Joshi A. Efficacy of erbium YAG laser-assisted autologous epidermal grafting in vitiligo. J Eur Acad Dermatol Venereol 2002; 16: 604-606.
10. Khandpur S, Sharma VK, Manchanda Y. Comparison of minipunch grafting versus split-skin grafting in chronic stable vitiligo. Dermatol Surg 2005; 31: 436-441.
11. M. J. Olsson. What Are the Needs for Transplantation Treatment in Vitiligo, and How Good Is It? Arch Dermatol 2004; 140, 1273-1274.
12. Gupta S, Jain VK, Saraswat PK. Suction blister epidermal grafting versus punch skin grafting in recalcitrant and stable vitiligo. Dermatol Surg 1999; 25: 955-958.



Figures 1 Suction device block

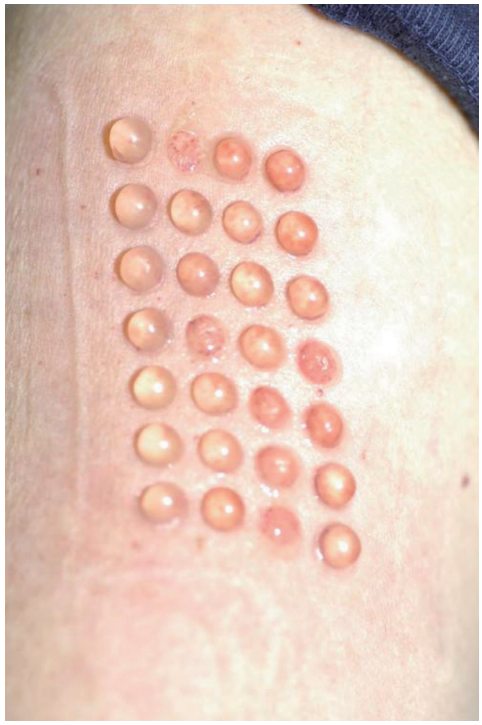


Figure 2 Evoking blisters by suction.

Figure 3 Erosions made by ablation with an Erbium-YAG laser

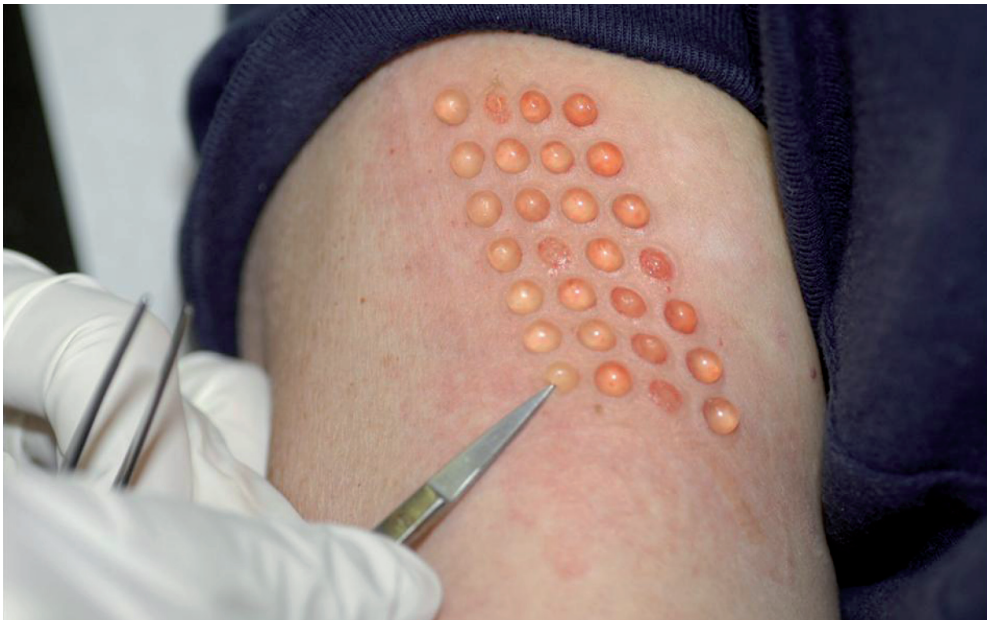


Figure 4. Removal of the blister roofs with forceps and scissors.

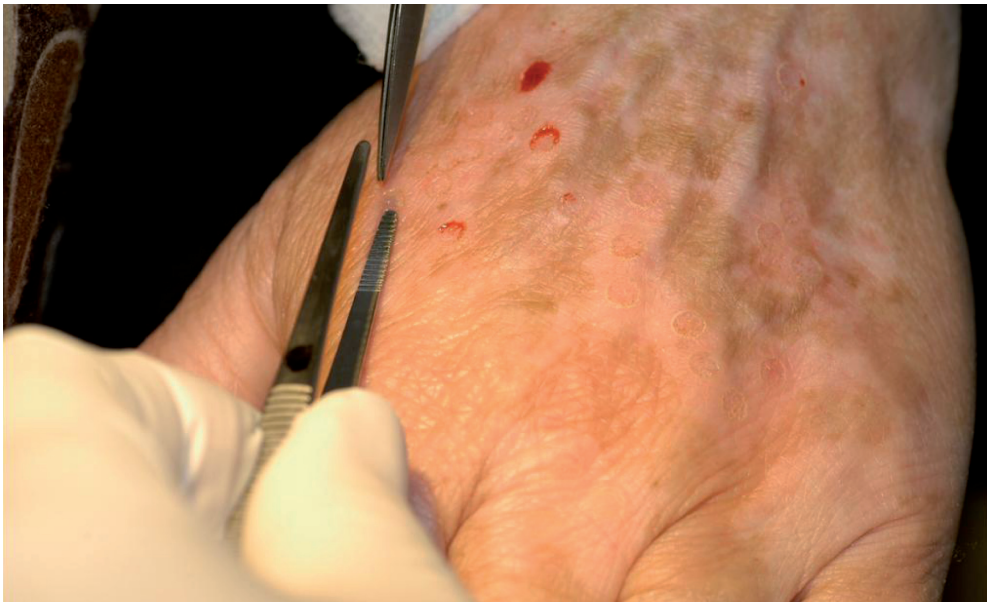


Figure 5. Transplantation of the blister roofs onto the erosions on the recipient area.

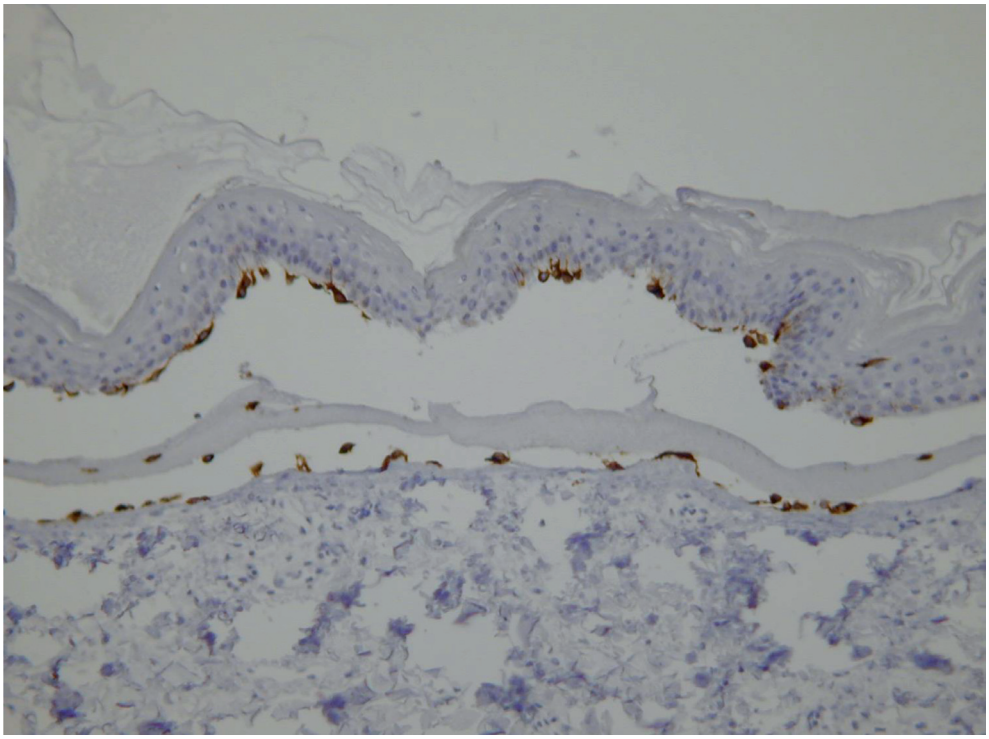


Figure 6. Distribution of the melanocytes in the roof and the bottom of the blister (Melan-a stain) (original magnification x 40).

Chapter 11

Treatment of vitiligo with khellin liposomes, ultraviolet light and blister roof transplantation.

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Submitted for publication

Abstract

Background: Various surgical and non-surgical methods are available to treat vitiligo. Surgical techniques such as epidermal blister graft transplantation may be effective for the re-pigmentation of stable, but refractory vitiligo areas. Khellin has phototherapeutic properties that are similar to those of the psoralens, but with substantially lower phototoxic effects and DNA mutation effects. Its penetration into the hair follicles is enhanced by encapsulating it into liposomes. This facilitates the stimulation of melanocytes in the hair follicles by ultraviolet light-activated khellin.

Objective: The first objective was to evaluate the surplus value of combining blister roof transplantation (BRT) with treatment using khellin in liposomes and ultraviolet light (KLUV) in the treatment of recalcitrant vitiligo patches. The second objective was to assess patients' satisfaction.

Methods and materials: Nineteen patients with vitiligo lesions non-responding to at least 1 year of KLUV treatment were included for BRT followed by KLUV. Locations where randomly assigned. The transplantation was performed by creating the blisters with a suction device, preparation of the recipient with Erbium laser ablation and the actual transplantation. In each subject non-transplanted vitiligo patches were used as controls. A blinded observer established the results.

Results: Seventy-five percent of the patients were satisfied with the cosmetic result. All of the patients would recommend the treatment to other vitiligo patients. Fifty-five percent of the patients showed more than 75% re-pigmentation of vitiligo areas as was concluded from the single blinded evaluation of the photographs taken before and after the treatment.

Keywords
Vitiligo, khellin, liposomes, ultraviolet light, blister roof transplantation.

Introduction
Vitiligo is an acquired disease characterized by sharply demarcated macules of de-pigmentation. It is a relatively common disease with a prevalence of about 1-2%. The course of vitiligo is usually one of slow progression, but it may exacerbate rapidly or stabilize. Spontaneous re-pigmentation may also occur sometimes. It is widely accepted that melanocytes disappear from the involved skin areas, but the exact pathogenesis has not yet been established.[1] During the de-pigmenting process in vitiligo, the ultimate consequence of all the pathogenic mechanisms is the destruction of melanocytes and thus the absence of pigmentation. Initially, only the epidermal melanocytes are affected, but as the condition progresses, the most important pigment cell reservoir, the hair follicle, may also become involved and leucotrichia develops. The hair follicle is unable to provide new melanocytes for pigmentation from that moment.[2] However, the melanocytes in the hair follicles are generally not affected. During the process of re-pigmentation, these melanocytes proliferate and mature to an active state as they migrate into the epidermis.[3] Vitiligo is commonly considered to be only a cosmetic problem, but the implications of vitiligo are beyond the limits of a cosmetic disease. Its impact on the quality of life of the patient is comparable with psoriasis on the emotional and socially functioning scales.[4-7] Therapeutic approaches in vitiligo are aimed at reversing the progressive loss of melanocytes and reconstituting the normal skin coloration. However, none of the approaches is uniformly effective and side effects may occur. Most common methods for treatment are topical corticosteroids, tacrolimus, systemic and topical psoralen ultraviolet-A light (PUVA), broad- and narrow-band ultraviolet-B (BB-UVB, NB-UVB) and topical khellin with UVA (KUVA).[1,8.9,10.11] Khellin, a furanochromone with a chemical structure resembling that of the psoralen family is activated by UVA and UVB.[12] Khellin has been used to treat vitiligo,[12,13] because it has phototherapeutic properties that are similar to those of the psoralens, but it has substantially lower phototoxic and mutagenic effects.[14,15] The current status on the position of khellin in the treatment of vitiligo was recently reported and it was concluded that the research into this area remains inconclusive and at times contradictory.[11] In a study comparing khellin or placebo under the influence of natural sunlight, 12 of the 30 patients with vitiligo in the khellin group showed more than 50% re-pigmentation compared with none in the placebo group.[16] A left versus right study in 72 patients comparing khellin with UVA versus UVA alone concluded that re-pigmentation was because of the UVA and not the khellin.[17] There was no difference between the khellin and the placebo groups in a left versus right study comparing khellin plus sunlight with vehicle plus

sunlight in 41 vitiligo patients.[18] However, in a later study the same authors compared a khellin gel and UVA with UVA alone and reported that both groups responded, but khellin plus UVA was superior to UVA alone ($P < 0.01$).[19] A study in 33 patients compared topical khellin plus UVA with PUVA and reported that khellin plus UVA may induce re-pigmentation that was comparable with that induced by systemic PUVA, but required a longer period.[20]

The selection of the vehicle in which khellin is incorporated is highly important for the adequate availability of khellin in the skin because of its very low solubility in water.[19] Liposomes are small vesicles of membrane lipids.[21,22] They are used as microscopic carrier capsules for the selective delivery of drugs to a specific disease site such as the hair follicles.[23,24] Encapsulating khellin in phosphatidylcholine liposomes enhances the availability of khellin in the hair follicles facilitating the stimulation of the melanocytes. The MultiCare SP 04 light source (Figure 1a) has emission peaks at 311 nm and 365 nm and is used as a handheld device, which makes it suitable for treatment at home. The advantage of home therapy is that the high social and economic costs of hospital visits are reduced. The disadvantage of home therapy is that medical supervision is less frequent.[25] In a retrospective, single blind, comparative study 74 vitiligo patients were treated with a spray containing khellin encapsulated in liposomes in combination with a UVA and UVB emitting light source (KLUV group). The control group consisted of 30 patients and was treated with the same light source and the same photo treatment schedule, but not in combination with khellin liposomal spray (UV group). After one year of treatment, re-pigmentation of 75%-100% was achieved in 61% of the KLUV patients (excluding the results of the hands). The patients in the control (UV) group hardly showed any re-pigmentation. No side effects were observed.[26]

Surgical treatment is limited to vitiligo maculae, refractory to the aforementioned treatments. An important condition for surgical therapy is the stability of the disease.[2] In stable vitiligo (such as segmental unilateral vitiligo) the outcome of transplantation therapy is usually excellent. In some cases, transplantation may indeed be the only effective treatment.[27] Unfortunately, to date there is no reliable test to predict the activity and the outcome of melanocyte transplantation treatment in patients with vitiligo. Vitiligo can be active in one skin area and inactive or even in regression in another area at the same time.[27] Thus, it may be difficult to draw conclusions from patient's anamnesis and from performing a few test grafts prior to more extensive treatments as an approach to predict stability. While there is no consensus on definite parameters for stability, various recommendations indicate a period of disease inactivity ranging from 6 months to two years.[28]

Methods of surgical modalities for vitiligo include both tissue grafts and cellular grafts. Both these techniques have their own advantages and disadvantages according

to the recently published guidelines (Table 1).[28] Tissue grafts comprises of punch grafting, suction blister epidermal grafting and split-thickness grafting. Cellular grafts require specialized trained personnel and appropriate laboratory facilities and are expensive.[28,29] In vitiligo surgery the most frequently reported causes of failure are the lack of take, the survival of the grafts and the absence of re-pigmentation in spite of the good take. De-pigmentation of grafts after initial pigmentation may also occur (often as a result of vitiligo instability). Another problem may be the quality of the preparation of the melanocytes before transplantation. In vitro cultured autologous skin cells have been chemically and mechanically prepared and/or cultured. For cell preparation it is important to use a medium containing a minimum of foreign proteins and if they have to be used, they should be from the safest possible source. These precautionary measures are taken in order to reduce the risk of transmitting slow viruses, prions and similar agents to the recipient site.[27] Liquid nitrogen, sandpaper, suction devices, topical methoxsalen followed by PUVA, CO_2 laser and Erbium-YAG lasers have been used for preparing the recipient site.[30,31] The disadvantage of preparing the recipient site with liquid nitrogen and topical methoxsalen followed by PUVA is that it is difficult to control the final result. Today, lasers are a good means to ablate the skin in a controlled fashion. It was reported that the Erbium-YAG laser-assisted ablation resulted in more pigment spread around the graft than areas prepared with topical methoxsalen followed by UVA.[31]

The aim of this study was to evaluate the efficacy and the patient satisfaction of epidermal blister graft transplantation in combination with khellin encapsulated in liposomes and ultraviolet light (KLUV) treatment before and after transplantation in patients with vitiligo.

Subjects and methods

Inclusion

Nineteen patients (9 women and 6 men), mean age 44 years (25 – 68 years) who were treated with KLUV for at least one year and who showed refractory and stable vitiligo lesions on the hands, the arms (in one patient also the shoulders), the neck, and the face were included for blister roof transplantation after providing informed consent (Table 2). Exclusion criteria were pregnancy, lactation, history of allergy to sunlight, photo-induced diseases, medication with drugs known to have potency for light toxicity or light sensitization, phenylketonuria, impaired hepatic and renal functions, malignant skin diseases, history of exposure to arsenates and ionizing radiation. In each patient two lesions where selected to be suited for blister roof transplantation. Of these lesions one was randomly chosen to be treated with blister roof transplantation combined with KLUV therapy and the other lesions being treated with a KLUV mono-therapy.

KLUV treatment

The patients were instructed to apply a spray containing khellin at a concentration of 0.005% encapsulated in phosphatidylcholine liposomes, stabilized with L-phenylalanine 0.1%, twice a day in the morning and in the evening. UV phototherapy was carried out with the MultiCare SP 04 light source (MultiCare, Hoge Naarderweg 7^{h}, Hilversum, The Netherlands). This device has peak emissions at 311 nm and 365 nm and is used as a handheld device (Figure 1a), which is in contact with the skin resulting in a constant lamp-skin distance. The illumination schedule started with one minute 3 times a week raising the exposure time by 50% every 2 treatments until redness appears on the treated skin area, which lasted for more than 24 hours (erythematous dose). From then onwards, the light treatment was continued 3 times a week with the sub-erythematous dose (the dose of light just below the erythematous dose). The spray was applied in the morning and half an hour prior to the UV treatment for the 3 days a week of light therapy. The exposure time per session was never more than 15 minutes. The treatment schedule was gradually reduced from 3 times a week to 1 time a week or stopped as soon as a marked improvement was achieved. Patients were advised to avoid sun exposure on the days of the UV treatment.

Blister roof transplantation

The blister roofs were the donor source of melanocytes in epidermal blister graft transplantation. The transplantation was performed in three phases. These were creating the blisters, preparation of the recipient site and the actual transplantation. In the first phase the blisters were created with a suction device connected to a rectangular plastic block (Figure 1b). This block divides the negative pressure over 28 holes each with a diameter of 5-millimetres (mm), and is attached to a skin area with normal pigmentation, preferentially the patient's upper arm (Figure 1b). It takes about one to one and a half hours to obtain manageable blisters using a vacuum of 300-mm of mercury (Figure 1c). This procedure is painless and topical anesthesia is unnecessary. The second phase consisted of making superficial erosions with diameters of 5- to 6-mm in the recipient area by ablation with an Erbium-YAG laser (Dornier Medilas E, Meditech, Munich, Germany), with a 4-mm diameter spot, energy 400- to 600 mJ/cm2 and frequency of 3- to 5 Herz (Figure 1d). The laser ablation was stopped at the moment pin-point haemorrhages appear indicating, that the ablation had reached the papillary dermis. This procedure required topical anesthesia with lidocaine 1%-2% (without adrenalin). In the third phase the blister roofs were removed with a pair of scissors and forceps and transferred to the erosions in the recipient area. Smooth forceps were used in order to avoid that the blister roofs sticked to the serrated forceps, which makes it difficult to remove the blister from the forceps.

Finally, the transplants in the recipient area were protected with sillicone sheeting (Mepitel Mölnlycke Health Care AB, Göteborg, Sweden), which was covered with standard gauze and fixated with an adhesive bandage (Fixomull stretch BSN medical GmbH, Hamburg, Germany) to prevent it from shifting. The blister roof grafts were too thin to be stitched to the laser ablation erosions. The bandage technique has to be optimal to keep the transplants in place and movements of the recipient body site have to be restricted because it is important that the grafts remain stationary during the first post-operative week. Concave body areas were filled up with synthetic cotton (Artiflex BSN medical Ltd, Hull, England). The duration of the whole procedure for the transplantation of 28 blister roofs is one and a half hour for the patient and half an hour for the doctor. The bandage was carefully removed after a week using plenty of water to detach the silicone sheets from the grafts because wet silicone sheets detach more readily from the underlying grafts (Figure 2). Then a new bandage with paraffin gauze and standard gauze was placed to protect the grafts for another week after which the bandages are no longer used. The KLUV treatment was re-started with a light dose that is 4 steps back in the UV treatment schedule followed by an increase of the light dose with one step a week until the optimal dose was reached once again (Figure 2).

Results

Three patients dropped out because they did not fill-in the questionnaire on the patient's satisfaction concerning the efficacy and the tolerance of the treatment. The remaining 16 patients (84%) filled-in this questionnaire. The results of the combined therapy can be broken up as follows: A repigmentation of 70-100% was achieved in 2 patients (after 1 and after 6 transplantation sessions). A re-pigmentation of 50-75% was achieved in 6 patients (after 1 to 10 transplantation sessions). Thus, an improvement of 50% or more was achieved in 8 (50%) of the patients. If necessary 10 (63%) of the patients would undergo re-treatment. There was no graft take in one patient, caused by improper immobilization of the recipient body site. Seventy-five percent of the patients were satisfied with the cosmetic result and reported that the treatment had more advantages than disadvantages. All of the participating patients would recommend the treatment to other vitiligo patients (Table 2).

The blinded evaluation of the photographs taken before and after treatment by a dermatologist showed an average repigmentation of 67.2 % (st. deviation = 22.4) of the lesions treated with the blister roof transplantation and KLUV. According to Student's paired T-test the improvement was statistically significant ($p<0.01$). The change in pigmentation of the control lesions treated with KLUV as mono-therapy showed no significant change. Additionally the difference in the change in pigmentation of the combined blister roof transplantation and KLUV therapy and that

of the KLUV mono-therapy was statistically significant ($p<0.01$). (Figures 2, 3 and 4). The donor sites showed spontaneous re-pigmentation (Figure 5). Scars are rarely seen after the epidermal blister transplantation procedure. A temporary hypo- and hyper-pigmentation at the donor site is common.

Discussion

Recent guidelines on the treatment of vitiligo recommended the use of potent or very potent topical steroids for a trial period of no more than 2 months.[11]$_1$ Although benefits were observed, skin atrophy was a common side effect. In such cases topical pimecrolimus is an alternative to the use of a topical steroid.[11] Narrow-band UVB phototherapy should be considered for the treatment of vitiligo only in patients who cannot be adequately managed with more conservative treatments, who have widespread vitiligo or have localized vitiligo associated with a significant impact on the patient's quality of life (QoL).[11] Patients should be monitored with serial photographs.[11] Unfortunately, in many patients refractory areas may remain, particularly on the distal body sites such as the dorsal parts of the hands and the feet, but all body sites may be involved. Photo-medical treatment such as PUVA and KLUV may be efficacious where aforementioned treatment methods have failed.[14,17,18,24] It seems rational to prefer KLUV to PUVA, because khellin has phototherapeutic properties that are similar to those of the psoralens, but it has substantially lower phototoxic an mutagenic effects than the psoralens.[14,15] A potential concern is the hepatotoxic side effect of khellin. A mild elevation of liver transaminases was observed in 7% to 28% of the patients on oral treatment.[13,32] The low active ingredient concentration of 0.005% khellin in the topically applied spray would tend to exclude this risk. KLUV results in disease stability in almost all cases,[24] which is an important requirement for the success of surgical therapy.[2]

A variety of techniques have been tried for blister roof transplantation. Liquid nitrogen and suction devices were used for preparing the donor site.[33] Liquid nitrogen is a painful procedure that may damage the melanocytes. Blister roofs are the donor of melanocytes in epidermal blister graft transplantation. This is because the melanocytes in suction blisters are more or less equally distributed between the blister roof and the bottom of the blister as is seen in the histology of a biopsy of a suction blister (Figure 6).[34] Due to this phenomenon approximately the same number of melanocytes is transported to the recipient area as is left behind in the donor area. This results in spontaneous re-pigmentation of the donor site, albeit after a period of erythema, followed by transient hypo-pigmentation and hyper-pigmentation. The choice for the medial side of the upper arm as the donor site is because this area is a relatively inconspicuous site in case of a temporary discoloration and also because this area is easily accessible. Patients can move freely during the creation

of the blisters with the block on the arm and the suction device with a cord over the shoulder. In our experience, the medial side of the upper arm yields superior blisters in a shorter period than the lateral side of the arm because of the difference in the skin thickness.

After transplantation, melanocytes induce pigmentation in their immediate vicinity, but are unable to proliferate continuously until the whole achromic defect becomes re-pigmented. Therefore, depending on the size of the de-pigmented area, a number of grafts have to be transplanted followed by phototherapy in combination with psoralen or khellin to stimulate the peripheral spreading of the pigment until the whole defect becomes repigmented(Figure 2).[2,35] A larger area can be treated as compared with the total sum of the individual graft surface areas because of this peripheral spreading of the pigment. A blister roof with a diameter of 5-mm corresponds with a surface area of 0.2 cm^2. Generally, the peripheral pigment spread around a transplanted graft is 1-mm to 2-mm resulting in an expansion rate of the surface area with a factor 2 to 3. In case that grafts with a diameter of 10-mm are used, corresponding with a surface area of 0.79 cm^2, the peripheral spreading of pigment of 1-mm to 2-mm increases the expansion rate of the surface area with a factor 1.4 to 1.9. Thus, 5-mm grafts provide optimum tissue and also have the advantage that they are easier to handle than the 10-mm grafts. The blister roof grafts are placed into the erosions (made by laser ablation in the de-pigmented areas) that are about 2-mm apart from each because the pigment expands into the periphery. The Erbium laser ablation is stopped at the moment pin-point haemorrhages appear indicating that the ablation has reached the papillary dermis. Laser ablation is painful and requires topical anesthesia with lidocaine 1%-2%. Adrenalin is not used to prevent masking of the pin-point hemorrhages. Blister roofs are too thin to be stitched on to the laser ablation erosions. Careful bandaging and moderate immobilization of the recipient body site are important for keeping the grafts in place during the first week. The bandage is carefully removed after a week and replaced by a new bandage for another week. The recipient areas initially show white skin areas with brown dots (Figure 9). Post-surgical photo-medical treatment is necessary to obtain confluence of pigmentation (Figure 9) and to maintain disease stability.

In the research setting the vitiligo area-scoring index (VASI) and the Vitiligo European Task force (VETF) assessment tools offer a more accurate measurement of the extent of the disease than only the simple clinical photography.[11] In therapeutic trials relating to vitiligo, researchers should make the patient's improvement in his/her Quality of life (Qol) as the most important outcome measure.[11] Serial photographs should still be used to record the progress in clinical practice among other things because patients may forget their initial condition.[11] This may also explain the discrepancy between the patient's view on the treatment result and the evaluations of the photographs

by the researchers. Therefore, the judgment whether the therapy is efficacious is somewhere between the physician's observation and the patient's view.[36]

Improvements in the blister roof transplantation technique may be achieved by:

- Selecting the patients prior to BRT according to the Vido classification (Table 3).[37]
- Application of a corticosteroid cream or a calcineurin cream to the recipient area after the grafts have been taken in order to avoid activation of the vitiligo process caused by the ablation procedure (Köbner phenomenon) or spontaneous aggravation of vitiligo.[37]
- Using a medical intervention for the underlying causes of melanocyte destruction.[27] Efalizumab may be such remedy.[38]
- Suction blister roof ablation of the recipient area instead of the ablation with the Erbium laser in order to obtain the same thickness of removed skin and transplanted skin, but this technique will be limited to smooth skin areas only.

The policy of treatment of vitiligo in this study (Figure 7) was to start with KLUV for stabilizing vitiligo, to reduce phototoxic reactions caused by sunlight and to achieve re-pigmentation of the white patches. Additional surgery was considered after 1 year of KLUV treatment for refractory vitiligo patches, especially situated in the 'emotionally charged' body sites such as the face, the head, the neck and the hands. Post-surgery photo-medical treatment (KLUV) is necessary to obtain enough peripheral spread of the pigment to cover the entire area of de-pigmentation (Figures 2, 3 and 4) .

This within-patient controlled blinded study demonstrated, that transplantation of suction blister roof grafts on erosions made by Erbium laser ablation in combination with before and after KLUV is a safe and reliable method to treat refractory and stable vitiligo areas. The combined therapy of blister roof transplantation and KLUV therapy had better results than KLUV alone. This procedure is, with lower costs, easier to perform than epidermal cell suspensions. The most important limitations of this method are the (moderate) immobilization of the treated body site and the fixation of the grafts to the erosions in the skin using bandages.

References

1. Kovacs S. Continuing medical education: vitiligo.. J Am Acad Dermatol 1998; 38: 647-666.
2. Falabella R. Surgical treatment of vitiligo: why, when and how. J Eur Acad Dermatol Venereol 2003; 17: 518–520.
3. Cui J, Shen L, Wang G. Role of hair follicles in the repigmentation of vitiligo. J Invest Dermatol 1991; 97: 410-416.
4. Kent G, al-Abadie M. Factors affecting responses on Dermatology Life Quality Index items among vitiligo sufferers. Clin Exp Dermatol 1996; 21: 330-333.
5 Parsad D, Pandhi R, Dogra S. Dermatology Life Quality Index score in vitiligo and its impact on the treatment outcome. Br J Dermatol 2003; 148: 373-374.
6. Sampogna F, Picardi A, Chren MM. Association between poorer quality of life and psychiatric morbidity in patients with different dermatological conditions. Psychosom Med 2004; 66: 620-624.
7. Ongenae K, van Geel N, De Schepper S, Naeyaert JM. Effect of vitiligo on self-reported health-related quality of life. Br J Dermatol 2005; 152: 1165-1172.
8. Westerhof W, Nieuweboer-Krobotova L. Treatment of vitiligo with UV-B (311 nm) radiation versus topical psoralen plus UV-A. Arch Dermatol 1997; 133: 1525-1528.
9. Njoo MD, Bos JD, Westerhof W. Treatment of generalized vitiligo in children with narrow-band (TL-01) UVB radiation therapy J Am Acad Dermatol 2000; 42: 245-253.
10. Antoniou G, Katsambas A. Guidelines for the treatment of vitiligo. Drugs 1992; 43: 490-498.
11. Gawkrodger DJ, Ormerod AD, Shaw L, Mauri-Sole I, Whitton ME, Watts MJ, Anstey AV, Ingham J, Young K. Guideline for the diagnosis and management of vitiligo. Brit J Dermatol 2008; 159: 1051-1076.
12. Morliere P, Hönigsmann H, Averbeck D, Dardalhon M, Hüppe G, Ortel B, Santus R, Dubertret L. Phototherapeutic, Photobiologic and Photosensitizing properties of khellin. J Invest Derm 1988; 90: 720-724.
13. Ortel B, Tanew A, Honigsmann H. Treatment of vitiligo with khellin and ultraviolet A. J Am Acad Dermatol 1988; 18: 693-701.
14. Abdel-Fattah A, Aboul-Enein MN, Wassel GM, El-Menshawi BS. An approach to treatment of vitiligo by khellin. Dermatologica 1982; 165: 136–40.
15. Procaccini EM, Riccio G, Montfrecola G. Ineffectiveness of topical khellin in photochemotherapy of vitiligo. J Dermatol Treatment 1995; 6: 117–120.
16. Orecchia G, Perfetti L. Photochemotherapy with topical khellin and sunlight in

vitiligo. Dermatology 1992; 184: 120-123.
17. Orecchia G, Sangalli ME, Gazzaniga A., Giordano F. Topical photochemotherapy of vitiligo with a new khellin formulation: preliminary results. J Dermatol Treatment 1998; 9: 65-69.
18. Valkova S, Trashlieva M, Christova P. Treatment of vitiligo with local khellin and UVA: comparison with systemic PUVA. Clin Exp Dermatol 2004; 29: 180-184.
19. Hope MJ, Kitson C.N. Liposomes: A Perspective for Dermatologists. Dermatol Clin 1993; 11: 143-154.
20. Egbaria K, Weiner N. Liposomes as a drug delivery system. Adv Drug Del Syst.1990; 5: 287-300.
21. Lieb L, Ramchandran C, Egbaria K, Weiner N. Topical delivery enhancement with multilamellar liposomes into pilosebaceous units. J Invest Dermat 1992; 99: 108-113.
22. Hoffman RM. Topical liposome targeting of dyes, melanins, genes, and proteins selectively to hair follicles. J Drug Target 1995: 67-74.
23. Sarkany RPE, Anstey A, Diffey BL, Jobling R, Langmack K, McGregor JM. Home phototherapy: report on a workshop of the British Photodermatology Group, December 1996. Br J Dermatol 1999; 140: 195-199.
24. De Leeuw J, van der Beek N, Maierhofer G, Neugebauer WD. A case study to evaluate the treatment of vitiligo with khellin encapsulated in L-phenylalanine stabilized phosphatidylcholine liposomes in combination with ultraviolet light therapy. Eur J Dermat 2003; 13: 474-477.
25. Olsson MJ. What are the needs for transplantation treatment in vitiligo, and how good is it? Arch Dermatol 2004; 140: 1273-1274.
26. Parsad D, Gupta S. Standard guidelines of care for vitiligo surgery. Indian J Dermatol Venereol Leprol 2008; 74: s37-45.
27. Falabella R. Surgical therapies for vitiligo. Clin Dermatol 1997; 15: 927-939.
28. Oh CK, Cha JH, Lim JY, Jo JH, Kim SJ. Treatment of vitiligo with suction epidermal grafting by the use of an ultrapulse CO2 laser with a computerized pattern generator. Dermatol Surg, 2001; 27(6): 565-568.
29. Pai GS, Vinod V, Joshi A. Efficacy of erbium YAG laser-assisted autologous epidermal grafting in vitiligo. J Eur Acad Dermatol Venereol, 2002. 16: 604-606.
30. Hann SK, et al., Treatment of stable vitiligo with autologous epidermal grafting and PUVA. J Am Acad Dermatol, 1995. 32: 943-948.
31. Assen YJ, de Leeuw J, Bjerring P, Neumann HAM. Epidermal blister grafts for vitiligo. Submitted to Clin Exp Dermat 2009.

32. Awad SS, Abdel-Raof H, Hosam El-Din W, El-Domyati M. Epithelial grafting for vitiligo requires ultraviolet A phototherapy to increase success rate. J Cosmet Dermatol 2007; 6:119-124.
33. Ongenae K, Beelaert L, van Geel N, Naeyaert J-M. Psychosocial effects of vitiligo. J Eur Acad Dermatol Venereol, 2006; 20: 1-8.
34. Njoo MD, Das PK, Bos JD, Westerhof W. Association of the Kobner phenomenon with disease activity and therapeutic responsiveness in vitiligo vulgaris. Arch Dermatol 1999; 135: 407-413.
35. Wakkee M, Thio HB. Neumann HAM. Efalizumab: effectief bij vitiligo? Ned T Dermatol Venereol 2008; 18: 131-132.

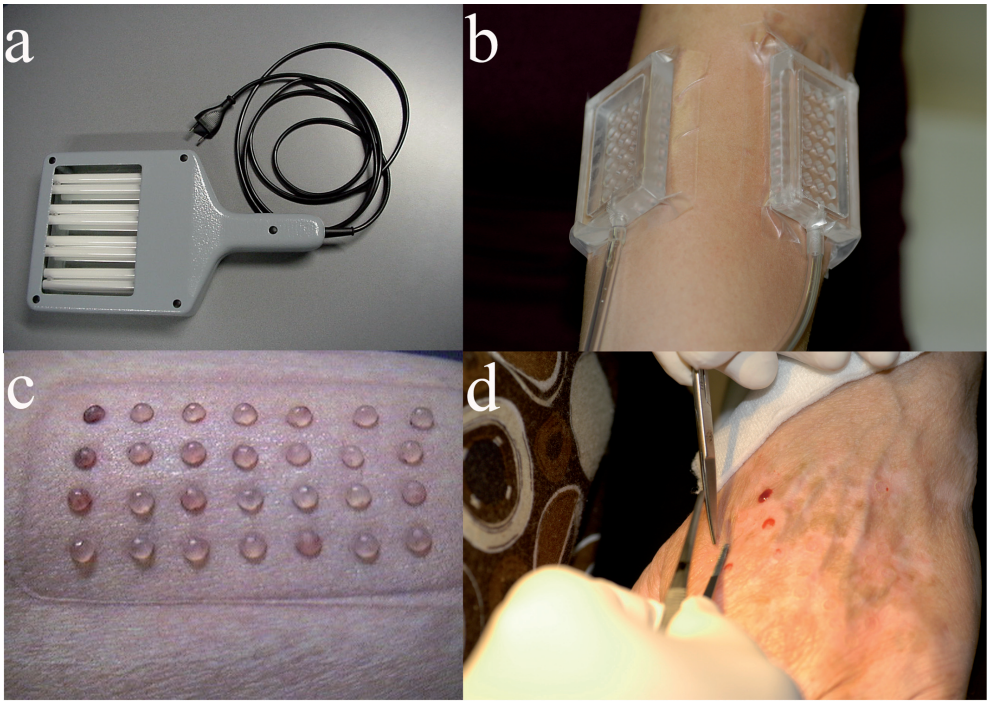

Figure 1.

a. The MultiCare SP 04 light source.
b. Suction device attached to the skin.
c. After about 1 hour the blisters are ready for harvesting.
d. Transplantation of the blister roofs into the laser ablated erosions in a vitiligo area.

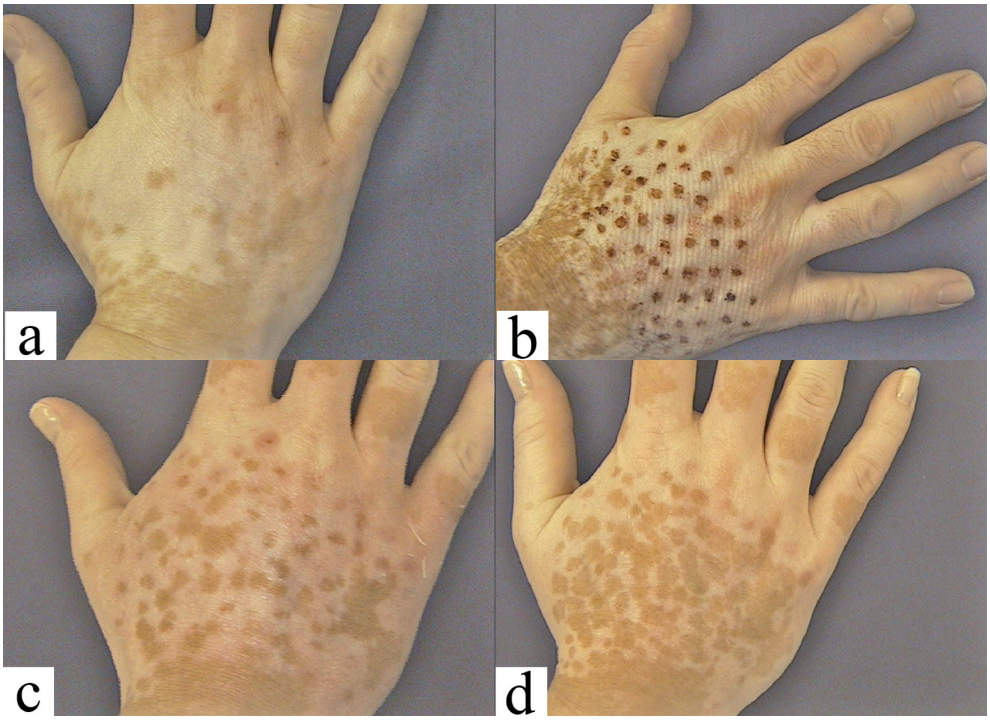

Figure 2. Blister roof transplantation (1 session) followed by KLUV 3 times a week:

a. Before transplantation

b. One week after transplantation = replacement of bandage

c. Four weeks after transplantation

d. Twelve weeks after transplantation (note peripheral spreading of pigment).

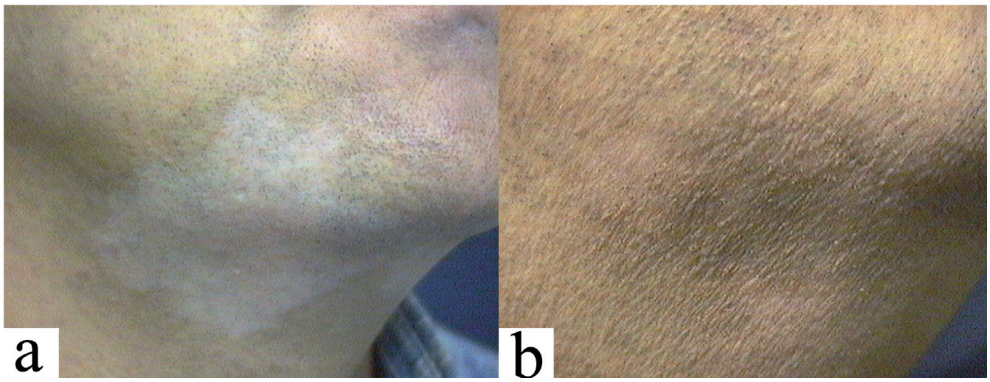

Figure 3. Results BRT and KLUV 6 months after 1 transplantation session.

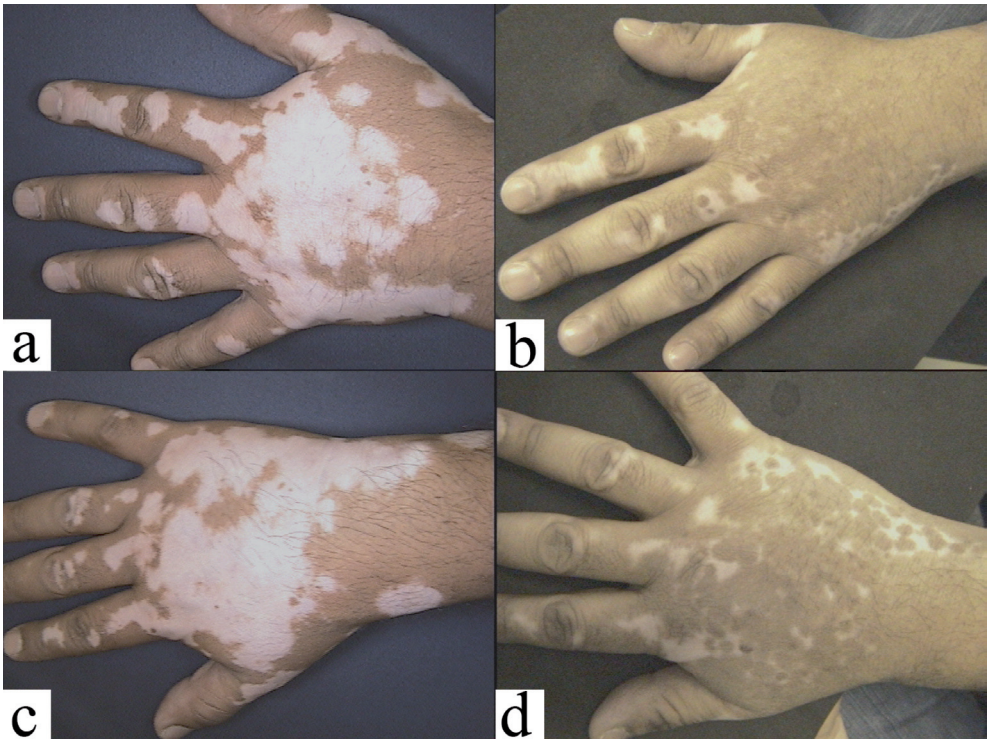

Figure 4. Results of BRT and KLUV after 5 transplantation sessions.

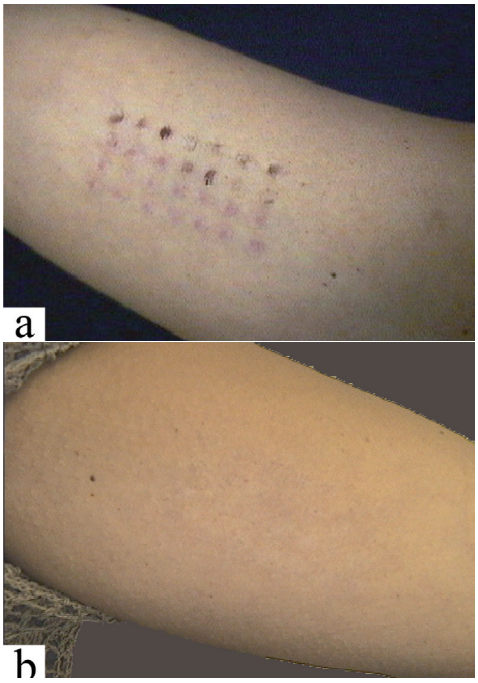

Figure 5. The donor site shows spontaneous repigmentation

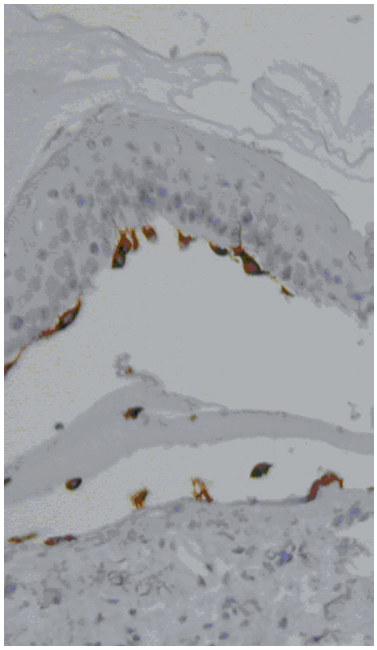

Figure 6. Histology of suction blister the melanocytes are more or less equally distributed

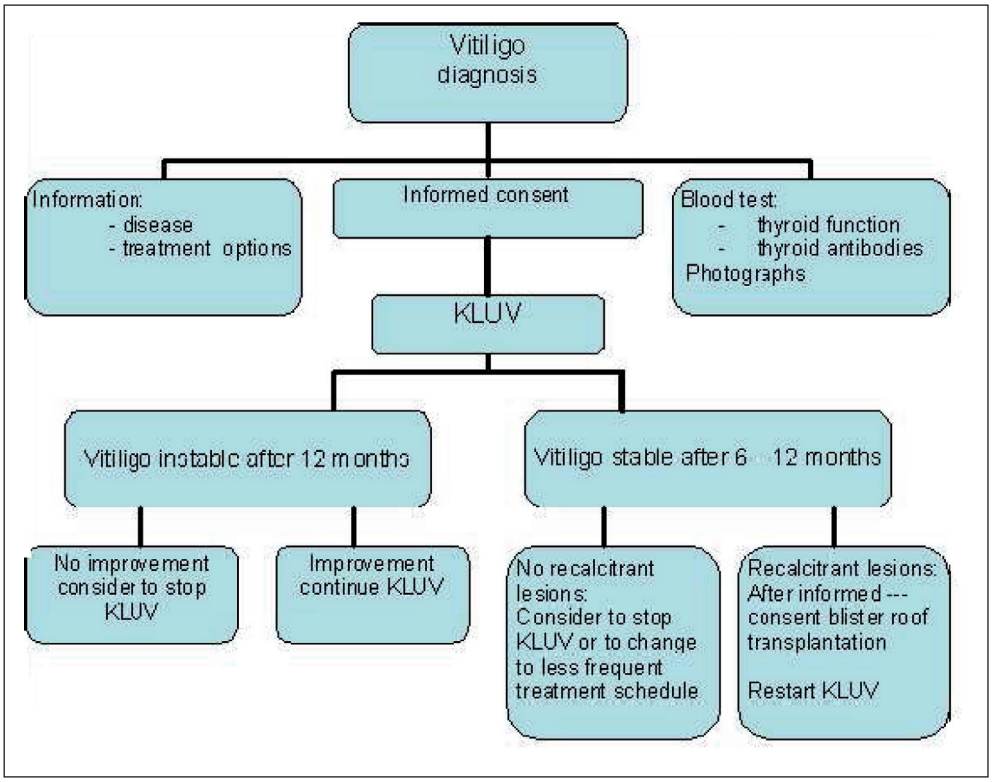

Figure 7. Flow diagram for the treatment of vitiligo in the present study

Chapter 12

Photodynamic Therapy of acne vulgaris using 5-ALA 0.5% liposomal spray and Intense Pulsed Light in combination with topical keratolytic agents.

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Abstract

Background: Increasing antibiotic resistance of Propionibacterium acnes and growing awareness on the side effects of topical and systemic drugs in the treatment of acne vulgaris by physicians and patients have paved the way for a search into new efficacious and safe treatment modalities such as photodynamic therapy (PDT). Although the efficacy of PDT using 20% 5-aminolevulinic acid (ALA) cream has been established, phototoxic side effects limit its use. The 5-ALA concentration can be lowered by a factor of 40 by changing the vehicle of 5-ALA from a moisturizing cream to liposome encapsulation.

Objectives: Assessment of the efficacy and the safety of PDT using 5-ALA 0.5% in liposomal spray and Intense Pulsed Light (IPL) in combination with topical peeling agents (Li-PDT-PC) in acne vulgaris.

Materials and Methods: Thirty-two patients suffering from acne participated in this randomized, prospective, single blind study. All patients were treated with Li-PDT-PC.) During the study nine patients were additionally treated with topical or systemic antibiotics (Li-PDT-PC-AT. These patients were removed from the study although their results were recorded.

Results: After a mean period of 7.8 months and a mean number of 5.7 treatments the mean total number of lesions dropped from 34.6 lesions to 11.0 lesions, resulting in a mean improvement of 68.2%. Side effects were minimal. Additionally, an intention to treat analysis was conducted.

Conclusion: Photodynamic therapy of acne vulgaris using 5-ALA 0.5% liposomal spray and Intense Pulsed Light in combination with topical peeling agents is safe and efficacious, even in patients with acne recalcitrant to standard therapy.

Introduction

Acne often improves after exposure to sunlight or artificial light sources.[1] Propionibacterium acnes produces protoporphyrin IX (PpIX), which generates reactive oxygen species, upon exposure to light, inducing phototoxic reactions and ultimately bacterial destruction[2] and damage to the sebaceous glands.[3] The activation of PpIX is visualized by the phenomenon of PpIX-fluorescence with fluorescence photography (Figures 1 and 2). The theoretically most effective wavelength for photoactivation of PpIX is 415 nm. Red light (635 nm) is less absorbed by photo-activating porphyrins, but penetrates more deeply into tissue and may also have anti-inflammatory effects by influencing the release of cytokines from macrophages.[4] Initially, light-based systems for treating acne emitted blue light, [5] red light or the combination of blue- and red light. [2] However, clinical improvement was limited and multiple sessions were required. Photodynamic therapy (PDT) with 5-aminolevulinic acid (ALA) or methylaminolaevulinate (MAL) was introduced to achieve better result with fewer treatment sessions. [6] Studies on the efficacy and the tolerability of PDT differ in the used light source, the treatment schedules, the parameters and the concentration of 5-ALA. [7-13] In those studies side effects such as pain, erythema, crusts (Figure 3) and post-treatment hyperpigmentation were noticed, highlighting the need for optimizing PDT for treatment of acne. Split-face studies using 5-ALA and Intense Pulsed Light (IPL) have demonstrated that the combination of 5-ALA and IPL is more effective than IPL alone. [6,7,9]

Liposomes are used in the treatment of hair follicle- and sebaceous gland-associated disorders because of their potential to carry lipophilic drugs and hydrophilic drugs such as 5-ALA into the pilosebaceous structures.[14--17] Promising results were obtained with liposomal encapsulated drugs in the treatment of acne. [18-21] Christiansen et al performed a study on the fluorescence distribution patterns of normal skin after topical application of 20% 5-ALA in a moisturizing cream and after application of low concentrations of 5-ALA (0.5% and 1%) encapsulated in liposomes. The 5-ALA concentration could be lowered by a factor of 40 by changing the vehicle for delivering the 5-ALA from a moisturizing cream to liposome encapsulation, and still induce the same level of fluorescence in the skin. The need for occlusion was eliminated at the same time. The low post-treatment fluorescence also significantly reduced the risk of post-treatment phototoxicity.[22] It has not been substantiated that follicular obstruction is reduced by PDT. [23] Therefore, in this PDT study concomitant treatment was carried out using tretinoin cream at night because peeling of the skin is an essential part of acne treatment. [24]

The first objective of this study was to determine the efficacy and the safety of 5-ALA 0.5% Liposomal PDT in combination with a Peeling Cream (Li-PDT-PC) in patients with acne vulgaris. The second objective was to establish the ability of Li-PDT- PC to

replace topical- and systemic standard anti-acne treatments.

Patients and methods

Patients

From October 2005 until August 2008, patients with acne vulgaris grade 4-6 on the Burton scale [25] (Table 1) were consecutively recruited into the study. They received information about all the aspects of acne, including advantages and disadvantages of treatment modalities before inclusion. After taking all options into account, the patients could freely make up their minds to enroll into the study or to choose for a traditional treatment modality. The study protocol (MC 2007-2) was approved by the medical ethics committee of the institute prior to the study. After providing informed consent 34 patients with acne vulgaris on the face were included in the study. These patients were divided into grade 4 (25 patients), grade 5 (8 patients) and grade 6 (1 patient) using the Burton grading scale (Table 1). Exclusion criteria were pregnancy, lactation, history of allergy to sunlight or 5-aminolevulinic acid, patients with diseases aggravated by sunlight (such as lupus erythematosus, porfyria cutanea tarda), patients using anti-inflammatory drugs and drugs known to have potency for photo-toxicity or photosensitization, topical and/or systemic antibiotics treatment during the half year prior to inclusion, and systemic isotretinoin treatment during one year before the start of the study (Table 2). The treatments were performed once a month. The endpoint of the study in each individual patient was the moment at which no further improvement was observed after two consecutive treatments.

Materials

In the present study, an emulsion containing 0.5% 5-ALA encapsulated in 50 nm sized unilamellar liposomes, which are the preferred structures for drug delivery, was used.[21] The liposomal solution was prepared according to the pharmacy-protocol written by Dianorm GmbH, München, Germany. These preparations were chemically stable for at least 24 months.

Intense Pulsed Light was delivered by an Intense Pulsed Light (IPL) device (Ellipse® Flex type 9DER0290 Ellipse A/S, Agem Allé 11, DK-2970 Hørsholm, Denmark). The IPL consisted of a high-energy flash lamp and a set of dual mode water-cooled filters, which enlarges the therapeutic energy window and therefore, requires no changing of filters according to the skin type and the degree of actual pigmentation. The skin was cooled during treatment with the Cryo 5 (Zimmer Elektromedizin, Neu Ulm, FRG). Although not an integral part of PDT, cream containing between 0.02% and 0.05% tretinoin was applied as a skin peeling agent. Patients who had irritation from the tretinoin cream were treated with adapalene gel (1 patient) or salicylic acid 5% cream (1 patient).

Procedure

Patients were asked not to apply make-up on the involved area of the skin on the day of the treatment. The involved skin area was degreased with acetone before treatment. After rinsing and drying the skin, 5-ALA liposomal spray was applied every 5 minutes for one hour to the involved skin area. During this phase, the patient remained in an artificially lit room at low intensity. After 1 hour of applying the spray, excessive spray on the skin was rinsed off and the skin was dried with gauze. The skin was covered by a thick layer of an optical index-matching gel to ensure an effective optical coupling between the IPL's light guiding crystal and the skin before the start of IPL treatment. Parameters of IPL treatment were chosen as follows: the acne applicator (530 nm to 750 nm) in 2 pulse trains with a pulse duration of 2.5 ms and an interpulse delay of 10 ms (acne-PDT) using fluences ranging from 2 x 4.0 J/cm^2 (sensitive skin) to 2 x 6.0 J/cm^2 in patients with skin type I to IV. Alternatively, the PL applicator (400-nm to 720- nm was used with fluences ranging from 3 x 3.0 J/cm^2 up to 3 x 4.0 J/cm^2) in patients with skin type V in order to the risk of side effects. Different treatment schedules were used depending on the skin type and the occurrence of pain. During the IPL treatment the skin was air-cooled using the Cryo 5 in order to reduce heat-induced pain sensation. After finishing the PDT treatment the gel residue on the involved skin was rinsed off, the skin was dried with paper tissue and a photo-protective cream containing 5% titanium dioxide was applied. The procedure took about 1 hour and 15 minutes, including the 15 minutes of active participation by the physician. The patients were advised to protect the treated skin areas against sunlight for 8 hours after the treatment Photographs of each patient were taken before the first treatment and 4 weeks after the last treatment. These photographs were used to evaluate the results of the treatments by a blinded counting of the acne lesions on the face by an observer (trained dermatologic assistant B.HS). The number of acne lesions per patient was divided into three categories: total lesions, inflammatory lesions and non-inflammatory lesions. Acne severity was additionally graded using the Burton scale (Table 1).

Clinical improvement was also assessed according to the patient's subjective response to the treatment, using a questionnaire ranking the level of improvement as poor = less than 50% (1 patient), as moderate = 50% to 75% (3 patients), as good to very good = 75% to 100% 19 patients (Table 3).

Results

Eleven patients dropped out of the study, one patient because of pregnancy and one patient because of traveling considerations. Nine patients received additional treatments during the study, thus violating the study protocol. The results of these patients were recorded irrespective of the violation of protocol. The remaining Li-

PDT-PC group consisted of 23 patients (12 females and 11 men), Fitzpatrick photo skin types II to V, acne grade 4 (17 patients) and grade 5 (6 patients) on the Burton scale, completed the study. Mean age of the patients was 24 years (range 15 – 42 years, SD = 7). Mean duration of acne prior to inclusion was reported by the patients to be 7.2 years (range 1 – 23 years, SD = 4.5). Both an outcome based analysis and an intention to treat analysis was conducted.

According to the analysis of outcome, the Li-PDT-TC treatment required a mean period of 7.8 months (SD =4.3) and a mean number of 5.7 treatments (SD =1.8). The change in the severity of the acne according to the Burton scale is shown in Table 3. As the Burton scale is an ordinal scale, we used the non-parametric Wilcoxon signed ranks test, which showed that the difference in the reported acne severity was statistically significant (p-value<0.05). Thus, it was safe to conclude that the Li-PDT-TC therapy resulted in a reduction in the severity of acne. The mean total number of lesions dropped from 34.6 lesions (SD=25.1) to 11.0 lesions (SD = 10.5), resulting in a decrease of 68.2%. The lesions were divided into two subsets based on the presence of an inflammatory component. The mean number of inflammatory lesions decreased by 70.6%, the mean pre-treatment inflammatory lesions being 18.0 (SD = 14.7) and the post-treatment mean being equal to 5.3 lesions (SD = 5.7). The mean number of non-inflammatory lesions decreased by 66.1%, starting from a mean pre-treatment lesions of 16.7 (SD = 15.1), with a mean number of 5.7 lesions (SD= 5.9) remaining after the treatment. The results are presented in table 3. The samples fit the normal distribution according to the Kolmogorov – Smirnov test, so that the significance of the difference in the samples can be tested with Student's T-test for paired samples. The improvement in the total number of lesions and the number of inflammatory lesions is statistically significant for p-value<0.01. The difference in the number of non-inflammatory lesions is statistically significant as well for p<0.05. An improvement of 75% to 100% in all lesions was achieved in 12 of the patients, in the inflammatory lesions in 12 of the patients and in the non-inflammatory lesions in 8 patients (Table 3).

The number of drop-outs is considerable (32.3%). This group did not significantly differ from the remaining group of patients both in terms of pre-treatment acne severity and post-treatment acne severity (non-parametric Mann-Whitney test (p>0.05)). As the results of all patients were recorded a intention to treat analysis was performed to assure that the results are not influenced by a bias in the drop-outs. This implies merging the results of the drop-out group with the results of those patients who did finish the therapy. The results of this analysis are as follows: Within this group of 34 patients, 11 did not follow the protocol and are considered treatment failure, although the data extracted from them was used in the analysis. The change in mean number of lesions before and after treatment, including standard deviation and

improvement percentage is 36.0 lesions (SD=30.1) to 10.5 lesions (SD = 10.6) and 70.9% for all lesions, 21.7 (SD = 24.8) to 5.0 lesions (SD = 5.6) and 77.1% for the inflammatory lesions and 14.3 (SD = 14.3) to 5.5 lesions (SD= 5.4) and 61.7% for the non-inflammatory lesions. The improvement in terms of the change in Burton acne severity scale) was significant, as was the change in the number of lesions (table 3) . Remarkably, the outcome of the intention to treat analysis and that of the outcome based analysis above could not be shown to differ significantly. The outcome based analysis was not influenced by a selection bias in the drop-outs.

The patient self-assessment of the therapy is also shown in table 3. The reported improvements exceeded those of the objective counting of lesions, with all patients reporting an improvement of at least 50%. More importantly, again using the Wilcoxon signed ranks test, the self-assessment of the investigated method was shown to be statistically different from the self-assessment of previous treatments reported at the outset of the study (Tables 2 and 4). All calculations where performed using SPSS® 15, SPSS inc.

All patients experienced a burning sensation during treatment. Pain symptoms, mostly related to the treatment with the acne applicator and not with the PL applicator, were generally well tolerated without any topical anesthesia. A reduction of the fluence was necessary in 3 patients because of pain. All patients showed post-treatment erythema for half an hour to one hour. One patient mentioned erythema and desquamation, which lasted up to 3 days. Some patients reported acneiform exacerbation after the first treatment. Serious side effects were not noted. Data on the remission period and the incidence of relapse are not yet available.

Discussion

Phototherapy in the treatment of acne was initially focused on the endogenous production of porphyrins by bacteria, such as P. acnes, as a by-product of their metabolism. [26] Fluorescence photographs may show no- or some fluorescence from acne lesions caused by protoporphyrins produced by the P. acnes (Figure 1a). Increased fluorescence is seen after 5-ALA liposomal spray has been applied over 5 minutes intervals for one hour, indicating the increase of PpIX in the involved skin area (Figures 1b and 2). Split-face studies demonstrated significant higher reduction in inflammatory lesion count from ALA-PDT and MAL-PDT compared with placebo PDT. [6,9,12,27] However, PDT using 20% 5-ALA under occlusion is often complicated by side-effects such as pain, erythema, crusts (Figure 3) and post -treatment hyperpigmentation.[13] Therefore, optimizing PDT for the treatment of acne is needed. Reduction of side effects is possible by reducing light fluences, reducing 5-ALA concentrations and/or 5-ALA application times. Another possibility is to enhance the penetration of 5-ALA into the sebaceous gland. In this study 5-ALA

was incorporated into liposomes, which have the capacity to transport incorporated drugs into the sebaceous glands (Figures 1 and 2), at the same time reducing the concentration of 5-ALA to 0.5%. The application of the 5-ALA liposomal spray augmented the fluorescence from the acne lesion (Figure 1b). Following subsequent PDT, the fluorescence in the acne lesion disappeared as a result of the phenomenon of photo-bleaching (Figure 2). The mechanism of action of 5-ALA liposomal PDT and IPL on acne is assumed to be an exogenous PDT response occurring in the presence of oxygen and light with appropriate wavelengths and intensity. This PDT reaction is very fast and is specific to the sebaceous gland. [9,28] Another plausible explanation for the observed efficacy is that PpIX acts as a fluorophore which absorbs the light energy and subsequently transports the energy to the P. acnes and the sebaceous glands. This may result in the destruction of the bacteria and minimize the production of sebum by thermal damage to the sebaceous gland.

In a previous unpublished pilot study, it was observed that acne-PDT may cause phototoxicity with crusts and exfoliation in patients with skin type V. In these patients no phototoxicity occurred with PL-PDT because of the longer pulse duration compared with acne-PDT and the spreading of the light energy over 3 passes with lower fluence per pass. Serious side effects of PL-PDT and acne-PDT were not observed. Some patients reported acneiform exacerbation after the first treatment with 5-ALA 0.5% liposomal PDT, comparable with that after the start of systemic isotretion therapy.

Standard treatment currently consists of peeling the skin and reducing P.acnes and inflammation by using topical peeling agents, topical and systemic antibiotics or oral isotretinoin. However, side effects may limit their use (table 4) and the efficacy of antibiotic treatment is progressively reduced by increasing resistance of the bacteria to the used antibiotics. The efficacy of most of the previous standard acne treatments used by the patients in this study was poor, except for isotretinoin (Table 2). The results and the safety aspects of Li-PDT-PC were very promising (Table 3, Figure 4). The first objective of the study was met because the severity of the acne in the patients receiving Li-PDT-PC was significantly reduced after treatment and the safety profile was high. Most of the patients who had experienced poor results from previous treatments benefited from Li-PDT-PC. Examples of the treatment results are provided in figures 5 and 6. The second objective of the study was met because the patient self-assessment score of the investigated method was significantly higher than the reported efficacy of previously received anti-acne treatments. The patient self-assessment score was even better than the blinded assessment by the observer. This highlighted the appreciation of the patients for the efficacy of the treatment and the paucity of side effects in addition to their appreciation of being treated with a topical treatment modality instead of systemic treatment. The results of this study

suggest that the described treatment has the potential to replace systemic therapy and even isotretoin or could serve as a supporting treatment thereby shortening the duration of the treatment and exposure to systemic drugs.

The intention to treat analysis shows that Li-PDT-PC may be a good basis for other combination treatments. The fact that the response of the group of nine patients receiving additional treatment was comparable with the results of the patients receiving Li-PDT-PC treatment is surprising as only 2 of the 9 patients were treated with systemic antibiotics for acne vulgaris grade 4 to 6. The 7 other patients would have, according to current acne treatment guidelines, qualified for systemic antibiotics or isotretinoin from the beginning of the treatment.[24] This would at least indicate that further studies comparing systemic antibiotics and/or isotretinoin with Li-PDT-PC are of interest.

At present, it is not yet possible to provide information on the remission period and the incidence of relapse. But up till now, it turned out to be that the frequency of treatments could be gradually lowered after about 4 treatments.

In spite of the use of topical keratolytic agents, the improvement of the non-inflammatory lesions in this study was not better than the improvement of the inflammatory lesions. This may indicate that 5-ALA 0.5% liposomal PDT does not contribute to the change in the keratinocyte shedding and hyperkeratosis as was previously assumed.[23] Therefore the addition of concomitant treatment with keratolytic agents to PDT seems rational. The results of Li-PDT-PC with an improvement in total acne lesions of 68.2% and improvement in inflammatory acne lesions of 70.4%, are more or less comparable with the results of other studies using 5-ALA 20% PDT: improvement in total lesions of 68% [29] improvement in total lesions of 60% and in inflammatory lesions of 65% [30], improvement in inflammatory lesions of 69%[15] [8] and improvement in total lesions of 72% [31]. The results of Li-PDT-PC surpassed those reported in 16% MAL-PDT studies with an improvement in total lesions of 54%, [11] and improvement in inflamed lesions of 68% [11] .Better results may be obtained by performing double passes with the acne-PDT on inflammatory lesions and by reducing treatment intervals to 2 or 3 weeks.

The reported higher appreciation of the current method when compared to earlier treatments suggests that indeed Li-PDT-PC could be an effective alternative for traditional acne treatment modalities.

Conclusions: Photodynamic therapy of acne vulgaris using 5-ALA 0.5% liposomal spray and intense pulsed light in combination with topical peeling agents is safe and effective, even in patients with acne recalcitrant to standard therapy and excels in paucity of side effects. In many acne patients this topical treatment has the potential to replace systemic therapy (even isotretion) or to reduce its duration in case additional systemic therapy is still required for optimum results. Target groups for Li-PDT-PC

are patients who have poor results from standard topical treatment and who dislike to try systemic therapy and patients with known side effects of standard topical or systemic remedies.

References

1. Cunliffe WJ, Goulden V. Phototherapy and acne vulgaris. Br J Dermatol 2000; 42, issue 5: 855-856.
2. Papageorgiou P, Katsambas A, Chui A. Phototherapy with blue (415 nm) and red (660) nm light in the treatment of acne vulgaris. Br J Dermatol 2000; 142: 973-978.
3. Divaris DXG, Kenedy JC, Poittier RH. Phototoxic damage to sebaceous glands and hair follicles of mice after systemic administration of 5-aminilevulinic acid correlates with localized protoporphyrin IX fluorescence. Am J Pathol 1990; 136: 891-897.
4. Young S, Bolton P, Dyson M. Macrophage responsiveness to light therapy. Lasers Surg Med 1989; 9: 497-505.
5. Tzung TY, Kuan-Hsing W, Huang ML. Blue light phototherapy in the treatment of acne. Photodermatol Photoimmunol Photomed 2004; 20: 266-269.
6. Rojanamatin J, Choawawanich P. Treatment of inflammatory facial acne vulgaris with intense pulsed light and short contact of topical 5-aminolevuleinic acid: a pilot study. Dermatol Surg 2006; 32: 991-99
7. Gold MH. A multi-center study of photodynamic therapy in the treatment of moderate to severe inflammatory acne vulgaris with topical 20% 5-aminolevulinic acid and a new intense pulsed light source. J Am Acad Dermatol 2004; 50 (s1): 14
8. Pollock B, Turner D, Stringer MR, Bojar RA, Goulden V, Stables GI, Cunliffe WJ. Topical aminolaevulinic acid-photodynamic therapy for the treatment of acne vulgaris: a study of clinical efficacy and mechanism of action. Br J Dermatol 2004; 151: 616-622.
9. Santos MA, Belo VG, Santos G. Effectiveness of photodynamic therapy with topical 5-aminolevulinic acid and intense pulsed light versus intense pulsed light alone in the treatment of acne vulgaris: comparative study. Dermatol Surg 2005; 31: 910-915.
10. Wiegell SR, Wulf HC. Photodynamic therapy of acne vulgaris using 5-aminolevulinic acid versus methyl aminolevulinate. J Am Acad Dermatol 2006; 54: 647-651.
11. Wiegell SR, Wulf HC. Photodynamic therapy of acne vulgaris using methylaminolaevulinate: a blinded, randomized, controlled trial. Br J Dermatology 2006; 154: 969-976.

12. Hörfelt C, Funk J. Frohm-Nilsson M, Wiegleb Edström D, Wennberg AM. Topical methyl aminolaevulinate photodynamic therapy for treatment of facial acne vulgaris: results of a randomized, controlled study. Br J Dermatol 2006; 155: 608-613.
13. Hörfelt C, Stenquist B, Larkö O, Faergemann J, Wennberg AM. Photodynamic therapy for acne vulgaris: a pilot study of the dose-response and mechanism of action. Acta Derm Venereol 2007; 87: 325-329.
14. Hope MJ, Kitson C.N. Liposomes: A Perspective for Dermatologists. Dermatol Clin 1993; 11: 143-154.
15. Lieb L, Ramchandran C, Egbaria K, Weiner N. Topical delivery enhancement with multilamellar liposomes into pilosebaceous units. J. Invest Dermat 1992; 99: 108-113.
16. Bernard E, Bubois JL, Wepeirre J. Importance of sebaceous glands in cutaneous penetration of an antiandrogen: target effect of liposomes. J Pharm Sci 1997; 86: 573-578.
17. De Leeuw J, de Vijlder HC, Bjerring P, Neumann HAM. Liposomes in Dermatology Today, review article. J Eur Acad Dermatol Venereol 2009; 23: 505-516.
18. Schäfer-Korting M, Korting HC, Ponce-Pöschl E. Liposomal tretinoin for uncomplicated acne vulgaris. Clin Invest 1994; 72: 1086-1091.
19. Patel VB, Misra AN, Marfatia YS. Topical liposomal gel of tretinoin for the treatment of acne: research and clinical implications. Pharm Dev Technol 2000; 5: 455-464.
20. Patel VB, Misra AN, Marfatia YS. Preparation and comparative clinical evaluation of liposomal gel of benzoylperoxide for acne. Drug Dev Ind Pharm 2001; 27: 863-870.
21. Fluhr JW, Barsom O, Gehring W, Gloor M. Antibacterial efficacy of benzoylperoxide in phospholipids liposomes. A vehicle-controlled, comparative study in patients with papulopustular acne. Dermatology 1999; 198: 273-277.
22. Christiansen K, Bjerring P, Troilius A. 5-ALA for photodynamic photorejuvenation-optimization of treatment regime based on normal-skin fluorescence measurements. Lasers in Surg and Med 2007; 39: 302-310.
23. Hongcharu W, Taylor CR, Aghasi D, Suthamjariya K, Anderson RR. Topical ALA-photodynamic therapy for the treatment of acne vulgaris. J Invest Dermatol 2000; 115: 183-192.
24. Strauss JS, Krowchuk DP, Leyden JJ, Lucky AW, Shalita AR, Siegfried EC, Thiboutot DM, Van Voorhees AS, Beutner KA, Sieck CK, Bhushan R. Guidelines of care for acne vulgaris management. J Am Acad Dermatol

2007: 651-663.
25. Elman M, Lebzelter J. Light therapy in the treatment of acne vulgaris. Dermatol Surg 2004; 30: 139-146.
26. Mollie A, MacCormack MD. Photodynamic Therapy in Dermatology: An update on applications and outcomes. Seminars Cut Med Surg 2008; 27 (1): 52-62.
27. Akaraphant R, Kanjanawanitchkul W. Gritiyarangsan P. Efficacy of ALA-PDT vs blue light in the treatment of acne. Photodermatol Photoimmunol Photomed 2007; 23:186-190.
28. Gold MH. Acne and PDT: new techniques with lasers and light sources. Lasers Med Sci 2007; 22: 67-72.
29. Goldman MP, Boyce S. A single-center study of aminolevulinic acid and 417 nm photodynamic therapy in the treatment of moderate to severe acne vulgaris. J Drugs Dermatol 2003; 2: 393- 396.
30. Gold MH. The utilization of ALA-PDT and a new photoclearing device for the treatment of severe inflammatory acne vulgaris – results of an initial clinical trial. J Lasers Surg Med 2003; 15(s): 46.
31. Gold MH, Bradshaw VL, Boring MM, Bridges TM, Biron JA, Carter LN. The use of a novel intense pulsed light and heat source and ALA-PDT in the treatment of moderate to severe inflammatory acne vulgaris. J Drugs Dermatol 2004; 3: 15-19.

Grade 0:	Total absence of lesions
Grade 1:	Sub-clinical acne with few comedones visible only on close examination
Grade 2:	Acne comedonicus: comedones with slight inflammation
Grade 3:	Mild acne with inflamed papules and erythema
Grade 4:	Moderate acne with many inflamed papules and pustules
Grade 5:	Severe nodular acne: inflamed papules and pustules with several deep nodular
Grade 6:	Severe cystic acne with many nodular cystic lesions with scarring

Table 1. Burton scale for grading acne

Efficacy and safety of previous treatments in the study population patients (nr 23)	Poor Less than 50%	Moderate 50% to 75%	GOOD > 75%	Medication stop because of side effects
Topical treatment				
Tretinoin cream	7	1		
Adapalene cream				
Benzoylperoxide gel	3			
Salicylic acid cream				
Erythromycin lotion	10	1		
Clindamycin lotion	1	1		
Antibiotic lotion not specified	1			
Systemic treatment				
Tetracycline	1			
Minocycline	3	4		1
Erythromycin		2		1
Antibiotic non-specified	1			
Cyproterone/estradiol	3	1		
Isotretinoin	1		5	1
Results of 46 previous treatments in 23 patients	67,39%	21,74%	10,87%	5%

Table 2

	Mean number of lesions (standard deviation) outcome analysis	Mean number of lesions and (standard deviation) intention to treat analysis
Pre-treatment lesions	34.6 (25.1)	36.0 (30.1)
Pre-treatment inflammatory lesions	17.9 (14.7)	21.7 (24.8)
Pre-treatment non-inflammatory lesions	16.6 (15.1)	14.3 (14.3)
Post-treatment lesions	11.0 (10.5)	10.5 (10.6)
Post-treatment inflammatory lesions	5.3 (5.7)	5.0 (5.6)
Post-treatment non-inflammatory lesions	5.7 (5.9)	5.5 (5.4)

Results according to the Burton scale (no. of patients in class)							
Class	6	5	4	3	2	1	0
Pre-treatment (outcome)	0	6	16	1	0	0	0
Post-treatment (outcome)	0	0	0	0	9	9	0
Pre-treatment (i.t.t.)	1	7	23	1	0	0	0
Post-treatment (i.t.t.)	0	0	0	0	15	12	0

Results according to the patient's subjective response to the treatment			
Improvement in %	Less than 50 %	50% to 74%	75% to 100%
Number of the patients	1	5	26

Table 3. Results

Topical drugs					
	salicylic acid	benzoyl-peroxide	Azelaic acid	tretinoin, adapalene	erythromycin, clindamycin
Skin and mucosae	Irritation, dermatitis, contact allergic eczema	Burning, itching, erythema, dry skin, scaling, cracks, crusting, blistering, allergic contact dermatitis	Burning, itching, erythema, desquamation, contact allergy, hypopig-mentation, photo-sensitization	Burning, itching, dry skin, erythema, desquama-tion, contact allergy, crustation, hypo- and hyperpig-mentation, photo-sensitization	Burning, itching, dry skin, erythema, desquamation, contact allergy, gram-negative folliculitis
Gastro-intestinal	Intoxica-tion following application on large skin areas				Clindamycin: Belly ache, diarrhea, colitis
Blood	Intoxica-tion following application on large skin areas				
Pregnancy and lactation	Treatment of large areas and under occlusion contra-indicated in pregnan-cy. Lactation no problem	No problem	No data available	Small quantities probably no problem. Treatment of large areas and under occlusion contra-indicated in pregnancy and lactation	No data available
Carcinogenicity		Concern about carcinogenesis in the long-term			
Others		White staining of clothes			

		Systemic drugs		
	PDT 5-ALA 10% to 20% cream	tetracyclin, doxycyclin, minocyclin	erythromycin	isotretinoin
Skin and mucosae	Pain, stinging, itching erythema, oozing, crustae and pustules. Phototoxicity 48 hours after treatment	Urticaria, angio-edema, phototoxical- and photoallergic reactions, Sweet's syndrome.	Pruritus, urticaria, exanthema, toxic epidermal necrosis, Stevens-Johnson-syndrome	Xerosis cutis, pruritus, dermatitis, exanthema, epistaxis, conjunctivitis-, blepharitis- and cheilitis sicca, alopecia, paronychia, nail dystrofia, hyperhidrosis, hirsutism, vasculitis
Gastro-intestinal		Nausea, vomiting, diarrhea, glossitis, hairy tongue, stomatitis, pseudo-membranous colitis, prutitus ani, hepar dysfunction	Anorexia, nausea, vomiting, cramps, diarrhea, pseudo-membranous colitis, hepatitis, pancreatitis	Nausea, gastro-intestinal bleeding, intestinal inflam-mation, hepar dysfunction, hepatitis, pancrea-titis, malaise.
Blood		Anemia, thrombocytopenia, neutropenia. Doxycycline: induction of hypoglycemia		anemia thrombo-cytopenia, neutropenia, hyperglycemia, elevated cholesterol- and triglyceride levels, low HDL, thrombosis
Kidneys		renal dysfunction		glomerulonephritis
Neurological and psychologica		Benign intracranial hypertensiona	Impaired hearing	Headache, benign intracranial hypertension, photophobia, blurred vision, disturbance dark adaptation, cataract, impaired hearing, convulsions, somnolence, mental depression, psychosis, suicide.
Pregnancy and lactation	Pregnancy category C	Contra-indicated during pregnancy and lactation Unreliability of oral contraceptive devices	No problem	Absolutely contra-indicated during pregnancy and lactation
Carcinogenicity		Increased risk of incident and fatal breast cancer	Increased risk of incident and fatal breast cancer	
Other		From minocin also reported: -lupus pneumonia -hypersensitivity -pneumonia -progressive respiratory failure		Increased risk of incident and fatal breast cancer

Table 4. Side effects of anti-acne drugs.

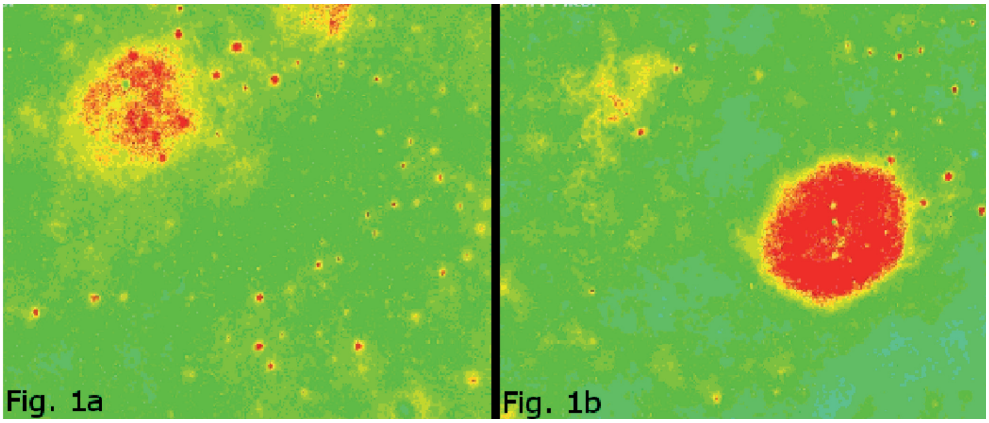

Figure 1. Fluorescence photography of a sebaceous gland.
1a. Spontaneous auto-fluorescence from PpIX
1b. Increased fluorescence after application of 5-ALA liposomal spray

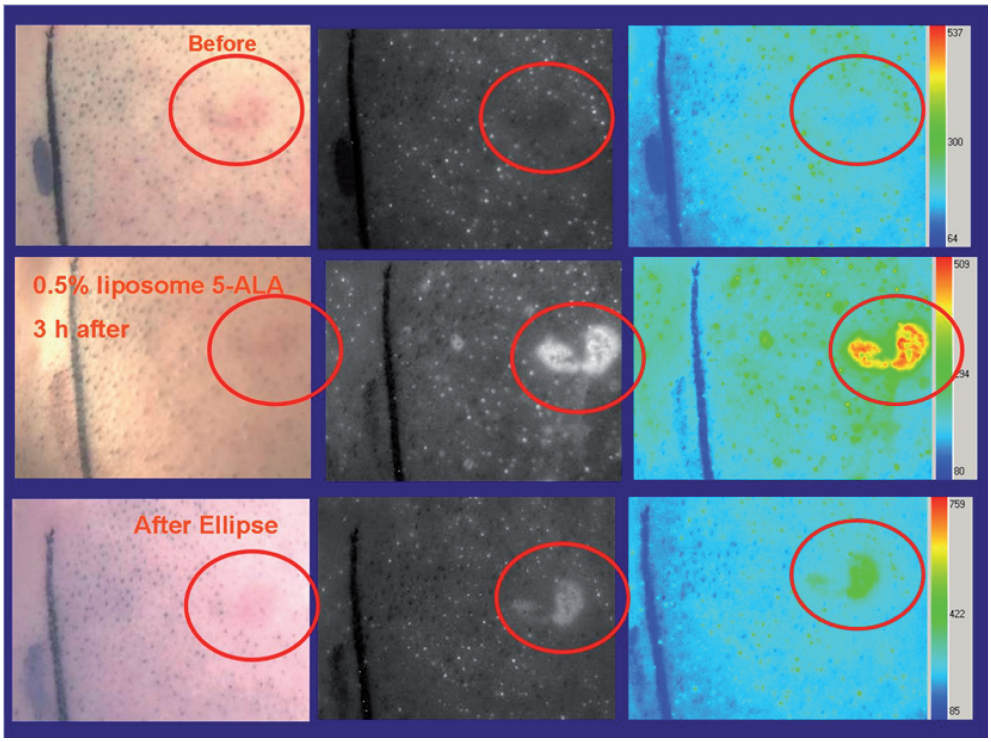

Figure 2. Acne nodule: left clinical images, middle fluorescence images, right pseudo-color images.
Top right before application of 5-ALA liposomal spray: no auto-fluorescence.
Middle right: after 1 hour of treatment with 5-ALA liposomal spray: clear fluorescence mainly located in the acne nodule.
Bottom right: decreased fluorescence as a result of photo-bleaching by IPL treatment.

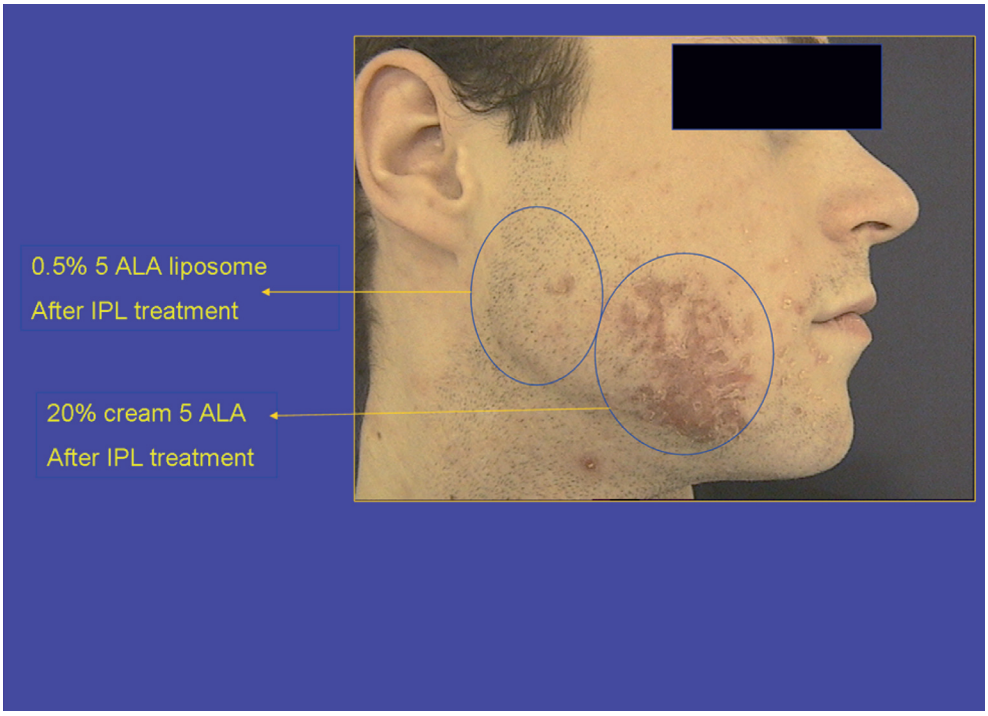

Figure 3. Left: 1 day after PDT with 1 hour application of 5-ALA 0.5% liposomal spray without occlusion (no side effects).
Right: 1 day after PDT with 5-ALA 20% cream under occlusion (redness and crusts). The parameters of the IPL treatment were the same for both areas.

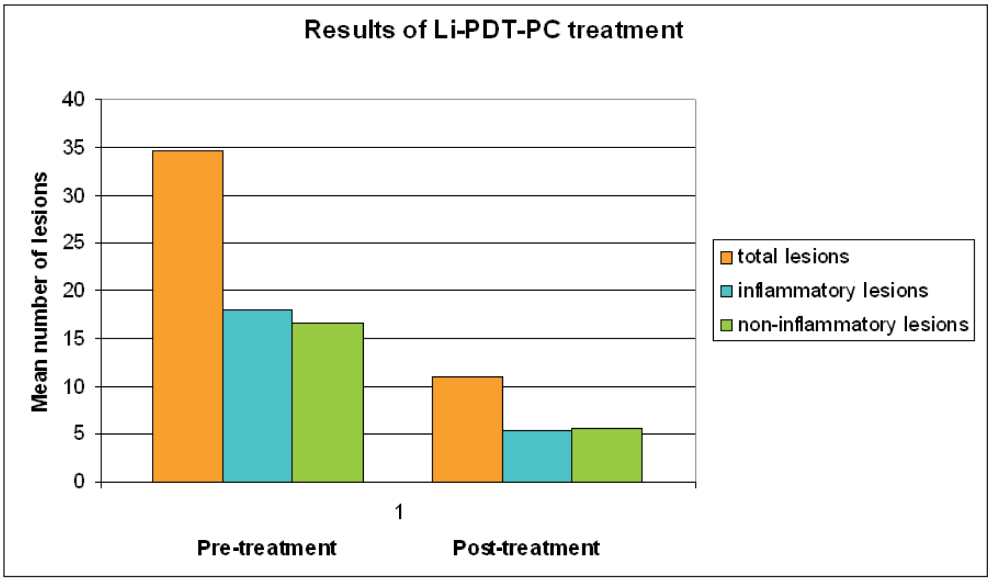

Figure 4. Reduction of the mean number of all lesions 68.2% ($p<0.05$), of inflammatory lesions 70.6% ($p<0.05$) and of non- inflammatory lesions 66.1% ($p<0.05$).

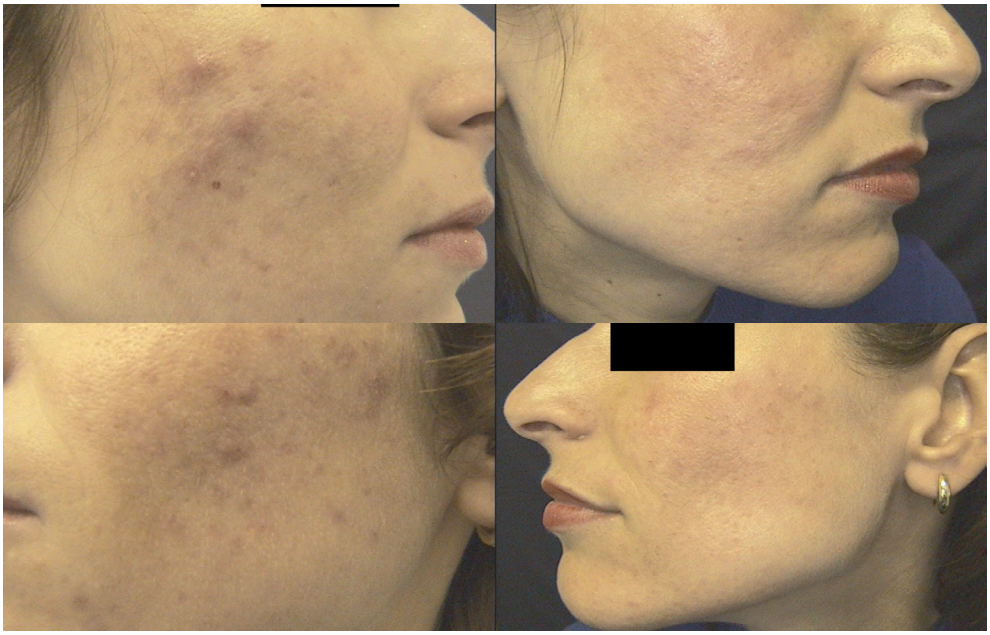

Figure 5. Left: Before Li-PDT-PC.
Right: After 6 Li-PDT-PC treatments in 7 months (improvement 80%). No side effects. No effect from previous treatments with erythromycin lotion, systemic minocycline and systemic isotretinoin.

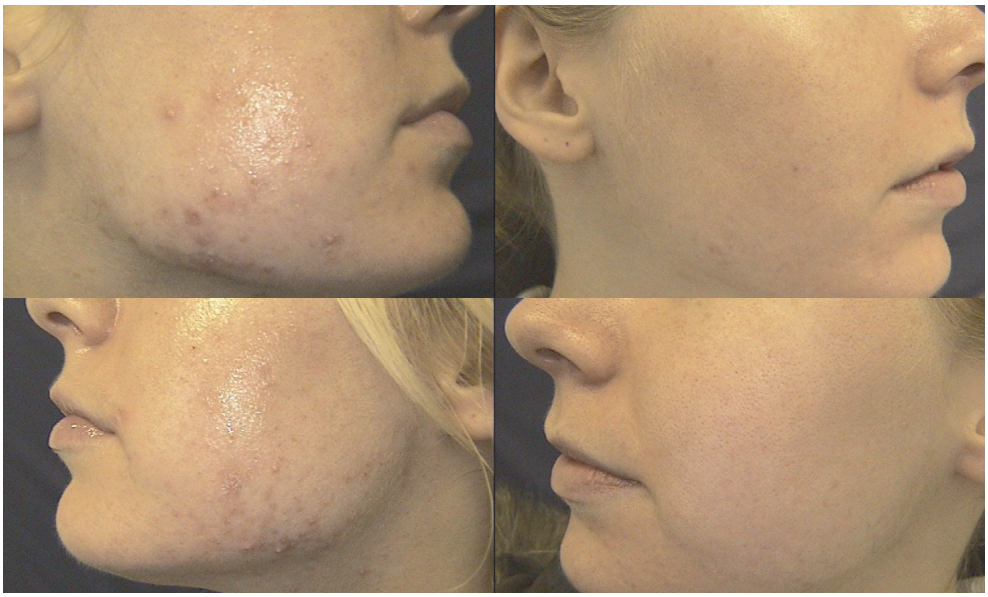

Figure 6. Left: Before Li-PDT-PC.
Right: After 6 Li-PDT-PC treatments in 6 months (improvement 90%). No side effects. No effect from previous treatment with cyproteron/ethinylestradiol and systemic minocycline.

Chapter 13

Published as

Fluorescence detection and diagnosis of non-melanoma skin cancer at an early stage.

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Abstract

Background: The occurrence of non-melanoma skin cancer (NMSC), including actinic keratosis (AK) is increasing all over the world. The detection and diagnosis of NMSC is not optimal in clinical practice. The need for complementary methods for detection and accurate demarcation of NMSC at an early stage is large in order to limit the damage caused by tumours.

Objective: The purpose of the present study was to use a large area skin fluorescence detection system to detect early NMSCs (clinical visible as well as non-visible lesions) in the face, neck, chest, back and hands of patients treated with UV and outdoor workers.

Methods: Fluorescence detection with a purpose-made digital camera and software (Dyaderm®) combined with 5-aminolevolinic acid (5-ALA) encapsulated in liposomes.

Results: In 93 consecutively referred patients positive skin fluorescence was detected in 61 patients. After histological examination the positive fluorescence appeared to be correlated to benign lesions in 28 patients (sebaceous gland hyperplasia in 22 patients) and to (pre-) malignant lesions in 33 patients (actinic keratosis in 29, BCC in 3 and SCC in 1 patient). False negative fluorescence was found in only one lesion. In 5 patients the FD technique used in this study appeared to be more sensitive for the identification of (pre-) malignant lesions than the clinical examination in contrast to FD techniques used in previous studies.

Conclusion: Diagnostic skin fluorescence using liposomal encapsulated 5-ALA and a specialized computerized detection and visualization system offers the possibility for detection of NMSC at an early, pre-clinical stage. The technique is well suited to

examine large areas of skin. It also identifies areas of most interest for performing confirmatory skin biopsies, as well as pre-operative assessment of boundaries of skin malignancies, and finally, the technique is applicable in the control and follow-up of skin cancer treatment.

Introduction

Nonmelanoma skin cancer (NMSC) is the most common cancer in Caucasian populations and its incidence is of epidemic proportions worldwide.[1-5] Although, the term NMSC covers all cutaneous cancers excluding melanomas, it is normally used to refer to two major types of skin cancer: basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). These two forms of skin cancer account for more than 95% of all NMSC.[5] BCCs arise de novo, which means there are no known precursor lesions.[5] SCC's may arise from actinic keratoses (AKs). Often, these lesions have been considered to be pre-malignant precursors[6-8], but according to other authors, AKs are true epithelial neoplasmata from the beginning.[3,9] From this standpoint of view, the evolution from AK to SCC represents progression rather than transformation, and therefore, AK should be recognized as incipient SCC.[10,11] It has been estimated, that 6% to 10% of AKs develop into invasive SCC.[12,13] This highlights the value of AK as a marker for SCC.[3] Thus, if these high-risk patients are monitored closely, invasive tumors that develop could be treated at an early stage when cure rates are high, morbidity low, mortality nonexistent, and costs of treatment are limited.[3] The average annual increase of NMSC in white populations in Europe, the United States, Canada and Australia has been 3% to 8% since the 1960s.[4] Nowadays, NMSC incidence is greater than all other cancers combined and NMSC is among the five most costly cancers reported in the USA .[14] There are many reasons for the worldwide increase in NMSC incidence. Etiological factors that underlie the development of skin cancer are of endogenous origin as well as of exogenous origin.[4] Major endogenous factors are age, genetic predisposition, such as skin type (in particular skin type I, II and III) and genetic diseases (for example xeroderma pigmentosa).The key environmental risk factor for NMSC is ultraviolet (UV) light radiation from exposure to sunlight, artificial tanning lamps[15-18] and iatrogenic exposure to psoralen and ultraviolet A (PUVA), and to ultraviolet B (UVB), modalities often used for the treatment of psoriasis and vitiligo.[19,20] Persons with occupational or recreational outdoors exposure, such as commercial fisherman, construction workers, park rangers, farmers, aircraft pilots, yachtsmen, golfers, skiers, mountaineers and sun-addicts, have higher incidence rates of AKs than indoor living people.[21-24] Other exogenous factors include chemical carcinogens (such as arsenic, pesticides, tar, certain industrial oils, dyes and solvents), ionizing radiation, human papilloma viral infections[25] and immunosuppression, especially in organ transplant recipients.[26] Tobacco smoking has also been recently

linked to SCC.[27] In 2002, more than 1 million cases of NMSC were diagnosed in the United states.[28] Squamous cell carcinoma accounts for more than 20% of NMSC and most metastatic disease and death due to NMSC. Therefore, the ability of the physician to distinguish precancerous lesions with a high degree of accuracy is of great importance and will decrease the morbidity and mortality of SCC.[29] The clinical features of AK's are nonspecific, usually described as red, scaly papules or plaques, 2- to 10- mm in diameter, occurring in sun-exposed sites. They may bleed and may become hypertrophic, at which point a biopsy should be performed to evaluate for progression to SCC.[29] The differential diagnosis of AK includes many benign and malignant lesions (Table 1).[13,29] This broad differential underscores the highly nonspecific clinical features of AKs.[29] The diagnostic accuracy or positive predictive value (PPV) of the clinical diagnosis of AKs made by specialized doctors appeared to be only 74% after histological diagnosis.[29] A sound public health policy adapted to the challenges of the 21st century must strive to prevent skin cancer development through risk factor modification (primary prevention) and improved disease surveillance and earlier detection (secondary prevention).[30] The need for complementary methods for detection and accurate demarcation of skin cancers at an early stage is large, to limit the damage caused by tumours.[31] .

Besides its usefulness in photodynamic therapy (PDT), 5-aminolevulinic acid (5-ALA) and methylaminolevulinic cream (MAL, Metvix®, Galderma) also can be exploited for diagnostic purposes. The mechanism of 5-ALA use in PDT is based on intracellular transformation of 5-ALA to protoporphyrin IX (PpIX), followed by light exposure, which induces phototoxicity. The transformation of ALA can be monitored non-invasively by detection of PpIX fluorescence at 634-nm. Fluorescence from the skin after application of 5-ALA is a complex interaction of ALA penetration into the skin, cellular absorption of ALA, and metabolic transformation of ALA into PpIX. For topical application of ALA relatively high concentrations of ALA are necessary, because of the low rate of penetration of free 5-ALA into the skin. Generic preparations of 5-ALA 20% in a moisturizing cream (pharmacy preparations) or methylaminolevulinate (MAL) 16% cream (Metvix® Galderma) are used, under an impermeable plastic occlusion film to enhance and standardize absorption. Following treatment with this high concentration preparations photosensitization remains for at least 32 hours. Exposure to ambient light within this time interval may cause severe clinical signs of phototoxicity e.g. swelling, erythema and scaling. Therefore, patients receiving this type of treatment are advised to avoid bright light and sun exposure for 24 to 48 hours after treatment.[32]

Liposomes are microscopic vesicles consisting of concentric bilayers formed by phospholipids, enclosing an aqueous core. Therefore, liposomes contain lipophilic compartments within the bi-layer membranes and hydrophilic compartments

between the membranes. Lipophilic substances can be stored into the lipid phase and water soluble substances into the water phase. Therefore, liposomes can be used as carriers for lipophilic as well as hydrophilic drugs.[33] Three mechanisms have been described concerning the penetration of liposomes into the epidermis: penetration via the pilosebaceous units[34], via lateral diffusion of liposomes in the stratum corneum[35], and via a transepidermal osmotic gradient and hydration force.[36] The similarity of lipid composition of liposomes and epidermis enables the liposomes (and also the drugs encapsulated in them) to penetrate into the epidermal barrier to a greater extend than lotions, creams and ointments do. In addition, liposomes bind to cells and deliver their contents to the cytoplasm of cells by fusion with the outer cell membrane or by endocytosis, where upon they are concentrated in lysosomal sacs.[37] These capabilities make liposomes very useful for the enhancement of the penetration of lipophilic as well as hydrophilic drugs (5-ALA is hydrophlic) into the epidermis.[38] An optimal penetration of liposomes requires a dry skin surface, under occlusion (maximal hydration) no penetration of liposomes occurs.[36]

The fluorescence of skin areas treated for more than 1 hour with 20% ALA cream under occlusion appears to be very uniform and the fluorescence intensity continues to increase after the end of 5-ALA application. The maximum fluorescence is reached at 8 hours after the end of 20% 5-ALA application.[32] The fluorescence of skin areas following non-occluded topical application of liposome-encapsulated 0.5% 5-ALA was heterogeneous distributed (heterogeneous distribution shows high fluorescence intensity from diseased tissue and low fluorescence intensity from normal skin) and reached a saturation level after 2 hours. The fluorescence decays linearly shortly (within 15 minutes) after the end of application of 0.5% 5-ALA liposomes and was back to baseline within 8 hours.[32] The average skin surface fluorescence induced by the liposome-encapsulated 0.5% 5-ALA applied for longer than 2 hours, is found to be equal to the average measured skin surface fluorescence obtained after 30 minutes exposure to 20% 5-ALA cream.[32] Changing the 5-ALA vehicle from a moisturizing cream to liposome encapsulation, the 5-ALA concentration can be lowered by a factor of 40, and still induce the same skin fluorescence and at the same time eliminates the need for occlusion.[32] Following topical application of 5-ALA, PpIX is induced selectively in cells with high metabolism, such as in epithelial tumor cells. Upon irradiation with light the tumor becomes visible and can be delineated from the surrounding tissue.[39] This method is called fluorescence diagnosis (FD). By using a highly light sensitive charged couple device (CCD) camera system together with a specially designed digital imaging (Dyaderm system, Biocam GmbH, Regensburg, Germany), the contrast of the acquired fluorescence images can be significantly enhanced.[39-41] This allows the determination of the optimal site for a directed biopsy and/or may indicate the boundaries of the tumour for preoperative planning when Mohs' surgical

treatment is scheduled.[39] Moreover, FD is also a helpful tool to prove the efficacy of PDT.[39] Although promising, the results till now were limited due to technological constraints both in terms of application of 5-ALA, illumination and observation of fluorescence.[42] The current technology deviates from preceding methods in three ways. Firstly, the previously used flashlamp is replaced by a homogeneous LED light source. Secondly, the 5%-ALA in a moisturizing cream under occlusion is replaced by liposomal encapsulated 5-ALA 0.5%, without occlusion. Thirdly, the Dyaderm® system with its relatively limited skin area (which can be investigated in one picture), is replaced by a specialized version of the Dyaderm® system. This new system enables investigation of large skin areas in one picture, resulting in less time consuming procedures. The aim of this study was to establish FD, using liposomal encapsulated 5-ALA and a specialized version of the Dyaderm© system, as a reliable guide for the detection of NMSCs (including AKs). An additional objective was to asses the diagnostic efficacy of heterogeneous fluorescence following application of 5-ALA 0.5% liposomes without occlusion in contrast to homogeneous fluorescence from 16% 5-methylaminolevulinic cream (MAL, Metivix©, Galderma) under occlusion.

Materials and Methods

Patients

The study was conducted on a consecutively recruited group of 93 persons with a history of chronic UV exposure, such as outdoor professions and long term UV therapy, or with a history of treatments for NMSC. The group consisted of 43 females and 50 males with an average age of 59 years (standard deviation 11 years). Pregnancy, a history of epilepsy, recent peeling or scrubbing or other rejuvenation techniques of the skin, and allergy to 5-ALA were exclusion criteria. The study areas were limited to highly UV exposed areas such as the face, the scalp, the chest, the upper back and the back of the hands. Prior to the FD procedure, the test areas of the skin were degreased by wiping with gauze moistened with acetone.

5-ALA Preparation

In the present study an emulsion was used containing 0.5% 5-ALA encapsulated in 50 nm sized unilamellar liposomes, which are the preferred structures for drug delivery.[33] The liposomal solution was prepared according to a pharmacy-protocol from Dianorm GmbH (Munich, Germany). The preparation is chemically stable for at least 24 months. The concentration of 0.5% 5-ALA appeared to be sufficient for fluorescence detection, meanwhile strongly reducing the risk of post procedure phototoxicity.[32]

The only known side effect of 5-ALA liposomal preparation is that it may induce a slight erythema and scaling when applied to skin which has recently been treated with a peeling or scrubbing agent or skin which is very dry where the epidermal

barrier function is not intact. Attention was given to these potential side-effects during application.

Fluorescence detection system

5-ALA is metabolized in mammalian cells to heme, producing protoporphyrin IX (PpIX), which absorbs optical energy when subjected to illumination with visible light. Absorption peaks exist at 409nm, 509nm, 544nm, 584nm, and 634nm. Fluorescence is emitted in the red part of the optical spectrum, with emission peaks at 636nm and 708nm. In cancer cells, the concentration of PpIX is significantly higher than in normal skin cells, and the intensity of the fluorescence emitted from the cancerous region will be higher than that emitted from normal skin tissue.[31] In this study fluorescence measurements were performed with a specially designed version of the DyaDerm® fluorescence detection system, which is a highly sensitive digital fluorescence imaging system intended for the analysis of larger skin areas. Each image dimension is 14 by 18 centimetres, giving an imaging skin size of 252 $cm^{2.}$ A high brightness LED pulsed light source is used, which emits blue light with a peak wavelength of 405 nm. Pulse duration is 5 ms and repetition frequency is 1 Hz. The resulting fluorescence signal is recorded by a 10 bit CCD camera mounted to an adjustable stand and coupled to a computer system where image acquisition and processing is controlled by the DyaDerm software. Because of the short pulsed excitation photo-bleaching of PpIX is minimized. As PpIX fluorescence emission consists of light in the red spectrum, the red pixels of the CCD camera are used to generate a fluorescence image. The DyaDerm® system can capture and display color images of the skin and their corresponding fluorescence images in a live video mode while artifacts due to camera motion are suppressed because of using a fixed stand. These images can be superimposed and pseudo-colored to better highlight relevant parts of increased fluorescence due to malignant or pre-malignant lesions. Because images are captured in video mode using both white and blue conditions, the system is triggered to capture every fifth image under fluorescent light. In this way, both color and fluorescence images are captured and transferred to the display. After interpolation, both images can be superimposed to allow a physician to localize any high-fluorescence spots at the examined skin. It is possible to present the fluorescence intensity in pseudo-color. The human eye distinguishes colors better than different shades of brightness, thus with a pseudo-color image a tumor is much easier to localize. By comparing the red light resulting from the red fluorescence of PPIX and the green auto-fluorescence, which is also excited by the used light source, an accurate and detailed image of the lesions is created, including a relative measurement of fluorescence intensity on a cardinal ranking, making intra-patient fluorescence comparisons possible. By computing the relative intensities of the

PPIX and auto-fluorescence image, inhomogeneities in the fluorescence intensity due to imperfections of the excitation light field and inhomogeneities due to the curvature of the observed object are automatically corrected. The resulting image is referred to as “PPIX filtered” and is also presentable in pseudo-color (Fig.1 a-c).[43] These presentations support, amongst others, easy detection of AK’s, as they are clearly visible with a well defined compact core of high intensity, shown in red, and a surrounding halo of intermediate fluorescence levels which is also well defined with borders sharply demarcated against the normal tissue.

Comparison of the fluorescence properties of 5-ALA 0.5% liposomal spray with 5-methylaminolevulinic 16% cream (MAL, Metvix©, Galderma).

MAL16% cream was applied under occlusion on the right side of the forehead for 3 hours. Liposomal 5-ALA 0.5% spray was applied every 5 minutes on the left side of the forehead without occlusion for 2½ hours, followed by a pause of ½ hour to allow the 5-ALA liposomes to be absorbed completely into the skin. Immediately after this, fluorescence pictures were made of both sides of the forehead.

Establishment of the efficacy of FD, using liposomal encapsulated 5-ALA and a specialized version of the Dyaderm system for the detection of NMSC (including AK)

After admission, the patients were situated in a room with dimmed light, where they applied the 5-ALA spray every 5 minutes for two and a half hours to the involved skin area. Thereafter, the spray was left to be absorbed into the skin for half an hour more. Although the intensity of the blue pulsed light emitted from the fluorescence detection system is not harmful, theoretically it may trigger a seizure in patients with epilepsy. Therefore, patients who either suffered from epilepsy or had a close relative suffering from epilepsy were examined wearing dark eye goggles. Subsequently, pictures were taken using the fluorescence detection system. In order to minimise any disturbance from ambient light, the pictures were taken in a completely darkened room. The pictures were evaluated by a trained dermatologist. The general threshold level for demarcating tumor from normal skin (fluorescence ratio) was found to be 1.37 times the mean value of the fluorescence in the ALA treated area using 4 hours of ALA cream application.[44] In this study skin areas showing a red centre surrounded by a yellow halo (intensity of fluorescence in the red centre above 1.5 compared with the fluorescence of the surrounding normal skin) were considered to have a positive fluorescence pattern. From these skin areas of most interest, biopsies were taken under local anaesthesia with 1% lidocaine-adrenalin for histological diagnosis.

Ethics

The study protocol and associated information were submitted to and approved by

an ethics committee prior to the study. All investigations are in accordance to all applicable legal norms and non-legal standards.

Results

The fluorescence image of the right (MAL- treated) side of the forehead showed very high and homogeneous fluorescence intensity in most of the studied skin areas with low discrimination between normal and diseased skin (Fig. 2). The left (ALA-liposome treated) side showed low autofluorescence of the normal skin and moderate, but distinct fluorescence of actinic keratoses, resulting in a high discrimination between the normal and the diseased skin (Fig. 3). The 5-ALA-liposome solution seems to be highly superior to MAL for discrimination between normal skin and NMSC lesions (Fig 2 and 3). Moreover, the significantly lower uptake of 5-ALA in liposomes into the normal skin results in lesser degree of photosensitivlty and -toxicity and the known faster clearance of 5-ALA ensures a much better safety profile of the 0.5% 5-ALA lipsome preparation compared with MAL cream (8 versus 36 hours of photosensitivity) (Fig. 4.).

A positive fluorescence intensity was detected from 287 (100%) lesions in 61 (65.6%) of the patients. After histological examination the positive fluorescence appeared to be correlated to 212 (73.9%) benign lesions in 28 (30.2%) of the patients and to 75 (26.1%) (pre-) malignant lesions in 33 (35.5%) of the patients (Table 2). The benign lesion were histologically identified as sebaceous gland hyperplasia (204 = 71.1%) in 22 (23.7%) of the patients (fig. 5). Other benign lesions (8 = 2.8%) included viral warts, a benign lichenoid inflammation. Dysplastic melanocytic nevi, were found in 6 (6.5%) of the patients. The (pre-) malignant lesions were histological diagnosed as actinic keratosis (71 = 24.7%) in 29 (31.2%) of the patients. Notably, these lesions could readily be recognised by a high intensity core with well circumscribed peripheral medium intensity fluorescence (fig. 6 and 7). In 4 (4.3%) patients 4 (1.4%) malignant lesions were histologically confirmed to be 3 BCCs (Fig.1) and 1 SCC.

False negative fluorescence was found in only one lesion (a negative result by fluorescence detection of a lesion clinically suspected and histologically confirmed to be an AK). This lesion, situated on the vertex of the head was noted by the patient because of a thick hyperkeratosis and was clinical identified as actinic keratosis by the examining doctor (Fig. 8 and 9).

In 5 (5.4%) of the patients AKs, not noted by the patients nor by the examining doctors were identified by fluorescence detection and later confirmed by histological investigation. This means that in contrast to previous studies[42] the number of tumours assessed by the FD technique used in this study was higher than the number of tumours determined by clinical diagnosis.

Discusion

The occurrence of NMSC is increasing all over the world[2] and field cancerization, in which there are multiple (pre)malignant lesions diffusely spread over ultraviolet-exposed skin in particular, is an increasing problem. Up till now, physicians often need to take multiple biopsy specimens of the clinically malignant-suspect lesions, which can be a significant burden for the patient.[41] The need for complementary methods for detection and accurate demarcation of these skin cancers at an early stage is large in order to limit the damage caused by tumours.[41] In fluorescence diagnosis (FD) PpIX exhibits red fluorescence when excited with blue (405 nm) light after incubation of the skin with 5-ALA cream or MAL cream. PpIX accumulates to a higher extent in malignant lesions than in nonlesional skin.[41] The selective accumulation of Pp IX in the tumour tissue after ALA application is dependent on the permeability of ALA through the stratum corneum[45,46], the diffusivity and cellular uptake of ALA in the epidermis and dermis[45-47], the biosynthesis of PpIX[48] and the clearance time of ALA and PPIX.[45,46] In order to obtain a good fluorescent demarcation of tumors by FD it is important that the fluorescence contrast between the tumor and the normal skin is as high as possible.[31] Liposomes are very efficacious as drug carriers for introducing both water and fat soluble substances into the skin.[38, 49] The fluorescence from skin areas treated with 20% ALA cream under occlusion is very uniform, and hence there is only very low discrimination between normal and diseased skin. The fluorescence from skin areas treated with non-occluded liposome-encapsulated 0.5% 5-ALA is heterogeneously distributed, allowing for a high discrimination between normal and diseased skin.[32] Moreover, the lower uptake of 5-ALA-liposomes into the normal skin results in a lower risk of post-procedure phototoxicity and the faster clearance of 5-ALA from the tissues results in a shorter duration of this phototoxicity compared with MAL cream (8 versus 36 hours) (Fig. 4). This means, that patients are less restricted from outdoor activities following application of 5-ALA-liposomes compared with MAL cream.

In this study liposomal encapsulated 5-ALA and a specialized version of the Dyaderm® fluorescence detection system were used to detect positive fluorescence in 65.6 % of the patients. After histological examination the positive fluorescence appeared to be correlated to benign lesions in 30.2% of the patients and to (pre-) malignant lesions 35.5% of the patients (Table 2). The benign lesions were histologically identified as sebaceous gland hyperplasia 23.7% of the patients (figure 5). This is not surprising, because sebaceous hyperplasia is a benign lesion characteristic of ultraviolet damaged skin and liposomes penetrate very well into the sebaceous glands.[50] The benign lesions displaying positive fluorescence, could easily be identified as sebaceous gland hyperplasia on clinical examination because by stretching the skin they become clearly visible as yellow-white translucent papules. Other benign

lesions including viral warts, a lichenoid inflammation and dysplastic melanocytic nevi were found in 6.5% of the patients, indicating, that thickness and pigmentation of the epidermis does not considerably interfere with 5-ALA-liposomal FD. This is in contrast with a negative relation shown between the fluorescence emitted from the skin and the thickness of the epidermis using 5-ALA in a cream base.[41]

The (pre-) malignant lesions were histologically diagnosed as actinic keratosis in 31.2% of the patients, as BCC in 3.03% and as SCC in 1.01%. Notably in FD, these lesions usually show up as an irregularly defined medium to high intensity core with a well circumscribed surrounding medium intensity halo (figures 6 to 7). In 5 of the patients AKs not noted by the patients and also not by the examining doctors were identified by fluorescence detection and confirmed by histological diagnosis. Therefore, the FD technique used in this study appeared to be superior for the identification of (pre-) malignant lesions than clinical examination in contrast to the FD technique used in previous studies.[42] Only one patient showed a false negative fluorescence. This lesion, situated on the vertex, was noted by the patient because of a thick hyperkeratosis and clinical identified as actinic keratosis by the examining doctor and confirmed by histology (Figure 8). The fluorescence image of this patient showed no distinct discrimination between the lesion and the surrounding skin, very probably caused by the abundant fluorescence of the surrounding hairs and may be also by difficult penetration of the ALA into the highly keratinized lesion (Figure 9). This makes it likely, that FD is not an appropriate method to examine skin lesions situated on dense hairy sites of the body. Interference may also occur from dermal infections, viral verrucae and inflammatory diseases, like psoriasis.

This new non-invasive technique is time saving for the doctor, because it can be performed by auxiliary personnel presenting the physician with images which can be assessed at a glance. It is advisable to apply the current system in a professional environment. A custom robotic arm can be used during the fluorescence detection operation and a totally darkened room is needed. In addition, special rooms with dimmed light where the patients can apply the 5-ALA spray are required. This makes the system less suitable for the daily practice in a general hospital. The creation of specialized detection centres would make sense, especially given the large population which are at risk of acquiring a NMSC.

Future studies may optimize the correlation between fluorescence patterns and clinical and histological pictures, especially concerning the discrimination of penetrating from non-penetrating malignancies.

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Conclusions

In the field of fluorescence detection 5-ALA-liposomes appear to be superior to MAL cream for discrimination between normal skin, benign lesions and NMSC lesions.

Moreover, 5-ALA-liposomes are safer to use due to lesser- and shorter duration of post-procedure photo-toxicity compared with MAL cream.

A substantial percentage (23.7%) of the patients appeared to have positive fluorescence caused by sebaceous gland hyperplasia. Because sebaceous gland hyperplasia can easily be identified on clinical examination they did not interfere in the detection of NMSC. Interference may also occur from dermal infections, viral verrucae and inflammatory diseases, like psoriasis.

The FD technique used in this study appeared to be more sensitive for the identification of (pre-) malignant lesions than clinical examination in contrast to the fluorescence techniques used in previous studies.[42]

False negative fluorescence was found in only one lesion (1.01%), situated on the hairy scalp of the patient, indicating that FD may not to be an appropriate method for examining skin lesions situated on dense hairy sites of the body.

FD using liposomal encapsulated 5-ALA and a specialized version of the Dyaderm® system offers the possibility for detection of NMSC at an early, pre-clinical stage, and the technique can easily examine large areas of skin. It also identifies areas of most interest for performing confirmatory skin biopsies, as well as pre-operative assessment of boundaries of skin malignancies, and finally the technique is well suited for control of treatment.

References

1. Marks R. An overview of skin cancers. Incidence and causation. Cancer 1995; 75: 607-612.
2. English DR, Armstrong BK, Kricker A, Fleming C. Sunlight and cancer. Cancer Causes Control 1997; 8: 271-283.
3. Salasche SJ. Epidemiology of actinic keratoses and squamous cell carcinoma. J Am Acad Dermatol 2000; 42: S4-7.
4. Diepgen TL, Mahler V. The epidemiology of skin cancer. Br J Dermatol 2002; 146, Suppl 61: 1-6.
5. Trakatelli M, Ulrich C, del Marmol V, Euvrard S, Stockfleth E, Abeni D. Epidemiology of nonmelanoma skin cancer (NMSC) in Europe: accurate and comparable data are needed for effective public health monitoring and interventions. Br J Dermatol 2007; 156, S 3: 1-7.
6. Marks R, Rennie G. Malignant transformation of solar keratoses to squamous cell carcinoma. Lancet 1988: 795-797.
7. Jorizzo JL, Carney PS, Ko WT, Robins P. Treatment options in the management of actinic keratosis. Cutis 2004; 74 (S 6): 9-17.
8. Epstein E. Quantifying actinic keratosis: assessing the evidence. Am J Clin Dermatol 2004; 5: 141-144.

9. Ackerman AB, Mones JM. Solar (actinic) keratosis is squamous cell carcinoma. Brit J Dermatol 2006; 155: 9-22.
10. Ceilly RI. Modalities and mechanisms of action. In: The field treatment of actinic keratosis. Skin Aging 2004; October (suppl.): 3-5.
11. Oppel T, Korting HC. Actinic keratosis: the key event in the evolution from photodaged skin to squamous cell carcinoma. Skin Pharmacol Physiol 2004; 17: 67-76.
12. Dodson JM, DeSpain J, Hewett JE, Clark DP. Malignant potential of actinic keratoses and the controversy over treatment: a patient-oriented perspective. Arch Dermatol 1991; 127: 1029-1031.
13. Rossi R, Mori M, Lotti T. Actinic keratosis. Int J Dermatol 2007; 46: 895-904.
14. Housman TS, Feldman SR, Williford PM, Fleisher AB. Skin cancer is among the most costly of all cancers to treat for Medicare population. J Am Acad Dermatol 2003; 48: 425-429.
15. Armstrong BK, Kricker A. The epidemiology of UV induced skin cancer. J Photochem Photobiol B: Biol 2001; 63: 8-18.
16. Young AR. Tanning devices-fast track to skin cancer? Pigment cell Res 2004; 17: 2-9.
17. MacKie RM. Long-term health risk to the skin of ultraviolet radiation. Prog Biophys Mol Biol 2006; 92: 92-96.
18. The international agency for research on cancer working group on artificial ultraviolet light and skin cancer. The association of use of sunbeds with cutaneous malignant melanoma and other skin cancers: a systemic review. Int J Cancer 2006; 120: 1116-1122.
19. Nijsten TEC, Stern RS. The increased risk of skin cancer is persistent after discontinuation of psoralen + ultraviolet A: a cohort study. J Invest Dermatol 2003; 121: 252-258.
20. Lim JL, Stern RS. High levels of ultraviolet B exposure increase the risk of non-melanoma skin cancer in psoralen and ultraviolet A-treated patients. J. Invest Dermatol 2005; 124: 505-513.
21. Vitasa BC, Taylor HR, Strickland PT, Rosenthal F, West S. Association of non-melanoma skin cancer and actinic keratosis with cumulative solar ultraviolet exposure in Maryland watermen. Cancer 1990; 65: 2811-2817.
22. Buja A, Lange JH, Perissinotto E, Rausa G, Grigoletto F, Canova C, Mastrangelo G. Cancer incidence among male military and civil pilots and flight attendants: an analysis on published data. Toxicol Ind Health 2005; 21: 273-282.
23. Moehrle M. Outdoor sports and skin cancer. Clin Dermatol 2008; 26: 12-15.
24. Schwartz RA, Bridges TM, Butani AK, Ehrlich A. Actinic keratosis: an

occupational and environmental disorder. J Eur Ac Dermatol Venereol 2008: 1-9.
25. Masini C, Fuchs PG, Gabrielli F, Starks S. Evidence for the association of human papillomavirus infection and cutaneous squamous cell carcinoma in immunocompetent individuals. Arch Dermatol 2003; 139: 890-894.
26. Euvrard S, Kanitakis J, Claudy A. Medical progress: Skin cancers in organ transplant recipients. N Engl J Med 2003; 348: 1681-1691.
27. De Hertog SAE, Wensveen CAH, Bastiaens MT, Kielich CJ, Leiden skin cancer study. Relation between smoking and skin cancer. J Clin Oncol 2001; 19: 231-238.
28. Jemal A, Thomas A, Murray T, Thun M. Cancer statistics, 2002. Ca Cancer J Clin 2002; 52: 23-47.
29. Venna SS, Lee D, Stadecker MJ, Rogers GS. Clinical recognition of actinic keratoses in a high-risk population. Arch Dermatol 2005; 141: 507-509.
30. Smits T, Kleinpenning MM, Blokx WAM, Kerkhof PCM, van Erp PEJ, Gerritsen MP. (2007). Fluorescence diagnosis in keratinocytic intraepidermal neoplasias. J Am Acad Dermatol 2007; 57: 824-831.
31. Trakatellis A, Gerochristos I, Trakatelli M. The programme of community action in the field of health (2007-2013). Pharm Policy Law 2005, 2006; 8: 3-11. IOS Press.
32. Ericson MB, Sandberg C, Gudmundson F, Rosen A, Larkö O. Fluorescence contrast and threshold limit: implications for photodynamic diagnosis of basal cell carcinoma. J Photochem Photobiol B: Biology 2003; 69: 121-127.
33. Svaasand LO, Wyss P, Wyss MT, Tadir Y, Tromberg BJ, Berns MW. Dosimetry model for photodynamic therapy with topically administered photosensitizers. Lasers Surg Med 1996; 18: 139-149.
34. Moan J, Ma LW, Iani V. On the pharmacokinetics of topically applied 5-aminolevulinic acid and two of its esters. Int J Cancer 2001; 92: 139-143.
35. Ackermann G, Abels C, Baumler W, Langer S, Landthaler M, Lang EW, Szeimies RM. Simulations on the selectivity of 5-aminolevulinic acid-induced fluorescence in vivo. J Photochem Photobiol B: Biol 1998; 47: 121-128.
36. Hua Z, Gibson SL, Forster TH, Hilf R. Effectiveness of delta-aminolevulinic acid-induced protoporphyrin as a photosensitizer for photodynamic therapy in vivo. Cancer Res 1995; 55: 1723-1731.
37. Christiansen, K., Bjerring, P., & Troilius, A. (2007). 5-ALA Photodynamic photorejuvenation-optimization of treatment regume based on normal-skin fluorescence measurements. Lasers Surg Med 2007; 39: 302-310.
38. Hope M.J., Kitson C.N. Liposomes: A Perspective for Dermatologists.

Dermatol Clin 1993; 11: 143-154.
39. Lieb L, Ramchandran C, Egbaria K, Weiner N. Topical delivery enhancement with multilamellar liposomes into pilosebaceous units. J. Invest Dermat 1992; 99: 108-113.
40. Artmann C, Roding J, Ghyczy M, Prazel HG. Liposomes from soya phospholipids as percutaneous drug carriers. Arzeim Forsch 1990; 40: 1365-1368.
41. Cevc G, Blume G. Lipid vesicles penetrate into intact skin owing to the transepidermal osmotic gradients and hydration force. Biochim Biophys Acta 1992; 1104: 226-232.
42. Plessis J, Egbaria K, Weiner N. Influence of formulation factors on the deposition of liposomal components into the different strata of the skin. J Soc Cosmet Chem 1992; 43: 93-100.
43. Ostro M. Liposomes. Sci Am 1987; 256: 102-111.
44. Verma DD, Verma S, Blume G, Fahr A. Liposomes increase skin penetration of entrapped and nonentrapped hydrophilic substances into human skin: a skin penetration and confocal laser scanning microscopy study. Eur J Pharm Biopharm 2003; 55: 271-277.
45. Bäumler W, Abels C, Szeimies RM. Fluorescence diagnosis and todynamic therapy in dermatology. Med Laser Appl 2003; 18: 47-56.
46. Fauteck J, Ackermann G, Birkel M, Breuer M, Moor ACE, Ebeling A, Ortland C. Fluorescence characteristics and pharmacokinetic properties of a novel self-adhesive 5-ALA patch for photodynamic therapy of actinic keratoses. Arch of Dermatol Res 2008; 300: 53-60.
47. Gambichler T, Moussa G, Altmeyer P. A pilot study of fluorescence iagnosis of basal cell carcinoma using a digital flash light-based imaging system. Photodermatol Photoimmunol Photomed 2008; 24: 67-71
48. Internet information: http// :www DyaDerm.
49. Wennberg AM, Gudmundson F, Stenquist B, Ternesten A, Molne L, Rosen A, Larkö O. In vivo detection of basal cell carcinoma using imaging spectroscopy. Acta Dermatol Venereol 1999; 79: 54-61.
50. Bernard E, Bubois JL, Wepeirre J. Importance of sebaceous glands in cutaneous penetration of an antiandrogen: target effect of liposomes. J Pharm Sci 1997; 86: 573-578.
51. Zaballos P, Ara M, Puig S, Malvehy J. Dermoscopy of sebaceous hyperplasia. Arch Dermatol 2005; 141: 808.
52. Stolz W, Braun-Falco O, Bilek P, Landthaler M, Burgdorf WHC, Cognetta AB. Color Atlas of Dermatoscopy, second edition. Blackwell Wissenschafts-Verlag Berlin. Blackwell Plubishing Company 2002: 31-35.

Differential diagnosis of actinic keratosis	
benign	malignant
kerarosis (seborrheic, stucco, arsenical, lichenoid)	Bowen's disease
porokeratosis	basal cell carcinoma
keratoacanthoma	squamous cell
carcinoma	
lentigo solaris	
discoid lupus erythematodes	
psoriasis	

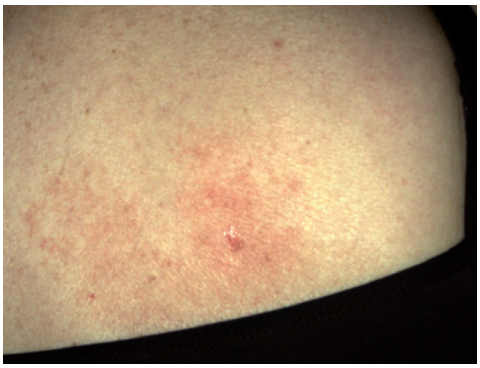
Table 1.

Total 93 patients	Number of lesions	% of lesions	Number of patients	% of Patients
No fluorescence	0	0	32	34,3
Positive fluorescence	287	100%	61	65,7
Sebaceous	204	71,1%	22	23,7
gland hyperplasia				
Other benign lesions	8	2,8%	6	6,5
Actinic Keratosis	71	24,7%	29	31,2
Malignant lesions	4	1,4%	4	4,3

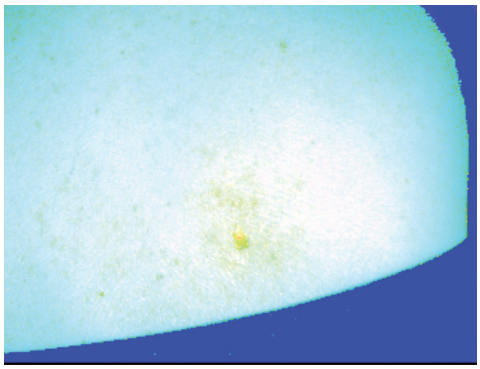
Table 2.

Figure 1.

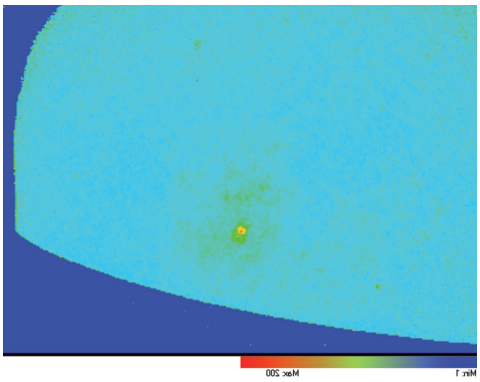
The sequence of images from the skin of a female patient with a histologically confirmed basal cell carcinoma on the upper back:



a. Colour image from the upper back



b. Fluorescence image showing the skin tumor position.



c. Processed image, where in pseudo-color the tumor position, known from the fluorescence image, is superimposed and blended into the original image. Irregularly defined fluorescence showing medium (yellow) intensity.

Figure2

Fluorescence image right side of the forehead, 3 hours after application of MAL cream under occlusion: High fluorescence in most of the studied skin area, showing low discrimination between normal and diseased skin.

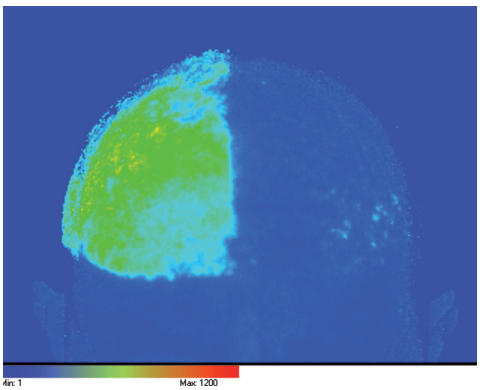


Figure 3
Fluorescence image left side of the forehead 3 hours after application of 5-ALA 0.5% liposomal spray (every 5 minutes for 2 ½ hours and ½ hour more for optimal absorption of 5-ALA into the skin) without occlusion: Low fluorescence from the normal skin in the studied skin area and distinct fluorescence from actinic keratoses, resulting in a high discrimination between normal and diseased skin.

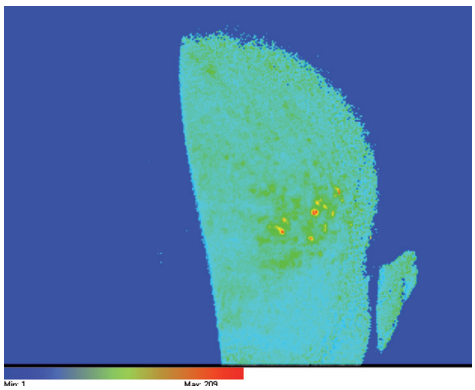


Figure 4
Fluorescence image forehead 36 hours after MAL cream right side and 36 hours after 5-ALA liposomal spray left side. The fluorescence of 5-ALA liposomal spray is of lower intensity and of lower duration compared to MAL cream.

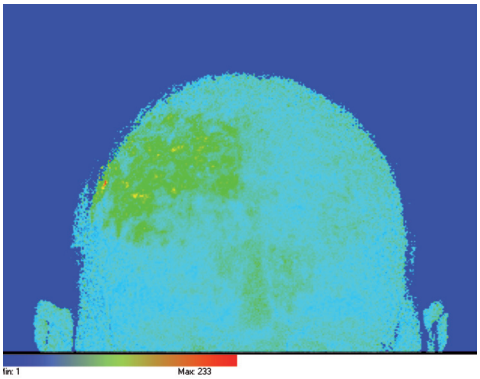
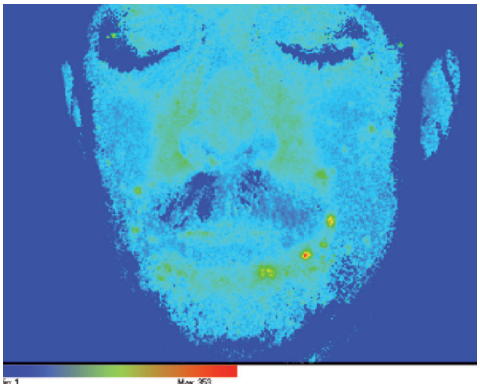


Figure 5
Fluorescence image from sebaceous gland hyperplasia: Circumscribed, regularly defined fluorescence showing medium (yellow) to high (red) intensity.



Chapter 14

Summary, general discussion and recommendations for further investigations

14.1 *Introduction*

Topical treatment has been used for centuries in skin diseases because they are visible and directly accessible. Since the introduction of arsenic in the 19th century as a systemic treatment for psoriasis, an increasing range of systemic drugs are available for the treatment of numerous skin diseases. However, none of the topical and systemic agents is effective in all patients and adverse effects limit their use. Therefore, an ideal drug for most skin diseases still remains to be developed. Although on the one hand, systemic drugs have a higher therapeutic potency than topically applied drugs, on the other hand, they cause more serious adverse effects.[1] The central subject of this thesis was to promote patient safety, because it should be a key focus of every dermatologic practice.[2,3] The aims of the investigations that were pursued are summarized in **chapter 1.**

14.2 *Phototherapy, fluorescence detection and liposomes*

Phototherapy and photo-chemical therapy are positioned between topical therapy and systemic therapy because they have topical- and systemic effects. The dilemma in the classic light treatment using different ultraviolet (UV) light sources is the risk of skin cancer. This has led to a conflicting situation in which dermatologists, on the one hand, advice the public to limit the exposure of their skin to UV light and on the other hand, advice patients suffering from certain skin disorders to undergo treatment with UV light. Long-term observations of psoriasis patients who were first treated with UV light and later on with cyclosporine ran a higher risk of Non-melanoma skin cancers (NMSCs) as compared with controls.[4] This highlights that the occurrence of iatrogenic complications, particularly iatrogenic tumors have to be minimized. In the same context, it is the dermatologist's duty to detect skin cancer at an early stage in order to avoid unnecessary damage to the patients from the complications of advanced tumors or by the complications of extensive surgery or chemotherapy.

A new approach is the use of light not of the wavelengths from the UV spectrum provided by lasers, intense pulsed light (IPL) systems and light emitting diodes (LED). These devices may provide the possibility to banish the risk of carcinogenesis from UV light treatment. The basis of the pulsed dye laser (PDL) therapy of vascular lesions is the destruction of abnormal vessels by the absorption of laser energy

without damaging the surrounding tissues (selective photothemolysis).
The goal of photodynamic therapy (PDT) is the selective destruction of targeted abnormal cells while preserving the normal cells. The key principle is the preferential accumulation of 5-aminolaevulinic acid (5-ALA), after application to the skin, in cells with an elevated metabolism (such as tumor cells). The higher concentration of 5-ALA, which is not a photosensitizer by itself, results in a higher concentration of protoporfyrin IX (PpIX), which causes a cascade effect of radical oxygen species after illumination with the proper wavelengths. The higher PpIX concentration ratio in tumor cells compared with normal cells is used in PDT to destroy selectively the diseased tissue and in fluorescence detection to visualize the diseased cells. Until recently, clinically approved indications were restricted to actinic keratoses (AK), superficial and nodular basal cell carcinoma (BCC) and Bowen's disease (intra-epithelial carcinoma). The range of indications has been expanding continuously. Nowadays, PDT is also used for treating non-malignant disorders such as acne vulgaris, verrucae vulgaris, leishmaniasis and skin ageing. Various guidelines on PDT have been published, but complete consensus has not yet been reached. Liposomes are microscopic vesicles formed from phospholipids as biological membranes and may be used as carriers for hydrophilic as well as lipophilic therapeutic agents. Low concentrations of drugs in liposomes may be as efficacious as higher doses in conventional preparations, but with reduced adverse effects from topical as well as from systemic absorption. The basic aspects of light absorption, photodynamic therapy, fluorescence detection and liposomes are reviewed in **chapter 2.**

14.3 *Major light-related dermatoses*

Psoriasis, vitiligo, acne and NMSCs have a relationship with light and all of them are commonly encountered at a general dermatological practice. The epidemiology, the clinical aspects and the pathogenesis of these diseases together with the advantages and the disadvantages of their treatments are described in **chapter 3.**

14.4 *PDL treatment of plaque type psoriasis*

Although psoriasis is an immune-mediated disease, the prominent increase in the dermal microvasculature in the lesional skin indicates that psoriasis is also angiogenesis-dependent. Traditional therapies for psoriasis have generally focused on the inhibition of epidermal proliferation and /or inflammation. The abnormities in the capillary vasculature have been therapeutically neglected. Partial- and also total clearance of psoriasis after selective photothermolysis of the dermal vasculature by the PDL was reported in several studies. This laser was used to treat 74 patients with chronic plaque type psoriasis unresponsive to conventional topical therapies. After laser treatment, improvement appeared to be good to very good in 73% of patients as described in **chapter 4**.

14.5 *Psoriasis palmoplantaris*

Psoriasis of the hands and the feet is a distressing and disfiguring disease, often resistant to treatment, especially in case of pustulosis palmoplantaris. A group of 41 patients with therapy-resistant psoriasis in the hands and in the feet were treated once every 4 to 6 weeks with PDL. Concomitant keratolytic treatment with calcipotriol ointment or salicylic acid 5% to 10% ointment was used. After an average of 4.2 treatment sessions, more than 70% clearance was achieved in 76% of the patients. Transient purpura occurred after each treatment session, crusts were noted in only 3% of the patients. The mean duration of remission after the final treatment was 10.7 months. A relapse was defined as recurrence of psoriasis to 30% or more of the baseline. In many clinical studies, ‘‘remission’’ and ‘‘relapse’’ are not actively defined. Not all the investigators tend use the same definitions even when these terms are defined. The least stringent definition of relapse involves a recurrence of psoriasis to 50% of the baseline. The strictest definition involves any recurrence of psoriasis after clearing. The results of this open prospective study showed that concomitant treatment with PDL and topical calcipotriol or salicylic acid was a satisfactory modality for treating psoriasis in the hands and in the feet with a subjective improvement in the symptoms and the quality of life in all patients as described in **chapter 5.**

14.6 *PDL compared with UVB in the treatment of plaque type psoriasis*

Recent breakthroughs in the treatment of psoriasis have led to a better understanding of the pathogenesis of this disease. The traditional systemic psoriasis therapies such as Methotrexate (MTX), Cyclosporine (Cs) and mycophenolate mofetil (MMF) may be associated with an increased risk of lymphoproliferative disorders during treatment. The risk of malignancy with biologic therapy, such as TNF-α blockers, is still unclear. However, the majority of studies examining this carcinogenic risk reported that TNF-α inhibitors may cause a slightly increased risk of cancer, including NMSCs and hematological malignancies.[5,6] The status of TNF-α inhibitors in relation with pan-epidemic threat and multi-resistance of tuberculosis is not yet clear, but is a subject of concern. Narrow-band ultraviolet-B light therapy (UVB) is highly efficacious against psoriasis. In contrast to UVB and psoralen ultraviolet-A (PUVA) therapy, PDL has no carcinogenic capacity. UVB targets primarily the structures in the epidermis, whereas PDL light passes through the epidermis and targets primarily the superficial vessels in the dermis indicating that UVB and PDL clear psoriasis via different pathways. The aim of the study described in **chapter 6** was to evaluate the efficacy and the safety of PDL treatment in plaque type psoriasis, to compare the results of PDL treatment with those of the UVB treatment and with those of the PDL+UVB treatment, and to look for a synergistic effect of PDL+UVB treatment on

psoriasis. PDL and UVB, as well as the combination therapy PDL+UVB, resulted in a statistically significant improvement. PDL was as effective as UVB treatment. A combination of PDL and UVB treatment had no additional value because of the lack of synergism. Surprisingly, no statistically significant differences were noted between UVB treatment and PDL treatment on the one hand, and the neighboring non-treated (NT) sides, on the other hand. There is no clear explanation for this, but an unexpected "carry-over" effect from the neighboring PDL- and UVB-treated sides is supported by the observation of an inverse relationship between improvement of NT sides and the distance between the PDL treated sides and the NT sides. These findings warrant further studies, because they indicate that overlapping spot sizes may not be necessary and this implies multiple benefits such as a reduction in the duration of treatment, less discomfort to the patients, and lower costs. Another possibility is a systemic effect on the NT sides due to the treatment of the remaining plaques. After all, it seems likely that dividing of plaques is not the best approach to study the efficacy of topical therapies, but if pursued, the treatment must be strictly restricted to the study plaques without treatment of other remaining plaques. In the present study, patients with a complete clearance of the target lesion appeared to be divided into either PDL responders or UVB responders indicating that patients who do not respond to UVB may respond to PDL and vice versa. Treatment with PDL resulted only in mild and transient adverse effects and therefore appeared to be safe. PDL treatment should not be used in widespread psoriasis or in patients who respond adequately to simple topically applied treatments. PDL treatment should be reserved for patients with persistent plaque type psoriasis, not responding to usual treatments (particularly on ''non-covered body'' sites), plaque type psoriasis complicated by adverse effects of medication, psoriasis palmoplantaris, psoriasis inversa, patients not willing to use topical corticosteroids or systemic treatment, and patients at risk for skin cancer caused by actinic damage or immunosuppression. Most patients in our study preferred PDL therapy above UVB.

The results of PDL treatments may be improved by:

- Increasing the number of sessions.
- Reducing the time intervals between treatments.
- Increasing the fluence and the pulse width to, respectively, 10 J/cm^2 and 1.5 msec.
- Application of optical coupling gel immediately prior to the PDL treatment.
- Concomitant treatment with calcipotriol ointment.

Further studies may provide additional insights for optimizing the treatment.

14.7 *Cellular and molecular effects of pulsed dye laser and narrow-band UVB therapy in psoriasis*

In a new psoriatic plaque, dilation and proliferation of the dermal papillary microvasculature is one of the early changes seen. Increase in the dermal microvasculature facilitates trafficking of immune cells into the skin, and is therefore important for maintenance of inflammation in psoriasis. Reduction of the hyper-proliferation of the papillary microvasculature by selective photothermolysis using PDL may lead to a reduction of immune cell trafficking between the vasculature and the interstitium of the skin, resulting in clearance of psoriasis plaqes. From the studies described in **chapter 7**, it appeared that clinical improvement of psoriasis after PDL treatment is accompanied by alterations in some classic markers of psoriasis disease activity. Clinically high responders to PDL treatment showed decreased expression of VEGFR2, VEGFR3 and E-selectin within 24 hours after PDL treatment. After 2 PDL treatments IL-23 and TNF-α mRNA and E-selectin protein expression were significantly decreased, whereas after 4 treatments all epidermal markers and dermal T cell infiltrates normalized. In UVB-treated plaques, epidermal activation markers and E-selectin expression were significantly decreased after 13 weeks. In responders to PDL and NB-UVB treatment, the effects observed at the mRNA level were comparable. The lower clinical efficacy of UVB in our study (52% improvement versus minimally 60% reported for total body irradiation) may be explained by the fact that UVB treatment was used focally on selected psoriasis plaques instead of total body irradiation. It is assumed that total body UVB irradiation also exerts a systemic effect. At the end of the treatment period both PDL and UVB resulted in reduced expression of epidermal activation markers and reduced dermal T cell infiltrates. An interesting observation was the down regulation of IL-23 during PDL treatment, because it was recently reported that Th17 cells played a major role in the pathogenesis of psoriasis. This T helper cell is stimulated by IL-23. A new biological blocks IL-23 and seems to be effective in psoriasis, indicating that PDL and this biological share the same target. The down-regulation of TNF-α and IL-23p19 may contribute to the efficacy of PDL treatment in psoriasis.

14.8 *Liposomes*

Liposomes have a high potential for topical therapy in Dermatology. Although the skin is an organ, which is directly accessible for topical application of drugs, it does not imply that drugs incorporated in conventional transport media such as lotions, creams and ointments are delivered at the optimal concentration to the right target structure in the skin. Liposomes can be used as carriers for hydrophilic as well as lipophilic therapeutic agents. They may improve stabilization of instable drugs by encapsulating them and serve as penetration enhancers facilitating the transport

of compounds through the epidermis that otherwise cannot penetrate the skin. Liposomes will help in reducing skin irritation by sustaining the release of the drugs and by hydration of the epidermis. They also have the potential to target drugs into the pilosebaceous structures and hence they can be used for treating disorders associated with sebaceous glands and hair follicles. Low concentrations of drugs in liposomes are as effective as higher doses in conventional preparations, but with reduced adverse effects from topical as well as from systemic absorption of drugs. Promising results have been obtained with liposomal encapsulated drugs in the treatment of acne, xerosis cutis, atopic dermatitis, psoriasis, vitiligo, superficial vein thrombosis and hair removal. 'Empty' liposomes (without encapsulated drugs) hydrate the skin simply by contributing lipids to the stratum corneum. Moreover, the water content of the liposomes promotes further hydration of the skin. Thus, liposomes may replace the classic emollients. Liposomes will also help to reduce skin irritation to drugs (e.g. tretinoin) by hydration of the epidermis, resulting in better acceptance and reliability by the patients.

PDT is widely used to treat AK and superficial BCC. In addition, PDT is increasingly used to treat acne and for skin-rejuvenation. Side-effects limit patient's acceptance, especially in case of non-malignant skin disorders. Changing the 5-aminolevulinic acid (ALA) vehicle from a moisturizing cream to liposome encapsulation lowers its concentration by a factor of 40 still inducing the same skin fluorescence and at the same time eliminating the need for occlusion. Another advantage is reduction of costs. The in and outs of liposomes are dealt with in **chapter 8**

The following studies on liposomes may be of value:

- 5-ALA liposomes and daylight for the treatment of acne.
- 5-ALA liposomes and daylight for the prevention of skin aging, AKs and superficial BCCs.
- 5-ALA liposomes and IPL or PDL for skin rejuvenation.
- T4-endonuclease-V in liposomes for the treatment and the prevention of NMSC.
- Cyclosporine in liposomes for the treatment of severe psoriasis and atopic eczema.
- Methotrexate in liposomes for the treatment of severe psoriasis.
- "Empty" liposomes or fillagrin containing liposomes for the treatment of dry skin and atopic dermatitis.

14.9 *Treatment of vitiligo with liposomal khellin in combination with UV light therapy*

Vitiligo is a non-life threatening dermatosis, which has a serious psychological impact and affects of the quality of life of the patient. Vitiligo is histologically characterized by

the absence of melanocytes in the epidermis. However, the melanocytes in the hair follicles are generally not affected. Therapeutic approaches are aimed at reversing the progressive loss of melanocytes and reconstituting normal skin coloration. During the process of re-pigmentation, the follicular melanocytes proliferate and migrate into the epidermis. Common methods for treatment are topical corticosteroids, tacrolimus, systemic and topical PUVA, UVB, and topical khellin with UVA (KUVA). However, none of these is uniformly effective. Khellin has phototherapeutic properties that are similar to those of the psoralens, but with substantially lower phototoxic effects and DNA mutation effects. Data on the current status of khellin in the treatment of vitiligo were inconclusive. The selection of the vehicle in which khellin is incorporated is highly important for the adequate availability of khellin in the skin. Encapsulating khellin in phosphatidylcholine liposomes enhances the availability of khellin in the hair follicles facilitating the stimulation of the melanocytes. The efficacy and the safety of the treatment with khellin encapsulated in L-phenylalanine stabilized phosphatidylcholine liposomes in combination with UVA/UVB light therapy was evaluated in an open, retrospective study described in **chapter 9**. A re-pigmentation response of 50% to 100% was achieved in 72% of treated locations after a mean treatment period of 12 months. The re-pigmentation was not equal for different parts of the body.

14.10 *Blister roof transplantation (BRT) for vitiligo*

Surgical techniques are generally reserved for stable and refractory vitiligo. The transplantation of epidermal blister grafts on the de-pigmented areas is safe, easy to use, has a maximum expansion rate of pigmentaion and is without any scarring as described in the study in **chapter 10.**

14.11 *Combination of liposomal khellin and UV light treatment with blister roof transplantation for refractory vitiligo*

Recent guidelines on the treatment of vitiligo recommended UVB therapy only in patients who cannot be adequately managed with conservative treatments, who have widespread vitiligo or have localized vitiligo associated with a significant impact on the patient's quality of life (QoL). However, in many patients refractory areas may remain. Photo-medical treatment such as PUVA and khellin with ultraviolet light (KLUV) may be efficacious where other treatments have failed. It seems rational to prefer KLUV because khellin has phototherapeutic properties that are similar to those of the psoralens, but substantially lower phototoxic effects and DNA mutation effects. Moreover, KLUV results in disease stability in almost all case, which is an important requirement for the success of surgical therapy. The procedure and the results of patients who had been treated for at least 1 year with KLUV followed by BRT for

refractory vitiligo lesions are described in **Chapter 11**. Post-surgery photo-medical treatment (KLUV) is necessary to obtain enough peripheral spread of the pigment to cover the entire de-pigmented area. Further studies may evaluate the individual role of BRT, UV and Khellin. Improvements in the blister roof transplantation technique may be achieved via:

- Applying a corticosteroid cream or a calcineurin cream to the recipient area after the grafts have been taken in order to avoid activation of the vitiligo process caused by the ablation procedure (Köebner phenomenon) or spontaneous aggravation of vitiligo.
- A medical intervention for the underlying causes of melanocyte destruction (e.g. Efalizumab).
- Suction blister roof ablation of the recipient area instead of the ablation with the Erbium laser in order to obtain the same thickness of the removed skin and the transplanted skin.

14.12 *PDT of acne vulgaris using 5-ALA 0.5% liposomal spray and IPL in combination with topical keratolytic agents*

The search for new efficacious and safe treatment modalities for acne is justified because of the increasing antibiotic resistance of Propionibacterium acnes and the growing awareness on the adverse effects of conventional topical and systemic anti-acne drugs. Although, the efficacy of PDT using 20% 5-ALA cream has been established, phototoxic adverse effects limit its use. Liposomes have the potential to carry lipophilic drugs and hydrophilic drugs such as 5-ALA into the pilosebaceous structures. It was reported that by changing the vehicle of 5-ALA from a moisturizing cream to liposome encapsulation the 5-ALA concentration can be lowered to 0.5% providing a similar level of induced skin fluorescence and eliminating the need for occlusion. The low post-treatment fluorescence also significantly reduced the risk of post-treatment phototoxicity. A study to assess the efficacy and the safety of PDT using 5-ALA 0.5% in liposomal spray and IPL in combination with a peeling cream (Li-PDT-PC) in acne vulgaris is described in **chapter 12.**

The results of this treatment may be improved by:

- Using double passes with the IPL on inflammatory lesions.
- Reducing the treatment intervals to 2 or 3 weeks.

14.13 *Non-melanoma skin cancer and fluorescence detection*

Non-melanoma skin cancer (NMSC) is the biggest challenge for the next two decades in Dermatology. The expected dramatic rise in the prevalence of skin cancer will lead to a work-overload at all the dermatological practices if no serious attempts are made to educate the general public on prevention to curb the problem. Even with the

foreseen increase of the number of dermatologists in the Netherlands, this problem is too immense to handle if we continue to carry on in the same way and the settings. Screening patients for (pre-) malignant lesions will allow early detection and prompt treatment. Diagnostic skin fluorescence using liposomal encapsulated 5-ALA and a specialized computerized detection and visualization system offers the possibility for detection of NMSC at an early (pre-clinical) stage. This fluorescence detection (FD) technique is well-suited to examine large areas of the skin. It also identifies areas of most interest for obtaining confirmatory skin biopsies, as well as pre-operative assessment of the boundaries of skin malignancies. The technique is also useful in the control and the follow-up of skin cancer treatment. The technique and the results of FD are described in **chapter 13.**

Further studies on fluorescence detection are essential to optimize the correlation between the fluorescence patterns and the clinical and the histological pictures, especially for distinguishing the penetrating from the non-penetrating malignancies. Additional studies on the efficacy of PDT using IPL or PDL of AKs and NMSC directly after the fluorescence detection in the same session may offer a highly efficient treatment modality.

Samenvatting, algemene discussie en aanbevelingen voor verder onderzoek.

14.14 *Introductie*

Uitwendige geneesmiddelen zijn eeuwenlang gebruikt voor de behandeling van huidziekten, omdat de huid zonder hulpmiddelen direct toegankelijk is. Sinds de introductie van arsenicum in de 19e eeuw voor de behandeling van psoriasis zijn er vele systematisch toepasbare geneesmiddelen ter beschikking gekomen om talrijke huidziekten te behandelen. Geen van deze locale en systematische middelen zijn bij alle patiënten effectief en bijwerkingen kunnen het gebruik ervan beperken. Voor de meeste huidziekten moet daarom een ideaal geneesmiddel nog worden ontwikkeld. Systematische geneesmiddelen hebben doorgaans een grotere therapeutische potentie dan locale middelen, maar kunnen aan de andere kant ook ernstiger bij werkingen hebben.[1] Het centrale thema van deze thesis is om de veiligheid van de patiënt te bevorderen, omdat dit beschouwd moet worden als de voornaamste doelstelling van iedere behandeling en dus ook in de dermatologische praktijk.[2,3] De doelstellingen van de onderzoekingen die werden uitgevoerd zijn samengevat in **hoofdstuk 1.**

14.15 *Phototherapie, fluorescentie detectie en liposomen*

Phototherapie en photochemotherapie hebben locale en systeem effecten en zijn daarom gepositioneerd tussen locale therapie en systematische therapie. Het dilemma in de klassieke licht therapie, waarbij ultraviolet (UV) lichtbronnen worden gebruikt, is het risico op huidkanker. Dit heeft geleid tot een conflictueuze situatie, waarin dermatologen aan de ene kant het publiek adviseren om de blootstelling van de huid aan UV licht te beperken en aan de andere kant patiënten met bepaalde huidziekten juist adviseren om behandelingen met UV licht te ondergaan. Observaties op langere termijn van psoriasis patiënten, die eerst zijn behandeld met UV licht en later met cyclosporine, hebben aangetoond, dat deze patiënten een groter risico lopen op het krijgen van non-melanoma huid kanker (NMSC) dan de controle patiënten.[4] Deze bevindingen onderstrepen dat het optreden van iatrogene complicaties, vooral van iatrogene tumoren geminimaliseerd dienen te worden. In dezelfde context is het de taak van de dermatoloog om huidkanker in een vroeg stadium op te sporen met het doel om onnodige schade van de patiënt door complicaties van uitgebreide tumoren of door de complicaties van uitgebreide chirurgie of chemotherapie te vermijden. Een nieuwe benadering is het gebruik van licht zonder de UV golflengten, dat geproduceerd wordt door lasers, intense pulsed light (IPL) systemen en light emitting diodes (LED). Deze apparaten maken het mogelijk om het risico op huidkanker door UV licht uit te bannen. De basis van de pulsed dye laser (PDL) behandeling van bloedvatafwijkingen is de destructie van abnormale bloedvaten door absorptie van laser licht energie zonder schade aan te brengen aan de omringende weefsels (selectieve photothermolysis).

Het doel van photodynamische therapie (PDT) is de selectieve destructie van abnormale cellen waarbij de normale cellen gespaard blijven. Het basis principe hiervan is, dat 5-aminolevulinezuur (5-ALA) na applicatie op de huid in hogere concentraties wordt opgenomen door cellen met verhoogde stofwisseling (bv. tumor cellen) dan door normale cellen. De hogere concentratie van 5-ALA, dat zelf geen photosensitieve eigenschappen bezit, leidt tot hogere concentratie in de abnormale cellen van protoporfyrine IX (PpIX), dat na belichting met de juiste golflengten een cascade effect teweegbrengt door de vorming van radicale zuurstof species. De hogere concentratieratio van PpIX in tumorcellen ten opzichte van normale cellen wordt gebruikt in de PDT om selectief het aangetaste weefsel te vernietigen en in de fluorescentie detectie (FD) om de aangetaste cellen zichtbaar te maken. Tot voor kort waren de geautoriseerde klinische indicaties van PDT beperkt tot actinische keratoses (AK), superficiële en nodulaire basaalcel carcinomen (BCC) en M. Bowen (intra-epitheliaal carcinoom). De rij van indicaties wordt continue uitgebreid. Tegenwoordig wordt PDT ook gebruikt bij de behandeling van niet kwaadaardige huidaandoeningen, zoals acne vulgaris, verrucae vulgares, leishmaniasis and

huidveroudering. Meerdere richtlijnen op het gebied van de PDT zijn gepubliceerd, maar complete concensus is nog niet bereikt.
Liposomen (microscopische blaasjes, die gemaakt zijn van dezelfde phospholipiden als waaruit biologische membranen zijn opgebouwd) kunnen gebruikt worden als dragers van zowel in water als van in vet oplosbare geneesmiddelen. Lage concentraties van geneesmiddelen in liposomen kunnen even effectief zijn als hogere doses in conventionele preparaten, maar met minder kans op bijwerkingen van zowel locale als systeem absorptie. De basis aspecten van licht absorptie, PDT, FD en liposomen zijn beschreven in **hoofdstuk 2.**

14.16 *Belangrijke aan licht gerelateerde dermatosen*
Huidaandoeningen zoals psoriasis, vitiligo, acne en NMSC's hebben een relatie met licht en komen veelvuldig voor in een algemene dermatologische praktijk. De epidemiologie, de klinische aspecten en de ontstaanswijze van deze ziekten worden, samen met de voordelen en de nadelen van de behandelingen, besproken in **hoofdstuk 3**.

14.17 *PDL behandeling van plaque type psoriasis*
Hoewel psoriasis in eerste instantie veroorzaakt wordt door erfelijk bepaalde afwijkingen in het afweersysteem wijst de opvallende toename van de kleine bloedvaatjes in de bovenste laag van de lederhuid ter plaatse van de psoriasis plekken erop dat psoriasis mede veroorzaakt wordt door afwijkingen in deze bloedvaatjes. Traditionele behandelingen voor psoriasis zijn gericht op de remming van het afweersysteem en de daardoor veroorzaakte ontsteking in de opperhuid en lederhuid en de verhoogde celdeling in de opperhuid. De afwijkingen in de bloedvaatjes in de lederhuid werden aanvankelijk therapeutisch genegeerd. In meerdere studies werd echter gedeeltelijke en ook totale verdwijning van psoriasis plekken vermeld na selectieve photothermolyse door de PDL van de verwijde bloedvaten in de lederhuid. **In hoofdstuk 4** wordt een studie beschreven, waarin de PDL werd toegepast bij de behandeling van 74 patiënten met chronische plaque type psoriasis, die niet had gereageerd op conventionele locale behandelingen. Na PDL behandeling werd een goede tot zeer goede verbetering bereikt bij 73% van de patiënten..

14.18 *Psoriasis palmoplantaris*
Psoriasis van handen en voeten is een invaliderende en ontsierende aandoening, die vaak slecht reageert op behandeling, vooral in het geval van een pustulosis palmoplantaris (psoriasis variant, die gepaard gaat met gele en bruine puisten). Een groep van 41 patiënten met therapie resistente psoriasis van handen en voeten werd iedere 4 tot 6 weken behandeld met PDL in combinatie met calcipotriolzalf

of 5% tot 10% salicylzuurzalf. Een verbetering van meer dan 70% werd bereikt in 76% van de patiënten na een gemiddeld aantal van 4.2 behandelingen. Tijdelijke purpura trad, zoals gebruikelijk, op na iedere PDL behandeling, korsten werden gezien bij slechts 3% van de patiënten. De gemiddelde remissie duur na de laatste behandeling bedroeg 10.7 maanden. Een recidief van psoriasis werd gedefinieerd als het wederom optreden van psoriasis van 30% of meer ten opzichte van de baseline score. In vele klinische studies worden remissie en recidief verschillend of helemaal niet gedefinieerd. De minst strenge definitie van recidief is het opnieuw optreden van psoriasis van 50% ten opzichte van de baseline score, terwijl de strengste definitie uitgaat van ieder zichtbaar recidief na een remissie. De resultaten van deze open prospectieve studie laten zien, dat gecombineerde behandeling met PDL en locaal calcipotriol- of salicylzuurzalf een bevredigend resultaat kan opleveren bij de behandeling van psoriasis van handen en voeten met een duidelijke verbetering van de symptomen en de quality of live, zoals is beschreven in **hoofdstuk 5.**

14.19 *PDL vergeleken met UVB in de behandeling van plaque type psoriasis*

Recente doorbraak in de behandeling van psoriasis heeft geleid tot een beter begrip van de pathogenese van deze ziekte. Traditionele systemische behandelingen van psoriasis, zoals methotrexaat (MTX), cyclosporine (Cs) en mycophenolaat mofetil (MMF) kunnen gepaard gaan met een toename van het risico op lymphoproliferatieve aandoeningen. Het risico op maligniteit met de moderne biologicals, zoals TNF-α blokkers is nog niet duidelijk. Het merendeel van de studies naar het carcinogene risico van biologicals noemen een gering verhoogd risico op kanker, inclusief NMSK's en hematologische malignitieten.[5,6] De status van TNF-α remmers in relatie tot pan-epidemische dreiging en multi-resistente tuberculose bacteriën is nog niet duidelijk, maar is wel reden tot waakzaamheid. Narrow-band ultraviolet licht (UVB) is zeer effectief tegen psoriasis. PDL heeft geen carcinogene eigenschappen in tegenstelling tot UVB en psoralen ultraviolet-A (PUVA) therapie. UVB pakt primair structuren aan in de epidermis, terwijl PDL de epidermis passeert zonder noemenswaardige schade aan te richten en primair de verwijde oppervlakkige bloedvaatjes in de dermis uitschakelt. Dit wijst erop, dat UVB en PDL psoriasis via verschillende routes kunnen laten verdwijnen. Het doel van de studie beschreven in **hoofdstuk 6** was om de effectiviteit en de veiligheid van PDL behandeling in plaque type psoriasis te evalueren, de resultaten van PDL behandeling te vergelijken met die van UVB behandeling en met die van PDL+UVB behandeling en om een eventueel synergistisch effect van PDL +UVB behandeling op psoriasis vast te stellen. Zowel PDL, UVB als de combinatie behandeling PDL+UVB resulteerde in een significante verbetering. PDL bleek even effectief te zijn als UVB. De combinatie van PDL en UVB had geen additioneel effect, tengevolge van het ontbreken van synergie. Een

onverwachte bevinding was, dat er geen significante verschillen werden aangetoond tussen UVB- en PDL behandelde gedeelten van de psoriasis plaques en de niet behandelde (NT) gedeelten. Hiervoor is nog geen duidelijke verklaring gevonden, maar een niet verwacht " carry-over" efect vanuit de UVB- en PDL behandelde gedeelten van de psoriasis plaques wordt ondersteund door de bevinding van een omgekeerde relatie tussen de verbetering van de NT delen en de afstand tussen de PDL behandelde gedeelten en de NT gedeelten van de psoriasis plaques. Met andere woorden: de verbetering in de NT gedeelten nam af naarmate de afstand tussen de NT gedeelten en de met PDL behandelde gedeelten groter was. Deze bevindingen maken verdere studies waardevol, omdat hieruit blijkt, dat het overlappen van laser spot sizes waarschijnlijk niet nodig is en dat zou een aantal voordelen opleveren, zoals verkorting van de behandeltijd, minder ongemak voor de patiënten en lagere kosten. Een andere mogelijkheid is, dat er sprake was van een systemisch effect op de NT gedeelten, tengevolge van de behandeling van de overige psoriasis laesies. Achteraf lijkt het erop, dat het verschillend behandelen van delen van een individuele psoriasis laesie niet de optimale methode is om de effectiviteit van locale therapieën te bestuderen. Indien deze methode toch wordt gebruikt is het aan te bevelen om de behandeling te beperken tot de voor de studie geselecteerde laesies, zonder behandeling van de overige laesies. In de onderhavige studie bleek, dat de patiënten met een volledige verdwijning van de psoriasis laesies of goed reageerden op PDL of op UVB. Dit wijst erop dat patiënten bij wie UVB faalt wel goed kunnen reageren op PDL en vice versa. De behandeling met de PDL bleek veilig te zijn, omdat er alleen milde en tijdelijke bijwerkingen werden waargenomen. De PDL dient niet te worden toegepast voor de behandeling van uitgebreide psoriasis of bij patiënten, die goed reageren op eenvoudige locale middelen. Behandeling met de PDL kan met succes ingezet worden bij patiënten met persisterende plaque type psoriasis (vooral op niet met kleding bedekte lichaamsdelen), plaque type psoriasis met bijwerkingen van de gebruikelijke middelen, psoriasis palmoplantaris, psoriasis inversa. PDL kan ook worden toegepast bij patiënten die bezwaar maken tegen het gebruik van locale corticosteroiden of systemische behandeling en bij patiënten met verhoogd risico op huidkanker, tengevolge van zonlicht beschadiging of immunosuppressie. De meeste patiënten in deze studie gaven de voorkeur aan PDL behandeling boven behandeling met UVB.

De resultaten van PDL behandeling kunnen worden bevorderd door:

- Toename van het aantal behandelingen.
- Vermindering van het tijdsinterval tussen de behandelingen.
- Verhoging van de energie en de pulsduur tot respectievelijk 10 J/cm^2 en 1.5 msec.
- Applicatie van optical coupling gel kort voor de laser behandeling.

- Combinatie met calcipotriolzalf behandeling.

Toekomstige studies kunnen informatie verschaffen om de resultaten te optimaliseren.

14.20 *Cellulaire en moleculaire effecten van pulsed dye laser en narrow-band UVB behandeling op psoriasis*

In een nieuwe psoriasis plaque behoren dilatatie en proliferatie van de dermale, papillaire capillairen tot de vroegst zichtbare veranderingen. Toename van de dermale microvascularisatie faciliteert het transport van immuun cellen naar de huid en speelt daardoor een belangrijke rol bij het in stand houden van de ontsteking in de psoriasis huid. Reductie van de hyperproliferatie van de papillaire microvascularisatie via selectieve photothermolyse door PDL behandeling kan leiden tot een vermindering van het transport van immuun cellen tussen bloedvaten en huid met als beoogd doel dat de psoriasis plaques verdwijnen.

Uit de studies, die beschreven zijn in **hoofdstuk 7**, is gebleken dat klinische verbetering van psoriasis na PDL behandeling vergezeld ging van veranderingen van enkele klassieke markers van psoriasis activiteit. Patiënten die goed reageerden op PDL behandeling toonden verminderde expressie van VEGFR2, VEGFR3 en E-selectine binnen 24 uur na PDL behandeling. Na 2 PDL behandelingen was de expressie van IL-23, TNF-α mRNA en E-selectin proteine significant verlaagt, terwijl na 4 behandelingen alle epidermale markers en dermale infiltraten genormaliseerd waren. In psoriasis plaques, die met UVB werden behandeld was de expressie van de epidermale activatie markers en E-selectine na 13 weken significant verlaagd. De effecten op de mRNA waarden van patiënten die goed op PDL en UVB therapie reageerden bleken vergelijkbaar te zijn. De geringere klinische effectiviteit van UVB in deze studie (52% verbetering versus 60% voor totale lichaamsbelichting) kan worden verklaard door het feit, dat in de studie UVB alleen werd gebruikt op een beperkt aantal geselecteerde psoriasis plaques en niet als totale lichaams behandeling. Uit recent onderzoek is namelijk gebleken, dat totale lichaams behandeling een additioneel systemisch effect heeft. Aan het einde van de behandel periode toonden zowel PDL als UVB een reductie in de expressie van epidermale markers en van de dermale T cell infiltraten. Een interessante observatie in deze studie was de benedenwaartse regulatie van IL-23 gedurende PDL behandeling, omdat recent werd gerapporteerd dat Th17 cellen een belangrijke rol spelen in the ontstaanswijze van psoriasis. Deze T helper cellen worden gestimuleerd door IL-23. Een recent ontwikkelde biological blokkeert IL-23 en blijkt effectief te zijn bij de behandeling van psoriasis. Het lijkt er dus op, dat PDL en deze nieuwe biological eenzelfde aangrijpingspunt hebben. Daarnaast kan de benedenwaartse regulering van TNF-α en IL-23p19 bijdragen tot de effectiviteit van de PDL behandeling op psoriasis.

14.21 *Liposomen*

Liposomen zijn veelbelovend voor locale behandeling in de dermatologie. Hoewel de huid een orgaan is, dat direct toegankelijk is voor locale applicatie van geneesmiddelen, wil dat niet zeggen dat de geneesmiddelen verwerkt in conventionele bases, zoals lotions, crèmes en zalven in de optimale concentraties en op de juiste plaatsen in de huid terecht komen. Liposomen kunnen gebruikt worden als dragers van zowel hydrofiele als lipofiele geneesmiddelen. De stabiliteit van instabiele middelen kan worden bevorderd door ze in te bouwen in liposomen. Daarnaast versterken liposomen de penetratie in de epidermis van stoffen, die niet op een andere manier in de huid kunnen doordringen. Liposomen dragen ertoe bij, dat de kans op huidirritatie wordt verminderd door vertraging van het vrijkomen van geneesmiddelen en door een vochtinbrengende werking op de epidermis. Ze hebben ook het vermogen om geneesmiddelen te concentreren in de haar/talgklier structuren, waardoor ze inzetbaar zijn bij aandoeningen van haarfollikels en talgklieren. Lage concentraties van geneesmiddelen in liposomen zijn even effectief als hogere doses in conventionele preparaten, maar met minder bijwerkingen zowel locaal als door systemische absorptie van het geneesmiddel. Veel belovende resultaten zijn bereikt met in liposomen ingekapselde geneesmiddelen bij de behandeling van acne, xerosis cutis, atopic dermatitis, psoriasis, vitiligo, oppervlakkig veneuze trombose, en verwijdering van overtollige haargroei. “Lege” liposomen (zonder ingekapselde geneesmiddelen) hydrateren de huid, eenvoudig weg door de toevoeging van vetten en water aan de epidermis en kunnen de plaats innemen van de klassieke emolients bij de behandeling van bijvoorbeeld eczeem. Hierdoor wordt ook huidirritatie door bepaalde geneesmiddelen (bijvoorbeeld tretinoin) verminderd, waardoor de acceptatie en de therapietrouw van de patiënt toeneemt.

PDT wordt veelvuldig gebruikt om AK’s en oppervlakkige BCC’s te behandelen. Verder wordt PDT in toenemende gebruikt om acne te behandelen en huidveroudering te bestrijden. Bijwerkingen beperken echter het gebruik ervan, vooral in geval van benigne huidaandoeningen. Door de 5-ALA in een crèmebasis te vervangen door 5-ALA in liposomen kan de concentratie van 5-ALA verminderd worden met een factor 40, waarbij het fluorescerend vermogen, dat een maat is voor de potentiële effectiviteit, behouden blijft en waardoor tevens de noodzaak tot occlusie overbodig wordt. Een bijkomstig voordeel is een kostenverlagend effect. De eigenschappen van liposomen worden verder besproken in **hoofdstuk 8**.

De volgende studies op het gebied van liposomen kunnen waardevol zijn:

- 5-ALA liposomen en daglicht voor de behandeling van acne.
- 5-ALA liposomen en daglicht voor de preventie van huidveroudering, AK’s en oppervlakkige BCC’s.
- 5-ALA liposomen en IPL of PDL voor huidverjonging.

- T4-endonuclease-V in liposomen voor de behandeling en preventie van NMSC, vooral bij patiënten met immunosuppressie.
- Cyclosprine in liposomen voor de behandeling van ernstige vormen van psoriasis en atopisch eczeem.
- Methotrexaat in liposomen voor de behandeling van ernstige psoriasis.
- “Lege liposomen” of fillagrine bevattende liposomen voor de behandeling van xerosis cutis en atopisch eczeem.
-

14.22 *Behandeling van vitiligo met liposomale khelline in combinatie met UV licht therapie*

Vitiligo is een niet levensbedreigende huidaandoening, die echter wel een ernstige psychologische impact heeft met voor de patiënt een sterk negatieve invloed op de kwaliteit van leven. Vitiligo wordt histologisch gekenmerkt door de afwezigheid van pigmentcellen (melanocyten) in de epidermis. De melanocyten in de haarfollikels worden echter bij vitiligo meestal niet in het proces betrokken. Therapeutische maatregelen beogen een ommekeer van het progressieve verlies van melanocyten en het terugbrengen van de normale huidskleur. Gedurende het proces van repigmentatie prolifereren de folliculaire melanocyten en migreren naar de epidermis. Gebruikelijke middelen hiervoor zijn locale corticosteroiden, tacrolimus, systemische en locale PUVA, UVB en locaal khelline met UVA (KUVA). Geen van deze middelen is echter in alle gevallen effectief en bijwerkingen kunnen het gebruik ervan beperken. Khelline heeft phototherapeutische eigenschappen, die overeenkomen met die van de psoralenen, maar met veel minder kans op phototoxische effecten en DNA mutaties. Literatuur gegevens betreffende de huidige status van khelline bij de behandeling van vitiligo waren niet overtuigend. De selectie van de basis waarin khelline wordt opgelost bleek zeer belangrijk te zijn voor een juiste beschikbaarheid van khelline in de huid. Inkapseling van khelline in phophatidylcholine liposomen verhoogt de beschikbaarheid van khelline in de haarfollikels, waardoor de stimulatie van de melanocyten wordt bevorderd. De effectiviteit en de veiligheid van de behandeling met khelline in L-phenylalanine gestabiliseerde phophatidylcholine liposomen in combinatie met UVA/UVB licht werd geëvalueerd in een open, retrospectieve studie, die beschreven wordt in **hoofdstuk 9**. Een repigmentatie van 50% tot 100% werd verkregen in 72% van de behandelde vitiligo plekken na een gemiddelde behandelingsperiode van 12 maanden. De repigmentatie bleek niet gelijk te zijn voor verschillende lichaamsdelen.

14.23 *Blaardak transplantatie (BRT) voor vitiligo*

Chirurgische technieken worden in het algemeen gereserveerd voor stabiele, maar therapie resistente vitiligo. De transplantatie van epidermale blaardak grafts op de

gedepigmenteerde gebieden is veilig, gemakkelijk uitvoerbaar, geeft een maximale expansie van pigment en veroorzaakt geen littekens. Dit wordt beschreven in **hoofdstuk 10**. Deze techniek heeft een aantal voordelen boven de melanocyten suspensie transplantatie techniek.

14.24 *Combinatie van liposomaal khelline en UV licht behandeling met blaardak transplantatie bij therapie resistente vitiligo*

In recente richtlijnen betreffende de behandeling van vitiligo wordt UVB behandeling alleen aanbevolen bij patiënten, die niet adequaat behandeld kunnen worden met conservatieve middelen, patiënten met zeer uitgebreide vitiligo of patiënten met gelocaliseerde vitiligo en significante impact op de kwaliteit van het leven. Maar bij vele patiënten blijven, ondanks deze maatregelen, hardnekkige vitiligo plekken bestaan. Photo-medicamenteuze behandeling zoals PUVA en khelline met ultraviolet licht (KLUV) kan effectief zijn waar andere behandelingen falen. Het lijkt rationeel om de voorkeur te geven aan KLUV boven PUVA, omdat khelline overeenkomstige phototherapeutische eigenschappen heeft, met veel minder kans op phototoxische effecten en DNA mutaties. Daar komt bij, dat KLUV behandeling bij vrijwel alle patiënten leidt tot stabiliteit van de vitiligo en dat is een belangrijke voorwaarde voor het succes van chirurgische maatregelen. De procedure en de resultaten van BRT bij patiënten, die na minstens 1 jaar behandeling met KLUV nog steeds stabiele, hardnekkige vitiligo plekken hadden wordt beschreven in **hoofdstuk 11**. Na de transplantatie is photo-medicamenteuze (KLUV) noodzakelijk om voldoende uitbreiding van de pigmentatie rond het blaardak transplantaat te bereiken en daarmee de repigmentatie van het gehele witte gebied. Verdere studies kunnen de individuele rol van BRT, UV en khelline evalueren.

Verbeteringen in BRT techniek kunnen worden bereikt via:

- Applicatie van een corticosteroidcrème of een calcineurinecrème op het getransplanteerde gebied, nadat de transplantaties zijn aangeslagen, om te voorkomen dat de vitiligo weer actief (instabiel) wordt door de operatie (Köbner fenomeen) of door spontane verergering.
- Een medicamenteuze interventie van de onderliggende oorzaken van de melanocyten destructie (b.v. Efalizumab).
- Suction blister ablatie van het te behandelen gebied in plaats van ablatie met de Erbium laser om te bereiken dat de verwijderde huid en de getransplanteerde huid dezelfde dikte hebben.

14.25 *PDT van acne vulgaris met gebruikmaking van 5-ALA 0.5% liposomale spray en IPL in combinatie met locale peeling middelen.*

De zoektocht naar nieuwe en veilige therapeutische methoden voor acne wordt

gerechtvaardigd door de toename van de resistentie van de Propionibacterium (Pr) acnes en het groeiend besef van de bijwerkingen van conventionele locale en systemische anti-acne middelen. Hoewel de effectiviteit van PDT waarbij gebruik wordt gemaakt van 20% 5-ALA crème onder afsluitend verband is aangetoond, wordt het gebruik ervan belemmerd door phototoxische bijwerkingen. Liposomen hebben als eigenschap om hydrofiele geneesmiddelen, zoals 5-ALA te transporteren naar de talgklieren, die bij acne ontstoken zijn. Door 5-ALA in te bouwen in liposomen kan de concentratie ervan verlaagd worden tot 0.5% met gelijk blijven van de geïnduceerde huid fluorescentie en waadoor tevens een afsluitend verband tijdens de inwerkingfase niet meer nodig is. Daarnaast is de huid fluorescentie en daarmee de kans op phototoxiciteit na de behandeling veel sneller verdwenen dan na 20% 5-ALA crème. Een studie, waarin de effectiviteit en de veiligheid worden bepaald van PDT met 5-ALA 0.5% in liposomale spray en IPL in combinatie met een peeling crème (Li-PDT-PC) wordt beschreven in **hoofdstuk 12**.
De resultaten van deze behandeling kunnen wellicht verbeterd worden door:

- Toepassing van dubbele IPL belichtingen op ontstoken laesies.
- Vermindering van de intervallen tussen de behandelingen tot 2 of 3 weken.

14.26 *Non-melanoma huidkanker en fluorescentie detectie*

Non-melanoma huidkanker (NMSC) is de grootste uitdaging voor de komende 2 decennia in de Dermatologie. De te verwachten stijging van huidkanker zal leiden tot een werkoverlast in alle dermatologische praktijken als er geen serieuze pogingen worden gedaan tot preventie educatie van het publiek om het probleem te beteugelen. Zelfs met de voorziene toename van het aantal dermatologen in Nederland, zal dit probleem te groot worden om te behappen als we op dezelfde weg doorgaan met dezelfde middelen. Screening van patiënten op (pre-) maligne huidafwijkingen maakt vroege detectie en daarmee tijdige behandeling mogelijk. Diagnostische huidfluorescentie met 5-ALA in liposomen en een speciaal gecomputeriseerd detectie en visualisatie systeem biedt de mogelijkheid om NMSC te ontdekken op een vroeg (preklinisch) stadium. Deze fluorescentie detectie (FD) techniek is goed geschikt om grote huidgebieden te onderzoeken, alsmede om nauwkeurig de plaatsen op de huid te identificeren om huidbiopsieën te verrichten en ook om pre-operatief de grenzen van maligniteiten van de huid vast te stellen. Daarnaast is deze techniek te gebruiken bij de controle en de follow-up na behandeling van huidkanker. De techniek en de resultaten van deze FD worden beschreven in **hoofdstuk 13**.
Verdere studies op het gebied van fluorescentie detectie zijn nodig om de correlatie tussen de fluorescentie patronen en de klinische en histologische beelden te optimaliseren, vooral om penetrerende maligniteiten te kunnen onderscheiden van

niet-penetrerende maligniteiten. Additionele studies betreffende de effectiviteit van PDT met IPL of PDL van AK's en NMSC direct uitgevoerd in dezelfde sessie na FD zou een zeer efficiente methode tot behandeling kunnen opleveren.

References

1. Lebwohl M. A clinician's paradigm in the treatment of psoriasis. J Am Acad Dermatol 2005; 53: s59-69
2. Elston DM, Taylor JS, Coldiron B, Hood AF, Read SI, Resneck JS, Kirsner RS, Maize JC, Sullivan S, Laskas J, Hanke CW. Patient safety Part I. Patient safety and the dermatologist. J Am Acad Dermatol 2009;61:179-190.
3. Elston DM, Erik Stratman, Johnson-Jahangir H, Alice Watson A, Swiggum S, Hanke W. Patient safety. Part II. Opportunities for improvement in patient safety. J Am Acad Dermatol 2009; 61: 193-205.
4. Marcil I, Stern RS. Squamous-cell cancer of the skin in patients given PUVA and ciclosporin: nested cohort crossover study. Lancet 2001; 358: 1042–1045.
5. Patel RV, Clark LN, Lebwohl M, Weinberg JM. Treatments for psoriasis and the risk of malignancy. J Am Acad Dermatol 2009;60:1001-1017.
6. Linthorst Homan MW, Spuls PI, de Korte J, Bos JD, Sprangers MA, Wietze van der Veen J PW. The burden of vitiligo: Patient characteristics associated with quality of life. J Am Acad Dermatol article in press.

Abbreviations

ADCC	antibody-dependent cell-mediated cytotoxicity
Ag	antigen
AK	actinic keratosis
ALA	aminolaevulinic acid
AP	activator protein
APC	antigen presenting cell
BBUVB	broadband ultraviolet-B
BCC	basal cell carcinoma
Bcl2	B-cell lymphoma/ leukemia-2 gene
BSA	bovine serum albumin
BPO	benzoyl peroxide
BRT	blister roof transplantation
CCD camera	bharged couple device
CD	cluster of differentiation
CHS	contact hypersensitivity
Cm	centimetre
CLA	cutaneous lymphocyte antigen
CO2	carbon dioxide
CptT	critical phase transition temperature
CTLA	cytotoxic T-lymphocyte antigen
CW	contineous wave
CsA	cyclosporin A
DC	dendritic cell
DNA	deoxyribonucleic acid
E	energy (photon)
EM	electromagnetic
FD	fluorescence detection
H	hour
HBD2	human β-defensin-2
HEV	high endothelial venule
IFN-γ	interferon-γ
HLA	human leucocyte antigen
ICAM	intercellular adhesion molecule
IL	interleukin
IPL	intense pulsed light
J/cm2	Joule/square centimetre
KLUV	khellin liposomal ultraviolet
KPLUV	khellin phenylalanine liposome ultraviolet
KRT	keratin
KUVA	khellin ultraviolet-A
Laser	light Amplification by the Stimulated Emission of Radiation
LC	langerhans cell
LED	light emitting diode
LFA	lymphocyte function antigen
Li-PDT-PC	liposomal 5-ALA PDT in combination with peeling cream
LMV	large multi-lamellar vesicle
LMWH	low molecular weight heparin
LUV	large uni-lamellar vesicle
MAL	methyl aminolaevulinate
MED	minimal erythema dose
MHC	major histocompatibility complex
Mm	millimetre
MMP	matrix metalloproteinase
Msec	millisecond
MTX	methotrexate
MW	milliWatt
Nd:YAG	neodymium:yttrium aluminium garnet
NF- κB	nuclear factor kappa B
NK cell	natural killer cell
Nm	nanometre
NBUVB	narrowband ultraviolet-B
NMSC	non-melanoma skin cancer
NT	non treated
P	propionibacterium
PAMP	pathogen-associated molecular pattern
PASI	psoriasis area and severity index
PBS	phosphate buffered saline
PDC	plasmacytoid dendritic cell
PDL	pulsed dye laser
PDT	photodynamic therapy
PL	pigmented lesions
PGA	physician's global assessment
PPAR	peroxisome proliferator-activated receptor
PpIX	protoporphyrin IX
PPV	ositive predictive value

PR	photorejuvenation
PUVA	psoralen ultraviolet A
QoI	quality of life
RNA	ribonucleic acid
RT-PCR	real time-polymerase chain reaction
ROS	reactive oxygen species
*S1	singlet excited state
Sec	second
SCC	squamous cell carcinoma
SD	standard deviation
Str. bas.	stratum basale
Str. corn	stratum corneum
Str. gran.	stratum granulosum
Str. spinosum	stratum spinosum
SUV	small uni-lamellar vesicle
T	time = week
*T1	triplet state
TCR	T cell receptor
TDS	thermal Damage Time
TGF-α	T cell growth factor-α
TGK	transglutaminase
Th1cell	T helper 1 cell
Th2 cell	T helper 2 cell
TKS	thermo-kinetic selectivity
TLR	Toll like receptor
T4N5	T4 endonuclease V
TNF	tumor necrosis factor
TRAF	tumor necrosis factor-associated factor
TRT	thermal relaxation time
UCA	uranic acid
UVA	ultraviolet A
UVB	ultraviolet B
UVC	ultraviolet C
VASI	vitiligo area-scoring index
VCAM	vascular cell adhesion molecule
VEGF	vascular endothelial growth factor
VETF	vitiligo European task force
VEGFR	vascular endothelial growth factor receptor
VIDAscore	vitiligo disease activity score
Vs	versus
VWf	von Willebrand factor
W	Watt
µm	micrometre
µsec	mcrosecond
h	Planck's constant
V	frequency
Λ	wavelength

Curriculum vitae

Op 7 december 1939 werd Jaap de Leeuw geboren te Den Haag. Vanaf 1954 tot 1959 doorliep hij de middelbare school op de Dalton HBS-B te Den-Haag, waar de vriendschappen ontstonden met Arnold Boegborn en Wim de Bie. In 1959 startte hij met de studie geneeskunde aan de rijksuniversiteit in Leiden. Na het doctoraalexamen in 1965 werden de co-assistentschappen gelopen aan de Stichting Klinisch Hoger Onderwijs in Rotterdam, waar in 1968 de studie geneeskunde werd afgerond met het behalen van het artsexamen. In deze periode ontstonden de vriendschappen met Rob Tubbergen en Henk Menke. Nog tijdens de studie op 11 augustus 1967 trad hij in het huwelijk met Fernanda Kersten. De militaire dienstplicht werd als officier van gezondheid vervuld van 1968 tot 1970 in het Militair Hospital Utrecht, Afdeling Anaesthesie en Oogheelkunde. In deze periode werden dochter Andrea en zoon Michiel geboren.

In 1970 startte hij de opleiding tot dermatoloog bij Prof.dr. C. Beek in het Academisch Ziekenhuis Dijkzigt Rotterdam. De opleiding werd afgerond in 1974 met de registratie in het Specialisten Register Dermatologie. Hij denkt aan de opleidingstijd met veel genoegen terug, mede dankzij Jan Overbeke, Babs van Hussen en Dig Tazelaar.

Na de opleiding nam hij in augustus 1974 de praktijk over van Dr. H. van Zwijndrecht, dermatoloog in Dordrecht. Er volgden jaren met veel werk als fulltime dermatoloog in het Albert Schweitzer Ziekenhuis in Dordrecht en Zwijndrecht. Na aanvankelijk als solist te hebben moeten werken ontstond er later een maatschap met de collegae Koos Schuller, Ids Boersma en Annick van Rengen. Veel steun, zowel beroepsmatig als vriendschappelijk ondervond hij van de refereerclub ”De Trouwe Rotterdammers”.

Sinds 1996 is hij medisch adviseur bij Multicare, ZBC voor Dermatologie en Lasertherapie in Hilversum. Op verzoek van prof.dr. Neumann verliet hij per 1 januari 2002 de praktijk in Dordrecht om de functie van chef policlinique van de afdeling Dermatologie Erasmus MC Rotterdam op zich te nemen. Zijn werkzaamheden zijn sinds 2005 gefaseerd overgegaan van het Erasmus MC naar het ZBC Multicare in Hilversum.

Dankwoord

Na vele jaren met grote voldoening mij gewijd te hebben aan de directe patiëntenzorg was ik verheugd toen prof.dr. Martino Neumann mij vroeg om als chef policlinique mee te gaan naar het Erasmus MC. Daarmee ging een nog sluimerende wens van mij om universitair werk te verrichten en om wetenschappelijk onderzoek te doen alsnog in vervulling. Martino, ik ben je er zeer dankbaar voor dat je mij deze kans hebt geboden. Met veel genoegen kijk ik op deze universitaire jaren terug, ik voelde me op de afdeling al snel thuis, vooral door de prettige sfeer op de werkvloer. Ik heb de begeleiding van de arts-assistenten en de samenwerking met de collega dermatologen als zeer stimulerend ervaren. Ik dank alle betrokkenen voor de goede en prettige samenwerking. Ik begin jullie nu al te missen.
In de periode voor mijn overgang van het Albert Schweitzer Ziekenhuis in Dordrecht naar het Erasmus MC in Rotterdam was mijn belangstelling voor de behandeling van psoriasis met de Pulsed Dye Laser gewekt door de positieve ervaringen die collega Brian Zelickson ervan meldde in de Journal of the American Academy of Dermatology van 1996. De klinische resultaten van PDL behandeling in combinatie met calcipotriol- of salicylzalf bij 74 psoriasis patiënten leken de ervaringen van Zelickson te bevestigen. Deze studie werd uitgevoerd bij MultiCare in samenwerking met Dieter Neugebauer en gepubliceerd in het Nederlands Tijdschrift voor Dermatologie en Venereologie. Een vervolg studie van dezelfde behandeling bij psoriasis palmoplantaris, werd gestart in MultiCare en afgerond in het Erasmus MC en ook dit onderzoek leverde bemoedigende resultaten op. Ik dank Dieter Neugebauer en Suzanne Koetsveld voor hun bijdragen aan de totstandkoming van deze artikelen.
Naast mijn werkzaamheden als chef policlinique in het Erasmus MC ben ik gestart met een studie met als eerste doel om de plaats te bepalen van de PDL behandeling bij psoriasis en als tweede doel om een onderzoek te doen naar de immunologische veranderingen van PDL behandeling bij psoriasis. Rosanne van Lingen was betrokken bij de eerste fase van het onderzoek en met groot genoegen denk ik terug aan onze samenwerking. Bij het oplossen van de start problemen, die het onderzoek met zich meebracht, ben ik onder de indruk gekomen van je grote intellectuele en communicatieve vaardigheden, die je combineerde met een prijzenswaardig goed humeur en goed gevoel voor humor. Ik dank je zeer voor de steun, die ik aan je had. Rosanne, ik voorzag voor jou een briljante toekomst in de geneeskunde. Dat juist de geneeskunde jou in de steek heeft gelaten komt op mij over als een schrijnende, extreme oneerlijkheid van het noodlot. Hilde Both was betrokken bij de tweede fase van bovengenoemd onderzoek. Ook jou wil ik bedanken voor je vakkundige en plezierige bijdrage. Bij het afronden van het onderzoek en de daarop volgende poging tot publicatie trad er een probleem op met de statistische bewerking van de onder-

zoeksresultaten. Dit probleem werd opgelost door Tamar Nijsten, waardoor het artikel alsnog geplaatst kon worden in Dermatologic Surgery. Tamar, ik ben je hiervoor zeer erkentelijk.
In MultiCare hadden we sinds 1996 ervaringen opgedaan met liposomen en in de loop van de tijd ben ik steeds meer geïnteresseerd geraakt in de bijzondere mogelijkheden, die liposomen kunnen bieden. In eerste instantie heeft dit geleid tot een studie die, in goede samenwerking met Dieter Neugebauer, Nick van der Beek en Günher Maierhofer, werd uitgevoerd naar de effectiviteit van khelline liposomen met ultraviolet licht (KLUV) bij vitiligo . De resultaten van deze studie zijn gepubliceerd in de European Journal of Dermatology. Hardnekkige vitiligo lesies reageren echter niet altijd op photo-medicamenteuze therapie en daarom werd, in samenwerking met Peter Bjerring, een blaardak transplantatie techniek ontwikkeld. Dit heeft geleid tot de totstandkoming van een artikel waarin deze techniek beschreven wordt. Daarna zijn de resultaten van de behandeling met KLUV gecombineerd met blaardak transplantaties gebundeld in een ander artikel. Bij beide artikelen is Yvette Assen nauw betrokken geweest. Yvette, ik dank je voor bijdrage.
Inmiddels had de studie van de PDL bij psoriasis om diverse redenen vertraging opgelopen. Dit bood mij de mogelijkheid om dieper in de liposomen te duiken en dat heeft geleid tot een review artikel over liposomen in de dermatologie. Ik dank Hannah de Vijlder, die in een vorige functie ook al met liposomen in aanraking was geweest, voor haar medewerking aan dit artikel, dat geplaatst werd in de Journal of the European Academy of Dermatology and Venereology (JEADV). Steeds meer werd bij MultiCare duidelijk, dat liposomen multi-inzetbaar zijn. Een studie naar een photodynamische behandeling met 5-ALA liposomen bij acne leverde, naast bemoedigende resultaten, ook een publicatie op, eveneens in de JEADV. Last but not least, heeft ons onderzoek met liposomen geleid tot de ontwikkeling van een nieuwe methode van fluorescentie detectie bij non melanoma huid kanker. Deze methode is gepubliceerd in Lasers in Surgery and Mecicine.
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Bibliography

De Leeuw J, Cohen FEP. Een geval van granulomatosis van Wegener. Ned Tijdschr Geneesk 1973; 117, nr. 30, 1118-1123.
De Leeuw J, Leiker DL. Een patient met een zwelling in de hals. Ned Tijdschr Geneesk 1975; 119, nr. 14, 551-555.
De Leeuw J, den Hollander P. A patient with a contact allergy to jogging cream. Cont Dermat 1987; 17: 260-261.
Van Joost Th, de Leeuw J. Een opvallend cutaan reactiepatroon als bijwerking van penicillamine: elastosis perforans serpiginosa. Ned Tijdschr Geneesk 1988; 132, nr.11, 501-503.
De Leeuw J, van Zonneveld TH, Boersma IH, Schuller JL. Schimmelinfecties van de huid van het gelaat. Ned Tijdschr Geneesk 1994; 138, nr. 47, 2350-2353.
De Leeuw J, van Zonneveld TH, Boersma IH, Schuller JL. Erosieve pustuleuze dermatose van de hoofdhuid. Ned Tijdschr Dermatol Venereol 1994; vol. 4, 368-369.
De Leeuw, Boersma IH. Het Gianotti-Crosti-Syndroom geassocieerd met Denque fever. Ned Tijdschr Dermatol Venereol 1995; vol 5: 322.
Van Oers JAH, de Leeuw J, van Bommel EFH. Nephrotic syndrome associated with isotretion. Nephrol Dial Transplant 2000; 15: 923-924.
De Leeuw. Dermatomycose. Modern Med 2000; nr. 4: 354-357.
De Leeuw J. Neugebauer WD. Behandeling van chronische plaque psoriasis met de pulsed dye laser. Ned Tijdschr Dermatol Venereol 2001; 11, nr 8: 3-7.
De Leeuw J, van der Beek N, Maierhofer G, Neugebauer WD. A case study to evaluate the treatment of vitiligo with khellin encapsulated in L-phenylalanin stabilized phosphatidylcholine liposomes in combination with ultraviolet light therapy. Eur J Dermatol 2003; 13: 474-477.
Racz E, de Leeuw J, van Lingen R, van Tuyll van Serooskerken A, Both H, Prens EP, van der Fits L. De effecten van pulsed-dye laser (PDL) in vergelijking met UVB-TL-01 behandeling in gelokaliseerde chronische plaque psoriasis. Ned Tijdschr Dermatol Venereol 2006; 16 (1): 11-13.
De Leeuw J, Tank B, Bjerring P, Koetsveld S, Neumann HAM, Concomitant treatment of psoriasis of the hands and feet with pulsed dye laser and topical calcipotriol, salicylic acid, or both: A prospective open study in 41 patients. J Am Acad Dermatol 2006; 54: 266-271.
De Leeuw J, van Lingen RG, Both H, Tank B, Nijsten T, Neumann HAM. A Comparative Study on the Efficacy of Treatment with 585 nm Pulsed Dye Laser and Ultraviolet B-TL01 in Plaque Type Psoriasis. Dermatol Surg 2009 ;35: 80–91.
De Leeuw J, de Vijlder HC, Bjerring P, Neumann HAM. Liposomes in dermatology today, J Eur Acad Dermatol Venereol 2009; 23: 505–516.
De Leeuw J, van der Beek N, Dieter Neugebauer WD, Peter Bjerring P, Neumann HAM, Fluorescence Detection and Diagnosis of Non-Melanoma Skin Cancer at an Early Stage. Lasers in Surgery and Medicine 2009; 41:96–103.
Bjerring P, Christiansen K, Troilius A, Bekhor P, de Leeuw J. Skin Fluorescence controlled Photodynamic Photorejuvenation II (Wrinkle Reduction). Lasers Surg Med 2009. In press.
De Leeuw J, van der Beek N, Peter Bjerring P, Neumann HAM. Photodynamic Therapy of acne vulgaris using 5-ALA 0.5% liposomal spray and Intense Pulsed Light in combination with topical keratolytic agents. J Eur Acad Dermatol Venereol. Accepted for publication 2009.
Rácz E, de Leeuw J, Baerveldt EM, Kant M, Neumann HAM, van der Fits L, Prens EP. Cellular and molecular effects of pulsed dye laser and narrow-band UVB therapy in Psoriasis. Submitted.Assen YJ, de Leeuw J, Bjerring P, Neumann HAM. Epidermal blister grafts for vitiligo. Submitted. De Leeuw J, Assen YJ, Peter Bjerring P, Neumann HAM. Treatment of acrofacial vitiligo using khellin in liposomes and ultraviolet light in combination with transplantation of suction blister roof epidermal grafts**.** Submitted.