Is a single day patient friendly methyl aminolevulinate photodynamic therapy illumination scheme for superficial basal cell carcinoma feasible? A randomized multicenter pilot trial

K. P. Nguyen, G. J. Knuiman, W. A. M. Blokx, L. Hoogedoorn, T. Smits & M. J. P. Gerritsen

To cite this article: K. P. Nguyen, G. J. Knuiman, W. A. M. Blokx, L. Hoogedoorn, T. Smits & M. J. P. Gerritsen (2019) Is a single day patient friendly methyl aminolevulinate photodynamic therapy illumination scheme for superficial basal cell carcinoma feasible? A randomized multicenter pilot trial, Journal of Dermatological Treatment, 30:2, 194-199, DOI: 10.1080/09546634.2018.1484558

To link to this article: https://doi.org/10.1080/09546634.2018.1484558
Is a single day patient friendly methyl aminolevulinate photodynamic therapy illumination scheme for superficial basal cell carcinoma feasible? A randomized multicenter pilot trial

K. P. Nguyen, G. J. Kuiniman, W. A. M. Bloks, L. Hoogedoorn, T. Smits and M. J. P. Gerritsen

Department of Dermatology, Radboud University Medical Center, Nijmegen, The Netherlands; Department of Pathology, Radboud University Medical Center, Nijmegen, The Netherlands; Department of Dermatology, Maxima Medical Center, Eindhoven, The Netherlands

ABSTRACT

Background: Topical methyl aminolevulinate photodynamic therapy (MAL-PDT) is highly effective for the treatment of superficial basal cell carcinoma (sBCC). Current European treatment protocol requires two hospital visits, which is costly and unpractical. The aim of this study was to evaluate the efficacy of fractionated MAL-PDT, using two light fractions at 3 and 4 h compared to illumination at 3 and 5 h after MAL-application.

Methods: Thirty patients were randomized into two groups. The first group received illumination at 3 and 4 h (20 ± 55 J/cm²) after MAL-application (3/4 group). In the other group, two light fractions were performed at 3 and 5 h (20 ± 55 J/cm²) after MAL-application (3/5 group). The lesion response was evaluated at 3 and 12 months posttreatment.

Results: In the 3/5 group, 70.0% showed a complete response (CR) at 3 months compared to 63.6% in the other group. At 12 months, 100% showed a CR in the 3/5 group compared to 80.0% in the other group. However, most failures/recurrences were eventually due to the presence of a more aggressive BCC subtype, mostly caused by sampling error of the primary punch biopsy.

Conclusion: Single day protocol for MAL-PDT for sBCC is feasible and this study shows promising results.

Introduction

Basal cell carcinoma (BCC) is the most common type of skin cancer with an increasing incidence worldwide, becoming an important health problem accompanied with rising health care costs (1–3). While nodular BCC (nBCC) is the most common type, a significant increase in the superficial subtype is noticed (4). Although surgery is an appropriate treatment option, superficial BCC (sBCC) is also suitable for nonsurgical treatment modalities, since it is easy accessible with topical treatment. Photodynamic therapy has been recommended as a first-line treatment for sBCC by an international consensus (5). Photodynamic therapy (PDT) involves the application of a topical photosensitizer or its prodrug, in most cases aminolevulinic acid (ALA) or its methylated ester methyl aminolevulinate (MAL).

MAL is the photosensitising agent approved for PDT for sBCC and/or nBCC (6). The current European protocol for MAL-PDT for sBCC consists of two light fractions (37 J/cm²) 1 week apart, repeated at 3 months if required (5,7–9). However, the double procedure is unpractical and the required day care visits result in high treatment costs (10). Therefore, a MAL-PDT protocol using two illumination fractions on the same day would be more practical and cheaper.

In ALA-PDT, multiple studies have shown the benefit of splitting the illumination into two light fractions over a single illumination session (11–13). This is due to re-synthesis of PpIX during the dark interval between two light fractions (14–16). Also in MAL-PDT, there is re-synthesis of PpIX after illumination (17). Further studies have tried to optimise fractionated ALA-PDT (18,19).

De Bruin et al. (17) investigated the response of MAL-PDT using a single- and a two-fold illumination scheme and compared that to ALA-PDT in normal mouse tissue. Four hours after ALA or MAL application, the skin was illuminated using either a single light fraction (100 J/cm²) or a two-fold illumination scheme (5 + 95 J/cm²) with a 2-h interval. They showed that fractionated illumination did not enhance the clinical efficacy of MAL-PDT, as was the case when using ALA. However, the optimum illumination scheme for MAL-PDT is 3 h after application and not 4, as is the case in ALA-PDT (20). Furthermore, this study was performed on normal mouse tissue, and not on human tumor tissue and MAL is known to be more tumor selective than ALA (21,22).

Therefore, the aim of this study was to evaluate fractionated illumination of MAL-PDT in patients with sBCC lesions. We compared two light fractions (20 + 55 J/cm²) of MAL-PDT with 1 or 2 h interval: illumination at 3 and 4 h compared to illumination at 3 and 5 h after MAL-application. The total light dose was 75 J/cm², according to the standard MAL-PDT protocol for sBCC. Furthermore, we studied the accuracy of histological examination in sBCC punch biopsies for detecting the correct BCC subtype.

Materials and methods

This is a prospective, single-blinded, randomized multicenter pilot trial, which was performed from June 2013 to October 2016 at
the Radboud University Medical Center (Radboudumc), Nijmegen and Maxima Medical Center (MMC), Eindhoven, the Netherlands. The study has full ethical approval (NL41859.091.12) and was executed according to the Declaration of Helsinki.

**Study population**

Eligible patients were those above the age of 18 years with a histological proven (3 mm punch biopsy) primary sBCC were included. From each patient, one sBCC was included. Exclusion criteria were patients with a known allergy to MAL or related compounds, participation in other clinical studies, received treatments in the last 12 weeks for skin cancer in the area to be treated, usage of chronic immunosuppressive medication and patients who were pregnant or breastfeeding.

**Intervention**

Patients were randomized into two groups in a 1:1 ratio using a sealed envelope system generated by a research nurse. The first group received illumination at 3 and 4 h (3/4 group) after MAL cream application. The second group was illuminated at 3 and 5 h (3/5 group) after application of MAL. No control group with illuminations 1 week apart was used, as many studies have analyzed the effect of this already approved protocol. Randomization occurred prior to pretreatment of the lesion. The research physician, who enrolled the patients and assessed the lesion response, was blinded to the assigned treatment. Patients and treating physicians were not masked for the assigned therapy. Lesion sizes were determined clinically. An ellipse formula \( \pi ab/4 \) was used to calculate the lesion area from the smallest \( a \) and largest dimension \( b \).

**MAL-PDT treatment protocol**

Salicylic acid (10%) in petrolatum daily for 1 week or an adhesive dressing (Duoderm\textsuperscript{©}, ConvaTec Inc., Deeside, UK) was applied prior to PDT if necessary. A MAL cream was used (Metyl\textsuperscript{©}, 160 mg/g, Galderma). First, a layer of MAL cream (approximately 1 mm thick) was applied to the lesion and to the surrounding 10 mm of normal skin. The tumor site was covered with an adhesive, occlusive dressing (Tegaderm\textsuperscript{©}, 3 M Health Care Ltd, Bracknell, UK) and tinfoil to prevent influence of light. Three hours after application, the cream was wiped off and the tumor was illuminated. The lesions were clinically evaluated at 3 and 12 months after treatment. At each visit, the clinical treatment response (complete, partial, and no response), lesion reduction and possible adverse events were evaluated. Complete responses (CR) were defined as 100% clinical visual clearance of the sBCC. Partial responses (PR) were defined as \( \geq 50\% \) reduction in the greatest diameter. No responses (NR) were assessed as \(< 50\% \) reduction in the greatest diameter. 

Photographs were taken at each follow up visit, unless no change was observed. A punch biopsy was performed in case of suspicion of a residual or recurrent BCC. If necessary, the choice of an additional treatment was determined by the treating physician.

**Histopathological examination process**

During the study, all punch biopsies were routinely histological examined with hematoxylin and eosin (H&E) stained tissue sections obtained from one level (at approximately 1000 \( \mu \)m). Superficial BCCs were histologically defined as nests of basaloid cells residing high in the dermis, usually in a multifocal pattern (23). After the PDT study, all punch biopsies were sectioned in four additional levels with an interval of 200 \( \mu \)m, in order to evaluate whether more aggressive BCC subtypes might have been missed using the routine protocol. After every 200 \( \mu \)m, 10 sections of 4 \( \mu \)m each were sectioned of which two sections were stained with H&E and evaluated by a pathologist-in-training (G.J.K.) and pathologist (W.A.M.B.). BCC subtype classification was based on the Dutch guideline (24). Tumor thickness was measured by calculating the basaloid nest from the stratum granulosum up until the deepest point of invasion.

**Statistical analysis**

Descriptive statistics, including median and range for continuous variables and percentages for categorical data, were used to explore patient and tumor characteristics. The Mann–Whitney U-test and Fisher’s exact test were used to compare continuous and categorical variables between groups, respectively. The Spearman’s rank-order correlation was used to assess whether there was an association between the amount of Acetaminophen used prior to illumination and the VAS score after the first and second illumination. In case a more aggressive BCC subtype was detected in the punch biopsies, that were sectioned in additional levels, follow up time was calculated from date of MAL-PDT treatment to date of second treatment (excision or Imiquimod) or last (poststudy) follow up (until January 2017). A \( p \)-value of \(< 0.05 \) was
regarded statistically significant. Statistical analyses were performed using IBM SPSS Statistics 22.0 (SPSS Inc., NY).

### Results

Between June 2013 and July 2015, 30 patients with sBCCs were enrolled; 16 patients of the Radboudumc and 14 of the MMC. Eight patients were excluded due to nonadherence to the protocol [no punch biopsy obtained (n = 6), more than one lesion per patient illuminated (n = 2)] and one patient was lost to follow up. The remaining 21 patients were included in the analyses. Patient and tumor characteristics were comparable in both groups (Table 1).

In 3 months posttreatment, both groups showed CR rates between 63.6% and 70.0% (Table 2). In the 3/4 group, 7 out of 11 sBCCs (63.6%) showed a CR after 3 months, while three sBCCs (27.3%) showed PR (Table 2). A punch biopsy was obtained from one of the partial responsive lesions, which revealed a sBCC. This lesion was marked as a treatment failure (9.1%) and treated with Imiquimod, no excision occurred. In one patient the partial response and treatment failure were detected at an extra visit 5 months after illumination.

Histological confirmation of response 1 (of the PR) 2 (of the PR) –

Excision: Nbcc 0 0 1 (5.6)

Recurrence (%)

Recurrence: presence of tumor tissue detected in follow-up after previous tumor clearance. This patient had a partial response at 3 months, complete response at 6 months, and partial response at 12 months after illumination.

Histological confirmation of response 1 (of the PR) 2 (of the PR) –

Excision: sBCC 1 (10.0) 0 .918 1 (5.6)

Median lesion size reduction in percentage (range) 1

Median lesion size reduction in mm² [range] 2

Recurrence (%)

Recurrence: presence of tumor tissue detected in follow-up after previous tumor clearance. This patient had a partial response at 3 months, complete response at 6 months, and partial response at 12 months after illumination.

Histological confirmation of response 1 (of the PR) 2 (of the PR) –

Excision: Nbcc 0 0 1 (5.6)

Recurrence (%)

Recurrence: presence of tumor tissue detected in follow-up after previous tumor clearance. This patient had a partial response at 3 months, complete response at 6 months, and partial response at 12 months after illumination.

Histological confirmation of response 1 (of the PR) 2 (of the PR) –

Excision: sBCC 1 (10.0) 0 .918 1 (5.6)

Median lesion size reduction in percentage (range) 3

Median lesion size reduction in mm² [range] 4

### Discussion

Current MAL-PDT treatment for sBCC requires two treatment sessions on separate days, and repeated after 3 months if necessary (5,7–9). This requires at least two hospital visits, which is unpractical and costly. Therefore, the purpose of this study was to evaluate two different MAL-PDT protocols in which two light fractions were performed on a single day, with 1 or 2 h interval.
This is the first study using fractionated MAL-PDT in sBCCs in human tissue. Overall, this study shows that two sessions of illumination on a single day leads to CR rates of 80.0–100% at 12 months. However, three of the five failures/reurrences were eventually due to the presence of a more aggressive BCC subtype, mostly caused by sampling error of the primary punch biopsy and, in a lesser degree, to underdiagnosis of the primary punch biopsy.

There are two prospective studies that used the approved MAL-PDT protocol and had a follow-up at 3 and 12 months (25,26). They show comparable CR rates at 12 months compared to our study, although their studies included a larger study population. Basset-Seguin et al. showed comparable results at 3 months when one single session of MAL-PDT (light dose 75 J/cm²) was given after sBCCs were treated with MAL-cream 3 h prior to illumination (27). This leads to the question whether it might be easier to perform one illumination session of 75 J/cm² instead of re-illumination at 4 and 5 h. However, the incomplete responders in the study of Basset-Seguin et al. were, thereafter, treated with 2 MAL-PDT illumination sessions 1 week apart at 3 months. For that reason, there is no information on the long-term effect of a single illumination session. Therefore, a study with a larger population comparing a single MAL-PDT illumination session (75 J/cm²) and fractionated MAL-PDT (20 + 50 J/cm²) with a longer follow-up period is recommended.

In this study, some lesions showed less erythema after PDT compared to before treatment, or mild remaining erythema possible due to scarring of the biopsy location or after PDT treatment. According to our strict definitions of ‘lesion response’, these lesions were marked PR or NR. However, not all these lesions were clinically suspect for a treatment failure or recurrence. Therefore, not all of them were biopsied. This is also the reason why some lesions, which appeared as PR or NR at 3 months, showed CR at 12 months.

All adverse events that were reported in this study were in accordance with other studies (25,28). No serious adverse reactions occurred. Arits et al. showed in their study that serious adverse events only occurred in patients treated with Imiquimod and 5-FU but not in the MAL-PDT treatment group (25). More importantly, a generally better cosmetic outcome is observed after PDT treatment of sBCC compared to other treatment options (25,26). Furthermore, the treatment regime for Imiquimod and topical 5-FU is intensive and long (4–6 weeks) (29). In daily practice, not all patients will be motivated or able to apply a cream for such a long period. For these patients, hospital-based treatments such as MAL-PDT and surgical excision might be preferable. In case, both treatments can take place during one visit, MAL-PDT may have the benefit over surgical excision, especially when cosmetic outcome and problematic healing sites are taken into consideration.

The five treatment failures/reurrences in the present study were mostly due to the presence of a more aggressive BCC subtype in the excision. In three lesions, other BCC subtypes (nodular and infiltrative) were detected in the excision specimen. Although, MAL-PDT is also effective in nBCC, poorer clearance rates and higher recurrences were seen in these tumors compared to sBCCs (26,30,31). Moreover, MAL-PDT is not registered for the treatment of iBCC. Histological underdiagnosis of the primary biopsy may have led to an increased number of treatment failures.

---

**Table 3. Histopathological evaluation.**

| Patients with punch biopsies | BCC subtype(s) detected in first punch biopsy | Treatment effect | BCC subtype detected in second punch biopsy | Treatment effect | BCC subtype detected in 3 months | BCC subtype detected in 12 months | Excision |
|-----------------------------|---------------------------------------------|-----------------|---------------------------------------------|-----------------|----------------------------------|----------------------------------|---------|
| 1                            | sBCC                                        | CR              | sBCC                                        | CR              | sBCC                             | sBCC                             | CR      |
| 2                            | sBCC                                        | CR              | sBCC                                        | CR              | sBCC                             | sBCC                             | CR      |
| 3                            | sBCC                                        | CR              | sBCC                                        | CR              | sBCC                             | sBCC                             | CR      |
| 4                            | sBCC                                        | CR              | sBCC                                        | CR              | sBCC                             | sBCC                             | CR      |
| 5                            | sBCC                                        | PR              | sBCC                                        | PR              | sBCC                             | sBCC                             | CR      |
| 6                            | sBCC                                        | CR              | sBCC                                        | CR              | sBCC                             | sBCC                             | CR      |
| 7                            | sBCC                                        | PR              | sBCC                                        | PR              | sBCC                             | sBCC                             | CR      |
| 8                            | sBCC                                        | PR              | sBCC                                        | PR              | sBCC                             | sBCC                             | CR      |
| 9                            | sBCC                                        | PR              | sBCC                                        | PR              | sBCC                             | sBCC                             | CR      |
| 10                           | sBCC                                        | CR              | sBCC                                        | CR              | sBCC                             | sBCC                             | CR      |
| 11                           | sBCC                                        | CR              | sBCC                                        | CR              | sBCC                             | sBCC                             | CR      |
| 12                           | sBCC                                        | CR              | sBCC                                        | CR              | sBCC                             | sBCC                             | CR      |
| 13                           | sBCC                                        | CR              | sBCC                                        | CR              | sBCC                             | sBCC                             | CR      |
| 14                           | sBCC                                        | CR              | sBCC                                        | CR              | sBCC                             | sBCC                             | CR      |
| 15                           | sBCC                                        | CR              | sBCC                                        | CR              | sBCC                             | sBCC                             | CR      |
| 16                           | sBCC                                        | CR              | sBCC                                        | CR              | sBCC                             | sBCC                             | CR      |
| 17                           | sBCC                                        | CR              | sBCC                                        | CR              | sBCC                             | sBCC                             | CR      |
| 18                           | sBCC                                        | CR              | sBCC                                        | CR              | sBCC                             | sBCC                             | CR      |
| 19                           | sBCC                                        | CR              | sBCC                                        | CR              | sBCC                             | sBCC                             | CR      |
| 20                           | sBCC                                        | CR              | sBCC                                        | CR              | sBCC                             | sBCC                             | CR      |
| 21                           | sBCC                                        | PR              | sBCC                                        | PR              | sBCC                             | sBCC                             | CR      |

---

iBCC: infiltrative basal cell carcinoma; nBCC: nodular basal cell carcinoma; sBCC: superficial basal cell carcinoma. *–* denotes Punch biopsy or surgical excision was not performed.

bNo additional treatments were given after the initial MAL-PDT treatment.

*This punch biopsy was obtained at an extra visit 5 months after illumination.

---
due to undertreatment. In order to reduce this risk, additional sectioning of the primary punch biopsy can be performed (32,33). This might result in a more accurate diagnosis of the primary punch biopsy for adequate treatment. In our study, additional sectioning yielded the detection of four BCCs with a more aggressive BCC component in the primary biopsies, which were initially diagnosed as sBCCs. Two of these lesions were treatment failures and removed by surgical excisions. The other two did not show any clinical signs of treatment failure/recurrence after MAL-PDT (median follow up period more than 2 years). In these two lesions, MAL-PDT seemed to be an effective treatment. A hypothesis for this might be that MAL-PDT is more effective in nBCCs with a small tumor thickness (Table 3; patient 17) compared to nBCCs with a larger tumor thickness (patients 6 and 8). On the other hand, one nBCC lesion with a large tumor thickness (patient 12) had a good clinical effect after MAL-PDT (follow-up approximately 2 years). There is a chance that this lesion might recur after an extended follow-up period, like in the study of Roozeboom et al., where they noticed recurrences 3 years after MAL-PDT (34). Therefore, this patient remains in (poststudy) routine clinical follow-up. Another hypothesis is that the most aggressive part of the mixed type BCC was removed by the punch biopsy, leaving only the superficial type which responded well to the MAL-PDT treatment.

One mixed type BCC was not detected after additional sectioning of the primary punch biopsy (Table 3; patient 4). This may have been a result of sampling error of the primary punch biopsy. The usage of noninvasive diagnostic techniques, such as the reflectance confocal microscopy (RCM), may reduce sampling errors as they offer the possibility to image the whole lesion and distinguish different BCC subtypes (35,36). Overall, various options are available to reduce sampling errors resulting in higher cost-effectiveness, because they prevent the need for repeated biopsies and subsequent treatment.

In conclusion, this study shows that MAL-PDT, given in a two-fold illumination scheme with 1 or 2 h interval, is feasible and shows promising results in the treatment of sBCC. Moreover, our study shows the added value of a more thorough histological examination in detecting the BCC subtype(s) in punch biopsies. The next step would be to perform a larger clinical study to evaluate the benefit of fractionated MAL-PDT over a single MAL-PDT session and the regular MAL-PDT protocol.

Trial registration

This trial was not registered in a trial registry because when this study started (in 2013) it was not very common practice to prospectively register trials. When future studies on this subject are conducted, we will prospectively register these studies in a trial registry.

Disclosure statement

Nguyen and Hoogendoorn received financial support from Galderma for performing clinical trials.

Smits received speaker’s honoraria from Galderma for organising PDT-related workshops and financial support for performing clinical trials.

Gerritsen received speaker’s honoraria from Galderma, 3 M and Medac and joined Galderma and Leo Pharma advisory board. Furthermore, she received financial support from PhotoCure, Galderma, Leo Pharma and 3 M for performing clinical trials.

Knuiman and Blokx have no conflict of interest.

Funding

This work was funded by Galderma S.A., Switzerland.

References

1. Flohil SC, de Vries E, Neumann HA, et al. Incidence, prevalence and future trends of primary basal cell carcinoma in the Netherlands. Acta Derm Venereol. 2011;91:24–30.
2. Lomas A, Leonardi-Bee J, Bath-Hextall F. A systematic review of worldwide incidence of nonmelanoma skin cancer. Br J Dermatol. 2012;166:1069–1080.
3. Housman TS, Feldman SR, Williford PM, et al. Skin cancer is among the most costly of all cancers to treat for the Medicare population. J Am Acad Dermatol. 2003;48:425–429.
4. Arits AH, Schlangen MH, Nelemans PJ, et al. Trends in the incidence of basal cell carcinoma by histopathological subtype. J Eur Acad Dermatol Venereol. 2011;25:565–569.
5. Braathen LR, Szeimies RM, Basset-Seguin N, et al. Guidelines on the use of photodynamic therapy for nonmelanoma skin cancer: an international consensus. International Society for Photodynamic Therapy in Dermatology. 2005. J Am Acad Dermatol. 2007;56:125–143.
6. Morton C, Szeimies RM, Sidoroff A, et al. European Dermatology Forum Guidelines on topical photodynamic therapy. Eur J Dermatol. 2015;25:296–311.
7. Morton CA, Szeimies RM, Sidoroff A, et al. European guidelines for topical photodynamic therapy part 1: treatment delivery and current indications – actinic keratoses, Bowen’s disease, basal cell carcinoma. J Eur Acad Dermatol Venereol. 2013;27:536–544.
8. Trakatelli M, Morton C, Nagore E, et al. Update of the European guidelines for basal cell carcinoma management. Eur J Dermatol. 2014;24:312–329.
9. Morton CA, McKenna KE, Rhodes LE. Guidelines for topical photodynamic therapy: update. Br J Dermatol. 2008;159:1245–1266.
10. Arits AH, Spoorenberg E, Mosterd K, et al. Cost-effectiveness of topical imiquimod and fluorouracil vs. photodynamic therapy for treatment of superficial basal-cell carcinoma. Br J Dermatol. 2014;171:1501–1507.
11. de Haas ER, Kruitj B, Sterenborg HJ, et al. Fractionated illumination significantly improves the response of superficial basal cell carcinoma to aminolevulinic acid photodynamic therapy. J Invest Dermatol. 2006;126:2679–2686.
12. Star WM, van’t Veen AJ, Robinson DJ, et al. Topical 5-aminolevulinic acid mediated photodynamic therapy of superficial basal cell carcinoma using two light fractions with a two-hour interval: long-term follow-up. Acta Derm Venereol. 2006;86:412–417.
13. de Vijlder HC, Sterenborg HJ, Neumann HA, et al. Light fractionation significantly improves the response of superficial basal cell carcinoma to aminolevulinic acid photodynamic therapy: five-year follow-up of a randomized, prospective trial. Acta Derm Venereol. 2012;92:641–647.
14. van der Veen N, van Leengoed HL, Star WM. In vivo fluorescence kinetics and photodynamic therapy using 5-aminolevulinic acid-induced porphyrin: increased damage after multiple irradiances. Br J Cancer. 1994;70:867–872.
15. Van der Veen N, De Brujin HS, Star WM. Photobleaching during and re-appearance after photodynamic therapy of topical ALA-induced fluorescence in UVB-treated mouse skin. Int J Cancer. 1997;72:110–118.
16. Robinson DJ, de Bruijn HS, de Wolf WJ, et al. Topical 5-aminolevulinic acid-photodynamic therapy of hairless mouse skin using two-fold illumination schemes: PpIX fluorescence kinetics, photobleaching and biological effect. Photochem Photobiol. 2000;72:794–802.

17. de Bruijn HS, de Haas ER, Hebeda KM, et al. Light fractionation does not enhance the efficacy of methyl 5-aminolevulinic acid mediated photodynamic therapy in normal mouse skin. Photochem Photobiol Sci. 2007;6:1325–1331.

18. Robinson DJ, de Bruijn HS, Star WM, et al. Dose and timing of the first light fraction in two-fold illumination schemes for topical ALA-mediated photodynamic therapy of hairless mouse skin. Photochem Photobiol. 2003;77:319–323.

19. de Bruijn HS, van der Ploeg-van den Heuvel A, Sterenborg HJ, et al. Fractionated illumination after topical application of 5-aminolevulinic acid on normal skin of hairless mice: the influence of the dark interval. J Photochem Photobiol B. 2006;85:184–190.

20. Angell-Petersen E, Sorensen R, Warloe T, et al. Porphyrin formation in actinic keratosis and basal cell carcinoma after topical application of methyl 5-aminolevulinate. J Invest Dermatol. 2006;126:265–271.

21. Peng Q, Moan J, Warloe T, et al. Build-up of esterified aminolevulinic-acid-derivative-induced porphyrin fluorescence in normal mouse skin. J Photochem Photobiol Biol B. 1996;34:95–96.

22. Fritsch C, Homey B, Stahl W, et al. Preferential relative porphyrin enrichment in solar keratoses upon topical application of delta-aminolevulinic acid methylester. Photchem Photobiol. 1998;68:218.

23. Marzuka AG, Book SE. Basal cell carcinoma: pathogenesis, epidemiology, clinical features, diagnosis, histopathology, and management. Yale J Biol Med. 2015;88:167–179.

24. Kelleners-Smeets NW dHEBR, Ingels KJAO, Corten EML, et al. Evidence based richtlijn basaalcelcarcinoom (modulaire update 2014). 2014.

25. Arits AH, Mosterd K, Essers BA, et al. Photodynamic therapy versus topical Imiquimod versus topical fluorouracil for treatment of superficial basal-cell carcinoma: a single blind, non-inferiority, randomised controlled trial. Lancet Oncol. 2013;14:647–654.

26. Szeimies RM, Ibbonson S, Murrell DF, et al. A clinical study comparing methyl aminolevulinate photodynamic therapy and surgery in small superficial basal cell carcinoma (8–20 mm), with a 12-month follow-up. J Eur Acad Dermatol Venereol. 2008;22:1302–1311.

27. Basset-Seguin N, IlbtsnoN S, Emtestam L, et al. Topical methyl aminolaevulinate photodynamic therapy versus cryotherapy for superficial basal cell carcinoma: a 5 year randomized trial. Eur J Dermatol. 2008;18:547–553.

28. Morton CA. Methyl aminolevulinate (Metvix) photodynamic therapy – practical pearls. J Dermatol Treat. 2003;14:23–26.

29. Telfer NR, Colyer GB, Morton CA. Guidelines for the management of basal cell carcinoma. Br J Dermatol. 2008;159:35–48.

30. Fantini F, Greco A, Del Giovane C, et al. Photodynamic therapy for basal cell carcinoma: clinical and pathological determinants of response. J Eur Acad Dermatol Venereol. 2011;25:896–901.

31. Rhodes LE, de Rie MA, Leifsdottir R, et al. Five-year follow-up of a randomized, prospective trial of topical methyl aminolevulinate photodynamic therapy vs surgery for nodular basal cell carcinoma. Arch Dermatol. 2007;143:1131–1136.

32. Hoogedoorn L, Hendriks JC, Knuiman GJ, et al. Treatment failure in superficial basal cell carcinoma following treatment with photodynamic therapy: is this a result of underdiagnosis? J Eur Acad Dermatol Venereol. 2017;31:e50–ee2.

33. Nguyen KP, Knuiman GJ, van Erp PE, et al. Standard step sectioning of skin biopsy specimens diagnosed as superficial basal cell carcinoma frequently yields deeper and more aggressive subtypes. Am Acad Dermatol. 2017;76:351–353.e3.

34. Roozeboom MH, Arits AH, Mosterd K, et al. Three-year follow-up results of photodynamic therapy vs. Imiquimod vs. fluorouracil for treatment of superficial basal cell carcinoma: a single-blind, noninferiority, randomized controlled trial. J Invest Dermatol. 2016;136:1568–1574.

35. Longo C, Lallas A, Kyrgidis A, et al. Classifying distinct basal cell carcinoma subtype by means of dermatoscopy and reflectance confocal microscopy. J Am Acad Dermatol. 2014;71:716–724.e1.

36. Peppelman M, Wolberink EA, Blokx WA, et al. In vivo diagnosis of basal cell carcinoma subtype by reflectance confocal microscopy. Dermatology (Basel). 2013;227:255–262.