Treatment of superficial basal cell carcinoma by topical photodynamic therapy with fractionated 5-aminolaevulinic acid 20% vs. two-stage topical methyl aminolaevulinate

BJD
British Journal of Dermatology

Treatment of superficial basal cell carcinoma by topical photodynamic therapy with fractionated 5-aminolaevulinic acid 20% vs. two-stage topical methyl aminolaevulinate: results of a randomized controlled trial*

J.P.H.M. Kessels,[1,2,3] H. Kreukels,[4] P.J. Nelemans,[5] M.H. Roozeboom,[1] H. van Pelt,[6] K. Mosterd,[1,3] E.R.M. de Haas[4] and N.W.J. Kelleners-Smeets[1,3]

[1]Department of Dermatology, Maastricht University Medical Centre, Maastricht, the Netherlands
[2]Department of Dermatology, Zuyderland Medical Centre, Heerlen, the Netherlands
[3]GROW School for Developmental Biology and Oncology and [5]Department of Epidemiology, Maastricht University, Maastricht, the Netherlands
[4]Department of Dermatology, Erasmus Medical Centre, Rotterdam, the Netherlands
[6]Department of Dermatology, VieCuri Medical Centre, Venlo/Venray, the Netherlands

Linked Comment: Ibbotson. *Br J Dermatol* 2018; **178**:1003.

Correspondence
J.P.H.M. Kessels.
E-mail: janneke.kessels@mumc.nl

Accepted for publication
31 August 2017

Funding sources
This study was funded by a grant from the Health Foundation Limburg, the Netherlands.

Conflicts of interest
None to declare.

J.P.H.M.K and H.K. contributed equally to the manuscript

*Plain language summary available online

DOI 10.1111/bjd.15967

Summary

Background Basal cell carcinoma (BCC) is the most common type of skin cancer and incidence rates are increasing. Photodynamic therapy (PDT) is a frequently used treatment, especially for superficial BCC (sBCC). Two topical photosensitizing agents are currently used to treat sBCC, namely 5-aminolaevulinic acid (ALA) and its ester, methyl aminolaevulinate (MAL). Previous research showed a high efficacy for ALA-PDT using a twofold fractionated illumination scheme in which two light fractions of 20 J cm^{-2} and 80 J cm^{-2} were delivered 4 h and 6 h after ALA application.

Objectives To evaluate whether twofold ALA-PDT is superior to conventional MAL-PDT for sBCC.

Methods We performed a single-blind, randomized, multicentre trial in the Netherlands.

Results Overall, 162 patients were randomized either to conventional MAL-PDT or twofold ALA-PDT. After 12 months, a total of six treatment failures occurred following ALA-PDT and 13 treatment failures occurred following MAL-PDT. The 12-month cumulative probability of remaining free from treatment failure was 92·3% [95% confidence interval (CI) (83·7–96·5)] for ALA-PDT and 83·4% (95% CI 73·1–90·0) for MAL-PDT (P = 0·091).

Conclusions The twofold ALA-PDT scheme resulted in fewer recurrences, although the difference between both treatment groups was not statistically significant. However, ALA-PDT resulted in higher pain scores and more post-treatment side-effects compared with MAL-PDT.

What's already known about this topic?

- Photodynamic therapy (PDT) is a well-known noninvasive therapy for superficial basal cell carcinoma (sBCC).
- In Europe, methyl aminolaevulinate (MAL) is the most frequently used photosensitizer.
- Previous research showed a high efficacy for a twofold fractionated illumination scheme using 5-aminolaevulinic acid (ALA) in which two light fractions of 20 J cm^{-2} and 80 J cm^{-2} were delivered 4 h and 6 h after ALA application.

What does this study add?

- This is the first randomized clinical trial to compare the twofold ALA-PDT scheme with conventional MAL-PDT.
- Twofold ALA-PDT resulted in fewer recurrences, although this was not statistically significant. Also, a higher cumulative probability of remaining free from treatment failure was found for ALA-PDT compared with conventional MAL-PDT.
- Twofold ALA-PDT should be considered as an effective treatment protocol.

Basal cell carcinoma (BCC) is the most common type of skin cancer and incidence rates are increasing.[1] BCC can be categorized into three histological subtypes, namely superficial, nodular and infiltrative.[2] Most BCCs are treated with surgical excision. However, one-third of BCCs are superficial and do not necessarily require excision. Topical treatments such as photodynamic therapy (PDT), 5-fluorouracil cream and imiquimod cream are frequently used. A recent randomized comparative study showed an efficacy for these treatments varying from 72·8% to 83·4%.[3]

Advantages of PDT, compared with topical ointments, include the short duration of treatment and a good cosmetic outcome.[4,5] Topical porphyrin precursors are applied to the skin and converted into protoporphyrin IX (PpIX). When exposed to oxygen and light in the appropriate wavelength, singlet oxygen is formed and the tumour cells are destroyed.[6–8] Two photosensitizers are currently used to treat superficial BCC (sBCC), namely 5-aminolaevulinic acid (ALA) and methyl aminolaevulinate (MAL). A systematic review by Peng *et al.* showed a weighted clearance rate (CR) of 87%.[9] Overall CRs of 72·8–84·0% have been observed 1 year post-treatment for MAL-PDT.[3,10,11] There is currently no randomized controlled trial that directly compares ALA-PDT with MAL-PDT for the treatment of sBCC.

A study by de Haas *et al.* investigated whether a twofold illumination scheme on 1 day after a single ALA application could lead to better efficacy. One year post-treatment, a CR of 97% was observed after treatment with this twofold ALA-PDT scheme.[12] In a recent retrospective study, a CR of 90·2% was reported after a 1-year follow-up.[13]

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

Materials and methods

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

Patients

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

Randomization and masking

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

Procedures

At the baseline visit, tumour and patient characteristics were recorded and patients were included in the study.

The 5-ALA 20% ointment (Tiofarma B.V., Oud-Beijerland, the Netherlands) was applied to the tumour surface at a thickness of 1–2 mm with a margin of 5 mm of healthy surrounding skin. The treatment area was then covered with an occlusive dressing (Tegaderm®, 3M, Leiden, the Netherlands), gauze and tinfoil to prevent illumination by ultraviolet radiation. After 4 h, the tumour was illuminated with a light-emitting diode light source (Aktilite, Galderma SA, Lausanne, Switzerland or Omnilux PDT, Phototherapeutics, London, U.K.). These light sources produced red light with an optimum wavelength of ~630 nm ± 5 nm and a fluence of 20 J cm^{-2} for a duration of 4 min. Subsequently, the treatment area was covered again in the same manner for 2 h,

whereupon a second illumination with a fluence of 80 J cm^{-2} for a duration of 18 min took place. Both illuminations were performed at an irradiance of 50 mW cm^{-2}.

Patients who received MAL-PDT were treated with Metvix® ointment (Galderma SA, Penn Pharmaceutical Services, Gwent, U.K.) that was applied to the tumour in the exact same way as 5-ALA ointment. The tumour was covered with an occlusive dressing and after 3 h was illuminated with either Aktilite or Omnilux with a fluence of 37 J cm^{-2} at an irradiance 75 mW cm^{-2} for a duration of 7 min. This regimen was repeated 1 week later.

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

Outcomes

The primary outcome was the probability of treatment success at 12-month follow-up. In the event of clinical suspicion of residual tumour at 3 months or recurrent tumour at 12 months, a biopsy was performed for histological examination. If a tumour was found, the case was considered to be a treatment failure.

Secondary outcomes were aesthetic outcome and adverse events. Aesthetic outcome was measured on a 4-point scale (poor, fair, good or excellent) and independently scored by two investigators who were blinded to treatment allocation. Treatment failures were scored as poor cosmetic outcome because, according to the protocol, these tumours had to be excised. Excision results in a scar, which generally compares unfavourably with cosmetic outcome after noninvasive treatment.

Patients completed diaries from which data on adverse events were extracted. Patients were asked to score adverse events on a 4-point scale (absent, mild, moderate or severe) 1 week after both illuminations. Pain and burning sensation were scored using a numerical rating scale (score 0–10), directly after both illuminations and 1 week later. The maximum pain scores for both illuminations were assessed. Occurrences of serious adverse events or suspected unexpected serious adverse reactions were registered.

Statistical analysis

The aim of this study was to assess whether the twofold 5-ALA illumination protocol is superior to conventional MAL-PDT. It was considered feasible to include 73 patients per group. This sample size allows for the detection of a clinically relevant difference of 15% between groups with a power of 80% (two-sided alpha = 5%). Taking into account a possible dropout rate of 10%, 162 patients were included.

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

For secondary outcomes, between-group differences in proportions were tested using the χ^2-test, and mean values of continuous variables were compared using the Student's *t*-test for independent samples or the nonparametric Mann–Whitney U-test. *P*-values $\leq 0{\cdot}05$ were considered to be statistically significant. All data were analysed using SPSS version 23.0 (IBM, Armonk, NY, U.S.A.), openepi.com or Stata version 14.0 (Stata Corp, College Station, TX, U.S.A.).

Results

Between September 2013 and May 2015 a total of 201 patients were recruited and assessed for eligibility. Overall, 39 patients refused participation either for personal reasons or because they had a strong preference for a treatment other than PDT. A total of 162 patients were enrolled in the study (62 from MUMC$^+$, 60 from EMC, 40 from VCMC), of which 80 patients were allocated to MAL-PDT and 82 to twofold ALA-PDT. After randomization, some patients expressed a preference for a treatment other than the one to which they had been allocated. In the MAL-PDT group, three patients expressed a preference for twofold ALA-PDT, two patients expressed a preference for twofold 5-fluorouracil ointment and one patient expressed a preference for twofold surgical excision. In the twofold ALA-PDT group, one patient was treated with MAL-PDT and one patient was treated with topical 5-fluorouracil. For these patients, data on the primary end point were available and they were included in the intention-to-treat analysis. The trial profile is provided in Figure 1.

Table 1 shows the distribution of baseline characteristics in the randomized groups. There were small imbalances with respect to study centre and tumour localization (Table 1).

Residual tumour after 3 months was seen in four patients treated with MAL-PDT and in three patients treated with ALA-PDT. At the 12-month follow-up another nine recurrences had occurred following MAL-PDT and three following ALA-PDT, resulting in a total of 13 treatment failures after MAL-PDT and six after ALA-PDT.

At 3 months the cumulative probability of treatment success was 96·2% (95% CI 88·7–98·8) for twofold ALA-PDT and 94·9% (95% CI 87·0–98·1) for MAL-PDT and at 12 months, the cumulative probability was 92·3% (95% CI 83·7–96·5) and 83·4% (95% CI 73·1–90·0), respectively ($P = 0{\cdot}091$) (Table 2). Univariate Cox regression analysis resulted in a crude HR for treatment failure of 2·17 (95% CI 0·82–5·70), indicating a higher risk of treatment failure after MAL-PDT. Multivariate Cox regression analysis was performed to adjust for the observed small imbalances in baseline characteristics between randomized groups. The adjusted HR from a model that included treatment, study centre, age, sex, tumour location and tumour size as independent variables was 2·35 (95% CI 0·84–6·53).

Additionally, a per protocol analysis was performed. Patients were analysed according to the treatment they actually

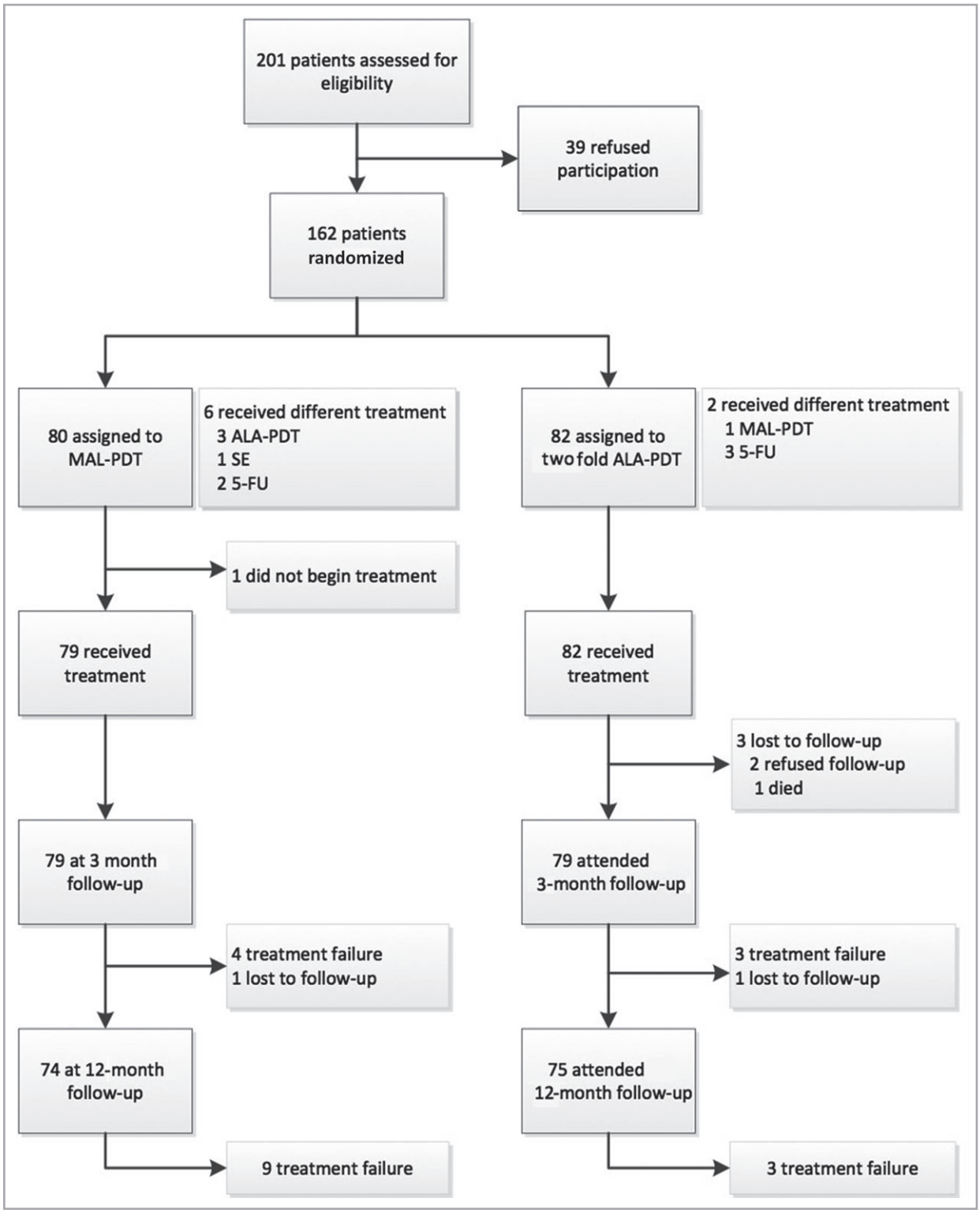

Fig 1. Trial profile flowchart. ALA, aminolaevulinic acid; MAL, methyl aminolaevulinate; PDT, photodynamic therapy; 5-FU, 5-fluorouracil ointment; SE, surgical excision.

received and four patients who were treated with topical 5-fluorouracil ointment (three) or surgical excision (one) were excluded from this analysis. The cumulative probability of treatment success at 12 months was 91·9% (95% CI 82·9–96·3) for ALA-PDT and 83·4% (95% CI 73·1–90·0) for MAL-PDT, P = 0·110. Crude and adjusted HR for treatment failure were 2·15 (95% CI 0·81–5·65) and 2·21 (95% CI 0·82–6·04), respectively.

Table 1 Distribution of baseline characteristics

	MAL-PDT (n = 80)	Twofold ALA-PDT (n = 82)
Mean age, years (range)	63·6 (28–83)	65·9 (38–85)
Sex, n (%)		
Male	35 (44)	40 (49)
Female	45 (56)	42 (51)
Study centre, n (%)		
MUMC$^+$	27 (34)	35 (43)
EMC	34 (43)	26 (32)
VCMC	19 (24)	21 (26)
Tumour location, n (%)		
Head/neck	1 (1)	7 (8)
Trunk	58 (73)	45 (55)
Upper extremities	7 (9)	16 (20)
Lower extremities	14 (18)	14 (17)
Mean tumour size, mm ± SD	11·2 ± 7·1	10·8 ± 5·3

ALA, aminolaevulinic acid; MAL, methyl aminolaevulinate; PDT, photodynamic therapy; MUMC$^+$, Maastricht University Medical Centre; EMC: Erasmus Medical Centre; VCMC: VieCuri Medical Centre.

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

	Proportion of patients without treatment failure after 3 months, n/N (%)	Proportion of patients without treatment failure within 3–12 months, n/N (%)	Cumulative probability of remaining free from treatment failure at 12 months[a] (95% confidence interval)
ALA-PDT	76/79 (96)	72/75 (96)	92·3% (83·7–96·5)
MAL-PDT	75/79 (95)	65/74 (88)	83·4% (73·1–90·0)

ALA, aminolaevulinic acid. MAL, methyl aminolaevulinate. PDT, photodynamic therapy. [a]Product of initial and sustained clearance rate. Log-rank test P = 0·091.

Adverse events

Table 3 presents the mean scores for pain and burning sensation after each illumination for both treatments. After the second illumination, mean pain scores were significantly higher in the twofold ALA-PDT group compared with patients treated with MAL-PDT, with mean pain scores of 3·36 ± 2·57 and 2·48 ± 2·57, respectively (P = 0·039). None of the patients discontinued treatment because of pain. Overall, patients who received ALA-PDT reported side-effects more often. Reported incidence of erythema, wounds/erosions and vesicles was significantly higher after ALA-PDT compared with MAL-PDT (Table 3). Furthermore, 16·4% in the ALA-PDT group vs. 5·8% in the MAL-PDT group reported the use of pain medication post-treatment.

Data on cosmetic outcome were available for 73 patients treated with ALA-PDT and 72 patients treated with MAL-PDT. Good-to-excellent cosmetic outcome was reported for 80% (58 of 73) of patients treated with ALA-PDT and 67% (48 of 72) of patients treated with MAL-PDT (P = 0·084). No serious unexpected adverse reactions were reported in either group. During the study, four serious adverse events occurred that were unrelated to the study treatment (three hospitalizations owing to transient ischaemic attack, chemotherapy for lung carcinoma and dizziness, and one patient died owing to cancer).

Discussion

Our data suggest that patients treated with the twofold ALA-PDT scheme have a higher cumulative probability of remaining free from treatment failure 1 year post-treatment compared with patients treated with conventional MAL-PDT (92·3% vs. 83·4%); however, the difference is not statistically significant. In addition, patients treated with ALA-PDT experienced more pain and local side-effects than those treated with MAL-PDT.

This is the first randomized controlled trial to compare MAL-PDT with a twofold ALA-PDT regimen. Many PDT studies investigating both MAL and ALA photosensitizers have been performed worldwide. In Europe, MAL is approved as Metvix (Galderma SA) for the treatment of BCC, actinic keratosis and Bowen disease.[14,15] In the U.S.A., Metvix is approved only for the treatment of AK.[16] MAL is more lipophilic and therefore has the theoretical benefit of a higher and faster intracellular absorption compared with ALA. MAL also has a higher selectivity for tumour cells, leading to fewer side-effects in normal healthy tissue.[17–20]

A systematic review studying the efficacy of several noninvasive treatments for sBCC showed a pooled estimate of 76·2% for tumour-free survival at 1 year for PDT, which included both ALA-PDT and MAL-PDT.[21] The majority of studies assessed efficacy of ALA-PDT after a single unfractionated illumination. A higher tumour-free survival was observed when PDT treatment was repeated (84%). A more recent study reported a 1-year CR of 72·8% after conventional MAL-PDT.[3]

To optimize efficacy, de Haas *et al.* studied a fractionated twofold ALA-PDT protocol for sBCC with a first dose of 20 J cm^{-2} followed by a dark interval and a second dose of 80 J cm^{-2}. They performed a prospective comparative study that reported a CR of 97% after twofold ALA-PDT vs. 89% after a single illumination 1 year post-treatment.[12] The superiority of the twofold ALA-PDT regimen was confirmed by de Vijlder *et al.* who reported a CR of 88% after twofold ALA-PDT 5 years post-treatment.[22]

The fractionated illumination protocol has been studied preclinically in a variety of models.[22,23] It is suggested that by applying two consecutive illuminations, there might be an additional utilization of PpIX owing to reoxygenation during

Table 3 Adverse events

	MAL-PDT	Twofold ALA-PDT	P-value
Pain score, mean NRS ± SD			
During first PDT session	2·25 ± 2·54	1·88 ± 2·36	0·369
During second PDT session	2·48 ± 2·57	3·36 ± 2·57	0·039[a]
Burning sensation score, mean NRS ± SD			
During first PDT session	3·12 ± 2·72	3·41 ± 2·37	0·457
During second PDT session	2·94 ± 2·72	4·49 ± 2·06	0·001[a]
Erythema, n/N (%)			
Absent/mild	37/73 (51)	13/80 (16)	
Moderate/severe	28/73 (38)	59/80 (74)	< 0·001[a]
Not available	8/73 (11)	8/80 (10)	
Swelling, n/N (%)			
Absent/mild	61/73 (84)	63/80 (79)	
Moderate/severe	5/73 (7)	9/80 (11)	0·406
Not available	7/73 (10)	8/80 (10)	
Wounds, n/N (%)			
Absent/mild	60/73 (82)	56/80 (70)	
Moderate/severe	4/73 (6)	16/80 (20)	0·014[a]
Not available	9/73 (12)	8/80 (10)	
Crusts, n/N (%)			
Absent/mild	60/73 (82)	57/80 (71)	
Moderate/severe	6/73 (8)	15 (19)	0·062
Not available	7/73 (10)	8/80 (10)	
Vesicles, n/N (%)			
Absent/mild	61/73 (84)	54/80 (68)	
Moderate/severe	5/73 (7)	18/80 (22)	0·011[a]
Not available	7/73 (10)	8/80 (10)	
Scaling, n/N (%)			
Absent/mild	59/73 (81)	57/80 (71)	
Moderate/severe	7/73 (10)	14/80 (18)	0·160
Not available	7/73 (10)	9/80 (11)	
Pruritus, n/N (%)			
Absent/mild	53/73 (73)	56/80 (70)	
Moderate/severe	13/73 (18)	16/80 (20)	0·835
Not available	7/73 (10)	8/80 (10)	

ALA, aminolaevulinic acid; MAL, methyl aminolaevulinate; PDT, photodynamic therapy; NRS, numeric rating scale. Mean NRS scores (0–10) were tested for statistical significance using the Student's t-test. Differences in categorical data were tested for statistical significance using the χ^2-test. [a]P-values ≤ 0·05 were considered to be statistically significant.

the dark interval.[22] Furthermore, there might be an enhanced local immune response when using light fractionation.[22] Previous PDT literature describes a relationship between vascular response, oxygen supply and an effective PDT response.[24–27] Middelburg *et al.* reported a higher accumulation of PpIX in endothelial vessel walls after ALA application, compared with MAL. After illumination more endothelial damage was observed.[28] Despite these various hypotheses, the exact explanation for the enhanced efficacy has not yet been fully elucidated.

The CR of 92·3% for twofold ALA-PDT as observed in this trial is lower compared with the previous results from de Haas *et al.*, whereas a higher CR after MAL-PDT was observed compared with the study by Arits *et al.*, who reported a CR of 72·8% at 1 year after treatment.[3] The differences between the study populations might be responsible for this variance. For instance, in our sample, only one patient in the MAL-PDT group had a BCC in the head and neck area, an area that is known to be associated with a higher risk of treatment failure, whereas in the trial reported by Arits *et al.* 12% of the patients had a BCC in this area.[29,30] Direct comparison of treatments within randomized trials is necessary to validate conclusions on comparative efficacy.

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

Previous literature demonstrated a favourable cosmetic outcome after PDT compared with surgery for BCC.[31,32] Despite a higher number of local skin reactions, we observed a trend towards a better cosmetic outcome after twofold ALA-PDT compared with MAL-PDT. The MAL-PDT results in our study are comparable with those of Arits *et al.*, who found a good-to-excellent cosmetic outcome in 62% of patients treated with MAL-PDT.[3]

The fact that PDT is an in-clinic treatment could be an advantage over other topical home-based treatments for specific patient categories, such as the elderly. An additional advantage of the twofold ALA-PDT vs. topical ointments or conventional MAL-PDT is that it can be performed within 1 day. Furthermore, the cost of PDT treatment is considered to be lower because the ointment needs to be applied only once and, in the Netherlands, ALA ointment is less expensive than MAL ointment (Metvix, Galderma).

A limitation of this trial is that the sample size enabled the detection of an absolute difference in proportion with treatment failure of 15% or more with a power of 80% (two-sided alpha = 5%). The expected difference of 15% was based on prior studies.[3,12] However, we observed a difference between ALA-PDT and MAL-PDT of 8·9% in favour of ALA-PDT and the power to detect this difference with 95% confidence was too low. Long-term follow-up and larger patient cohorts might be needed to detect a statistically significant difference between both treatments. An additional limitation is that post-treatment biopsies to confirm a lack of tumour were not performed and it is possible that clinical and dermoscopic examination missed some recurrences that had not yet surfaced. However, potential underreporting of recurrences is unlikely to affect the comparison of treatment success between both groups.

It should be kept in mind that this study did not only compare two different drugs (ALA and MAL), but additionally the treatment regimen was variable; the treatment with ALA was fractionated on 1 day, whereas MAL protocol consisted of two

illuminations with a 1-week interval. For future studies, it would be interesting to compare the twofold fractionated ALA-PDT regimen with a comparable fractionated MAL-PDT regimen. Although twofold MAL-PDT has not yet been studied in humans, previous studies using mouse models did not show a favourable response to fractionation in MAL-PDT.[33,34]

In conclusion, our findings suggest a trend towards better efficacy for twofold ALA-PDT compared with conventional MAL-PDT for the treatment of sBCC, although the difference is not statistically significant. However, the twofold ALA-PDT regimen entails a higher risk of pain and side-effects.

Acknowledgments

We would like to thank all the patients who participated and the nurses who performed the PDT treatments (Anja Ebus, Germaine Warnier, Marie-Paule de Droog, Marlene Curvers and Jolanda Sibtsen-van Herk). We thank Nicole Luckerhof for her outstanding administrative support.

References

1 Flohil SC, Seubring I, van Rossum MM *et al.* Trends in basal cell carcinoma incidence rates: a 37-year Dutch observational study. *J Invest Dermatol* 2013; **133**:913–18.
2 Rippey JJ. Why classify basal cell carcinomas? *Histopathology* 1998; **32**:393–8.
3 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**:647–54.
4 Martin I, Schaarschmidt ML, Glocker A *et al.* Patient preferences for treatment of basal cell carcinoma: importance of cure and cosmetic outcome. *Acta Derm Venereol* 2016; **96**:355–60.
5 Wang H, Xu Y, Shi J *et al.* Photodynamic therapy in the treatment of basal cell carcinoma: a systematic review and meta-analysis. *Photodermatol Photoimmunol Photomed* 2015; **31**:44–53.
6 Kennedy JC, Pottier RH. Endogenous protoporphyrin IX, a clinically useful photosensitizer for photodynamic therapy. *J Photochem Photobiology B* 1992; **14**:275–92.
7 Dolmans DE, Fukumura D, Jain RK. Photodynamic therapy for cancer. *Nat Rev Cancer* 2003; **3**:380–7.
8 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**:125–43.
9 Peng Q, Warloe T, Berg K *et al.* 5-Aminolevulinic acid-based photodynamic therapy. Clinical research and future challenges. *Cancer* 1997; **79**:2282–308.
10 Basset-Seguin N, Ibbotson SH, 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**:547–53.
11 Szeimies RM, Ibbotson 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**:1302–11.
12 de Haas ER, Kruijt 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**:2679–86.
13 Kessels J, Hendriks J, Nelemans P *et al.* Two-fold illumination in topical 5-aminolevulinic acid (ALA)-mediated photodynamic therapy (PDT) for superficial basal cell carcinoma (sBCC): a retrospective case series and cohort study. *J Am Acad Dermatol* 2016; **74**:899–906.
14 Bath-Hextall FJ, Perkins W, Bong J, Williams HC. Interventions for basal cell carcinoma of the skin. *Cochrane Database Syst Rev* 2007; **1**: CD003412.
15 Morton CA, Szeimies RM, Sidoroff A, Braathen LR. 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**:536–44.
16 Cohen DK, Lee PK. Photodynamic therapy for non-melanoma skin cancers. *Cancers (Basel)* 2016; **8**:E90.
17 Fritsch C, Lehmann P, Stahl W *et al.* Optimum porphyrin accumulation in epithelial skin tumours and psoriatic lesions after topical application of delta-aminolaevulinic acid. *Br J Cancer* 1999; **79**:1603–8.
18 Fritsch C, Homey B, Stahl W *et al.* Preferential relative porphyrin enrichment in solar keratoses upon topical application of delta-aminolevulinic acid methylester. *Photochem Photobiol* 1998; **68**:218–21.
19 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 B* 1996; **34**:95–6.
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**:265–71.
21 Roozeboom MH, Arits AH, Nelemans PJ, Kelleners-Smeets NW. Overall treatment success after treatment of primary superficial basal cell carcinoma: a systematic review and meta-analysis of randomized and nonrandomized trials. *Br J Dermatol* 2012; **167**:733–56.
22 de Vijlder HC, Sterenborg HJ, Neumann HA *et al.* Light fractionation significantly improves the response of superficial basal cell carcinoma to aminolaevulinic acid photodynamic therapy: five-year follow-up of a randomized, prospective trial. *Acta Derm Venereol* 2012; **92**:641–7.
23 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.
24 Gold MH. Fractionated aminolevulinic acid-photodynamic therapy (PDT) provides additional evidence for the use of PDT for nonmelanoma skin cancer. *J Eur Acad Dermatol Venereol* 2009; **23**:571–2.
25 Peng Q, Soler AM, Warloe T *et al.* Selective distribution of porphyrins in skin thick basal cell carcinoma after topical application of methyl 5-aminolevulinate. *J Photochem Photobiol B* 2001; **62**:140–5.
26 Sandberg C, Paoli J, Gillstedt M *et al.* Fluorescence diagnostics of basal cell carcinomas comparing methyl-aminolaevulinate and aminolaevulinic acid and correlation with visual clinical tumour size. *Acta Derm Venereol* 2011; **91**:398–403.
27 Henderson BW, Fingar VH. Oxygen limitation of direct tumor cell kill during photodynamic treatment of a murine tumor model. *Photochem Photobiol* 1989; **49**:299–304.
28 Middelburg TA, de Vijlder HC, de Bruijn HS *et al.* Topical photodynamic therapy using different porphyrin precursors leads to

differences in vascular photosensitization and vascular damage in normal mouse skin. *Photochem Photobiol* 2014; **90**:896–902.

29 Roozeboom MH, Nelemans PJ, Mosterd K *et al.* Photodynamic therapy vs. topical imiquimod for treatment of superficial basal cell carcinoma: a subgroup analysis within a noninferiority randomized controlled trial. *Br J Dermatol* 2015; **172**:739–45.

30 Vinciullo C, Elliott T, Francis D *et al.* Photodynamic therapy with topical methyl aminolaevulinate for 'difficult-to-treat' basal cell carcinoma. *Br J Dermatol* 2005; **152**:765–72.

31 Cosgarea R, Susan M, Crisan M *et al.* Photodynamic therapy using topical 5-aminolaevulinic acid vs. surgery for basal cell carcinoma. *J Eur Acad Dermatol Venereol* 2013; **27**:980–4.

32 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**:1131–6.

33 de Bruijn HS, de Haas ER, Hebeda KM *et al.* Light fractionation does not enhance the efficacy of methyl 5-aminolevulinate mediated photodynamic therapy in normal mouse skin. *Photochem Photobiol Sci* 2007; **6**:1325–31.

34 Middelburg TA, de Bruijn HS, van der Ploeg-van den Heuvel A *et al.* The effect of light fractionation with a 2-h dark interval on the efficacy of topical hexyl-aminolevulinate photodynamic therapy in normal mouse skin. *Photodiagnosis Photodyn Ther* 2013; **10**:703–9.