Non-invasive treatment of epidermal keratinocyte neoplasms

NON-INVASIVE TREATMENT MODALITIES IN SUPERFICIAL KERATINOCYT NEOPLASMS

Janneke Kessels

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Voor mijn ouders

Contents

Chapter 1

General introduction

" Science, my boy, is made up of mistakes, but they are mistakes which it is useful to make, because they lead little by little to the truth"

- Jules Verne, Journey to the Center of the Earth, 1864

In ancient Greek mythology, it was believed that Apollo was the god of sun and light, but could also bring sickness as well as cure.[1] Nowadays, scientists know that sunlight is necessary to life and evolution, but on the contrary, its ultraviolet radiation (UV) radiation causes direct damage to our genetic material. When atmospheric scientists focused their attention on the destruction of the ozone layer in the early 1970s, a rise in skin cancer was predicted.[2] Indeed, over the past decades, a tremendous increase in the amount of skin cancer and premalignant diseases has been observed.[3] This was not only due to the destruction of the ozone layer, but also because of an increase in sun exposure. A natural tan became a fashion item in western countries from the 1920s onward.[4,5] Despite the effort of governments and dermatologists in trying to change sun exposure through public education and media campaigns, sun exposure and a tan is still considered a "healthy" look in western countries.

Skin cancer can be divided into two subcategories: malignant melanoma and non-melanoma skin cancer (NMSC). Malignant melanoma is known as the most aggressive type of skin cancer with high mortality rates.[6] The group of NMSC encompasses many different skin malignancies, such as adnexal tumors, Merkel cell carcinomas and lymphomas, but is basically used to address the two most frequent skin malignancies: basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Recent literature has suggested the term keratinocyte cancer (KC) as the preferred term for these two common skin malignancies.[7,8]

Accompanied with the rise in skin cancer, an increase in premalignant skin diseases has been observed. Bowen's disease and actinic keratosis (AK) are known to be possible precursor lesions of SCC.[9]

This thesis discusses non-invasive treatments for the two most frequent intra-epidermal neoplasms in Caucasians: actinic keratosis (AK) and basal cell carcinoma (BCC). In this chapter, both neoplasms are introduced separately.

Actinic keratosis

Actinic keratosis (AK) is the most common premalignant skin disorder in Caucasians, caused by chronic UV radiation exposure. Several studies suggest that, if left untreated, AK might be capable of developing into squamous cell carcinoma (SCC). Percentages range between <1% and 16% per year.[9-14] The true number remains questionable, due to methodological heterogeneity. It is known that there is a high recurrence rate following treatment, often resulting in repetitive treatments.[15] Because of its frequent occurrence, the uncertain risk of progression into invasive SCC and the high recurrence rate, this skin disorder encompasses a substantial part of dermatological care and poses a burden on healthcare in general.[11,12,16]

Epidemiology

Cumulative sun exposure is the main risk factor for the development of AK. Its prevalence is highest among fair-skinned patients living at low latitudes.[17] As AK is not registered in skin cancer databases, we must depend on cohort studies to learn more about the prevalence and incidence.

Data on prevalence are mainly known for Australia and the USA. Prevalence varies from 60% in Australia to 11-26% in the USA.[12,18,19] European prevalence rates are lower, with numbers varying between 1.4-34.1%.[20-22] The reported differences are probably due to geographical differences and the variance in UV exposure this entails. A Dutch population-based cohort study with a mean age of 72 years described a prevalence of 49% in men and 28% in women.[23] Flohil et al. showed that male sex, age above 70, baldness/hair loss and light skin phototype are risk factors.[23] High sun exposure is also related to an increased risk of developing AK. It is therefore, that areas with higher sun exposure such as the face, scalp, and the dorsum of the hands and arms are affected the most. A personal history of SCC also predisposes patients to a risk of AK.[24] Furthermore, an immunosuppressed status, as seen in organ transplant recipients, is an important risk factor for developing AK.[25,26]

Pathogenesis

AK usually develops because of chronic exposure to UV radiation. UV-induced damage to the tumor suppressor gene TP53 is associated with the induction of cancer. AKs are strongly associated with changes in TP53. The literature reports that up to 50% of AK and up to 90% of SCC have TP53 mutations.[3,27-30] Most of the UV radiation that reaches the earth is UV-A. This type of UV penetrates the skin deeper, causing the formation of reactive oxygen species (ROS), thereby leading to oxidative damage to nucleic acids, membrane lipids and proteins.[31] There is increasing evidence that oxidative stress is implicated in

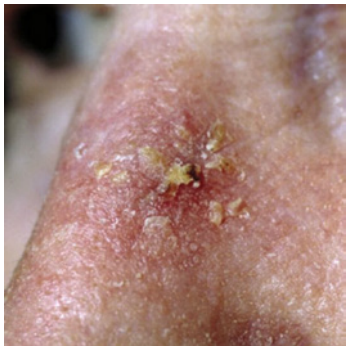
photocarcinogenesis. Excessive UV exposure also initiates activation of the Ras pathway. Ras genes are among the most frequently mutated genes in human cancers.[32]

Clinical presentation

Actinic keratosis (AK) generally presents as keratotic plaques and/or papules, often on an erythematous base, in sun-exposed skin such as the face, balding scalp and dorsum of the hands *(Figure 1)*.[33] Histologically atypical keratinocytes along the basal layer of the epidermis are found with large hyperchromatic nuclei, mitotic figures, inflammatory infiltrate and/or hyperkeratosis.[34] When examining a patient, it is important to realize that AKs can be more easily felt than seen.[35] Some are erythematous, others are pigmented. They can occur solitarily, but most frequently they present as multiple lesions in a large area.[36,37] This so-called field cancerization or field change consists both of clinically obvious AKs but also multiple subclinical (non-visible or non-palpable) AK lesions.

AKs are usually asymptomatic, but tenderness and itch can be reported, especially when lesions are thicker and multiple.[38] It is also not possible to predict which AK will develop into SCC and which will not. Prior research has shown that certain clinical features such as bleeding, size > 1 cm diameter, inflammation, induration, rapid growth, erythema and ulceration (IDRBEU or BEIRUD criteria) might predict a higher risk of SCC development in AK.[39] Whenever these features are present, a biopsy is often needed to differentiate between AK and invasive SCC. Interestingly, spontaneous regression of AK is also described.

Figure 1. Clinical presentation of actinic keratosis.



Treatment

The decision to treat AK can be made for several reasons: because the AK itself causes itching or is cosmetically disturbing, or to prevent development into SCC. The latter remains under debate. As the exact risk of progression of an AK into invasive SCC is not known, current guidelines advise to treat AKs. However, the risk of recurrence remains high.

The choice of a specific treatment depends on the extensiveness and severity of the AK. In case of several solitary lesions, lesion-directed therapy with cryotherapy, for example, is sufficient.[40] In the case of field change, field-directed treatment should be considered, as this additionally treats subclinical lesions in chronically sun exposed skin.[41] In the Netherlands, photodynamic therapy (PDT), 5-fluorouracil and imiquimod creams are among the most frequently used field-directed therapies. However, current guidelines lack clear treatment recommendations for clinical practice.[36,42-45] Gupta et al. conducted a network meta-analysis in which they compared the efficacy of eight different interventions and concluded that 5% 5-FU is the most effective treatment for AK.[46] However, randomized clinical studies are lacking, making it difficult to pose a more definite conclusion about the best and most cost-effective treatment.[47] Field-directed therapies for AK are discussed in a separate paragraph below as the majority of treatments are used for both AK and superficial basal cell carcinoma (sBCC).

Basal cell carcinoma

BCC is the most common skin cancer; it accounts for up to 80% of all NMSC.[48] BCC are indolent tumors that metastasize very rarely.[49,50] They can be locally invasive and, if ignored, can lead to substantial tissue destruction.[51]

Epidemiology

Because of large variability in registries between countries, the exact incidence of KC in general and BCC specifically is difficult.[3,52] In the Netherlands, BCC is registered in only one geographical region and only the first BCC is registered. A study performed in the Netherlands showed an estimated annual percentage of increase in BCC incidence between 2002 and 2009 of 6.8% for men and 7.9% for women. The same study showed that the European age-standardized (ESR) incidence rates quadrupled from 40 to 165 per 100,000 person-years for men and from 34 to 157 for women.[53] It is estimated that 30-50% of patients will develop at least one or more further BCC within 5 years after a primary tumor.[3] The life-time risk of developing a BCC for the Dutch population is estimated 1:5-6.[54]

BCC is considered a disease of the elderly population.[53,55] It is estimated that 80% of cases

occur in patients older than 60 years.[56] However, an increase in younger patients has been observed.[57] Men are more affected than women, except for the population younger than 40 years. In this age category, women develop more BCC than men.[58-61]

When studying the incidence rates of BCC by subtype, nodular BCC (nBCC) is the most common subtype, followed by superficial BCC (sBCC) and infiltrative subtypes. Interestingly, over the past decades, a large increase was observed for sBCC.[62] A Dutch registry-based study showed an increase in the proportion of sBCC from 17% in 1991 to 30% in 2007.[63]

The rising incidence can be explained by several factors: increased UV light exposure, depletion of the ozone layer in several geographical regions and an increased awareness that leads to more visits to healthcare professionals and therefore an increase in diagnosis. Besides UV, other factors such as the use of immunosuppressive or photosensitizing medication influence the susceptibility to develop KC.[64,65] Additionally, there is a genetic susceptibility that contributes to the development of BCC, as is known in basal cell nevus syndrome (BCNS) for example.[66]

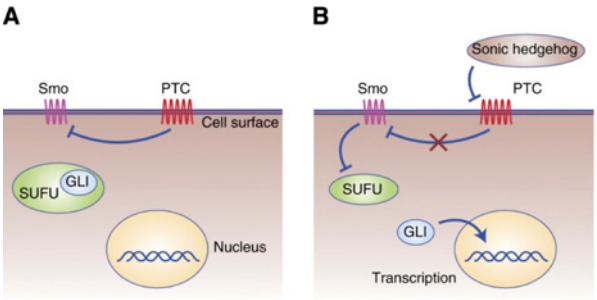
Pathogenesis

Until the late 1990s, the molecular biology of BCC was a "black box", but in the past decade, our understanding of its molecular pathogenesis has increased.[67] BCC belongs to the group of cancers in which deregulated Hedgehog (HH) signaling is of crucial importance.[67,68] BCNS patients appear to carry heritable mutations in the patched-1 (PTCH1) gene, a tumor suppressor gene that acts as an inhibitor of the HH pathway. It is now known that approximately 90% of sporadic BCC also have identifiable mutations in at least one allele of the PTCH1 gene.[67] In short, PTCH1 encodes for a transmembrane protein that can bind to the sonic hedgehog (SHH) protein. In normal conditions, the HH protein suppresses activation of the transmembrane protein smoothened (SMO). This suppression prevents SMO targeting of Gli transcription factors and activation of intra-nuclear target genes is hindered. In the case of activating mutations in SMO or loss of function mutations in PTCH1, no inhibition of downstream Gli transcription can take place, resulting in tumor proliferation. *Figure 2* schematically demonstrates HH signaling. Approximately 10% of BCC are caused by activating mutations in SMO.[67]

Under physiological conditions, SHH is responsible for the proliferative expansion of the follicle epithelium required for maturation of the follicle. It is suggested that BCC arise from cells of the upper infundibulum and inter-follicular epidermis.[69,70] The development of these epidermal cells and hair bud formation is regulated by canonical Wingless (WNT) signaling.[69] There are suggestions that deregulation of the Wnt pathway causes intra-nuclear accumulation of β-catenin, leading to further proliferation of HH pathway-driven

neoplasia, such as BCC.[71]

Figure 2. Schematic representation of hedgehog (HH) signaling.



The hedgehog inhibitor pathway. (A) In the absence of the sonic hedgehog ligand, the patched receptor (PTCH) inhibits the activity of smoothened (Smo), allowing suppressor of fused (SUFU) to bind and to inactivate Gli transcription factors. (B) Binding of the sonic hedgehog ligand to PTCH allows the activation of Smo, inhibiting the binding of SUFU to Gli. The Gli transcription factors are then able to enter the nucleus and modulate transcription of Hedgehog pathway-associated genes. Reprinted with permission of the Nature publishing group: Lear JT et al; Br. J. Cancer, 2014; 111(8):1476-81. Doi: 10.1038/bjc.2014.270.[72]

Clinical presentation and diagnosis

BCC mostly present as a translucent or pearly colored papules or plaques with telangiectasia. The clinical appearance depends on the histological subtype. Diagnosis can usually be made on its clinical appearance: approximately 72-80% of tumors are correctly diagnosed by clinical examination.[73-76] Around 8% of all BCC occur on sun-exposed areas such as the head and neck area.[77] BCC can be roughly divided in four categories: superficial, nodular, micronodular and infiltrative. These can be categorized in *non-aggressive* (superficial and nodular) and *aggressive* (infiltrative and micronodular) BCC. *Figure 3* shows the clinical presentation of superficial, nodular and infiltrative subtypes.

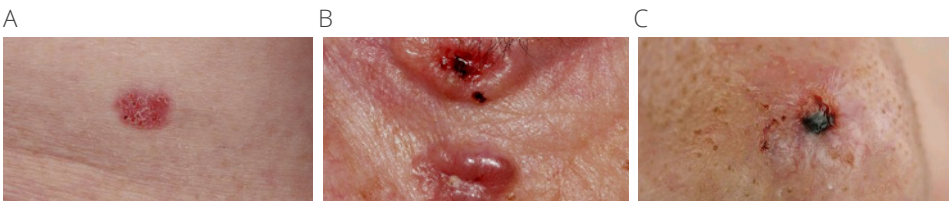
Superficial BCC present as an eczema-like plaque frequently located on the trunk. Sometimes it is accompanied by slight keratosis. Histologically atypical basaloid cells are seen that stay connected to the epidermal surface or hair follicle. A tumor cell palisade at the periphery of tumor nests and artificial retraction from surrounding stroma can be seen.[78,79] Nodular BCC is the most frequent subtype (approximately 60%) and generally presents as a round raised papule or nodule with well-defined edges, which has a translucent or pearly appearance with telangiectasia or sometimes pigmentation.[80] Histologically, this variant is characterized by nests of atypical basaloid cells in the papillary or reticular dermis, frequently accompanied by an artificial retraction from the surrounding stroma.[78,79] Tumor nests frequently show peripheral cell palisading.

Infiltrative (aggressive) and micronodular BCC present as shiny pale or white patches

or plaques with poorly defined borders and might have an atrophic appearance with induration. Histologically, this subtype typically spreads further than can be observed clinically.[81] Basaloid cells invading the deeper dermis are observed histologically. In infiltrative tumors, peripheral palisading and stromal retraction are fairly rare.[79] Some BCC can contain melanin pigmentation within basaloid cells and macrophages, causing a pigmented appearance. This may occur in all subtypes.

It is important to distinguish the different subtypes of BCC, as a different therapeutic approach is necessary to prevent recurrence of tumors. The use of dermatoscopy and taking a biopsy specimen for histopathological examination increase the diagnostic accuracy for the BCC subtype.[82,83] It is advised to take a punch biopsy to determine the histological subtype, but clinicians should be aware that a substantial part of BCC consist of more than one subtype, so called mixed-type BCCs.[84-86] A punch biopsy might detect only one histological subtype of BCC, leading to a possible under-treatment in cases where the most aggressive part is not identified in the biopsy.

Figure 3. Clinical presentation of superficial (a), nodular (b) and infiltrative (c) BCC subtypes



Treatment

Treatment of BCC can be divided into invasive and non-invasive therapies. Up to now, surgery has been considered the gold standard treatment for all BCC subtypes because of the associated high cure rates and histological confirmation.[87-89] Because of its superficial growth, sBCC are especially accessible to non-invasive therapy such as topically applied treatments and photodynamic therapy.

Topical treatment of actinic keratosis and superficial basal cell carcinoma

In this paragraph, non-invasive topical treatments for sBCC and field-directed treatments for AK are discussed together.

5-fluorouracil

5% 5-Fluorouracil (5-FU) cream was introduced and approved by the US Food and Drug association (FDA) in 1957 as a new anti-tumor treatment.[90,91] Its approval was based on the first study in which a 93% success rate was described for sBCC. Other studies confirmed its efficacy and tolerability.[91,92] For AK, several studies describe a complete clearance rate varying from 43-96% with a recurrence percentage of 43-44% after 12-36 months.[47,93,94]

The mechanism of action mainly depends on its cytotoxic effect. After entering the cell, enzymatic reactions lead to conversion in three important metabolites: fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP) and fluorouridine triphosphate (FUTP). These metabolites interfere with DNA and RNA through the inhibition of thymidylate synthetase (TS), which is required for DNA synthesis and repair. Eventually 5-FU influences DNA repair mechanisms, ultimately leading to cell death.[95] In dermato-oncology, 5% 5-FU (Efudix®) is mainly used for intra-epithelial (pre)malignant skin diseases such as AK, Bowens disease and sBCC. It is not indicated to treat high-risk BCC or nodular BCC because of low cure rates.[96] Possible side effects are pain, a burning sensation, erythema, crusting, erosions and edema. Side effects generally occur on the treated and diseased skin. This is supported by research showing that healthy skin cells are fairly resistant to the described cytotoxic effects of 5-FU compared to rapidly proliferating tumor cells.[91,97] 5-FU cream is produced in two different dosages: 0.5% and 5%. The latter is the only available concentration in the Netherlands (Efudix®, Meda Pharma). For both sBCC and AK, the 5% 5-FU ointment is applied twice daily for 3-4 weeks until erosions and crusts develop.

Imiquimod

Imiquimod is another topical cream registered to treat (pre)malignant skin disorders. In the Netherlands, it is used to treat actinic keratosis and sBCC.[98] It was first approved to treat small primary sBCC in 2004 by the U.S. FDA. Studies have reported success rates of 87-88% for sBCC.[99,100] A more recent study revealed that 5% Imiquimod cream was inferior to surgery for sBCC, but showed acceptable cure rates of 83.6% and 82.5% at 3 and 5 years follow-up.[101,102] For AK, complete clearance rates vary between 24% and 73% after 5% imiquimod.[93,103] In general, studies that assessed efficacy are very heterogenous; follow-up periods vary and there are only a few randomized comparative studies. More recently, imiquimod 3.75% (Zyclara ®) was developed. A complete clearance rate of 34%

and a median reduction from baseline in number of AK of 80% were described in a placebo controlled study.[104]

Imiquimod is a synthetic nucleoside analogue of the imidazoquinoline family.[105] It acts as an immunomodulator with antitumoral and antiviral efficacy. Its activity is initiated by toll-like receptor (TLR) 7 and 8 signaling cascades, leading to the release of inflammatory cytokines and other mediators. The release of natural killer cells is also stimulated and Th1 cytokines are produced. Subsequently, tumor-directed cellular immune responses are activated.[106] It is also reported to induce apoptosis. Furthermore, a recent study by Wolff et al. demonstrated a tumor-specific mechanism of action by repressing HH signaling by negatively modulating Gli activity in BCC.[105] For sBCC, the treatment regimen consists of once daily application, five days a week, for a period of 6 consecutive weeks. To treat AK, the ointment should be applied once daily, 3 days a week (e.g. Monday-Wednesday-Friday) for 4 weeks. Evaluation after four weeks is indicated and, in case of an insufficient effect, this should be repeated for an additional period of 4 weeks. The new formulation imiquimod 3.75%, registered for field AK, has a different dosing regimen: it should be applied once daily for two weeks. After an interval of two weeks without treatment, the same regimen is repeated. The side effects of imiquimod are comparable to those of 5-FU: pain, a burning sensation, erythema, erosions and edema. Because of its immune-modulatory effect, patients can report flu-like symptoms during treatment as well.

Photodynamic therapy

Photodynamic therapy (PDT), also called photochemotherapy, combines a light source with a photosensitizer. In dermato-oncology, PDT is primarily used to treat AK, sBCC and Bowens disease. To obtain an optimal photodynamic reaction, three components are needed: 1) a photosensitizer; 2) light that can be absorbed by the photosensitizer and 3) oxygen. When these components act together with the surrounding tissue, a photodynamic reaction occurs. Commonly available topical photosensitizers are 5-aminolevulinic acid (5-ALA) and its methyl ester methyl-aminolevulinate (MAL). Both photosensitizers are precursor drugs of the photosensitizer protoporphyrin IX (PpIX). PpIX accumulates in epidermal, metabolically active cells. When illuminated by a light source with the appropriate wavelength, the release of reactive oxygen radicals is observed.[107] Subsequently, this causes cellular (mitochondrial) damage, apoptosis and necrosis in tumor cells *(Figure 4)*. Because there is a higher conversion of PpIX in metabolically active cells, such as tumor cells, PDT is a tumor-specific treatment in which damage to healthy tissue is minimal. PDT is an in-hospital treatment, mostly performed by trained nurses. MAL is the most commonly used photosensitizer in the European Union for both BCC and AK treatment. On the other hand, 5-ALA is registered in the US for both BCC and AK, but in Europe for AK only.[41,108,109] For sBCC, the MAL treatment scheme used in most Dutch hospitals involves two ses-

sions, separated by one week. During each session, MAL (Metvix®, Galderma) ointment is applied to the tumor surface with a margin of 5 mm in the surrounding tissue. After a 3-hour dark interval, the skin is illuminated with a light emitting diode (LED) light source for 7 minutes with a fluence of 37 J/cm^2. Clearance rates between 72 and 84% are described after MAL-PDT.[108,110,111]

De Haas et al. developed a fractionated two-fold illumination schedule (two illuminations on one day) using 5-ALA cream to treat sBCC. This treatment regimen is used in several Dutch hospitals for sBCC, along with the more frequent used conventional MAL-PDT treatment. 5-ALA cream is applied in the same manner as MAL-PDT, and after 4 hours of waiting the tumor is illuminated with a fluence of 20 J/cm^2 for 4 minutes. Subsequently, the tumor area is covered and after 2 hours is illuminated again with 80 J/cm^2 for 18 minutes. Clearance rates described were 97% one year post-treatment, which is higher than for conventional MAL-PDT.[112]

Conventional MAL-PDT treatment using MAL cream (Metvix®, Galderma) for AK includes one illumination session. After a 3-hour dark interval, the skin is illuminated once with a red light emitting diode (LED) light source (~630 nm +/- 5 nm) for 7 minutes and a fluence of 37 J/cm^2. Placebo controlled studies report complete clearance rates in 69-91% of AK lesions 3 months post-treatment after one or two sessions with MAL-PDT.[113-116] When evaluating the efficacy of two versus one illumination session in MAL-PDT, Tarstedt et al. concluded that a single illumination is almost equally effective as two illuminations.[116] Recently, daylight PDT has been introduced. The initial trials from Scandinavia reported clearance rates varying from 75-79%.[118 117,119] Advantages are convenience for the patient and tolerability with less pain sensation compared to conventional PDT.[120] An important limitation can be the weather conditions.

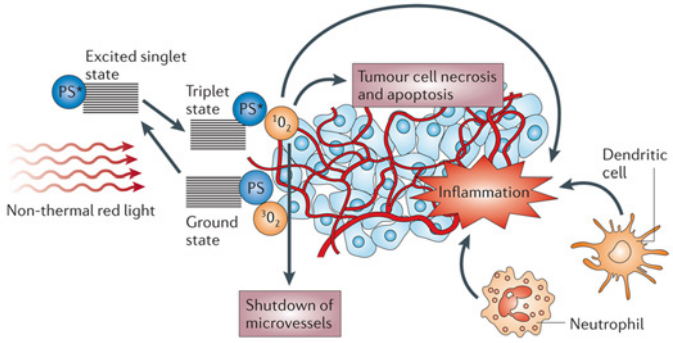
Most reported side effects during conventional PDT are pain or a burning sensation during illumination and post-treatment erythema, crusting and scaling. Sometimes, erosions can appear. Serious pain during illumination can be a drawback for patients, especially in AK patients.[121] Because of this, illumination schedules and light sources are being studied and optimized.

Ingenol mebutate

Ingenol mebutate is a relatively new topical treatment, approved for the treatment of AK by the FDA in 2012. It has been available in the Netherlands since 2013. The active substance is derived from the plant *Euphorbia peplus*. Its mechanism of action is still not fully understood, but it is hypothesized that it has a dual mechanism: immediate cytotoxicity with the occurrence of mitochondrial edema and an immunomodulatory effect through

the production of pro-inflammatory cytokines and the recruitment of neutrophils. [24] The first placebo-controlled clinical trial on ingenol mebutate showed a complete AK clearance rate of 42.2% in the head and neck area, compared to 3.7% in the placebo group, 8 weeks post-treatment. The median reduction in the number of AK was 83%. At long-term follow-up (12 months), the lesion reduction from baseline was 87.2% in the head and neck area and 86% for the trunk and extremities.

Figure 4. The mechanisms of action of photodynamic therapy in tumors.



The photosensitizer (PS) absorbs light and an electron moves to the first short-lived excited singlet state. This is followed by intersystem crossing, in which the excited electron changes its spin and produces a longer-lived triplet state. The PS triplet transfers energy to ground-state triplet oxygen, which produces reactive singlet oxygen ($^1O_2$). $^1O_2$ can directly kill tumor cells by the induction of necrosis and/or apoptosis, can cause destruction of the tumor vasculature and produces an acute inflammatory response that attracts leukocytes such as dendritic cells and neutrophils.
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Ingenol mebutate (Picato®, Leopharma) is available in two concentrations: 1) 150 µg/g, used for the face and scalp, applied once daily on three consecutive days and 2) 500 µg/g, used for trunk and extremities applied once daily on two consecutive days. The fact that treatment only lasts two to three days is a major advantage for patients, especially when compared to other topical ointments such as imiquimod or 5-FU. The side effects of ingenol mebutate are also comparable to those of 5-FU and imiquimod. Because of the short duration of treatment, this influences compliance in a positive way. There have been a few small studies reporting on the use of ingenol mebutate to treat sBCC; however, the studies were small and the evidence is limited.[123-125] sBCC is not yet an approved indication for ingenol mebutate treatment.

Aims and outline of this thesis

Nowadays, treatment of KC is not only a matter of offering the most effective treatment to a patient. Cosmetic outcome, the cost of treatment and patient preference are increasingly important, especially because of the growing incidence of (pre)malignant skin disorders in the younger population.[53,58] This stresses the need to determine the optimal therapy and to search for new non-invasive treatments.

For AK, few head-to-head trials are available, leading to a lack of evidence-based treatment recommendations for daily practice. The AK studies in this thesis concentrated on an alternative illumination source for PDT and a large multi-center randomized trial comparing the four most frequently applied therapies.

Treatment of sBCC with self-applicable creams is associated with local adverse effects and good compliance is inevitable to achieve good therapeutic results. Photodynamic therapy results in fewer side effects, but recent studies have shown that it is less effective compared to self-applied creams such as imiquimod or 5-fluorouracil.[110,126] In patients where compliance is doubtful, an in-hospital non-invasive treatment such as PDT could be desirable. However, the efficacy of PDT must increase. Therefore, the search for optimal light sources and illumination schemes continues. The studies on sBCC performed in this thesis focused on the optimization of PDT protocols and evaluated the efficacy of a possible new topical treatment.

This thesis aimed to answer the following questions regarding the effectiveness of:

- PDT using pulsed-dye laser illumination for AK *(chapter 2.2)*
- the four most frequently used field-directed therapies for AK (*chapter 2.3*)
- topical sinecatechin 10% ointment for sBCC *(chapter 3)*
- two-fold ALA-PDT compared to conventional MAL-PDT for sBCC *(chapter 4.1 and chapter 4.2)*
- ambulatory PDT for sBCC *(chapter 4.3)*

Outline of the thesis

In **chapter 2,** we discuss the available literature regarding the different treatment modalities for AK. The efficacy of laser illumination as an alternative PDT light source for AK is assessed. This chapter also contains the results of a large randomized controlled effectiveness trial comparing four common topical treatments for AK.

Chapter 3 describes a randomized study evaluating the efficacy of topical green tea ointment for sBCC.

Chapter 4 is devoted to photodynamic therapy for sBCC. Different illumination schedules and illumination sources are discussed, including the results of a randomized controlled trial comparing two different PDT protocols.

Chapter 5 provides a discussion of the results and the implications for clinical practice.

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Chapter 2

Evidence based treatment of actinic keratosis


2.1

Chapter 2.1

Evidence based treatment of actinic keratosis: review of the literature

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"There is nothing wrong with dirty data as long as you have a clear mind"
- Dr. Langan, EADV fostering resident course – Clinical research & epidemiology, 2016

Introduction

Skin cancer is a major public health issue, as its incidence is increasing rapidly. Actinic keratosis (AK) is the most prevalent precancerous chronic skin condition, caused by chronic UV radiation exposure. AK can persist, regress or transform into squamous cell carcinoma (SCC).[1,2] It is among the most common conditions treated by dermatologists.[3] Previous research by Flohil et al. showed a prevalence of 49% in men and 28% in women above the age of 45 in the Netherlands.[4] The number of newly diagnosed lesions in the Netherlands is currently estimated at 160.000/year. It is expected that the number of patients with AK will rise even further in the future, also due to already experienced sun exposure during life.[5] Furthermore, phenotypic characters of the Dutch population with light skin type have an increased risk for AK compared to darker skin types.

AKs have a high recurrence rate, often resulting in frequently repeated treatments. The current opinion is that recurrence of AK can be a result of field cancerization; AKs arise in a skin area that has diffuse precancerous damage, which may not be clinically visible. This phenomenon has been confirmed in mice.[6] Field-directed therapy offers a number of potential benefits over lesion directed therapy, such as better overall clearance with limited local skin responses and better cosmetic results. Because of the important advantage to treat subclinical lesions, it is therefore advised to treat areas, rather than solitary lesions.[7]

Currently, there is insufficient information in literature considering the risk of developing SCC in a pre-existing AK. Several studies state percentages varying between 0.025% - 16% per AK lesion per year.[1,2,8] Because of this uncertainty, the Dutch guideline for actinic keratosis advises to treat all AK.[9] There are however several treatment options, with variable (cosmetic) result and side effects.

Cryotherapy with liquid nitrogen is the most widely used therapy for actinic keratosis.[10] For solitary AKs cryosurgery is an effective, easily performed and cost-effective treatment. However, for field treatment cryotherapy is painful and may be associated with high recurrence rates.[11] Both national and international guidelines consider cryotherapy not to be a preferred treatment modality for field treatment.[12,13 9,14] There is a number of (placebo) controlled studies that compare different field-treatments, such as photodynamic therapy (PDT), topical 5-fluorouracil (Efudix), topical Imiquimod (Aldara), chemical peelings and laser surgery. However, treatment protocols and outcome parameters differ to a great extent. Moreover, follow-up periods often are limited. Many comparative trials are industry sponsored and give inconsistent results. Some studies strongly favor 5-fluorouracil over Imiquimod, while others give quite the opposite results. For that reason, those studies are an inadequate basis to come to an evidence based choice of treatment.

At the moment 5-fluorouracil and PDT are the two most commonly used treatments in the Netherlands.[15] However, the current Dutch guideline for AK does not recommend either those as a preferable treatment. [16] Hence, which treatment the patient will receive, generally relies on the preference of the physician instead of evidence based medicine.

In 2012 Gupta et al. concluded in their Cochrane systematic review, that more direct comparisons between frequently used field treatments are needed to determine the best therapeutic approach for patients with AK.[17] The most recent data to approach this question more explicitly, are provided very recently by the same group, who performed a network meta-analysis of eight interventions for AK as a follow-up of their Cochrane review (cryotherapy, topical Diclofenac 3%, 5-aminolevulinic acid (ALA) photodynamic therapy (PDT), 5-fluorouracil (5-FU) 0.5% or 5%, Imiquimod (IMI) 5%, Ingenol mebutate (IMB) 0.015%-0.05%, methyl-aminolevulinate (MAL)-PDT and placebo/vehicle).[18] Interventions were eventually ranked as follows: 5% 5-FU > 5-aminolevulinic acid(ALA)-PDT ≈ Imiquimod 5% ≈ Ingenol Mebutate (IM) ≈ Methylaminolevulinate (MAL)-PDT > Cryotherapie > Diclofenac 3%. Consequently, based on this outcome 5% 5-FU should be the treatment of choice. However, this statement has never been investigated in a prospective randomized controlled trial. General advantages of network meta-analyses over standard pairwise meta-analysis is that it facilitates indirect comparisons of multiple interventions that have not been studied in a head-to-head fashion. However, an important limitation of network meta-analyses in general is that they are perceived to be more complex and prone to misinterpretation.

In this article, the most frequently used therapies for field-AK are described and results are outlined in *Table 1-4*. There is a large heterogeneity in the outcome measures used in the different studies.

Search and results

The PubMed and Cochrane databases were searched (august 1976 – march 2014). The following search terms (including derivates) were used: *actinic keratosis, solar keratosis*, in combination with*: treatment, randomized controlled trial, clinical trial, review*. Further eligible publications were found from reference lists.

Cryosurgery

Cryosurgery or cryotherapy is the application of extreme cold liquid nitrogen, to destroy diseased tissue. The nitrogen is applied to the skin lesion for a few seconds, depending on the desired diameter and depth of freeze. Treatment is repeated once, the so called 'double freeze-thaw'.[19]

Even though cryosurgery is a frequently used treatment, randomized controlled studies are lacking. Percentages of complete remission in *solitary* AK lesions, vary between 75% and 98%.[20] Recurrence of lesions is estimated between 1.2% and 25% of lesions at 12 months follow-up. Szeimies *et al.* compared methylaminolevulinate (MAL)-PDT with cryosurgery in 193 patients with a total of 699 AK's. Complete response (CR) rates were assessed, resulting in 69% CR in MAL-PDT group versus 75% CR in cryosurgery group. This difference was not statistically significant. Both treatments were more effective for thin AK lesions (Olsen grade I, II) compared to thicker lesions (Olsen grade III). [21,22] Another study found that that the histologic clearance rate (32%) was considerably lower than the initial clinical clearance rate of 68%.[23] These authors also found that histologic clearance rate was higher with topical therapy (Imiquimod and 5-FU) for AK lesions on the head, neck or décolletage. After 1 year only 4% of patients who underwent cryotherapy had a sustained clinical clearance. These low efficacy rates do not support widespread use of cryotherapy. Furthermore, cryotherapy is not a desired treatment for multiple or subclinical lesions.[9,12-14] Treatment generally causes a grade 2 burn wound and can cause extensive scarring when a large area is treated.

Photodynamic therapy

Photodynamic therapy (PDT) is an in-hospital treatment in which either 5-aminolevulinic acid (ALA) or methyl-aminolevulinate (MAL) as topical agents are generally combined with a red non-cohorent light source. However, protocols vary and besides differences in photosensitizing agents, also different light sources, fractionation schedules, number of illuminations and pre-treatment modalities are described. MAL (metvix®) cream is the most frequently used topical photosensitizer in the Netherlands at the moment. Treatment according to the standardized MAL treatment protocol (Galderma ®) is advised. 5-ALA cream (Ameluz ®) is an alternative photosensitizer. Because there are no direct comparative studies between Metvix (MAL) and Ameluz yet. It is expected that they have the same efficacy, however the available 5-ALA studies use different formulations.

The cosmetic outcome after PDT treatment is usually excellent. Because of the discomfort patients may have in case of the long-term application of other topical agents and consequently the assumed poor compliance, many physicians prefer an in-hospital treatment. This is why in a significant amount of hospitals PDT is now the first-choice treatment.[17,24] However, PDT is more expensive and patients experience it as very painful, because of the burning sensation it causes. For this reason, patients often refuse further treatment.

Placebo-controlled randomized trials with MAL-PDT show complete lesion response in 69%-91%, 3 months post-treatment after one or two treatment sessions based on several clinical trials.[20,21,25,26] A randomized trial performed by Tarstedt et al. assessed the efficacy

of 2 versus 1 illumination for MAL-PDT treatment. All lesions with non-complete response were treated a second time after three months. Results show that overall, one illumination is as effective as two (81% versus 87% response, p= ns), 3 months post treatment. Thicker and non-responding lesions do benefit from 2 illuminations (70% response after one illumination, compared to 84% after two illuminations).[26] Thirty-seven (19%) lesions with a non-complete response after a single treatment were re-treated. Follow-up lasted until 3 months after the last treatment.

Unfortunately, many studies describe efficacy results based on two consecutive illuminations, one week apart and non-responding lesions are treated again after 3 months. This is not the standard MAL treatment protocol for AK in the Netherlands, which makes the studies described above less representative for our daily practice. Negative effects of PDT are the prior mentioned local pain, duration of the treatment, and the costs. Major advantages are selectivity of photosensitizing agents (e.g. when compared to 5-FU) and the possibility for field treatment.

New in the field of PDT is daylight-PDT, in which a photosensitizing agent is combined with daylight instead of an in hospital, non-coherent light source. Described advantages are less pain, patient comfort and lower costs. This treatment is not yet standard care in the Netherlands.

5-Fluorouracil

5-Fluorouracil (5-FU) is a topical chemotherapeutic agent. It is a well-known field directed treatment, which can be applied by the patient at home during 4 weeks twice daily.[27] Two different dosages (0.5% and 5%) are used. In Dutch practice only 5% 5-FU is registered.[28] When treating localized disease, patient complete clearance rates of 43%-96% are described.[16,23,29,30] Recurrence rates after 12-36 months vary from 43%-44%.[28] These clinical trials do not describe a repetitive treatment after 3 months.

The majority of clinical trials so far have assessed the 0.5% dosage. A randomized controlled trial comparing topical 5% Imiquimod, 5% 5-FU and cryosurgery showed initial clearance rates of 85%, 96% and 68% respectively.[23] After 1 year follow-up 28% of patients treated with cryosurgery had no residual or recurrence AK, compared to 54% in 5-FU group and 73% in Imiquimod group (p <0.01). A similar study comparing topical 5% 5-FU and 5% Imiquimod showed that 5% 5-FU is superior for field treatment. There was a reduction of final AK count (decline during 24-week follow-up) by 94% versus 66% in 5% 5-FU and 5% Imiquimod groups respectively (p <0.05). Patient complete clearance was achieved in 84% of 5% 5-FU patients compared to 24% of 5% Imiquimod patients (p < 0.01).[31] Kurwa *et al* examined the efficacy of a single PDT illumination versus 5% 5-FU cream twice daily for

3 weeks in a randomized right/left comparison, with no statistically significant difference between them.[32] A systematic review found that treatment with 5% 5-FU resulted in an average reduction of 79.5% in the mean number of lesions.[33] Krawtchenko et al. showed no statistical difference in both clinical and histological clearance rate, between Imiquimod 5% and 5% 5-FU at 3 months follow-up. [23]

Imiquimod

5% Imiquimod is also often used for generalized treatment of more disseminated disease. A once daily application for 3 days per week during a 4 weeks schedule is needed. Consequently a 4 week period of no application follows. If there is residual AK, the same schedule of 4 weeks can be repeated. However, comparable to MAL-PDT studies the clinical studies reviewed do not mention what amount of residual AK is needed for this follow-up treatment.

Several results considering the efficacy of Imiquimod have been described above. A study from 2005 showed a higher average patient complete clearance with imiquimod treatment (70% for Imiquimod versus 52% for 5-FU).[34] However 0.5% 5-FU was used. A study by Krawtchenko *et al*. showed a similar clinical and histological clearance rate between Imiquimod and 5% 5-FU, but Imiquimod had a superior sustained patient complete clearance rate at 12-month follow-up (73% for Imiquimod and 54% for 5% 5-FU).[23] However, the number of patients in each group was small. Alomar et al. studied 259 patients who were randomized for treatment with either 5%-Imiquimod or placebo. Imiquimod had a CR of 55%.[35] In 2004 Lebwohl *et al.* showed 45% patient complete clearance rate and 83.3% lesion reduction after 5% Imiquimod treatment.[36] Follow-up duration however was only 2 months.

Ingenol mebutate

Ingenol Mebutate (IM) gel is a novel topical product, FDA approved since January 2012 and approved by Dutch health care insurances since October 2013.[28,37,38] IM is a pleotropic effector inducing cell death and activates the immune response and is an effective treatment.[39] IM gel is suitable for field treatment. The main advantage of treatment with this self-applicable gel, is a shorter duration of treatment (2-3 consecutive days, depending on the location of AK, applied by the patient) and therefore also a shorter downtime, compared to 5-FU. This probably has a positive effect on the compliance.

Lebwohl *et al.* were the first to describe the clinical efficacy of topical Ingenol Mebutate (IM) in a randomized placebo controlled trial. Pooled analysis of face and trunk areas showed 42.2% of patients had complete patient clearance of AK at 8 weeks follow-up compared to 3.7% in placebo group (p <0.001). Partial clearance was observed in 63.9% for IM compared with 7.4% in placebo group (p < 0.001). A median reduction of 83% from baseline

in the number of actinic keratosis treated with IM was observed. At long term follow up one year post-treatment, the sustained lesion reduction rates compared to baseline were 87.2% for the face and scalp. For trunk and extremities this percentage was 86.8%.[40]

Conclusion

In conclusion, AK is a chronic skin condition with a high prevalence, which is expected to rise even further. There is a lack of good quality evidence to determine what is the best field treatment for AK. There is a need for a multi-center randomized controlled trial that enables head to head comparison of frequently used treatments for AK in the same study population with at least 1 year follow-up. Furthermore, the cost-effectiveness of treatments is not yet established in a large prospective trial. Our group previously showed that an old cheap treatment (5% 5-FU) is not inferior to the expensive new treatment (PDT) for treatment of superficial basal cell carcinoma.[41] This outcome will save the Dutch government millions each year. This issue and the expected cost-saving can be even larger in AK, since it is much more common than superficial basal cell carcinoma.

Table 1. Literature overview MAL-PDT. * *Complete clearance (100%); partial clearance (>75%)*

MAL-PDT	Study	Interventions	Study design	Outcome mean % lesion reduction from baseline	Outcome *lesion complete* clearance rate (CR)	Outcome *patient complete* clearance rate (CR)	Outcome *patient partial* clearance rate (PCR)	Number participants	Follow-up duration (months)	Industry sponsored
	Freeman *et al*. 2003	MAL-PDT vs Placebo-PDT vs cryotherapy **MAL-PDT two illuminations*	Multicenter randomized, double blind, placebo controlled study	X	91%	X	X	200 (88 PDT, 23 placebo, 89 cryotherapy)	3	Not mentioned
	Szeimies *et al*. 2002	MAL-PDT vs cryotherapy **MAL-PDT, one illumination*	Multicenter, randomized, open, active controlled study	X	69%	X	X	202	3	Yes
	Pariser *et al*. 2003	MAL-PDT vs placebo-PDT **MAL-PDT and placebo-PDT two illuminations*	Multicenter randomized placebo controlled study	X	89%	X	X	80 (42 MAL-PDT, 38 placebo-PDT)	3	Yes
	Pariser *et al*. 2008	MAL-PDT vs placebo-PDT **MAL-PDT and placebo-PDT two illuminations*	Multicenter randomized double blind study	X	86%	X	X	97 (49 MAL-PDT, 48 placebo-PDT)	3	Yes
	Tarstedt *et al*. 2005	MAL-PDT one illumination vs MAL-PDT two illuminations	Randomized open, active-controlled study	X	81% (one illumination) (*) 87% (two illuminations	X	X	211 (105 one illumination, 106 two illuminations)	3	Yes

Table 2. Literature overview 5-fluorouracil * *Complete clearance (100%); partial clearance (>75%)*

5% 5-fluorouracil	Study	Interventions	Study design	Outcome mean % lesion reduction from baseline	Outcome *lesion* complete clearance rate (CR)	Outcome *patient complete* clearance rate (CR)	Outcome *patient partial* clearance rate (PCR)	Number participants	Follow-up duration (months)	Industry sponsored
	Krawtchenko *et al*. 2007	5% 5-FU vs 5% IMI vs cryotherapy	Prospective randomized controlled trial	X	X	54% (sustained CR) 96% (intial CR)	X	75 (24 5-FU, 26 IMI, 25 cryo)	12 1	Yes
	Tanghetti *et al*. 2007	5% 5-FU vs 5% IMI	Single blind randomized study	94%	X	84%	X	36 (18 5-FU, 18 IMI)	6	Unknown
	Loven *et al*. 2002	0.5% 5-FU vs 5% 5-FU	Single blind randomized study	47%	X	43%	X	21 (11 0.5% 5-FU, 10 5% 5-FU)	1	No
	Kurwa *et al*. 1999	ALA-PDT vs 5% 5-FU	Randomized paired comparison open label study	X	X *outcome: reductional lesional area (70%)*	X	X	14	6	Unknown

Table 3. Literature overview Imiquimod * *Complete clearance (100%); partial clearance (>75%)*

5% Imiquimod	Study	Interventions	Study design	Outcome mean % lesion reduction from baseline	Outcome *lesion* complete clearance rate (CR)	Outcome *patient complete* clearance rate (CR)	Outcome *patient partial* clearance rate (PCR)	Number participants	Follow-up duration (months)	Industry sponsored
	Krawtchenko *et al.* 2007	5% 5-FU vs 5% IMI vs cryotherapy	Prospective randomized controlled trial	X	X	73% (% sustained CR) 85% (% initial CR)	X	75 (24 5-FU, 26 IMI, 25 cryo)	12 2	Yes
	Tanghetti *et al.* 2007	5% 5-FU vs 5% IMI	Single blind randomized study	66%	X	24%	X	36 (18 5-FU, 18 IMI)	6	Unknown
	Alomar *et al.* 2007	5% IMI vs placebo *both 3x/wk, 4 weeks on, 4 weeks off, repeated if not cleared	Multicenter, randomized double blind	75.7%	X	55%	X	259 (129 IMI vs 130 placebo)	4	Yes
	Jorizzo *et al.* 2004	5% IMI vs placebo	Randomized double-blind vehicle controlled study	74.4%	X	53.7%	X	84 (66 IMI, 18 vehicle)	12	Yes
	Stockfleth *et al.* 2003	5% IMI vs placebo *both 3x/wk 4 weeks on, 3 weeks off, repeated if not cleared	Randomized double-blind vehicle controlled study	X	84%	X	x	36 (25 IMI, 9 placebo)	3.5	Yes
	Lebwohl *et al.* 2004	5% imi VS placebo *IMI and placebo 2x/wk	Randomized double-blind placebo controlled study	83.3%	X	45%	59%	436 (215 IMI, 221 placebo)	2	Yes

Table 4. Literature overview Ingenol mebutate * *Complete clearance (100%); partial clearance (>75%)*

Ingenol mebutate	Study	Interventions	Study design	Outcome mean % lesion reduction from baseline	Outcome *lesion* complete clearance rate (CR)	Outcome *patient complete* clearance rate (CR)	Outcome *patient partial* clearance rate (PCR)	Number participants	Follow-up duration (months)	Industry sponsored
	Lebwohl *et al.* 2012	Ingenol mebutate vs placebo **once daily 3 consecutive days head/neck, 2 consecutive days trunk/ extremities*	Multi-center, double-blind study	83%	X	42.2% (head/ neck) 34.1% (trunk/ extremities	63%	Head/neck: 547 (277 IM, 270 placebo)	2	yes
	Lebwohl *et al.* 2013	IM 0.015% 3 consecutive days (head/neck) vs IM 0.05% 2 consecutive days (trunk/extremities)	Observational follow-up study (Lebwohl *et al.* 2012)	87.2% (head/ neck) 86.6% trunk/ extremities)	X	X	X	Head/neck: 100 Trunk/ extremities:71	12	yes

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2.2

Chapter 2.2

Laser mediated photodynamic therapy: an effective alternative treatment for actinic keratosis?

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"*Focus like a laser, not a flash light*"
- Michael Jordan

Abstract

Photodynamic therapy (PDT) with light emitting diode (LED) illumination is a frequently used treatment modality for actinic keratosis (AK) with excellent cosmetic outcome. A major disadvantage is the high pain score. Illumination using the pulsed dye laser (PDL) has been suggested. The long-term efficacy is unknown. In this split face study, we prospectively treated 61 AK patients, with both LED-PDT and PDL-PDT. The mean change in number of lesions between end of follow-up and start of therapy was -4.25 (95% CI [-5.07; -3.43]) for LED-PDT and -3.88 (95% CI [-4,76; -2.99]) for PDL-PDT with a non-significant difference (p=0.258) of -0.46 (95% CI [-1.28;0.35]). The percentage decrease from baseline in total number of AK was 55.8% and 47.8%, respectively, at 12 months follow-up. VAS pain score was lower after PDL (mean 2.64) compared to LED illumination (mean 6.47). These findings indicate that PDL-PDT is an effective alternative illumination source for AK when pain is a limiting factor for regular LED-PDT.

Introduction

Actinic keratosis (AK) is the most prevalent precancerous skin condition, resulting from chronic ultraviolet (UV) radiation exposure. It is predominantly located on chronically sun-exposed skin such as the head, neck and the dorsal aspects of the hands.[1] Prevalence is especially high among individuals with fair skin type or among individuals taking immunosuppressant medication.[2] Typically, multiple AKs co-exist in a photo-damaged area and recurrence tends to be high probably as a result of field cancerization.[3,4] This stresses the need for (repetitive) field-directed treatments.

Several interventions are currently used for the treatment of AK. Liquid nitrogen cryotherapy is the most frequently used therapy worldwide.[5] However, this is a lesion directed treatment and has limited use in areas of field cancerization. In contrast, field directed therapies have the potential to treat subclinical lesions resulting in lower recurrence rates.[6,7] Among the most frequently used field therapies are topical 5-fluorouracil cream, imiquimod cream, ingenol mebutate gel, diclofenac gel and photodynamic therapy (PDT).[8]

The mechanism of PDT is based on the interaction between photosensitizing agents such as 5-aminolevulinic acid (5-ALA) or methyl-aminolevulinate (MAL) and a light source.[9,10] For this purpose, non-coherent light emitting diodes (LED) are used in daily practice.[11]

A major disadvantage of non-coherent light sources is high pain experience, especially in patients with multiple AK. As a result, this forms a major drawback for follow-up treatments.[12]

Prior research aimed to optimize PDT by attempting to reduce pain, offer shorter treatment duration and shorter down times. One example is illumination with a long-pulsed pulsed dye laser (LP PDL).[13] Despite promising results regarding the equal efficacy with less side effects, it remains unclear whether this efficacy is maintained at long-term follow-up.[13-15] In our study, we compared treatment efficacy of LED-PDT and PDL-PDT with a long-term follow-up.

Methods

Patients

Participants were recruited and treated at a secondary dermatology referral center in the Netherlands. Inclusion criteria were age 18 years or older, Fitzpatrick skin type I-III and a clinical diagnosis of AK on the scalp and/or forehead (minimal area of 25 cm^2). Exclusion criteria were suspicion for malignancy in the treatment area, the use of immunosuppressive medication, any topical treatment in the past six months within the treatment area, known hypersensitivity for the photosensitizer or presence of other skin conditions in the treatment area. The study was approved by the local medical ethical review board.[16] All patients gave their written informed consent.

Procedures

The total number of target lesions in the treatment area of each individual participant was scored. Lesion severity was assessed using the Olsen scale (1 = mild (slightly palpable, more easily felt than seen); 2 = moderate (moderately thick, easy to see and feel); 3 = severe (very thick AK)). Two clinically equal treatment areas were assigned. Subsequently, both areas were pre-treated with slight curettage, followed by methyl-aminolevulinate cream (Metvix, Galderma ®) application. Both areas were covered with an occlusive and light blocking tape. After 3 hours, all participants received PDL illumination on the left side of the treatment area (595 nm Pulsed Dye Laser, Vbeam, Candela Corporation®, Wayland, MA, 7 mm spot size, fluence 7 J/cm^2, pulse duration 10ms, epidermal cooling with Dynamic Cooling Device (DCD spray/delay) 30/10ms, spots overlapping 50%) and regular LED illumination on the right side (Aktilite, Galderma®, 37 J/cm^2, 635 +/- 18 nm.

Outcome assessment

Follow-up visits were scheduled at 3, 6, 9 and 12. During each follow-up the number of target AK's was calculated. Adverse events were recorded by questionnaires. Pain scores were assessed using a visual analogue scale (VAS 0-10).

Statistical analysis

The primary outcome measure was defined as mean change in number of lesions between baseline and 12 months follow-up. A t-test for paired samples was conducted to test difference in mean decrease between treatments. The sample size of this study with 57 patients enabled detection of a between treatment difference (in mean decrease of AK lesions with a standard deviation of 3) of 1.6 or more with a power of 80%.

Other continuous outcomes were also tested for statistical significance with a t-test for paired samples. Differences in proportions between treatments were tested using the McNemar test for paired proportions. All analyses were performed on an intention to treat basis. P-values smaller than or equal to 0.05 were considered as a significant difference.

Results

Sixty-one male patients with a mean age of 73.7 years (range 57-87) and Fitzpatrick skin type I-III were included. Baseline characteristics are shown in table 1. A total of 1041 AKs (531 PDL, 510 LED) with a mean Olsen score of 2.02 and 2.07 for LED and PDL illumination respectively, were included. A total of 57 patients were treated and completed follow-up. The other four patients were lost to follow-up because traveling distance or because severe health problems restrained them from follow-up visits. One patient was not able to complete LED illumination, due to extensive pain sensation. This patient was subsequently treated with PDL illumination on both treatment areas, but was analyzed in the LED group. Two patients were illuminated with the same treatment regimen again at 3 months follow-up, because of no or little clinical response in both treatment areas.

Efficacy
The mean decrease in number of lesions from baseline to 12 months follow-up was -4.25 (95% CI [-5.07; -3.43]) for LED-PDT and -3.88 (95% CI [-4.76; -2.99]) for PDL-PDT. The negative sign indicates a decrease in number of AK lesions in both groups. Hence, the difference between treatments in mean number of lesions was -0.46 (95% CI [-1.28;0.35]) with a standard deviation (SD) of 3.04 (p = 0.258).

The relative decrease in total number of lesions from baseline (as percentage of the number of AK lesions at baseline) was 47.8% and 55.8% for PDL and LED illumination, respectively, at 12 months follow-up. Table 2 shows all the relevant outcome measurements. Figure 1 illustrates the percentage of patients with better, equal or worse efficacy of LED compared to PDL illumination.

The McNemar test showed no significant difference in global clinical improvement between both treatment groups (p = 0.625). A total of 89.3% of patients showed clinical improvement after PDL versus 92.9% after LED illumination.

Table 1. Relevant baseline characteristics for both pulsed dye laser (PDL) and light emitting diode (LED, Aktilite) illumination.

	PDL (n=61)	LED (n=61)
Age (y), mean ± SD	73.7 ± 7.5	73.7 ± 7.5
Sex		
Male	61 (100%)	61 (100%)
Female	0 (0%)	0 (0%)
Fitzpatrick skin type		
I	14 (22.9%)	14 (22.9%)
II	45 (73.8%)	45 (73.8%)
III	2 (3.3%)	2 (3.3%)
No. of lesions per patient		
1-5	14 (23%)	17 (27.8%)
6-10	32 (52.5%)	28 (46%)
11-15	11 (18%)	13 (21.3%)
>15	4 (6.5%)	3 (4.9%)
Total No. of lesions	531	510
Baseline total AK lesions (mean ± SD)	8.70 **±** 3.98	8.36 **±** 3.79
Olsen grade (No. of lesions)		
1	6 (9.8%)	9 (14.7%)
2	45 (73.8%)	42 (68.9%)
3	10 (16.4%)	10 (16.4%)
Lesion location		
Scalp	50 (82%)	50 (82%)
Forehead	11 (18%)	11 (18%)

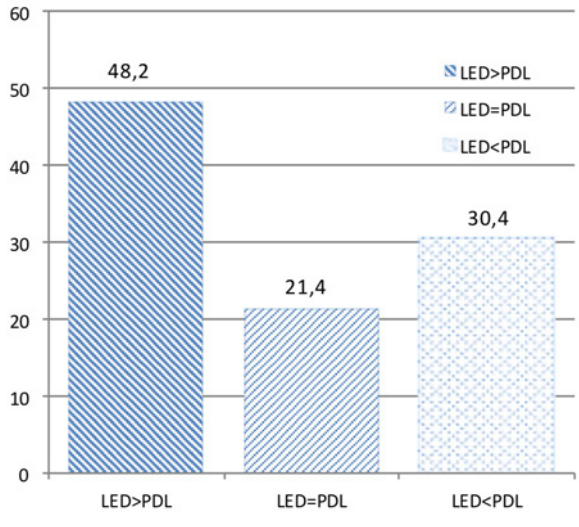
AK: actinic keratosis, SD: standard deviation.

Table 2. Relevant outcome measurements for both light emitting diode (LED) and pulsed dye laser (PDL) illumination during follow-up.

		Baseline	3 months	6 months	9 months	12 months
LED	Mean lesion decrease (95% CI)	-	-4.93 [-4.10; 5.76]	-5.17 [-5.97;-4.37]	-4.75 [-5.57; -3.93]	-4.25 [-5.07;-3.43]
	Total number AK	510	197	183	204	225
	Cured number AK	-	313	327	306	285
	Percentage decrease from baseline	-	61.4%	64.1%	60.0%	55.8%
PDL	Mean lesion decrease (95% CI)	-	-5.11 [-5.80;-4.42]	-5.29 [-6.15;-4.43]	-4.61 [-5.44;-3.78]	-3.88 [-4.76;-2.99]
	Total number AK	531	223	203	236	277
	Cured number AK	-	308	328	295	254
	Percentage decrease from baseline	-	58.0%	61.8%	55.6%	47.8%

AK: actinic keratosis, CI: confidence interval

Figure 1. Percentage of patients with better, equal or worse efficacy of light emitting diode (LED) compared to pulsed dye laser (PDL) illumination



Side effects

VAS pain score after PDL was significantly lower than after LED with a mean VAS-score of 2.64 (SD= 1.84) and 6.47 (SD = 2.17) for PDL and LED respectively. The mean difference (PDL minus LED) was -4.55 (95% CI = [-4.06;-5.05], $p < 0.01$). Mean treatment duration for PDL was 1.45 minutes, compared to a predetermined 7.23 minutes for LED illumination.

Table 3 demonstrates the percentages of patients who reported side effects. Burning sensation was reported significantly more often after LED illumination compared to PDL. Two patients developed a local skin infection in the LED treatment area, which was subsequently treated with topical antibiotic ointment (Fucidin cream, 20 mg/g, Leo Pharmaceuticals, the Netherlands). The skin healed without any residual changes in both patients.

Patient preferences

Of the patients treated with PDL illumination, 78.7% would definitely undergo this treatment again versus 32.8% of the patients treated with LED illumination ($p < 0.01$). Furthermore 4.9% of patients treated with PDL illumination would definitely not undergo another treatment versus 19.7% of the patients treated with LED illumination ($p = 0.013$).

Table 3. The frequency of adverse events 1 week post-treatment,

Adverse event	PDL, n (%)	LED n (%)	*p*-value*
Burning sensation	13 (21.3)	43 (70.5)	< 0.001
Erythema	54 (88.5)	59 (96.7)	0.095
Crusting	3 (4.9)	11 (18.0)	0.033
Infection	0 (0)	2 (3.3)	0.248

* McNemar test for paired proportions

Discussion

PDL illumination is a quick, patient friendly and safe treatment for mild to moderate AK.[13,14] To our knowledge, the present study is the first to demonstrate efficacy data with long-term follow-up. Our results indicate that both illumination sources result in a similar decrease of AK lesions between baseline and 12 months follow-up.

The similar effectiveness of PDL and LED is consistent with other studies that reported results after shorter follow-up duration. Aleksiades et al. studied the use of LP-PDL (595 nm) illumination after either 3 or 14 to 18 hours incubation time with topical 5-ALA cream.[13] They concluded that it is a safe and effective treatment, with minimal discomfort and rapid recovery times. Specifically, the mean percentage of lesions cleared after one treatment

at 8 months follow-up was 90.3%. However, the number of patients who completed the 8-month follow-up was small and little information about statistical analyses is given.[13,17-19] Other studies with a 1-3 month follow-up period reported no difference in efficacy between LED and PDL. These results however cannot be compared with our study as they had a shorter follow-up period and were performed within a smaller population.[14,15]

The exact mechanism behind the PDT response is not fully known yet. PDT is mediated by oxygen dependent photochemical reactions. In epithelial neoplasms, the topical photosensitizer is metabolized into protoporphyrin IX (PpIX) following illumination with visible light.[20] Excitation of photosensitizers such as 5-ALA or MAL results in formation of cytotoxic free radicals and singlet oxygen. These target cellular and mitochondrial membranes resulting in apoptosis and necrosis.[14,21,22] It is hypothesized that by dividing light exposure into several shorter pulses, there might be time for tissue re-oxygenation. This principle can be seen in pulsed laser systems. PDL illumination does trigger apoptosis, but because there is time for re-oxygenation in between pulses, there might be less tissue ischemia. A paper by Togsverd-Bo et al. describes the amount of photobleaching – the depletion in photosensitizer fluorescence intensity - using different light sources. They conclude that LED produces significantly higher photobleaching compared with LP-PDL. The median photobleaching percentages of LED at a dose of 37 J/cm^2 were 91% and 98%, compared to 43% and 52% after LP-PDL at 7.5 J/cm^2. This might explain the lower pain experience during PDL illumination.[23]

Pain is a major concern among physicians and a major drawback for patients to undergo new PDT treatments in the future. Our results show that pain scores are high following LED mediated PDT, while pain sensation during PDL illumination is significantly lower. Our results also indicate a higher patient preference for PDL over LED illumination.

In previous studies, several factors that might influence pain sensation during PDT have been described.[24,25] The presence of a dynamic cooling device, fast operation speed and the ability to work with longer pulse durations with non-purpuric effects, all may contribute to lower pain sensation in patients after PDL illumination.[17,18] Air-cooling for example reduces the level of pain sensation during illumination.[26,27] However, the amount of PpIX photobleaching is reduced when for example air-cooling devices are used, which might influence efficacy.[28] Wiegell et al. suggested that pain sensation was directly related to the amount of PpIX formation prior to illumination.[29] Another hypothesis is that photosensitizing agents are transported into peripheral nerve-endings, hereby triggering nerve stimulation.[30,31] The presence of apoptosis and necrosis, together with an inflammatory reaction, presumably contributes to the burning sensation as well. Beside a lower pain sensation, other studies do show that PDL illumination can result in side effects such

as erythema and burning sensation as well, albeit to a smaller extent.[14,17,18] Our results support these observations.

Despite the possible benefit of pain reduction, shorter treatment duration and less adverse events, PDL illumination has various disadvantages that should be taken into account. Relatively high costs, the need for special supplies and expertise to use the device are the most important ones. Not every hospital has a PDL device present. Both PDL and LED illumination are in-hospital treatments. Several studies have been done to assess efficacy of daylight as illumination source for the PDT response with the supposed advantage of less pain during the procedure.[32-35] These studies show non-significant differences between the efficacy of daylight and LED illumination and report high patient satisfaction, less pain sensation and a better time,- and cost effectiveness. Daylight PDT is therefore also a good alternative in cases in which pain is a limiting factor. However, in Europe it cannot be performed throughout the year. PDL-PDT is therefore a good alternative in winter times. The shorter total duration of PDL-PDT compared to daylight-PDT, is also an advantage.

Limitation of the present study is the open label non-randomized study design and the fact that the patients and investigator were not blinded. Also, newly developed lesions post-treatment were not differentiated from persistent lesions in analysis. Besides the presence of side effects, the duration of side effects was not assessed.

To conclude, AK is a chronic lifetime skin condition with frequent relapses. Our results show that PDL illumination can be performed rapidly, resulting in lower pain sensation and is an acceptable alternative illumination source when pain is a limiting factor for regular LED illumination.

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Supplementary methods

Study design

The current study is a prospective, open-label study with split-face design. Patients were treated with pulsed dye laser (PDL) mediated photodynamic therapy (PDT) on the left side of the face and conventional light emitting diode (LED)-mediated PDT on the right side of the face as was predefined in the study protocol. Both treatments took place on the same day with an interval of 1 min. Treatment order was counterbalanced to control for order effects. This was done to prevent an effect of treatment order on pain score for example. The primary outcome measure was mean change in the number of lesions between baseline and 12-month follow-up. Secondary outcome measures were pain sensation, qualitative clinical improvement and adverse events.

Patients

Participants were recruited and treated at the dermatology department of a secondary dermatology referral center in Eindhoven, the Netherlands, between November 2011 and August 2012. Patients were considered eligible if they were 18 years or older, had Fitzpatrick skin type I–III and a clinical diagnosis of actinic keratosis (AK) on the scalp and/or forehead. The AK had to cover a minimal area of 25 cm^2 with the potency to be divided into 2 equal halves. The diagnosis was based on clinical assessment. Exclusion criteria were suspicion for malignancy in the treatment area, the use of immunosuppressive medication, topical treatment of any kind in the past 6 months within the treatment area, known hypersensitivity for the photosensitizer or presence of other skin conditions in the treatment area. The study was performed in accordance with the principles of the Declaration of Helsinki and approved by the local medical ethics review board (16). Prior to enrolment, all patients gave their written informed consent.

Procedures

At baseline the total number of target lesions in the treatment area of each individual participant was scored. Furthermore, lesion severity was assessed using the Olsen scale, based on the thickness of AK: 1 = mild (slightly palpable, more easily felt than seen); 2 = moderate (moderately thick, easy to see and feel); 3 = severe (very thick and/or obvious AK). Color photographs were taken from the treatment area at baseline and at each follow-up visit. Furthermore, patients' concomitant medication and skin type according to the Fitzpatrick 6-point scale were registered. When the inclusion criteria were met, 2 clinically equal treatment areas were assigned. Subsequently, both areas were pre-treated with slight curettage of hyperkeratotic lesions, followed by methyl-aminolevulinate cream (Metvix®, Galderma Benelux, Rotterdam, The Netherlands) application. Both areas were covered with an occlusive dressing (Tegaderm®, 3M Health Care, Amsterdam, The Neth-

erlands), a gauze, tinfoil and light-blocking tape, in order to increase penetration of the photosensitizer and prevent light exposure. After a 3-h incubation time, the cream residue was removed. Subsequently, all participants received PDL illumination on the left side of the scalp and/or forehead (595 nm Pulsed Dye Laser, Vbeam, Candela Corporation®, Wayland, MA, USA, 7-mm spot size, fluence 7 J/cm^2 , pulse duration 10 ms, epidermal cooling with Dynamic Cooling Device (DCD spray/delay) 30/10 ms, spots overlapping 50%) and regular LED illumination on the right side (Aktilite®, Galderma), 37 J/cm^2 , 635 ± 18 nm, cooling was obtained with the incorporated fan. During illumination of either one of the areas, the other adjacent area was covered with occlusive dressing to prevent light exposure. Time required for both illuminations was registered. Because of the treatment nature patients were not blinded for treatment.

Outcome assessment

Follow-up visits were scheduled at 3, 6, 9 and 12 months post-treatment and were performed by the same investigator (JK). During follow-up, the remaining number of target AKs were calculated. Moreover, global clinical improvement in AK post-treatment was scored in a qualitative way as no clinical improvement vs. clinical improvement. In case of no treatment effect or an increase in AK after treatment, the same treatment(s) were repeated. When a histologically confirmed skin malignancy was confirmed within the treated area, this was registered. Patients were asked to complete a detailed diary in which they recorded adverse events (erythema, crusting, infection and burning sensation) during the first 2 weeks post-treatment. In this diary, concomitant medication, such as antibiotic treatment, used within 2 weeks after treatment, was registered. Pain scores were assessed immediately after both treatments using a visual analogue scale (VAS) ranging from 0 (no pain at all) to 10 (worst pain imaginable).

Statistical analysis

The primary outcome measure was defined as the mean change in the number of lesions between baseline and 12-month follow-up. The decrease in number of AK lesions per patient in the treatment area was calculated. A t-test for paired samples was conducted to test the difference in mean decrease between treatments. The sample size of this study with 57 patients enabled detection of a between-treatment difference (in the mean decrease in AK lesions with a SD of 3) of 1.6 or more with a power of 80%. Other continuous outcomes were also tested for statistical significance with a t-test for paired samples. Differences in proportions between treatments were tested using the McNemar test for paired proportions. All statistical analyses were performed on an intention-to-treat basis. Data were collected and analyzed with SPSS (version 19.9 for Windows). p-values smaller than or equal to 0.05 were considered as a significant difference.


2.3

Chapter 2.3

Topical ingenol mebutate versus 5% 5-fluorouracil versus 5% imiquimod versus photodynamic therapy in the treatment of actinic keratosis: a multi-center randomized controlled trial

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Submitted

"The true method of knowledge is experiment"
- William Blake

Abstract

Background

Actinic keratosis (AK) is the most frequent premalignant skin disease in the Caucasian population. In current guidelines, there are no clear recommendations about which treatment is preferred. We aimed to investigate the effectiveness of 5-fluorouracil, imiquimod, photodynamic therapy (PDT) and ingenol mebutate (IM) in patients with multiple AK in the head and neck area.

Methods

In this single-blind, randomized controlled multicenter trial, we enrolled patients with clinical diagnosis of ≥ 5 AK lesions in the head and neck area, involving one continuous area of 25-100 cm^2, in four Dutch hospitals. Patients were randomly assigned to 5-fluorouracil cream (twice daily for four weeks), 5% imiquimod cream (three days/week for four weeks), methylaminolevulinate (MAL-)PDT (one session), or 0·015% IM gel (three consecutive days). Data were collected by one observer blinded to treatment allocation. The primary outcome is the proportion of patients with ≥75% reduction of the number of AK counted at baseline, 12-months post-treatment, according to intention-to-treat analysis. Here we report the treatment success 3 months post-treatment.
This trial is registered as an International Randomized controlled trial (NCT 02281682).

Findings

A total of 624 patients were recruited between November 2014 and March 2017 and randomized for treatment with 5-fluorouracil (155), imiquimod (156), MAL-PDT (156), and IM (157). 3 months post-treatment, 149 patients treated with 5-fluorouracil, 148 with imiquimod, 154 with MAL-PDT, and 150 with IM were analyzed. The proportion of patients with treatment success was 90·6% (95% CI 84·7-94·4), 76·4% (95% CI 68·9-82·5), 76·0% (95% CI 68·6-82·1), and 67·3% (95% CI 59·5-74·3) for 5-fluorouracil, imiquimod, MAL-PDT and IM, respectively. The relative risk was 0·83 (95% CI 0·75-0·92 p<0·001), 0·84 (95% CI 0·76-0·93 p<0·001) and 0·74 (95% CI 0·66-0·84 p<0·001) for imiquimod, MAL-PDT and IM, respectively, with 5-fluorouracil as reference therapy.

Interpretation

Three months post-treatment it seemed that 5% 5-fluorouracil cream is more effective than 5% imiquimod cream, MAL-PDT and 0·015% IM gel in the treatment of patients with multiple grade I-III AK in the head,- and neck area.

Introduction

Actinic keratosis (AK) is the most frequent premalignant skin disease in the Caucasian population and is caused by UV exposure. A Dutch population based cohort study, reported a prevalence of 37·5% among the participants.[1] Extrapolation of these data suggests that approximately 1·4 million people above the age of 50 are affected in the Netherlands.[1] Hence, it is one of the most frequent reasons for patients to visit a dermatologist.[2,3] Several studies suggest that, if left untreated, AK might develop into squamous cell carcinoma (SCC).[4,5] However, there is little evidence which AK lesion is at risk and what proportion of AK will progress into a SCC, with percentages ranging between 0·025% and 16% per AK lesion per year.[6-8]

There is a high recurrence rate following treatment of AK and repetitive treatments are common. Solitary lesions can easily be treated with cryotherapy. However, AK patients often present with multiple lesions in one continuous area, so called field-change.[3,9] For these continuous areas field-directed therapies are preferred.

There are several treatment modalities such as creams (5-fluorouracil, imiquimod), gels (ingenol mebutate, diclofenac), photodynamic therapy (aminolevulinic acid (ALA)-, methylaminolevulinate (MAL)-, daylight), laser therapy, and chemical peelings. They have a different mode of application, side-effects, cosmetic appearance, costs, and probably also variable effectiveness.

In the current Dutch and British guidelines there are no clear recommendations about which treatment modality is preferred.[10-12] The most prescribed and studied treatment modalities are 5-fluorouracil cream, imiquimod cream, ingenol mebutate (IM) gel, and photodynamic therapy (PDT). 5-Fluorouracil cream is a topical chemotherapeutical agent that is self-applied twice daily for approximately 4 weeks. 5% Imiquimod cream is an immunomodulating agent, applied by the patient three times a week for 4 weeks once daily. IM gel has a dual mode of action, causing rapid lesion necrosis and specific activation of the immune system and requires only a few applications: once daily on two or three consecutive days depending on the location.[13,14] PDT, combining a photosensitising agent with light, is an in-clinic treatment and is performed in one single session.

Currently treatment considerations in field-directed therapy of AK are depending on lesion-, patient- and treatment- related factors and not based on proof of effectiveness gained with head-to-head studies. Very few randomized trials with direct comparisons between treatments and long-term follow-up have been published. In those trials predominantly grade I and II AK were included. A Cochrane systematic review performed by Gupta

et al. concluded that field directed treatments might have similar effectiveness, but there is a need for more trials with direct comparisons to determine best therapeutic approach.[15]

To the best of our knowledge, the current study is the first randomized controlled trial with direct comparison of 5-fluorouracil, imiquimod, IM, and MAL-PDT, in terms of effectiveness. This paper presents the effectiveness in terms of more than 75% reduction of lesions at 3-months post-treatment.

Methods

Study design

This multi-center single blinded randomized study was conducted at the dermatology department of four hospitals in the Netherlands. The trial was performed and coordinated at the Maastricht University Medical Center (MUMC+). Other participating centers were Zuyderland Medical Center Heerlen (ZMC), VieCuri Medical Center Venlo/Venray (VC), and the Catharina Hospital Eindhoven (CH).

The study was performed according to the declaration of Helsinki. The study protocol was approved by the local medical ethical committee of the MUMC+.

Patients

Patients older than 18 years with a clinical diagnosis of minimally 5 AK lesions in the head and neck area, involving one continuous area of 25-100 cm^2 were eligible for participation. All AK grades (Olsen grades I-III) were included. Patients were not able to participate if they had any treatment for AK in the study area in the past 3 months or using systemic retinoids or immunosuppressant drugs. Other reasons for exclusion were suspicion of malignancy in the target area, porphyria, allergy to study drugs or peanut/soy products, pregnancy or breastfeeding or a personal history of a genetic skin cancer disorder. All patients gave their written informed consent before being randomized.

Randomization and masking

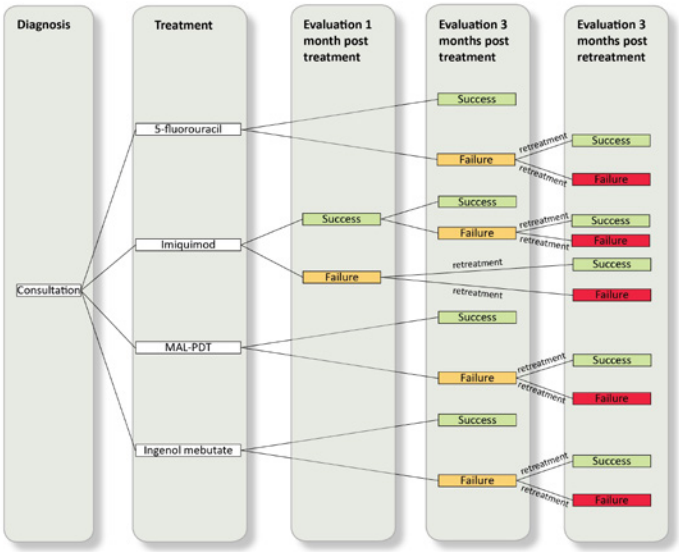
After written informed consent, patients were randomly assigned to one of four investigated treatments. Randomization was performed using minimisation.[16] Computer generated randomization lists were created using ALEA (ALEA version 2·2, Amsterdam, the Netherlands). Stratifying factors were center of treatment and severity of AK grade. Participants were randomly assigned to one of the four treatments in a 1:1:1:1 ratio.

All study visits were performed by one investigator who was blinded for treatment allocation (MJ) and recorded all study endpoints. A second investigator, not-blinded to treatment allocation (JK), was responsible for the distribution of study information and medication and took care of additional hospital visits in case of adverse events. Due to the nature of study medication patients could not be blinded for treatment allocation.

Procedures

Patients who were considered eligible to participate were randomly assigned to treatment groups. Treatment strategies consisted of a first treatment and if necessary a re-treatment in case of insufficient treatment response, defined as <75% lesion response at the 3-months follow-up visit. For patients assigned to 5-fluorouracil, IM or MAL-PDT, response was primarily evaluated at 3 months after the end of the first treatment. For patients assigned to imiquimod, treatment response was primarily evaluated at 1 month after the last treatment day, in accordance with the imiquimod summary of product characteristics (SPC) guidelines. *Figure 1* provides a schematic representation of the treatment strategies.

Figure 1. Schematic presentation of the treatment strategies



PDT treatment was performed by trained nurses. MAL cream (Metvix®, Galderma SA, Penn Pharmaceutical Services, Gwent, UK) was applied to the treatment area in a thin layer of 1 mm. Consecutively, the area was covered by light blocking aluminium foil and occlusive dressing (Tegaderm®, 3M, Leiden, the Netherlands), in order to prevent light

exposure. After 3 hours the excess cream was removed and the area was illuminated with a light emitting diode (LED): Aktilite® (Galderma, SA, Lausanne, Switzerland) or Omnilux® (Waldmann phototherapeutics, London, UK) with an optimum wavelength of 635 ± 18 nm, at a fluence of 37 J/cm^2 during 7.23 minutes. Directly after illumination the treatment area was covered up again to protect from light exposure during 24 hours. For each 25 cm^2 of treatment area, 2 grams of MAL cream were used.

IM 0·015% gel (Picato®, LEO Pharma A/S, Bellerup, Denmark) was applied once daily for 3 consecutive days. For each 25 cm^2 of treatment area, one tube of 0·47 gram per application was used.

For 5% imiquimod cream (Aldara®, Meda Pharma B.V., Solna, Sweden) patients were instructed to apply the cream once daily before going to sleep and wipe it off in the morning. This was performed 3 days a week (Monday-Wednesday-Friday), for 4 consecutive weeks. Per area of 25 cm^2 one sachet of 250 mg was used per application.

5% 5-Fluorouracil cream (Efudix®, Meda Pharma B.V., Amstelveen, the Netherlands) was instructed to apply twice daily for 4 weeks. Each patient received one tube of 40 grams independent of the treatment area size.

In all patients, slight curettage of hyperkeratotic lesions was performed prior to start of treatment. No occlusive dressing was allowed during treatment with any of the topical ointments.

Outcomes

The primary outcome of this study is the proportion of patients with ≥75% reduction of the number of AK counted at baseline, 12 months after the last treatment application. Secondary outcomes are the proportion of patients with ≥75% reduction of the number of AK counted at 3-months after the end of the last treatment compared to baseline, the proportion of patients with partial response (defined as 50-75% reduction in number of AKs counted at baseline) at 3 and 12 months, side effects, patient satisfaction, cosmetic results, compliance, and healthcare / treatment costs. Here we report the results at 3-months post-treatment.

Evaluation of outcomes

At the first study visit (baseline) the treatment area was defined by the physician (MJ). Lesion count and extent was performed by drawing all lesions with their exact location on a transparent sheet. Physical reference points such as hair line, ears or wrinkles were used as landmarks. The overall severity of each lesion was assessed using the Olsen scale

by grading AK in three categories: 1 = mild (slightly palpable, more easily felt than seen), 2 = moderate (moderately thick, easy to see and feel) or 3 = severe (very thick and/or obvious AK).[17] Digital photographs were taken at each study visit. The same physician (MJ) assessed treatment outcome by lesion count and by assessing the Olsen grade for each individual lesion at 3- and 12-months after the end of treatment. For logistical reasons, follow-up visits were planned within a window of one month prior or one month after the intended date.

All patients could receive a maximum of 2 allocated treatment courses depending on response to the first treatment course. In case of <75% clearance of AK at 3 months after the final treatment, these non-responding lesions were evaluated as treatment failures for final analysis.

Patients were asked to complete detailed diaries to obtain information about side effects, treatment compliance and treatment satisfaction. Pain and burning sensation were recorded using a Numeric Rating Scale (NRS) from 0-10, with 0 indicating no pain and 10 indicating unbearable pain. Pain and burning sensation scores were categorized into absent/mild (0·0 – 3·0), moderate (3·1-6·0) or severe (6·1-10·0). Patient-reported adverse events such as erythema, swelling, erosion, crusting, vesicles/bullae, squamae and itching were obtained through the diary, using a 4-point scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe). In the analysis, the severity of adverse events was classified into absent/mild and moderate/severe. For all adverse events, the maximum scores during treatment and post-treatment were used for analyses.

Data on treatment compliance were retrieved by telephone consultation two weeks after treatment by the investigator not blinded to treatment allocation (JK). Patients were asked if they completed the whole treatment schedule, and if not, on which day/week they stopped treatment. If patients stopped on advice of a physician, due to adverse events, this was also recorded by the same investigator.

Statistical analyses

The sample size of this study was based on the primary endpoint, ≥75% lesion reduction at 12 months. Based on previous studies we estimated that 65% of patients would have ≥75% lesion reduction at 12 months after treatment.[14,18] To enable detection of a 15% difference between treatment groups with a power of 80% and alpha=5%, 140 patients were required per treatment group. To account for a potential loss to follow-up of 10%, a total of 624 (4x156) patients needed to be included.

Endpoints were compared between the treatment groups. Between-group differences in proportions were compared using the Chi-square test or Fisher exact test. In case of continuous variables between-group differences were compared using analysis of variance (ANOVA) if normally distributed, or a non-parametric test for independent samples if not-normally distributed. Both modified intention-to-treat (ITT) and per-protocol (PP) analysis were performed. P-values ≤0·05 were considered statistically significant. Analyses were performed using SPSS version 23·0 (IBM Corp., Armonk, NY, USA) and www.openepi.com. The trial is registered on clinicaltrials.gov (NCT02281682).

Role of the funding source

The sponsor of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between November 2014 and March 2017, a total of 1174 patients were assessed for eligibility. From those, 550 patients refused to participate due to the following reasons: preference or disfavor for one or more of the studied treatments (n=197), old age or comorbidities (n=113), disapproval to receive a treatment by randomization (n=88), refusal to treat the AK (n=53), logistic reasons (n=24), anxious of possible side effects (n=24), treatment costs (n=9), and preference of treatment in a different hospital (n=3). One patient died before informed consent could be obtained and 38 patients did not give a reason for their refusal to participate.

A total of 624 patients were randomized in 4 hospitals: MUMC+ (247), CH (176), VC (108), and ZMC (93), of which 155 were treated with 5-fluorouracil, 156 with imiquimod, 156 with MAL-PDT, and 157 with IM *(Figure 2).* Fourteen patients did not start treatment and 9 patients were treated but did not attend the 3 months follow-up visit. Eight cross-overs occurred before the assigned treatment was started, all because patients preferred a different therapy. One patient assigned to 5-fluorouracil received MAL-PDT. Two patients (one assigned to imiquimod and one to IM) received 5-fluorouracil. Of 5 patients allocated to PDT, 3 received 5-fluorouracil, and 2 IM. No substantial imbalances in baseline characteristics were observed between treatment groups *(Table 1).*

Figure 2. Patient flow chart

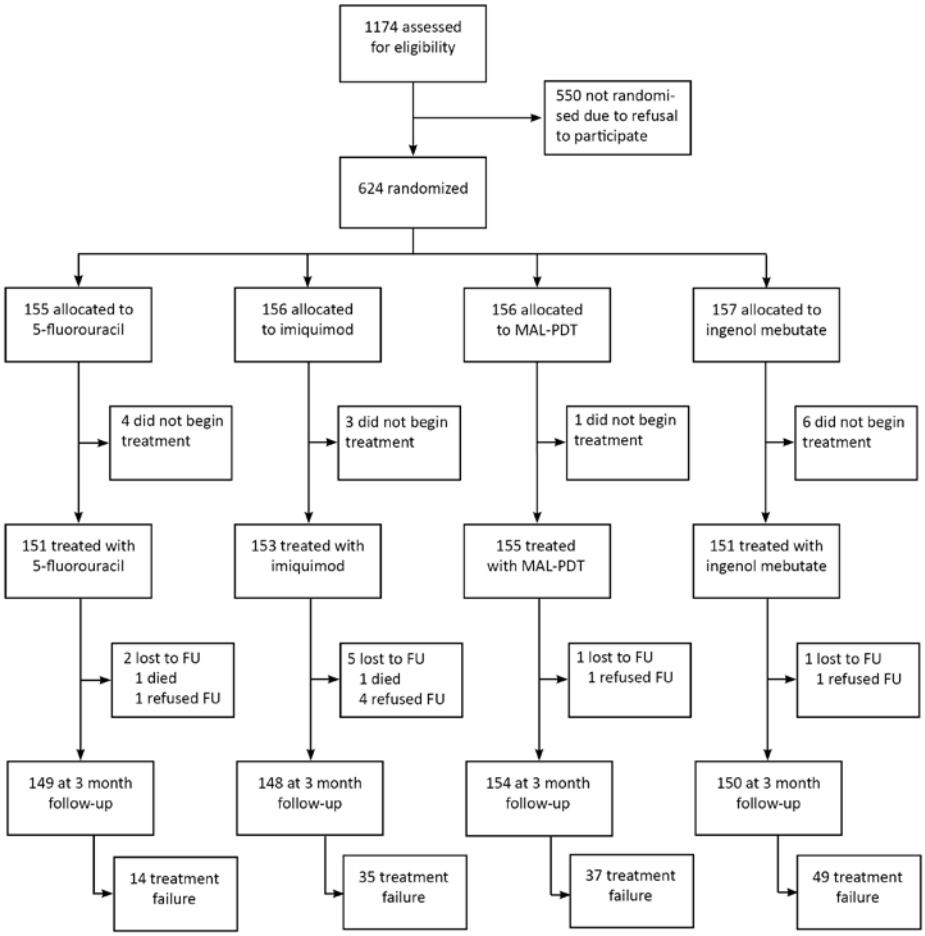


Table 1. Baseline characteristics of the modified intention-to-treat population

Characteristic	Total (n= 624)	5-FU (n=155)	Imiquimod (n=156)	MAL-PDT (n=156)	IM (n=157)
Sex					
Male	558 (89·4%)	136 (87·7%)	143 (91·7%)	140 (89·7%)	139 (88·5%)
Female	66 (10·6%)	19 (12·3%)	13 (8·3%)	16 (10·3%)	18 (11·5%)
Age in years median (range)	73 [48-94]	74 [48-90]	73 [59-89]	73 [55-90]	72 [51-94]
Skin type					
I	245 (39·3%)	63 (40·6%)	67 (42·9%)	54 (34·6%)	61 (38·9%)
II	333 (53·4%)	81 (52·3%)	79 (50·6%)	92 (59·0%)	81 (51·6%)
III	46 (7·4%)	11 (7·1%)	10 (6·4%)	10 (6·4%)	15 (9·6%)
History of AK					
Yes	487 (78%)	121 (78·1%)	129 (82·7%)	115 (73·7%)	122 (77·7%)
No	137 (22%)	34 (21·9%)	27 (17·3%)	41 (26·3%)	35 (22·3%)
History of (N)MSC					
Yes	353 (56·6%)	90 (58·1%)	82 (52·6%)	86 (55·1%)	95 (60·5%)
No	271 (43·4%)	65 (41·9%)	74 (47·4%)	70 (44·9%)	62 (39·5%)
Sun exposition					
Mild	19 (3·0%)	6 (3·9%)	5 (3·2%)	5 (3·2%)	3 (1·9%)
Moderate	283 (45·4%)	69 (44·5%)	73 (46·8%)	72 (46·2%)	69 (43·9%)
Severe	322 (51·6%)	80 (51·6%)	78 (50·0%)	79 (50·6%)	85 (54·1%)
History of immunosuppressive drugs					
Yes	84 (13·5%)	18 (11·6%)	25 (16·0%)	19 (12·2%)	22 (14·0%)
No	540 (86·5%)	137 (88·4%)	131 (84·0%)	137 (87·8%)	135 (86·0%)
Treated area in cm^2 median (range)	81 [25-100]	80 [27-100]	86.5 [25-100]	81 [25-100]	78 [25-100]
# AK lesions median (range)	16 [5-48]	16 [5-48]	16.5 [5-37]	16 [5-38]	15 [5-40]
Severity of AK					
Olsen Grade I and II	575 (92·1%)	144 (92·9%)	143 (91·7%)	144 (92·3%)	144 (91·7%)
>1 lesion Olsen Grade III	49 (7·9%)	11 (7·1%)	13 (8·3%)	12 (7·7%)	13 (8·3%)
Location					
Vertex	321 (51·4%)	78 (50·3%)	78 (50·0%)	80 (51·3%)	85 (54·1%)
Face	303 (48·6%)	77 (49·7%)	78 (50·0%)	76 (48·7%)	72 (45·9%)
Study site					
Maastricht	247 (39·6%)	61 (39·4%)	62 (39·7%)	62 (39·7%)	62 (39·5%)
Eindhoven	176 (28·2%)	43 (27·7%)	44 (28·2%)	44 (28·2%)	45 (28·7%)
Venlo	108 (17·3%)	27 (17·4%)	27 (17·3%)	27 (17·3%)	27 (17·2%)
Heerlen	93 (14·9%)	24 (15·5%)	23 (14·7%)	23 (14·7%)	23 (14·6%)

Definition of abbreviations: F, female; M, male; IM, ingenol mebutate; 5-FU, 5-fluorouracil; MAL-PDT, methylaminolevulinate photodynamic therapy; AK, actinic keratosis; (N)MSC, (non)melanoma skin cancer;

Effectiveness

Table 2 shows the proportion of treatment success 3 months after final treatment (including re-treatment) for all treatment groups based on modified ITT and PP analysis. The percentage of treatment success for 5-fluorouracil was 90·6% (95% CI 84·7-94·4). For imiquimod, PDT, and IM these percentages were 76·4% (95% CI 68·9-82·5), 76·0% (95% CI 68·6-82·1), and 67·3% (95% CI 59·5-74·3), respectively, according to the ITT analysis. 5-Fluorouracil cream was significantly more effective compared to IM gel, PDT, and imiquimod cream *(Table 2A)*. When comparing imiquimod and PDT to IM, statistically significant differences in favor of imiquimod and PDT were found *(Table 2B)*. The PP population consisted of patients who were treated according to the treatment protocol and patients with initial treatment failure who refused retreatment were excluded. Cross-overs were analyzed in the group of the treatment they actually received. According to the PP analyses, the proportion treatment success was higher in all treatment groups and differences in effectiveness became smaller (*Table 2A*).

There was treatment failure after one treatment cycle in 14·8% (23/155) of the patients after 5-fluorouracil, 35·9% (56/156) after imiquimod, 34·6% (54/156) after MAL-PDT and 47·8% (75/157) after IM. Higher proportions of patients with initial treatment failure refused retreatment in the imiquimod, MAL-PDT, and IM group than in the 5-fluorouracil group. For 5-fluorouracil 19 of 23 (82·6%) patients who had treatment failure were re-treated. For imiquimod this were 44 of 56 (78·6%) patients, for MAL-PDT 41 of 54 (75·9%) patients and for IM this were 60 of 75 (80·0%) patients.

When restricting the analysis to patients with Grade I and II AK, the percentages of treatment success for 5-fluorouracil and MAL-PDT were slightly higher: for 5-fluorouracil the percentage of treatment success was 91·4% (95% CI 85·5-95·2). For imiquimod, PDT, and IM these percentages were 76·5% (95% CI 68·6-82·9), 78·9% (95% CI 71·4-84·8), and 67·2% (95% CI 58·9-74·5), respectively.

Side effects

Data on adverse events were available for 135 patients treated with 5-fluorouracil, 122 with imiquimod, 117 with MAL-PDT, and 140 with IM. Serious adverse events (SAEs) related to treatment did not occur in any of the four treatment groups. *Table 3* shows the percentages of patients who reported adverse events during treatment or the two weeks after treatment. Severe pain and burning sensation were more often reported by patients treated with MAL-PDT, compared to patients treated with topical cream/gel.

When assessing side effects two weeks post-treatment erythema was observed significantly more often after PDT treatment and erosions occurred slightly more often after

5-fluorouracil. IM treatment led to a more frequent report of crusts, squamae, and itching. Patients treated with PDT and 5-fluorouracil reported more burning sensation post-treatment.

Table 2. Proportions of treatment success and relative risks with 95% CI at 3 months post-treatment for therapies, with 5-fluorouracil (2A) or Ingenol Mebutate (2B) as reference therapy

	Intention-to-treat			*Per protocol*		
2A	**Proportion treatment success***	**RR (95% CI)**	***p*-value**	**Proportion treatment success***	**RR (95% CI)**	***p*-value**
5-Fluorouracil	135/149 (90·6%; 84·7-94·4)	1		138/148 (93·2%; 87·9-96·4)	1	
Imiquimod	113/148 (76·4%; 68·9-82·5)	0·84 (0·76-0·93)	<0·001	109/135 (80·7%; 73·2-86·6)	0·87 (0·79-0·95)	<0·001
PDT	117/154 (76·0%; 68·6-82·1)	0·84 (0·76-0·93)	<0·001	114/138 (82·6%; 75·4-88·1)	0·89 (0·81-0·97)	0·003
IM	101/150 (67·3%; 59·5-74·3)	0·74 (0·66-0·84)	<0·001	102/135 (75·6%; 67·6-82·1)	0·81 (0·73-0·90)	<0·001

	Intention-to-treat			*Per protocol*		
2B	**Proportion treatment success***	**RR (95% CI)**	***p*-value**	**Proportion treatment success***	**RR (95% CI)**	***p*-value**
IM	101/150 (67·3%; 59·5-74·3)	1		102/135 (75·6%; 67·6-82·1)	1	
Imiquimod	113/148 (76·4%; 68·9-82·5)	1·13 (0·98-1.31)	0·043	109/135 (80·7%; 73·2-86·6)	1·07 (0·94-1·21)	0·154
PDT	117/154 (76·0%; 68·6-82·1)	1·13 (0·98-1·30)	0·048	114/138 (82·6%; 75·4-88·1)	1·09 (0·97-1·24)	0·078

95% CI; confidence interval, RR; relative risk, PDT; photodynamic therapy, IM; Ingenol Mebutate
*Data are n/N (%; 95% confidence interval)

Table 3. Adverse events

	5-Fluorouracil (n =135) n (%)	Imiquimod (n = 122) n (%)	MAL-PDT (n =117) n (%)	IM (n =140) n (%)	*p*-value
During treatment					
Erythema			n.a.		
Moderate/Severe	110 (81·5)	88 (72·7)		105 (75)	0·22
Absent/Mild	25 (18·5)	33 (27·3)		35 (25)	
Swelling			n.a.		
Moderate/Severe	41 (30·4)	53 (43·8)		59 (42·1)	0·050*
Absent/Mild	94 (69·6)	68 (56·2)		81 (57·9)	
Erosion			n.a.		
Moderate/Severe	54 (40·0)	58 (47·9)		42 (30·0)	0·01*
Absent/Mild	81 (60·0)	63 (52·1)		98 (70·0)	
Crusts			n.a.		
Moderate/Severe	77 (57·0)	83 (68·6)		53 (37·9)	<0·001*
Absent/Mild	58 (43·0)	38 (31·4)		87 (62·1)	
Vesicles/bullae			n.a.		
Moderate/Severe	33 (24·4)	38 (31·4)		59 (42·1)	0·007*
Absent/Mild	102 (75·6)	83 (68·6)		81 (57·9)	
Squamae			n.a.		
Moderate/Severe	60 (44·4)	51 (42·1)		50 (35·7)	0·31
Absent/Mild	75 (55·6)	70 (57·9)		90 (64·3)	
Itching			n.a.		
Moderate/Severe	84 (62·2)	74 (61·2)		58 (41·4)	0·001*
Absent/Mild	51 (37·8)	47 (38·8)		82 (58·6)	
Pain					
Severe	22 (16·3)	11 (9·1)	73 (62·4)	17 (12·1)	<0·001*
Moderate	21 (15·6)	21 (17·4)	20 (17·1)	40 (28·6)	
Absent /Mild	92 (68·1)	89 (73·6)	24 (20·5)	83(59·3)	
Burning sensation					
Severe	29 (21·5)	12 (9·9)	78 (66·7)	30 (21·4)	<0·001*
Moderate	34 (25·2)	30 (24·8)	22 (18·8)	42 (30·0)	
Absent /Mild	72 (53·3)	79 (65·3)	17 (14·5)	68 (48·6)	
Two weeks post-treatment					
Erythema					
Moderate/Severe	79 (58·5)	61 (50·4)	87 (74·4)	65 (46·4)	<0·001*
Absent/Mild	56 (41·5)	60 (49·6)	30 (25·6)	75 (53·6)	
Swelling					
Moderate/Severe	31 (23·0)	26 (21·5)	29 (24·8)	41 (29·3)	0·48
Absent/Mild	104 (77·0)	95 (78·5)	88 (75·2)	99 (70·7)	
Erosion					
Moderate/Severe	49 (36·3)	36 (29·8)	30 (25·6)	30 (21·4)	0·045*
Absent/Mild	86 (63·7)	85 (70·2)	87 (74·4)	110 (78·6)	
Crusts					
Moderate/Severe	66 (48·9)	68 (56·2)	49 (41·9)	87 (62·1)	0·008*
Absent/Mild	69 (51·1)	53 (43·8)	68 (58·1)	53 (37·9)	

Table 3. Continued

	5-Fluorouracil (n =135) n (%)	Imiquimod (n = 122) n (%)	MAL-PDT (n =117) n (%)	IM (n =140) n (%)	*p*-value
Two weeks post-treatment (Continued)					
Vesicles/bullae					
Moderate/Severe	28 (20·7)	17 (14·0)	22 (18·8)	35 (25·0)	0·17
Absent/Mild	107 (79·3)	104 (86·0)	95 (81·2)	105 (75·0)	
Squamae					
Moderate/Severe	77 (57·0)	46 (38·0)	70 (59·8)	93 (66·4)	<0·001*
Absent/Mild	58 (43·0)	75 (62·0)	47 (40·2)	47 (33·6)	
Itching					
Moderate/Severe	75 (55·6)	47 (38·8)	56 (47·9)	85 (60·7)	0·003*
Absent/Mild	60 (44·4)	74 (61·2)	61 (52·1)	55 (39·3)	
Pain					
Severe	9 (6·7)	7 (5·8)	12 (10·3)	10 (7·1)	0·09
Moderate	15 (11·1)	9 (7·4)	21 (17·9)	24 (17·1)	
Absent /Mild	111 (82·2)	105 (86·8)	84 (71·8)	106 (75·7)	
Burning sensation					
Severe	19 (14·1)	5 (4·1)	15 (12·8)	8 (5·7)	0·01*
Moderate	18 (13·3)	14 (11·6)	17 (14·5)	32 (22·9)	
Absent /Mild	98 (72·6)	102 (84·3)	85 (72·6)	100 (71·4)	

Definition of abbreviations: MAL-PDT, methylaminolevulinate photodynamic therapy; IM, ingenol mebutate
* p≤0·05 is considered statistically significant

Compliance

The percentage of patients with 100% compliance was the highest in the IM group with 149 out of 151 (98·7%) patients. For 5-fluorouracil this percentage was 88·7%, for imiquimod 88·2%, and for MAL-PDT 96·8%. In the 5 patients who were not compliant in the MAL-PDT group, treatment was stopped prematurely due to pain.

Discussion

This study revealed that 5% 5-fluorouracil cream is significantly more effective compared to imiquimod cream, MAL-PDT, and IM gel when assessing the proportion of treatment success at 3 months post-treatment. The observed differences in effectiveness at 3 months after end of treatment are substantial, about 15% or more according to the ITT analysis and about 10% or more according to the PP analysis.

Although there is substantial literature about different field- and lesion directed treatments, studies often lack head-to-head comparisons and are underpowered.[10,19-21] To our knowledge, this is the first randomized controlled trial comparing four field-directed treatments in a large multi-center trial.

Previously, two network meta-analyses have been published. One indicated that ALA-PDT using BF-200 ALA gel had the highest probability (75·8%) to achieve total clearance of AK lesions, compared to 0·5% 5-fluorouracil (59·9%), 5% imiquimod (56·3%) and MAL-PDT (54·8%) with a follow-up between 3 months and 1 year. However, this study did not include the 5% 5-fluorouracil, used in our study.[22] The other meta-analysis by Gupta et al. suggested that 5% 5-fluorouracil is the most effective treatment when assessing 'participant complete clearance'.[23] Based on expert opinion in the 2015 European Dermatology Forum guidelines, the majority of experts did not express a preference for any of the most commonly prescribed treatments. They agreed that 3·75% imiquimod, ALA,- or MAL-PDT, IM (0·015/0·050%) or 0·5% 5-fluorouracil were equally effective for patients with multiple AK lesions/field-cancerization. However, there was less agreement about the effectiveness of 5% 5-fluorouracil.[24]

The reported adverse events in this study are well known treatment related side-effects that have been described in the corresponding SPCs. No related serious adverse events occurred. Moderate to severe erythema was reported slightly more often in patients treated with 5-fluorouracil. However, overall, treatment with 5-fluorouracil was not associated with higher frequency of adverse events during and after treatment when compared with the other treatments. High scores for pain and burning sensation were reported most often during MAL-PDT treatment. Pain can be an important reason for a patient to refuse further treatment. In our study 3·2% (5/155) of the patients in the MAL-PDT group did not complete the entire PDT treatment due to pain.

The search for new therapies continues to increase effectiveness but also to search for treatments with less side effects. In this search, daylight-PDT has been introduced. Daylight-PDT has gained popularity over the past years. Studies show that compared to conventional PDT, daylight-PDT is more convenient for patients with lower pain scores and less in-hospital time, while being equally effective.[25-28] However, daylight-PDT was not yet registered in the Netherlands at the start of this study.

More recently, imiquimod 3·75% has become available. Previous clinical trials showed a lower effectiveness compared to 5% imiquimod data when applied in two 4-week cycles, but suggested a higher compliance.[29,30] Unfortunately, till date there are no clinical trials comparing 5% versus 3·75% concentrations. The results in the current study also indicate relatively low compliance for 5% imiquimod cream when compared to the other investigated treatments. A possible explanation is the application regimen of 3 days per week. Perhaps patients tend to forget applications more frequently compared to e.g 5-fluorouracil cream with a daily application.

Only about half of the patients assessed for eligibility were willing to participate in this study. About one third of the patients who refused to participate, did so because of personal preference for a specific therapy or disfavor for one of the therapies. Refusal to participate is a common problem in randomized trials and may threaten external validity. Refusal to undergo retreatment in case of initial treatment failure was also observed in 45 patients. The proportion of refusals was higher in the imiquimod, MAL-PDT, and IM group than in the 5-fluorouracil group. As a result, the PP analyses, which included only patients who completed treatment as planned and thus excluded patients with initial failure and no retreatment, are biased towards better results and smaller differences between treatment groups. However, the overall conclusion that 5-fluorouracil is most effective is robust.
An important gap in current literature is that most studies assessing the effectiveness of field-directed treatments exclude grade III AK. In this study, we decided to approach real-life practice and included all grades AK. The results indicated that exclusion of grade III AK was associated with only slightly improved success percentages in 5-fluorouracil and MAL-PDT.

The primary outcome of this study was the proportion of patients with ≥75% reduction compared to baseline at 12-months post-treatment. In this paper, we report on the 3-months results, which was a secondary outcome measure of the study. The expected lesion reduction 12-months post-treatment should be awaited, to conclude on whether this superior effectiveness of 5-fluorouracil is maintained. In our opinion, the very low response rate for IM at 3 months after treatment, that was observed in this study, makes it unlikely whether IM should be considered as a first-choice treatment for field-directed AK in the head and neck area.

Based on our 3-months follow-up results, 5% 5-fluorouracil appears to be the most effective field-directed treatment. From a cost perspective 5-fluorouracil is also an attractive option, because in the Netherlands costs of 5-fluorouracil are much lower than costs of the other studied treatments. The costs of a tube of 40 grams are €31·32 compared to €58·93 for 12 sachets of imiquimod 5% cream, €203·35 for a 2-gram tube of MAL-cream, and €77·67 for 3 tubes of 0·015% IM gel.[31]

Both dermatologists and primary healthcare providers are confronted with AK lesions very often, because of increased age and sun exposure but also because of its recurrence rate. It is estimated that 14% of dermatology patients consult due to AK, which comes along with 5 million dermatology visits in the United States only.[32,33] With a better understanding of the effectiveness of AK treatments, more uniformity in treatment choices and possibly a further shift from in-clinic care to home-based care can be achieved and general costs spent on AK can be reduced. By defining the most effective treatment, recurrences and

unnecessary retreatments can be prevented. Although it is a question of debate whether AK can develop into SCC, several international guidelines suggest treatment to prevent malignant transformation and facilitate early detection of keratinocyte carcinomas.

In conclusion, we showed that after 3-months follow-up 5% 5-fluorouracil cream is significantly more effective to 5% imiquimod cream, MAL-PDT, and 0·015% IM gel in the treatment of patients with multiple grade I-III AK in the head,- and neck area.

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Chapter 3

Can tea cure?
Topical sinecatechins ointment for superficial basal cell carcinoma

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"Look deep into nature and you will understand everything better"
- Albert Einstein

Abstract

Topical sinecatechins 10% ointment is known for its anti-viral properties, anti-proliferative effects and induction of apoptosis. It is suggested that the active constituent – EGCG – has anti-tumoral effects. We aimed to assess whether topical sinecatechins 10% ointment could lead to histological clearance of superficial basal cell carcinoma (sBCC) and we assessed whether it influences proliferation and apoptosis.

In this randomized, double blind, placebo-controlled trial, 42 patients were assigned to sinecatechins or placebo-ointment, which was applied for 6 weeks. All tumors were excised after 8 weeks. Histological tumor clearance and adverse events were evaluated. To determine the effect of sinecatechins on proliferation and apoptosis, we assessed the proportion of patients with decreased immunohistochemical expression of Ki-67 and Bcl-2 between excision and baseline biopsy.

Complete histological tumor clearance was seen in 1/21 (4.8%) and 2/21 (9.5%) patients of the sinecatechins and placebo group respectively (p >0.99). Decrease in Bcl-2 expression was observed more frequently in the sinecatechins group than in the placebo group (respectively 41.2% vs 23.5%, p = 0.163) and decrease in Ki-67 occurred in similar proportions (31.3% versus 29.4%, p = 0.909). The sinecatechins 10% ointment led to more local skin reactions compared to placebo.

We found no therapeutic effect of topical sinecatechins 10% ointment in treatment of sBCC.

This trial is registered on clinicaltrials.gov (NCT02029352).

Introduction

The rising incidence of basal cell carcinoma and the extensive workload this entails, creates a need for non-invasive, self-applicable treatments.[1,2] Superficial basal cell carcinomas (sBCC) grow down from the epidermis in the superficial dermis, making them accessible for topical treatment. However, tumor-free survival rates following topical treatments such as photodynamic therapy (PDT), imiquimod cream or 5-fluorouracil cream, are significantly lower than after surgical excision.[3,4] Thus, the search for a more effective non-invasive topical treatment is ongoing.

The exact molecular mechanism of BCC development remains to be clarified. Almost 90% of sporadic BCC have identifiable mutations in at least one allele of the patched 1 (PTCH 1) gene, an inhibitor of the Hedgehog (HH) pathway.[5] There is also some evidence that the Wingless (Wnt) pathway might be a component for development of HH pathway-driven neoplasia such as BCC and that there might be some cross-talk between both pathways.[6-8] In physiological circumstances, the Wnt pathway initiates hair bud formation and HH signaling subsequently promotes the proliferative expansion of follicle epithelium required for a mature follicle.[6] Deregulation of the Wnt pathway causes accumulation of nuclear β-catenin protein, which then leads to tumor proliferation through activation of Tcf/LEF transcription factors.[9]

The active constituents of green tea may be promising in the treatment of BCC because of their supposed anti-carcinogenic effects as described by several cell culture and animal studies.[10,11] Particularly the catechin epigallocatechin-3-gallate (EGCG) is considered an useful active constituent. It is assumed that it has a cytotoxic effect on skin cancer cells, inhibits cell growth and induces apoptosis.[12,13] Furthermore there is evidence that EGCG might play a role in inactivation of β-catenin signaling of the Wnt pathway.[12] In case of crosstalk between HH and Wnt signalling in BCCs, this effect of EGCG on β-catenin might indicate a target for BCC therapy.[14,15] Other studies demonstrate that EGCG causes an apoptotic effect by decreasing Bcl-2 expression.[16-18] EGCG has also been shown to decrease the expression of Cox-2, an enzyme involved in prostaglandin (PG) synthesis which is known to be elevated in BCC.[19]

Sinecatechins 10% ointment is an extract of green tea leaves of the Camellia sinensis species and contains EGCG. It is currently used in the treatment of condylomata acuminata (CA)[20,21] where it is known to have anti-viral properties, inhibits signal transduction and proliferation, and induces apoptosis.[22-24]

In this study, we aimed to assess whether topical Sinecatechins 10% ointment is effective in treatment of sBCC. We hypothesized, that topical Sinecatechins 10% application could lead to histological tumor clearance of sBCC. Furthermore, immunohistochemical stainings were used to assess expression of Ki-67 and Bcl-2. Ki-67 is a well-known proliferation marker.[25,26] Bcl-2 is a proto-oncogene, encoding a large anti-apoptotic cytoplasmic protein.[27,28] Both stainings have been previously used to determine the effect of a therapy on proliferation and apoptosis in BCC. To the best of our knowledge, there are no clinical trials on human subjects with topical EGCG on sBCC yet.

Materials and methods

Protocol

We performed a double blind, randomized, placebo-controlled clinical trial at the Maastricht University Medical Center+ (MUMC+), the Netherlands. Patients were recruited between November 2014 and September 2015 at the outpatient dermatology department.

Patients were eligible to participate if they had a primary, histologically proven sBCC with a diameter between 4mm and 20mm. sBCC was defined as small buds of basal cells with large, relatively uniform nuclei, growing down from the epidermis into the superficial dermis, whilst maintaining their attachment to the base of the epidermis.[29,30] One sBCC per patient was included to ensure independence of observations. If patients had more than one sBCC, the tumor with the most accessible location and / or largest diameter was chosen. Patients were excluded if they had a sBCC located in the H-zone (high risk zone in the face) or on the hairy scalp, were using immunosuppressive drugs or had genetic skin cancer disorders. Breast-feeding and pregnant women were also excluded.

The study was performed in accordance with the Declaration of Helsinki. The protocol was approved by local ethics committee (MUMC+) and registered on www.clinicaltrials.gov (NCT02029352). All patients provided written informed consent.

Outcomes

The primary outcome was the proportion of patients with complete histological tumor regression in the excision specimen post-treatment. Secondary outcomes were (1) proportion of patients with decreased expression of Ki-67 and Bcl-2, (2) change in tumor size pre- and post-treatment, (3) adverse events and (4) compliance. The change in immunohistochemical expression between the excision specimen and the biopsy specimen was categorized as increased, equal or decreased compared to baseline.

Data on compliance were obtained from the personal diary and compliance was calculated as number of actual applications divided by the number of prescribed applications. To assess the adverse events during treatment a subdivision in two categories was made: absent/mild or moderate/severe. We evaluated the proportion of patients with moderate to severe adverse local reactions.

Histopathologic assessment

From both biopsy and excision specimen, haematoxylin and eosin (HE) histological sections were obtained. We used immunohistochemical stainings to indicate the proliferative activity (Ki-67) and anti-apoptotic activity (Bcl-2). The immunohistochemical stains were prepared according to the laboratory protocols of the Department of Pathology, MUMC+. Stains were performed on 4 µm formalin fixed and paraffin embedded (FFPE) tissue sections of both biopsy and excision specimens using a Dako Autostainer Link 47™ (DAKO corporation, Carpinteria, CA, USA). The primary antibodies used were Ki-67 (IR614, clone MIB-1, mouse monoclonal, ready to use Antibody, DAKO) and Bcl-2 (IR626, clone 124, mouse monoclonal, ready to use Antibody, DAKO). For each immunohistochemical staining, all tissue specimens were stained in one session to prevent differences in staining intensities between the samples. Positive and negative controls were used.

All HE specimens were independently assessed by two dermato-pathologists who were blinded to treatment allocation, to evaluate presence of residual tumor and completeness of the excision. All HE slides were reviewed to assess which part of the excised specimen contained the largest amount of tumor. Consecutively the corresponding part of the paraffin block was further sliced and used for immunohistochemical staining. The slide with the largest amount of tumor visible and the highest staining intensity was chosen for further assessment, in order to assess the most representative part of the tumor. From each selected slide of both biopsy and excision specimens a photograph was taken using a Leica DFC320® digital camera that was attached to a Leica DM3000 microscope, while using a 10x objective. All images were stored as tagged image file (TIF).

Two trained investigators blinded to treatment allocation used all immunohistochemistry specimens to assess whether there was an increase, decrease or equal staining intensity when comparing expression of Ki67 and Bcl2 in the pre-treatment biopsy with expression in the post-treatment excision specimen. In case of disagreement between the investigators, consensus was reached.

Assignment and masking

Patients were randomized to either intervention or placebo using a computer-generated randomization list using random permuted blocks of four. Patients and investigators,

including the assessing pathologists, were blinded to treatment assignment until the end of the study. Sinecatechins 10%,- and placebo ointments were provided by Will Pharma B.V (Zwanenburg, the Netherlands). All study medication was labeled according to good manufacturing practice (GMP) guidelines with pre-assigned study numbers. Labeling of study medication was performed by the trial pharmacy of the Radboud university medical center Nijmegen. The vehicle of the placebo was equal to that of the sinecatechins 10% ointment. Additionally, the placebo contained colorants such as titanium dioxide, red iron oxide and yellow iron oxide, resulting in a color and consistency identical to the sinecatechins 10% ointment. To ensure blinding both ointments had an identical package.

Participant flow and follow-up
After patients gave their written informed consent, tumor size was measured and standardized photographs were taken during the first study visit. Consecutively, patients received the study ointment. Patients were instructed to apply the ointment in a thin layer to the tumor, including 5 mm of surrounding healthy skin, twice daily (morning and evening) for six weeks.

Patients returned for follow-up visits in week 3 and week 6 after treatment. All tumors were removed by surgical excision with a 3 mm margin in week 8 during the final study visit.
During the follow-up visits, tumor size and local skin reactions were assessed by the coordinating investigator. Patients reported on skin reactions once a week in a personal diary they received at the first study visit Severity of skin reactions during treatment (i.e. redness, swelling, erosions, crusts, vesicles, squamae, itching and tingling) were scored as absent, mild, moderate or severe. Compliance data were also obtained from the personal diary.

Statistical analysis

It was assumed that about 5% of sBCCs in the placebo group will show histological complete tumor regression in the excision specimen, because of a possible biopsy-induced tumor regression [31]. At least 50% in the sinecatechins 10% group had to show a complete tumor regression to consider this treatment as sufficiently effective for further evaluation. Based on an alpha=5%, and power of 80%, we calculated that a total of 38 patients (19 patients per group) would be required. To account for a loss-to-follow-up of 10%, a total of 42 patients (21 patients per group) had to be included.

Difference in proportions between the randomized groups was tested for significance using the Chi-square test or Fisher exact test (e.g. proportion of complete regression, proportion with decreased expression of tumor markers, adverse events). Mean continuous

variables (e.g. tumor diameter, compliance percentages) were compared between groups and tested for significance using the T-test for independent samples (when normally distributed) or the Mann-Whitney U test (when not normally distributed). Reported two-sided P values ≤ 0.05 are considered statistically significant. Statistical analysis was performed with SPSS version 23.0 software (SPSS, Chicago, IL, U.S.A).

Results

Between November 2014 and September 2015, 89 patients were approached to participate in the trial. A total of 42 patients were considered eligible and were willing to participate. They were randomly assigned to treatment with sinecatechins 10% or placebo. There were no major differences between both treatment groups. A total of 19 patients in the sinecatechins 10% and 20 in the placebo group completed the six weeks of study treatment. The primary endpoint was available for all randomized patients.

Tumor clearance

Complete histological tumor clearance was seen in 1/21 (4.8%) and 2/21 (9.5%) patients of the sinecatechins 10% and placebo group respectively (p >0.99).

Median decrease in tumor size between baseline and end of study was 0 mm (range -10 to +5) for placebo and -1.50 mm (range -14 to +3) for the intervention group (p = 0.153).

Immunohistochemical evaluation

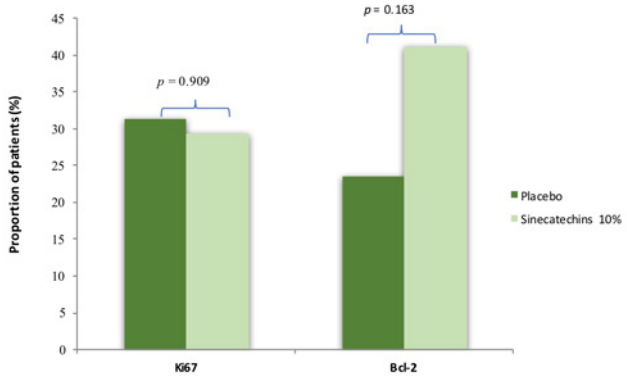
Figure 1 presents the proportion of patients with decreased expression of Ki-67 or Bcl-2. Complete data on Ki-67 and Bcl-2 were available for 33 and 34 patients, respectively. Not all tumors could be evaluated due to technical difficulties. Decrease in Bcl-2 expression was observed slightly more frequently in the sinecatechins 10% group than in the placebo group (respectively 41.2% vs 23.5%, p = 0.163) and decrease in Ki-67 occurred in similar proportions in both groups (29.4% versus 31.3%, p = 0.909). None of these differences were statistically significant.

Adverse events

One patient in the placebo group reported pain during treatment, compared to four patients (20%) in the sinecatechins 10% group. Most local skin reactions occurred in the sinecatechins 10% group in the fourth week of treatment and decreased afterwards. In week 4 there was a significantly higher proportion of patients reporting moderate to severe erythema (60% vs 10%, p = 0.004), edema (30% vs 0%), p = 0.029), erosions (35% vs 0%, p = 0.014), crusts (50% vs 0%, p = 0.001) and itching (65 vs 0%, p< 0.001) in the

sinecatechins 10% group compared to the placebo group. Figure 2 demonstrates the percentage of patients who reported moderate to severe side effects during treatment. Analyses using investigator-reported side effects gave similar results. One patient in the sinecatechins 10% group reported flu-like symptoms, but completed study treatment. One patient in the same group stopped treatment in week 4 due to local skin reactions. None of the patients reported a serious adverse event.

Figure 1: Proportion of patients (%) with decreased Ki-67 and Bcl-2expression post-treatment, compared to baseline.

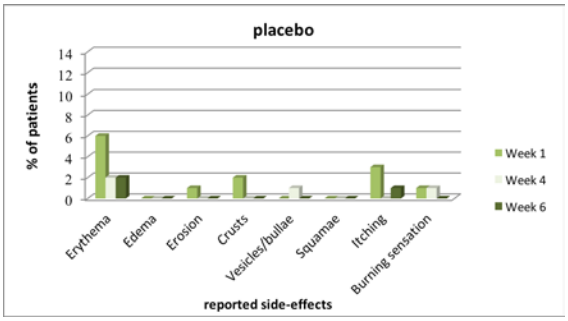


Compliance

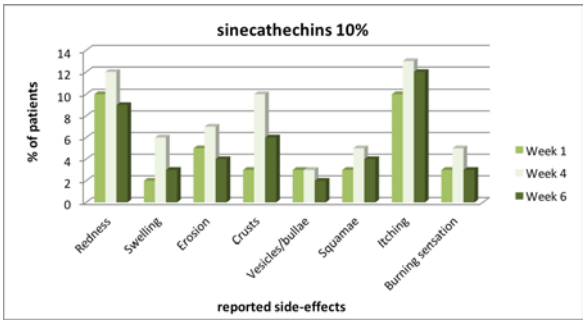
Median compliance was 100% in both groups, with a range of 83-100% in the placebo group compared to 65-100% in the intervention group.

Figure 2. Percentage of patients with moderate-severe local skin reactions, during treatment, n (%) for placebo group and sinecatechins 10% group.

2.1



2.2



Discussion

The current study was the first to assess clinical efficacy of topical sinecatechins 10% ointment in the treatment of sBCC. Our results show that there was no significant difference in histological tumor clearance between the sinecatechins 10% and the placebo group. In the intervention and in the placebo group, complete tumor remission occurred in 1 and 2 patients, respectively, possibly reflecting a biopsy-induced immune response. [31] Decrease in tumor size was slightly larger after sinecatechins 10% application than after placebo, but the difference was non-significant.

In the current trial we assessed the proportion of patients with decreased expression between baseline biopsy and excision specimen for Ki-67 and Bcl-2. Ki-67 is a proliferation marker, expressed in all cell proliferation phases, except G0.[27] Apoptosis is regulated by *Bcl-2,* expressed in the majority of BCC.[27,32] Both markers were used previously in studies to assess the efficacy of a therapy. [27,28,33,34] A decrease in the expression of Bcl-2 was found in a clinical trial assessing the effect of topical imiquimod application in BCC.[27] No significant changes for Ki-67 were observed in the same trial. Brinkhuizen et al. found a significantly decreased Bcl-2 and Ki-67 expression in sBCCs treated with topical Diclofenac, compared to baseline.[33]

In our study, a decrease in expression of Bcl-2 was observed slightly more frequently than in the placebo group. This difference was not statistically significant. We quite unexpectedly observed a decrease in Bcl-2 and Ki-67 expression in a proportion of patients in the placebo group. A previously hypothesized increase in apoptosis in biopsy induced tumor regression could play a role.[31]

A possible explanation for the observed lack of efficacy of the topical sinecatechins 10% in the current study might be insufficient EGCG uptake after usage of the current formula could explain the lack of efficacy of the topical sinecatechins 10% ointment in the current study. An in vitro and murine study by Fang et al. found that when EGCG was encapsulated in liposomes with deoxycholic acid and ethanol, the drug deposition was increased significantly compared to administration of free EGCG.[35]

In 2011 Tjeerdsma et al. reported a clinical case of a patient with basal cell nevus syndrome (BCNS) achieving a temporary (one year) arrest of developing new BCC shortly after she was receiving green tea body wraps once a month.[36] The wraps used in this clinical report consisted of green tea and four other plant extracts (ginger oil, algae, mustard oil and calendula oil). Whereas both brown algae and ginger have some anti-carcinogenic effects, green tea supposedly has the most powerful anti-carcinogenic capacity with epi-

gallocatechine-3-gallate (EGCG) being the most active constituent.[12,13,37-40] The suggested effect of green tea in this specific case was preventive rather than curative. Also in other studies, oral or topical administration of polyphenols in green tea has shown to reduce UV induced inflammation, photo-aging and immunosuppression.[16,22,39] Smith et al found that β-catenin was overexpressed in UVB-exposed mouse skin and keratinocytes, suggesting that this could be a target for skin cancer prevention.[18] However, the observed frequency of adverse events discourages preventive application with the current formula of the sinecatechins 10% ointment in the dosage we used in this trial (twice daily).

In conclusion, the results of this study do not corroborate the hypothesis that treatment of sBCC with topical sinecatechins 10% provides an alternative to already available topical treatments.

Acknowledgments

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Chapter 4

Treatment of superficial basal cell carcinoma: the role of PDT


4.1

Chapter 4.1

Efficacy of 5-aminolevulinic acid PDT for the treatment of superficial basal cell carcinoma: a retrospective study

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"Luckily however, life doesn't always follow tradition, but craves out a path of its own"
- Kader Abdolah, The house of the mosque

Abstract

Background

There is limited literature on efficacy, using a twofold illumination scheme in ALA-PDT treatment for sBCC.

Objectives

To determine the efficacy of ALA-PDT for sBCC using a 2-fold illumination scheme after a single ALA application. Treatment failure within 12 months post-treatment was assessed.

Methods

In this retrospective case series and cohort study, electronic files from patients treated between January 2010 and August 2011 were reviewed. Follow-up data were gathered until March 2014.

Results

A total of 323 sBCC were analyzed for recurrence. Cumulative probability of clinical recurrence free survival was 88.8% (95% CI [85.4; 92.4]), 81.8% (95% CI [77.3; 86.3]) and 77.1% (95% CI [71.0;83.6]) at 12, 24 and 48 months respectively. For histologically confirmed recurrences this was 90.2% (95% CI [86.9;93.5]), 85.4% (95% CI [75.5;89.3]) and 81.8% (95% CI [75.5;88.1]) respectively. A worse recurrence free survival for tumors in the head and neck area and tumors larger than 10 mm was observed.

Limitations

The retrospective nature and the lack of a control group.

Conclusions

ALA-PDT using a twofold illumination scheme might be a feasible treatment option with acceptable long-term results for small sBCC located outside the head and neck area.

Introduction

Basal cell carcinoma (BCC) is the most common skin cancer in Caucasians, with a rapidly increasing incidence.[1-5] The estimated annual percentage of change between 2002 and 2009 was 6.8% for men and 7.9% for women in the Netherlands.[4] Histologically, BCC may be subdivided into three subtypes: superficial, nodular and infiltrative.[6] Around 9% to 43% of BCC has a mixture of histological subtypes.[7,8] The most prevalent subtype is the nodular BCC.[9,10] Interestingly, a rise in the relative proportion of the superficial type of BCC (sBCC) has been observed, with an increase from 18% to 31% in the last 20 years.[11,12] This rise is especially seen in young women.[13]

Treatment by surgical excision has the lowest recurrence rate and therefore remains the gold standard for most BCC. Recurrence rates vary from 2-8% 5 years after surgery.[14-16] Surgical procedures however, tend to be invasive and lead to scar formation.

A prior study by Flohil et al. showed a linear increase of 6.3% in the incidence of BCC in women below the age of 40 years, since 1973. [17] Because of the increasing number of younger patients, cosmetic outcome is an important factor in treatment choice. Despite lower efficacy compared to surgery, alternative treatments such as cryotherapy, photodynamic therapy (PDT), topical imiquimod and 5-fluorouracil, are also frequently used.[18,19] PDT is especially known because of its excellent cosmetic outcome and the advantage of an office-based treatment, which ensures compliance.[18,20-22] It combines the use of topical porphyrin precursors as photosensitizers followed by illumination with visible light. Currently two topical precursors are used. 5-Aminolevulinic acid (ALA) was first approved by the Food and Drug Administration (FDA) in 2001. The more lipophilic methyl-aminolevulinate (MAL) was registered in most European countries for sBCC and actinic keratosis (AK) in 2004 and for the treatment of Bowen's disease in 2006.[23] It is not available in the United States.

Although PDT is an effective treatment, a systematic review by Roozeboom et al. described a recurrence rate of 16% in sBCC, based on pooled estimates at 12 months post-treatment when assessing both ALA and MAL-PDT and different light sources.[19] In order to improve its efficacy de Haas et al. developed a 2-fold illumination ALA-PDT protocol. Instead of the regular treatment scheme for sBCC using a fluence of 75 J/cm^2 4 hours after administration of 5-ALA, two illuminations were used with 20 and 80 J/cm^2, delivered respectively 4 and 6 hours after one single 5-ALA administration. [24] In this study, a recurrence rate of only 3% was reported 12 months post-treatment. This new, 2-fold illumination 5-ALA protocol was implemented in daily practice in our hospital. The present study evaluates the risk of recurrence after this 5-ALA PDT treatment and aims to identify prognostic factors for recurrence.

Methods

Patients

After receiving approval from the local ethical committee, patients were recruited retrospectively by performing a search within our electronic patient system at the department of Dermatology, Catharina hospital Eindhoven, the Netherlands. This identified all patients treated with PDT between January 1st 2010 and August 31st 2011. Consecutively, all these electronic patient files were reviewed. Included were patients with histologically confirmed sBCC that were treated with the 5-ALA PDT protocol using a twofold illumination between January 1st 2010 and August 31st 2011. Exclusion criteria were previous treatment of the index sBCC, absence of histological confirmation prior to treatment, other histological subtypes apart from superficial BCC in case biopsy was taken, genetic disorders causing skin cancer and immunosuppressive drug-use at time of treatment. The same examiner reviewed all patient files. In case a patient decided to end follow-up or consult a dermatologist in another facility for follow-up, we did not have access to these patient records. Results of these few patients were analyzed until their last moment of follow-up. If a patient had multiple eligible sBCC, the first reported tumor was included for analysis to ensure independence of observations. If reported, age, sex, Fitzpatrick skin type, number of tumors and dates of outpatient visits were recorded for all patients. Consecutively histology, localization and size were recorded for each tumor. Retrospective review of all patient files was completed on March 1st 2014.

Design

This single center study describes the 1-year probability of recurrence free survival in a case series. For the evaluation of factors that are prognostic for treatment failure, the study can be considered a cohort study, wherein potentially relevant prognostic factors represent exposure status (tumor size, tumor location etc.). Exposure groups are compared with respect to the probability of treatment failure.

All patients were treated with topical 20% 5-ALA cream. This was prepared using 20% 5-ALA (FLUKA, the Netherlands) in Neribas® (Bayer, Leverkusen, Germany) cream, prepared by the hospital pharmacy.[25-27] Before treatment, excessive scaling was removed by curettage using a wooden spatula. 5-ALA cream was then applied on the lesion with a margin of 1 centimeter and a thickness of 1-2 millimeters. Consequently, the treatment area was covered with a semi-permeable dressing (Tegaderm ®, 3M Healthcare, Leiden, the Netherlands), a gauze and aluminium foil in order to prevent light exposure. After a period of 3.5 hours, the occlusive material was removed, excess cream was gently removed and illumination took place, using a 630 nm light source (Aktilite, Galderma®, the Netherlands). A total light dose of 20 J/cm^2 was accomplished in 4.23 minutes. Subse-

quently, the treatment area was covered again with Tegaderm®, a gauze and aluminium foil for 2 hours, whereupon a second illumination of 18.8 minutes and a light dose of 80 J/cm^2 took place. This resulted in a PDT treatment with two illuminations on the same day with a total light dose of 100 J/cm^2.

Whenever burning sensation during treatment was too intense, wet gauzes were used to cool the skin. In accordance with the treatment protocol, this was allowed not prior to 2 minutes after initiation of the illumination. In case of excessive pain sensation, the treatment site could be locally infiltrated with a Lidocaine 2% without epinephrine injection. After the second illumination, a cooling ointment (Unguentum Leniens) was applied to the treated site. All patients were recommended to use the cooling ointment after the treatment, until the burning sensation ceased. Patients were advised to avoid direct sunlight two days post-treatment.

Statistical analysis

The primary outcome measure was the proportion of treatment failure within 12 months post treatment. Time-to-event analyses were used to take into account differences in follow-up between patients and success of treatment was presented in terms of recurrence free survival. Kaplan-Meier survival analysis was performed to calculate the cumulative probability of recurrence free survival at 12, 24 and 48 months post-treatment.[28]

Cox proportional hazards regression models were utilized to evaluate the independent effects of patient and tumor characteristics such as age, gender, tumor localization and tumor size on probability of recurrence. Hazard ratios with 95% confidence intervals associated with prognostic factors were calculated. The proportional hazards assumption was tested graphically and with use of Schoenfeld residuals.

All analyses were performed using SPSS version 21 (IBM statistics, U.S.A) and STATA version 11.0 (STATA Corp, College Station, TX, U.S.A). A two-sided *p*-value of <0.05 was considered as statistically significant.

Results

A total of 379 patients with 562 sBCC's were treated with the described twofold ALA-PDT protocol between January 2010 and August 2011. A total number of 56 patients were excluded from analysis because of loss to follow-up directly after treatment (n=6), lack of histopathological confirmation of the diagnosis prior to treatment (n =17) and the index BCC being a recurrent tumor (n=33). A total of 323 patients remained for analysis. Of these patients, 223 (69%) had one sBCC and 31% had more than one sBCC.

The majority of sBCC's were located on the back (*n*=96, 29.7%) and chest / abdomen (*n*=72, 22.3%) *(Table 1)*. Tumor size was dichotomized into: ≤ 10 mm or > 10 mm diameter. More than half of all included tumors were less than or equal to 10 mm (n=165, 51.1%). A minority had a size larger than 20 mm (n=9, 2.8%).

The median follow-up time was 28 months (range 1-48), with 192 patients (59%) having a follow-up period of more than 2 years. During follow-up visits, treatment failure was clinically observed in 58 patients. In 46 patients, histopathological examination was performed and recurrence was histologically confirmed in 44 patients. The majority of histological subtype in case of recurrence was nodular (54%) and superficial (41%). In 12 cases the treating physician was convinced of a recurrent BCC or the patient refused biopsy, so histological confirmation was lacking in these cases.

All patients completed both illuminations. Two patients received local anesthesia because of excessive pain during illumination. The cumulative probability of recurrence free survival was 88.8% (95% CI [85.4;92.4]), 81.8% (95% CI [77.3;86.3]) and 77.1% (95% CI [71.0;83.6]) at 12, 24 and 48 months respectively. When defining treatment failure as a histologically confirmed recurrence (leaving out clinically observed recurrences without histological verification) the 12 month cumulative probability of recurrence free survival was 90.2% (95% CI [86.9;93.5]). At 24 and 48 months, cumulative probabilities were 85.4% (95% CI [81.3;89.3]) and 81.8 % (95% CI [75.5;88.1]) respectively *(Figure 1, Table 2)*.

Table 1. Baseline characteristics

	n (%)
Age (y), mean (range)	63.6 (25-90)
Sex	
Male	156 (48.3)
Female	167 (51.7)
Fitzpatrick skin type	
I	80 (24.8)
II	128 (39.6)
III	21 (6.5)
Unknown	94 (29.1)
No. of lesions per patient	
1	223 (69.0)
2	65 (20.1)
3	21 (6.5)
4	7 (2.2)
≥ 5	7 (2.2)
Location sBCC	
Head/neck area	52 (16.1)
Upper extremity	49 (15.2)
Lower extremity	54 (16.7)
Back	96 (29.7)
Chest/abdomen	72 (22.3)
Size (mm)	
< 10	165 (51.1)
10-20	100 (30.9)
>20	9 (2.8)
Missing	49 (15.2)

Table 2. Cumulative probability of recurrence free survival for both clinically observed and histologically confirmed recurrences.

Follow-up time (months)	Proportion recurrence free survival histological recurrences (%), [95% CI]	Proportion recurrence free survival clinical recurrences (%), [95% CI]
3	95.8% [93.6;97.9]	95.0 % [94.8;95.2]
6	93.7% [90.9;96.4]	92.1% [91.8;92.4]
12	90.2% [86.9;93.5]	88.8% [85.4;92.4]
24	85.4% [81.3;89.3]	81.8% [77.3;86.3]
48	81.8% [75.5;88.1]	77.1% [71.0;83.6]

Figure 1. Kaplan-Meier survival curve

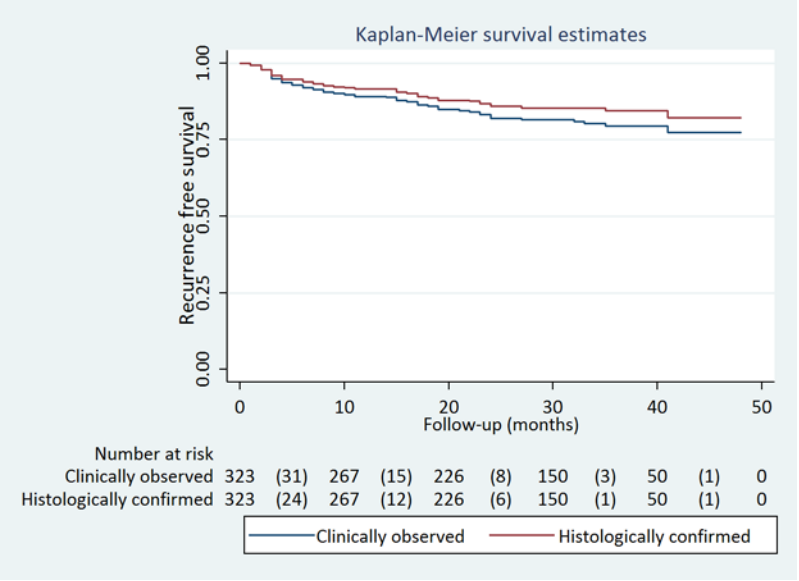
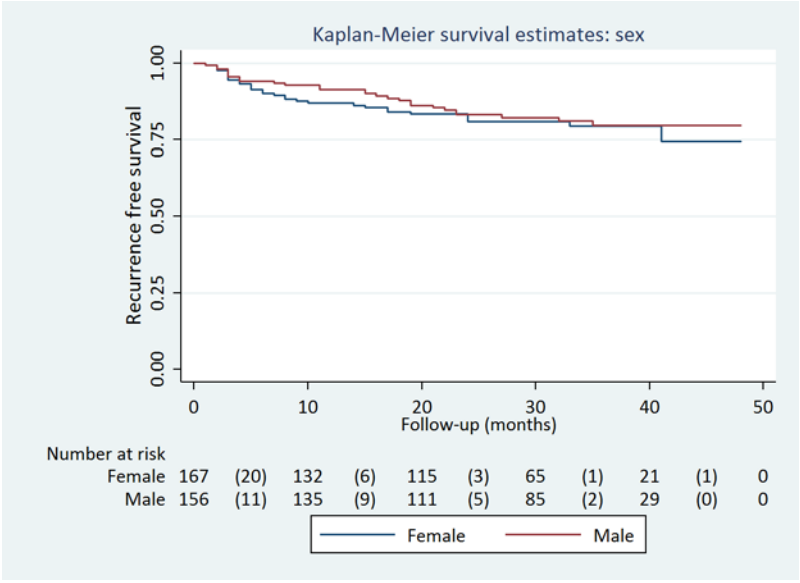


Table 3 presents the hazard ratios for recurrence according to baseline characteristics (male-female, age groups ≤60 and > 60 years and tumor size > 10 mm and ≤ 10 mm). The test of the proportional hazard assumption was not significant for both clinical and histological recurrences (respectively p= 0.692 and p= 0.389). Tumor localization in the head and neck area is associated with a significantly higher risk of clinical treatment failure compared to tumor localization elsewhere (HR = 2.55 (95% CI [1.43;4.57], p = 0.002). In the head and neck area we found 8.3% superficial, 83.3% nodular and 8.3% infiltrative recurrent tumor subtypes. In the non-head and neck area these percentages were respectively 53.1%, 43.8% and 3.1%.

Furthermore, a worse recurrence free survival was observed for large tumors (i.e. >10 mm) compared to small tumors (HR = 1.81 (95% CI [1.07; 3.06], p = 0.026). When defining treatment failure as a histologically confirmed recurrence, the HR estimates were similar, but only tumor location in the head and neck area was associated with significantly higher risk of recurrence.

Figure 2. Kaplan-Meier survival according to sex, tumor localization (head/neck versus other), tumor size (>10mm versus ≤ 10mm) and age (>60 versus ≤60).

2.1



2.2

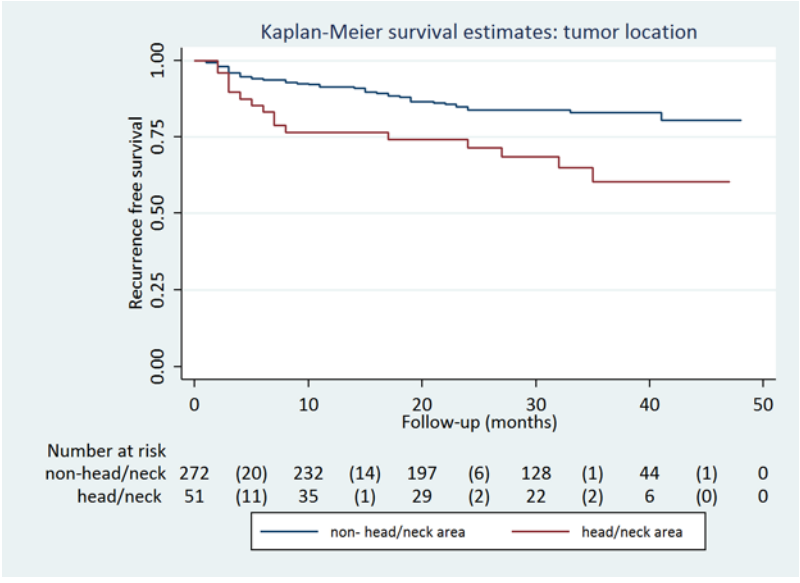
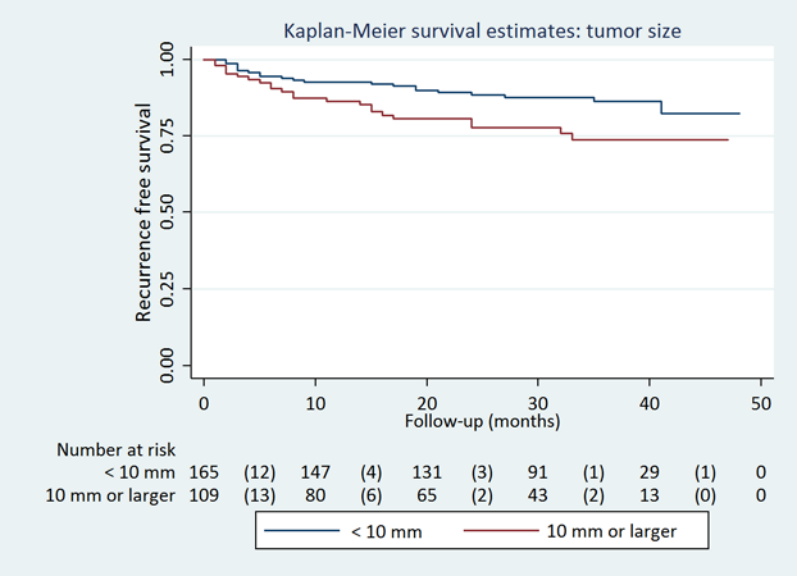


Figure 2. Continued.

2.3



2.4

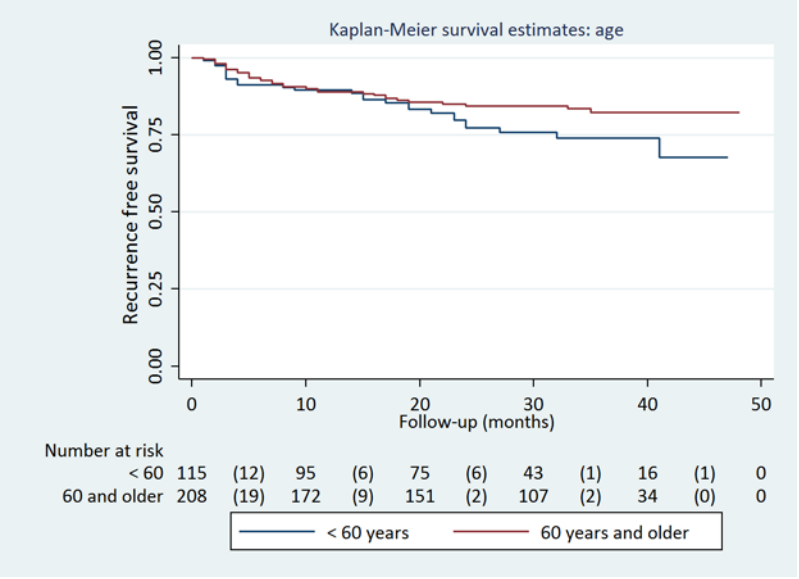


Table 3: Cox proportional hazard ratios for recurrence for sex, tumor location (head/neck versus other), size (>10mm versus ≤ 10mm) and age (>60 years versus ≤60 years). * p-value <0.05

	Clinically observed recurrences (n=58)		***Histologically confirmed recurrences (n=44)***	
	HR for recurrence (95% CI)	*p-value*	*HR for recurrence (95% CI)*	*p-value*
Sex (female versus male)	0.85 [0.51; 1.43]	0.547	0.99 [0.54;1.79]	0.968
Tumor location (head/neck versus non-head/neck)	2.55 [1.43; 4.57]	0.002*	2.44 [1.25; 4.76]	0.009*
Tumor size (>10mm versus ≤ 10 mm)	1.81 [1.07; 3.06]	0.026*	1.61 [0.89; 2.94]	0.117
Age (>60 vs ≤ 60)	0.59 [0.35; 1.00]	0.053	0.644 [0.35; 1.18]	0.155

Discussion

This retrospective study confirms prior observations, that using 20% 5-ALA PDT followed by a twofold illumination scheme might be an effective treatment modality for sBCC with a tumor free survival of approximately 90% after 12 months.

When ALA was introduced as a topical photosensitizer, results were promising.[29-31] In a systematic review, Peng *et al*. reviewed 12 studies in which sBCC were treated with ALA-PDT, and showed a weighted clearance rate (CR) of 87%.[32] A more recent review by Roozeboom *et al.* described an overall CR of 79% at 12 weeks post-treatment based on pooled estimates derived from 28 studies using both ALA as well as MAL as a photosensitizer. The majority of studies investigated ALA-PDT after a single illumination. Tumor-free survival was higher in studies that repeated illumination compared to a single illumination. This could indicate that repeated illumination results in improved clinical outcome.[19]

There are only a few studies that report follow-up data of ALA-PDT after a twofold illumination scheme.[23,24,33,34] Star *et al.* reported a pilot clinical study in 15 patients and found a CR of 88% at 12 months follow-up after twofold 5-ALA illumination (45 J/cm^2 + 45 J/cm^2 separated by a two-hour dark interval).[33] In a large prospective comparative study from the same group, the twofold protocol (20 + 80 J/cm^2 with a two hour dark interval) was compared to single illumination (75 J/cm^2). This resulted in a CR of respectively 97% and 89% at two-year follow-up.[23] At long-term follow-up (5 years), a CR of 88% is still observed compared to 75% after a single illumination.[34]

Our data show a CR comparable with Star *et al*. 12 months post-treatment. However, the CR of 97% described by de Haas *et al*. was much higher than our results, even though we performed the same two-fold illumination treatment. An important difference between both studies is the study design. The lower treatment success might be due to the retrospective nature of our study. Even though this treatment is performed by a nurse/physician and compliance is expected to be high, protocol deviations (e.g. the exact time between cream application and illumination) might not be reported as accurate as in a prospective study design.

The choice for a 2-fold illumination scheme is based on extensive pre-clinical research. Several studies, including mouse models, demonstrated re-synthesis of PpIX after a first illumination. Between two cycles of illumination there is time for tissue re-oxygenation. It is hypothesized that the heme synthesis cycle is still intact, which leads to further conversion of ALA into PpIX.[35-38] This post-illumination increase in PpIX has both been observed after PDT with systemic and topical ALA.[38-40] These observations might explain the described increase in PDT efficacy after a second illumination. The optimal time interval between both illuminations has also been studied extensively. Maximal PpIX concentration following ALA application was observed 6 hours after the end of the application time. A two-hour interval between both illuminations results in a significant increase in PDT response.[24,37] Compared to topical 5-fluorouracil cream and imiquimod cream for which the patient has to apply a cream for several weeks or MAL-PDT which includes 2 separate illuminations, the advantage of this 2-fold illumination is the fact that it is a one day treatment.

Results suggest that sBCC with a diameter smaller than or equal to 10 mm and sBCC that are located outside the head and neck area have a better recurrence free survival compared to tumors larger in diameter and located in the head and neck area. This is consistent with other studies that showed an increased risk for recurrence in larger tumors and located in the mid-face and ears.[41,42] In our sample there might have been more mixed tumor types in the head and neck tumors, which might also explain a worse outcome.

Across Europe, the lipophilic methyl-aminolevulinate (MAL) is frequently used (Metvix®, Galderma).[22] Szeimies *et al*. describe a lesion response of 92.2% (118/128) 3 months after treatment of sBCC with MAL-PDT. Among these cleared lesions 9.3% recurred at 12 months follow-up.[43] Basset-Seguin et al. reported a recurrence rate of 22% after 5 years follow-up and more recently Arits et al. describe a recurrence rate of 27.2% after 1 year follow-up.[44,45] Differences in the reported outcomes can be explained because of the use of different illumination schedules, the number of illuminations and whether patients were re-illuminated in case of treatment failure. Up to date there is very limited literature describing a 2-fold illumination schedule with MAL-PDT. There is only one mouse study,

in which the twofold illumination does not increase efficacy.[46] As there is very limited literature on the described 2-fold ALA-PDT protocol, it might be of additional interest to compare this protocol with illuminations using for example routine MAL-PDT in future prospective research.

Important limitations of our study are the retrospective nature, the lack of a control group and analysis of single center data. Furthermore, the ALA cream was produced in our hospital pharmacy, which might be difficult to reproduce. In our center PDT is a routine therapy for sBCC. Patients were able to choose their preferred treatment (topical imiquimod cream, 5-fluorouracil cream, surgical excision or PDT) after explanation of the treatments by their physician. This may have caused selection bias.

In conclusion, our results imply that ALA-PDT using a twofold illumination protocol for sBCC has acceptable long-term efficacy rates. It may be less effective in the head and neck area and in tumors with a diameter larger than 10 mm.

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4.2

Chapter 4.2

Treatment of superficial basal cell carcinoma with fractionated 5-aminolevulinic acid 20% versus two stage topical methyl-aminolevulinate: a multi-center randomized controlled trial

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"Absence of evidence is not evidence of absence"
- Altman et al; BMJ, 1995 (311):485

Abstract

Background

Basal cell carcinoma (BCC) is the most common type of skin cancer with growing incidence rates. Photodynamic therapy (PDT) is a frequently used treatment, especially for superficial BCC (sBCC). Two topical photosensitizing agents are currently used to treat sBCC: 5-aminolevulinic acid (ALA) and its methyl-ester (MAL). Previous research showed a high efficacy of ALA-PDT using a 2-fold fractionated illumination scheme in which two light fractions of 20 and 80 J/cm^2 are delivered, four and six hours after ALA application.

Objectives

To evaluate whether this 2-fold ALA-PDT is superior to conventional MAL-PDT for sBCC.

Methods

We performed a single blind, randomized multi-center trial in the Netherlands.

Results

162 patients were randomized to either conventional MAL-PDT or 2-fold ALA-PDT. After 12 months a total of 6 treatment failures occurred after ALA-PDT and 13 after MAL-PDT. The 12 months cumulative probability of remaining free from treatment failure was 92.3% (95% CI [83.7-96.5]) and 83.4 (95% CI [73.1-90.0]), respectively (p=0.091).

Conclusions

The 2-fold ALA-PDT scheme resulted in fewer recurrences, although the difference between both treatment groups was not statistically significant. On the contrary, it resulted in higher pain scores and more post-treatment side-effects compared to MAL-PDT.
Clinical trial registry number: NCT01491711

Introduction

Basal cell carcinoma (BCC) is the most common type of skin cancer with growing incidence rates.[1] BCC can be categorized into three histological subtypes: superficial, nodular, and infiltrative.[2] Most BCCs are treated with surgical excision. However, one third of BCCs are superficial and do not necessarily require excision. Topical treatments such as photodynamic therapy (PDT), 5-fluorouracil cream and imiquimod cream are frequently used. A recent randomized comparative study showed an efficacy of these treatments varying from 72.8%-83.4%.[3]

Compared to topical ointments, the advantage of PDT is the short duration and a good cosmetic outcome.[4,5] Topical porphyrin precursors are applied to the skin and converted into protoporphyrin IX (PpIX). When exposed to oxygen and light in the appropriate wave length, singlet oxygen is formed and the tumor cells are destroyed.[6-8] Two photosensitizers are currently used to treat sBCC: 5-aminolaevulinic acid (ALA) and methyl-aminolaevulinate (MAL). A systematic review by Peng et al. showed a weighted clearance rate (CR) of 87%.[9] Overall CR one year post-treatment of 72.8-84.0% are observed for MAL-PDT.[3,10,11] There is no randomized controlled trial that directly compares treatment with ALA- and MAL-PDT for sBCC.

De Haas et al. investigated whether a 2-fold illumination scheme on one day after a single ALA application could lead to a better efficacy. One year post-treatment, a CR of 97% was observed after treatment with this 2-fold ALA-PDT scheme.[12] In a recent retrospective study, a CR of 90.2% after one year follow up was found.[13]

The current study aims to assess whether 2-fold ALA-PDT is more effective than MAL-PDT for the treatment of sBCC.

Materials and methods

This single blinded randomized controlled trial was performed at the outpatient departments of two Dutch university hospitals (Maastricht University Medical Center (MUMC$^+$) and Erasmus Medical Center Rotterdam (EMC)) and one regional hospital (VieCuri Medical Center Venlo/Venray (VCMC)).

Patients

Patients aged 18 years and older, with a primary histologically confirmed sBCC were eligible for inclusion. In case a patient had more than one eligible sBCC, the tumor with the largest diameter was included. Exclusion criteria were the use of immunosuppressive drugs, presence of a genetic skin cancer disorder, prior treatment at the same site, porphyria, pregnancy and breastfeeding and a known allergy to one of the ointment components. sBCC localized in the high-risk area of the face (H-zone), the hairy scalp and convex or concave areas such as the ears or fingers, were also excluded because of a known inferior efficacy of PDT in these areas. All patients received written information about the study and gave their informed consent prior to treatment. The study was approved by the medical ethical board of the Erasmus Medical Center Rotterdam and was performed in accordance with the Declaration of Helsinki. The trial was registered at clinicaltrials.gov (NCT01491711).

Randomization and masking

Patients were randomized using computer-generated lists, using permuted blocks of 6. Research physicians did not have access to the randomization lists. These lists were saved in a closed closet at the department of dermatology of the Maastricht University Medical Center. Only one secretary had access to this list. All study visits (baseline, 3 and 12 months post-treatment) were performed by two investigators blinded for treatment allocation. Patients could not be blinded for treatment allocation because of the different illumination schemes.

Procedures

A baseline visit was planned in which tumor and patient characteristics were recorded and patients were included in the study.

The 5-ALA 20% ointment (Tiofarma B.V., Oud-Beijerland, the Netherlands) was applied to the tumor surface 1-2 mm thick with a margin of 5 mm healthy surrounding skin. The treatment area was then covered with an occlusive dressing (Tegaderm ®, 3M, Leiden, the Netherlands), a gauze and tinfoil to prevent illumination by UV light. After 4 hours, the tumor was illuminated with a light emitting diode (LED) light source (Aktilite, Galderma SA, Lausanne, Switzerland, or Omnilux, Waldmann phototherapeutics, London, United

Kingdom). These light sources produce red light with an optimum wavelength of ~630 nm +/- 5 nm and a fluence of 20J/cm^2 during 4 minutes. Subsequently the treatment area was covered again in the same manner for 2 hours, whereupon a second illumination with 80 J/cm^2 during 18 minutes took place. Both illuminations took place at an irradiance of 50 mW/cm^2.

Patients who received MAL-PDT were treated with Metvix® ointment (Galderma SA, Penn pharmaceutical services, Gwent, United Kingdom) that was applied to the tumor in the exact same way as 5-ALA ointment. The tumor was covered with an occlusive dressing and after 3 hours illuminated with either Aktilite or Omnilux, 37 J/cm^2, 75 mW/cm^2, during 7 minutes. This regimen was repeated one week later.

All treatments were performed by qualified and trained nurses. All study medication was prepared and labelled according to good manufacturing practice (GMP) guidelines.

Outcomes

Primary outcome was the probability of treatment success at 12 months follow-up. In case of clinical suspicion of residual tumor at 3 months or recurrent tumor at 12 months, a biopsy was taken for histological examination. If tumor was found this was considered a treatment failure.

Secondary outcomes were aesthetic outcome and adverse events. Aesthetic outcome was measured on a four-point scale (poor, fair, good or excellent) and scored independently by two investigators blinded to treatment allocation. Treatment failures were scored as poor cosmetic outcome, because according to the protocol these tumors had to be excised. Excision results in a scar which generally compares unfavorably with cosmetic outcome after non-invasive treatment.

Patients completed diaries from which data on adverse events were extracted. Patients were asked to score adverse events on a four-point scale (absent, mild, moderate or severe), one week after both illuminations. Pain and burning sensation were scored using a numerical rating scale (score 0-10), directly after and one week post both illuminations. The maximum pain scores of both illuminations were assessed. Occurrences of serious adverse events (SAEs) or suspected unexpected serious adverse reactions (SUSARs) were registered.

Statistical analysis

The aim was to assess whether the 2-fold 5-ALA illumination protocol is superior to conventional MAL-PDT. It was considered feasible to include 73 patients per group. This

sample size allows detection of clinically relevant difference of 15% between groups with a power of 80% (two-sided alpha=5%). Taking into account a possible drop-out of 10%, 162 patients were included.

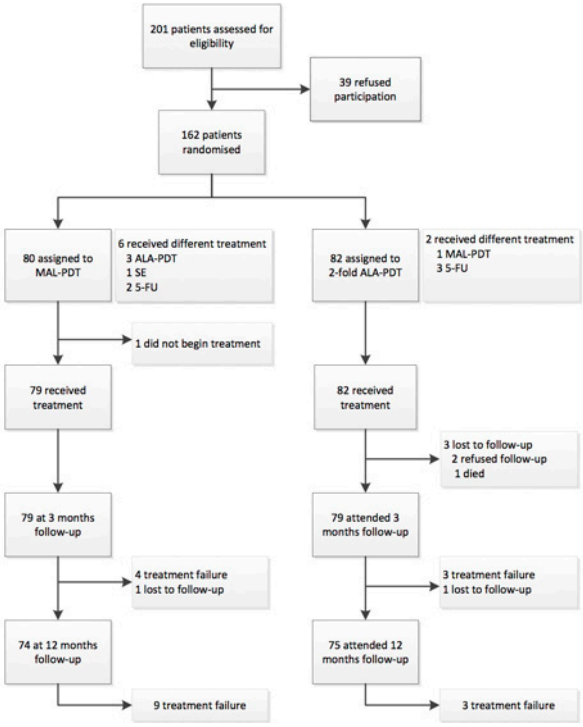
Kaplan Meier survival analysis was performed to estimate the cumulative probability of recurrence free survival at 12 months follow-up. Hazard ratios (HR) for treatment failure with 95% confidence intervals were calculated with Cox proportional hazard models. If necessary, multivariate Cox regression analysis was used to adjust for imbalances in baseline characteristics between randomized groups.

For secondary outcomes, between group differences in proportions were tested using the Chi square test and mean-values of continuous variables were compared using the Students t-test for independent samples or the non-parametric Mann-Whitney-U test. P-values ≤ 0.05 were considered statistically significant. All data were analyzed using SPSS version 23.0 (SPSS, Chicago, IL, U.S.A), openepi.com or Stata version 14.0 (Stata Corp, College Station, TX, U.S.A).

Results

Between September 2013 and May 2015, 201 patients were recruited and assessed for eligibility. Thirty-nine patients refused participation for personal reasons or because they had a strong preference for a treatment other than PDT. A total of 162 patients were enrolled in the study (62 MUMC^{+}, 60 EMC, 40 VCMC), of which 80 patients were allocated to MAL-PDT and 82 to 2-fold ALA-PDT. After randomization, some patients preferred another treatment than the allocated treatment. In the MAL-PDT group three patients preferred 2-fold ALA-PDT, two patients 5-fluorouracil ointment and one patient surgical excision. In the 2-fold ALA-PDT group one patient was treated with MAL-PDT and one with topical 5-fluorouracil. For these patients, data on the primary endpoint were available and they were included in the intention-to-treat analysis. Figure 1 demonstrates the trial profile. Table 1 shows the distribution of baseline characteristics in the randomized groups. There were small imbalances with respect to study center and tumor location (*Table 1)*.

Figure 1. Flow chart.



ALA = aminolevulinic acid. MAL = methylaminolevulinate. PDT = photodynamic therapy. 5-FU = 5-fluorouracil ointment. SE = surgical excision.

Table 1. Distribution of baseline characteristics.

	MAL-PDT (n=80)	2-fold ALA-PDT (n=82)
Mean Age, years (range)	63.6 (28-83)	65.9 (38-85)
Sex, n (%)		
Male	35 (44%)	40 (49%)
Female	45 (56%)	42 (51%)
Study center, n (%)		
MUMC+	27 (34%)	35 (43%)
EMC	34 (43%)	26 (32%)
VCMC	19 (24%)	21 (26%)
Tumor location, n (%)		
Head/neck	1 (1.3%)	7 (8.5%)
Trunk	58 (73%)	45 (55%)
Upper extremities	7 (8.8%)	16 (20%)
Lower extremities	14 (18%)	14 (17%)
Mean tumor size in mm ± SD	11.2 ± 7.1	10.8 ± 5.3

ALA: aminolevulinic acid. MAL: methyl-aminolevulinate. PDT: photodynamic therapy.
MUMC: Maastricht university Medical Center; EMC: Erasmus Medical Center; VCMC: VieCuri Medical Center.

Residual tumor after 3 months was seen in 4 patients treated with MAL-PDT and in 3 patients after ALA-PDT treatment. At 12 months follow-up another 9 recurrences had occurred following MAL-PDT and 3 following ALA-PDT resulting in a total of 13 treatment failures after MAL-PDT and 6 after ALA-PDT.

At 3 months the cumulative probability of treatment success was 96.2% (95% CI [88.7-98.8]) for 2-fold ALA-PDT and 94.9% (95% CI [87.0-98.1]) for MAL-PDT. At 12 months, the cumulative probability was 92.3% (95% CI [83.7-96.5]) and 83.4 (95% CI [73.1-90.0]), respectively (p=0.091) *(Table 2).* Univariate Cox regression analysis resulted in a crude hazard ratio (HR) for treatment failure of 2.17 (95% CI [0.82-5.70]), indicating a higher risk of treatment failure after MAL-PDT. Multivariate Cox regression analysis was performed to adjust for the observed small imbalances in baseline characteristics between randomized groups. The adjusted HR from a model including treatment, study center, age, sex, tumor location and tumor size as independent variables was 2.35 (95% CI [0.84-6.53]).

Table 2: Estimated initial and sustained clearance rate according to intention-to-treat analysis.

	Proportion of patients without treatment failure after 3 months	Proportion of patients without treatment failure within 3-12 months	Cumulative probability of remaining free from treatment failure at 12 months* [95% confidence interval]
ALA-PDT	76/79 (96.2%)	72/75 (96.0%)	92.3% [83.7-96.5]
MAL-PDT	75/79 (94.9%)	65/74 (87.8%)	83.4% [73.1-90.0]

Data are n/N (%). Log-rank test: $p = 0.091$.
* product of initial and sustained clearance rate.
ALA = aminolevulinic acid. MAL = methylaminolevulinate. PDT = photodynamic therapy.

Additionally, a per protocol analysis was performed. Patients were analyzed according to the treatment they actually received and 4 patients who were treated by topical 5-fluorouracil ointment (3) or surgical excision (1) were excluded from this analysis. The cumulative probability of treatment success at 12 months was 91.9 (95% CI [82.9-96.3]) for ALA-PDT and 83.4 (95% CI [73.1-90.0]) for MAL-PDT, $p = 0.110$. Crude and adjusted HR for treatment failure were 2.15 (95% CI [0.81 – 5.65]) and 2.21 (95% CI [0.82-6.04]), respectively.

Adverse events

Table 3 presents the mean scores for pain and burning sensation after each illumination for both treatments. After the second illumination, mean pain scores were significantly higher in the 2-fold ALA-PDT group compared to patients treated with MAL-PDT, with mean pain scores 3.36 ± 2.57 and 2.48 ± 2.57, respectively ($p = 0.039$). None of the patients discontinued treatment because of pain. Overall, patients who received ALA-PDT more often reported side-effects. Reported incidence of erythema, wounds/erosions and vesicles was significantly higher after ALA-PDT compared to MAL-PDT *(Table 3)*. Furthermore, 16.4% in the ALA-PDT versus 5.8% in the MAL-PDT group reported the use of pain medication post-treatment.

Data on cosmetic outcome were available for 73 patients treated with ALA-PDT and 72 patients with MAL-PDT. Good to excellent cosmetic outcome was reported in 79.5% (58/73) of ALA-PDT patients and 66.7% (48/72) of MAL-PDT patients ($p = 0.084$). No serious unexpected adverse reactions were reported in both groups. During the study, four serious adverse events occurred (3 hospitalizations due to transient ischemic attack, chemotherapy for lung carcinoma and dizziness, and 1 patient died due to cancer), which were un-related to the study treatment.

Table 3: Adverse events

	MAL-PDT	2-fold ALA-PDT	*p*-value
Pain score, mean NRS ± SD			
During first PDT session	2.25 ± 2.54	1.88 ± 2.36	0.369
During second PDT session	2.48 ± 2.57	3.36 ± 2.57	0.039*
Burning sensation score, mean NRS ± SD			
During first PDT session	3.12 +/- 2.72	3.41 +/- 2.37	0.457
During second PDT session	2.94 +/- 2.72	4.49 +/- 2.06	0.001*
Erythema, n/N (%)			
absent/ mild	37/73 (50.7%)	13/80 (16.3%)	
moderate/ severe	28/73 (38.4%)	59/80 (73.8%)	<0.001*
not available	8/73 (11%)	8/80 (10%)	
Swelling, n/N (%)			
absent/ mild	61/73 (83.6%)	63/80 (78.8%)	
moderate/ severe	5/73 (6.8%)	9/80 (11.3%)	0.406
not available	7/73 (9.6%)	8/80 (10%)	
Wounds, n/N (%)			
absent/ mild	60/73 (82.2%)	56/80 (70%)	
moderate/ severe	4/73 (5.5%)	16/80 (20%)	0.014*
not available	9/73 (12.3%)	8/80 (10%)	
Crusts, n/N (%)			
absent/ mild	60/73 (82.2%)	57/80 (71.3%)	
moderate/ severe	6/73 (8.2%)	15 (18.8%)	0.062
not available	7/73 (9.6%)	8/80 (10%)	
Vesicles, n/N (%)			
absent/ mild	61/73 (83.6%)	54/80 (67.5%)	
moderate/ severe	5/73 (6.8%)	18/80 (22.5%)	0.011*
not available	7/73 (9.6%)	8/80 (10%)	
Scaling, n/N (%)			
absent/ mild	59/73 (80.8%)	57/80 (71.3%)	
moderate/ severe	7/73 (9.6%)	14/80 (17.5%)	0.160
not available	7/73 (9.6%)	9/80 (11.3%)	
Pruritus, n/N (%)			
absent/ mild	53/73 (72.6%)	56/80 (70%)	
moderate/ severe	13/73 (17.8%)	16/80 (20%)	0.835
not available	7/73 (9.6%)	8/80 (10%)	

NRS: numeric rating scale (0-10), SD: standard deviation

Mean NRS scores were tested for statistical significance using the student's t-test. Differences in categorical data were tested for statistical significance using the Chi-square test.

Discussion

Our data suggest that patients treated with the 2-fold ALA-PDT scheme have a higher cumulative probability of remaining free from treatment failure one year post-treatment, compared to patients treated with conventional MAL-PDT (92.3% versus 83.4%), although the difference is not statistically significant. In addition, patients treated with ALA-PDT experienced more pain and local side-effects.

This is the first randomized controlled trial comparing MAL-PDT with a 2-fold ALA-PDT regimen. World-wide many PDT studies are performed investigating both MAL and ALA photosensitizers. In Europe, MAL is approved as Metvix® (Galderma SA, Penn pharmaceutical services, Gwent, United Kingdom) for treatment of BCC, actinic keratosis and Bowens disease.[14,15] In the U.S.A Metvix® is only approved for the treatment of AK.[16] MAL is more lipophilic and therefore has the theoretical benefit of a higher and faster intracellular absorption compared to ALA. MAL also has a higher selectivity for tumor cells leading to fewer side effects in normal healthy tissue.[17-20]

A systematic review studying the efficacy of several non-invasive treatments for sBCC showed a pooled estimate of tumor-free survival at one year of 76.2% for PDT, including both ALA and MAL-PDT.[21] The majority of studies assessed efficacy of ALA-PDT after a single unfractionated illumination. A higher tumor free survival was observed when PDT treatment was repeated (84%). A more recent study reported a one year CR of 72.8% after conventional MAL-PDT.[3]

To optimize efficacy, de Haas et al. studied a fractionated 2-fold ALA-PDT protocol for sBCC with a first dose of 20 J/cm^2 followed by a dark interval and a second dose of 80 J/cm^2. They performed a prospective comparative study in which a CR of 97% after 2-fold ALA-PDT versus 89% after a single illumination was reported one year post-treatment.[12] The superiority of the 2-fold ALA-PDT regimen was confirmed by de Vijlder et al. who described a CR of 88% after 2-fold ALA-PDT 5 years post-treatment.[22]

The fractionated illumination protocol has been studied pre-clinically in a variety of models.[22,23] It is suggested that by applying two consecutive illuminations, there might be an additional utilization of PpIX due to re-oxygenation during the dark interval.[22] Furthermore, there might be an enhanced local immune response when using light fractionation.[22] Previous PDT literature describes a relation between vascular response, oxygen supply and an effective PDT response.[24-27] Middelburg et al. reported a higher accumulation of PpIX in endothelial vessel walls after ALA application, compared to MAL. After illumination, more endothelial damage was observed.[28] Despite these described hypotheses, the exact explanation for an enhanced efficacy is not yet fully elucidated.

The CR of 92.3% for 2-fold ALA PDT as observed in this trial is lower compared to the previous results from de Haas et al. whereas a higher CR after MAL-PDT was observed compared to the study by Arits et al, who reported a CR of 72.8% at 1 year after treatment.[3] Differences between study populations might be responsible. For instance, in our sample only one patient in the MAL-PDT group had a BCC in the head and neck area, an area that is known to be associated with higher risk of treatment failure, whereas in the trial of Arits et al. 12% of the patients had a BCC in this area.[3,29] Direct comparison of treatments within randomized trials is necessary to validate conclusions on comparative efficacy.

Additionally, we found significantly higher pain scores after 2-fold ALA-PDT compared to MAL-PDT. Erythema, wounds and vesicles occurred significantly more frequently in the 2-fold ALA-PDT group. The reported stronger effect on vascular endothelium and local immune response might be an explanation for these observations.

Previous literature demonstrated a favorable cosmetic outcome after PDT compared to surgery for BCC.[30,31] Despite of more local skin reactions, we observed a trend towards a better cosmetic outcome after 2-fold ALA-PDT compared to MAL-PDT. MAL-PDT results are comparable with Arits et al., who found good-excellent cosmetic outcome in 62% of patients treated with MAL-PDT.[3]

The fact that PDT is an in-clinic treatment could be an advantage over other topical home-based treatments for specific patient categories such as the elderly. An additional advantage of the 2-fold ALA-PDT versus topical ointments or conventional MAL-PDT is that it can be performed within one day. Furthermore, costs of the PDT treatment are supposed to be less because the ointment needs to be applied only once and in The Netherlands ALA ointment is less expensive than MAL (Metvix®, Galderma) ointment.

A limitation of this trial is that the sample size enabled detection of an absolute difference in proportion with treatment failure of 15% or larger with a power of 80% (alpha=5%). The expected difference of 15% was based on prior studies.[3,12] However, we observed a difference between ALA-PDT and MAL-PDT of 8.9% in favor of ALA-PDT and the power to detect this difference with 95% confidence was too low. Long-term follow-up and larger patient cohorts might be needed to detect a statistically significant difference between both treatments. An additional limitation is that post-treatment biopsies were not performed to confirm a lack of tumor and it is possible that clinical and dermoscopic examination missed some recurrences that had not yet surfaced. However, potential underreporting of recurrences is unlikely to affect the comparison of treatment success between both groups.
It should be kept in mind that this study does not only compare two different drugs (ALA and MAL) but additionally the regimen is variable: the treatment with ALA was fractionated

on one day whereas MAL protocol consisted of two illuminations with one week interval. For future studies it would be interesting to compare the 2-fold fractionated ALA-PDT with a comparable fractionated MAL-PDT regimen. Although 2-fold MAL-PDT has not yet been studied in humans, previous studies on mouse-models did not show a favorable response of fractionation in MAL-PDT.[32,33]

In conclusion, our results suggest a trend towards a better efficacy of 2-fold-ALA-PDT compared to conventional MAL-PDT for the treatment of sBCC, although the difference is not statistically significant. The 2-fold ALA-PDT regimen however entails a higher risk of pain and side-effects.

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4.3

Chapter 4.3

Treatment of superficial basal cell carcinoma with ambulatory photodynamic therapy: a retrospective data study

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"Darkness cannot drive out darkness: only light can do that.
Hate cannot drive out hate: only love can do that"
- Martin Luther King Jr.

Introduction

Photodynamic therapy (PDT) is a non-invasive treatment for several (pre)malignant superficial skin cancers, such as superficial basal cell carcinoma (sBCC), Bowen's disease (BD) and actinic keratosis (AK).[1,2] PDT is traditionally known as an in-clinic treatment that can be time-consuming for both patients and healthcare personnel. Prior studies have also shown that conventional PDT (cPDT) can be painful.[3] In order to optimize comfort during treatment, new photosensitizing agents and light sources have been studied over the past decades.

Moseley et al. reported on the use of a portable low-irradiance illumination source for sBCC.[4] This ambulatory PDT (aPDT) device delivers a standard light dose at low irradiance (7 mW/cm^2) over a prolonged period of time, compared to other devices such as the Aktilite (80-90 mW/cm^2, Galderma SA, Lausanne, Switzerland).[5] It is considered a patient friendly, out of clinic treatment with lower pain scores compared to regular PDT.[4-6]

In this study we retrospectively evaluated the risk of recurrence in patients treated with aPDT for primary sBCC and the effect of tumor size on recurrence.

Materials and methods

Medical files of patients treated with aPDT between February 1st 2012 and May 31st 2013 in the Catharina hospital, the Netherlands, were retrospectively reviewed. Eligible for this study, were patients with a histologically confirmed primary sBCC with a maximum diameter of 2 centimeters (due to size limitation of the portable PDT device). Excluded were patients with genetic disorders causing skin cancer and patients using immunosuppressive medication. The primary outcome measure was 1-year probability of remaining tumor-free. Treatment failure was defined as the presence of residual or recurrent tumor during follow-up visits. Follow-up visits were scheduled according to the local hospital protocol 3 and 12 months post-treatment. Secondary outcome measures were cumulative probability of recurrence free survival at 6 and 18 months and incidence of adverse events.

Treatment procedure
In case of slight hyperkeratosis, lesions were prepared by curettage using a wooden spatula to remove scales and crusts, to increase penetration of the active agent. Consecutively methyl-aminolevulinate (Metvix, Galderma SA, Penn pharmaceutical services, Gwent, UK) was applied to the tumor itself and a 5 mm margin of surrounding normal tissue. A transparent occlusive bandage (Tegaderm®, 3M Healthcare, Minnesota, USA), was applied,

after which the portable PDT device (Ambulight®, Ambicare Health, Livingston, Scotland) was attached. The device remained switched off for 3 hours. After that, it switched on automatically and remained switched on for another 3 hours, hereby delivering a total light dose of 75 J/cm^2, with 7 mW/cm^2 irradiance.

Statistical analysis

The distribution of baseline characteristics was described by absolute numbers and percentages for categorical variables and mean ± standard deviation for age. Kaplan Meier survival analyses were used to assess the cumulative probability of recurrence free survival with 95% confidence intervals at 6, 12 and 18 months. Differences in recurrence free survival between groups were tested for significance using the log-rank test. Follow-up ended at the date of a treatment failure or the date of the last follow-up visit. A two-sided *p*-value ≤0.05 was considered to indicate statistical significance. Analyses were performed using SPSS version 23.0 (SPSS, Chicago, IL, U.S.A) and STATA version 14.0 (STATA Corp, College Station, TX, U.S.A).

Results

During the study period 125 patients with 143 sBCC were treated with aPDT. The first diagnosed tumor per patient was included for analysis. In case a patient was treated for two or more primary sBCC on the same day, the largest tumor was chosen for analysis. A total of 104 patients had a histologically confirmed primary sBCC. Three patients were lost to follow-up directly post-treatment, because they preferred follow-up elsewhere. Thus, 101 patients remained for analysis. Baseline characteristics are shown in *table 1*.

Median follow-up time was 13 months (range 2-23) with 59.4% of patients having completed a follow-up time of at least 12 months and 27% more than 18 months. In 11 patients treatment failure was observed based on clinical observation. Eight of the clinically suspect recurrences were confirmed by histopathological examination, three tumors were retreated without histological confirmation. Eleven recurrences were included in the analysis.

At 3 months there were no patients with residual tumor. At 6, 12 and 18 months the cumulative probability of recurrence free survival was 93.6% (95% CI [86.3-97.1]), 89.9% (95% CI = [81.5-94.7]) and 87.6% (95% CI [77.4-93.3]) .For 74 patients data on tumor size was available. These patients were categorized according to tumor size: ≤ 5 mm, 6-10 mm and > 10 mm. The 1-year probability of recurrence free survival was 100% for the ≤ 5 mm size group and 92% (95% CI [78.5-97.6]) and 72.9 (95% CI [42.6-89.0]) for the 6-10 mm and > 10 mm groups respectively, *p*-value = 0.014 *(Figure 1 and Table 2).*

Table 1. Baseline characteristics.

Age (years), mean ± SD	63.6±11.7
Sex, *n* (%)	
Male	53 (52.5)
Female	48 (47.5)
Tumor size, *n* (%)	
<5 mm	10 (9.9)
5-10 mm	48 (47.5)
10-20 mm	16 (15.8)
Unknown	27 (26.7)
Lesion location, *n* (%)	
Head / neck	1 (1.0)
Upper extremity	22 (21.8)
Lower extremity	13 (12.9)
Back	41 (40.6)
Chest/abdomen	24 (23.8)

SD: standard deviation

Figure 1. Kaplan Meier survival curve according to tumor size.

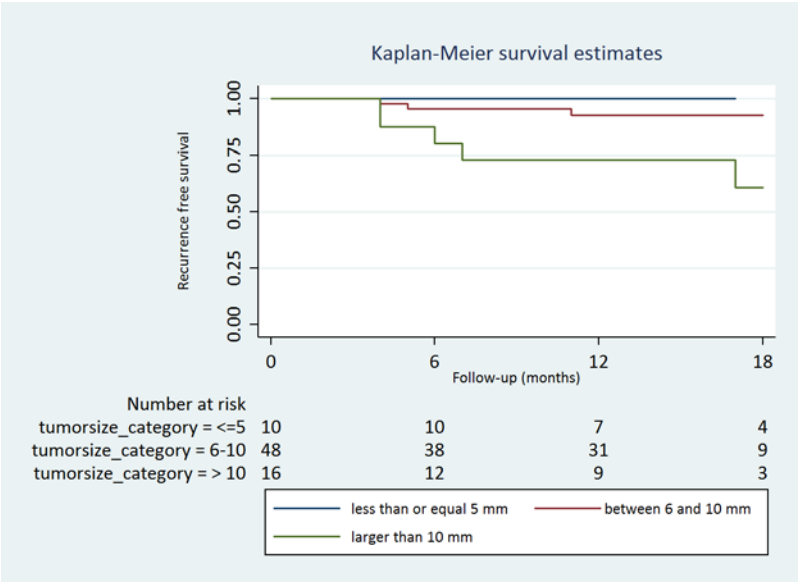

Table 2. 1-year survival data for recurrence.

Tumor size	1 year risk of recurrence free survival (95% CI), %	*p*-value
≤ 5 mm	100	
6-10 mm	92.6 (78.5-97.6)	
> 10	72.9 (42.6-89.0)	0.014*

CI, confidence interval; **p*-value <0.05

Adverse events were reported in two patients: one patient reported blistering and erosions post-treatment and the other patient had a bacterial skin infection, treated with topical antibacterial ointment.

Discussion

The current study suggests that aPDT seems to be an effective treatment for primary sBCC with clearance rates of 89.9% at 12 months follow-up. It is most effective in sBCC smaller than 10 mm.

The probability of recurrence free survival following aPDT compares favorably to results reported by studies on cPDT. Roozeboom et al. found a 1-year cumulative probability of 84% (95% CI [78-90]) based on pooled estimates of recurrence free survival in a systematic review on cPDT treatment of sBCC.[7] In a recent prospective randomized controlled trial probability of recurrence free survival of 72.8% (95% CI [66.8-79.4]) was reported at 12 months following MAL-PDT.[8] A possible explanation for better results of aPDT might be the different irradiance in aPDT. The aPDT device emits red light at low irradiance over a longer period of time. It is hypothesized that this low irradiance is more cytotoxic and has a greater photobleaching efficiency and therefore could lead to a higher efficacy.[9,10] However, the lack of a control group in this study prohibits direct comparison with cPDT.

An interesting finding in the current study is that aPDT is especially effective in tumors <10 mm. The decrease in treatment success in larger tumors has already been reported by Atilli et al. in a small open pilot study with aPDT. They observed that lesions larger than 1.5 centimeter were more likely to show recurrence.[6] One could argue that smaller sBCC, in general, respond better to treatment compared to larger ones. However, PDT literature is not consistent regarding the association between tumor size and effectiveness of PDT .[11-15]

An important limitation of this study is the retrospective nature of the study. Reports were often brief and post-treatment photography was usually not conducted. For this

reason, recurrences may have been misclassified and adverse events may have been underreported.

Currently, aPDT is not widely implemented in daily practice in Dutch hospitals. A limitation of the device is the inability to treat tumors located on convex or concave areas (e.g. nose, fingers) or tumors > 2 centimeters. Since there are viable and more cost-effective alternative therapeutic options such as imiquimod or 5-fluourouracil, the position of aPDT has to be established. aPDT could be a preferred mode of PDT for the working population, for whom in-clinic treatment might not be preferable and for patients who are not able or willing to apply a cream. Another advantage could be the good tolerance during illumination, in contrast to cPDT in which a burning sensation is more often reported.[5,6]

Thus far, there is insufficient evidence to implement aPDT on a wide scale and comparison to other existing effective treatments in a randomized controlled setting is warranted.

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Chapter 5

General discussion and Valorization

"It is more important to know what sort of person has a disease than to know what sort of disease a person has"
- Hippocrates

This thesis describes the outcomes of several studies conducted on non-invasive interventions for actinic keratosis (AK) and superficial basal cell carcinoma (sBCC). For AK, it focuses on the comparison of the most frequently used field directed treatments. For superficial BCC, it focuses on one specific non-invasive therapy – photodynamic therapy (PDT) – and compares the efficacy of different PDT modalities.

In this chapter, I will outline the major conclusions of this research, discuss and interpret the results and reflect on the relevance for scientists, clinicians and patients.

Relevance of the research

Over the past decades, there has been a rise in the incidence of keratinocyte skin cancer and its precursor lesions such as Bowen's disease and actinic keratosis. The life-time risk of developing BCC in the Dutch population is estimated 1:5-6.[1] A recent Dutch study showed that the European age-standardized incidence rate (ESR) for basal cell carcinoma (BCC) in the Netherlands quadrupled between 1973 and 2009.[2] It is important to realize that there is a lack of BCC registries in most countries.[3,4] Because of this, the incidence can only be estimated. For premalignant conditions such as AK, the number of studies investigating its incidence is even fewer. Similar to BCC, there is no registry system for AK either and there is a need for decent cohort studies. Flohil et al. were the first to describe the prevalence of AK in the Netherlands in a population-based cohort study. In their study population, 37.5% of the participants had at least one AK.[5] Based on extrapolation of these data, they suggested that a total of 1.4 million Dutch citizens aged 50 years or older, have AK.[5] It is estimated that 12% of the consultations in Dutch dermatology practice are because of AK.[6,7] Moreover, people continue to attain higher ages; estimates indicate that in 2040 there will be 4.6 million people aged 65 years and older in the Netherlands.[8] On the other hand, there is a trend towards the development of innovative treatments, new technologies and more empowered healthcare consumers. This has resulted in awareness of increasing healthcare costs, while maintaining a high-quality standard of care. In this context, we need to remain critical regarding our treatment choices.

Treatment of actinic keratosis

Whenever we discuss treatment options for AK, the question raises: is it necessary to treat? There is still some controversy about the exact risk of developing an invasive SCC from a pre-existing AK. Some state that AK is an in situ variant of a squamous cell carcinoma (SCC) that needs to be treated to prevent further development into an invasive

SCC.[9] Rates in the literature vary from 0.025% up to 20% of AK progressing into SCC per year.[10-14] Several studies on SCC estimate that 40-80% develop from pre-existing AK.[10,12,15] Holmes et al. summarized this as follows: we do not know how often AK progresses into SCC, but the majority of SCC develops from a pre-existing AK.[16] On the other hand, there is the opinion that treatment is not required to prevent development into SCC and that the available evidence arguing this malignant potential is of insufficient strength.[17] Furthermore, Werner et al. concluded that estimates of the frequency of AKs developing into SCC are not reliable.[18] When individual lesions were assessed, it was found that treatment is not essential to prevent malignant progression.[19] A Cochrane review could not observe a reduction in SCC after treatment of AK.[20] The presence of AK was merely presented as an indicator for sun damage and therefore relevant as a predictor for the risk of developing further AK and KC.[20] Other than possible malignant transformation, the question to treat or not to treat also depends on patient complaints related to their AK. AK can lead to itching, mechanic irritation, cosmetic impairment or pain, all of which can be relieved by treatment.

When it is desirable to treat AK, there is a great number of treatment options. Relatively invasive treatments are available, such as laser resurfacing, cryotherapy and dermabrasia. In addition, less invasive treatments such as 5-fluorouracil (5-FU) cream, imiquimod cream, photodynamic therapy (PDT), ingenol mebutate (IM) gel or diclofenac sodium 3% gel can be used. In the Netherlands, the most frequently used lesion-directed therapy is cryotherapy. For field-directed therapies, 5% 5-FU cream, 5% imiquimod cream, PDT and IM gel are registered.

Current AK guidelines do not provide clear advice on the treatment of primary choice. The 2010 Dutch guidelines advised treating all patients with AK, primarily because of the uncertain malignant potential.[21] The 2017 updated version states there is no direct oncological need to treat AK, but factors such as complaints or cosmetic outcomes can be reasons to treat.

One AK treatment for all?
Different patient categories might have different needs and require distinctive therapeutic approaches. The younger (working) population, for example, might prefer treatments with a shorter down-time such as PDT or IM gel. The elderly population, on the other hand, might have trouble with a self-applied treatment. As AK tends to have a high recurrence rate and repetitive treatments are often needed, compliance is an important factor in treatment choice.

Because of its in-clinic setting, PDT is an example of a treatment that might be preferred by patients who are not able to complete a self-applied treatment or prefer a one-day

treatment. Unfortunately, pain is an important limiting factor during PDT treatment, especially in AK patients.[22] In ***chapter 2.2***, we compared conventional methyl-aminolevulinate (MAL)-PDT with laser mediated MAL-PDT using a pulsed dye laser (PDL). Previous literature indicated that PDL illumination leads to lower pain scores.[23,24] We aimed to assess whether the efficacy is comparable to conventional MAL-PDT and whether pain scores are lower after PDL illumination. In a split-face study, we found no statistically significant differences between the two illumination sources when assessing the mean change in the number of lesions from baseline, one year post-treatment. However, pain scores were significantly lower after laser-mediated PDT. Furthermore, patients indicated a preference for PDL illumination (78.8%) above conventional PDT (32.8%). Unfortunately, in our study, newly developed lesions were not differentiated from pre-existing lesions when counting the AK numbers post-treatment, a frequently observed problem in AK studies. Because of this, it was not possible to conclude on the exact effect of these two treatments on the lesions at baseline. An important drawback of the treatment itself is the limited availability of laser devices in clinical practice and the costs. Besides the high expenses for the laser device, the treatment is still a complete in-clinic treatment which involves additional costs, e.g. for healthcare workers.

Another solution for the pain during PDT is daylight PDT, a treatment presented for grade I-II AK a few years ago.[25-28] Exposure to daylight after application of a photosensitizer leads to a continuously lower amount of protoporphyrin IX (PpIX) formation, instead of a rapid peak with conventional illumination devices. In daylight PDT, there should be continuous exposure to daylight for 2 consecutive hours instead of a couple of minutes in conventional PDT. The use of daylight leads to lower pain scores, fewer side effects and comparable cure rates to those after conventional PDT.[27,28,29] However, the use of daylight comes with weather and season uncertainties. Perhaps due to these practical concerns, currently there is only a small number of hospitals that offer daylight PDT in the Netherlands.

What field-directed treatment should be preferred?

The majority of patients will choose the most effective treatment. Ideally this coincides with a short treatment duration, a minimum of side effects, a short down-time and of course low costs. With the background of increasing costs in medical care, however, this is challenging. In ***chapter 2.1*** we described a literature review in which we concluded that most AK studies are very heterogeneous in terms of the study population and outcome measures. It is desirable to support treatment choices with more evidence, preferably with head-to-head trials comparing the most frequently used therapies for AK. The gap in the available literature led to the initiation of a large multi-center randomized controlled effectiveness trial at our center, described in ***chapter 2.3*** We included 624 patients with the aim of determining the

most effective field-directed treatment for AK. Data after three months of follow-up indicated that 5-FU appears to be the most effective field-directed treatment. The proportion of treatment success at three months of follow-up was significantly higher for 5% 5-FU cream (90.6%), compared to 5% imiquimod cream (76.4%), MAL-PDT (76.0%) and 0.015% IM gel (67.3%). In the 5-FU group, 4 patients who had initial treatment failure refused re-treatment. For imiquimod, PDT and IM this occurred for 12, 13 and 15 patients. When assessing the side effects, our data show that patients treated with MAL-PDT reported significantly more pain and burning sensations compared to patients treated with the other topical ointments. Patients treated with 5% 5-FU, on the other hand, experienced erosions more frequently in the two-week post-treatment period, compared to the other treatments.

What are the implications of these results for physicians?
The primary outcome of this trial was the proportion of patients with ≥75% lesion clearance at 12 months follow-up. Hence, it is too early to draw definite conclusions; the lesion reduction 12-months post-treatment will provide insight into whether the superior efficacy of 5-FU is sustained.

In case of insufficient initial response, patients received a maximum of one re-treatment. It is remarkable that to achieve a high effectiveness, only 14.8% of the patients treated with 5-fluorouracil needed a second treatment cycle, compared to 35.9%, 43.6% and 47.8% after imiquimod, MAL-PDT and IM, respectively. Furthermore, a substantial larger number of patients refused re-treatment after imiquimod, PDT and IM, compared to 5-FU, which might indicate that 5-FU is better tolerated. Factors as the need for an extra treatment and the way a treatment is tolerated are important considerations to address when a treatment strategy is discussed with a patient.

Based on the current available three-month follow-up data, 5-FU should be considered the field treatment of first choice. The study we performed is the first large multi-center randomized controlled study comparing the four most common treatments head to head. This trial is also one of the few AK trials that included Olsen grade III AK and a large treatment area (up to 100 cm^2). With this, we tried to simulate daily clinical practice in a trial setting, as in daily practice AK patients usually do not present with only grade I-II AK in a limited area. The outcomes of our study are the first data to provide insight into the most effective treatment for field AK, including severe AK lesions.

Along with conclusions about the effectiveness of therapies at 12 months post-treatment, this study will also provide insight into the number of AK lesions that progress into SCC. We performed a specific lesion follow-up that allows us to register all SCC that will develop in the study area during follow-up. Because of the mapping performed in the study, we

will be able to tell if the SCC developed from a pre-existing AK. It will be of future interest to analyze the number of SCCs that develop in the study area five years post-treatment and to evaluate whether there are interesting correlations, such as the previous grade of AK at that specific location or the administered treatment.

In my opinion, until there is no consensus regarding the risk of development into SCC, patients should be informed about the uncertain malignant potential and the decision to treat or not should be based on shared decision making between the physician and the patient. AK can be left untreated, when the patient is adequately informed to return to their dermatologist in case of complaints such as pain, growth of a lesion or bleeding. When deciding to treat field change, I would primarily advise 5% 5-FU cream, based on its efficacy and tolerability at three months of follow-up in our randomized controlled trial.

Treatment of basal cell carcinoma

Can tea cure?

Besides established treatments for sBCC, it is important to gain knowledge about possible new topical treatments to strive for an optimization in efficacy. The future of cancer medicine is increasingly focusing on molecular medicine and targeted therapies by blocking tumor growth more specifically by targeting particular molecules or pathways. The exact molecular mechanism of BCC development has not been fully elucidated. It is known that the vast majority of sporadic BCC contain mutations in the patched 1 (PTCH 1) gene, which is an inhibitor of the Hedgehog (HH) pathway.[30] It remains debatable whether there is a role for the Wingless (Wnt) pathway in neoplasia in a manner that is HH-pathway driven or whether there might be cross-talk between both pathways.[31-33] Wnt plays a role in hair bud formation; HH subsequently promotes the maturation of hair follicles. Deregulation of the Wnt pathway leads to the accumulation of nuclear b-catenin, which consequently leads to tumor cell proliferation. There are some data suggesting that epigallocatechin-3-gallate (EGCG) – an active constituent of green tea – might lead to the inactivation of b-catenin signalling through the Wnt pathway.[34] Other data have demonstrated an anti-apoptotic effect of EGCG by decreasing the anti-apoptotic Bcl-2 proto-oncogene.[35-37]

Sinecatechin 10% ointment (currently available as Veregen® and indicated to treat condyloma acuminatum) contains EGCG. In ***chapter 3***, we aimed to investigate the efficacy of sinecatechin 10% ointment to treat sBCC in a randomized controlled trial. We hypothesized that this ointment would lead to histological tumor clearance; however, we found no significant differences in histopathological clearance post-treatment between the intervention and placebo groups. We also assessed whether there was decreased expression

of Ki-67 (proliferation) or Bcl-2 (anti-apoptosis) by immunohistochemical staining pre- and post-treatment. We could not observe statistically significant differences, but we did observe a tendency towards a greater decrease in Bcl-2 expression in the sinecatechin 10% group compared to placebo (41.2% versus 23.5%). A decrease in Ki-67 was observed in similar proportions (29.4% versus 31.3%). This study could not confirm the theoretically hypothesized efficacy of sinecatechin 10% ointment in sBCC. It remains debatable as to why decreased Bcl-2 expression was observed in the intervention group. Is it just coincidence? Perhaps the current formula contains insufficient EGCG to be effective to treat sBCC. Another explanation could be that the ointment was unable to reach the nucleus of tumor cells and thereby failed to increase apoptosis and decrease proliferation sufficiently to lead to histological tumor clearance. We can conclude that, based on our study, there is no need to further assess the use of topical sinecatechin 10% ointment in the current formula to treat sBCC. More pre-clinical (laboratory and animal studies) might be needed to assess the effect of EGCG on BCC tumor cells and to determine the optimal EGCG dosage.

Personalized medicine: a niche for PDT?

The gold standard treatment for all BCC is surgical excision. In recent decades, research has shown that non-invasive therapies (e.g., topical 5-FU cream, imiquimod cream or PDT), result in acceptable clearance rates for sBCC. An important disadvantage of non-invasive treatments is the lack of histological control. On the contrary, they lead to a lower workload, might be more patient friendly and – in the case of topical creams – lead to fewer in-clinic treatments. In the 20th century, PDT gained popularity as an alternative to surgery. This rise was based on a few small studies reporting on efficacy. However, with rising patient numbers, the need for alternative non-invasive treatments was high. In the Netherlands, PDT used to be a popular treatment because of reported good cosmetic results, the excellent reimbursement for the dermatologist and an international consensus mentioned PDT as first-line non-invasive treatment for sBCC.[38] Interestingly, in 2013, Arits et al. demonstrated that imiquimod cream was superior and 5-flurouracil was non-inferior and more cost-effective compared to PDT after one year of follow-up in a large multi-center randomized trial studying sBCC.[39] Recently, five-year follow-up data showed that 5% imiquimod cream is superior to both MAL-PDT and 5% 5-FU cream.[40]

Several studies have been conducted to assess whether the effectiveness of PDT could be improved by different photosensitizers, illumination sources or treatment schemes. De Haas et al. were the first to describe a two-fold 5-aminolevulinic acid (ALA)-PDT scheme, in which two illuminations were performed with 20 and 80 J/cm^2, 4 and 6 hours after a single application of aminolevulinic acid (ALA) in a randomized controlled trial. The results were promising, with 12-month clearance rates (CR) of 97% compared to 89% after a single ALA application.[41] These CR, however, were not replicated by other study groups.

We tried to assess the replicability of these CR published by de Haas et al. and described the results in ***chapter 4.1 and 4.2***. We initially performed a retrospective study investigating the efficacy of this two-fold ALA-PDT scheme. We assessed a total number of 323 primary sBCC for recurrence, derived from data in electronic patient files at the outpatient department of a single non-university hospital. Our results indicate a cumulative probability of recurrence-free survival based on clinical observations of 88.8%, 81.8% and 77.1% after 12, 24 and 48 months post-treatment, respectively. Our results are comparable to a study by Star et al. showing a clearance rate of 88% at 12 months follow-up using two-fold illumination with 45 J/cm^2.[42] However, we could not confirm the high one-year clearance rate reported by de Haas et al.[41] The limitations of this study were its retrospective nature and the lack of a control group.

We tried to solve these limitations by performing a prospective multi-center randomized trial comparing the two-fold illumination ALA-PDT scheme with the conventional MAL-PDT scheme, which is mostly used by dermatologists in Europe. Our results showed a lower probability of treatment success in the conventional MAL-PDT group compared to the two-fold ALA-PDT group at 12 months follow-up (92.3% versus 83.4%). Even though not statistically significant (p=0.091), these results do show a trend towards better efficacy of the two-fold ALA PDT scheme and support the previous findings from the group of de Haas et al.[41,43] We also observed a higher pain score and more local adverse events after two-fold ALA-PDT compared to conventional MAL-PDT. Pain during PDT is a well-known drawback for patients. This is especially observed in AK patients, but it is also reported in BCC patients. Our findings might be explained by the double illumination on one day. Middelburg et al. found a significantly higher degree of PpIX in the dermal vasculature after ALA compared to MAL.[44] It is suggested that, by fractionation of the illumination, there might be an additional use of PpIX because of re-oxygenation during the dark interval.[45] The reported stronger effect of ALA on the vasculature and an increased local immune response might be an explanation for the higher degree of pain sensation experienced in our sample.

The trade-off between treatment efficacy and side effects is a personal choice: a higher probability of treatment success after two-fold ALA-PDT or fewer side effects and lower efficacy after MAL-PDT. We did not perform a cost-effectiveness analysis, but in the Netherlands ALA cream is cheaper than MAL cream. Furthermore, an important advantage of two-fold ALA-PDT over conventional MAL-PDT is the one-day treatment, which leads to lower costs and workload. Our five-year follow-up data will reveal whether the lower probability of treatment failure after two-fold ALA-PDT will be maintained in the long term. In this same context of researching possible improvements for PDT, we assessed the efficacy of an ambulatory MAL-PDT device in a retrospective study, described in ***chapter 4.3***.

The results showed a clearance rate of 89.9% at 12 months follow-up, with the highest efficacy in tumors smaller than 10 mm in diameter. Ambulatory PDT emits red light at a low irradiance compared to conventional PDT and over a longer period of time. It is hypothesized that this leads to a greater cytotoxic effect and photobleaching efficiency.[46-48] Photobleaching is a phenomenon describing the decrease in the fluorescence signal in irradiated tissue due to photosensitizer destruction. In this process, protoporphyrin IX (PpIX) fluorescence is reduced. The amount of photobleaching during PDT is correlated with the amount of PDT-induced damage.[49] These factors might be an explanation for the greater effectiveness compared to conventional PDT. As our study was a retrospective data study and the literature is lacking in prospective, randomized controlled comparative trials, there is not enough evidence to implement the ambulatory PDT device widely in dermatology practice.

Finally, we considered the role of PDT in dermatology practice in the Netherlands. After the publication of the randomized trials by Arits et al.[39] and Roozeboom et al.,[50] reimbursement for PDT to treat sBCC was questioned, because of its proven lower efficacy and higher costs compared to 5% imiquimod cream and 5% 5-FU cream. This thesis focused on the use of PDT for sBCC, so the question is: should PDT return to the stage? In my opinion, PDT should not be eliminated as a treatment for sBCC. It is of great importance to look at the individual patient when determining a therapeutic approach. Even though less effective, PDT may still be preferred for a certain category of patients. One can think of the elderly, who are sometimes unable to apply a topical ointment themselves and/or in whom surgery might not be preferred. Also, younger patients might have a profession, which holds them back from applying a topical ointment for 4-6 weeks with a long down-time. At this moment, we cannot draw conclusions on the comparison of the two-fold ALA PDT scheme and topical creams such as imiquimod or 5-FU. A direct comparison of these treatments through randomized trials is necessary to validate conclusions on their comparative efficacy. It would also be of interest to determine the long-term outcomes of the two-fold ALA PDT regimen at three or five years follow-up.

Conclusion

In conclusion, in this thesis, different randomized controlled trials are reported in which we aimed to investigate several non-invasive treatment modalities for two keratinocyte neoplasms: AK and sBCC. Randomized controlled trials are essential to create valuable evidence regarding the efficacy of treatments. Our data show that 5% 5-FU should be the first-choice treatment for field AK. In case an in-clinic treatment is preferred, PDT using the pulsed dye laser could be an alternative to conventional PDT, when pain is a limiting factor.

Despite the fact that recent studies showed that MAL-PDT is inferior compared to 5% imiquimod for sBCC, we aimed to search to optimize its efficacy. The two-fold ALA-PDT regimen is promising. In our study, it led to fewer recurrences compared to conventional MAL-PDT. PDT might remain valuable for a certain subgroup of patients. We should realize that treating patients does not only involve choosing the most effective treatment. Although all treating physicians should be aware of the most (cost) effective approach, the treatment choice also relies on the needs and possibilities for our individual patients. As Hippocrates once stated: "*It is more important to know what sort of person has a disease than to know what sort of disease a person has*".

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Chapter 6

Summary
Samenvatting

Summary

Over the past years there has been a rise in the incidence of skin cancer. The work presented in this thesis focused on non-invasive treatment modalities for both actinic keratosis (AK) and superficial basal cell carcinoma (sBCC): several retrospective data studies and prospective randomized trials were performed.

Chapter 1 gives a general introduction to both AK and sBCC. *Chapter 2* discusses treatment options for AK. In *chapter 2.1* an overview of the available literature is presented, in which the most frequently used therapies are summarized. Photodynamic therapy (PDT), for example, is a well-established treatment for several keratinocyte carcinoma (KC) and its precursors. It is known that AK patients experience pain during PDT illumination. This frequently leads to discontinuation of treatment or refusal of future follow-up treatments. In a split face study *(chapter 2.2*), we investigated whether the use of a pulsed dye laser (PDL) device as illumination source during PDT, was associated with lower pain scores compared to conventional LED-PDT. We found that patients reported lower pain scores and side effects after PDL illumination. The PDL device is however expensive and not available in all clinics, which makes it only an alternative treatment option in case in-clinic treatment is desired and pain is a limiting factor.

In the literature review of chapter 2.1, we found that studies were methodologically heterogeneous and there was a need for more head-to-head trials comparing the most frequently used treatments. It was not possible to draw a solid conclusion on what treatment is the most effective for a continuous area with multiple AKs (so called field-AK). This initiated a large randomized controlled study in *chapter 2.3,* that compared the effectiveness of topical 5-fluorouracil, imiquimod, ingenol mebutate and methylaminolevulinate photodynamic therapy (MAL-PDT) with one another. A total of 624 patients in 4 different hospitals participated. Three months follow-up data show that 5-fluorouracil cream is superior to the other mentioned treatments for field AK: 90.2% of patients treated with 5-fluorouracil had ≥75% reduction in AK lesions, compared to baseline. Results for imiquimod, PDT and ingenol mebutate were respectively 76.4%, 76.0% and 67.3%. In this study patients could only receive one re-treatment in case of <75% lesion reduction. Only 14.8% of the patients treated with 5-fuorouracil needed a second treatment cycle due to initial insufficient effectiveness, before the 3 months assessment was done. For imiquimod, MAL-PDT and ingenol mebutate these percentages were 35.9%, 43.6% and 47.8%, respectively.

Various treatments are available for BCC, with surgical treatment as gold standard. As invasive treatment is not always the preferred treatment, especially in the case of the superficial BCC, many topical non-invasive treatments are used. Nowadays, cancer medi-

cine increasingly aims to focus on molecular medicine and targeted therapies in order to block tumour growth more specifically. The majority of BCC contains patched 1 (PTCH1) mutations, which lead to an inhibition of the Hedgehog (HH) pathway. There is some evidence that there might be a role for the Wingless (WNT) pathway in BCC. Dysregulation of the WNT pathway leads to the stacking of nuclear b-catenin enhancing tumour cell proliferation. We aimed to investigate whether topical sinecatechin ointment (which consists among others of epigallocatechin-3-gallate), could lead to tumour proliferation arrest or tumour clearance. In a randomized placebo controlled study we could not find a statistically significant clinical, histological or immunochemical effect of the topical sinecatechin ointment on BCC (*chapter 3).*

Previous studies showed that when comparing PDT with topical 5-fluorouracil and imiquimod in the treatment of sBCC, PDT was less effective both at one and three and five-year follow-up in sBCC. It might however still be a desired treatment for a certain subcategory of patients. One can think of the elderly, patients who are unable to complete a self-applicable treatment etcetera). Previous studies showed a high efficacy using a fractionated ALA PDT scheme. We performed a retrospective study *(chapter 4.1)* assessing 323 primary sBCC treated with this fractionated 2-fold aminolevulinic acid 20% (ALA) PDT. With this scheme, two light fractions of 20 and 80 J/cm^2 are delivered, 4 and 6 hours after 5-ALA application, respectively. Our results showed a cumulative probability of recurrence free survival of 88.8% after 12 months, 81.8% after 24 months and 77.1% after 48 months follow-up. In order to further assess the effectiveness in a more controlled setting and in order to compare it to the conventional MAL-PDT scheme, we performed a multi-centre randomized controlled trial in which 162 patients with primary sBCC participated *(chapter 4.2)*. Although the difference was not statistically significant, the 2-fold ALA-PDT scheme did lead to fewer recurrences at 12 months follow-up compared to MAL-PDT: 6 versus 13. Another advantage of this scheme above the conventional MAL scheme is that the one day treatment is more efficient than two illuminations one week apart, as is the case with the conventional MAL scheme.

In *chapter 4.3* we retrospectively assessed the effectiveness of an ambulatory PDT (aPDT) device to treat sBCC. Results show that aPDT is an effective treatment for primary sBCC with a clearance rate of 89.9% at 12 months follow-up. Limitations of the device are its costs and the inability of treating tumours on every location and tumours larger than 2 centimetres. In *chapter 5* results and implication for clinical practise are described and discussed in further detail.

Samenvatting

De afgelopen jaren is er een stijging gezien in de incidentie van huidkanker, zowel in Nederland als de rest van de wereld. Dit proefschrift richt zich op de niet-chirurgische behandelingen van twee veel voorkomende aandoeningen: actinische keratose (AK) en het oppervlakkige (superficieel) basaalcelcarcinoom (sBCC) en laat de resultaten zien van zes studies.

Hoofdstuk 1 geeft een algemene introductie over beide aandoeningen. *Hoofdstuk 2* richt zich op de behandeling van AK. Allereerst wordt in *hoofdstuk 2.1* een overzicht van de literatuur gegeven waarin de meest voorkomende behandelingen op een rij gezet worden. Photodynamische therapie (PDT) bijvoorbeeld, is een gekende behandeling binnen de dermato-oncologie. Omdat pijn tijdens de PDT behandeling voor veel AK patiënten een beperkende factor is, hebben we in *hoofdstuk 2.2* onderzocht of de pijn minder is als er belicht wordt met een pulsed dye laser (PDL). In een zogeheten 'split-face' studie hebben wij de conventionele PDT vergeleken met PDL gemedieerde PDT. Resultaten lieten zien dat patiënten minder pijn rapporteerden na PDL belichting. Het PDL apparaat is echter duur in de aanschaf en niet iedere kliniek beschikt over deze apparatuur. Dit maakt het daarom enkel een alternatieve behandeloptie als behandeling in het ziekenhuis gewenst is en pijn een beperkende factor is.

Uit de literatuurstudie van *hoofdstuk 2.1* bleek ook dat studies naar AK erg wisselen in studie-opzet, onderzochte patiënt populatie en uitkomstmaten. Het is dus op basis van de huidige literatuur niet mogelijk een goede conclusie te trekken over wat de meest effectieve behandeling is voor een gebied met meerdere AK's (zogeheten veld-AK). Om die reden hebben wij een prospectieve gerandomiseerde studie opgezet waarvan de resultaten in *hoofdstuk 2.3* worden gepresenteerd. In deze studie hebben wij de vier meest toegepaste behandelingen van veld-AK onderzocht: 5-fluourouracil crème, imiquimod crème, ingenol mebutate gel en photodynamische therapie (PDT). Een totaal van 624 mensen, verdeeld over 4 centra namen deel. Resultaten laten zien dat na 3 maanden follow-up 5-fluourouracil crème de meest effectieve behandeling is, waarbij 90.2% van de patiënten ≥75% reductie had van het aantal AK's ten opzichte van begin van de studie. Voor imiquimod, ingenol mebutate en PDT was dit significant minder met respectievelijk 76.4%, 76.0% en 67.3%. In deze studie mochten mensen maximaal één herbehandeling ondergaan, alvorens de eindevaluatie plaatsvond. Deze herbehandeling werd verricht indien er <75% reductie was van het aantal AK's. Slechts 14.8% van de patiënten die met 5-fluorouracil crème werden behandeld, hadden een tweede behandelcyclus nodig. Deze percentages waren 35.9%, 43.6% en 47.8%, voor imiquimod crème, PDT en ingenol mebutate gel respectievelijk.

Naast het onderzoeken van reeds bestaande therapieën, richt onderzoek binnen de oncologie zich steeds meer op moleculaire mechanismen van tumoren. Het exacte mechanisme achter het ontwikkelen van BCC's is nog niet volledig gekend, maar we weten wel dat de grote meerderheid van de BCC's ontstaan ten gevolge van een mutatie in het patched 1 (PTCH 1) gen, onderdeel van de hedgehog (HH) pathway. Een andere pathway, de Wingless (WNT) pathway, zou mogelijk ook een rol hebben bij de tumorgroei door toename van nucleair b-catenine eiwit en er zijn aanwijzingen dat de HH en WNT pathway met elkaar communiceren. Er zijn data die suggereren dat een bestanddeel van groene thee – epigallocatechin-3-gallate (EGCG), een in-activatie van b-catenine tot gevolg zou kunnen hebben. In een gerandomiseerde placebo gecontroleerde studie hebben wij bij 42 patiënten onderzocht of Sinecatechin 10% zalf (die o.a. bestaat uit EGCG), leidde tot het verdwijnen van de BCC cellen. Resultaten lieten geen klinisch, histologisch of immunohistochemisch effect zien van de zalf op de BCC's *(hoofdstuk 3)*.

Eerdere studies naar sBCC hebben laten zien dat vergeleken met 5-fluourouracil en imiquimod crème, PDT tot de meeste recidieven leidt, zowel 1, 3 en 5 jaar na behandeling. PDT kan echter toch een gewenste behandeloptie zijn bijvoorbeeld voor ouderen of mensen die niet in staat zijn een crème zelf thuis aan te brengen.

Wij hebben daarom geprobeerd te zoeken naar andere en mogelijk betere belichtingsschema's waarmee de effectiviteit van PDT verhoogd zou kunnen worden. Eerdere studies hebben laten zien dat de effectiviteit van PDT bij BCC's vergroot kan worden door de belichtingen te fractioneren na aanbrengen van aminolevuline zuur (ALA). In *hoofdstuk 4.1* hebben wij een retrospectieve dossierstudie verricht en 323 primaire sBCC's onderzocht welke met gefractioneerd ALA PDT behandeld zijn. Bij dit behandelschema worden op één dag twee belichtingen gegeven van 20 en 80 J/cm^2, respectievelijk 4 en 6 uur na aanbrengen van de ALA-crème. Onze resultaten laten zien dat 12 maanden na de behandeling, 88.8% van de patiënten geen recidief tumor had. Na 24 en 48 maanden zijn deze cijfers 81.8% en 77.1%. Om de effectiviteit in een meer gecontroleerde setting te beoordelen en om de vergelijking te maken met het veel toegepaste MAL-PDT schema, hebben wij een multi-center gerandomiseerde studie verricht waaraan 162 patiënten deelnamen in 3 verschillende centra *(hoofdstuk 4.2)*. Ondanks dat het verschil niet statistisch significant was, leidde het gefractioneerde ALA-PDT schema tot minder recidieven 12 maanden na behandeling ten opzichte van de conventionele MAL-PDT: 6 versus 13. Een belangrijk voordeel van het ALA-PDT schema is dat dit op één dag uitgevoerd kan worden. Het conventionele MAL-PDT schema wordt op twee dagen met één week tussenpauze verricht.

In *hoofdstuk 4.3* hebben wij de effectiviteit van een draagbaar PDT apparaat (aPDT) in een retrospectieve studie onderzocht. De resultaten laten zien dat aPDT een effectieve behan-

deling is voor primaire sBCC's waarbij 89.9% van de patiënten 12 maanden na behandeling nog tumorvrij is. Beperkingen van het apparaat zijn de kosten en het feit dat niet op iedere lokalisatie behandeld kan worden en ook tumoren groter dan 2 centimeter niet behandeld kunnen worden. *Hoofdstuk 5* beschrijft en interpreteert de resultaten en conclusies van het gedane onderzoek en bediscussieerd de relevantie voor de dagelijkse praktijk.

Appendix

Curriculum Vitae
List of publications and presentations
Dankwoord
Cover

Curriculum vitae

Janneke Kessels werd op 10 maart 1987 geboren te Kerkrade. Nadat zij in 2005 haar gymnasium diploma behaalde aan het Grotius college te Heerlen startte zij in ditzelfde jaar met de studie geneeskunde aan de Universiteit van Maastricht. Gedurende haar studie volgde zij het honours programma 'international medicine'. Dit leidde o.a. tot een bestuursfunctie bij de Mustangh foundation Maastricht. Tijdens het doorlopen van de coschappen, heeft zij zich binnen de studievereniging ingezet voor de kwaliteit van het medisch onderwijs op de universiteit Maastricht. Gedurende het coschap dermatologie in het Maxima Medisch Centrum te Veldhoven werd de interesse voor de dermatologie gewekt. Daartoe besloot zij haar klinische en wetenschappelijke vaardigheden verder te ontwikkelen binnen de dermatologie in het laatste jaar van haar artsexamen. In 2011 behaalde zij haar artsexamen en startte in het Catharina ziekenhuis te Eindhoven als arts-assistent, hetgeen in 2013 leidde tot een opleidingsplaats dermatologie in het Maastricht Universitair Medisch Centrum. In ditzelfde jaar besloot zij verder te gaan met wetenschappelijk onderzoek en startte haar promotie traject onder begeleiding van promotor prof. dr. P.M. Steijlen en co-promotoren mevr. dr. N.W.J. Kelleners-Smeets en mevr. dr. K. Mosterd. Zij schreef een onderzoeksvoorstel voor een gerandomiseerde multi-center studie naar de behandeling van aktinische keratosen, waarvoor door ZonMw een subsidie werd toegekend. Daarnaast werkte zij aan verschillende andere studies naar de behandelingen van superficiële basaalcelcarcinomen en actinische keratose, hetgeen leidde tot dit proefschrift.

Vanaf 1 april 2017 werkt zij als dermatoloog in het Zuyderland medisch centrum te Heerlen.

List of publications

JPHM Kessels, JU Ostertag; "The use of Tumescent local anaesthesia for Erbium:YAG Laser treatments: clinical results in 8 cases", *J. Eur. Acad. Dermatol. Venereol., 2012, 26(11): 1456-7*

JPHM Kessels, ET Hamers, JU Ostertag, "Superficial hemangioma: pulsed dye laser versus wait-and-see policy, *Dermatol. Surg, 2013, vol. 39: 414-21*

JPHM Kessels, NWJ Kelleners-Smeets, K. Mosterd, "Behandelingen van aktinische keratosen: een overzicht", *NTVD, 2014, vol. 24 (9): 558-561*

JPHM Kessels, G.A.M. Krekels, K. Mosterd, N.W.J. Kelleners-Smeets, P.J. Nelemans, J.U. Ostertag, "Laser mediated photodynamic therapy: an alternative treatment for actinic keratosis?"*Acta Derm. Venereol. 2015, doi:10.2340/00015555-2278*

JPHM Kessels, J.C.J. Hendriks, P.J. Nelemans, K. Mosterd, N. Kelleners-Smeets, "Two-fold illumination in topical 5-aminolevulinic acid (ALA)-mediated photodynamic therapye (PDT) for superficial basal cell carcinoma (sBCC): a retrospective case series and cohort study", *J. Am. Acad. Dermatol. 2016, 74(5):899-906*

JPHM Kessels, N. Dzino, P.J. Nelemans, K. Mosterd, N. Kelleners-Smeets, "Ambulatory photodynamic therapy for the treatment of superficial basal cell carcinoma: an effective light source?" *Acta Derm. Venereol.,2017; 97(5):649-650*

JPHM Kessels, H. Kreukels, P.J. Nelemans, K. Mosterd, E. de Haas, N. Kelleners-Smeets, "Treatment of superficial basal cell carcinoma by topical photodynamic therapy with fractionated 5-aminolevulinic acid 20% versus two stage topical methylaminolevulate". *Br. J. Dermatol. 2017 Sep 8, doi: 10.1111/bjd.15967. [Epub ahead of print]*

JPHM. Kessels, K.J.A. Frencken, L. Voeten, P.J. Nelemans, V. Winnepenninckx, P. Steijlen, K. Mosterd, N. Kelleners-Smeets; "Topical sinecathechins ointment in treatment of primary superficial basal cell carcinoma". *JAMA Dermatol. 2017, 153(10):1061-63*

MHE Jansen, **JPHM Kessels**, PJ Nelemans, N. Kouloubis, AHMM Arits, JPA van Pelt, PJF Quaedvlieg, BA Essers, PM Steijlen, NWJ Kelleners-Smeets, K. Mosterd, "Topical ingenol mebutate versus 5% 5-fluorouracil versus 5% imiquimod versus photodynamic therapy in the treatment of actinic keratosis: a multi-center randomized controlled trial", *submitted*

R.C. Beljaars, W. Bergman, H.A.M. Neumann, 2014, Handboek dermato-oncologie, **JPHM Kessels**, GAM Krekels: "Huidkanker en UV protectie" (chapter)

Grant

ZonMw grant, Goed Gebruik Geneesmiddelen, 2014. *Project:* " Topical Ingenol mebutate versus 5% 5-fluorouracil versus 5% Imiquimod versus Photodynamic therapy in the treatment of actinic keratosis: a multi-center randomized efficacy and cost-effectiveness study (AKTI trial), €319.859,-

Oral and poster presentations

Wat is nieuw in PDT en field-cancerization. *Oral presentation*. DIS symposium, Amsterdam, 2012

Pulse dye laser illumination for PDT. *Oral presentation*. VBEAM expert and user meeting, Dalton Medical, Amsterdam, 2012.

PDL in childhood hemangioma. *Oral presentation*. Laser Innsbruck congress, 2012

Erbium:YAG to treat lentigo maligna. *Oral presentation*. Laser Innsbruck congress, 2012

Laser mediated photodynamic therapy for actinic keratosis. *Oral presentation*. Euro-PDT conference, Madrid, 2013

Een onuitsprekelijke tumor: blastair plasmacytoid dendritische cel neoplasma. *Oral presentation*. Regional evening seminar Dermatology, Maastricht, 2013

Laser-mediated photodynamic therapy for actinic keratosis. *Poster presentation*. NVED congress, Lunteren, 2014

Een misplaatste afwijking? Leg type lymfoom. *Oral presentation*. Regional evening seminar Dermatology, Maastricht 2014

Door de ooievaar gebracht, vaatafwijkingen bij pasgeborenen. *Oral presentation*. Regional evening seminar Dermatology, Maastricht 2015

Topical sinecatechin ointment in the treatment of superficial basal cell carcinoma. *Poster presentation.* NVED congress, Lunteren 2017

Treatment of superficial basal cell carcinoma by topical photodynamic therapy with fractionated 5-aminolevulinic acid 20% versus two stage topical methylaminolevulate. *Poster presentation*. Euro-PDT conference, Munchen, 2017

Met de spijker op de kop, hobnail hemangioma. *Oral presentation*. Regional evening seminar Dermatology, Maastricht 2017

Fractionated 5-ALA PDT versus conventional MAL-PDT for sBCC, *Oral presentation*, Euro-PDT conference, Nice, 2018

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Cover

Op de cover ziet u een ruwe diamant. Aan de bovenzijde is een brede lichtbundel te zien en aan de onderzijde een smallere schaduw. De achterliggende gedachte bij deze cover is dat de ruwe – grove – diamant de aandoeningen weerspiegelt die dit proefschrift behandelt: actinische keratose en het superficieel basaalcelcarcinoom. Actinische keratose is een huidaandoening die zich presenteert als plekken met ruw aanvoelende schilfers. Bij het basaalcelcarcinoom worden vaak vertakkende bloedvaatjes gezien, welke in de diamant terug te zien zijn als 'cracks'. Voor de oplettende kijker kan er ook een groen blad gezien worden, refererend aan de studie die wij verricht hebben naar groene thee zalf bij het basaalcelcarcinoom.

Zowel het basaalcelcarcinoom als actinische keratose ontstaan door zonschade. In dit proefschrift worden meerdere niet-invasieve behandelingen besproken voor beide aandoeningen. Eén van die behandelingen betreft photodynamische therapie (PDT), ook wel licht therapie genoemd. De lichtbundel aan de bovenzijde van de diamant weerspiegelt enerzijds het zonlicht en anderzijds de lichtbundel van PDT. In de conclusie van dit proefschrift wordt benadrukt dat er op de eerste plaats naar de meest (kosten)effectieve behandeling voor deze huidaandoeningen gekeken moet worden, maar dat niet iedere behandeling geschikt is voor de individuele patiënt. Dit wordt op de cover weergegeven als een smalle schaduw aan de onderzijde van de diamant. De gebroken stukjes diamant op de achterzijde van de cover weerspiegelen de diversiteit aan patiënten en behandelindicaties.