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Diagnosis and treatment of basal cell carcinoma

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DIAGNOSIS AND TREATMENT OF BASAL CELL CARCINOMA

Marieke H. Roozeboom

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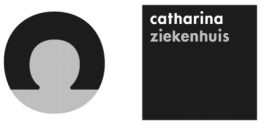

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Diagnosis and treatment of basal cell carcinoma

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CHAPTER 1

General introduction

Skin cancer

Skin cancer is the most common cancer amongst Caucasians.[1] It can be divided into three major types, from least to most aggressive: basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and melanoma. BCC and SCC, the non-melanoma skin cancers, are the most common skin cancers and can usually be well cured.[2,3] Melanoma is the least common, but most severe type that is capable of metastasizing and can lead to death.[4]

BCC accounts for approximately 80% of non-melanoma skin cancers and arises within sun-damaged skin.[5] This tumor is a slowly growing, malignant neoplasm of keratinocytes that resides within the basal layer of the epidermis. It is presumed that it develops from epidermal stem cells of the outer root sheath of the hair follicle.[6] Nevertheless, the precise origin of BCC is yet unknown. Despite its indolent nature and low mortality rate, BCC left untreated or inadequately treated can cause substantial morbidity by tissue destruction and cosmetic disfigurement, especially in the chronic sun-exposed head and neck region.[5]

All types of BCC show increasing incidence rates worldwide including The Netherlands.[7,8] The largest increase has been observed for the histological superficial subtype. In 1991, superficial basal cell carcinoma (sBCC) constituted 17.6% of all BCC but this proportion increased to 30.7 in 2007.[9] During the last decades, the substantial rise in incidence of sBCC has been accompanied by increasing use of non-invasive treatment options for this subtype. These trends require evaluation of diagnostic and treatment strategies for optimal management of sBCC, which will be the focus of this thesis.

Epidemiology

BCC incidence rates are difficult to determine as only a few cancer registries collect BCC information. In addition, if there is a registration, only the first BCC in a patient is usually registered.[10] Thus, available incidence rates are definitely underestimating the occurrence of BCC. The incidence has risen dramatically last decades with 3-10% annually, without signs of a future plateau phase.[7,11] Although the nodular subtype still remains the most common variant (~40%), an increase in both superficial (~30%) and aggressive (~30%) subtypes has been observed over the last decades.[9] Based on the obtainable data, there is a marked geographical variation in incidence rates worldwide, with the highest

incidence in Australia.[7] The closer Caucasians live to the equator, the higher the risk of developing a BCC. A decade ago, De Vries et al. predicted that the European Standardized Rate (ESR) for BCC in The Netherlands would increase from 92 per 100,000 person-years for men in 2005 to 122 per 100,000 in 2015.[12] The ESR in women was predicted to increase from 79 to 119. However, an unexpected acceleration in the ESR took place. In 2009, the ESR was already 164 per 100,000 person-years for men and 157 for women.[13] More recent predictions for The Netherlands by Flohil et al. expect that the ESR will be 234 per 100,000 person-years for men in 2020 and 226 for women.[14]

BCC was formerly a disease of the elderly but nowadays a larger number of young people are also affected as a result of recreational sun exposure.[15] This increase is regardless of growing public awareness campaigns targeting the harmful effects of ultraviolet (UV) exposure. The incidence is higher in males than in females with a ratio of 2:1. However, it is remarkable that in the younger population (< 40 years) women are more affected than men.[16-19] Currently, one in every 5-6 individuals will develop a BCC during their life.[14] In addition, persons with a history of BCC are at an increased risk of developing subsequent lesions.[20,21] A meta-analysis found a 29% risk of developing a second BCC after the first tumor.[22] This finding suggests a genetic predisposition besides the presence of other risk factors. These alarming incidence trends warrant the optimization and development of diagnostic tools and treatment options to reduce the burden on health care services.

Risk factors

Many Caucasians have a Fitzpatrick skin type I or II (light skin, blond or red hair, light colored eyes), frequently caused by single nucleotide polymorphisms in the melanocortin 1 receptor (MC1R) gene.[17,19] Their light colored skin with low amounts of melanin makes them vulnerable to the carcinogenic effect of ultraviolet (UV) light exposure. UV light exposure is regarded as the major risk factor for BCC but the precise relationship remains obscure.[23] UVA is less carcinogenic than UVB. UVA (320-400 nm) penetrates deep into the dermis where it mainly causes a skin ageing effect. Its carcinogenic effect is 10,000 lower compared to UVB and results from photo-oxidative stress leading to DNA mutations.[24,25] UVB (290-320 nm) is mostly absorbed in the epidermis and directly damages both DNA and RNA. By the cumulative effect of every single UV-light exposure DNA repair mechanism will eventually fail and skin cancer develops.[26] Intermittent recreational sun exposure appears to be important in the development of sBCC,

while chronic sun exposure may be an etiologic factor in nodular (nBCC) and aggressive BCC (aBCC).[27] The recreational sun seeking behavior might explain the increasing incidence rates in younger population and women. UV light exposure can take place 20-50 years before the development of a BCC. Therefore, it is not surprising that an older age is a risk factor for BCC. Further risk factors are male gender, exposure to phototherapy, ionizing radiation, arsenic or coal tear, immunosuppressive therapy and genetic predisposition.[5,28]

Pathogenesis

The pathogenesis of BCC is complex and includes a disruption of the hedgehog (HH) signaling pathway and mutations in the tumor suppressor gene p53. The HH-pathway was initially discovered in *Drosophila melanogaster*. This pathway is important in embryonic development as it regulates cell growth, pattering and differentiation.[29] In adults, the pathway is usually turned off except in hair, skin and stem cells.[30] A human homologue of the Drosophila is the patched 1 (PTCH1) gene. The PTCH gene product is an inhibitor of the HH-pathway. PTCH1 is part of a receptor for the Sonic Hedgehog (SHH) protein, a protein involved in the embryonic development. When SHH binds to PTCH1, the transmembrane signaling protein smoothened (SMO) is released. SMO is a HH-pathway activator. It is responsible for transducing HH-signaling to downstream GLI-transcription factors, resulting in cell growth and differentiation of the embryo. In adults, the HH-pathway is normally turned off by the inhibited effect of PTCH1. However, the pathway may be turned on by mutations or deletions in PTCH1 or SMO. Sporadic BCC results from inactivating mutations in PTCH1 (90%) or activating mutations (10%) in SMO. If PTCH is unable to inhibit SMO or SMO is activated, downstream target genes are overexpressed and may result in the development of a sporadic BCC.[31] Germ-line mutations in the PTCH1 gene on chromosome 9q22 are associated with the development of the hereditary basal cell nevus syndrome (BCNS).[32]

UV-light exposure can cause DNA damage and mutations in the tumor-suppressor gene p53.[33] In sporadic BCC this mutation is found in approximately 50% of cases.[34] p53 is normally involved in many diverse cellular processes, such as cell-cycle arrest, DNA repair and apoptosis. Mutated p53 allows the proliferation of damaged abnormal cells by UV-light. Mutations of p53 are not specific for BCC and are found in almost all human cancers.

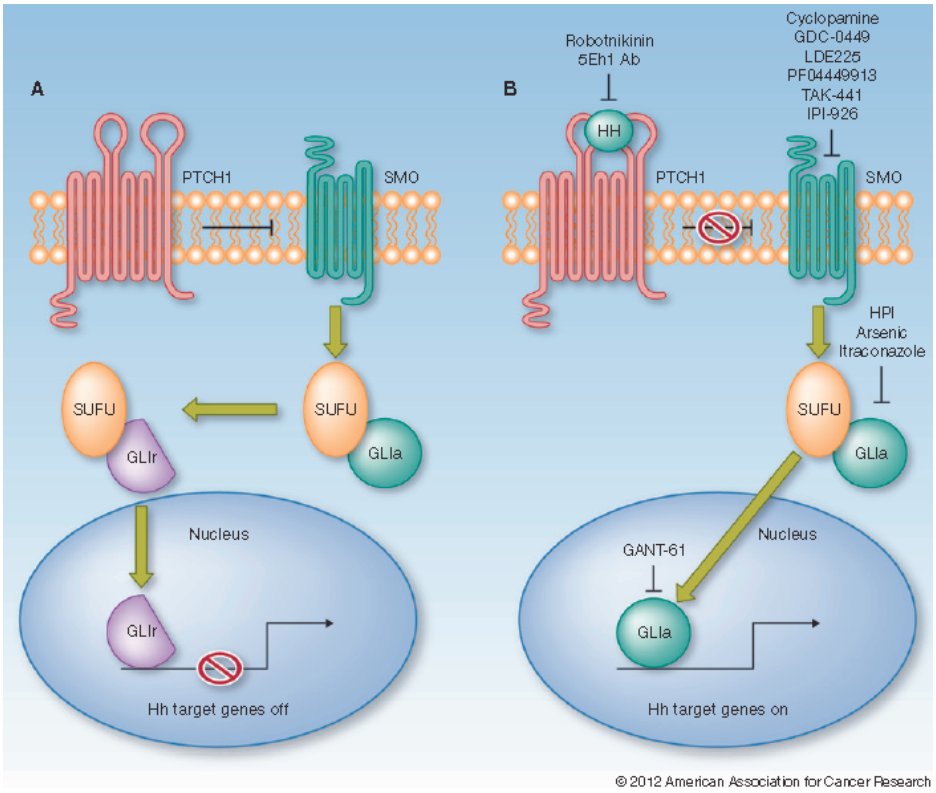

Fig. 1 The Hedgehog (HH) signaling pathway. Positive and negative regulatory components are depicted in green and red, respectively. **A** In the absence of HH ligand, Patched 1 (PTCH1) inhibits smoothened (SMO) allowing the GLI processing complex containing suppressor of fused (SUFU) to generate transcriptional repressors (GLIr). **B** HH ligand binding to PTCH depresses SMO and generates activated GLI factors (GLIa) that induce the expression of HH target genes. Clinical and preclinical inhibitors of pathway signaling are listed at their sites of pathway activity.
Reprinted with permission from the American Association for Cancer Research: McMillan, Molecular pathways: the hedgehog signaling pathway in cancer, Clin Cancer Res, 2012 Sep 15;18(18):4883-8. doi: 10.1158/1078-0432.CCR-11-2509.[35]

Clinical and histological presentation

The majority of BCC develop in the sun-exposed areas with 56% arising on the head/neck region, followed by the trunk and extremities.[9] However, BCC have also been reported in unusual sites like the genitalia and axillae.[36,37] BCC have several clinical features, corresponding with different histological subtypes. More than 20 histological subtypes have been described in the past, which made implications for clinical practice

difficult.[38] Therefore, a simplified and more practical classification is available in current international guidelines.[39,40] This classification distinguishes between non-aggressive BCC (superficial and nodular subtypes) and aggressive BCC.[41,42]

Superficial BCC are predominantly located on the trunk and manifest as an erythematous macule or thin plaque that can mimic actinic keratosis, Morbus Bowen, squamous cell carcinoma or eczema (Fig. 2a).[43] Atrophy, hypopigmentation and ulceration may be present. Dermatoscopy may be helpful to identify arborizing blood vessels, blue-grey ovoid nests or ulceration.[44] sBCC occur at a younger age than other BCC variants, particularly in women.[16,18] sBCC are histologically characterized by nests of basaloid cells that are attached to the epidermis and are confined to the papillary dermis (Fig. 3a). Tumor nests may be surrounded by fibrous stroma with a lymphocytic infiltrate and an increase in thin-walled vessels.[42,45]

Fig. 2 *Clinical presentation of BCC subtypes.* 2a. Superficial BCC on the back. 2b. Nodular BCC on the nose. 2c. Aggressive BCC on the angle of the eye.

Nodular BCC are predominantly located in the head and neck region.[27] nBCC generally occur in older people as a raised, translucent papule or nodule with teleangiectasias (Fig. 2b).[43] In large lesions, tissue destruction and ulceration may be present. nBCC are composed of round/oval nests of basaloid cells in the dermis, often with epidermal attachment (Fig. 3b). These nests differ in shape and size but are often large. The peripheral cells are in a palisade arrangement and artificial retraction between tumor nests and the surrounding stroma is often present.[42,45]

Aggressive BCC appear as flat, slightly atrophic macules or plaques without well-demarcated borders (Fig. 2c).[43] The actual tumor size is often larger than the clinically visual borders. This subtype is mainly located in the face and is red or whitish in color, sometimes with overlying telangiectasia.[18] The lesion is typically indurated and can be difficult to differentiate from a scar. aBCC consist of infiltrative / morpheaform, mi-

cronodular and basosquamous BCC. Infiltrative / morpheaform BCC present as narrow elongated basaloid strands of only a few cell layers thick that infiltrate between collagen bundles. The micronodular type resembles nBCC, but tumor nests are smaller, peripheral palisading is often absent and the subcutis can be infiltrated (Fig. 3c). Basosquamous BCC are composed of both basaloid and squamoid cells, are more aggressive and have metastatic potential.[42,45] The aggressive subtype is more destructive than the other types, because of its tendency to infiltrate more deeply and to grow with subclinical extensions.

BCC may consist of more than one histological subtype (mixed subtypes) within the same lesion. Mixed subtypes are present in 18% to 54% of BCC.[46,47]

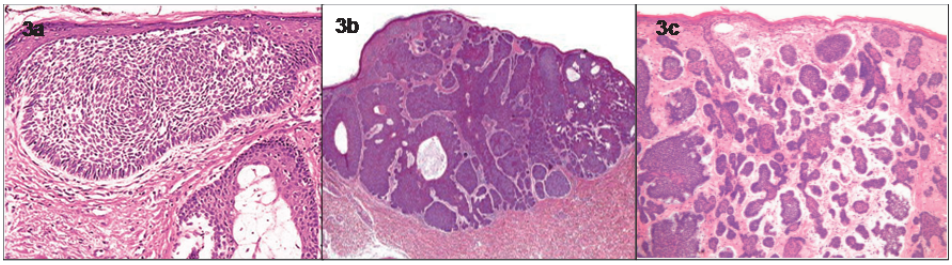

Fig. 3 *Histological presentation of BCC subtypes.* 3a. Superficial BCC. 3b. Nodular BCC. 3c. Aggressive (micronodular) BCC.

Diagnosis

Clinical

BCC are clinically well identifiable by dermatologists because of the typical clinical tumor features. Clinical aspects associated with a BCC diagnosis are the presence of pigmentation in 81%, telangiectasias in 65% and a pearly border in 67%.[48] Previous studies demonstrated that physicians were able to differentiate a BCC clinically from other skin diseases with a sensitivity of 64-89%.[49-51] The use of a dermatoscope improves the clinical diagnosis of BCC with a sensitivity of 97%, although significant differences in specificity and positive predictive values were found.[52,53] Dermatoscopy even enables the detection in an early stage, when the BCC is clinically still unremarkable. Several dermatoscopic criteria of BCC correspond with the histological subtype.[44,54] A new non-

invasive imaging technique called reflectance confocal microscopy is still thoroughly investigated as a diagnostic tool for BCC and its subtypes.[55]

Biopsy

Currently, international guidelines recommend a biopsy for all clinically suspected BCC in order to confirm the diagnosis and to identify the histopathological subtype.[39,40] Four types of skin biopsies are available: punch, shave, excision and incision biopsy.[56]
Amongst these, punch biopsies with a diameter of 3 mm are most commonly used in The Netherlands. Biopsies should be taken from the clinically most aggressive area in order to identify the most aggressive subtype. Several histopathological subtypes can be present in one tumor. This so called 'mixed histological BCC subtype' is present in 18-54% of lesions.[46,47,57] The majority of mixed subtypes contains an aggressive subtype.[58] Treatment choice will be primarily determined by the most aggressive histopathological BCC subtype that is present in the biopsy specimen. Although a punch biopsy is recommended in clinically suspected BCC, about 7% of BCC are not histologically proven prior to treatment.[59] These BCC are often suspected for a superficial growth pattern and are often located on the trunk. A reason to omit a punch biopsy before treatment might be that physicians have got a high confidence in their clinical diagnosis of the BCC subtype. Another reason for this trend of omitting punch biopsies might be the disadvantages of discomfort for the patient and the associated time and costs.[60]

Treatment

The primary goal of BCC treatment is tumor clearance. Treatment choice depends on several factors; patient characteristics (age, previous BCC treatments) and tumor characteristics (location, size and histological subtype seen on prior punch biopsy). In the growing (young) population with BCC, cosmetic results and health care costs are becoming more important. It might be difficult for the treating physician to deal with all these factors and the confusing mass of treatment options. There are only a few head-to- head comparison studies to guide physicians in the decision making of BCC treatment.[2] Many therapies are compared with different treatment regimens instead of a different therapy or studies have a short follow-up period. Treatment options can be divided into invasive and non-invasive modalities. Invasive treatments are surgical excision, Mohs' micrographic surgery (MMS), curettage and electrodessication/cautery and cryosurgery.[40]

Surgery remains the gold standard in treatment of BCC. The advantage of surgery is the histopathological examination to confirm tumor clearance. Non-invasive treatment modalities are radiotherapy, photodynamic therapy (PDT), imiquimod cream and 5-fluorouracil (5-FU) cream. These last three therapies are good alternatives for surgery in sBCC as the superficial growth pattern makes the tumor well accessible for PDT and creams. These treatments are increasingly used and have the advantage of less scarring, which is frequently seen after surgery. In addition, they can relieve the busy dermatology practice. However, treatment response has to be verified and treatment success rates are lower (73-83%) compared to surgery.[61-63] BCC rarely metastasizes and any residues or recurrences following non-invasive therapy can easily be retreated with surgery. Patients with metastatic or locally advanced BCC for whom surgery or radiation is not a therapeutic option, can be treated with vismodegib (a systemic inhibitor of the HH-pathway).[40]

Because the focus of this thesis is on surgical excision, PDT, imiquimod and 5-FU, these therapies will be further discussed below.

Surgical excision

Surgical excision is regarded as the gold standard for all three histological BCC subtypes: superficial, nodular and aggressive.[2,40,64] In The Netherlands, a clinical excision margin of 3 mm is required for primary BCC ≤ 10 mm and a 5 mm margin for BCC >10 mm, aggressive subtypes or recurrent tumors.[64] Surgical excision permits histologic assessment, although less than 1% of the excised tissue margins is being assessed.[65] Excision of BCC ≤ 20 mm with a 3 mm margin results in tumor clearance in 85% of cases. This percentage increases to 95% when a surgical margin of 4-5 mm is used.[66,67] Large BCC and aggressive subtypes require larger excision margins in order to obtain tumor clearance.

Photodynamic therapy

PDT causes tumor destruction by the use of a photosensitizer, light and oxygen. The first step in PDT is application of a photosensitizing cream on the tumor. Most commonly used photosensitizers are 5-aminolaevulinic acid (5-ALA) and the more lipophilic methylaminolevulinate (MAL). Both agents are normally present in all human cells and are a precursor in the haem biosynthesis pathway. 5-ALA and MAL can be converted into protoporphyrin IX (PpIX). This conversion occurs more rapidly in neoplastic cells compared to normal cells. Porphyrins are photoactive, fluorescing com-

pounds and, upon light activation of an appropriate wavelength in the presence of oxygen, singlet oxygen is formed which causes damage to cellular compartments, in particular the mitochondria (Fig. 4). Light activation of accumulated porphyrins leads to a photochemical reaction and thereby phototoxicity to the light-exposed target cells.[68,69]

World-wide, MAL is registered for the use of topical PDT in sBCC while 5-ALA is registered in the European Union for actinic keratosis only.[2,70] In The Netherlands, both fractionated 5-ALA 20% and MAL-PDT in 2 sessions are used as treatment for sBCC. MAL has the theoretical benefit of being more and faster absorbed in the cell than 5-ALA 20% and, should therefor generate a higher production of PpIX. In addition, MAL has higher selectivity for tumor cells, inducing fewer side-effects in normal tissue.[71,72]

The MAL-PDT protocol recommends application of the cream for 3 hours, after which the area is illuminated with a LED light source (wavelength of 570-670 nm and a total light dose of 75 J/cm^2). After one week, the same procedure is repeated. PDT is a hospital administered treatment with a good cosmetic result compared to surgery.[63] A disadvantage of PDT is the unpredictable burning pain sensation that might influence completing the treatment.[73]

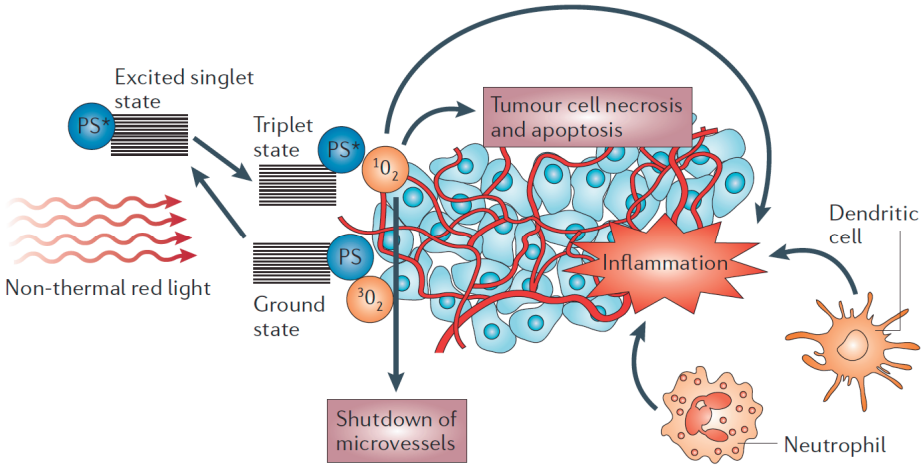

Fig. 4 Mechanism of photodynamic therapy (singlet oxygen, 1O2; triplet oxygen, 3O2; PS, photosensitizer). Reprinted by permission from Macmillan Publishers Ltd: Nat Rev Cancer [68], copyright (2006).

Imiquimod

Imiquimod 5% cream (Aldara®, Meda Pharmaceuticals) is an immune response modifier that is approved since 2004 by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of small, primary sBCC on low risk sites in immune competent adults. Although the exact working mechanism is not fully understood, previous studies have suggested that imiquimod has antiviral and antitumor potentials by activating both the innate and acquired immune system. Binding of imiquimod to Toll-like receptors 7 and 8 on antigen presenting cells induces activation of the central transcription factor nuclear factor-κB. As a result, pro-inflammatory cytokines, chemokines and other mediators are released. The induction of IFN-α is mainly responsible for the innate immune response. In addition, imiquimod also stimulates the innate response by stimulation of natural killer cells. The cellular arm of the acquired immune response is indirectly activated by production of the Th1 cytokine IFN-γ by naïve T-cells. Imiquimod can suppress the humoral arm of the acquired immune response by inhibition of Th2 cytokines (IL-4, IL-5). An additional effect of imiquimod is the activation and migration of Langerhans cells from the epidermis to the regional lymph nodes, resulting in antigen presentation to T-cells.[74,75] A recent study revealed that imiquimod also acts as a potent inhibitor of the oncogenic Hedgehog/GLI signaling via an unexpected signaling route involving adenosine receptors and protein kinase A.[76]

The currently approved imiquimod treatment regimen for sBCC in the EU and USA is once daily, five times a week for a period of 6 weeks. The cream has to be applied on the tumor and 5-10 mm of the surrounding area. Side effects usually develop in the last weeks of treatment and include application side and skin reaction: burning, itching, pain, erythema, erosions, ulceration and edema.[77] Interestingly, an increase in severity of skin reactions is associated with a better tumor clearance.[78] As imiquimod is an immunomodulator, flu-like symptoms such as fever, fatigue and muscle plain can develop during treatment. All side effects are generally mild and resolve after medication is withdrawn.

5-fluorouracil

5% 5-FU (Efudix® or Efudex®, Meda Pharmaceuticals) cream was the first topical therapy approved by the U.S. FDA for the treatment of sBCC. It is a pyrimidine analogue that is intracellulary converted into three metabolites: fluorodeoxyuridine monophosphate, fluorodeoxyuridine triphosphate and fluorouridine triphosphate.[79] These metabolites

can interfere with DNA and RNA synthesis. DNA synthesis is also blocked by inhibiting thymidylate synthetase (TS), a nucleoside required for DNA replication. TS methylates deoxyuridine monophosphate (dUMP) to form the cytotoxic thymidine monophosphate (dTMP). The imbalance between dUMP and dTMP causes cell cycle arrest and apoptosis. Rapidly proliferating cells are most sensitive to this cytotoxic effect, while normal cells are minimally penetrated by 5-FU and are relatively resistant to its effect.[80]

Topical 5-FU should be applied twice daily up to 4 weeks as tolerated. Patients have to apply a thin layer of cream on the tumor and 5-10 mm of surrounding tissue. Like imiquimod, 5-FU can cause burning, itching, pain, erythema, erosions, ulceration and edema. However, the side effects are more predictable as the treatment depends on the presence of tumor cells and not on the body's ability to mount an immune response.[81]

Prognosis

The prognosis of most patients with BCC is excellent as these tumors grow slowly and rarely metastasize. However, BCC can cause a significant morbidity due to local tissue destruction and cosmetic disfigurement by tumor invasion of skin, nerves, muscles and bones. BCC can be divided into 'low' and 'high' risk tumors based on their ability to recur (Table 1). High risk tumors include aggressive subtypes, localization in the H-zone of the face, tumor diameter > 2 cm and recurrent BCC.[64] These are all risk factors for recurrence following surgery. Little is known about possible predictors of treatment failure after non-invasive therapies in BCC. Knowledge of treatment effectiveness in subgroups of patients and tumors would be of great value in clinical practice as these non-invasive treatments are used on a large scale. Defining clinical or histological predictors for tumor residue or recurrence is of importance to select the most effective treatment for an individual patient with BCC. Six non-comparative studies (4 on PDT, 2 on imiquimod) have investigated patient and tumor characteristics as possible determinants of treatment failure in BCC.[78,82-86] The results on possible determinants such as gender, age, tumor size, tumor localization and tumor thickness are contradictory.

Table 1. Factors associated with BCC recurrence.[64]

	Low risk	High risk
Histological growth pattern	Superficial, nodular	Aggressive
Localization	Trunk	H-zone (eyes, ears, lips, nose)
Size	< 2 cm	> 2 cm
Previous treatment	Primary	Recurrent

Follow-up

Follow-up of patients with BCC are highly considered in order to evaluate treatment effects, detect local recurrences and detect new skin cancers.[86] Most local recurrences occur within two years post treatment, although recurrences have been described after 10 years.[4,86,87] About 30% of patients with one BCC subsequently develop another primary BCC within 5 years.[17] Patients with a BCC also have a higher risk on developing a SCC or melanoma, 3 and 2.5 times, respectively.[18]

There is no international consensus on frequency or duration of follow-up of patients with BCC: regimens range from annual visits for at least 3 years up to lifelong.[36,37,88] The Dutch BCC guideline recommends annual follow-up visits of patients with high risk BCC or local recurrent BCC in the face.[63] The guideline states that for some patients a more frequent follow-up scheme might be required. However, exact frequencies or total duration of follow-up is unclear.

Objectives of this thesis

The aim of this thesis was to contribute to the improvement of diagnosis and treatment of BCC. First, the punch biopsy as diagnostic tool for detecting the most aggressive BCC subtype will be discussed. Second, treatment modalities for superficial and nodular subtypes are evaluated. In addition, determinants of non-invasive treatment failure will be discussed. The following questions are answered in this thesis:

Diagnosis of BCC

What is the agreement between histological BCC subtype on punch biopsy and the subsequent surgical excision of primary BCC? (Chapter 2.1)

Histological confirmation of a clinically suspected BCC by biopsy is recommended before treatment. Treatment choice is among others based on the most aggressive histopathological subtype on punch biopsy. Histological misdiagnosis may result in over- or undertreatment. A shave biopsy of the tumor surface can identify the correct subtype in 76-81% of primary BCC. We hypothesized that the deeper punch biopsy is a better diagnostic tool to predict BCC subtype. To confirm this hypothesis, we investigated the agreement between histological subtype on punch biopsy and the subsequent surgical

excision in primary BCC. In addition, the proportion of BCC in which punch biopsy enables identification of the most aggressive subtype was evaluated.

What is the diagnostic accuracy of clinical assessment of BCC subtype compared to histological diagnosis on punch biopsy? (Chapter 2.2)
Although a punch biopsy is recommended before treatment in European guidelines, some experts argue that omitting a biopsy might be acceptable or even preferable in some cases. Disadvantages of a punch biopsy are discomfort for the patient and the associated time and costs for the physician. In contrast, clinical diagnosis is a painless, time- and possibly money-saving procedure. We compared the diagnostic accuracy of clinical assessment and histological diagnosis by punch biopsy for subtyping of BCC. Furthermore, we evaluated the impact of omitting the punch biopsy on treatment recommendations.

Treatment of BCC

What is the effectiveness of the most frequently used treatments for sBCC? (Chapter 3.1)
An increase in the superficial BCC subtype has been observed the last decades. Although surgical excision is still recommended as first choice treatment in sBCC, non-surgical therapies such as PDT, imiquimod and 5-FU are alternatives with a better cosmetic outcome and lower costs compared to surgery. However, there is no consensus on the treatment of sBCC. We systematically reviewed the literature in order to compare success probabilities for primary sBCC treated with frequently used therapies. Results were combined in a meta-analysis in order to obtain more reliable results.

Is the effectiveness of imiquimod and 5-FU comparable to that of MAL-PDT in the treatment of primary sBCC? (Chapter 3.2)
PDT is the most investigated non-invasive treatment for sBCC, with treatment success rates ranging between 60-90%. We already know that imiquimod and 5-FU are non-inferior at one year post treatment. We wonder whether this is still true three years post treatment. A non-inferiority, multicenter, randomized controlled trial was performed to compare the efficacy of these three therapies.

Do patient and tumor characteristics influence treatment response in sBCC treated by MAL-PDT or imiquimod? (Chapter 3.3)

As PDT and imiquimod have a different pharmacological working mechanism, treatment response may depend on certain patient and tumor characteristics. Identification of subgroups of patients that differ in treatment response is very valuable in order to select the most effective treatment in the individual patient with sBCC. We therefore explored whether the relative treatment effect of MAL-PDT and imiquimod is consistent across subgroups defined by patient and tumor characteristics.

Are tumor thickness and adnexal extension of sBCC determinants of non-invasive treatment failure? (Chapter 3.4)

sBCC grow continuously with the epidermis but tumor nests can reach within the papillary dermis or grow deep along hair follicles. We hypothesized that thick sBCC and tumors with adnexal extension fail to respond to the superficial working mechanisms of PDT, imiquimod and 5-FU. To support this hypothesis, we compared histopathological slides of sBCC with and without treatment failure one year after non-invasive therapies.

What is the effectiveness of surgical excision in nBCC at the long-term and how is tumor thickness related to PDT treatment failure? (Chapter 3.5)

Although surgical excision has proven to be more effective than PDT in nBCC after five years of follow-up, long-term follow-up studies are lacking. However, they could be valuable as surgically excised BCC can recur many years after treatment. International guidelines recommend surgical excision as first choice treatment in nBCC and PDT as alternative in thin nBCC. However, it is still debatable what exact tumor thickness is appropriate for PDT treatment. We hypothesized that only extremely thin nBCC are suitable for PDT. We performed a prospective, randomized controlled trial with at least five year follow-up on fractionated 5-ALA-PDT following partial debulking versus surgical excision in nBCC.

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CHAPTER 2

Diagnosis of basal cell carcinoma

CHAPTER 2.1

Agreement between histological subtype on punch biopsy and surgical excision in primary basal cell carcinoma

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Abstract

Background: Diagnosis of clinically suspected basal cell carcinoma (BCC) by histological confirmation by punch biopsy is recommended before treatment. Even shave biopsy has been proposed as useful to predict the correct subtype in primary BCC in 76-81%, whereas the agreement between histological BCC subtype on punch biopsy and subsequent excision specimens in recurrent BCC is 67.1%. However, no large studies on the agreement between histological BCC subtype seen on punch biopsy and the following surgical excision are performed in primary BCC.

Objectives: The aims of this study were (1) to establish the agreement between histological BCC subtype on punch biopsy and the subsequent surgical excision of primary BCC and; (2) to investigate the proportion of primary BCC in which punch biopsy enables identification of the most aggressive growth pattern.

Methods: Retrospective analyses of 243 primary BCC with both punch biopsy and subsequent surgical excision. Analyses were based on the most aggressive histological subtype of the tumor.

Results: The agreement between BCC subtype on punch biopsy and the subsequent surgical excision of primary BCC was 60.9%. A punch biopsy can predict the most aggressive growth pattern of primary BCC in 84.4%. Seventy-four percent of all primary BCC consisted of more than one histological subtype.

Conclusions: Dermatologists and other physicians have to be aware of the limited diagnostic value of a punch biopsy to determine the histological BCC subtype of the whole lesion. Misdiagnosis of the subtype will lead to undertreatment in one out of six primary BCC.

Introduction

Basal cell carcinoma (BCC) is a major problem in Caucasians worldwide. Incidence rises at an alarming rate by 3-10% annually.[1,2] One in six persons will develop a BCC during their life and this puts a heavy burden on health care systems.[3,4] Treatment of BCC is based on the histopathological BCC subtype, its location on the body and whether it concerns a primary or recurrent BCC.[5,6]

Three histopathological subtypes can be distinguished for determining treatment: superficial (sBCC), nodular (nBCC) and aggressive BCC (aBCC).[6] The subgroup aBCC contains the infiltrative / morpheaform, micronodular and basosquamous BCC subtypes.[7] The first choice treatment for all BCC is surgical excision.[5,6] However, sBCC in low-risk areas can also be treated by non-invasive modalities like photodynamic therapy, imiquimod or 5-fluorouracil. For nBCC and aBCC surgical excision is the most used treatment modality with a 3 mm margin or a 5 mm margin, respectively.[8] BCC located in cosmetically sensitive areas and recurrent facial BCC, require Mohs' micrographic surgery (MMS).[8,9]

To guide decisions on optimal treatment, accurate diagnosis and treatment of histological BCC subtype is essential. Prior to treatment, clinically suspected BCC are biopsied for histological diagnosis with a punch, shave or incision biopsy.[8] The goal of the biopsy is twofold; to confirm the diagnosis and to determine the histopathological BCC subtype.

Prior to treatment, punch and shave biopsies have been proposed to confirm the clinical BCC diagnosis and to determine the histopathological BCC subtype.[8] The most aggressive histological BCC subtype that is identified by biopsy will be an important determinant of treatment choice. Detecting the most aggressive subtype can be difficult as 18-49% of BCC consist of more than one subtype.[7-9] Failure to detect aggressive subtypes may result in undertreatment. For this reason, it is important to know in what proportion of patients a biopsy enables identification of the most aggressive histological subtype of BCC. Three previous studies, one on punch biopsies in recurrent BCC and two on primary BCC that were mostly biopsied by a shave biopsy, have suggested that the agreement between histological subtype on biopsy and the subsequent surgical excision is limited.[10-12] The aim of this study is to evaluate the agreement between punch biopsy and excision on the most aggressive histological BCC subtype in primary BCC and, thereby, determine the true utility of punch biopsies in the pre-surgical planning.

Materials and methods

In this retrospective study we analyzed the agreement between histological BCC subtype and most aggressive component on punch biopsy and the subsequent surgical excision. Eligible were patients aged 18 years or older, attending the department of Dermatology at the Maastricht University Medical Centre, The Netherlands, between July 1st and December 31st 2009, and who underwent both a punch biopsy and a subsequent surgical excision of a histological proven primary BCC. Histological data were retrieved from the PALGA database, a national pathology registration database. Exclusion criteria were shave biopsy, BCC excised without prior punch biopsy, biopsied BCC without following excision, patients with genetic skin cancer disorders and recurrent BCC.

Punch biopsies were taken in all BCC from the clinically most suspected area for thick and infiltrative growth and had a diameter of 3-4 mm. Surgical excision was performed under local anesthesia with a standard clinical safety margin of 3 mm in sBCC and nBCC, while aBCC were excised with a 5 mm margin. In case a tumor was located in a cosmetically sensitive area, MMS was performed.[13] MMS specimens were excluded from further analysis because BCC subtype can only be judged at the surgical margins and not in the central tumor part.

Data were collected and the following characteristics were registered: gender, age, tumor location, tumor diameter, histological BCC subtype on punch biopsy and surgery specimen, and type of surgery (surgical excision or MMS). Based on the most aggressive subtype noted on histology, all BCC were divided into three subgroups, namely sBCC, nBCC and aBCC. Infiltrative / morpheaform, micronodular and basosquamous BCC are categorized as aBCC.[6,7] Histological subtype of BCC was judged by different pathologists according to standard defined histopathological features.[7,14-16]

Mixed histological subtypes were defined as tumors with lack of agreement between the punch biopsy and the excision specimen on histological subtype or presence of more than one subtype on either punch biopsy or excision. A basic assumption underlying the analysis is that the most aggressive histological subtype of the tumor, seen on either punch biopsy or surgical excision, defines the definite histological subtype of the tumor. This means that, on the one hand, if the punch biopsy specimen identifies an aggressive subtype that is not observed by excision specimen, we assume that the aggressive component of the tumor was removed by biopsy. On the other hand, if the excision specimen identifies an aggressive subtype that is not observed on punch biopsy specimen, we assume that it was missed by the punch biopsy.

Statistical analysis

Descriptive results are given as number and percentages. BCC tumors are used as unit of analysis. Diagnoses of histological subtype by punch biopsy and excision were compared separately for all BCC tumors and for tumors composed of more than one subtype on histopathological examination (mixed histological subtypes). Proportions with concordant results were calculated as the proportion of tumors with the same diagnosis in punch biopsy and excision specimens. Proportions with discordant results were calculated as the proportion of tumors in which the diagnosis according to punch biopsy differed from that by surgical excision. With respect to discordant results, a distinction is made between cases where punch biopsy detected a less aggressive subtype than excision and vice versa. Data analyses were performed with SPSS version 18.0 (SPSS, Chicago, IL, USA).

Results

Patient characteristics

We included and analyzed 243 BCC of 191 patients (111 men, 80 women). Twenty-eight patients had two BCC, nine had three BCC and two had four BCC. The mean age was 70.1 years (range 36-93 years).

Tumor characteristics

BCC were most frequently located in the head/neck region (n=152, 62.6%). Sixty-one tumors (25.1%) were located on the trunk, 20 (8.2%) on the lower extremities and 10 (4.1%) on the upper extremities. The mean tumor diameter was 8.6 mm (range 2-30 mm). Seventy-two percent of BCC was 10 mm or smaller.

Histological subtypes

On punch biopsy, 61.7% of BCC was histologically nodular (n=150), 34.6% aggressive (n=84) and 3.7% (n=9) superficial. Surgical excision specimens demonstrated percentages of 45.7% (n=111), 38.7% (n=94) and 15.6% (n=38) for nodular, aggressive and superficial subtypes, respectively.

A total number of 180 BCC (74.1%) was composed of mixed histological subtypes on either punch biopsy or the surgical excision. Ten different mixed subtypes were observed. The three most common mixes were 'superficial/nodular' (n=65, 36.1%), 'nodular/micronodular' (n=29, 16.1%) and 'nodular/infiltrative' (n=23, 12.8%). Tumors with a single subtype were seen in 25.9% (63/243) of cases, of which nBCC was the most common one (n=53, 84.1%).

Agreement between punch biopsy and surgical excision of BCC subtype

The agreement between histological subtype seen on punch biopsy specimen and excision specimen was 60.9% (148/243 concordance cases, Table 1). The proportion of cases where punch biopsy detected a less aggressive subtype than surgical excision was 15.6% (38/243) and in 23.5% (57/243) punch biopsy detected a more aggressive subtype. Considering the assumption that the most aggressive histological subtype of the tumor, seen on either punch biopsy or surgical excision, defines the definite histological subtype of the tumor, it can be concluded that punch biopsy enables diagnosis of the most aggressive component in 84.4% (205/243).

The discrepancy between diagnosis on punch biopsy and excision has important consequences. In total, 120 (49.4%) BCC demonstrated an aggressive subtype either on punch biopsy or on the subsequent surgical excision. In 36 of the 120 (30%) aggressive BCC, aggressive growth was absent in the punch biopsy but present on surgical excision.

In the 180 mixed histology tumors, the most aggressive component present in the tumor was missed by punch biopsy in 21.1% (38/180) (Table 2).

Table 1. Histological diagnosis of the most aggressive BCC subtype on punch biopsy compared to surgical excision.

Biopsy	Surgical excision			
	Superficial BCC	Nodular BCC	Aggressive BCC	Total
Superficial BCC	**5 (2.1)**	2 (0.8)	2 (0.8)	9 (3.7)
Nodular BCC	31 (12.8)	**85 (35.0)**	34 (14.0)	150 (61.7)
Aggressive BCC	2 (0.8)	24 (9.9)	**58 (23.9)**	84 (34.6)
Total	38 (15.6)	111 (45.7)	94 (38.7)	243 (100)

Data are given as numbers (percentages). Bold numbers indicate concordant cases. BCC; basal cell carcinoma.

Table 2. Histology of the mixed BCC (n=180) on punch biopsy compared to surgical excision.

Biopsy	Surgical excision	Most aggressive subtype missed on biopsy	Most aggressive subtype missed on surgical excision
Superficial (4)	Nodular (1)	100% (4/4)	0% (0/4)
	Nodular + superficial (1)		
	Mixed with aggressive (2)		
Nodular (54)	Superficial (10)	48% (26/54)	19% (10/54)
	Nodular + superficial (18)		
	Aggressive (4)		
	Mixed with aggressive (22)		
Aggressive (31)	Superficial (1)	0% (0/31)	32% (10/31)
	Nodular (7)		
	Nodular + superficial (2)		
	Aggressive (4)		
	Mixed with aggressive (17)		
Nodular + superficial (43)	Superficial (21)	19% (8/43)	49% (21/43)
	Nodular (3)		
	Nodular + superficial (11)		
	Aggressive (2)		
	Mixed with aggressive (6)		
Mixed with aggressive (48)	Superficial (1)	0% (0/31)	35% (17/48)
	Nodular (10)		
	Nodular + superficial (6)		
	Aggressive (5)		
	Mixed with aggressive (26)		
Total (180)		21% (38/180)	32% (58/180)

Discussion

The proportion of punch biopsies that correctly predicts the most aggressive growth pattern of the entire BCC is 84.4%. As treatment choice of BCC is based on the most aggressive histological subtype seen on punch biopsy, this means that in one out of six BCC the most aggressive growth pattern is missed in an adequately taken punch biopsy. These tumors might not be properly treated and a higher percentage of re-excision or recurrences may occur.

Only three previous studies reported on the agreement between histological BCC subtype on biopsy and the subsequent surgical excision in BCC.[10-12] These studies included mainly shave biopsies in primary BCC or reported on punch biopsy in recurrent BCC. However, shave biopsy of the tumor surface might fail to identify BCC subtypes located deeper in the dermis. In this study, for the first time the agreement between the most aggressive histological subtype seen on punch biopsy and the subsequent surgical excision was examined in a large population with 243 primary BCC.

We found that a punch biopsy can identify the most aggressive growth pattern of primary BCC in 84.4%. A similar percentage (84.2%) was found by Mosterd et al. in recurrent BCC judged by one blinded dermatopathologist.[12] A slightly higher percentage (91.8%) was obtained when histopathological specimens were independently and blinded judged by only two dermatopathologists.[11]

The percentage of concordant cases of BCC subtype on punch biopsy compared to surgical excision specimens was 60.9%. This is in contrast to the agreement of 81% Russell et al. and 89% Haws et al. reported in the subgroup of primary BCC that received a punch biopsy (instead of a shave biopsy).[10,11] An explanation for our lower agreement might be the high percentage of mixed histological subtypes (74.1%) compared to 18-49% in other studies, that may have influenced results [7,12,17] In case of a mixed histological BCC, one subtype might be totally biopsied and is therefore not seen on surgical excision.

Another explanation might be the fact that verification bias is present due to the non-invasively treated sBCC. sBCC are normally treated non-surgically in our clinic. This group probably reflects a group were there was doubt about the superficial growth and was therefore excised. Furthermore, histological specimens were not judged by one or two blinded dermatopathologists. However, our study reflects daily clinical practice in which different pathologists, each with a slightly different reference frame of BCC subtypes, judge histological specimens. To improve the interobserver agreement between pathologists that evaluate BCC subtype, it is advisable that histopathological slides are judged in a standardized manner by dermatopathologists qualified in evaluating BCC subtypes.

To our knowledge this is to date the first study to determine the agreement between punch biopsy and surgical excision to predict the histological BCC subtype in a large population with primary BCC. The results of this study indicate that 3 and 4 mm punch biopsies are a generally fair diagnostic tool to detect the most aggressive growth pattern of the complete tumor. Treatment is based on the most aggressive subtype seen on punch biopsies and the chance of undertreatment is still present in one out of six BCC. Even when a punch biopsy is taken from the clinically most aggressive part, clinicians

have to be aware of this discrepancy. Therefore, we might also have to rely on the clinical diagnosis. In the past, several studies demonstrated that the clinical diagnosis of BCC is accurate in 70-89%.[18-21]

However, a correct clinical BCC diagnosis does not imply a correct clinical diagnosis of histological subtype. It would be interesting to know whether the clinical BCC subtype is as reliable as or even more reliable than the histological BCC subtype determined on a punch biopsy.

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CHAPTER 2.2

Subtyping basal cell carcinoma by clinical diagnosis versus punch biopsy

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Abstract

Background: Basal cell carcinoma (BCC) guidelines recommend a punch biopsy prior to treatment to identify the histological subtype for optimal treatment selection. However, clinical ability to differentiate between BCC subtypes has not been studied.

Objectives: To compare the diagnostic accuracy of clinical assessment and histological diagnosis by punch biopsy for subtyping BCC and to evaluate the impact of omitting the punch biopsy on treatment recommendations.

Methods: Sensitivity, specificity, positive predictive value and negative predictive value, with corresponding 95% confidence intervals were calculated for discrimination between (i) superficial versus nodular/aggressive BCC and (ii) aggressive versus nodular BCC. The gold standard was the histological subtype on the subsequent surgical excision.

Results: 152 histologically confirmed BCC were included. Detection of the most aggressive BCC subtype by punch biopsy was better than by clinical diagnosis. Omission of punch biopsy may result in overstaging of 1 in 4 superficial BCC and in understaging of 1 in 4 aggressive BCC.

Conclusions: Punch biopsy is more accurate in BCC subtyping than clinical diagnosis. Omitting a punch biopsy may lead to over- and understaging.

Introduction

International guidelines on diagnosis and treatment of basal cell carcinoma (BCC) recommend a punch biopsy in the majority of clinically suspected BCC prior to treatment. This is to confirm diagnosis and to identify the histological subtype (superficial, nodular, aggressive), which is necessary to know for optimal treatment selection.[1,2] A punch biopsy can detect the most aggressive subtype in 84-92% of cases, but has the disadvantages of discomfort for the patient and costs for the health care system.[3-5] In contrast, clinical diagnosis is a painless, and possibly money-saving procedure.[6] However, the difference in diagnostic accuracy of BCC subtyping between punch biopsy and clinical diagnosis has never been evaluated. This study compares the diagnostic accuracy of clinical assessment and histological diagnosis by punch biopsy for subtyping BCC. Furthermore, we evaluated the impact of omitting the punch biopsy on treatment recommendations.

Materials and methods

Eligible patients attending the outpatient department of Dermatology of the Maastricht University Medical Centre (MUMC) and the Erasmus Medical Centre Rotterdam (Erasmus MC), The Netherlands, were included between August 2011 and August 2012. Included were patients aged 18 years or older with a clinically suspected primary BCC that was histologically confirmed on surgical excision specimen. Exclusion criteria were: genetic skin cancer syndromes and use of immunosuppressive drugs. All patients gave written informed consent for participation. The trial was approved by the Medical Ethics and Scientific Committee of the MUMC.

Clinical diagnosis of the most aggressive BCC subtype was made by one of the dermatologists specialized in oncology (three at MUMC, two at Erasmus MC), based on the criteria by Crowson et al.[7] A distinction was made between superficial, nodular and aggressive BCC. Subsequently, a 3 mm punch biopsy was obtained from the clinically most aggressive tumor area. Superficial and nodular BCC were surgically excised with a 3 mm margin, aggressive BCC with 5 mm. Incompletely excised BCC were re-excised and Mohs' micrographic surgery was performed in facial high risk BCC.[8]

All biopsy and excision specimens were haematoxylin and eosin stained. Biopsies were (partially) cut in serial sections of 150 µm. Four serial section of 4-5 consecutive slices were made. Excision specimens were cut at 2 mm, completely imbedded and once slice per section was made. Histopathological slides were evaluated by two dermato-

pathologists, who were unaware of the diagnosis of the other pathologist and blinded to the clinical diagnosis. The most aggressive BCC subtype was recorded following histological criteria.[7,9] Aggressive BCC comprised infiltrative/morpheaform, micronodular and basosquamous BCC.

This study focused on the ability to discriminate clinically and histologically (by punch biopsy) between: i) superficial BCC vs. nodular/aggressive BCC and; ii) aggressive vs. nodular BCC. These distinctions were considered most relevant for optimal treatment selection, as superficial BCC can be treated non-invasively and aggressive BCC require a larger surgical margin than nodular BCC.[10] The primary outcomes were sensitivity and specificity of clinical assessment and histological diagnosis by punch biopsy. The gold standard for BCC subtyping was the histological subtype on the subsequent surgical excision. False positive and false negative results have an impact on treatment recommendations. False positive results are associated with overstaging: clinical diagnosis or punch biopsy classified a BCC as more aggressive than the histological diagnosis on subsequent surgical excision. False negative results are associated with understaging: clinical diagnosis or punch biopsy classified a BCC as less aggressive than the gold standard.

Diagnostic values with corresponding 95% confidence intervals were calculated for discrimination between (i) and (ii). Differences in proportions were tested using the McNemar test for paired proportions. P-values ≤ 0.05 were considered to indicate statistical significance. Statistical analyses were performed with SPSS-pc version 20.0 (SPSS, Chicago, IL, USA).

Results

Biopsies were performed in 285 clinically suspected primary BCC. A total of 191 BCC were histologically confirmed, 152 of which were in the 116 patients who agreed to participate (64 men, 52 women). Mean age was 68 years (range 33-92 years). Prevalence of superficial, nodular and aggressive BCC on surgical excision were 16.4% (25/152), 52.0% (79/152) and 31.6% (48/152), respectively (Table 1).

Superficial versus nodular/aggressive BCC

Table 2 shows the diagnostic parameters for discrimination between superficial vs. nodular/aggressive BCC. Sensitivity to detect nodular/aggressive BCC was similar for clinical diagnosis and punch biopsy (89.0% vs. 92.1%, p=0.38), but punch biopsy was more

specific than clinical diagnosis (88.0% vs. 64.0%, p=0.11); i.e. the percentage of superficial BCC that was falsely diagnosed as nodular/aggressive decreased from 36% to 12%. Thus, omitting a punch biopsy would have resulted in overstaging in an extra 24.0% of superficial BCC.

Table 1. Tumor characteristics of 152 basal cell carcinoma.

Characteristic	n (%)
Tumor localization	
Head/neck	79 (52)
Trunk	53 (35)
Upper extremities	12 (8)
Lower extremities	8 (5)
BCC subtype on surgical excision	
Superficial	25 (16)
Head/neck	1 (4)
Trunk	18 (72)
Upper extremities	5 (20)
Lower extremities	1 (4)
Nodular	79 (52)
Head/neck	41 (52)
Trunk	26 (33)
Upper extremities	6 (8)
Lower extremities	6 (8)
Aggressive	48 (32)
Head/neck	37 (77)
Trunk	9 (19)
Upper extremities	1 (2)
Lower extremities	1 (2)
Mean tumor diameter in mm (range)	8.6 (3-25)

Definition of abbreviations: BCC, basal cell carcinoma.

Table 2. Diagnostic parameters of clinical diagnosis and histological diagnosis by punch biopsy for detection of the most aggressive histological BCC subtype on surgical excision.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	OR (%)
Superficial vs. nodular/aggressive BCC					
Clinical diagnosis	89.0 (113/127) [0.85-0.92]	64.0 (16/25) [0.45-0.79]	92.6 (113/122) [0.89-0.96]	53.3 (16/30) [0.38-0.66]	14.4 [4.8-43.8]
Punch biopsy	92.1 (117/127) [0.89-0.94]	88.0 (22/25) [0.71-0.97]	97.5 (117/120) [0.94-0.99]	68.8 (22/32) [0.56-0.76]	85.8 [19.4-442.3]
Aggressive vs. nodular BCC					
Clinical diagnosis	56.3 (27/48) [0.45-0.67]	77.2 (61/79) [0.70-0.84]	60.0 (27/45) [0.47-0.71]	74.4 (61/82) [0.68-0.81]	4.4 [1.9-10.12]
Punch biopsy	85.4 (41/48) [0.75-0.93]	84.8 (67/79) [0.78-0.89]	77.4 (41/53) [0.68-0.84]	90.5 (67/74) [0.84-0.95]	32.7 [10.8-103.9]

Data in parentheses are numbers used to calculate the percentage and data in brackets are 95% confidence intervals. Definition of abbreviations: BCC, basal cell carcinoma; PPV, positive predictive value; NPV, negative predictive value; OR, odds ratio.

Aggressive versus nodular BCC

Punch biopsy is more sensitive (p=0.002) and more specific (p=0.29) for discrimination between aggressive and nodular BCC (Table 2) than clinical diagnosis. The proportion of aggressive BCC that was understaged as nodular or superficial was 43.8% (21/48) after clinical diagnosis and 14.6% (7/48) after punch biopsy. Thus, omission of a punch biopsy would have resulted in understaging of aggressive BCC in an extra 29.2% of cases.

ROC-curves

The ROC-curves showed a higher ability for punch biopsy than for clinical diagnosis for differentiating between superficial versus nodular/aggressive BCC (Fig. 1) and aggressive versus nodular BCC (Fig. 2).

We repeated the analyses with restriction to BCC on the trunk and extremities. These analyses showed similar results.

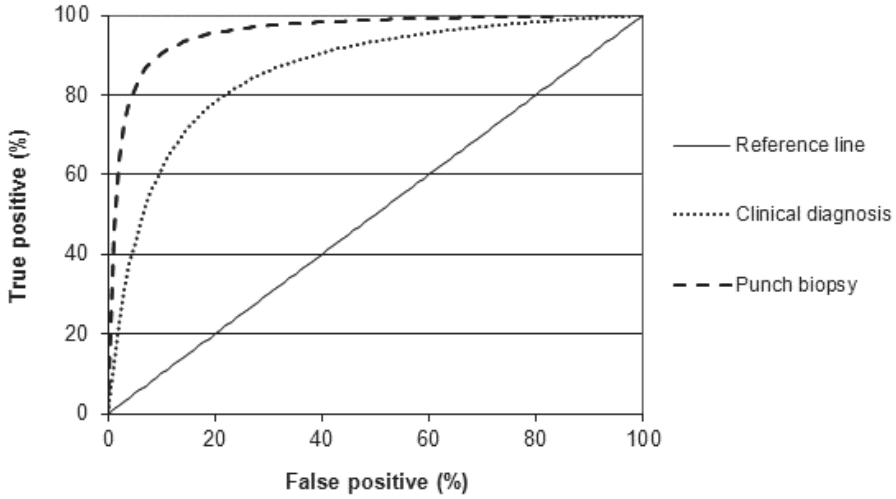

Fig. 1 The ability of clinical diagnosis and the histological diagnosis on punch biopsy to discriminate between superficial and nodular/aggressive basal cell carcinoma.

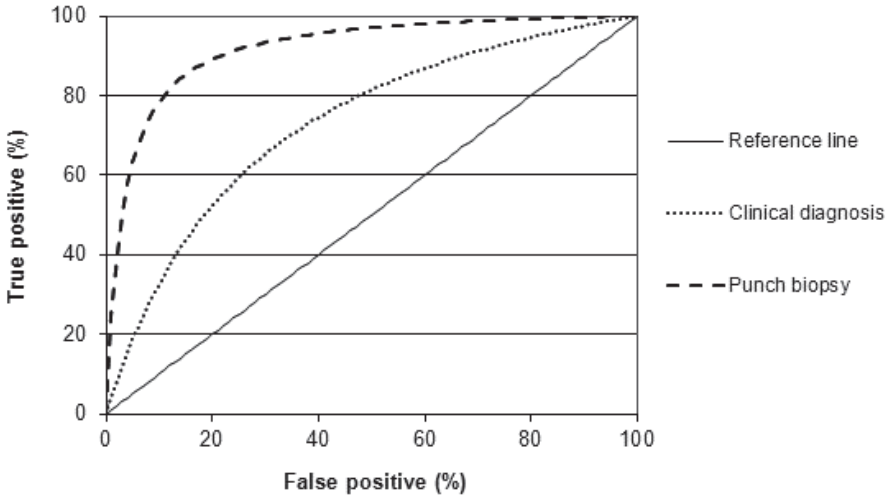

Fig. 2 The ability of clinical diagnosis and the histological diagnosis on punch biopsy to discriminate between aggressive and nodular basal cell carcinoma.

Discussion

These findings indicate that a punch biopsy is a better diagnostic tool than the clinical diagnosis for detection of the histological BCC subtype, i.e. essentially in line with international guidelines.[1,2] However, some experts argue that omitting a biopsy might be acceptable or preferred in some cases.[1,2,6] We showed that, when a punch biopsy is omitted, there is a risk of overstaging superficial BCC as more aggressive in an extra 24% of cases. In such a case, the physician will probably advise surgical excision and deny the patient the choice of less invasive alternatives (photodynamic therapy, imiquimod or 5-fluorouracil). Superficial BCC comprises approximately 30% of the total BCC population.[11] Thus, if treatment is based on clinical diagnosis, only a small minority (7%) of all patients with BCC would receive a more invasive therapy than strictly required.

Another consequence of omitting a punch biopsy is a significantly increased risk of understaging an aggressive BCC as nodular BCC in approximately a quarter of cases. These patients run the risk of having their tumor excised with too small margins, resulting in a re-excision or a recurrence.[12]

Considering these findings, it may be justified that physicians choose to omit the punch biopsy if they have high confidence in their diagnosis on the subtype of BCC, especially when using a dermatoscope.[13] Histological confirmation by punch biopsy might then be reserved for diagnoses that are made with less confidence and also for BCC in the head/neck region because recurrences in this area are not retreated that easily and can cause great morbidity. Nevertheless, the consequences of omitting a punch biopsy need to be discussed with the patient.

A limitation of the study is that patients with superficial BCC who preferred non-invasive therapies did not participate. For this reason, the absolute estimates of sensitivity and specificity (for both clinical diagnosis and punch biopsy) may be subject to verification bias, which results in overestimation of sensitivity and underestimation of specificity.[14] Secondly, some results lack statistical significance, probably due to the relatively small number of superficial BCC in this study. Thirdly, the level of confidence in the clinical diagnosis of BCC subtype was not recorded and, therefore, it was not possible to evaluate the level of over- and understaging in case of highly confident diagnoses.

In summary, punch biopsy is more accurate in BCC subtyping than clinical diagnosis. The impact of over- or understaging on treatment recommendations must be weighed against extra time, cost and patient discomfort associated with a punch biopsy.

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CHAPTER 3

Treatment of basal cell carcinoma

CHAPTER 3.1

Overall treatment success after treatment of primary superficial basal cell carcinoma: a systematic review and meta-analysis of randomized and non-randomized trials

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Abstract

Background: Several non-invasive treatment modalities are available for superficial basal cell carcinoma (sBCC).

Objectives: This systematic review aims to determine residue, recurrence and tumor-free survival probabilities of patients with primary sBCC treated with the currently most frequently used therapies.

Methods: The Pubmed (January 1946 to October 2010), EMBASE (January 1989 to October 2010), Cochrane (January 1993 to October 2010) databases, and reference lists were searched without date restriction. Inclusion criteria were studies that included primary, histologically proven sBCC, that reported on residue and/or recurrence probabilities after treatment, and had a minimum follow-up period of 12 weeks. Both randomized and non-randomized studies were included. The primary and secondary outcomes were the probability of complete response and tumor-free survival, respectively. Two independent reviewers selected 36 studies (14 randomized and 22 non-randomized), and extracted residue, cumulative recurrence and tumor-free survival probabilities.

Results: Pooled estimates of percentages of sBCC with complete response at 12 weeks post treatment, derived from 28 studies, were 86.2% (95% CI 82-90%) for imiquimod treatment, and 79.0% (95% CI 71-87%) for photodynamic therapy (PDT). With respect to tumor-free survival at one year, the pooled estimates derived from 23 studies were 87.3% for imiquimod (95% CI 84-91%) and 84.0% for PDT (95% CI 78-90%). Only a small number of studies reported on results of sBCC treatment with 5-fluorouracil (two), surgical excision (one) and cryotherapy (two).

Conclusions: Pooled estimates from randomized and non-randomized studies showed similar tumor-free survival at one year for imiquimod and PDT. The PDT tumor-free survival was higher in studies with repeated treatments. However, these results were largely derived from non-randomized studies, and randomized studies with head-to-head comparison of imiquimod and PDT are lacking. There is a need for head-to-head comparison studies between PDT, imiquimod and other treatments with long-term follow-up to enable better recommendations for optimal sBCC treatment.

Introduction

Basal cell carcinoma (BCC) is the most common skin cancer in the white population, with a remarkable increase in incidence of 3-10% annually.[1-3] Treatment of BCC is becoming a major health care problem, causing enormous health care costs and an increased workload for dermatologists and many other physicians. In the past it was a disease of elderly patients, but as a consequence of recreational sun exposure and tanning beds, a larger number of young people develop skin cancer as well.[4,5] People who develop one BCC are at a 10-fold increased risk of developing subsequent BCC at other body sites.[6,7] A study from The Netherlands in 2005 predicted that the number of patients with BCC would increase 78% in 2015.[1]

The histopathological diagnosis of BCC can be assessed by punch biopsy.[8] There are three major histological subtypes corresponding with several clinical manifestations. The most common subtype is the nodular BCC (40% of BCC cases). However, a rapid rise in the relative proportion of the superficial subtype of BCC has been observed last 20 years, with an increase from 18% to 31% of total cases.[9] Surgical excision remains the standard of care in most BCC because histopathological examination ensures tumor clearance. However, a good cosmetic outcome is becoming more important with the growing affected young population. Superficial BCC (sBCC) is the least aggressive subtype, requiring less destructive therapeutic options. Nowadays, sBCC can be treated by a variety of non-surgical techniques, such as photodynamic therapy (PDT), immunotherapy, local chemotherapeutic cream and cryotherapy.[8,10] These treatments avoid scarring, which is frequently seen after surgical excision. Furthermore, they can relieve the busy dermatologist's practice. However, there is no consensus on the treatment of sBCC. Which treatment the patient will receive relies on some tumor characteristics (localization and size) and patient characteristics (age, history of previous treatments and general health),[11] but mostly on the preference of the treating physician.[12] In addition, the cosmetic outcome, aftercare and costs should be taken into consideration.

The objective of this study was to systematically review published studies in order to compare the percentage of cases with complete response (primary outcome) and long-term tumor-free survival (secondary outcome) for patients with primary sBCC between the different frequently used treatments. Combined results from a meta-analysis result in a more reliable estimate than results from individual studies. Cosmetic outcome, aftercare and costs will not be the subject of this review.

Materials and methods

This systematic review was performed according to the PRISMA statement for systematic reviews and meta-analyses.[13] No online review protocol was made prior to this study.

Search

A systematic search for papers in the English language was performed in three well-established databases: Pubmed (January 1946 to October 2010), EMBASE (January 1989 to October 2010) and Cochrane Library (January 1993 to October 2010). PubMed is a free search engine for accessing the MEDLINE database of citations and abstracts of biomedical research articles. EMBASE is an abstract and indexing database also specialized in the biomedical field. The Cochrane Library is a collection of databases, and contains Cochrane reviews, which represent the highest level of evidence. The following search terms (including derivatives and analogues) were used: *carcinoma, basal cell, superficial* in combination with *therapy* using the following limits *randomized controlled trial, clinical trial* and *human.* There was no date restriction and the last search was run on the 1 October 2010. Further eligible publications were subsequently identified from the reference lists and were used for this systematic review.

Data sources and study selection

Eligible for review were studies which met the prespecified inclusion criteria. Studies had to report on treatment success of a modality for treatment of sBCC and had to include only patients with primary and histologically proven sBCC. Furthermore, studies had to have a follow-up period of at least 12 weeks after treatment. Data on the number of patients treated and the number of patients with treatment failure had to be available for at least one time point during follow-up. Because there was a limited number of randomized controlled trials (RCTs), both randomized and non-randomized studies were considered eligible, as well as prospective and retrospective studies, in order to include studies with results after long-term follow-up. We excluded case series, case reports, reviews, studies in which the recurrence probability of sBCC was not described separately from the other histological BCC subtypes, and studies in which results of two or more treatments were combined. Different papers by the same author or research group were included for review only when it was obvious that a different sample of patients was used. Two independent reviewers carefully screened titles and abstracts for

relevancy. If necessary, authors were contacted by email or telephone to obtain and confirm their data.

Data extraction

Two reviewers independently read the full text of the articles that were considered for inclusion using structured forms for extraction of relevant data. Any disagreements between reviewers were discussed with a third reviewer and further resolved by consensus. From studies which reported results of treatment of different BCC subtypes, only the subset of sBCC data was included. The outcomes that were considered of interest in this review were: probability of residual tumor/incomplete response, cumulative probability of recurrence, and probability of tumor-free survival (sustained clearance) at the end of follow-up. If any tumor tissue was present at the first control visit after treatment, the response to treatment was considered to be incomplete and the lesion was regarded as a residual tumor. Recurrence was defined as the presence of tumor tissue that was detected during follow-up in patients who had no residual tumor tissue. Tumor-free survival was defined as absence of both residual tumor and recurrence.

Information was extracted from individual studies regarding (i) treatment modality, including type, dose, frequency, duration, margin of cream application, illumination source and its tuning; (ii) data required for estimation of probability of residual tumor/incomplete response, cumulative probability of recurrence and probability of tumor-free survival at the end of follow-up; (iii) methodological aspects including sample size, selection of study population (such as restriction to tumors ≤ 2 or > 2 cm in diameter), study design (randomized vs. non-randomized), duration of follow-up, verification of residual tumor by clinical or a combined clinical and histopathological examination, use of time-to-event analysis (yes vs. no) , sponsored by pharmaceutical industry (yes vs. no), type of comparison (with placebo, other treatment or no treatment); (iv) distribution of patients' characteristics including age, comorbidities and tumor characteristics such as localization and size.

Risk of bias

To evaluate the methodological quality of the studies we scored eight items that are related to (i) representativeness of the study population, (ii) the description of the intervention, (iii) the evaluation of the clinical outcome, (iv) design-specific sources of bias, and (v) the analysis of data (Table 1).[14] The scores range from 0 to 8 with higher scores indicating better methodological quality and less potential for biased estimates.

Table 1. Items scored to evaluate methodological quality of non-randomized controlled trials.

Item	Score
1. Use of a representative study population without restriction to subgroups	Yes/no
2. Use of a well-described and standardized intervention	Yes/no
3. Evaluation of treatment response by histological verification	Yes/no
4. Definition of success as complete response (instead of partial response)	Yes/no
5. Prospective design	Yes/no
6. Inclusion of all treated patients in evaluation of long-term prognosis	Yes/no
7. Percentage lost to follow up	< 10% or ≥ 10%
8. Use of survival analysis	Yes/no

Non-randomized controlled trials were given a quality score ranging from 0 (poor) to 8 (excellent) points.

Statistical analysis

Percentages of patients with complete response to treatment were derived from the individual studies. The number of patients with no residual tumor at the first control visit after treatment was divided by the number of patients that were included for treatment, irrespective of whether patients were actually treated and/or finished treatment. Patients with adverse events (e.g. local skin reactions to imiquimod) were not considered to be treatment failures. This approach was chosen according to the intention-to-treat principle to prevent biased estimates due to selective loss to follow-up.

Valid estimation of the long-term risk of recurrence and tumor-free survival after treatment requires time-to-event analysis. If studies had not performed survival analysis, such as Kaplan-Meier analysis or life table analysis, efforts were made to extract the data required for life table analysis. The number of patients at risk at the start of each 1-year interval, as well as the number lost to follow-up and the number having recurrence during each interval, were used to calculate cumulative probabilities of sustained clearance. Only patients with complete response to treatment were considered for these analyses. The probability of tumor-free survival at the end of follow-up was calculated by multiplying the probability of complete response by the cumulative probability of sustained clearance. Standard errors were calculated using the Peto formula.[15]

For pooling, a random effects model as proposed by DerSimonian and Leird was performed using the inverse of the standard errors of the percentages from the individual studies as weights.[16] The I^2 index was used to test for heterogeneity between study results. The significance of this index indicates that differences between studies cannot solely be attributed to sampling variation, and that differences in study population, design and analysis are responsible for variation between study results. The I^2 index

ranges from 0 to 100%. Statistical heterogeneity was defined as a I^2 index > 50%.[17] Exploratory subgroup analyses were performed to identify sources of variation between study results. These subgroup analyses were not prespecified, but studies were categorized into subgroups according to several study characteristics that can potentially affect study results. Publication bias was examined statistically using the Egger's test.[18] All analyses were performed using STATA version 11.0 (STATA Corp, College Station, TX).

Results

The literature search identified 903 papers from which 36 studies fulfilled the inclusion criteria (Fig. 1). Two papers could not be obtained.[19,20] Included studies were conducted in the USA, Europe, Australia, New-Zealand and Brazil and were published between 1994 and 2010. The selected studies often excluded patients with sBCC in the anogenital area or areas within 1 cm of the H-zone, or pigmented or morpheaform BCC. Patients with dermatologic conditions that interfere with treatment, those who had genetic skin disorders, those who were receiving immunosuppressive therapy, or who were pregnant or breastfeeding were also often excluded.

Fourteen studies were RCTs in which two or more treatment protocols were directly compared (Table 2). Eight RCTs were dose-finding studies,[21-25] of which three also included a placebo study arm.[26-28] The other six RCTs compared imiquimod with placebo,[29] or PDT with another treatment.[30-34] The remaining 22 studies were non-RCTs and reported results of one treatment modality.[11,35-55] One study compared imiquimod with methylaminolevulinate (MAL)-PDT in a non-randomized trial.[45]

The majority of studies provided data on the results of treatment with PDT (16)[11,30-34,43,45,47-49,51-55] or imiquimod (15).[21-24,27-29,35-37,40,42,44-46] Other treatments that were evaluated were pulsed dye laser[38,39,50], 5-FU[25,41], cryotherapy[30,33], surgical excision[31] and PEP005.[26] The latter is also known as ingenol mebutate.[56] Pooling of results was restricted to PDT and imiquimod studies.

Probabilities of complete response after treatment could be derived from 34 of 36 studies,[11,21-32,34-42,44-55] and information on recurrence probabilities during follow-up was available in 23 of 36 studies.[11,21,30-37,39,40,42-45,47-49,51-53,55] Four studies did not use a single standardized treatment protocol, but presented study results of combinations of treatment regimens. Subgroup analyses were not performed.[11,33,36,44] The follow-up period for evaluation of recurrence differed from three months to five years after the first control visit. Probability of tumor-free survival at one year follow-up could be derived from 14 studies,[21,30,31,33-37,39,40,42,44,49,55], but estimates at two, three, four and five years of follow-up

were only available in six [30,34,35,37,40,44], five [30,35,40,43,44], three [30,35,40] and three [30,35,40] studies, respectively.

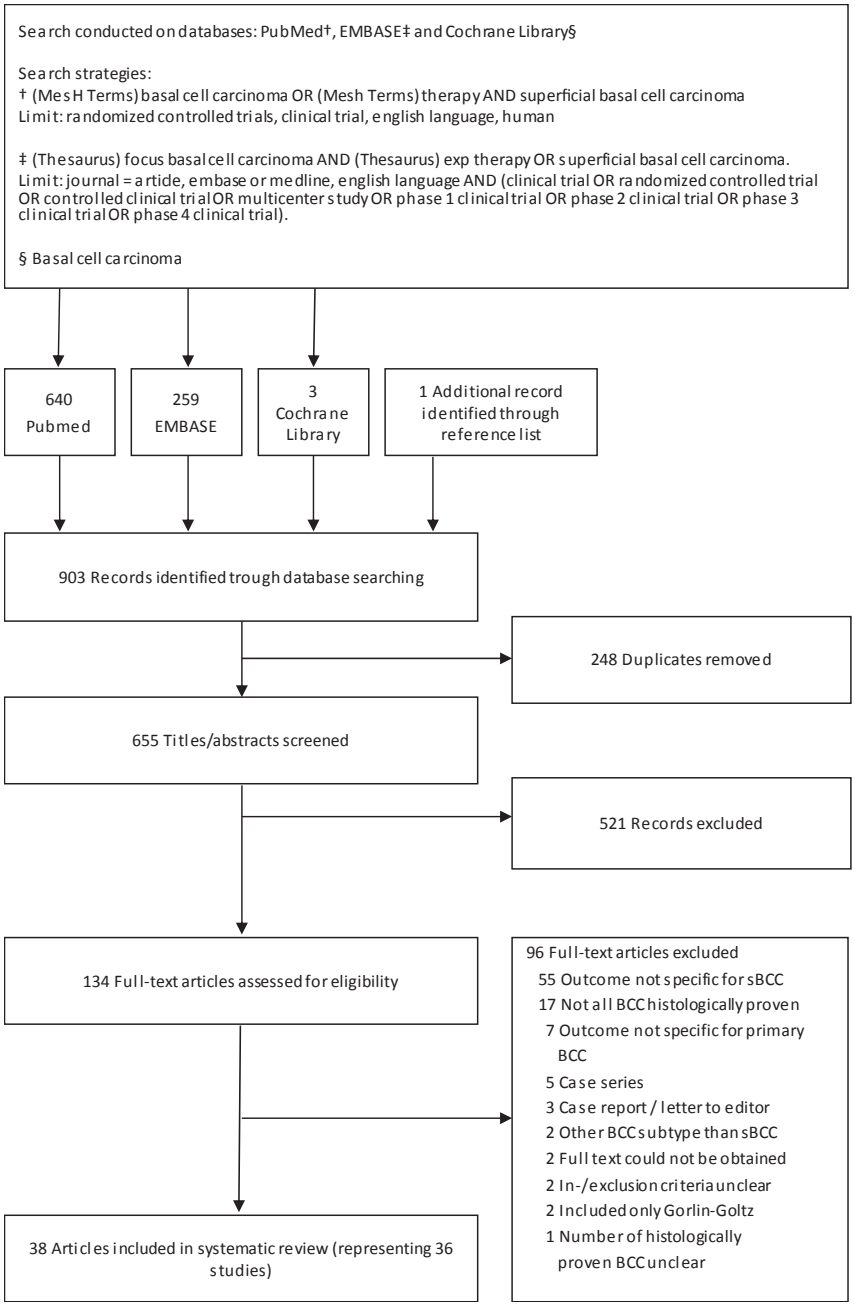


Fig. 1 Flow diagram of study selection in systematic review.

Table 2. Characteristics and extracted data of included studies.

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Imiquimod							
Quirk 2010/2006[35,60]	Australia and New-Zealand. Phase 3, prospective, multicenter, single-arm, open-label study.	No RCT Quality score 6 Funding: yes.	Standard. In: sBCC ≥ 0.5 cm^2, ≤ 2 cm in diameter.	169 participants (169 sBCC), 96 M and 73 F, mean age 57 yrs (range 26-86). Localization: 45% trunk, 31% upper extremities, 24% other. Median tumor size 1.0 cm^2 (range 0.4-4.0).	7x/week for 6 weeks	10 (5.9%) at 12 weeks post treatment, clinical verification.	157 sBCC entered long-term follow-up. 3 months: 1 (0.6%) 6 months: 2 (1.3%) 1Y: 6 (3.8%) 2Y: 14 (8.9%) 3Y: 15 (9.6%) 4Y: 18 (11.5%) 5Y: 20 (12.7%) Clinical verification. Total withdrawals 18.
Alessi 2009[36] *	Brazil. Retrospective study.	No RCT Quality score 3 Funding: unknown.	Not mentioned	39 participants (51 sBCC). Patient and tumor characteristics not specified for sBCC.	3-7x/week for 6-12 weeks (median 6.9 weeks)	12 (23.6%) at 12 weeks post treatment, clinical verification.	39 sBCC entered long-term follow-up of median 15.2 months (range 3-37). 1Y: 0 (0%) Clinical or combined clinical/histological verification. No withdrawals.

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Ruiz-Villaverde 2009[37]	Spain. Prospective, single-arm study.	No RCT Quality score 5 Funding: no.	In: rejected surgical treatment, accepted imiquimod treatment, contraindication to surgery.	82 participants (82 sBCC), 36 M and 46 F, age range 30-99 yrs. Tumor diameter: 9% < 0.5 cm, 27% 0.5-1.0 cm, 44% 1.0-1.5 cm, 20% >1.5 cm.	3x/week for 4 weeks	8 (9.8%) at 10 weeks post treatment, combined clinical/histological verification.	74 sBCC entered long-term follow-up. 1Y: 0 (0%) 2Y: 0 (0%) Clinical verification. Total withdrawals 4.
Gollnick 2008/2005[40,61]	Europe. Phase 3, prospective, multicenter, single-arm, open-label study.	No RCT Quality score 6 Funding: yes.	Standard. In: sBCC ≥ 0.5 cm^2, ≤ 2 cm in diameter.	182 participants (182 sBCC), 120 M and 62 F, median age 65 yrs (range 21-89). Localization: 63% trunk, 23% upper extremities, 14% other. Median tumor size 1.0 cm^2 (range 0.2-9.0).	5x/week for 6 weeks	19 (10.4%) at 12 weeks post treatment, clinical verification.	162 sBCC entered long-term follow-up. 3 months: 4 (2.5%) 6 months: 4 (4.9%) 1Y: 10 (6.2%) 2Y: 14 (8.6%) 3Y: 14 (8.6%) 4Y: 16 (9.9%) 5Y: 18 (11.1%) Clinical verification. Total withdrawals 9.

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Ezughah 2008[21] † ‡	United Kingdom. Prospective, randomized, investigator-blind study.	RCT Quality score 5 Funding: yes.	Standard. In: sBCC ≥ 0.5 cm^2, depth ≤ 2 mm.	32 participants randomized, 30 treated (36 sBCC). -T1: 14 treated (16 sBCC), 8 M and 6 F, mean age 68 yrs (range 50-83). Localization: 56% trunk, 25% extremities, 19% head/neck. Mean surface area 0.8-5.0 cm^2. -T2: 16 treated (20 sBCC), 9 M and 7 F, mean age 62 yrs (range 49-81). Localization: 75% trunk, 15% extremities, 10% head/neck. Mean surface area 0.7-4.0 cm^2.	-T1:7x/week for 8 weeks on alternate weeks -T2: 7x/week for 5 weeks with 1 week interval	- T1: 5 (35.7%) - T2: 3 (18.8%) At 19 weeks post treatment, clinical verification.	22 patients entered long-term follow-up. -T1: 3 (21.4%) at 1Y -T2: 2 (12.5%) at 1Y Clinical verification. Total withdrawals not mentioned.
Schiessl 2007[42]	Austria. Prospective, single-arm study.	No RCT Quality score 5 Funding: yes.	Not mentioned	41 participants with 47 BCC (15 sBCC). Mean surface area 0.5-24 cm^2. Localization: 53% trunk, 40% head/neck, 7% arm. Other patient characteristics not specified for sBCC.	5x/week for 6 weeks	0 (0%) At 6 weeks post treatment, combined clinical/histological verification.	15 sBCC entered long-term follow-up of median 10 months (range 2-17). 1Y: 0 (0%) Clinical verification. Total withdrawals 8.

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Vun 2006[44] *	Australia. Retrospective, single-arm study.	No RCT Quality score 4 Funding: yes.	In: sBCC ≤ 2 cm in diameter.	7 participants (11 sBCC). Location: 100% face. Other patient and tumor characteristics not specified for sBCC.	7x/week for mean 6 weeks (range 3-9)	1 (9.1%) At 12 weeks post treatment, clinical or combined clinical/histological verification.	10 sBCC entered long-term follow-up of mean 39 months (range 33-51). 1Y: 0 (0%) 2Y: 0 (0%) Clinical or combined clinical/histological verification. Total withdrawals 0.
Schulze 2005[29]	Europe. Phase 3, prospective, multicenter, randomized, vehicle-controlled, double-blind study.	RCT Quality score 7 Funding: yes.	Standard. In: sBCC ≥ 0.5 cm^2, ≤ 2 cm in diameter.	166 participants (166 sBCC), overall median tumor size 1.0 cm^2 (range 0.3-6.3). -T1: 84 participants, 50 M and 34 F, median age 67 yrs (range 25-83). Localization: 70% trunk, 20% extremities, 1% face, 8% other. Tumor size not specified to treatment group. -T2: 82 participants, 51 M and 31 F, median age 68 yrs (range 31-86). Localization: 61% trunk, 29% extremities, 6% face, 4% other. Tumor size not specified to treatment group.	-T1: 7x/week for 6 weeks -T2: vehicle 7x/week for 6 weeks	T1: 17 (20.2%) T2: 77 (93.9%) At 12 weeks post treatment, combined clinical/histological verification.	Not performed

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Nikkels 2005[45]	Belgium. Prospective, two-arm study.	No RCT Quality score 5 Funding: unknown.	Not mentioned	8 participants (34 sBCC), 5 M and 3 F, mean age 63 yrs (range 41-78). Localization: 75% trunk, 25% head/neck. Tumor size range 10-100 mm in diameter.	3x/week for 3 weeks followed by 1 stop week. Repeated for 3 times.	2 (5.9%) At 12 weeks post treatment, combined clinical/histological verification.	32 sBCC entered long-term follow-up. 6 months: 0 (0%) Clinical verification. Total withdrawals 0.
Shumack 2004[46]	Australia and New-Zealand. Phase 2, prospective, multicenter, open-label study.	No RCT Quality score 5 Funding: yes.	In: sBCC ≥ 2 cm in diameter.	66 participants (66 sBCC), median tumor size 4.3 cm^2 (range 2.0-48.0). Other patient and tumor characteristics not specified for sBCC	5x/week for 6 weeks	11 (16.7%) at 12 weeks post treatment, combined clinical/histological verification.	Not performed
Marks 2004[22]	Australia. Phase 2, prospective, multicenter, randomized, double-blind study.	RCT Quality score 5 Funding: yes.	Not mentioned	67 participants (208 sBCC). -T1: 97 sBCC, localization: 52% trunk, 47% extremities, 1% neck. -T2: 111 sBCC, localization: 55% trunk, 42% extremities, 3% neck.	Both treatments during 6 weeks. -T1: 7x/week -T2: 5x/week	-T1: 22 (22.7%) -T2: 25 (22.5%) At 12 weeks post treatment, combined clinical/histological verification.	Not performed

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Geisse 2004[27]	USA. Prospective, multicenter, randomized, vehicle-controlled, double -blind study.	RCT Quality score 7 Funding: yes.	Standard. In: sBCC 0.5-4.0 cm^2, diameter 0.4-2.0 cm.	724 participants (724 sBCC), overall tumor size 0.2-5.8 cm^2. -T1: 185 participants, 116 M and 69 F, median age 59 yrs (31-89). Localization: 49% trunk, 46% extremities, 5% other. -T2: 179 participants, 106 M and 73 F, median age 58 yrs (29-88). Localization: 49% trunk, 44% extremities, 7% other. -T3:179 participants, 120 M and 59 F, median age 61 yrs (35-85). Localization: 50% trunk, 45% extremities, 5% other. -T4: 181 participants, 103 M and 78 F, median age 58 yrs (32-84). Localization: 43% trunk, 52% extremities, 5% other.	All treatments during 6 weeks. -T1: 5x/week -T2: 7x/week -T3: vehicle 5x/week -T4: vehicle 7x/week	-T1: 33 (17.8%) -T2: 37 (20.7%) -T3 +T4: 349 (96.9%) At 12 weeks post treatment, combined clinical/histological verification.	Not performed

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Sterry 2002[24]	Europe. Prospective, multicenter, randomized, open-label study.	RCT Quality score 7 Funding: yes.	Standard. In: sBCC 0.5-2.0 cm^2.	93 participants (93 sBCC). -T1: 23 participants, 15 M and 8 F, mean age 69 yrs. Localization: 70% trunk, 30% extremities. Median tumor size 1.2 cm^2. -T2: 25 participants, 14 M and 11 F, mean age 61 yrs. Localization: 68% trunk, 32% extremities. Median tumor size 1.0 cm^2. -T3: 21 participants, 14 M and 7 F, mean age 63 yrs. Localization: 67% trunk, 29% extremities, 5% other. Median tumor size 1.5 cm^2. -T4: 24 participants, 16 M and 8 F, mean age 69 yrs. Localization: 58% trunk, 29% extremities, 13% other. Median tumor size 1.0 cm^2.	All treatments during 6 weeks. -T1: 3x/week with occlusion -T2: 3x/week without occlusion -T3: 2x/week with occlusion -T4: 2x/week without occlusion	-T1: 3 (13.0%) -T2: 6 (24.0%) -T3: 12 (57.1%) -T4: 12 (50.0%) At 6 weeks post treatment, combined clinical/histological verification.	Not performed

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Geisse 2002[28]	USA. Phase 2, prospective, multicenter, randomized, vehicle-controlled, double-blind study.	RCT Quality score 7 Funding: yes.	Standard. In: sBCC 0.5-2.0 cm^2.	128 participants (128 sBCC). -T1: 10 participants, 8 M and 2 F, mean age 59 yrs (range 51-85). Localization: 4 trunk, 5 extremities, 1 head/neck. Median tumor size 1.0 cm^2. -T2: 31 participants, 20 M and 11 F, mean age 56 yrs (range 35-85). Localization: 18 trunk, 11 extremities, 2 head/neck. Median tumor size 0.7 cm^2. -T3: 26 participants, 18 M and 8 F, mean age 55 yrs (range 38-84). Localization: 16 trunk, 9 extremities, 1 head/neck. Median tumor size 0.6 cm^2. -T4: 29 participants, 16 M and 13 F, mean age 62 yrs (range 36-85). Localization: 19 trunk, 9 extremities, 1 head/neck. Median tumor size 1.0 cm^2. -T5: 32 participants, 20 M and 12 F, mean age 58 yrs (range 38-85). Localization: 15 trunk, 14 extremities, 3 head/neck. Median tumor size 0.8 cm^2.	All treatments during 12 weeks. -T1: twice daily -T2: once daily -T3: 5x/week -T4: 3x/week -T5: vehicle with protocols T1-T4.	-T1: 0 (0%) -T2: 4 (12.9%) -T3: 5 (19.2%) -T4: 14 (48.3%) -T5: 28 (81.2%) At 6 weeks post treatment, combined clinical/histological verification.	Not performed

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Marks 2001[23]	Australia and New-Zealand. Phase 2, prospective, multicenter, randomized, open-label study.	RCT Quality score 7 Funding: yes.	Standard. In: sBCC 0.5-2.0 cm^2.	99 participants (99 sBCC), 72 M and 27 F, mean age 61 yrs (range 23-83). Localization: 40% head/neck, 32% upper extremities, 28% trunk. -T1: 3 participants, median tumor size 1.5 cm^2. -T2: 33 participants, median tumor size 0.7 cm^2. -T3: 30 participants, median tumor size 0.9 cm^2. -T4: 33 participants, median tumor size 1.0 cm^2.	All treatments during 6 weeks. -T1: twice daily -T2: once daily -T3: twice daily 3x/week -T4: once daily 2x/week	-T1: 0 (0%) -T2: 4 (12.1%) -T3: 8 (26.7%) -T4: 10 (30.3%) At 6 weeks post treatment, combined clinical/histological verification.	Not performed
Photodynamic therapy							
Basset-Seguin 2008/2005[30,62]	Europe. Prospective, multicenter, randomized, open label study.	RCT Quality score 4 Funding: yes.	Standard. In: sBCC diameter 0.6-1.4 cm on face/scalp, ≤ 2.0 cm on extremities and neck, ≤ 3.0 cm on trunk.	62 participants randomized, 60 treated (114 sBCC) 58 completed protocol (103 sBCC).39 M and 19 F, mean age 62 yrs (range 25-86). Localization: 72% trunk/neck, 22% extremities, 6% face/scalp. Tumor diameter: 43% 0.5-1.0 cm, 42% 1.1-1.9 cm, 16% ≥ 2.0 cm.	MAL 20%, 5 mm margin, application for 3 hours. Illumination: Curelight®, 570-670 nm, 75 J/cm. -T1: one cycle (on day 1 and 8) -T2: two cycles (incomplete responders at 3 months received 2 further MAL-PDT sessions on day 1 and 8)	-T1: 33 (38.9%) -T2: 1 (2.3%) -Overall: 14 (12.3%) At 12 weeks post treatment, clinical verification.	100 sBCC (56 participants) entered long-term follow-up. T1 and T2 together: 1Y: 9 (9.0%) 2Y: 17 (17.0%) 3Y: 22 (22.0%) 4Y: 22 (22.0%) 5Y: 22 (22.0%) Clinical or combined clinical/histological verification. Total withdrawals 16 sBCC.

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Szeimies 2008[31]	Europe and Australia. Prospective, multicenter, randomized, open label study.	RCT Quality score 5 Funding: yes.	Standard. In: sBCC 0.8-2.0 cm in diameter.	100 participants (135 sBCC). 64 M and 36 F. Mean age 65 (range 33-85). Localization: 60% trunk/neck, 29% extremities, 11% face/scalp. Mean tumor diameter 1.3 cm.	MAL 20%, 5-10 mm margin, application for 3 hours. Illumination with Aktilite®, 37 J/cm^2. -T1: once cycle (on day 1 and 8) -T2: two cycles (Incomplete responders at 3 months received 2 further MAL-PDT sessions on day 1 and 8)	-T1: 38 (29.7%) -T2: 10 (26.3%) -Overall: 17 (12.6%) At 12 weeks post treatment, clinical verification.	118 sBCC entered long-term follow-up. 1Y: 11 (9.3%) Clinical verification. Total withdrawals not mentioned.
Schleier 2007[32]	Germany. Prospective, randomized, double blind study.	RCT Quality score 6 Funding: no.	Standard. In: sBCC depth ≤ 2 mm.	24 participants (112 sBCC). Mean tumor diameter 0.7 cm (range 0.3-1.2) -T1+2: 13 participants, 7 M and 6 F, mean age 70 yrs (range 42-96). Localization: 81% head/neck, 19% other. -T3+4: 11 participants, 7 M and 4 F, mean age 72 yrs (range 49-88). Localization: 90% head/neck, 10% other. Note: 3 patients with Gorlin-Goltz included.	-T1: single session ALA 10% -T2: single session mALA 10% All crèmes 3 mm margin, application for 3 hours. Illumination with Ceralas®, 120 J/cm^2, 0.1 W/cm^2.	-T1: 28 (38.9%) -T2: 17 (42.5%) At 12 weeks post treatment, clinical verification.	67 sBCC (44 T1 and 23 T2) entered long-term follow-up. -T1: 6 months: 8 (11.6%) -T2: 6 months: 5 (12.8%) Clinical verification. Total withdrawals not mentioned. Note: 2 Gorlin-Goltz patients showed recurrences.

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Star 2006[43]	The Netherlands. Prospective, single-arm study.	No RCT Quality score 4 Funding: no.	Standard	15 participants (86 sBCC), 8 M and 7 F, mean age 61 yrs (range 35-83). Localization: 44% head/neck, 33% trunk, 23% extremities. Mean tumor diameter 1.3 cm (range 0.4-5.0).	ALA 20%, 5 mm margin, application for 4-6 hours. Fractionated illumination with 2-hours interval: Argon dye laser, 633 ± 1 nm, 45 J/cm^2, 50 mW/cm^2.	Not performed	72 sBCC (15 participants) entered long-term follow-up of median 58 months (range 44-82). 1Y: 7 (8.1%) 2Y: 10 (11.6%) 3Y: 10 (11.6%) 4Y: 11 (12.8%) 5Y: 12 (14.0%) Clinical or combined clinical/histological verification. Total withdrawals 14 sBCC (2 patients).
Baptista 2006[11] *	Portugal. Prospective, single-arm study.	No RCT Quality score 5 Funding: unknown.	Standard	59 participants (64 sBCC). Localization: 44% head/neck, 34% trunk, 22% extremities. Mean tumor diameter 1.3 cm (range 0.4-5.0). Patient characteristics not specified. Note: only 1 sBCC per patient histological proved before treatment.	ALA 20%, 10 mm margin, application for 4-6 hours. Single illumination with Waldmann 1200 L, 630 nm, 100 J/cm2, 100 mW/cm^2, *or* Versalight®, 600-720 and 1250-1550 nm simultaneously. Repeated if necessary every month up to 5 times.	15 (23.4%) At 1-5 months post treatment, clinical verification.	49 sBCC entered long-term follow-up of median 38 months (range 3-84). 2Y: 6 (10.9%) Clinical verification. Total withdrawals not mentioned.

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Nikkels 2005[45]	Belgium. Prospective, two-arm study.	No RCT Quality score 5 Funding: unknown.	Not mentioned	7 participants (10 sBCC), 5 M and 2 F, mean age 76 yrs (range 68-84). Localization: 50% trunk, 50% head/neck. Tumor size range 15-65 mm in diameter.	MAL 20%, application for 3 hours. Double illumination on day 1 and 8. LED light, 634 nm, 37 J/cm^2.	0 (0%) At 4 weeks post treatment, combined clinical/histological verification.	10 sBCC entered long-term follow-up. 6 months: 0 (0%) Clinical verification. Total withdrawals 0.
Naidenov 2004[55]	Bulgaria. Prospective, single-arm study.	No RCT Quality score 3 Funding: unknown.	Standard	39 participants (39 sBCC). Participants and tumor characteristics not specified for sBCC.	ALA 20%, application for 4 hours. Single illumination with Waldmann 1200 L *or* slide projector. Both 635 nm, 150 J/cm^2.	Not performed	39 sBCC entered long-term follow-up. 1Y: 4 (10.3%) 2Y: 6 (15.4%) Clinical verification. Total withdrawals not mentioned.
Dijkstra 2001[47]	The Netherlands. Prospective, single-arm study.	No RCT Quality score 4 Funding: unknown.	Standard	34 participants (35 sBCC). Tumor diameter range 0.3-4.5 cm	ALA 20%, 8-10 mm margin, application for 8 hours. Illumination: Philips HPM-10 violet light, 400-450 nm, 10-20 J/cm^2, 5.4-10.8 mW/cm^2. -T1: single session -T2: double session, repeated after 3 months.	-T1: 8 (22.9%) -T2: 0 (0%) At 12 weeks post treatment, combined clinical/histological verification.	27 sBCC entered long-term follow-up of mean 6 months (range 4-12). -T1: 3 months: 0 (0%) -T2: 3 months: 0 (0%) Clinical verification. Total withdrawals not mentioned.

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Wang 2001[33] *	Sweden. Phase 3, prospective, randomized, open-label study.	RCT Quality score 6 Funding: yes.	Standard. In: age 20-90 yrs.	22 participants (22 sBCC). Participants and tumor characteristics not specified for sBCC.	ALA 20%, 10 mm margin, application for 6 hours. Illumination with YAG laser, 635 nm, 60 J/cm^2, 80±20 mW/cm^2. Note: 30% of sBCC retreated with second PDT after 3 months.	Data not mentioned in article.	21 sBCC entered long-term follow-up. 1Y: 8 (38.1%) Combined clinical/histological verification. Total withdrawals not mentioned.
Langmack 2001[48]	United Kingdom. Prospective, single-arm study.	No RCT Quality score 5 Funding: no.	Standard. In: sBCC depth ≤ 1 mm.	22 participants (32 sBCC), 11 M and 11 F, mean age 65 yrs (41-86). Localization: 34% extremities, 28% head/neck, 38% trunk. Median tumor size 10.0 cm^2 (range 1.6-60.0)	ALA 20%, application for 6 hours. Illumination: YAG laser, 635 nm, 12.6 J/cm^2, 7 mW/cm^2. Repeated every month if no complete response. -T1: single session -T2: double session -T3: triple session -T4: quadruple session	-T1: 27 (84.4%) -T2: 6 (22.2%) -T3: 3 (50.0%) -T4: 0 (0%) At 4 weeks post treatment, clinical verification.	32 sBCC entered long-term follow-up. All treatment arms together: 6 months: 3 (9.4%) 1Y: 5 (15.6%) Clinical verification. Total withdrawals not mentioned.
Haller 2000[49]	United Kingdom. Prospective, single-arm study.	No RCT Quality score 4 Funding: no.	Standard	6 participants (26 sBCC), 6 M and 0 F, mean age 75 yrs (45-85). Localization: 81% trunk, 4% face, 15% extremities. Median tumor size 0.8 cm^2 (range 0.3-4.9)	ALA 20%, application for 4 hours. Double illumination on day 1 and 8. Paterson non-laser, 630 ± 15 nm, 120-134 J/cm^2, 50-100 mW/cm^2.	0 (0%) At 4-8 weeks post treatment, clinical verification.	26 sBCC entered long-term follow-up of median 27 months (range 15-45). 1Y: 0 (0%) Clinical verification. Total withdrawals not mentioned.

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Soler 2000[34]	Norway. Prospective, randomized, single- blind study.	RCT Quality score 5 Funding: unknown.	Standard. In: sBCC ≤ 3.0 diameter, depth ≤ 1 mm.	83 participants (245 sBCC). -T1: 41 participants (111 sBCC), 14 M and 27 F, mean age 62 yrs. Localization: 81% trunk, 4% face, 15% extremities. Median tumor size 0.8 cm^2 (range 0.3-4.9) -T2: 42 participants (134 sBCC), 25 M and 17 F, mean age 62 yrs. Localization: 81% trunk, 4% face, 15% extremities. Median tumor size 0.8 cm^2 (range 0.3-4.9)	ALA 20%, DMSO 2%, EDTA 2%. Application for 3 hours. Single illumination. - T1: laser light, 630 nm, mean 102 J/cm^2, 120-150 mW/cm^2. - T2: broadband light, 570-740 nm, mean 192 J/cm^2, 100-180 mW/cm^2. Note: 2 sBCC in T1 and 5 sBCC in T2 were treated twice.	-T1: 16 (14.4%) -T2: 24 (17.9%) At 26 weeks post treatment, clinical verification.	205 sBCC (95 in T1, 110 in T2) entered long-term follow-up. - T1 1Y: 2 (2.1%) 2Y: 4 (4.2%) - T2 1Y: 0 (0 %) 2Y: 5 (4.5%) Clinical verification. Total withdrawals: 32 (T1) and 41 (T2) sBCC.
Hurlimann 1998[51]	Switzerland. Phase 2, prospective, single-arm study.	No RCT Quality score 5 Funding: unknown.	Not mentioned	19 participants (54 sBCC), 14 M and 5 F, mean age 65 yrs (range 32-93). Localization: 67% trunk, 20% head/neck, 12% extremities. Mean tumor diameter 1.3 cm (range 0.4-4.5)	ALA 10%, 20 mm margin, application for 6 hours. Single illumination with unfiltered halogen lamp, peak 800 nm, 240 J/cm^2, 120-200 mW/cm^2.	5 (19.2%) At 26 weeks post treatment, combined clinical/histological verification.	21 sBCC entered long-term follow-up. 1Y: 1 (4.8%) Clinical or combined clinical/histological verification. Total withdrawals not mentioned.

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Wennberg 1996[52] ‡	Sweden. Prospective, single-arm study.	No RCT Quality score 5 Funding: unknown.	Not mentioned	37 participants (190 sBCC), mean age 65 yrs (range 28-83). Mean tumor size 1.7 cm^2 (range 0.2-16.0). Number of participants in both treatment groups and tumor localization not mentioned.	ALA 20%, 10-20 mm margin, application for 3 hours. Single illumination with filtered xenon lamp, 620-670 nm. -T1: 75 J/cm^2. -T2: 100 J/cm^2.	-T1: 10 (11.9%) -T2: 3 (4.1%) At 12 weeks post treatment, combined clinical/histological verification.	144 sBCC entered long-term follow-up. -T1: 6 months 0 (0%) -T2: 6 months 0 (0%) Clinical or combined clinical/histological verification. Total withdrawals not mentioned.
Cairnduff 1994[53]	United Kingdom. Phase I, prospective, single-arm study.	No RCT Quality score 4 Funding: unknown.	Not mentioned	14 participants (16 sBCC). Median tumor diameter 2.1 cm (range 1-7). No other participants and tumor characteristics mentioned. Note: only 1 sBCC per patient histological proved before treatment.	ALA 20%, 7.5 mm margin, application for 3-5 hours. Single illumination with copper vapor/dye laser, 630 nm, 125-250 J/cm^2, 150 mW/cm^2.	2 (12.5%) At 8 weeks post treatment, clinical or combined clinical/histological verification.	14 sBCC entered long-term follow-up of median 17 months (range 4-21). 6 (42.9%) in overall follow-up period. Clinical or combined clinical/histological verification. Total withdrawals not mentioned.
Svanberg 1994[54]	Sweden. Prospective, single-arm study.	No RCT Quality score 4 Funding: unknown.	Not mentioned	18 sBCC. Participants and tumor characteristics not specified for primary sBCC.	ALA 20%, 10-20 mm margin, application for 4-6 hours. Single illumination with YAG-pumped dye laser, 630 nm. 60 J/cm^2, 110 mW/cm^2.	0 (0%) At 12 weeks post treatment, clinical verification.	Not performed

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Pulse dye laser							
Shah 2009[38]	USA. Prospective, single-arm study.	No RCT Quality score 6 Funding: unknown.	Not mentioned	9 sBCC, only males. Localization: 78% trunk, 22% upper extremities. Mean tumor diameter 1.3 cm.	VBeam, 4 sessions, 2 week interval, 595 nm, 15 J/cm^2.	2 (22.2%) At 2 weeks post treatment, combined clinical/histological verification.	Not performed
Campolmi 2008[39]	Italy. Prospective, single-arm study.	No RCT Quality score 4 Funding: unknown.	Standard	20 participants (20 sBCC), 8 M and 12 F, mean age 63 (range 49-72). Localization: 56% extremities, 44% neck/trunk. Median tumor diameter 0.8 cm	Dermobeam 2000, 5 sessions, 3 week interval. 5-10 passes, 595 nm, 6.5-7.5 J/cm^2.	2 (10%) At 12 weeks post treatment, clinical verification.	18 sBCC entered long-term follow-up of 12-24 months. 6 months: 2 (11.8%) 1Y: 2 (11.8%) Clinical verification. Total withdrawals 0.
Humphreys 1998[50]	USA. Prospective, two-arm study.	No RCT Quality score 6 Funding: unknown.	Standard. In: sBCC ≥ 5 mm in diameter.	17 participants (17 sBCC), mean age 57. Localization: 47% trunk, 35% extremities, 12% head/neck. Mean tumor diameter 11 mm, mean thickness 0.34 mm. -T1: 8 participants (8 sBCC) -T2: 9 participants (9 sBCC)	High-energy pulsed CO_2 laser, 500 mJ, 2-4W. -T1: 2 passes -T2: 3 passes	-T1: 5 (62.5%) -T2: 0 (0%) Direct after treatment combined clinical/histological verification.	Not performed

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
5-Fluorouracil							
Gross 2007[41]	USA. Prospective, single-arm study.	No RCT Quality score 6 Funding: yes.	Standard. In: sBCC diameter 0.5-2.0 cm.	29 participants (31 sBCC). Tumors on trunk and limbs. Participants and tumor characteristics not mentioned.	5% 5-Fluorouracil, topical twice daily -T1: during 6 weeks -T2: during 9 weeks -T3: during 12 weeks	-T1: 27 (87.1%) -T2: 22 (81.5%) -T3: 3 (13.6%) At 3 weeks post treatment, combined clinical/histological verification.	Not performed
Miller 1997[25]	USA. Prospective, randomized, multicenter, open-label study.	RCT Quality score 6 Funding: yes.	Standard. In: sBCC diameter 0.6-1.5 cm.	38 participants (38 sBCC). Participants and tumor characteristics not specified for sBCC.	Intralesional 5-Fluorouracil /epinephrine -T1: 1.0 ml 1x/week for 6 weeks. -T2: 0.5 ml 1x/week for 6 weeks. -T3: 1.0 ml 2x/week for 3 weeks. -T4: 0.5 ml 2x/week for 3 weeks. -T5: 0.5 ml 2x/week for 4 weeks. -T6: 0.5 ml 3x/week for 2 weeks.	6 regimes together: 5 (13.2%) At 12 weeks post treatment, combined clinical/histological verification.	Not performed

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Cryotherapy							
Basset-Seguin 2008/2005[30,62]	Europe. Prospective, multicenter, randomized open label study.	RCT Quality score 4 Funding: yes.	Standard. In: sBCC 0.6-1.5 cm in diameter on face/scalp, ≤ 2.0 cm on extremities and neck, ≤ 3.0 cm on trunk.	58 participants randomized, 57 treated (98 sBCC). 30 M and 27 F., mean age 64 yrs (range 38-90). Localization: 76% trunk/neck, 20% extremities, 4% face/scalp. Tumor diameter: 42% 0.5-1.0 cm, 42% 1.1-1.9 cm, 16% ≥2.0 cm.	Liquid nitrogen spray, double freeze-thaw cycle. -T1: one treatment -T2: two treatments, with 3 months interval.	-T1: 33 (31.4%) -T2: 3 (7.5%) At 12 weeks post treatment, clinical.	93 sBCC (52 participants) entered long-term follow-up. T1 and T2 together: 1Y: 12 (14.0%) 2Y: 18 (22.0%) 3Y: 18 (22.4%) 4Y: 18 (22.8%) 5Y: 19 (24.2%) Clinical or combined clinical/histological verification. Total withdrawals 10 sBCC.
Wang 2001[33]	Sweden. Phase 3, prospective, randomized, open-label study.	RCT Quality score 6 Funding: yes.	Standard. In: age 20-90 yrs.	17 participants (17 sBCC). Patients and tumor characteristics not specified.	Liquid nitrogen spray, double freeze-thaw cycle. 3% retreated with second cycle.	Data not mentioned in article.	16 sBCC entered long-term follow-up. 1Y: 1 (6.3%) Combined clinical/histological verification. Total withdrawals not mentioned.

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
Surgical excision							
Szeimies 2008[31]	Europe and Australia. Prospective, multicenter, randomized, open label study.	RCT Quality score 5 Funding: yes.	Standard. In: sBCC 0.8-2.0 cm in diameter.	96 participants (132 sBCC), 66 M and 30 F, mean age 63 (range 31-92). Localization: 71% trunk/neck, 25% extremities, 5% face/scalp. Mean tumor diameter 1.3 cm.	Excision with 3 mm margin.	15 (11.4%) At 12 weeks post treatment, clinical verification.	117 sBCC entered long-term follow-up. 1Y: 0 (0%) Clinical verification. Total withdrawals 0.

Study	Setting	Quality assessment	Inclusion and exclusion criteria	Participants and tumor characteristics	Treatment protocol	Residue	Cumulative recurrence at long-term follow-up
PEP0005							
Siller 2010[26]	Australia. Phase 2a, prospective, multicenter, randomized, vehicle-controlled study	RCT Quality score 7 Funding: yes.	Standard. In: sBCC 0.4-1.5 cm in diameter, depth ≤ 4 mm.	- T1+2: 16 participants (16 sBCC), 11 M and 5 F, mean age 56 (range 34-86). Localization: 56% extremities, 44% neck/trunk. Median tumor diameter 0.8 cm. -T3+4: 16 participants (16 sBCC), 13 M and 3 F, mean age 61 (range 48-80). Localization: 56% extremities, 38% neck/trunk, 6% face. Median tumor diameter 0.9 cm. -T5+6: 16 participants (16 sBCC), 10 M and 6 F, mean age 61 (range 43-83). Localization: 56% extremities, 44% neck/trunk. Median tumor diameter 0.9 cm. -T7: 12 participants (12 sBCC), 10 M and 2 F, mean age 59 (range 36-77). Localization: 67% extremities, 33% neck/trunk. Median tumor diameter 0.8 cm.	Day 1 and 2 -T1: 0.0025% -T2: 0.01% -T3: 0.05% Day 1and 8 -T4: 0.0025% -T5: 0.01% -T6: 0.05% -T7: vehicle	-T1: 8 (100%) -T2: 6 (75%) -T3: 3 (38%) -T4: 7 (87%) -T5: 8 (100%) -T6: 5 (62%) -T7: 11 (91.5%) At 85 days post treatment, combined clinical/histological verification.	Not performed

Data are given as number of sBCC and percentages.
Definition of abbreviations: RCT, randomized controlled trial; sBCC, superficial basal cell carcinoma; M, male; F, female; Y, year; T, treatment arm; DMSO, dimetylsulfoxide; EDTA, ethylendiaminetetraacetic acid; ITT, intention to treat.
Standard: inclusion when ≥ 18 yrs, primary histologically confirmed sBCC, not previously treated. Exclusion: anogenital area, areas within 1 cm of H-zone, pigmented or morpheaform sBCC, dermatologic conditions that interfere with treatment, genetic skin disorders, immunosuppressive therapy, pregnant or breastfeeding women.
† In number of patients. ‡ Only number of sBCC that actually finished treatment available, not how many were included initially. * Not one single standardized treatment protocol.

In most studies, analyses were performed on tumor level. One study on treatment with imiquimod reported results from analysis solely on a patient level.[21] Ezughah et al. and Wennberg et al. calculated residue probability by dividing the number of residues by the number of patients who actually finished treatment instead of the number who were included at the start of the study.[21,52] In all studies, response to treatment was verified by clinical verification. An additional histopathological verification was performed in 18 of 34 studies that reported residue probabilities, of which most were studies on imiquimod.[22-29,37,38,41,42,45-47,50-52] From the 23 studies that reported recurrence probabilities, two studies performed an additional histopathological verification of the diagnosis.[33,44]

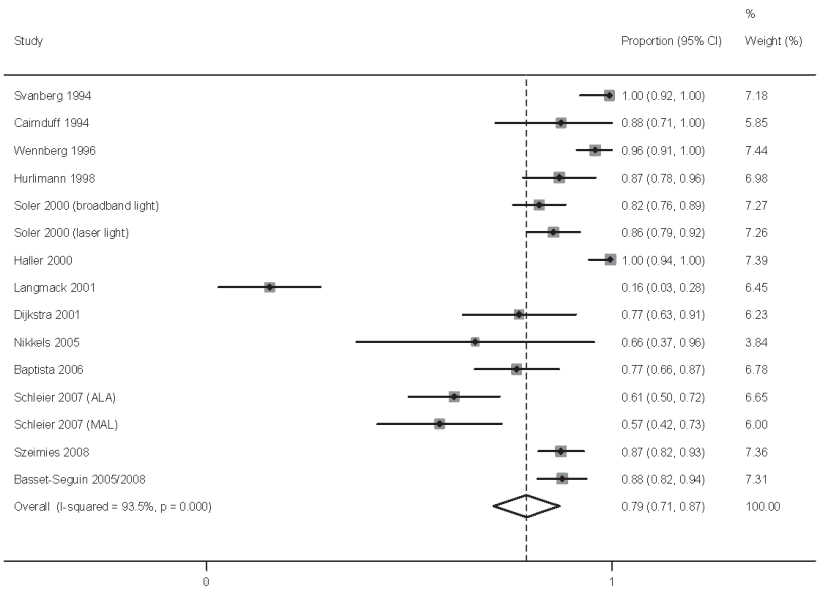
Probability of complete response after treatment with imiquimod or photodynamic therapy

Probabilities of complete response after treatment with imiquimod could be derived from 15 studies with a total of 1088 sBCC.[21-24,27-29,35-37,40,42,44-46] From six dose-finding studies, data were used from only one study arm.[21-24,27,28] The preferred arm had a dosing regimen most similar to the widely used protocol of imiquimod 5 days a week during 6 weeks. Schedules differed from three times a week to once daily every day. Treatment duration ranged from 4 to 12 weeks and the first control visit to evaluate response to treatment was scheduled at 6 and 19 weeks post treatment. Fig. 2a shows a forest plot with proportions of patients with complete response and 95% confidence interval (CI) for individual imiquimod studies. The pooled estimate was 86.2% (95% CI 82-90%), with large heterogeneity (I^2 index = 72% and $p<0.0001$).

Probabilities of complete response after treatment with PDT could be derived from 13 studies with a total of 934 sBCC.[11,30-32,34,45,47-49,51-54] From three illumination dose-finding studies, data were used from only one study arm.[47,48,52] The arm most similar to the current regimen was preferred; 100 J/cm^2, one time 20% aminolevulinic acid (ALA)-PDT illumination or one cycle (on day 1 and 8) 20% MAL-PDT illumination. Despite

this aim, the frequency of illumination ranged from one to four times. Two studies compared different light sources and light-sensitive agents.[32,34] From each study both study arms were used for analysis.

a)



b)

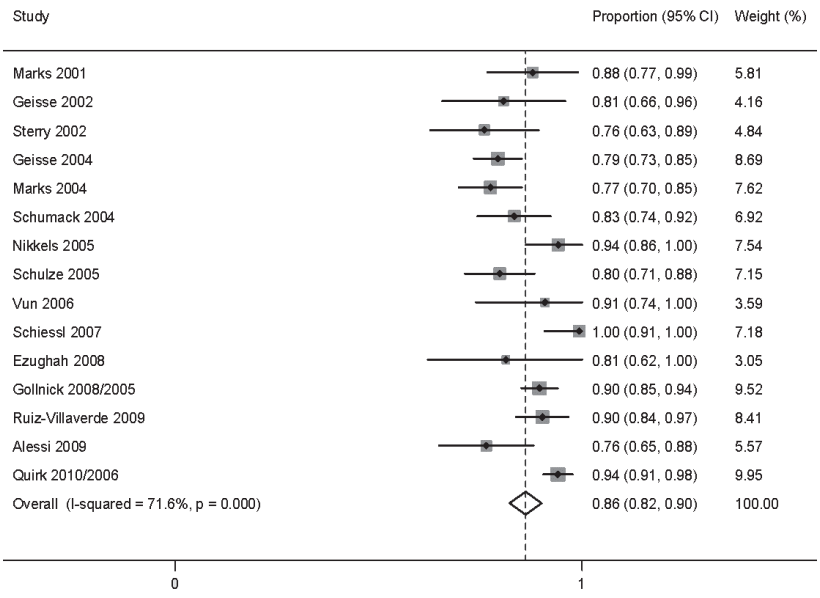


Fig. 2 Forest plots of results from (a) imiquimod and (b) photodynamic therapy studies on complete response probability at first control visit.

The majority of PDT studies used 20% ALA as a light-sensitive agent. The first control visit to evaluate response to treatment was scheduled 4 to 26 weeks post treatment. Fig. 2b shows a forest plot with proportions of sBCC with complete response and 95% CI for individual PDT studies. The pooled estimate for the proportion of sBCC with complete response to treatment was 79.0% (95% CI 71-87%) with large heterogeneity (I^2 index = 94% and p<0.0001). The difference of 7.2% in pooled estimates of complete response probability between imiquimod and PDT did not reach statistical significance (p=0.171). This pooled estimate included the complete response probabilities of Szeimies et al. (87.4%) and Basset-Seguin et al. (87.7%) after one or two cycles of MAL-PDT.[30,31] An analysis which excluded patients who received two cycles in these studies resulted in a pooled estimate of 75.6%, which was not significantly different from the estimate of 86.2% for imiquimod (p=0.057).

Cumulative probabilities of recurrence and tumor-free survival

The cumulative probabilities of recurrence and tumor-free survival that could be derived from the studies for one, two and five years post treatment are given in Table 3. Fig. 3a and 3b show forest plots with proportions of patients with tumor-free survival and 95% CI for individual imiquimod and PDT studies, respectively. The pooled estimate at one year after imiquimod treatment was 87.3% (95% CI 84-91%) without significant heterogeneity (I^2 index = 22% and p=0.263). PDT studies showed a lower pooled estimate of 84.0% (95% CI 78-90%) with large heterogeneity (I^2 = 73% and p=0.005).

The difference of 3.3% in pooled estimates of tumor-free survival at one year between imiquimod and PDT was not statistically significant (p=0.469).

Tumor-free survival after other treatments

There are not enough studies on pulse dye laser, 5-FU, cryotherapy, surgical excision and PEP005 in the treatment of sBCC to perform a meta-analysis (Table 2).[25,26,30,31,33,38,39,41,50] Tumor-free survival could be calculated for three individual studies (Table 3).[30,31,39] The study of Campolmi et al. on treatment with pulsed dye laser resulted in 79% tumor-free survival after 1 year.[39] Patients treated with one or two double freeze-thaw cycles of cryotherapy in Basset-Seguin's study showed a tumor-free survival of 67% at 5 years.[30] Surgical excision by Szeimies et al. demonstrated a high tumor-free survival with 89% (117/135) at 1 year.[31] However, in this study 17 of the included sBCC received no surgical excision because of patients' request. Tumor-free survival at one year was 99% (117/118) for patients actually receiving surgical excision.

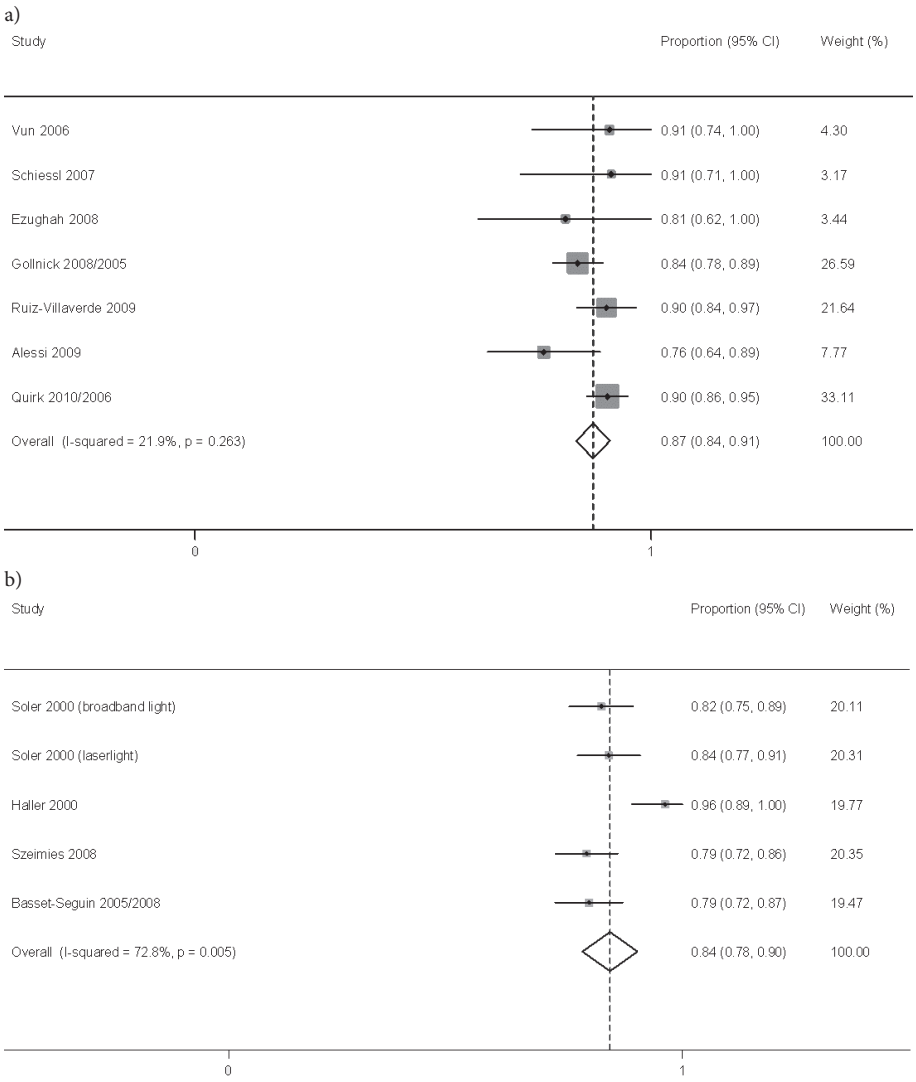


Fig. 3 Forest plots of results from (a) imiquimod and (b) photodynamic therapy studies on tumor-free survival at one year.

Publication bias

Egger's test shows that for PDT and imiquimod studies the intercepts were -5.94 (p=0.012) and -1.92 (p=0.082), respectively. This deviation of the intercept from zero indicates that smaller studies showed higher success rates than larger studies and thus publication bias is likely to be present.

Table 3. Cumulative probability of sustained clearance and tumor-free survival at long-term follow-up.

Study	Study design	Complete response ± 3 months	Cumulative probability of sustained clearance			Cumulative probability of tumor-free survival
			1 year	2 year	5 year	
Imiquimod						
Quirk 2010/2006[35,60]	Phase 3, prospective, multicenter, single-arm, open-label study	0.94	0.96	0.91	0.85	1Y 0.90 2Y 0.85 5Y 0.80
Alessi 2009[36]	Retrospective study	0.77	1.00	-	-	1Y 0.77
Ruiz-Villaverde 2009[37]	Prospective, single-arm study	0.90	1.00	1.00	-	1Y 0.90 2Y 0.90
Gollnick 2008/2005[40,61]	Phase 3, prospective, multicenter, single-arm, open-label study	0.90	0.94	0.91	0.87	1Y 0.84 2Y 0.82 5Y 0.78
Ezughah 2008[21]	Prospective, randomized, investigator-blind study	0.81	1.00	-	-	1Y 0.81
Schiessl 2007[42]	Prospective, single-arm study	1.000	1.00	-	-	1Y 1.00
Vun 2006[44]	Retrospective, single-arm study	0.91	1.00	1.00	-	1Y 0.91 2Y 0.91
Photodynamic therapy						
Basset-Seguin 2008/2005	Prospective, multicenter, randomized, open label study	0.88	0.91	0.81	0.73	1Y 0.79 2Y 0.71 5Y 0.64
Szeimies 2008	Prospective, multicenter, randomized, open label study	0.87	0.90	-	-	1Y 0.79
Haller 2000[49]	Prospective, single-arm study	1.00	1.00	-	-	1Y 1.00

Study	Study design	Complete response ± 3 months	Cumulative probability of sustained clearance			Cumulative probability of tumor-free survival
			1 year	2 year	5 year	
Soler 2000 *Broadband light*[34]	Prospective, randomized, single blind study	0.82	1.00	0.94	-	1Y 0.82 2Y 0.77
Soler 2000 *Laser light*[34]	Prospective, randomized, single blind study	0.86	0.98	0.95	-	1Y 0.84 2Y 0.81
Pulsed dye laser						
Campolmi 2008[39]		0.90	0.88	-	-	1Y 0.79
Cryotherapy						
Basset-Seguin 2008/2005[30,62]	Prospective, multicenter, randomized, open label study	0.89	0.86	0.78	0.76	1Y 0.76 2Y 0.69 5Y 0.67
Surgical excision						
Szeimies 2008[31]	Prospective, multicenter, randomized, open label study	0.89	1.00	-	-	1Y 0.89

Y, years. Complete response probability is calculated for all patients who were included at start of study. Years in long-term follow up were calculated from the complete response date and assigned per the following: 1 year, 2 years and 5 years. The number of patients at risk at the start of each 1-year interval as well as the number loss to follow-up and the number having no recurrence during each interval were used to calculate cumulative probabilities of recurrence. Tumor-free survival was calculated by multiplying the complete response probability by the sustained clearance probability.

Subgroup analyses

Subgroup analyses were performed to identify sources of heterogeneity in study results. Results are presented in Tables 4 and 5 for imiquimod and PDT studies, respectively. For imiquimod studies, the pooled estimates for complete response from randomized studies were lower than for non-randomized studies (79.8% vs. 90.7%). For PDT studies, pooled estimates differed substantially between subgroups of studies with lower and higher scores for methodological quality (92.0 % vs. 72.5%) However, these study characteristics do not fully explain the observed heterogeneity between study results, because within subgroups of studies the I^2-index remained high.

Sensitivity analyses

The robustness of the results of this review was evaluated by performing sensitivity analyses.

With respect to a complete response at the first control visit after treatment, results of all imiquimod (15) and PDT (13) study arms were pooled. A first sensitivity analysis was performed by including only patients who actually finished treatment. Using these percentages, the pooled estimate for complete response of 1065 sBCC treated with imiquimod therapy was higher, with 87.6% (95% CI 84-91%, $I^2 = 67\%$, $p<0.0001$) instead of 86.2%. A higher pooled estimate of 82.3% (95% CI 75-90%) instead of 79.0% was also found for the patients with 914 sBCC, who actually finished treatment with PDT ($I^2 = 94\%$, $p<0.0001$).

Secondly, we pooled the results of five imiquimod studies with a dosing regimen of 5 days per week during 6 weeks.[22,27,40,42,46] A similar pooled estimate for proportion with a complete response of 85.8% (95% CI 79-93%, $I^2 = 82\%$, $p<0.0001$) was found compared with 86.2% (95% CI 82-90%) for all imiquimod studies.

In the third sensitivity analysis, we excluded the PDT study by Haller et al. wherein patients were illuminated not once but twice with ALA-PDT.[49] The pooled estimate for proportion with complete response decreased from 79.0% to 77.3% and for the proportion with tumor-free survival from 84.0% to 81.1%. In that case, the difference of 6.4% in pooled estimates of tumor-free survival at one year between imiquimod and PDT was statistically significant (p=0.033).

Fourth, MAL-PDT studies by Szeimies et al. and Basset-Seguin et al. re-treated patients who at 3 months had not responded to treatment with one PDT cycle (day 1 and 8).[30,31] When including only results of BCC treated with one PDT cycle in these two studies the complete response probabilities were 70.3% (90/128) and 61.4% (70/114) in Szeimies et al. and Basset-Seguin et al., respectively.[30,31] The pooled estimate of PDT

complete response probability decreased from 79.0% to 75.6%, which was not significantly lower than that for imiquimod (p=0.057). Restriction of the analysis to PDT studies with exactly one year follow-up resulted in a decrease of the pooled estimate of tumor-free survival from 84.0% to 76.2% (95% CI 62-90%, I^2 = 95%).

Table 4. Imiquimod studies. Pooled estimates of proportion with complete response (with 95% confidence intervals) in subgroups of studies according to study and tumor characteristics.

Imiquimod (n=15 studies)		
RCT	Yes	No
	79.8 (76.2 – 83.3), I^2 0.0%	90.7 (86.8 – 94.6), I^2 58.1%
	n=7 [21-24,27-29]	n=8 [35-37,40,42,44-46]
Publication year	≤ 2003	> 2003
	82.5 (75.0 – 89.9), I^2 0.0%	86.9 (82.5 – 91.2), I^2 75.9%
	n=3 [23,24,28]	n=12 [21,22,27,29,35-37,40,42,44-46]
Funding	Yes	No
	85.6 (80.9 – 90.8), I^2 74.8%	88.0 (79.5 – 96.6), I^2 67.6%
	n=12 [21-24,27-29,35,40,42,44,46]	n=3 [36,37,45]
Sample size sBCC	≤ 30	> 30
	86.7 (76.0 – 97.4), I^2 66.0%	85.8 (81.4 – 90.2), I^2 76.3%
	n=5 [21,24,28,42,44]	n=10 [22,23,27,29,35-37,40,45,46]
tumor diameter (inclusion criteria)	≤ 2 cm	> 2 cm
	87.4 (83.1 – 91.6), I^2 70.9%	80.6 (75.8 – 85.4), I^2 0.0%
	n=12 [22-24,28,29,35-37,40,42,44,45]	n=3 [21,27,46]
Fixed treatment protocol	Yes	No
	86.6 (82.4 – 90.7), I^2 74.9%	82.3 (68.4 – 96.2), I^2 47.0%
	n=13 [21-24,27-29,35,37,40,42,45,46]	n=2 [36,44]
Treatment duration	≤ 6 weeks	> 6 weeks
	86.8 (82.2 – 91.4), I^2 77.3%	84.1 (75.5 – 92.7), I^2 58.2%
	n=10 [21-23,27,29,35,37,40,42,46]	n=5 [24,28,36,44,45]
Histological residue verification	Yes	No
	85.3 (80.2 – 90.3), I^2 70.4%	88.7 (83.1 – 94.4), I^2 60.9%
	n=10 [22-24,27-29,37,42,45,46]	n=5 [21,35,36,40,44]
Quality score	≤ 5	> 5
	87.2 (81.0 – 93.4), I^2 69.3%	85.0 (79.3– 90.7), I^2 78.0%
	n= [21,22,36,37,42,44-46]	n=7 [23,24,27-29,35,40]

RCT, randomized controlled trial; I^2, index for heterogeneity; sBCC, superficial basal cell carcinoma. Data are given as pooled estimates of proportion with complete response (95% confidence interval).

Table 5. Photodynamic therapy studies. Pooled estimates of proportion with complete response (with 95% confidence intervals) in subgroups of studies according to study and tumor characteristics.

PDT (n=13 studies)		
RCT	Yes	No
	80.3 (70.8 – 89.8), I^2 89.9%	79.4 (65.8 – 93.0), I^2 95.4%
	n=4 [30-32,34]	n=9 [45,47-49,51-54]
Publication year	≤ 2003	> 2003
	81.5 (69.0 – 94.1), I^2 95.9%	77.2(66.0 – 88.4), I^2 87.5%
	n=8 [34,47-49,51-54]	n=5 [11,30-32,45]
Funding	Yes	No
	87.6 (83.5 – 91.7), I^2 0.0%	78.1 (66.8 – 89.8), I^2 954%
	n=2 [30,31]	n=11 [11,32,34,45,47-49,51-54]
Sample size sBCC	≤ 30	> 30
	74.9 (50.5 – 99.3), I^2 56.4%	83.2 (75.9 – 90.4), I^2 94.2%
	n=4 [45,49,53,54]	n=9 [11,30-32,34,47,48,51,52]
Fixed treatment protocol	Yes	No
	80.1 (70.7 – 89.5)	-
	n=12 [30-32,34,45,47-49,51-54]	n=1 [11]
Photosensitizer	Aminolaevulinic acid	Methylaminolevulinate
	79.0 (67.7 – 90.4), I^2 95.5%	78.8 (67.6 – 89.9), I^2 80.6%
	n=10 [11,32,34,47-49,51-54]	n=4 [30-32,45]
Light source	Standard	Special
	84.8 (75.8 – 93.7), I^2 90.5%	72.3 (51.7 – 92.9), I^2 97.2%
	n=8 [11,30-32,45,49,51,54]	n=5 [34,47,48,52,53]
Histological residue verification	Yes	No
	81.7 (72.2 – 91.3), I^2 26.2%	80.1 (69.8 – 90.4), I^2 95.8%
	n=3 [45,47,51]	n=10 [11,30-32,34,48,49,52-54]
Quality score	≤ 4	> 4
	92.0 (84.2 – 99.8), I^2 77.7%	72.5 (59.4 – 85.6), I^2 95.9%
	n=5 [30,47,49,53,54]	n=8 [11,31,32,34,45,48,51,52]

RCT, randomized controlled trial; I^2, index for heterogeneity; sBCC, superficial basal cell carcinoma. Data are given as pooled estimates of proportion with complete response (95% confidence interval).

Discussion

This review of the literature on the treatment of sBCC in RCTs and non-RCTs provides evidence that treatment with imiquimod or PDT results in nearly equal tumor-free survival rates one year after treatment. However, this review reveals that RCTs with direct comparisons of imiquimod with PDT are totally lacking and that the number of studies with follow-up longer than one year after treatment is very limited. Studies reporting on the effect of treatment after pulsed dye laser, 5-FU, cryotherapy, surgical excision and PEP005 were scarce. Therefore, reliable evaluation of the results of these treatment modalities was not possible.

The extensive search of the literature revealed that evidence on the comparative effectiveness of treatment modalities of sBCC is not widely available. We experienced various difficulties with inclusion of possible relevant studies in this systematic review. Many studies used heterogeneous study populations, including patients with genetic skin disorders, different BCC subtypes, both histologically and clinically proven sBCC prior to treatment, and both primary and recurrent sBCC, whereas subgroup analyses to provide results for separate groups were not performed. Therefore, we had to exclude these studies. Most of the studies included in the review had limited follow-up. Long-term follow-up is essential to predict the probability of treatment success after many years. Although most recurrences occur within the first two years after treatment, treatment failure is also reported after a longer follow-up period.[30,31,35]

This review reveals a lack of head-to-head comparison RCTs, in which the effects of treatment with imiquimod and PDT are directly compared.[57] Such RCTs provide the most rigid and valid evidence on the relative effects of different interventions. The results from the present review are based on indirect comparison of both treatment modalities and can be biased by differences in study population and design between imiquimod and PDT studies. The literature search points to a need for well-designed RCTs comparing one or more treatment modalities with long-term follow-up. Such trials guarantee comparability of patients who are assigned to different treatments and allow for more valid conclusions on the relative effectiveness of competing treatment options.

A common problem in meta-analysis is the large heterogeneity of study results. The subgroup analyses that were performed to identify the sources of the large variation in success rates suggest that several study characteristics may have led to overly optimistic estimates. An interesting finding in this respect is that imiquimod studies using histological verification of tumor clearance reported a higher failure rate than those with only a clinical verification.[40,42,58] This discrepancy was not found in PDT studies. The most

accurate way to determine tumor clearance is complete excision, as in a single biopsy sample error is still possible. However, long-term follow-up and evaluation of non-invasive treatment effects are impossible after surgical excision. In daily practice, clinical examination will reveal residues and recurrences as sBCC will eventually develop into a visible tumor on long-term follow-up.

An interesting finding of this systematic review is that the effectiveness of PDT may strongly depend on the number of cycles used. When repetitive PDT treatments are used, the pooled estimate increased for both PDT complete tumor response (75.6% to 79.0%) and tumor-free survival (76.2% to 84.0%). Therefore, PDT illumination might result in optimizing clinical outcome of treatments by fractionating ALA-PDT or providing two cycles of MAL-PDT.[30,31,43,48,49,59] Disadvantages of more frequent illuminations will be higher costs and more frequent treatment appointments. As not all patients are capable or willing to visit the hospital multiple times for PDT treatment, and other patients are not able or willing to apply imiquimod cream for six weeks, treatment choice will also depend on patient preferences and tumor characteristics.

Both the subgroup analyses and the Egger's test indicate that studies with smaller sample size showed higher success rates than studies with a larger sample size. This finding is suggestive of publication bias. This is a widespread problem and arises from the fact that small studies showing favorable results are more likely to be published and submitted for publication than small studies showing less favorable results. The presence of publication bias may imply that the actual benefit from PDT and imiquimod is lower than suggested by the studies included in this review.

In conclusion, treatment of sBCC with imiquimod or PDT results in similar long-term tumor-free survival probabilities. Treatment results after PDT might be optimized by repetitive treatments. Better designed large RCTs with a head-to-head comparison of current treatment modalities for sBCC with long-term follow-up are needed to establish the relative effectiveness of the various therapeutic options. Information from such trials enable more evidence-based recommendations in treatment of sBCC in the future.

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CHAPTER 3.2

Three year follow-up results of photodynamic therapy versus imiquimod versus fluorouracil for treatment of superficial basal cell carcinoma: a single blind, non-inferiority, randomized controlled trial

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Abstract

Background: We previously assessed in a randomized controlled trial whether the effectiveness of imiquimod and fluorouracil cream were non-inferior to methylaminolevulinate (MAL) photodynamic therapy (PDT) in patients with superficial basal cell carcinoma (sBCC). The results indicated that imiquimod was superior and fluorouracil not inferior to MAL-PDT in terms of one year tumor-free survival.

Objectives: Because part of the treatment failure occurs after more than one year post treatment, the tumor-free survival of imiquimod and fluorouracil versus MAL-PDT was evaluated at three years post treatment.

Methods: Patients who had participated in a single-blinded, non-inferiority, multicenter randomized controlled trial and had been tumor-free at one year post treatment, were approached for evaluation of treatment success after three years follow-up. Participants had been randomly assigned to MAL-PDT (two sessions with one week interval), imiquimod (once daily, five times a week for a period of 6 weeks), or fluorouracil (twice daily for 4 weeks). A research physician who was blinded to the assigned therapy clinically assessed the treated lesion for signs of treatment failure in patients who had been tumor-free at one year post treatment. Clinical treatment failures were histologically confirmed by a 3 mm punch biopsy. Cox proportional hazard models were used for calculation of hazard ratios with 95% confidence intervals. We used a pre-specified non-inferiority margin of 10% and performed an intention-to-treat as wells as per-protocol analysis. Additional subgroup analyses were performed to explore whether the relative treatment effect between therapies is consistent across subgroups defined by gender, age, tumor location, and tumor size. The trial is registered as ISRCTN 79701845.

Results: 601 patients were randomized, of which 442 were tumor-free at one year follow-up. Three years post treatment, 66 of 196 patients treated with MAL-PDT, 34 of 189 treated with imiquimod and 50 of 198 treated with fluorouracil had a treatment failure. The probability of tumor-free survival at three years post treatment was 58.0% for MAL-PDT (95% CI 47.8-66.9), 79.7% for imiquimod (95% CI 71.6-85.7), and 68.2% for fluorouracil (95% CI 58.1-76.3). The hazard ratio (HR) for treatment failure comparing imiquimod with MAL-PDT was 0.50 (95% CI 0.33-0.76, p=0.001). Comparison of fluorouracil with MAL-PDT resulted in a HR of 0.73 (95% CI 0.51-1.05, p=0.092), and comparison of fluorouracil with imiquimod in a HR of 0.68 (95% CI 0.44-1.06, p=0.091). Subgroup analysis showed a higher probability of treatment success for imiquimod versus MAL-PDT in all subgroups with an exception of elderly patients with a sBCC on the lower extremities. In this latter subgroup, the risk difference in tumor-free survival was 57.6% in favor of MAL-PDT.

Conclusions: Imiquimod is superior to MAL-PDT in treatment of sBCC. Fluorouracil is not-inferior to MAL-PDT but does not compare favorably with imiquimod. Therefore, imiquimod should be considered as first choice non-invasive therapy for most primary sBCC. MAL-PDT might be preferred in elderly with sBCC on the lower extremities.

Introduction

Basal cell carcinoma (BCC) is the most common type of skin cancer and its increasing incidence puts a large burden on health-care services worldwide.[1-3] BCC can be categorized into three main histological subtypes: superficial, nodular and aggressive.[4] While the majority of subtypes require surgery, superficial BCC (sBCC) can also be treated topically with alternatives such as photodynamic therapy (PDT), imiquimod cream, fluorouracil cream, cryosurgery or electrodessication and curettage.[5] The main advantages of non-invasive therapies like PDT, imiquimod and fluorouracil are a good cosmetic outcome, preservation of surrounding tissue and potential for home application of both creams.[6] However, to date there is a lack of randomized controlled trials with a long-term follow-up that compare effectiveness of non-invasive treatment modalities.[7] Therefore, in international BCC guidelines, no consensus has been reached on the first choice of non-invasive therapy for sBCC.[5]

To assess the effectiveness of imiquimod and fluorouracil versus methylaminolevulinate photodynamic therapy (MAL-PDT) for treatment of sBCC, a randomized controlled trial was conducted. The one year follow-up results of this non-inferiority study showed that imiquimod was superior and fluorouracil not inferior to MAL-PDT.[8] As long-term follow-up studies are lacking and part of sBCC may recur between one and three years' post treatment, a longer follow-up is required to evaluate the sustained treatment success.[7,9,10] We now report the three year follow-up results of this study. In addition, we have performed exploratory (not driven by prior hypotheses) subgroup analysis within this non-inferiority trial at three years follow-up.

Materials and methods

Study design

This study compared long-term results between treatment with MAL-PDT and imiquimod or fluorouracil cream in patients with sBCC who participated in a non-inferiority, randomized controlled trial that was conducted in seven hospitals in the southern part of The Netherlands.[8] Patients were recruited at the departments of Dermatology between March 2008 and August 2010. Eligible patients had a histologically proven, primary sBCC.

Randomization and masking

Participants were randomly assigned to either MAL-PDT, imiquimod, or fluorouracil in a 1:1:1 ratio. Randomization was stratified by age (≤ 60 years vs. > 60 years) and tumor location (head/neck vs. other). The research physician who assessed the treated lesions for signs of treatment failure was blinded to the assigned treatment modality and was not involved in the treatment. Patients and treating physicians were not masked for the assigned therapy. Statistical analysis was performed by two investigators not blinded to allocation.

Procedures

Patients randomized to MAL-PDT were treated with one cycle of two treatments with one week interval. Imiquimod treatment required a period of 6 weeks in which patients applied the cream once daily (evening) for 5 consecutive days a week. Patient randomized to fluorouracil had to apply the cream twice daily (morning and evening) for a duration of 4 weeks. Additional treatment details have been described previously.[8]

Data collection

The primary outcome was the probability that a patient was free of clinical evidence of tumor at all three follow-up visits, which is referred to as the three year probability of tumor-free survival. The need for retreatment after histological verification was considered as treatment failure. During follow-up a physician blinded to treatment assignment clinically assessed lesions for signs of treatment failure. Clinically observed treatment failures were histologically confirmed by a 3 mm punch biopsy. Relevant baseline patient and tumor characteristics were used for definition of subgroups. The full study design and procedures have been previously described.[8,11]

Follow-up information

Patients who were tumor-free at one year follow-up, were invited for a follow-up visit to enable evaluation of a three year probability of tumor-free survival. For logistical reasons, follow-up visits were planned within a window of three months prior or three months subsequent to the actual three year follow-up date.

The study was performed in accordance with the Declaration of Helsinki. The national authority and the ethics committees of all participating centers approved the

study protocol. All patients gave written, informed consent before participation and the trial was registered as International Standard Randomized Controlled Trial (ISRCTN 79701845).

Statistical analysis

Recorded baseline patient and tumor characteristics were summarized per treatment with descriptive statistics. The original sample size was estimated at 197 patients per treatment group. This sample size enabled detection of an absolute difference in one year recurrence-free survival of 10% (non-inferiority margin) with a power of 80% and one-sided type I error of 5%. Hereby it was assumed that the proportion with no tumor recurrence at one year after treatment with MAL-PDT would be 80%.[12,13]

Time-to-event analyses were performed to account for differences in follow-up between patients. Data were censored at diagnosis of a treatment failure or when loss-to-follow-up occurred. Both intention to treat and per protocol analysis was performed. The cumulative probability of recurrence free survival at three years' post treatment was estimated using Kaplan Meier survival analysis. Cox proportional hazards models were used to calculate hazard ratios (HRs) for treatment failure with 95% confidence intervals (95% CI). To facilitate interpretation of HRs, the non-inferiority margin of 10% for absolute differences in survival probability was translated to a non-inferiority threshold on the relative risk scale based on the observed three year tumor-free survival probability (p0) in the MAL-PDT group (log 1-p0/log p0).[14] Reported p-values are two-tailed corresponding with a one-sided significance level of 2.5% for testing non-inferiority.

Subgroup analyses were performed for subgroups defined by patient and tumor characteristics such as gender, age, tumor location, and tumor size. Additionally, Cox regression models including terms for characteristic by therapy interaction were used to test for statistical significance. Therapy was coded by two dummy variables. All data were analyzed using SPSS version 22.0 (SPSS, Chicago, IL, USA) and STATA version 11.0 (STATA Corp, College Station, TX, U.S.A.).

Results

A total of 601 patients were randomized to MAL-PDT (n=202), imiquimod (n=198), or fluorouracil (n=201). The three study groups had a similar distribution of baseline characteristics, except for tumor size (Table 1). The mean tumor size of tumors treated with MAL-PDT was smaller than the mean size in both the imiquimod and fluorouracil

group. Of the 601 enrolled patients, 590 started treatment (Fig. 1). Eleven patients did not start treatment because they refused, withdrew, died, or were unresponsive on call. Following randomization, five cross-overs occurred before the assigned treatment was started, due to a strong preference for a different treatment group. One patient did not receive the allocated MAL-PDT but was treated with imiquimod, while one patient allocated to imiquimod and three patients allocated to fluorouracil received MAL-PDT. No treatment failures occurred between the three months and one year follow-up visits. During follow-up, protocol deviations occurred in four patients where the treating physician decided that there was a need for extra treatment due to clinical suspicion of tumor recurrence; surgical excision in two patients and non-invasive treatment in two patients. No histological evidence of tumor recurrence was found in the excised specimens and no histological verification was available for the patients treated with MAL-PDT. As these four patients could not be (further) assessed for recurrence during follow-up the were considered as lost to follow-up (Fig. 1).

At the end of follow-up, data were missing for 43 patients (7.4%) who were lost to follow-up (7 patients at three months, 19 patients at one year, and 17 patients at three years follow-up).

Three years post treatment, 66 patients treated with MAL-PDT, 34 patients treated with imiquimod, and 50 patients treated with fluorouracil had a treatment failure (Fig. 1). Twenty-nine treatment failures (15 PDT, 3 imiquimod, 11 fluorouracil) were diagnosed after more than one year follow-up. The median follow-up period in the study was 35 months (range 1-54).

Table 1. Distribution of patient and tumor characteristics

Characteristic	MAL-PDT (n=202)	Imiquimod cream (n=198)	Fluorouracil cream (n=201)
Sex, M/F	96/106	102/96	106/95
Median age in years (range)	63 (26-87)	62 (30-91)	64 (35-86)
Tumor location			
Head/neck	24 (12%)	23 (12%)	31 (15%)
Trunk	119 (59%)	121 (61%)	120 (60%)
Upper extremities	32 (16%)	26 (13%)	27 (13%)
Lower extremities	27 (13%)	28 (14%)	23 (11%)
Median tumor size in mm^2 (range)	52 (5-1382)	63 (5-1413)	63 (9-5472)

F, female; M, male; MAL-PDT, methylaminolevulinate photodynamic therapy

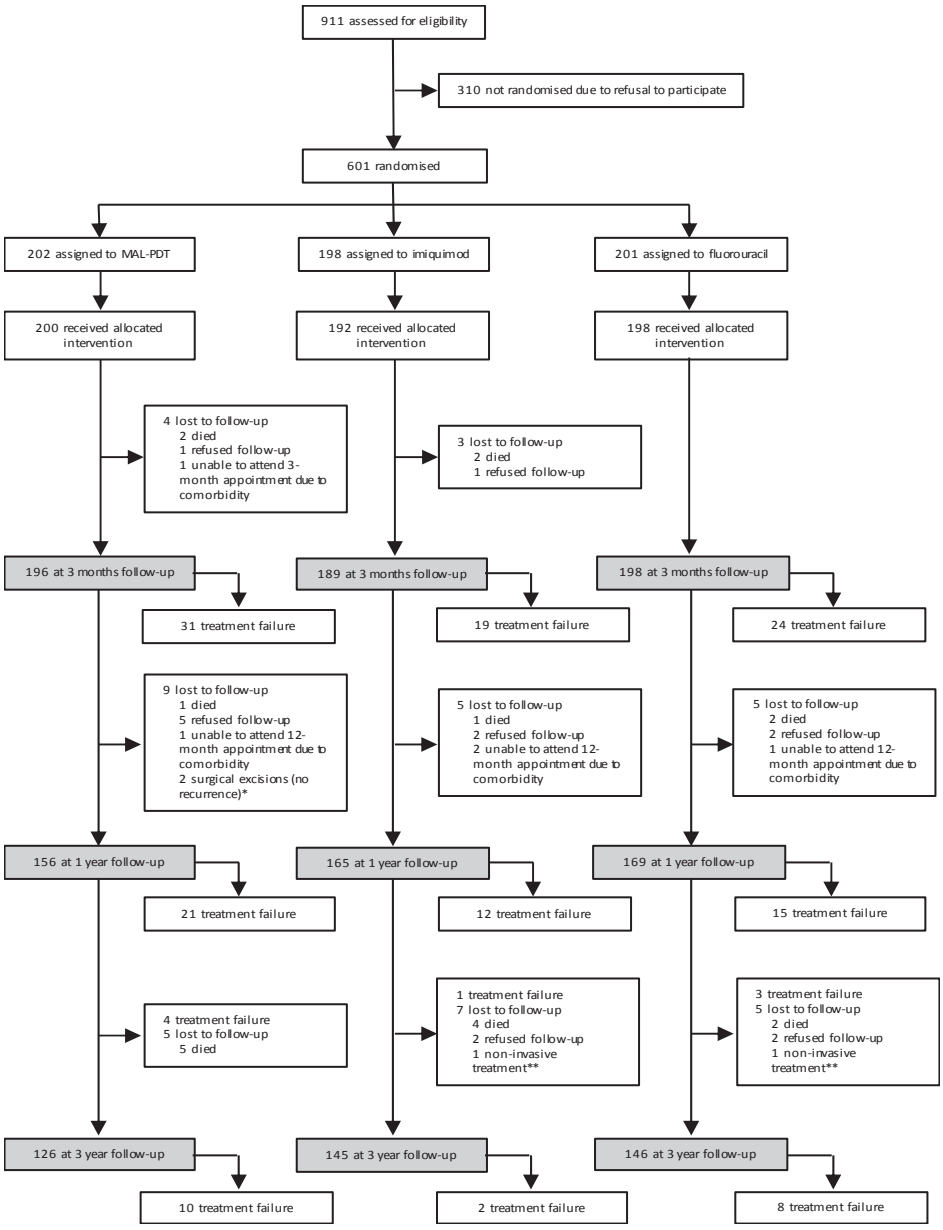

Fig. 1 Flow chart.

No treatment failures occurred between the three months and one year follow-up visits.

* Surgical excision was done in two patients due to a strong clinical suspicion of recurrence. Since these patients could not be assessed for recurrence during follow-up, they were considered lost to follow-up.

** Non-invasive treatments were given in two patients due to a strong clinical suspicion of recurrence. Since these patients could not be assessed for recurrence to the primary non-invasive treatment during follow-up, they were considered lost to follow-up.

Probability of tumor-free survival

Estimates of the one year and three year cumulative probability of tumor-free survival are presented in Table 2. According to the intention-to-treat analysis, probability of tumor-free survival at three years was 58.0% for MAL-PDT (95% CI 47.8-66.9), 79.7% for imiquimod (95% CI 71.6-85.7), and 68.2% for fluorouracil (95% CI 58.1-76.3).

Based on an estimated three year tumor-free survival probability of 58% for the MAL-PDT group, the non-inferiority margin of 10% for absolute differences in survival probability translates to a non-inferiority threshold for the HR of 1.35 (log 0.48/log 0.58).[14] Consequently, non-inferiority of both creams to MAL-PDT can be concluded if the 95% CI of the corresponding HRs are entirely below 1.35. Superiority of both creams to MAL-PDT can be concluded if the 95% CI of the corresponding HRs are entirely below 1.0. Based on an estimated three year tumor-free survival probability of 79.7% for imiquimod, the non-inferiority margin of 10% for absolute differences in survival probability between imiquimod and fluorouracil translates to a non-inferiority threshold for the HR of 1.59 (log 0.697/log 0.797). Superiority of imiquimod to fluorouracil can be concluded if the 95% CI of the corresponding HRs are entirely below 1.0.

Table 2. Cumulative probability of tumor-free survival at one and three year post treatment.

	One year		Three year	
	ITT	PP	ITT	PP
MAL-PDT	72.8% (66.8-79.4)	72.8% (65.9-78.6)	58.0% (47.8-66.9)	58.3% (48.3-67.1)
Imiquimod	83.4% (78.2-88.9)	83.4% (78.2-88.9)	79.7% (71.6-85.7)	79.7% (71.6-85.7)
Fluorouracil	80.1% (74.7-85.9)	79.5% (73.1-84.6)	68.2% (58.1-76.3)	68.1% (57.8-76.4)

ITT, intention-to-treat; MAL-PDT, methylaminolevulinate photodynamic therapy; PP, per-protocol.

At three years post treatment, the HR for treatment failure comparing imiquimod with MAL-PDT was 0.50 (95% CI 0.33-0.76, p=0.001) (Table 3). Comparison of fluorouracil with MAL-PDT resulted in a HR of 0.73 (95% CI 0.51-1.05, p=0.092), and comparison of fluorouracil with imiquimod in a HR of 0.68 (95% CI 0.44-1.06, p=0.091).

These results were nearly identical for the per protocol analysis: imiquimod compared with MAL-PDT showed a HR of 0.50 (95% CI 0.33-0.76, p=0.001), fluorouracil compared with MAL-PDT a HR of 0.73 (95% CI 0.51-1.06, p=0.095), and fluorouracil with imiquimod a HR of 0.69 (95% CI 0.44-1.06, p=0.095) (Table 3).

Table 3. Absolute differences and hazard ratios with 95% CI of tumor-free survival at three year follow-up.

	Intention to treat analysis			Per protocol analysis		
	Difference (%)	HR (95% CI)	p	Difference (%)	HR (95% CI)	p
Imiquimod vs. MAL-PDT	21.7	0.50 (0.33 - 0.76)	0.001	21.4	0.50 (0.33 - 0.76)	0.001
Fluorouracil vs. MAL-PDT	10.2	0.73 (0.51 - 1.05)	0.092	9.8	0.73 (0.51 - 1.06)	0.095
Fluorouracil vs. imiquimod	-11.5	0.68 (0.44 – 1.06)	0.091	-11.6	0.69 (0.44 – 1.06)	0.095

CI, confidence interval; HR, hazard ratio; MAL-PDT, methylaminolevulinate photodynamic therapy; p, significance; vs., versus.

Subgroup analysis for treatment success

The relative effect of imiquimod versus MAL-PDT was dependent on age, sex, localization and sBCC size. Imiquimod was superior to MAL-PDT in the subgroups of females, patients aged ≤ 60 years, sBCC on the head/neck and trunk, and tumors sized > 60 mm^2 (Table 4). In the other subgroups, imiquimod was also associated with a higher probability of treatment success (but no superiority). An exception was found for the subgroup of sBCC localized to the lower extremities, where a significant lower probability of treatment success for imiquimod versus MAL-PDT was found with a difference in success percentage favoring MAL-PDT of 25.2% (HR 2.07, 95% CI 0.94 - 4.57, p=0.070). This finding was further explored by stratifying patients with sBCC on the lower extremities by age (≤ 60 years vs. > 60 years). Within the subgroup of older patients with sBCC on the lower extremities, three year tumor-free survival was lower after imiquimod (36.2%) than after MAL-PDT treatment (93.8%). Within the subgroup of younger patients, treatment success was observed at 100% subsequent to imiquimod compared with 55.6% following MAL-PDT.

Table 4. Cumulative probability of tumor-free survival, between group differences and hazard ratios with 95% CI at three years post treatment.

Characteristic	Cumulative probabilities of treatment success (%, 95% CI)		Difference (%)	HR (95% CI)	
	PDT (n=196)	Imiquimod (n=189)	Imiquimod vs. MAL-PDT	Imiquimod vs. MAL-PDT	p
Gender					
Male	57.6 (41.5-7.08)	74.2 (64.0-82.0)	16.6	0.94 (0.58 - 1.52)	0.786
Female	58.4 (45.1-69.6)	85.4 (70.8-93.1)	27.0	0.30 (0.16 - 0.59)	<0.001
				Interaction p=0.029	
Age					
≤ 60 years	51.0 (35.3-64.7)	86.6 (76.5-92.5)	35.6	0.30 (0.15 - 0.58)	<0.001
> 60 years	63.8 (50.7-74.3)	75.0 (62.8-83.7)	11.2	0.90 (0.56 - 1.46)	0.677
				Interaction p=0.049	
Tumor location					
Head/neck	49.7 (28.7-67.6)	79.2 (53.5-91.6)	29.5	0.42 (0.15 - 1.22)	0.112
Upper extremities	71.5 (48.8-85.5)	75.0 (39.3-91.5)	3.5	0.73 (0.23 - 2.25)	0.578
Lower extremities	81.2 (56.0-92.8)	56.0 (35.5-72.3)	-25.2	2.07 (0.94 - 4.57)	0.070
Trunk	52.1 (37.8-64.5)	87.6 (79.9-92.4)	35.5	0.38 (0.21 - 0.67)	0.001
				Interaction p=0.001	
Tumor size[a]					
≤ 60 mm^2	60.7 (47.1-71.9)	74.4 (60.0-84.3)	13.7	0.77 (0.46 - 1.30)	0.323
> 60 mm^2	54.9 (39.7-67.7)	84.1 (75.1-90.1)	29.2	0.43 (0.24 - 0.77)	0.005
				Interaction p=0.84	

CI, confidence interval; HR, hazard ratio; MAL-PDT, methylaminolevulinate photodynamic therapy; p, significance; vs., versus. P-values in bold did not reach the non-inferiority threshold of the hazard ratio. [a] Data on tumor size were available in 192 of 196 superficial basal cell carcinoma (sBCC) treated with MAL-PDT and in 186 of 189 sBCC treated with imiquimod.

Discussion

Our results showed that imiquimod is superior and fluorouracil not inferior to MAL-PDT in treatment of sBCC at three years follow-up. Therefore, imiquimod should be considered as a first choice treatment for sBCC in terms of efficacy.

The finding that around 80% of patients with sBCC are tumor-free after imiquimod treatment at three years follow-up is in accordance with results from previous studies. A recent randomized controlled trial by Bath-Hextall et al., that compared surgical excision with imiquimod, found that imiquimod was successful in 85.1% of sBCC at three

years post treatment.[15] Two other studies reported a three year cumulative probability of tumor-free survival of 82% and 85%.[9,16]

In the present study, the probability of tumor-free survival three years following MAL-PDT treatment was 58.0%. To our knowledge, only one other randomized controlled trial on MAL-PDT with at least three years of follow-up has been performed to enable comparison of results.[17] This study included 114 histologically proven primary sBCC that were treated with one or two MAL-PDT cycle. Results showed a higher cumulative probability of tumor-free survival of 70% at three years after MAL-PDT treatment, which may be explained by the fact that incomplete responders received two further MAL-PDT sessions (n=20) 3 months subsequent to the first treatment. In our study, sBCC were treated with the most current European protocol for MAL-PDT: one MAL-PDT cycle consisting of two treatments with one week interval.[12] Incomplete or non-responders were not retreated. While a second treatment in the case of a non-responder three months post treatment might increase tumor-free survival, a second PDT cycle will further increase the already high treatment costs and requires two additional patient visits. [7,18]

Only one previous study by Gross et al. investigated the effectiveness of topical fluorouracil twice daily for six to 12 weeks in 31 sBCC.[19] The histological clearance rate at 3 weeks post treatment was 90%. No long-term follow-up studies have, as yet, been reported.

A previous systematic review and a network geometry review have shown that there is a lack of head-to-head comparison studies for treatment of sBCC.[7,20] The present study fulfills the need for head-to-head comparison studies by investigating the relative treatment effects between non-invasive therapies. Furthermore, it is the first study with a long-term follow-up of fluorouracil in treatment of sBCC. Currently, according to the European BCC guideline by Trakatelli et al., imiquimod and PDT are both considered good treatment options for low-risk sBCC.[5] In addition, surgical excision (effectiveness of 89-98%) is considered reasonable but not essential.[5,15,21] Based on our findings, we suggest that imiquimod should be considered as first choice treatment in most primary, low-risk sBCC.

In order to optimize treatment success, it is of great value to select the most effective treatment for an individual patient with a sBCC. We have previously reported subgroup analyses showing that imiquimod is more effective than MAL-PDT in most sBCC, with the exception of sBCC localized on the lower extremities in older patients.[11] At three years of follow-up, conclusions remain unaltered: MAL-PDT may be preferable in elderly patients with sBCC on the lower extremities. MAL-PDT may also be an alternative therapy in a small group of patients for whom cream application is not feasible for practical reasons.

If imiquimod is the treatment of first choice, where stands fluorouracil? Both creams have an equal cosmetic outcome and risk of local adverse events.[8] Fluorouracil has the advantage of lower costs compared to imiquimod. In The Netherlands, the costs for a tube fluorouracil (40 grams) are €29.19 and for imiquimod (36 sachets) €170.87.[22] At 12 months follow-up, both creams were cost-effective compared to MAL-PDT although the cost-savings were larger for fluorouracil compared to imiquimod.[18] However, at three years follow-up, the additional cost-savings of fluorouracil compared to imiquimod may decrease as the higher number of treatment failures require an increase in surgical excisions. Perhaps even more important is the fact that between one and three year follow-up, more recurrences were diagnosed in the fluorouracil group compared with the imiquimod group. This higher number of recurrences may necessitate a longer yearly follow-up for patients who are treated with fluorouracil. Consequently, the additional cost-savings of fluorouracil compared to imiquimod will in all probability decrease substantially since the costs of a yearly outpatient visit per patient have to be taken into account. Our study showed that the majority of imiquimod treatment failures (31/34) already had occurred within one year after therapy. We therefore suggest that one follow-up visit at one year post treatment is sufficient in patients with a solitary sBCC treated with imiquimod. These patients should be informed and instructed about self-examination of the skin after one year because there remains a small risk of a recurrence and they have an increased risk of developing multiple BCC.

One limitation to a randomized controlled trial is that only patients who are willing to participate in a trial, and who have no specific treatment preference, can be randomized. In daily practice, not all patients can be motivated or are able to apply a cream daily for six weeks. For those patients, a hospital based treatments such as surgical excision, curettage and electrodessication, or PDT might be preferable. An additional limitation is that post treatment biopsies were not performed to confirm lack of tumor in this study. The study was designed to make long-term clinical follow-up possible, in accordance with daily clinical practice, and large biopsies would have interfered with a clinical follow-up. However, it is possible that clinical exam missed some subtle recurrences that will manifest after the end of the study. Furthermore, as patient preferences are very important in choosing an individual treatment, another limitation is that we have not evaluated the patient reported outcomes on preference three years post treatment.

In summary, our study has shown that imiquimod is superior to MAL-PDT and that treatment outcome of fluorouracil compares unfavorably with results after imiquimod treatment in patients with sBCC. Imiquimod should be considered as the first choice non-invasive treatment in most primary, low-risk sBCC. MAL-PDT might be preferred in elderly with sBCC on the lower extremities.

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CHAPTER 3.3

Photodynamic therapy versus topical imiquimod for treatment of superficial basal cell carcinoma: a subgroup analysis within a non-inferiority randomized controlled trial

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Abstract

Background: A recent non-inferiority randomized controlled trial (RCT) indicated that imiquimod can be considered as superior to methylaminolevulinate photodynamic therapy (MAL-PDT) in treatment of superficial basal cell carcinoma (sBCC). Knowledge of treatment effectiveness in subgroup of patients is of great value in clinical practice to select the most effective treatment for an individual patient with sBCC.

Objectives: To explore whether the relative treatment effect of MAL-PDT and imiquimod is consistent across subgroups defined by patient and tumor characteristics.

Methods: Data were derived from a single-blinded, non-inferiority, multicenter RCT comparing MAL-PDT, topical imiquimod and fluorouracil (ISRCTN79701845). Treatment success was defined as free of tumor recurrence at 12-months follow-up. Subgroup analyses were performed for subgroups defined by sex, age, tumor location and tumor size.

Results: Two hundred and two patients received MAL-PDT and 198 received imiquimod. The superiority of imiquimod vs. MAL-PDT was observed in subgroups of females, sBCC on the trunk and large tumors with risk differences in favor of imiquimod of 18.4% (95% CI 7.8-29.0%), 21.0% (95% CI 10.9-31.1%), 18.9% (95% CI 7.1-30.7%), respectively. Higher probability of treatment success for imiquimod vs. MAL-PDT was consistently found in all other subgroups with exception of sBCC localized on the lower extremities in older patients. In the latter subgroup, the risk difference at the expense of imiquimod was -57.3% (95% CI -81.7 to -32.9%).

Conclusions: Imiquimod remains the first choice treatment for sBCC in terms of effectiveness. In older patients with sBCC on the lower extremities MAL-PDT might be preferred. Results should be interpreted carefully as subgroup analyses were exploratory and not driven by prior hypotheses.

Introduction

Worldwide, the incidence rates of non-melanoma skin cancers (NMSC) are growing to epidemic proportions. The incidence of NMSC varies worldwide but true rates are estimated to be much higher due to incomplete registration.[1] Approximately 80% of these NMSC are basal cell carcinoma (BCC) and in The Netherlands, 1 in every 5-6 persons will develop a BCC during their life.[2] Therefore, BCC puts a large burden on health care services.[3]

The gold standard for treatment of BCC is surgery.[4,5] However, non-invasive treatment modalities like photodynamic therapy (PDT) and imiquimod have been thoroughly investigated and seem good alternatives for surgery in superficial BCC (sBCC).[4-7] We recently published a randomized controlled trial (RCT) showing that imiquimod was superior to methylaminolevulinate (MAL)-PDT for treatment of sBCC.[7] In addition, imiquimod is a more cost-effective therapy compared to MAL-PDT in treatment of sBCC.[8] In this trial, a non-inferiority margin of 10% was used because a between group difference in recurrence risk at 1 year after treatment within these limits was considered as acceptable. MAL-PDT and imiquimod are both associated with a lower success rate (73-83%) compared to surgery (89-100%) but have the benefits of reducing the burden on treating physicians, treatment costs and patient discomfort.[7,9-11]

MAL-PDT and imiquimod have a different pharmacological mode of action and treatment effects may depend on baseline characteristics, such as sex, age, tumor location and tumor size.[12,13] Identification of subgroups of patients that differ in response to MAL-PDT and imiquimod is of great value in clinical practice to select the most effective treatment for an individual patient with sBCC. For this reason, the objective of this study was to explore whether the relative treatment effect of MAL-PDT and imiquimod is consistent across subgroups defined by such baseline characteristics.

Materials and methods

Study design

Data were derived from a single-blinded, non-inferiority, randomized controlled multicenter trial (ISRCTN 79701845) on treatment success in sBCC of patients who were randomly assigned to MAL-PDT or topical imiquimod treatment.[7] The study design and procedures have been described elsewhere. In summary, the study population consisted of patients attending the dermatology departments of seven participating hospi-

tals in the southern part of The Netherlands, who had a primary, histologically proven sBCC on a 3 mm punch biopsy. A sBCC was defined as a tumor composed of basaloid cell nests which are attached intermittently along the epidermis. One sBCC per patient was included. Patients were excluded when using immunosuppressive drugs or suffering from genetic skin cancer disorders. In addition, breastfeeding or pregnant women were excluded. Also, recurrent tumors, pigmented BCC and sBCC located in the H-zone were not eligible. The Institutional Review Boards approved the study protocol and all patients gave written informed consent before participation. The trial was conducted according to the Declaration of Helsinki Principles.

Randomization and masking

Between March 2008 and August 2010, patients were randomly assigned to MAL-PDT and imiquimod in a 1:1 ratio. In order to ensure concealment of allocation, randomization was done by phone with a computer-generated numbered list with random permuted blocks of six. The list was prepared by an investigator who was not clinically involved in the trial. Patients were stratified by age (≤ 60 year and > 60 years) and tumor location (head/neck region and other location). An independent investigator, who was blinded to the assigned treatment modality and was not involved in the treatment, evaluated the tumors for treatment response. Patients and treating physicians were not masked for the assigned therapy. Statistical analysis was performed by two investigators who were blinded to allocation.

Follow-up

Follow-up visits took place 3 and 12 months after last treatment day by an independent investigator. Treatment failure was defined as residue or recurrence within 1 year post treatment that was histologically confirmed by a 3 mm punch biopsy.

Procedures

Prior to PDT treatment, lesions received a non-traumatic surface preparation. MAL 16% cream (Metvix, Galderma SA, Penn Pharmaceutical Services, Gwent, UK) was applied on the sBCC and 5-10 mm of the surrounding skin. The area was covered with an occlusive dressing (Tegaderm, 3M, Leiden, The Netherlands) and aluminum foil. Three hours after application the area was illuminated for around 7 minutes (630 nm, 37 J/cm^2) with a light emitting diode (LED) light source, Omnilux (Waldmann, Photo-

therapeutics, London, UK) or Aktilite (Galderma SA, Lausanne, Switzerland). The treatment was repeated after 1 week.

Participants randomized to 5% imiquimod (Aldara, Meda AB, Solna, Sweden) had to apply the cream once daily for five consecutive days during six weeks. Patients were instructed to apply the cream on the tumor and 5-10 mm of surrounding healthy skin.

Data collection

Baseline data on patient and tumor characteristics such as sex, age, tumor location and tumor size, were used for definition of relevant subgroups. Tumor surface area was calculated in mm^2 by multiplying the short tumor axis by the long axis.

Statistical analysis

A pre-specified margin of 10% was used to evaluate non-inferiority for imiquimod to MAL-PDT in the treatment of sBCC. This margin was chosen based on a previous study in which an increase in recurrence risk by maximally 10% was considered acceptable.[11] sBCC is a non-aggressive slowly growing skin cancer and residue or recurrent tumor is easily treated with surgical excision. In order to establish non-inferiority with a power of 80% and one-sided type I error of 5%, a sample size of 197 participants per group was acquired. We presumed that the proportion of treatment success one year after MAL-PDT is 80%.[5,6] The distribution of patient and tumor characteristics was summarized by descriptive statistics. Absolute numbers and percentages were used for categorical data and median values with range for continuous data. Subgroups were defined by patient and tumor characteristics at baseline. Within these subgroups, proportions with treatment success and differences in success percentages between treatment groups with corresponding 95% confidence intervals (CIs) were calculated. Subgroup-treatment effect interaction was tested for statistical significance using logistic regression analysis with treatment outcome as dependent variable and baseline characteristic, treatment group and an interaction term as independent variables. Baseline characteristics with more than one category were entered as dummy variables. P-values ≤ 0.05 were considered to indicate statistical significance. All data were analyzed by using SPSS version 20.0 (SPSS, Chicago, IL, USA) and STATA version 11.0 (STATA Corp, College Station, TX, U.S.A.).

Results

Patients and tumors

A total of 400 patients were randomized to MAL-PDT (n=202) or imiquimod (n=198) (Fig. 1). Relevant endpoints were available for 385 patients (MAL-PDT n=196 and imiquimod n=189). Table 1 presents the distribution of patient and tumor characteristics according to treatment group.

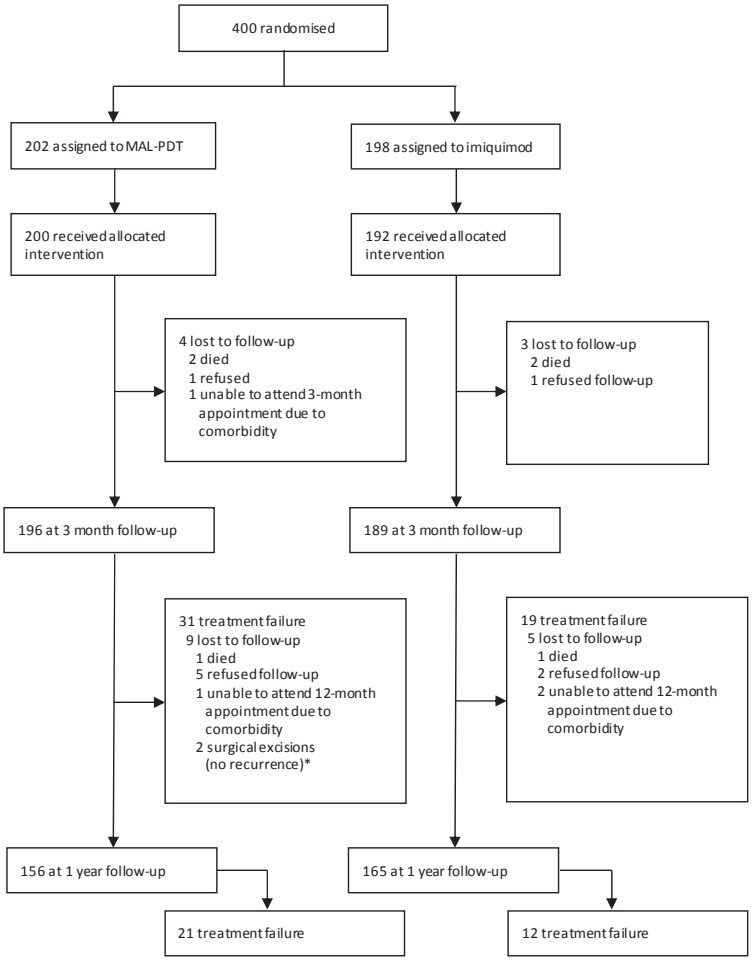

Fig. 1 Flow chart modified from Arits et al.[7] MAL-PDT, methylaminolevulinate photodynamic therapy. *Surgical excision was done in two patients due to a strong clinical suspicion of a recurrence. Because these patients could not be assessed for recurrence during follow-up, they were considered lost to follow-up.

Table 1. Patient and tumor characteristics by treatment group.

	MAL-PDT (n=202)	Imiquimod cream (n=198)
Mean age; years (range)	63 (26 – 87)	62 (30 – 91)
Tumor location		
Head/neck	24 (12)	23 (12)
Trunk	119 (59)	121 (61)
Upper extremities	32 (16)	26 (13)
Lower extremities	27 (13)	28 (14)
Men	96 (48)	101 (51)
Tumor size; mm2 (range)	52 (5–1382)	63 (5–1413)
BCC in medical history	102 (50)	96 (48)

BCC, basal cell carcinoma; MAL-PDT, methylaminolevulinate photodynamic therapy. Continuous variables are expressed as median (range) and categorical variables as n (%).

Subgroup analysis for treatment success

Superiority of imiquimod versus MAL-PDT was observed in subgroups of females, patients aged ≤ 60 years, sBCC on the trunk and tumors with size > 60 mm^2 with differences in success percentages favoring imiquimod of 18.4% (95% CI 7.8-29.0%), 20.5% (95% CI 8.3-32.7%), 21.0% (95% CI 10.9-31.1%) and 18.9% (95% CI 7.1-30.7%), respectively (Table 2). A higher probability of treatment success (but no superiority) of imiquimod versus MAL-PDT was found for males, patients > 60 years, tumors on the head/neck and upper extremities and smaller sBCC. Logistic regression models including terms for treatment by subgroup interaction yielded statistically significant p-values for sex (p=0.029), age (p=0.032), tumor location (p<0.001) and tumor size (p=0.043)

Imiquimod was significantly less effective than MAL-PDT in sBCC localized on the lower extremities, with a difference in proportion with treatment success of -35.2% (95% CI -56.2. to -14.2%, p=0.003). We therefore performed additional analyses stratifying for sBCC location (lower extremities vs. other body sites).

Within the subgroup of sBCC on the lower extremities, treatment success after imiquimod was lower than after MAL-PDT in patients > 60 years (Table 3); treatment success was achieved in only 36.8% for imiquimod and 94.1% for MAL-PDT with a difference in success rate of -57.3% (95% CI -81.7 to -32.9%). This higher probability of treatment success for MAL-PDT compared to imiquimod was not seen in younger patients with a sBCC on the lower extremities.

Table 2. Proportions with treatment success and between group differences with 95% confidence intervals (CIs).

Characteristic	Probabilities of treatment success (%)		Between group differences (95% CI)	
	PDT (n=196)	Imiquimod (n=189)	Imiquimod vs. PDT	p
Sex				
Male	75.3 (70/93)	77.3 (75/97)	2.0 (-10.1 – 14.1)	0.746
Female	71.8 (74/103)	90.2 (83/92)	18.4 (7.8 – 29.0)	0.001
			Interaction p=0.029	
Age				
≤ 60 years	69.1 (56/81)	89.6 (69/77)	20.5 (8.3 – 32.7)	0.002
> 60 years	76.5 (88/115)	79.5 (89/112)	3.0 (-7.7 – 13.7)	0.586
			Interaction p=0.032	
Tumor location				
Head/neck	62.5 (15/24)	80.0 (16/20)	17.5 (-9.3 – 43.6)	0.205
Upper extremities	83.9 (26/31)	88.0 (22/25)	4.1 (-14.1 – 22.2)	0.663
Lower extremities	92.3 (24/26)	57.1 (16/28)	-35.2 (-56.2 – -14.2)	0.003
Trunk	68.7 (79/115)	89.7 (104/116)	21.0 (10.9 – 31.1)	<0.001
			Interaction p<0.001	
Tumor size[a]				
≤ 60 mm^2	78.3 (83/106)	80.0 (72/90)	1.7 (-9.7 – 13.1)	0.771
> 60 mm^2	68.6 (59/86)	87.5 (84/96)	18.9 (7.1 – 30.7)	0.002
			Interaction p=0.043	

PDT, photodynamic therapy. P-values <0.05 were considered to be statistically significant. [a]Data on tumor size were available in 192 of 196 superficial basal cell carcinomas (sBCC) treated with PDT and in 186 of 189 sBCC treated with imiquimod.

Table 3. Proportions with treatment success and between group differences with 95% confidence intervals for superficial basal cell carcinoma on lower extremities.

Characteristic	Probabilities of treatment success (%)		Between group differences (95% CI)	
	PDT (n=26)	Imiquimod (n=28)	Imiquimod vs. PDT	p
Sex				
Male	100.0 (4/4)	46.7 (7/15)	-53.3 (-79.0 – -28.1)	0.055
Female	90.9 (20/22)	69.2 (9/13)	-21.7 (-49.5 – 6.1)	0.100
Age				
≤ 60 years	88.9 (8/9)	100.0 (9/9)	11.1 (-9.4 – 31.6)	0.304
> 60 years	94.1 (16/17)	36.8 (7/19)	-57.3 (-81.7 – -32.9)	<0.001
Tumor size[a]				
≤ 60 mm^2	89.5 (17/19)	60.0 (9/15)	-29.5 (-57.9 – -1.1)	0.044
> 60 mm^2	100.0 (7/7)	54.5 (6/11)	-45.5 (-74.9 – -16.1)	0.036

CI, confidence interval; PDT, photodynamic therapy. P-values <0.05 were considered to be statistically significant. [a]Some incomplete data as noted in Table 2.

For sBCC not localized on the lower extremities, the relative benefits of imiquimod over MAL-PDT for males, older patients and small sBCC remained smaller than those for females and larger sBCC, but the interaction term for age by treatment was no longer statistically significant (p=0.580) (Table 4).

Table 4. Proportions with treatment success and between group differences with 95% confidence intervals after exclusion of superficial basal cell carcinoma on lower extremities.

Characteristic	Probabilities of treatment success (%)		Between group differences (95% CI)	
	PDT (n=170)	Imiquimod (n=161)	Imiquimod vs. PDT	p
Sex				
Male	74.2 (66/89)	82.9 (68/82)	8.7 (-3.5 – 20.9)	0.167
Female	66.7 (54/81)	93.7 (74/79)	27.0 (15.4 – 38.6)	<0.001
			Interaction p=0.022	
Age				
≤ 60 years	66.7 (48/72)	88.2 (60/68)	21.5 (8.1 – 34.8)	0.002
> 60 years	73.5 (72/98)	88.2 (82/93)	14.7 (3.8 – 25.6)	0.010
			Interaction p=0.580	
Tumor size[a]				
≤ 60 mm^2	75.9 (66/87)	84.0 (63/75)	8.1 (-4.1 – 20.3)	0.202
> 60 mm^2	65.8 (52/79)	91.8 (78/85)	26.0 (14.0 – 38.0)	<0.001
			Interaction p=0.042	

CI, confidence interval; PDT, photodynamic therapy. P-values <0.05 were considered to be statistically significant. [a]Some incomplete data as noted in Table 2.

Discussion

Our results consolidate superiority of imiquimod to MAL-PDT in sBCC in females, sBCC localized on the trunk and large tumors. In most other subgroups, imiquimod also resulted in better treatment results but the differences with MAL-PDT were less pronounced. Interestingly, imiquimod was less effective than MAL-PDT in patients > 60 years with a sBCC on the lower extremities.

The higher treatment success of imiquimod in females compared to males might be explained by the imiquimod modified immune response. The female estrogen has positive hormonal effects on cytokines in the immune response.[14] Estrogen stimulates the secretion of several cytokines, of which certain ones are also induced by the imiquimod

induced immune response.[15] Differences in adherence to medication regimens between men and women were excluded on the basis of the diaries patients completed.

The better treatment response of imiquimod in larger tumors is possibly due to the imiquimod induced immune response that reaches far into the surrounding tissue, even in subclinical tumor cells that may be present at the border. MAL-PDT might be less effective in larger sBCC because the light can scatter at the periphery. Therefore, peripheral tumor cells may be treated suboptimal by MAL-PDT and can remain.

The most remarkable finding of this study is that imiquimod was less effective than MAL-PDT in patients > 60 years with a sBCC on the lower extremities. We thought of a plausible explanation for this finding. There was no difference in adherence to medication regimen between young and old patients who applied imiquimod on the lower extremities. A hypothesis might be that elderly are less capable of leaning down when they apply the cream on their legs and, consequently, imiquimod is applied inadequately.

The results indicate that in patients > 60 years with a sBCC on the lower extremities treatment with MAL-PDT might result in more effective treatment than imiquimod treatment. Another reason for a preference of MAL-PDT instead of imiquimod in this specific subgroup might be the wound healing on the lower legs. It is well known that wound healing is often delayed on the lower extremity, especially on the lower legs. A result of treatment with imiquimod is erosion and ulceration. In older patients, often suffering from venous insufficiency, local wound infections, crural ulceration or erysipelas might be expected more frequently. In this trial only one local wound infection in a patient with a sBCC on the chest occurred during treatment with imiquimod.

Apart from the recently published RCT by Arits et al., there are no head-to-head comparison studies on the relative treatment success between MAL-PDT and imiquimod cream in sBCC to compare with our results.[7,9] However, there are a few non-comparative studies, four on PDT and two on imiquimod, that investigated possible determinants of treatment failure in BCC.

Fantini et al. examined possible clinical and pathological determinants of MAL-PDT response of BCC and found, in contrast to our results, that tumor location on the limbs was associated with higher risk of treatment failure.[16] In that study, patient age and sex were no determinants of PDT treatment response. Christensen et al. found that male sex was associated with treatment failure after 5-aminolaevulinic acid PDT.[17] Two other studies found that treatment failure occurred more often in large tumors.[18,19] These studies are different from our study as inclusion was not restricted to sBCC, which is likely to have influenced the results.

Two RCTs on imiquimod reported no significant association between age, tumor location or tumor size and treatment response.[20,21] However, the authors did not report whether this lack of significance was due to lack of effect or a small sample size. One of these studies reported better treatment response in women (95%) compared to men (87%), which is in line with our findings.[21]

We explored the relative treatment effect of MAL-PDT and imiquimod across subgroups in a randomized controlled trial. We emphasize that this result should be interpreted carefully as the subgroup analyses in this study were not driven by prior hypotheses and should be considered as exploratory. Our findings need to be validated in larger studies.

In conclusion, treatment success of imiquimod cream versus MAL-PDT was consistently higher across nearly all pre-specified subgroups. This implies that imiquimod cream remains the first choice treatment in most patients presenting with sBCC. However, in the subgroup of patients > 60 years with a sBCC on the lower extremities, MAL-PDT seems preferable, probably because of application difficulties.

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CHAPTER 3.4

Tumor thickness and adnexal extension of superficial basal cell carcinoma as determinants of treatment failure for methylaminolevulinate photodynamic therapy, imiquimod and 5-fluorouracil

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Abstract

Background: Non-invasive treatments are frequently used in treatment of superficial basal cell carcinoma (sBCC) because of better cosmetic results, lower costs, and less burden on health care services when compared with surgical excision. However, probability of treatment failure is higher after non-invasive therapies and may depend on histological tumor characteristics.

Objectives: We sought to investigate whether tumor thickness and adnexal extension are determinants of treatment failure in sBCC treated with topical methylaminolevulinate-photodynamic therapy (MAL-PDT), imiquimod or 5-fluorouracil (5-FU).

Methods: Data were derived from a randomized controlled trial on the effectiveness of MAL-PDT, imiquimod and 5-FU for treatment of sBCC (ISRCTN79701845). For tumors with treatment failure (n=112) and a randomly selected control group of tumors without treatment failure (n=224) data on tumor thickness and adnexal extension were retrospectively collected. Treatment failure was defined as a clinically and histologically persistent or recurrent tumor within one year post treatment.

Results: Tumor thickness of included patients ranged from 0.2 to 1.0 mm. Tumor thickness and adnexal extension of sBCC were not significantly associated with treatment failure of MAL-PDT, imiquimod or 5-FU.

Conclusions: There seems to be no need to determine tumor thickness or adnexal extension in sBCC before treatment.

Introduction

Basal cell carcinoma (BCC) is the most common type of skin cancer with a rapidly rising incidence worldwide.[1] Surgical excision is regarded to be the gold standard for treatment.[2,3] To reduce the burden on physicians and decrease health care costs, superficial BCC (sBCC) can also be treated with less invasive techniques such as photodynamic therapy (PDT), imiquimod, 5-fluorouracil (5-FU), cryosurgery, or electrodessication and curettage. In addition, PDT, imiquimod and 5-FU have the benefit of a better cosmetic outcome compared with surgical excision.[4] However, recurrence rates of non-invasive treatments are higher compared with surgery and range from 13% to 27% at one year post treatment.[5,6] Little is known about histological characteristics of sBCC that may influence treatment response of non-invasive therapies.

A sBCC is a basaloid tumor that grows continuously with the epidermis but tumor nests can reach within the papillary dermis.[7] The tumor sometimes grows deeply along hair follicles. We therefore hypothesized that thicker sBCC and tumors with extension along the hair follicle might partially fail to respond to the superficially working non-invasive therapies and lead to residue or recurrence of BCC. A few previous PDT studies have reported on tumor thickness as a histological determinant of treatment failure in BCC.[8-12] However, results were contradictory, possibly because of the fact that inclusion was not restricted to sBCC subtypes. Only one study focused solely on sBCC and showed that after treatment with imiquimod a tumor with a thickness > 0.4 mm was significantly more likely to reoccur than a thinner tumor.[13]

The objective of this study was to investigate whether increased tumor thickness and adnexal extension are determinants of treatment failure in sBCC treated with methylaminolevulinate (MAL)-PDT, imiquimod or 5-FU.

Methods

Patients and tumors

Data were derived from a recently published non-inferiority, randomized controlled trial on treatment success after treatment of sBCC with MAL-PDT, imiquimod or 5-FU (ISRCTN79701845). Details of the study design and procedures have previously been reported.[6] In summary, the study was conducted in 7 hospitals in the southern part of The Netherlands. Patients aged 18 years or older attending the department of Dermatology with a primary, histologically proven sBCC were eligible to participate in this

trial. A sBCC was defined as a tumor with small, islands of basaloid cells attached to the epidermis and restricted to the papillary dermis.[14] One tumor per patient was included. Patient related exclusion criteria were use of immunosuppressive drugs, genetic skin cancer disorders, breastfeeding and pregnancy. Recurrent sBCC, pigmented sBCC and tumors located in the H-zone of the face were also excluded. In addition, we also excluded patients who were at risk in terms of precautions, warnings and contraindications as indicated in the package insert for MAL-PDT, topical imiquimod, and topical 5-FU. The trial was approved by the Medical Ethics and Scientific Committee of the Maastricht University Medical Centre, The Netherlands. All patients provided written informed consent.

Follow-up

Treatment failure was evaluated by an investigator who was blinded to treatment allocation at 3 and 12 months after the last treatment day. The treatment area was examined to detect clinical residue or recurrent tumor. Clinical treatment failure was histologically confirmed by a 3 mm punch biopsy specimen.

Treatments

Participants randomized to PDT received a non-traumatic tumor surface preparation before treatment. MAL 16% cream (Metvix, Galderma SA, Penn Pharmaceutical Services, Gwent, United Kingdom) was applied on the tumor and 5-10 mm of the surrounding skin. The area was subsequently covered with an occlusive dressing (Tegaderm, 3M, Leiden, The Netherlands) and aluminum foil. After three hours, the area was illuminated with a light emitting diode light source, (Omnilux, Waldmann Phototherapeutics, London, United Kingdom) or Aktilite (Galderma SA, Lausanne, Switzerland), with an optimum wavelength of 630 nm. The same procedure was repeated after one week.

Patients who were treated with 5% imiquimod (Aldara, Meda AB, Solna, Sweden) had to apply the cream during six weeks, once daily (evening) for five following days. Patients randomized to 5% 5-FU (Efudix, Meda Pharmaceuticals, Amstelveen, The Netherlands) had to apply the cream for four consecutive weeks twice daily (morning and evening). Both creams had to be applied on the tumor and 5-10 mm of the surrounding skin.

Data collection

This was designed as a case-control study, where 112 patients with treatment failure were selected as cases and 224 patients with treatment success were randomly selected as control subjects. Hematoxylin-eosin stained histopathological slides of tumors were retrieved to obtain additional data on tumor thickness and adnexal extension. The histopathological slides of the initial diagnostic punch biopsy were retrospectively and independently judged by two observers who were blinded to case-control status: a resident in dermatology (M.H.R.) and a sixth-year medical student (L.v.K.). Both observers were intensively trained by a dermatopathologist (V.J.L.W.) in judging histological sections of sBCC. Maximum tumor thickness was measured with a 0.1 mm precise ocular micrometer from the top of the stratum granulosum to the deepest located tumor nest. The mean tumor thickness measured by the two observers was used for analysis. Adnexal extension of sBCC was recorded as present or absent and was defined as tumor cells along hair follicles growing deeper than the deepest located tumor nest (Fig. 1).

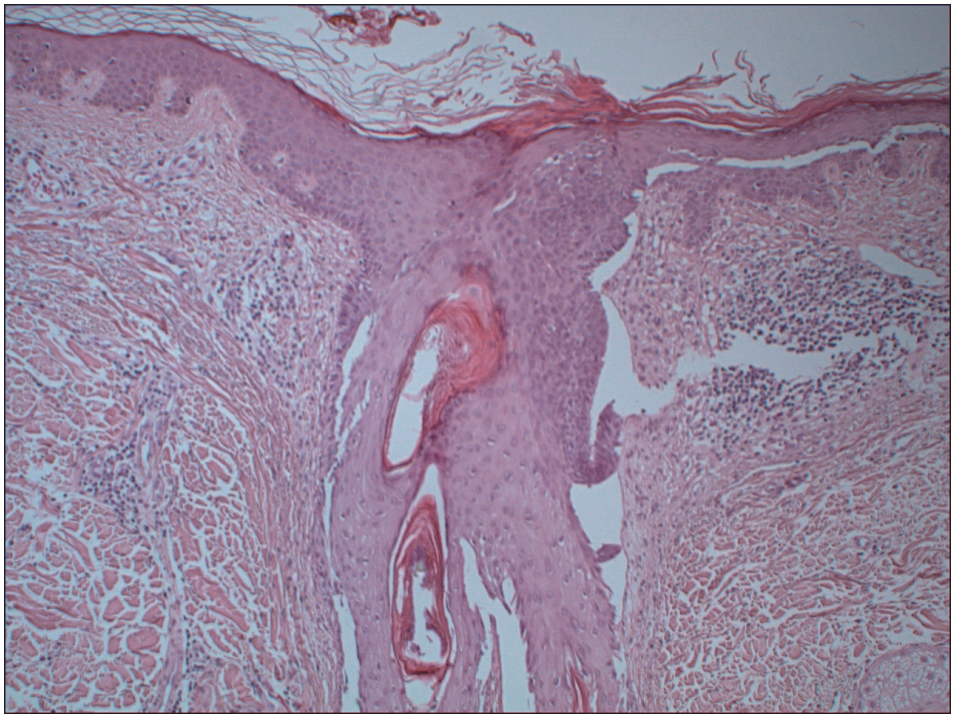

Fig. 1 Histologic superficial basal cell carcinoma with adnexal extension (Hematoxylin-eosin stain; original magnification: x100).

Statistical analysis

Histologic data were available for 112 of 122 tumors with treatment failure. Based on the assumption that the proportion of controls with adnexal extension was 10% and a case:control ratio of 1:2, a sample of 224 patients without treatment failure was randomly selected to enable detection of an odds ratio (OR) ≥ 2.5 with a power of 80% and a two-sided alpha of 5%.

For each treatment modality, the association between tumor thickness as a continuous variable and treatment failure was visually explored using scatter plots. The association between treatment failure with tumor thickness and adnexal extension was estimated with univariate and multivariate logistic regression models. The multivariate model adjusted for potential confounders such as gender, age, type of treatment, tumor surface area and tumor location. ORs with 95% confidence intervals (CI) (unadjusted and adjusted) were derived by exponentiation of regression coefficients corresponding with tumor thickness and adnexal extension. P-values ≤ 0.05 were considered to indicate statistical significance. All data were analyzed using SPSS version 20.0 (SPSS, Chicago, IL, USA) and STATA version 11.0 (STATA Corp, College Station, TX, U.S.A.).

Results

Patients and tumors

A total of 601 patients were included and randomized between March 2008 and August 2010. The study population of the present study consisted of 112 tumors with treatment failure (cases) and 224 tumors with treatment success (controls). The distribution of baseline characteristics was similar among cases and controls, with the exception of tumor surface area (Table 1). Among cases with treatment failure, median tumor thickness was 0.35 mm (range 0.20-0.85) and adnexal extension was present in 10% (11/112). Among controls with treatment success, median tumor thickness was 0.35 mm (range 0.20-1.00) and adnexal extension was present in 14% (32/224). sBCC with treatment failure showed a larger median tumor surface area (56 mm^2) compared to tumors with treatment success (47 mm^2). Scatter plots show a lack of association between tumor thickness as a continuous variable and treatment failure (Fig. 2). A similar distribution of thickness for tumors with and without treatment failure is observed for each treatment modality. For this reason, we performed no separate analyses per treatment modality, but combined all tumors in one dataset.

Table 1. Patient and tumor characteristics for cases (treatment failure) and controls (treatment success).

Characteristics	Treatment failure (n=112)	Treatment success (n=224)	p-value
Gender, n (%)			0.64
Male	57 (51)	107 (48)	
Female	55 (49)	117 (52)	
Age, years			0.21
Mean	60	62	
Median (range)	61 (36-88)	61 (26-87)	
Tumor location, n (%)			
Head/neck, reference	17 (15)	26 (12)	
Upper extremities	11 (10)	37 (16)	0.09
Lower extremities	19 (17)	26 (12)	0.80
Trunk	65 (58)	135 (60)	0.38
Tumor surface area, mm^2			0.08
Median	66	47	
Tumor thickness, mm			0.90
Mean (range)	0.39 (0.20-0.85)	0.39 (0.20-1.00)	
Median	0.35	0.35	
≤ 0.4 mm, n (%)	61 (54)	115 (51)	0.64
> 0.4 mm, n (%)	51 (46)	109 (49)	
Adnexal extension, n (%)			0.30
Absent	101 (90)	192 (86)	
Present	11 (10)	32 (14)	
Treatment, n (%)			
MAL-PDT, reference	49 (44)	72 (32)	
Imiquimod	29 (26)	76 (34)	0.04
5-FU	34 (30)	76 (34)	0.13

P-values <0.05 were considered to be statistically significant. 5-FU, 5-fluorouacil; MAL-PDT, methylaminolevulinate photodynamic therapy.

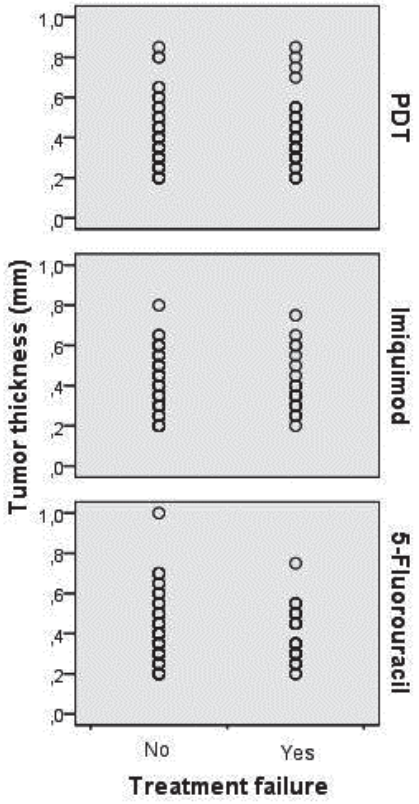

Fig. 2 Scatter plot for tumor thickness and treatment failure per treatment. PDT, photodynamic therapy.

Tumor thickness

Tumor thickness of included patients ranged from 0.20 to 1.00 mm. With respect to treatment failure, the OR per unit increase of tumor thickness was 1.001 (95% CI 0.985-1.017). Using a cut-off point of ≤ 0.4 mm versus > 0.4 mm, the association with treatment failure was 0.88 (95% CI 0.56-1.39, p=0.598) (Table 2). After correction for potential confounders (gender, age, tumor surface area, tumor location and type of treatment) in a multivariate logistic regression analysis, the adjusted OR was 0.87 (95% CI 0.54-1.41, p=0.568).

Table 2. Association between treatment failure and tumor thickness and adnexal extension.

	Unadjusted OR	95% CI	p	Adjusted OR	95% CI	p
Tumor thickness > 0.4 mm vs. ≤ 0.4 mm	0.88	0.56 – 1.39	0.598	0.87	0.54 – 1.41	0.568
Adnexal extension	0.65	0.32 – 1.35	0.251	0.66	0.31 – 1.42	0.290

P-values <0.05 were considered to be statistically significant. The multivariate model adjusted for potential confounders: gender, age, type of treatment, tumor surface area and tumor location. CI, confidence interval; OR, odds ratio.

Adnexal extension

In univariate analysis, the presence of adnexal extension was not significantly associated with higher treatment failure (OR 0.65, 95% CI 0.32-1.35, p=0.251) (Table 2). Similar results were obtained after correction for potential confounders in a multivariate logistic regression analysis; the adjusted OR was 0.66 (95% CI 0.31-1.42, p=0.290).

Discussion

The results of this study do not indicate that tumor thickness and adnexal extension are predictors of treatment failure in sBCC treated with MAL-PDT, imiquimod, or 5-FU. We found no evidence that these histological parameters have to be taken into account before deciding whether non-invasive treatment is adequate for a patient presenting with sBCC.

The Cochrane review on BCC interventions hypothesized that imiquimod response depends on tumor thickness.[4] This hypothesis was based on a study by Shumack et al., who suggests that longer treatment times of imiquimod are needed for nodular BCC compared to sBCC.[15] A recent study by McKay et al. examined imiquimod response rates in 127 sBCC with a mean thickness of 0.30 mm (range 0.09-1.41 mm).[13] In contrast to our study, a tumor thickness > 0.4 mm was found to be a predictor of treatment failure. McKay et al. also recommended that adnexal extension should be measured in pathology reports, but no statistical analyses were reported to support this finding. Based on our analyses we found no evidence that adnexal extension is a determinant of non-invasive treatment failure in sBCC. However, our results were obtained in a population of sBCC with a mean size of 47-66 mm^2. It is unclear whether these results are applicable for larger sBCC.

Previous PDT studies have shown that BCC do not respond with tumor thickness beyond 1.3-2.2 mm.[9,16] These studies included superficial, nodular and aggressive BCC

subtypes. The maximum tumor thickness of sBCC in the current study was 1.0 mm and therefore the data do not allow conclusions about tumors thicker than 1.0 mm. However, previous findings of McKay et al. indicate that thickness of the majority of sBCC does not exceed 1.0 mm.[13]

Currently, the main indication for non-invasive treatment is a histological superficial growth pattern on punch biopsy specimen.[2,3] The histologic definition of a sBCC is multiple lobules of basaloid cells attached to the epidermis and restricted to the papillary dermis, while maintaining their attachment to the epidermis, irrespective of how deep the deepest tumor nest is located.[17] If this definition is maintained, measurement of sBCC thickness and adnexal extension seems redundant.

Tumor thickness was measured on 3 mm punch biopsy specimens. As a punch biopsy represents only a part of the total tumor, the tumor thickness on punch biopsy might not represent the thickest part of the entire tumor. However, our study reflects daily clinical practice in which a biopsy is taken from the clinically thickest part of the tumor. Furthermore, a previous study showed that if sBCC measure ≤ 1.0 mm in depth on punch biopsy specimen, the discrepancy between tumor thickness on punch biopsy specimen and the subsequent surgical excision is small.[18]

The analysis was restricted to a one year follow-up period, and patients in the control group may still be at risk of developing a recurrence during later follow-up. However, we believe that the majority of treatment failures was captured, because 73-78% of failures after non-invasive treatment are known to occur within one year post treatment.[19-21] Furthermore, it is unlikely that late recurrences in the control group would selectively occur in thicker tumors.

In conclusion, in this study with sBCC with a maximum tumor thickness of 1.0 mm, we found no evidence that the histological characteristics tumor thickness and adnexal extension are associated with treatment failure in sBCC treated with MAL-PDT, imiquimod, or 5-FU. Therefore, there seems to be no need to determine tumor thickness or adnexal extension in sBCC before treatment.

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CHAPTER 3.5

Fractionated 5-aminolaevulinic acid photodynamic therapy following partial debulking versus surgical excision in nodular basal cell carcinoma: a randomized controlled trial with at least five year follow-up

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Abstract

Background: Although effective in superficial basal cell carcinoma (BCC), the treatment effect of photodynamic therapy (PDT) in nodular BCC (nBCC) is still questionable. The relation between tumor thickness and PDT failure is unclear.

Objectives: We sought to compare long-term effectiveness of fractionated 20% 5-aminolaevulinic acid (ALA)-PDT with prior partial debulking versus surgical excision in nBCC. The effect of tumor thickness on ALA-PDT failure was analyzed.

Methods: 173 primary, histologically proven nBCC in 151 patients were randomized to fractionated ALA-PDT (n=85) or surgical excision (n=88). Two PDT illuminations were performed with a 1-hour interval. Follow-up was at least 5 years post treatment. Clinical recurrences were confirmed histologically.

Results: A total of 171 nBCC were treated and had a median follow-up of 67 months (range 0-106). At 60 months, 23 tumors had recurred in the ALA-PDT group and 2 tumors in the surgical excision group. Cumulative recurrence probabilities 5 year post treatment were 30.7% (95% CI 21.5%–42.6%) for ALA-PDT and 2.3% (95% CI 0.6%–8.8%) for surgical excision ($p<0.0001$). Two tumors in the ALA-PDT group recurred at 72 and 91 months post treatment. Cumulative probability of recurrence-free survival following ALA-PDT was 65.0% (95% CI 51%-76%) for nBCC measuring
> 0.7 mm in thickness and 94.4% (95% CI 67%–99%, $p=0.018$) for tumors ≤ 0.7 mm.

Conclusions: In nBCC, 5-year cumulative probability of recurrence after surgical excision is lower than after fractionated ALA-PDT with prior debulking. Although surgical excision remains golden standard of treatment, fractionated ALA-PDT might be an alternative for inoperable patients with thin (≤ 0.7 mm) nBCC.

Introduction

The incidence of basal cell carcinoma (BCC) is rapidly increasing worldwide.[1-3] Therefore, effective treatment of this major health problem is essential.

Surgical excision is the gold standard for treatment of all three major histological BCC subtypes; superficial, nodular and aggressive.[4-6] However, the increasing BCC incidence results in a demand for alternative treatments to reduce the workload for physicians and health care costs. Currently, superficial BCC are therefore frequently treated with non-invasive treatment modalities such as photodynamic therapy (PDT), imiquimod and 5-fluorouracil.[6,7] Among these treatments, most experience has been gained with PDT. The treatment effect of PDT in nodular BCC (nBCC) is still questionable as studies on the long-term efficacy are scarce and the effect of tumor thickness on treatment failure has not been well established.[4,6]

According to the guidelines by Braathen et al., PDT is suggested as a treatment option for thin nBCC.[7] This recommendation is based on a study by Soler et al. in which PDT failure was evaluated 35 months post treatment.[8] However, in this study distinction between thin (< 2 mm) and thick (> 2 mm) nBCC lesions was based on a clinical evaluation instead of a histological evaluation. We therefore investigated the relation between PDT treatment failure and the histological nBCC thickness on punch biopsy specimen. We previously described the results of this randomized controlled trial (RCT) on PDT and surgical excision in nBCC.[9] Our interim analysis with a three year follow-up period showed that: (1) tumor thickness was not significantly related to treatment failure after PDT; and (2) surgical excision is more effective than PDT. Because it is known from literature that BCC treated with PDT can recur years after treatment, long-term results are of great importance for conclusive recommendations on nBCC thickness and PDT.[10-12]

Here, we report the results of a prospective RCT with at least 5 years of follow-up and address the relation between tumor thickness and PDT treatment failure.

Materials and methods

Patients and tumor characteristics

Patients with nBCC were recruited from the outpatient department of Dermatology of the Maastricht University Medical Center, The Netherlands. The study design and the procedures have been described in a prior report.[9] In summary, included were patients

aged 18 years or older with a primary, histologically proven tumor on a 3 mm diagnostic punch biopsy specimen of the clinically thickest tumor part. BCC had to be exclusively of the nodular subtype. Patient related exclusion criteria comprised pregnancy, a life expectancy of less than 5 years, any known skin cancer diseases, the use of phototoxic/photosensitive drugs, and hypersensitivity to light or 5-aminolaevulinic acid (5-ALA) cream. Tumor related exclusion criteria comprised recurrent or pigmented BCC, histological subtypes other than nodular (e.g. micronodular) and mixed nodular/aggressive histological subtypes. Tumors located on extremely concave areas (e.g. alar-facial junction, ear) or hairy skin were also excluded in order to guarantee an equal light distribution and a good light absorption in non-pigmented areas, respectively.[13,14] Tumors of patients who gave written informed consent were randomly assigned in a 1:1 ratio to either ALA-PDT or surgical excision using a computer-generated random allocation scheme. Blinding of patient or physicians to treatment allocation was not possible because the practical execution of both treatments extremely differs. The trial was approved by the Medical Ethics and Scientific Committee of the Maastricht University Medical Center.

Photodynamic therapy

Before PDT, tumors were partially debulked by removing all tumor tissue above the skin level with a 4 mm ring curette (Stiefel Laboratories LTD, Sligo, Ireland). The intention was to remove any protuberant tumor and to remove the surface skin barrier. More deeply located tumor tissue was not removed in order to prohibit converting the lesion into a tumor more suitable for PDT. A pressure bandage was applied in case of bleeding. Three weeks after debulking, tumors were treated with fractionated 20% 5-ALA-PDT under occlusion. The entire treatment protocol was based on previously reported preclinical in-vivo studies.[15-17] The tumor was illuminated with a broadband metal-halogen light source (585-720 nm) twice for 15 minutes with an interval of 60 minutes (Photodemarcation System 1, Prototype 5 Medeikonos AB®, Götenborg, Sweden; or Waldmann PDT 1200®, Waldmann Medizintechnik, Villingen-Schwenningen, Germany). This single fractionated treatment on the same day had a total light dose of 150 J/cm^2. Incomplete response or recurrent tumor was registered as treatment failure and retreated surgically.

Surgical excision

nBCC were excised including a 3 mm clinically tumor-free margin with local anesthesia. In case of a histologically proven residual tumor, it was registered as treatment failure and one or more re-excisions were performed until all margins were free of tumor.

Tumor thickness

In the PDT group, tumor thickness was retrospectively measured by an independent investigator on the initial diagnostic punch biopsy specimen, by using a microscope and a 0.1 mm precise liner. A total of 30 biopsies specimens (35%) was randomly re-examined by a pathologist whose measurements were compared with those of the investigator to confirm the accuracy.

Follow-up

Follow-up visits took place 1-2 weeks after surgical excision because of removal of stitches. At 3, 6, 12, and 18 months post treatment, and after 2, 3, 4, and 5 years all patients were subsequently examined for recurrence of the tumors. Some patients even had a follow-up of 6, 7, or 8 years. If recurrent tumor was suspected at clinical examination, a 3 mm punch biopsy specimen was obtained for histological confirmation. All lesions within 5 mm of the scar were considered suspicious for recurrent tumor.[18] Patients who were lost to follow-up or died during the trial were censored at the date of last examination.

Statistical analysis

We aimed at including 175 tumors, based on our previously reported sample size calculation.[9] The analysis was performed on tumor level and according to the intention-to-treat principle. The primary outcome was 5-year cumulative probability of recurrence-free survival after ALA-PDT and surgical excision. Between-group comparisons were performed using Kaplan Meier survival analysis. Recurrence-free survival was defined as the absence of any local recurrence during follow-up. Follow-up time was calculated from the date of treatment to the date of recurrence or the date of last follow-up (censoring date). An exploratory subgroup analysis was performed to calculate the probability of recurrence-free survival according to tumor thickness within the group of patients who were treated by PDT. The log rank test was used to test for differences in recur-

rence-free survival between groups. In all analyses, a p-value <0.05 was considered statistically significant. Data were analyzed using SPSS-pc version 18.0 (SPSS, Chicago, IL, U.S.A.) and STATA version 11.0 (STATA Corp, College Station, TX).

Results

Patient and tumor characteristics

Between August 2002 and February 2006, 151 patients with 173 nBCC were recruited. In this study, 171 nBCC in 149 patients were treated, 88 with surgical excision and 83 with ALA-PDT. Baseline characteristics of patients and tumors were similar in both groups (Table 1).

Table 1. Distribution of patient and tumor characteristics.

Characteristic	ALA-PDT (n=83)	SE (n=88)	Total (n=171)
Sex, M/F	43/40	44/44	87/84
Mean age, years (range)	64.0 (24-83)	65.1 (21-91)	64.6 (21-91)
Tumor localization in the face, n* (%)	44 (53)	45 (51)	89 (52)
Tumor localization rest of the body, n* (%)	39 (47)	43 (49)	82 (48)
No. of nBCC (in no. of patients)			
1	-	-	132
> 1	-	-	17
Mean tumor size (mm) ± SD	8.9 ± 4.0	9.3 ± 4.3	9.1 ± 4.1

Mean tumor size of the largest diameter is measured before the diagnostic punch biopsy. ALA-PDT, aminolaevulinic acid photodynamic therapy; F, female; M, male; nBCC, nodular basal cell carcinoma; SE, surgical excision. *No. of lesions.

Follow-up

The median follow-up of the total study population was 67 months (range 0-106). Before the start of PDT treatment two patients, each with one tumor, dropped out. One patient died before the treatment and one patient turned out to have a recurrent BCC. After randomization, three tumors randomized to ALA-PDT were treated with surgical excision and two tumors randomized to surgical excision were treated with ALA-PDT because the patients preferred the opposite treatment. The tumors in these patients were analyzed according to the intention-to-treat principle. After a median follow-up period of 67 months, 33 patients with 40 tumors were lost to follow-up (Fig. 1). In all, 25 of

these patients with 32 tumors died due to causes unrelated to the tumor or treatment. Of the other patients who were lost to follow-up 6 refused further participation in the study and 4 were untraceable. A total of 106 tumors (62.0%) had a follow-up period equal to or longer than 60 months. Of those, 48 tumors (28.1%) had a follow-up period equal to or longer than 84 months and 18 tumors (10.5%) had a follow-up period exceeding 96 months.

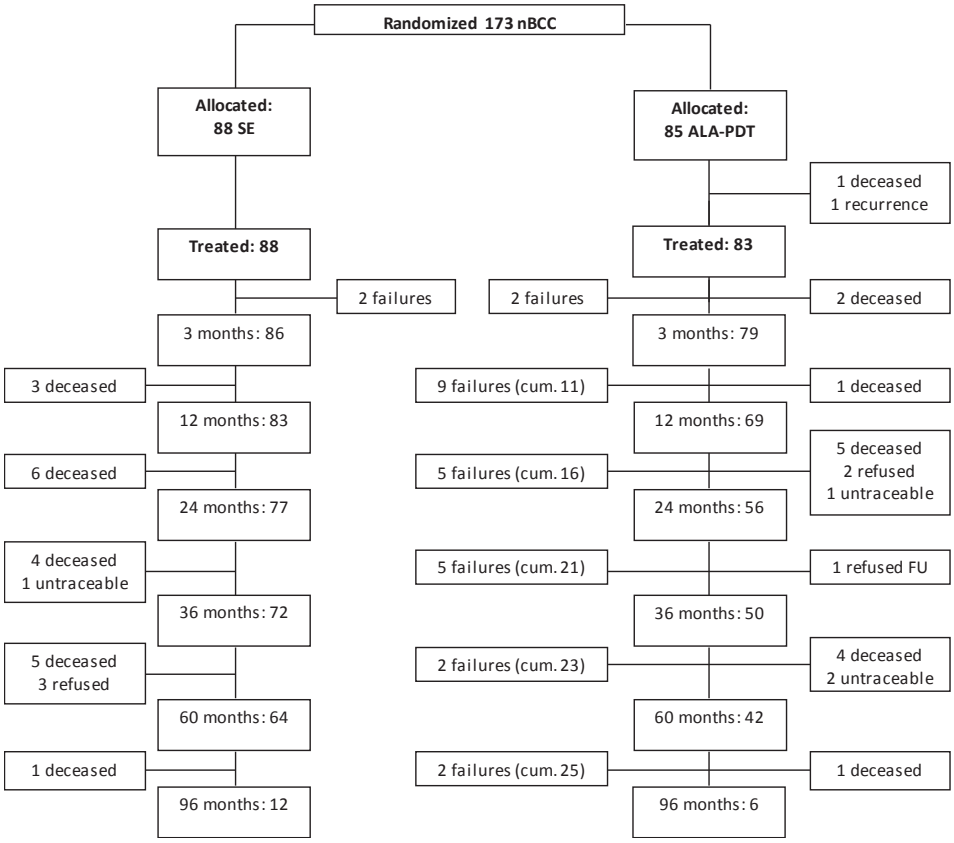
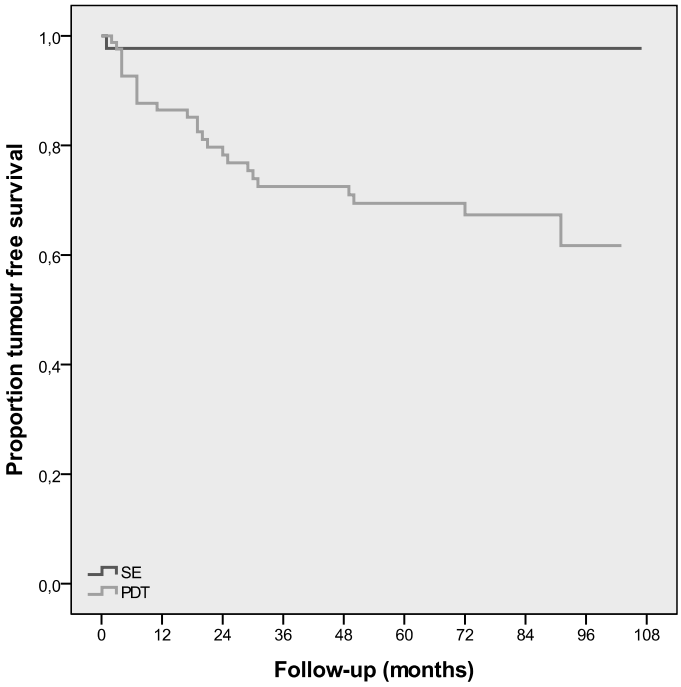


Fig. 1 Flow chart. ALA, aminolaevulinic acid; cum, cumulative; FU, follow-up; nBCC, nodular basal cell carcinoma; PDT, photodynamic therapy; SE, surgical excision.

Treatment failure

During the long-term follow-up, treatment failure occurred in 2 tumors in the surgical excision group and 25 tumors in the ALA-PDT group. Cumulative probabilities of treatment failure, based on Kaplan-Meier survival analysis, are summarized in Fig. 2. After 60 months post treatment, 23 tumors (30.7%) showed treatment failure in the

ALA-PDT group (95% CI 21.5% – 42.6%) whereas the treatment failure in the surgical excision group still was 2.3% (95% CI 0.6% – 8.8%, p<0.0001) (Table 2). Two more tumors in the ALA-PDT group recurred after a longer period than the 60 months follow-up period, at 72 and 91 months post treatment, and were not included in our analyses. The two failures in the surgical excision group were both caused by incomplete excision of the tumors. The first tumor remained incompletely excised after two more excisions and finally was completely removed as nBCC after Mohs' micrographic surgery. The other tumor remained incompletely excised because the patient refused further treatment. However, in this patient no clinical residual tumor was present at the last follow-up visit 22 months post treatment.



Patients in follow-up (n)

SE	88	83	77	72	69	64	41	30	12	0
PDT	83	69	56	50	48	42	33	18	6	0

Fig. 2 Kaplan–Meier graph: survival analysis in the aminolevulinic acid photodynamic therapy (PDT) and in the surgical excision (SE) groups.

Table 2. Cumulative probabilities of treatment failure after fractionated aminolevulinic acid photodynamic therapy with prior curettage versus surgical excision.

	Cumulative recurrence probability (95% CI)	
Follow-up, months	ALA-PDT	Surgical excision
12	13.6 (7.8 – 23.2)	2.3 (0.6 – 8.8)
24	20.4 (13.0 – 31.2)	2.3 (0.6 – 8.8)
36	27.6 (18.9 – 39.2)	2.3 (0.6 – 8.8)
48	27.6 (18.9 – 39.2)	2.3 (0.6 – 8.8)
60	30.7 (21.5 – 42.6)	2.3 (0.6 – 8.8)

ALA-PDT, aminolaevulinic acid photodynamic therapy; CI, confidence interval.

Recurrence-free survival according to tumor thickness

The effect of tumor thickness on treatment failure was analyzed for nBCC treated with ALA-PDT. In 78 (94%) initial punch biopsy specimens tumor thickness was retrospectively measured without interobserver discrepancy. Median tumor thickness was 1.3 mm (range 0.3 – 3.1 mm). Use of this median value of 1.3 mm as cut-off point resulted in probabilities of recurrence-free survival of 77.4% (95% CI 60% - 88%) and 66.8% (95% CI 49% - 80%, p=0.14) for tumor thickness < 1.3 mm and ≥ 1.3 mm, respectively. We also used the lowest quartile of 0.7 mm as cut-off point and found a significant lower probability of recurrence-free survival for nBCC measuring > 0.7 mm in thickness (65.0%, 95% CI 51% - 76%) compared with tumors growing ≤ 0.7 mm deep (94.4%, 95% CI 67% - 99%, p=0.018) (Table 3).

Table 3. Relationship between tumor thickness on diagnostic punch biopsy specimen an cumulative probability of recurrence-free survival 60 months after photodynamic therapy treatment.

Tumor thickness, mm	Cumulative percentage	Cumulative recurrence-free survival (95% CI)
≤ 0.7	23.1	94.4 (67 - 99)
0.8 – 1.2	48.7	61.9 (36 - 80)
1.3 – 1.7	75.6	69.0 (43 - 85)
> 1.7	100	64.3 (37 - 85)

Data are given in percentages unless indicated otherwise. CI, confidence interval.

Discussion

The results of this RCT showed a significant difference in cumulative probability of treatment failure of 2.3% in the surgical excision group compared to 30.7% in the ALA-PDT group at 60 months of follow-up. Furthermore, success of treatment of nBCC with ALA-PDT depends on tumor thickness. Probability of recurrence-free survival is significantly lower for nBCC growing deeper than 0.7 mm compared with tumors with an infiltration depth ≤ 0.7 mm deep (65.0% vs. 94.4%, p=0.018). Notably, we found that nBCC treated with ALA-PDT can even recur after 91 months.

Our results indicated that ALA-PDT treatment success of nBCC depends on tumor thickness corroborate the previous findings by Morton et al.[19] For the future this implies that: (1) diagnostic punch biopsies should be performed in the clinically thickest tumor part; and (2) pathologists should measure nBCC thickness on request when PDT is considered as treatment option. However, we realize that the agreement between corresponding tumor thickness measurement in punch biopsy and surgical excision specimens varies and discrepancy increases with increasing tumor thickness.[20]

The position of PDT as a treatment option for nBCC in today's practice is debatable. Although surgical excision is a more effective treatment than PDT, a non-invasive approach with PDT might be more appropriate for some patients.[4,6] PDT may be considered as an acceptable treatment option in a selected population with nBCC, for example elderly, inoperable patients or patients with a tumor thickness ≤ 0.7 mm. Notably, nBCC growing deeper than 0.7 mm, BCC with mixed nodular/aggressive histological subtypes and aggressive subtypes (e.g. micronodular) should not be treated with PDT.

We found that in primary thin nBCC (≤ 0.7 mm thick), the ALA-PDT cure rate of 94.4% is almost comparable to the 5-year cure rates of nBCC treated with surgical excision (97.7%). Those cure rates are even among the higher cure rates found in most studies on treatment of BCC. In a study investigating the effect of Mohs' micrographic surgery 5-year cure rates of 98% are found[21,22] and in a different study in electrodessication and curettage cure rates are 79%-99%.[23] The efficacy of imiquimod in nBCC is less investigated and varies between 42-100%.[24] Only a small imiquimod study had 5-year follow-up and showed a cure rate of 75%.[25] In fact, cure rates of different studies may not be compared, because of many differences in protocols. However it is important information, because, in case of comparable cure rates, other factors such as cosmetic outcome and costs will determine treatment choice. The best method to compare cure rates of therapies is of course a RCT.

A limitation of this study might be that 3 mm punch biopsy specimens may show a sample error. It is known from literature that a 3 mm punch biopsy specimen predicts

the most aggressive BCC subtype in only 84% of primary BCC.[26] As a consequence, other BCC subtypes than nodular might have been treated in this trial. However, deciding what treatment should be given on the histological subtype in the punch biopsy specimen is representative for daily practice.

In 2007 Braathen et al. reviewed the results of both randomized and non-randomized PDT studies in nBCC. The conclusion of this review was that methylaminolaevulinate (MAL)-PDT, as a treatment option for nBCC, is an effective and reliable method that is possibly preferable for thin lesions.[7] The advice for limits on thickness of tumors was based on a retrospective MAL-PDT study by Soler et al. that showed a 7% rate of recurrence in thin nBCC (< 2 mm thickness, n=82) compared with 14% in thick nBCC (> 2 mm thickness, n=86) after a mean follow-up period of 35 months.[8] We did not find significant differences in recurrence rate when using this cut-off point; only treatment of nBCC with a thickness ≤ 0.7 mm was associated with a low probability of treatment failure. Results may differ because Soler et al. determined tumor thickness clinically instead of performing a histological measurement via punch biopsy specimen.

PDT results depend on treatment protocols. Differences in photosensitizers, prior curettage, the use of the tissue penetration enhancer dimethylsulfoxide, or repetitive PDT cycles are all protocol variations that may result in different treatment outcomes.[27-30] Some authors claim that MAL-PDT is more effective than ALA-PDT. However, to date no study has compared both photosensitizers in a RCT and in the above mentioned studies MAL and ALA seem quite similar in treatment results.[31-33]

Fractionated illumination, which has been investigated in ALA-PDT, shows better clearance rates than treatment with only one illumination.[34,35] When ALA-PDT is used in combination with prior dimethylsulfoxide and curettage even lower recurrence probabilities were obtained.[36]

There are some new developments within the PDT field. Recent studies demonstrated that PDT with BF-200 ALA (Biofrontera Bioscience GmbH, Leverkusen, Germany) is a very effective treatment for both BCC and actinic keratosis.[37-40] Another new development that shows promising results in the treatment of actinic keratosis is patch PDT.[41,42] These developments could offer new insights into possible ways of optimizing PDT as therapy for BCC.

In conclusion, our study revealed that ALA-PDT is less effective than surgical excision in treatment of nBCC. Based on our results, ALA-PDT might be a second treatment choice for inoperable patients with nBCC growing ≤ 0.7 mm deep. We therefore suggest that when considering PDT as treatment for nBCC, tumor thickness should be taken into account. This implies that physicians should take a diagnostic punch biopsy

from the clinically thickest tumor part and pathologists should measure thickness of a nBCC on request.

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CHAPTER 4

Discussion and valorization

Despite skin cancer prevention campaigns on sun avoidance, little behavior change is seen and the incidence of skin cancer continues to rise. About 80% of skin cancers are basal cell carcinoma (BCC). Nowadays, the lifetime risk of developing a BCC before the age of 85 years is 1 in 5-6 persons.[1] Predictions for the future incidence are even more alarming. One should keep in mind that incidence studies are based on data from cancer registries which often only register the first, histologically confirmed BCC. Within 5 years after the first BCC diagnosis, 29% of patients will develop another BCC.[2] Therefore, only the tip of the iceberg is measured and actual incidence rates will be higher. BCC may be regarded as a chronic disease which poses a large burden on dermatologic health care services.

A century ago, surgery was the only treatment option for BCC and was mostly performed by general surgeons. Surgical excision is a treatment that enables histological verification of complete tumor removal. It therefore was (and still is) the most effective treatment in BCC. The first non-invasive option that emerged more than 50 years ago was 5-fluorouracil (5-FU). This therapy probably became abandoned as more dermatologists were expanding their surgical skills and a treatment was developed that resulted in nearly 100% cure rates (Mohs' micrographic surgery).[3,4] Furthermore, in the 1960-1980s, incidence of skin tumors was still not that high that alternative treatments were in high demand. It is conceivable that in those days patients were glad their tumor was removed and were not that concerned about the aesthetic outcome. The situation changed in the late 20th century, when the incidence rates of BCC increased rapidly, more young patients were affected and patients became more demanding. At that time, the second non-invasive treatment was developed: photodynamic therapy (PDT). The concept of PDT was already initiated 100 years earlier by Von Tappeiner: an interaction between light, a photosensitizer and oxygen.[5] It is interesting to observe how PDT suddenly gained popularity based on a few small-sized studies with a short follow-up period. One of the reasons was probably the strong need for non-surgical therapies that could be executed by nurses, relieving the busy dermatologic practice. PDT became a competitor of surgery with a promised high treatment success and excellent aesthetic outcome. PDT was incorporated in (inter)national guidelines on treatment of superficial BCC (sBCC). With the introduction of imiquimod in 2004 competition came into play. Imiquimod cream is less expensive than the in-hospital PDT. Another advantage of imiquimod is that patients can apply the cream themselves at home. Consequently, imiquimod became also incorporated in guidelines on BCC treatment. Guidelines agree that surgical excision should still be considered as first choice treatment for sBCC but excision is not always needed and often considered as overtreatment. Despite the

acknowledgement that in some instances non-invasive therapies may be preferred, there was no consensus on the best non-invasive treatment choice in sBCC.

In the last decades, the need for consensus has increased due to the observed shift towards sBCC.[6] sBCC can be treated non-invasively and this provides the opportunity to reduce the workload for dermatologists. As a result, non-invasive therapies such as PDT, imiquimod and 5-FU have become increasingly popular. These trends highlight the need for evaluation of diagnostic and treatment options for optimal management of sBCC. First, the development of these non-invasive therapies demands an accurate diagnostic tool with the ability to discriminate between BCC subtypes. Knowledge of subtypes is important as sBCC can be treated non-invasively while nodular BCC (nBCC) and aggressive BCC (aBCC) mostly require a surgical treatment. The need for distinction between sBCC and other types of BCC asks for diagnostic strategies that can meet this requirement. Secondly, the availability of various non-invasive treatment options for patients with sBCC asks for comparison of the effectiveness of these treatments and other aspects such as cosmetic results, adverse effects and costs. Such results are pivotal for reaching consensus on the most effective non-invasive treatment option in patients with sBCC. A third issue is whether treatment needs to be tailored based on patient and tumor characteristics. It may well be that subgroups of patients benefit more or less from specific treatment options.

Diagnosis

Optimal management of BCC relies on early and accurate diagnosis in order to optimize outcome and minimize morbidity. Current national and international guidelines on BCC recommend a punch biopsy of clinically suspected BCC prior to treatment to facilitate treatment choice based on the histological BCC subtype.[7-9] We performed two studies to evaluate the diagnostic accuracy of a punch biopsy. In one study on the agreement between the results of punch biopsy and surgical excision, we found that punch biopsies can detect the most aggressive subtype in 83.4% of cases when compared with surgical excision, resulting in a correct staging in 5 out of 6 primary BCC.[10] Our findings corroborate results of previous studies that also showed high percentages of 79-89%.[11,12] An explanation for the fact that the most aggressive subtype is not detected in all cases is that a punch biopsy, often 3-4 mm, represents only a small sample of the tumor. The majority of BCC (74%) consists of a 'mixed' histological subtype and the most aggressive component can be missed in a small tumor sample.[10] However, we can

conclude that a punch biopsy has a high sensitivity to identify nBCC and aBCC that qualify for surgery instead of a non-invasive treatment.

In another study, the accuracy of punch biopsy was compared with that of a clinical diagnosis by a dermatologist. The diagnosis based on the surgical excision specimen was used as gold standard. It is assumed that dermatologists can diagnose a BCC fairly well by their clinical observation.[13] The advantage of clinical diagnosis is that it is a painless, time saving and presumably also a cost saving procedure. But a relevant question is: how many tumors will be over- or understaged and consequently how many tumors would be over- or undertreated when omitting a punch biopsy? We performed a study to compare the diagnostic accuracy of clinical assessment and histological diagnosis by punch biopsy for subtyping BCC.[14] In this study, a punch biopsy appeared to be a more accurate diagnostic tool than the clinical diagnosis for detection of the histological BCC subtype. Omission of a punch biopsy resulted in overstaging of 1 in 4 sBCC as nodular or aggressive and in understaging in about 1 in 4 aBCC as nBCC. The question can be raised whether these diagnostic errors result in inadequate treatment. In case of overstaging, the physician will almost certainly advise surgery and the patient will be denied the choice between surgery and less invasive alternatives. However, some patients will be satisfied with a surgical excision as they are content that their tumor has been removed. In case of understaging, patients are at risk of having their BCC excised with too small margins. However, a BCC is a tumor with a low mortality rate and any residue or recurrence following therapy can be diagnosed and retreated easily in most cases. Based on these findings, it may be justified in a few cases to omit a punch biopsy, especially if the tumor is located on a low risk area. It would be interesting to find out whether the accuracy of clinical diagnosis approaches that of a punch biopsy when the degree of confidence in the diagnosis of BCC subtype is taken into account. In situations where physicians are very confident of their clinical diagnosis on BCC subtype, the need for a punch biopsy may be less obvious. Furthermore, the subtyping of BCC might further be improved by the use of a dermatoscope, which enables detection of certain clinical features that are characteristic for BCC subtypes.[15] The hypothesis, that a clinical diagnosis of BCC subtype made with a dermatoscope and high confidence may exclude the necessity of a punch biopsy prior to treatment, needs to be confirmed in a well-designed prospective study.

Treatment

The equivocal guidelines and the large web of treatment options presented in guidelines on sBCC can hamper physicians in making well-informed treatment choices. Due to the current lack of evidence on relative effectiveness of therapies, treatment choice is nowadays partly based on the doctors' own experience and education. More evidence on relative effectiveness of treatments is needed. We performed a systematic review and meta-analysis of published studies to compare the tumor-free survival of patients with primary sBCC treated with the most frequently used non-invasive therapies.[16] The majority of the included studies reported on treatment with imiquimod or PDT, which enabled a meta-analysis on treatment success of these therapies. The pooled estimates for one year tumor-free survival were 87.3% for imiquimod and 84.0% for PDT. However, restriction of the analysis to PDT studies with only one PDT cycle resulted in a lower pooled estimate of tumor-free survival of 76.2%. These results provided evidence that imiquimod and PDT are nearly equally effective in treatment of sBCC at one year post treatment. The meta-analysis also revealed that head-to-head comparison studies and studies with a long-term follow-up were lacking. Closing this gap in knowledge is essential to enable better recommendations in guidelines on sBCC treatment.

To fulfill the need for a head-to-head comparison study with long-term follow-up, a non-inferiority randomized controlled trial in 601 patients with sBCC treated with methylaminolevulinate (MAL)-PDT, imiquimod or 5-FU has been performed. The one year follow-up results indicated that both creams are not inferior to MAL-PDT in terms of effectiveness and that imiquimod was even superior to MAL-PDT.[17] One year tumor-free survival for imiquimod was 83.4% versus 72.8% for MAL-PDT with a difference of 10.6% (95% CI 1.5%-19.5%) favoring imiquimod. Extra data during a three year follow-up period were collected. At three years post treatment, imiquimod and 5-FU remained non-inferior to MAL-PDT, but the gap between imiquimod and MAL-PDT widened even more. Imiquimod showed a large advantage in three year tumor-free survival of 79.7% compared with 68.2% for 5-FU and only 58.0% for MAL-PDT. The conclusion is that based on effectiveness, imiquimod should be considered as the first treatment choice in primary, low-risk sBCC.

The trial was designed as a non-inferiority trial because before the start of the trial it was assumed that MAL-PDT was the most effective treatment and that both creams might be less effective in terms of tumor-free survival. However, it was expected that the creams would have advantages such as lower costs and reduction of workload on dermatologists, because creams can be applied by the patient at home. Because of these

advantages and the possibility to fall back on excision in case of a recurrent BCC, a loss in one year tumor-free survival by 10% was deemed acceptable. The choice of MAL-PDT as reference therapy was based on a review of the Cochrane Collaboration which concluded that surgical excision should be the first choice therapy for sBCC but that PDT can be considered as a good alternative because of better cosmetic outcomes and a greater patient tolerability.[18] It was assumed that the one year cumulative probability of tumor-free survival after PDT would be about 80%, based on the average of the estimates for PDT from the meta-analysis. However, the trial results seem to contradict the correctness of this assumption. This faulty assumption is partly due to the scarcity of randomized controlled trials comparing MAL-PDT with surgery. Before the start of the trial, only one randomized controlled trial had compared MAL-PDT with surgical excision at one year post treatment and estimates of treatment success after PDT may have been overoptimistic.[19] The one trial included 128 histologically proven primary sBCC that were treated with one or two MAL-PDT cycles. In this study, 90 of 128 (70.3%) sBCC responded to one MAL-PDT cycle, but the non-responders and incomplete responders received two additional MAL-PDT sessions after a period of three months. This increased the overall cumulative probability of sustained clearance to 90% at one year post treatment for MAL-PDT. A reason for the observed discrepancy with the expected 80% treatment success after PDT may be that the most frequently used European PDT protocol was applied very strictly in this trial. This protocol for MAL-PDT is advised by the company producing MAL and prescribes one MAL-PDT cycle consisting of two treatments with one week interval.[20] Repeating treatments would probably improve treatment success but increases the dermatologist's workload and treatment costs.

The trial results indicate that PDT is not the most effective non-invasive treatment option. However, PDT has the benefit of being a two-day treatment with less side effects and a more rapid recovery compared with imiquimod. In this respect, further research to improve PDT and its illumination protocol seems worthwhile.

Now we have concluded that imiquimod should be the first choice therapy in primary sBCC, the question raises whether imiquimod is the most effective therapy for every patient with a sBCC. Based on data derived from the above mentioned non-inferiority trial, subgroup analyses were performed to explore whether the relative treatment effect of MAL-PDT and imiquimod is consistent across subgroups of patients and tumors.[21] A higher probability of treatment success for imiquimod versus MAL-PDT at three year follow-up was confirmed in most subgroups. An interesting finding was that in patients >60 years with a sBCC on the lower extremities, MAL-PDT was more effective than imiquimod (tumor-free survival of 93.8% vs. 36.2%). This finding coming from an ex-

plorative analysis needs to be confirmed in future studies, but for the time being these results suggest that MAL-PDT can be considered as a treatment option for older patients with sBCC on the lower extremities, in whom the imiquimod induced erosion might cause local wound infections, ulcerations or erysipelas. There are other situations wherein imiquimod may not be the optimal choice for an individual patient. Imiquimod requires compliance with a treatment regimen which prescribes application once daily, five times a week for six weeks. Therefore, this treatment is not indicated when the sBCC is situated inconveniently on a body site that is out of reach of a patient or when there is another reason why patients are not capable of applying the cream. In these cases, PDT may be an option, but surgery may also be considered depending on the comparative assessment between effectiveness and cosmetic aspects.

It was also evaluated whether tumor thickness and adnexal extension might influence response to non-invasive treatments. The superficial growth pattern is easily accessible for topical treatments. We defined a sBCC as a tumor existing of small buds of proliferating basal cells that grow down from the epidermis into the superficial dermis, whilst maintaining their attachment to the base of the epidermis. However, it might be that there is a threshold thickness above which sBCC do not respond well to PDT, imiquimod and 5-FU. We measured tumor thickness in 336 sBCC with a median thickness of 0.35 mm and a range of 0.2-1.0 mm.[22] Our results showed that tumor thickness and adnexal extension of sBCC were not significantly associated with treatment failure following MAL-PDT, imiquimod or 5-FU.

International guidelines recommend PDT as treatment option for sBCC, but also for 'thin' nBCC that cannot be surgically excised. Thin tumors are likely to respond to the superficially working PDT. We analyzed the effect of nBCC thickness on treatment failure five years after treatment with fractionated aminolaevulinic acid (ALA)-PDT.[23] The cumulative probability of tumor-free survival after ALA-PDT was 65.0% for tumors measuring > 0.7 mm in thickness compared with 94.4% in tumors ≤ 0.7 mm. The finding that thin nBCC in this trial responded more often to PDT than the sBCC in the non-inferiority trial (treatment success of 94.4% vs. 58.0%) might be explained by the partial debulking prior to PDT.

Another explanation might be that nBCC were treated with ALA-PDT instead of MAL-PDT. According to the literature, the effectiveness in terms of clearance rates of sBCC is lower for MAL-PDT in two sessions compared with fractionated ALA-PDT: 73% vs.88% at five years post treatment.[24,25] Based on these results, fractionated ALA-PDT with partial debulking might be considered as an acceptable non-invasive treat-

ment option in a selected population of inoperable patients with nBCC measuring ≤ 0.7 mm in thickness.

Based on the data in this thesis, we can conclude that a punch biopsy is preferred in clinically suspected BCC in order to confirm the diagnosis but, even more important, to identify the most aggressive subtype. Omission of a punch biopsy may be justified in few cases, but the possible consequences should always be discussed carefully with the patient. First choice treatment for sBCC is imiquimod cream whereas PDT might be a treatment option in elderly patients with sBCC on the lower extremities or in patients for whom cream application is not feasible for practical reasons. It will remain a goal but also a challenge for dermatologist and other physicians to provide clear guidance in treatment of an individual patient with sBCC.

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CHAPTER 5

Summary / samenvatting

Summary

The rapid increase in basal cell carcinoma (BCC) incidence has prompted the need for new, non-invasive treatment modalities. These superficial working therapies are good alternatives for surgery in superficial BCC. As BCC are categorized in three main subtypes (superficial, nodular and aggressive, denoted as sBCC, nBCC and aBCC, respectively), it is of great importance to accurately discriminate between histological subtypes in order to select an appropriate treatment. sBCC can be treated non-invasively while nBCC and aBCC require a surgical treatment. This thesis addresses the results of seven studies on the diagnosis and treatment of BCC.

Chapter 1 gives an introduction to the thesis. It provides a short overview of the epidemiology, risk factors, pathogenesis, diagnosis and treatment of BCC. Furthermore, the objectives of the thesis are described in this chapter.

Chapter 2 describes two studies on the diagnosis of BCC. In **chapter 2.1** we present the results of a retrospective study that established the agreement between the histological BCC subtype and the most aggressive component on punch biopsy and diagnosis according to the subsequent surgical excision specimen. A total of 243 primary BCC in 191 patients were analyzed and the histological subtype present on punch biopsy and the subsequent surgical excision was recorded. As a substantial percentage of BCC consist of more than one subtype, our analyses were based the most aggressive histological subtype. The agreement between histological subtype observed on punch biopsy specimens and excision specimens was 60.9%. The proportion of punch biopsies that correctly identifies the most aggressive growth pattern of the entire BCC was 84.4%. This means that in one out of six BCC the most aggressive growth pattern is missed in an adequately taken punch biopsy. In addition, we found that 74% of all primary BCC consisted of more than one histological subtype. These results indicate that dermatologists and other physicians treating BCC should be aware of the limited diagnostic value of a punch biopsy to determine the histological BCC subtype of the entire lesion. Misdiagnosis of the subtype may lead to under- and overtreatment of BCC.

Some dermatologists argue that omitting a biopsy might be acceptable or even preferable in some cases. Disadvantages of a punch biopsy are discomfort for the patient and the associated time and costs for the physician. In contrast, clinical diagnosis is a painless, time- and possibly cost-saving procedure. In **chapter 2.2** we compare the diagnostic accuracy of clinical assessment and histological diagnosis by punch biopsy for sub-

typing BCC. The gold standard for subtyping BCC was the histological subtype on the subsequent surgical excision. In addition, we evaluate the impact of omitting the punch biopsy on treatment recommendations. This study was performed at the departments of Dermatology of the Maastricht University Medical Centre and the Erasmus Medical Centre Rotterdam. A total of 152 histologically confirmed BCC from 116 patients were evaluated for the most aggressive subtype by clinical diagnosis, the subsequent punch biopsy and surgical excision specimens. A distinction was made between superficial, nodular and aggressive BCC. The gold standard for BCC subtyping was the histological subtype on the subsequent surgical excision. The results show that omission of a punch biopsy may result in overstaging of 1 in 4 sBCC and in understaging of 1 in 4 aBCC. Thus, a punch biopsy is a better diagnostic tool than the clinical diagnosis for detection of the histological BCC subtype, which is in line with international guidelines. In case a physician decides to omit a punch biopsy, the impact of over- or understaging must be weighed against extra time, cost and patient discomfort associated with a punch biopsy.

Chapter 3 consists of five sections and presents several studies on the efficacy of different treatment modalities for low risk BCC. In addition, we aimed at identifying subgroups of patients and tumors that differ in treatment response in order to select the most effective treatment for individual patients depending on patient and tumor characteristics.

In **chapter 3.1** a systematic review and meta-analysis was performed to determine the residue-free, recurrence-free and tumor-free survival probabilities of patients with primary sBCC treated with the currently most frequently used treatment options. We searched for articles in the Pubmed, EMBASE and Cochrane databases, and reference lists of selected articles were retraced. Included were studies reporting on residue and/or recurrence probabilities after treatment of primary, histologically proven sBCC with a minimum follow-up of 12 weeks. Thirty-six studies (14 randomized and 22 non-randomized trials) were included. The pooled estimates of sBCC with tumor-free survival at one year post treatment, derived from 23 studies, were 87.3% (95% CI 84-91%) for imiquimod and 84.0% (95% CI 78-90%) for photodynamic therapy (PDT). The PDT tumor-free survival was lower in studies with a single treatment cycle. To date, only a few studies have reported on treatment of sBCC with 5-fluorouracil (5-FU) or surgical excision. Based on our meta-analysis, we can conclude that imiquimod and PDT are both good non-invasive treatment options for sBCC. However, there is a lack of head-to-head comparison studies between imiquimod, PDT and other therapies and long-term follow-up studies are scarce.

In **chapter 3.2** we present the results of a non-inferiority randomized controlled trial with three year follow-up that investigated the tumor-free survival of imiquimod and 5-FU versus methylaminolevulinate (MAL)-PDT in patients with sBCC. This study fulfills the need for head-to-head comparison studies with long-term follow-up and enables better treatment recommendations for non-invasive treatment of sBCC. A total of 601 patients with a primary sBCC were enrolled at seven departments of Dermatology in the southern part of The Netherlands. Participants had been randomly assigned to MAL-PDT (two sessions with one week interval), imiquimod (once daily, five times a week for 6 weeks), or 5-FU (twice daily for 4 weeks). A follow-up visit took place at three years post treatment by a research physician who was blinded to the assigned therapy. Clinical treatment failures were histologically confirmed by a 3 mm punch biopsy. A pre-specified non-inferiority margin of 10% was used. We showed that the probability of tumor-free survival at three years post treatment was 58.0% for MAL-PDT, 79.7% for imiquimod, and 68.2% for 5-FU. The hazard ratio for treatment failure comparing imiquimod with MAL-PDT was 0.50 (95% CI 0.33-0.76, p=0.001). Comparison of 5-FU with MAL-PDT resulted in a hazard ratio of 0.73 (95% CI 0.51-1.05, p=0.092), and comparison of 5-FU with imiquimod in a hazard ratio of 0.68 (95% CI 0.44-1.06, p=0.091). Thus, imiquimod is superior to MAL-PDT and guidelines on sBCC treatment should recommend imiquimod as first choice treatment option. 5-FU is not-inferior to MAL-PDT, but compares unfavourably with treatment success after imiquimod treatment. Both creams have a comparable cosmetic outcome and risk of local adverse events. Although 5-FU is less expensive than imiquimod, the additional cost-savings of 5-FU compared to imiquimod is small since the higher number of treatment failures require more additional treatments.

In **chapter 3.3** we explore whether this relative treatment effect of MAL-PDT and imiquimod is consistent across subgroups defined by certain patient and tumor characteristics. Identification of subgroups of patients that differ in response to MAL-PDT and imiquimod is of great value in clinical practice to select the most effective treatment for an individual patient with sBCC. Data were derived from the multicenter randomized controlled trial described in chapter 3.2. Treatment success was defined as tumor-free survival at one year post treatment. Subgroup analyses were performed for subgroups defined by sex, age (≤ 60 years vs. > 60 years), tumor location (head/neck, trunk, lower extremities or upper extremities) and tumor size (≤ 60 mm^2 vs. > 60 mm^2). Relevant endpoints were available for 196 patients treated with MAL-PDT and 189 patients treated with imiquimod. Imiquimod was superior to MAL-PDT in subgroups of females, sBCC on the trunk and large tumors with differences in success percentages favoring imiquimod of 18.4%, 21.0%, 18.9%, respectively. A higher probability of treatment suc-

cess (but no superiority) of imiquimod vs. MAL-PDT was consistently found for males, patients > 60 years, tumors on the head/neck and upper extremities, and smaller sBCC. Interestingly, within the subgroup of sBCC on the lower extremities, treatment success after imiquimod was much lower (36.8%) than after MAL-PDT (94.1%) in patients > 60 years. This higher probability of treatment success for MAL-PDT compared to imiquimod was not seen for younger patients with a sBCC on the lower extremities. In chapter 3.2 we showed that the results of these subgroup analyses remained unaltered at three years of follow-up. However, we emphasize that the results should be interpreted carefully as the subgroup analyses in this study were exploratory and not driven by prior hypotheses. Based on the results, imiquimod should remain the first choice treatment for sBCC in terms of effectiveness. However, MAL-PDT might be preferred in elderly with sBCC on the lower extremities.

In **chapter 3.4** we further investigate whether the histological characteristics tumor thickness and adnexal extension of sBCC may influence treatment response of non-invasive therapies. Again, we used the database of the non-inferiority randomized controlled trial described in chapter 3.2. The study was designed as a case-control study, where 112 patients with treatment failure were selected as cases and 224 patients with treatment success were randomly selected as control subjects to enable detection of an odds ratio ≥ 2.5 with a power of 80% (alpha = 5%). Histopathological slides of the initial diagnostic punch biopsies were retrieved to obtain additional data on tumor thickness and adnexal extension. Scatter plots showed a lack of association between tumor thickness as a continuous variable and treatment failure for all treatment groups. Therefore, no separate analyses per treatment modality was performed and all tumors were combined in one dataset. Tumor thickness of included patients ranged from 0.2 to 1.0 mm. The results showed that tumor thickness and adnexal extension of sBCC were not significantly associated with treatment failure of MAL-PDT, imiquimod or 5-FU. Similar results were obtained after correction for potential confounders (sex, age, type of treatment, tumor surface area and tumor location) in a multivariate logistic regression analysis. We therefore conclude that tumor thickness and adnexal extension do not have to be taken into account before deciding whether non-invasive treatment is adequate for a patient with sBCC.

In **chapter 3.5** we compare the long-term effectiveness of fractionated 20% 5-aminolaevulinic acid (ALA)-PDT with prior partial debulking versus surgical excision in nBCC. In addition, we analyze the effect of nBCC thickness on ALA-PDT failure. Patients presenting at the department of Dermatology at the Maastricht University Medical Centre with a primary, histologically proven nBCC were included and randomly assigned to treatment with fractionated ALA-PDT (n=85) or surgical excision with a

3 mm margin (n=88). Two PDT illuminations were performed with a one hour interval. Follow-up was at least 5 years post treatment and clinical treatment failures were histologically confirmed by a 3 mm punch biopsy. In the ALA-PDT group, tumor thickness was retrospectively measured by an independent investigator on the initial diagnostic punch biopsy specimens. The cumulative recurrence probability at 5 years post treatment was 30.7% for ALA-PDT and 2.3% for surgical excision ($p<0.0001$). Remarkably, two tumors treated with ALA-PDT even recurred after 72 and 91 months post treatment. The tumor thickness of nBCC ranged from 0.3-1.3 mm. We found that the cumulative probability of tumor-free survival after ALA-PDT was 65.0% for tumors measuring > 0.7 mm in thickness compared with 94.4% in tumors ≤ 0.7 mm. These findings confirm that surgical excision remains the gold standard for treatment of nBCC. However, ALA-PDT might be considered as an acceptable treatment option in a selected population of inoperable patients with nBCC measuring ≤ 0.7 mm in thickness. This implies that in case of a suspected nBCC, physicians should take a diagnostic punch biopsy from the clinically thickest tumor part and pathologists should measure the thickness of a nBCC on request.

Finally, **chapter 4** describes the discussion and valorization in which the results of this thesis are put into perspective. We discuss the position of our results within the current knowledge on diagnosis and treatment of BCC.

Samenvatting

De snelle incidentie stijging van het basaal cel carcinoom (BCC) heeft ervoor gezorgd dat er nieuwe, niet-invasieve behandelingen op de markt zijn gekomen. Deze oppervlakkig werkende therapieën zijn goede alternatieven voor chirurgie in het superficieel BCC. Omdat BCC worden onderverdeeld in drie grote subgroepen (superficieel, nodulair en agressief, afgekort als respectievelijk sBCC, nBCC en aBCC) is het van groot belang om accurate diagnostiek toe te passen met een goed vermogen om te discrimineren tussen de verschillende histologische subtypes zodat een gepaste behandeling kan worden gekozen. sBCC kunnen niet-invasief worden behandeld terwijl nBCC en aBCC een chirurgische behandeling vereisen. In dit proefschrift worden de resultaten weergegeven van zeven studies naar de diagnostiek en de behandeling van het BCC.

Hoofdstuk 1 geeft een algemene introductie van het proefschrift. Hierin wordt een kort overzicht gegeven van de epidemiologie, risicofactoren, pathogenese, diagnostiek en behandeling van het BCC. Daarnaast worden in dit hoofdstuk de doelstellingen van het proefschrift besproken.

Hoofdstuk 2 beschrijft twee studies naar de diagnostiek van het BCC. In **hoofdstuk 2.1** presenteren we de resultaten van een retrospectieve studie waarin wordt vastgesteld wat de overeenkomst is tussen het histologisch BCC subtype en de meest agressieve component op het punch biopt in vergelijking met de diagnose op de daaropvolgende chirurgische excisie. In totaal werden 243 primaire BCC bij 191 patiënten geanalyseerd en werd het histologisch subtype op het punch biopt en de daaropvolgende chirurgische excisie genoteerd. Omdat bekend is dat een aanzienlijk percentage van BCC uit meer dan één subtype bestaat, zijn de analyses gebaseerd op het meest agressieve histologisch subtype. De overeenkomst tussen histologisch subtype op het punch biopt en de chirurgische excisie was 60.9%. De proportie van punch biopten dat op een correct wijze het meest agressieve groeipatroon van het gehele BCC kan identificeren was 84.4%. Dit betekent dat in 1 op de 6 BCC het meest agressieve groeipatroon wordt gemist in een juist afgenomen punch biopt. Deze resultaten geven aan dat dermatologen en overige artsen die BCC behandelen zich bewust moeten zijn van de beperkte diagnostische waarde van een punch biopt bij het vaststellen van het histologisch BCC subtype van de gehele laesie. Foutieve diagnose van het subtype kan leiden tot onder- en overbehandeling van het BCC.

Sommige dermatologen betwisten of het achterwege laten van een biopt acceptabel is of zelfs de voorkeur geniet in sommige gevallen. Nadelen van een punch biopt zijn namelijk het ongemak voor de patiënt en de gerelateerde tijd en kosten voor de arts. De klinische diagnose is daarentegen een pijnloze, tijd- en kostenbesparende procedure. In **hoofdstuk 2.2** vergelijken we de diagnostische nauwkeurigheid van de klinische blik en de histologische diagnose middels punch biopt voor subtypering van het BCC. De gouden standaard voor subtypering van het BCC was het histologische subtype aanwezig in het chirurgisch excisie preparaat. Daarnaast evalueren we de gevolgen van het achterwege laten van het punch biopt op de behandelvoorstellen. Deze studie werd uitgevoerd op de afdeling dermatologie in het Maastricht Universitair Medisch Centrum en het Erasmus Medisch Centrum Rotterdam. In totaal werden 152 histologisch bewezen BCC van 116 patiënten beoordeeld op het meest agressieve subtype middels klinische blik, het daaropvolgende punch biopt en de chirurgische excisie. Er werd een onderscheid gemaakt tussen superficieel, nodulair of agressief BCC. De gouden standaard voor BCC subtypering was het histologisch subtype aanwezig in de chirurgische excisie. De resultaten laten zien dat het achterwege laten van een punch biopt kan resulteren in overstadiëring van 1 op de 4 sBCC en onderstadiëring van 1 op de 4 aBCC. Een punch biopt is dus een beter diagnostisch middel dan de klinische blik voor het vaststellen van het histologisch BCC subtype, hetgeen in overeenstemming is met internationale richtlijnen. In het geval dat een arts toch beslist om het punch biopt achterwege te laten, moet er een afweging worden gemaakt tussen de impact van over- en onderstadiëring en de extra tijd, kosten en ongemak voor de patiënt die gerelateerd zijn aan het nemen van een punch biopt.

Hoofdstuk 3 is onderverdeeld in vijf secties en beschrijft studies naar de effectiviteit van verschillende behandelopties voor laag-risico BCC. Bovendien streefden wij naar het identificeren van subgroepen van patiënten en tumoren die verschillen in behandelreactie om zodoende de meest effectieve behandeling te selecteren voor individuele patiënten gebaseerd op patiënt en tumor karakteristieken.

In **hoofdstuk 3.1** werd een systematische review en meta-analyse uitgevoerd om vast te stellen wat de residuvrije, recidiefvrije en de tumorvrije overlevingskansen zijn van patiënten met een primair sBCC behandeld met één van de huidige, meest frequente behandelopties. We zochten naar artikelen op de Pubmed, EMBASE en Cochrane database. Daarnaast werden referentielijsten van de geselecteerde artikelen nagetrokken. Studies werden geïncludeerd wanneer deze rapporteerden over residu en/of recidief kansen na behandeling van primair, histologisch bewezen sBCC met een minimale follow-up van 12 weken. Zesendertig studies (14 gerandomiseerde en 22 niet-gerando-

miseerde trials) werden geïncludeerd. De gepoolde uitkomst van sBCC met tumorvrije overleving één jaar na behandeling , verkregen uit 23 studies, was 87.3% (95% CI 84-91%) voor imiquimod en 84.0% (95% CI 78-90%) voor fotodynamische therapie (PDT). De PDT tumorvrije overleving was lager voor onderzoeken waarin werd behandeld met één enkele cyclus. In de literatuur zijn slechts enkele studies voorhanden die rapporteren over de behandeling van sBCC middels 5-fluorouracil (5-FU) of chirurgische excisie. Aan de hand van onze meta-analyse concluderen we dat imiquimod en PDT allebei goede niet-invasieve behandelopties zijn voor sBCC. Er is echter wel een gebrek aan head-to-head vergelijkende studies tussen imiquimod, PDT en andere behandelingen en lange termijn studies zijn schaars.

In **hoofdstuk 3.2** presenteren we de resultaten van een non-inferioriteit gerandomiseerde trial met drie jaar follow-up, waarin de tumorvrije overleving werd onderzocht van imiquimod en 5-FU ten opzichte van methylaminolevulaat (MAL)-PDT in patiënten met sBCC. Deze studie voldoet aan de behoefte naar head-to-head vergelijkende studies met een lange follow-up en maakt het mogelijk om betere aanbevelingen te doen voor niet-invasieve behandelingen van sBCC. In totaal werden 601 patiënten met een primair sBCC geïncludeerd op zeven afdelingen dermatologie in het zuiden van Nederland. Deelnemers werden gerandomiseerd naar MAL-PDT (twee sessies met één week interval), imiquimod (éénmaal daags, vijf dagen per week gedurende 6 weken) of 5-FU (tweemaal daags gedurende 4 weken). Een controle afspraak vond plaats drie jaar na behandeling door een arts-onderzoeker die geblindeerd was voor de toegewezen behandeling. Klinisch behandelfalen werd histologisch bevestigd met een 3 mm punch biopt. Een vooraf vastgestelde non-inferioriteits marge van 10% werd gehanteerd. We toonden aan dat de kans op tumorvrije overleving drie jaar na behandeling 58.0% was voor MAL-PDT, 79.7% voor imiquimod en 68.2% voor 5-FU. De hazard ratio voor behandelfalen tussen imiquimod en MAL-PDT was 0.50 (95% CI 0.33-0.76, p=0.001). De vergelijking tussen 5-FU met MAL-PDT resulteerde in een hazard ratio van 0.73 (95% CI 0.51-1.05, p=0.092) en de vergelijking tussen 5-FU en imiquimod in een hazard ratio van 0.68 (95% CI 0.44-1.06, p=0.091). Imiquimod is dus superieur aan MAL-PDT en richtlijnen over sBCC behandeling zouden imiquimod moeten aanbevelen als eerste keus behandeling. 5-FU is niet inferieur aan MAL-PDT, maar is ongunstig vergeleken met het behandelsucces na imiquimod behandeling. Beide crèmes hebben een vergelijkbaar cosmetisch resultaat en risico op lokale bijwerkingen. Ondanks het feit dat 5-FU minder duur is dan imiquimod, is de kostenbesparing van 5-FU vergeleken met imiquimod erg laag gezien het feit dat het hoge aantal behandelfalen meer aanvullende behandelingen vereist.

In **hoofdstuk 3.3** hebben we onderzocht of het relatieve behandeleffect van MAL-PDT en imiquimod consistent is binnen subgroepen gedefinieerd door bepaalde patiënt en tumor karakteristieken. Het definiëren van subgroepen patiënten die verschillen in reactie op MAL-PDT en imiquimod is van grote waarde in de klinische praktijk voor het selecteren van de meest effectieve behandeling voor een individuele patiënt met een sBCC. De benodigde gegevens werden verkregen uit de gerandomiseerde multicenter studie die in hoofdstuk 3.2 is beschreven. De behandeling werd als succesvol gedefinieerd bij een tumorvrije overleving één jaar na behandeling. Subgroep analyses werden uitgevoerd voor subgroepen gedefinieerd door geslacht, leeftijd (≤ 60 jaar vs. > 60 jaar), tumor locatie (hoofd/hals, romp, onderste extremiteiten en bovenste extremiteiten) en tumor grootte (≤ 60 mm^2 vs. > 60 mm^2). Relevante eindpunten waren beschikbaar voor 196 patiënten behandeld met MAL-PDT en 189 patiënten behandeld met imiquimod. Imiquimod was superieur aan MAL-PDT in de subgroepen vrouwen, sBCC op de romp en grote tumoren met een verschil in slagingspercentages in het voordeel van imiquimod van respectievelijk 18.4%, 21.0% en 18.9%. Een grotere kans op behandelslagen (maar geen superioriteit) van imiquimod versus MAL-PDT werd consequent vastgesteld bij mannen, patiënten > 60 jaar, tumoren in het hoofd/hals gebied en de bovenste extremiteiten en bij kleinere sBCC. Opmerkelijk is dat binnen de subgroep van sBCC op de onderste extremiteiten de kans op een succesvolle behandeling na imiquimod veel kleiner was (36.8%) dan na MAL-PDT (94.1%) in patiënten > 60 jaar. Deze hogere kans op behandelslagen voor MAL-PDT ten opzichte van imiquimod werd niet waargenomen bij jongere patiënten met een sBCC op de onderste extremiteiten. In hoofdstuk 3.2 hebben we laten zien dat de resultaten van deze subgroep analyses onveranderd blijven na 3 jaar follow-up. We willen echter wel benadrukken dat de resultaten voorzichtig geïnterpreteerd dienen te worden omdat de subgroep analyses in deze studie exploratief waren en niet aangestuurd door vooraf gedefinieerde hypothesen. Op basis van de resultaten zou imiquimod de eerste keus behandeling blijven voor sBCC in termen van effectiviteit. MAL-PDT zou echter de voorkeur kunnen hebben in ouderen met een sBCC op de onderste extremiteiten.

In **hoofdstuk 3.4** hebben we verder onderzocht of de histologische karakteristieken tumordikte en uitbreiding langs haarfollikels van sBCC invloed hebben op het behandelslagen van niet-invasieve therapieën. Wederom hebben we hiervoor gebruik gemaakt van de gegevens afkomstig uit de gerandomiseerde multicenter studie die in hoofdstuk 3.2 is beschreven. De studie werd opgezet als een case-controle onderzoek waarin 112 patiënten met behandelfalen werden geselecteerd als cases en 224 patiënten met behandelslagen willekeurig werden geselecteerd als controle personen. Hierdoor was het mogelijk om een odds ratio ≥ 2.5 te detecteren met een power van 80% (alfa = 5%). Histo-

pathologische coupes van de initiële diagnostische punch biopten werden opgevraagd om aanvullende informatie te verkrijgen over tumordikte en uitbreiding langs haarfollikels. Spreidingsdiagrammen toonden aan dat er geen verband is tussen tumordikte als continue variabele en behandelfalen voor alle behandelgroepen. Daarom zijn er geen aparte analyses per behandeling uitgevoerd maar zijn alle tumoren gecombineerd in één dataset. De tumordikte van alle geïncludeerde patiënten varieerde van 0.2 tot 1.0 mm. De resultaten lieten zien dat de tumordikte en uitbreiding langs haarfollikels van sBCC niet significant geassocieerd waren met behandelfalen na MAL-PDT, imiquimod of 5-FU. Vergelijkbare resultaten werden verkregen na correctie voor eventuele confounders (geslacht, leeftijd, type behandeling, tumor oppervlakte en tumor locatie) in een meervoudige logistische regressie analyse. We concluderen daarom dat tumordikte en uitbreiding langs haarfollikels niet in acht genomen hoeven te worden bij het besluit of niet-invasieve behandeling adequaat is voor een patiënt met sBCC.

In **hoofdstuk 3.5** vergelijken we de effectiviteit op lange termijn van gefractioneerd 20% 5-aminolevulinezuur (ALA)-PDT met voorafgaande debulking versus chirurgische excisie voor nBCC. Bovendien hebben we geanalyseerd wat het effect is van nBCC dikte op het behandelfalen van ALA-PDT. Patiënten die zich presenteerden op de polikliniek Dermatologie van het Maastricht Universitair Medisch Centrum met een primair, histologisch bewezen nBCC werden geïncludeerd en gerandomiseerd naar een behandeling met gefractioneerd ALA-PDT (n=85) of chirurgische excisie met een marge van 3 mm (n=88). Twee PDT belichtingen werden uitgevoerd met één uur interval. De follow-up bedroeg minimaal 5 jaar na behandeling en elk klinisch verdacht behandelfalen werd histologisch bevestigd met een 3 mm punch biopt. In de ALA-PDT groep werd de tumordikte retrospectief bepaald op het initiële diagnostische punch biopt door een onafhankelijk onderzoeker . De cumulatieve kans op behandelfalen 5 jaar na behandeling was 30.7% voor ALA-PDT en 2.3% voor chirurgische excisie ($p<0.0001$). Opvallend is dat twee tumoren behandeld met ALA-PDT nog recidiveerden 72 en 91 maanden na behandeling. De tumordikte van nBCC varieerde van 0.3-1.3 mm. De cumulatieve kans op tumorvrije overleving na ALA-PDT was 65.0% voor tumoren met een dikte > 0.7 mm vergeleken met 94.4% voor tumoren ≤ 0.7 mm. Deze bevindingen bevestigen dat chirurgische excisie de gouden standard moet blijven voor behandeling van nBCC. ALA-PDT zou daarentegen wel overwogen kunnen worden als aanvaardbare behandeling voor een selecte groep patiënten met een inoperabele nBCC met een dikte ≤ 0.7 mm. Dit betekent dat wanneer er een verdenking op een nBCC bestaat, artsen een diagnostisch punch biopt zouden moeten afnemen van het klinisch meest dikke deel van de tumor en dat pathologen op verzoek de tumordikte van nBCC zouden moeten bepalen.

Hoofdstuk 4 besluit met een discussie en valorisatie waarin wordt besproken welke plaats de resultaten van dit proefschrift innemen binnen de bestaande kennis over diagnostiek en behandeling van het BCC.

List of abbreviations

List of abbreviations

5-FU	5-fluorouracil
aBCC	aggressive basal cell carcinoma
ALA	aminolaevulinic acid
BCC	basal cell carcinoma
BCNS	basal cell nevus syndrome
CI	confidence interval
dUMP	deoxyuridine monophosphate
dTMP	deoxythymidine monophosphate
ESR	European Standardized Rate
FDA	Food and Drug Administration
HH	hedgehog
HR	hazard ratio
IL	interleukin
LED	light emitting diode
MAL	methylaminolevulinate
MC	Medical Centre
MC1R	melanocortin 1 receptor
MMS	Mohs' micrographic surgery
MUMC	Maastricht University Medical Centre
nBCC	nodular basal cell carcinoma
NMSC	non melanoma skin cancer
OR	odds ratio
PDT	photodynamic therapy
PpIX	protoporphyrin IX
PTCH	patched 1
RCT	randomized controlled trial
sBCC	superficial basal cell carcinoma
SCC	squamous cell carcinoma
SHH	sonic hedgehog
SMO	smoothened
TS	thymidylate synthetase
UV	ultraviolet light

Curriculum Vitae

Curriculum Vitae

Marieke Roozeboom werd geboren op 18 oktober 1985 in Waalre. Zij groeide daar op als oudste van een gezin met twee jongere broers. In 2003 behaalde zij haar VWO diploma aan het Lorentz Casimir Lyceum te Eindhoven. In hetzelfde jaar begon zijn aan de studie Geneeskunde in Maastricht. Na drie jaar studeren heeft zij haar studie een jaar onderbroken om onderzoek te kunnen doen bij de Kinderpulmonologie in het Maastricht Universitair Medisch Centrum (MUMC). Gedurende deze periode groeide haar interesse voor wetenschappelijk onderzoek. Vervolgens heeft zij haar reguliere coschappen in Nederland en 3 maanden in Kenia doorlopen. Tijdens haar coschap Dermatologie in het Maxima Medisch Centrum te Veldhoven werd haar belangstelling voor dit vak gewekt. Daarom heeft zij in het laatste jaar van haar opleiding tot basisarts ervoor gekozen om zich verder toe te leggen op klinische en wetenschappelijke ervaring bij de Dermatologie in het MUMC. Op 31 augustus 2010 behaalde zij het artsexamen en op 1 september van hetzelfde jaar is zij begonnen met haar promotieonderzoek bij de afdeling Dermatologie in het MUMC. Op 1 september 2012 startte zij naast haar promotieonderzoek met de opleiding tot Dermatoloog, eveneens in Maastricht. Zij werd in 2014 genomineerd voor de Pélerin wetenschapsprijs voor artsassistenten.

List of publications

List of publications

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Oral presentations

Lupus erythematosus. Multidisciplinary evening seminar, Elkerliek hospital, Helmond, February 2016.

Topical treatments for basal cell carcinoma. Skin cancer symposium, Centre of oncology MUMC+, Maastricht, January 2016.

Three year follow-up results of MAL-PDT versus imiquimod versus fluorouracil for treatment of superficial basal cell carcinoma: a single blind, non-inferiority, randomized controlled trial.

- EADO, Marseille, France, October 2015.
- Pélerin scientific symposium for residents at MUMC+, Maastricht, October 2014.

A skin problem as final part of the puzzle (Langerhans cell histiocytosis). Regional evening seminar dermatology, Southern Limburg, Maastricht, May 2015.

Shiitake dermatitis. Foundation Dutch Education of Dermatology and Venereology, Brussels, Belgium, November 2014.

Subtyping basal cell carcinoma by clinical diagnosis versus punch biopsy. Oncology in the pearl of the Ardennes, Spa, Belgium, March 2014.

Soft expected but still hard (subcutaneous fat necrosis of the newborn). Regional evening seminar dermatology, Southern Limburg, Maastricht, March 2014.

White with reddish dots (disseminated granuloma annulare). Regional evening seminar dermatology, Southern Limburg, Maastricht, May 2013.

Malignant peripheral nerve sheath tumor.

- Annular meeting of the Dutch Society for Dermatology and Venereology, Maastricht, June 2012.
- Regional evening seminar dermatology, Aachen, Germany, April 2011.
- Regional evening seminar dermatology, Southern Limburg, Maastricht, March 2011.

Clinical and histological prognostic factors for local recurrence and metastasis of invasive squamous cell carcinoma of the skin: Analysis of a varied population.

- Annular meeting of the Dutch Society for Experimental Dermatology, Lunteren, February 2012.
- Symposium of Integral Centre of Cancer Limburg on new developments in non-melanoma skin cancer, Sittard, March 2010.

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Dankwoord

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