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INVESTIGATIVE REPORT

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

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Photodynamic therapy (PDT) using topical porphyrin-precursors is a promising treatment for superficial basal cell carcinoma (sBCC), but it needs further optimization. The aim of this study was to compare 5-year lesion (complete) response rates of sBCC treated with topical aminolaevulinic acid (ALA)-PDT using a single illumination vs. ALA-PDT using a 2-fold illumination scheme. A prospective, randomized study was performed, in which 91 patients with 299 lesions were treated with a 2-fold illumination scheme with 2 light fractions of 20 and 80 J/cm^2 delivered 4 and 6 h after a single application of 20% ALA, and 106 patients with 274 lesions were treated with a single illumination of 75 J/cm^2 4 h after a single application of 20% ALA. All lesions were treated at a fluence rate of 50 mW/cm^2. An interim time to event analysis of complete response (CR) rates at 12 months showed encouraging results, and therefore lesions were followed for 5 years post-therapy. A third group of 50 patients with 172 lesions treated with 2-fold illumination were included after the initial period and analysed separately. The CR rate was significantly greater following the 2-fold illumination than the single illumination ($p=0.0002$, log-rank test). Five years after therapy the CR rate after 2-fold illumination was 88%, whereas the CR rate after single illumination was 75%. The CR rate in the third group of lesions, treated with 2-fold illumination was 97% and 88% at 12 months and 5 years after therapy, respectively. Long-term follow-up indicates superior efficacy in sBCC of ALA-PDT with 2-fold illumination compared with ALA-PDT with single illumination. ***Key words: ALA-PDT; PpIX; basal cell carcinoma; light fractionation; dosimetry.***

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Photodynamic therapy (PDT) is under development as an experimental therapy for many tumour types (1). Its effectiveness as a curative or palliative treatment in certain niche clinical applications is well documented (2–4). Treatment consists of 2 relatively simple procedures: the administration of a photosensitive drug or pre-cursor, and illumination of the tumour with visible light. This leads to the generation of reactive oxygen species, notably singlet oxygen (5), and results in the destruction of the tumour by a combination of direct cellular and secondary vascular effects (6). In addition, PDT is able to initiate an immune response against the remaining tumour cells (7). A successful outcome following PDT is reliant on each of these mechanisms, and the relative contribution of each depends on the photosensitizer and treatment regimen (8). Except for temporary skin photosensitization, there are no long-term side-effects if appropriate protocols are followed. Healing occurs with little or no scarring and the procedure can be repeated without cumulative toxicity. The main drawback of using PDT as frontline therapy is that PDT has generally been tested only in small-scale studies; long-term clinical response studies and large randomized trials are rare (1).

One approach to PDT is the use of porphyrin precursors, such as aminolaevulinic acid (ALA), which has been under investigation for over 2 decades (9, 10). ALA is itself not a photosensitizer but a precursor of protoporphyrin IX (PpIX). ALA is converted within cells into PpIX, via the heme cycle. ALA-PDT has been used topically for the treatment of non-melanoma skin cancer (NMSC) (11) and orally for cancer in the oral cavity (12) and gastrointestinal tract (13). The advantage of topical ALA-PDT over other types of PDT using preformed photosensitizers is the absence of widespread skin photosensitization, except for the application area. The short-term efficacy of topical ALA-PDT in NMSC has been demonstrated in many clinical trials (reviewed in 11) and ALA and one of its derivatives are now approved for clinical use (14).

While ALA-PDT provides good short-term results, long-term clinical results are sparse and much less impressive and show a considerable variation in recurrence rates (15). This has prompted investigators to search for approaches to improve the response to PDT.

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A number of factors limit response. While the most important of these is the rapid photobleaching of PpIX during illumination, which limits the maximum PDT dose that can be delivered (16), other factors play a role. The availability of ALA to cells in deeper regions of the skin lesions may be limited by the penetration of topically applied ALA (17). PpIX accumulation may be limited by the capacity of the heme synthesis pathway. The response to PDT can be limited by the availability of oxygen and the distribution of light in tissue (18). The uptake of ALA and/or the accumulation of PpIX can be improved by the use of iontophoresis (19), penetration enhancers (20) or iron chelators (21). Moreover, several alkyl esters of ALA have been developed with the intention of enhancing cell uptake and tissue penetration (22). Methyl aminolevulinate (Metvix® Galderma Benelux, Rotterdam, The Netherlands) was approved in the European Union in 2001 for the treatment of actinic keratosis (AK) and basal cell carcinoma (BCC) (23). Given these efforts to enhance PDT with porphyrin pre-cursors it is disappointing that few large-scale, long-term clinical studies have been performed to investigate whether these approaches are effective. In the small number of case studies that exist results have been disappointing (24).

We have been investigating an approach to enhance the response following ALA-PDT by changing the illumination parameters (25). We have performed numerous pre-clinical studies investigating the effect of splitting the illumination into 2 light fractions separated by a dark interval of several hours. We have shown that the response to this type of light-fractionated approach is enhanced over a single illumination (26), and that this increase is largest when a low-dose light fraction followed by a high-dose light fraction is applied, separated by a dark interval of 2 h. We have shown that the choice of fluence (rate) for the first fraction is critical, and a high fluence for the second light fraction and a 2-h dark interval is necessary for maximal tissue response (27). Based on these preclinical results, a randomized comparative prospective open clinical study was performed in the treatment of superficial basal cell carcinoma (sBCC), comparing traditional non-fractionated ALA-PDT using a single fluence of 75 J/cm^2 and a 2-fold illumination of 20+80 J/cm^2 (28) The choice of a single dose of 75 J/cm^2, compared with the cumulative dose of 100 J/cm^2, in the fractionated group is based on: (*i*) the fact that photobleaching of PpIX limits the PDT dose that can be delivered in a single fraction at fixed fluence rate (16); and (*ii*) our findings that a high fluence second light fraction is most effective (26). An interim analysis of CR rate time-to-event analysis at 12 months showed a significantly better result for fractionated PDT (28). In the present study we report on the 5-year follow-up data analysis of our randomized clinical trial.

MATERIALS AND METHODS

Patients

The study design and clinical procedure have been described in more detail previously (28). Briefly, all patients were diagnosed as having a sBCC within the department of dermatology of Erasmus MC in Rotterdam, The Netherlands. ALA-PDT was performed from July 2002 to November 2004 according to 2 treatments protocols, approved by the local ethics committee, in accordance with the principles of the Declaration of Helsinki. All patients gave informed consent. A total of 104 patients, who altogether had 274 lesions, were treated using a single illumination scheme, and 91 patients with a total of 299 lesions were treated using a 2-fold illumination scheme, as summarized in Fig. 1. A 12-month interim analysis of these 2 groups of patients resulted in a statistically significant increase in CR rate in the group receiving the 2-fold illumination. Given this result, a third group of 50 additional patients with 172 lesions that received only the 2-fold illumination were included between November 2004 and August 2005. All patients were followed for a period of 5 years. Diagnosis was determined histologically and clinically in approximately equal proportions within all 3 treatment groups. Patient characteristics in the 3 groups were comparable. All patients were adult Caucasians with a mean age of 56.7 years (range 31–88) in the single illumination group, a mean age of 56.9 years (range 32–84) in the group receiving a 2-fold illumination scheme from July 2002 to November 2004, and a mean age of 65.5 years (range 39–90) in the third group receiving a 2-fold illumination scheme from November 2004 to August 2005. The number of high-risk patients in each treatment group is shown in Table I.

Aminolaevulinic acid application and light sources

The topical ALA ointment used was prepared by our hospital pharmacy using 20% ALA (FLUKA, Zwijndrecht, The Netherlands) in Instilagel® (Medeco BV, Oud Beijerland, The Netherlands). The ointment was freshly prepared, stored in a refrigerator and used within 3 days. Before application of ALA ointment, crusts and scaling were gently removed using a disposable curette. Thereafter the lesion was covered with a margin of 1 cm and dressed with a semi-permeable dressing (Tegaderm 3M, The Netherlands) and light-protecting dressing (aluminium foil).

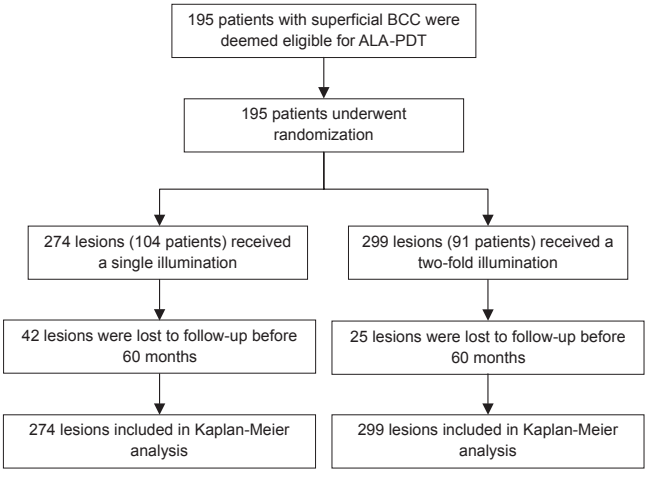

Fig. 1. Patient and lesion inclusion, allocation, follow-up, and data analysis of patients undergoing aminolaevulinic acid photodynamic therapy (ALA-PDT) using a single and a 2-fold illumination scheme.

Table I. *High-risk patients*

Patient group	75 J/cm²		20+80 J/cm²		20+80 J/cm²	
	Patients *n*	Lesions *n* (%)	Patients *n*	Lesions *n* (%)	Patients *n*	Lesions *n* (%)
Immunocompromised[a]	5	5 (1.8)	4	16 (5.3)	2	2 (1.2)
Previous radiotherapy	6	29 (10.6)	5	16 (5.4)	4	6 (3.5)
Goltz-Gorlin syndrome	5	46 (16.7)	5	31 (10.4)	3	33 (19.2)
High sun exposure[b]	3	18 (6.6)	3	29 (9.7)	2	10 (5.8)
Total high-risk	18	96 (35.0)	17	92 (30.8)	11	51 (29.7)

[a]Immunocompromised: HIV-positive, organ recipient, or using immunosuppressive drugs.
[b]Patients who have lived more than 15 years in tropical countries and had Fitzpatrick skin type 1.

The light sources used in this study were a 630-nm diode laser (Carl Zeiss, Oberkochen, Germany) and 2 broadband light sources: a broadband light source with a spectral output between 590 and 650 nm (Medeikonos, Gothenburg, Sweden) and a light-emitting diode (LED) light source with a spectral output centred on 633 nm and a bandwidth of 20 nm (Omnilux, Waldman, Tiel, The Netherlands). All 3 light sources were used to illuminate lesions with a margin of at least 5 mm at a constant measured fluence rate of 50 mW/cm². In the single illumination group, lesions were illuminated 4 h after the application of ALA ointment to a fluence of 75 J/cm². In both 2-fold illumination groups, lesions received light fractions of 20 and 80 J/cm², 4 and 6 h after the application of ALA ointment. ALA ointment was applied once. Both light fractions were delivered at a fluence rate of 50 mW/cm². During the 2-h interval between the light fractions, lesions were covered with light-protective dressing.

Response and follow-up

Patients were followed up during the 5-year interval between treatment and a final follow-up assessment between April 2009 and August 2010. The overwhelming majority of patients were seen in repeat visits to our department. Files of patients who were originally referred to our department for treatment in connection with our study and referred back to their peripheral primary dermatologist, were examined, if necessary, at our department. Out of a total of 354 patients, 31 were lost to follow-up, of whom 18 patients died. Follow-up was performed by staff members and residents within our department. A small number of patients were seen by peripheral dermatologists. One investigator (HdV) was responsible for the final clinical follow-up and was blinded for the treatment scheme delivered to each lesion.

Complete response (CR) was defined as the absence of clinically visible basal cell carcinoma. Histologically confirmed recurrences were identified via patient files and digital photographs. If lesions, histologically confirmed, occurred at the same body area as the original lesion, and no detailed report or/and photographs were present, they were considered as recurrences. Lesions in patients who were lost to follow-up for any reason were included appropriately in the statistical analysis. Recurrences and lesions with a partial response were re-treated with either fractionated PDT or surgical excision and were not included in the statistical analysis.

Statistics

Kaplan–Meier analysis was performed on relative CR rates after therapy and the log-rank test was used to compare significance of differences between the non-fractionated and fractioned groups included before November 2004 and to check the consistency of the data of the fractioned group, included thereafter. Data was right censored if lesions were lost to follow-up or were lost to follow-up because of death. A secondary Kaplan–Meier analysis was performed, assuming that lesions lost to follow-up had recurred at their time of last observation. Primary and overall response rates of lesions treated with different illumination schemes at specific time-intervals after therapy were compared using Fisher's exact test.

RESULTS

Two-fold illumination of 20+80 J/cm² with a 2-h dark interval results resulted in a significantly better clinical response to aminolaevulinic acid-photodynamic therapy compared with a single light fraction

The relative CR of lesions following ALA-PDT using a single light fraction and a 2-fold illumination scheme is shown is Fig. 2. The relative CR using a 2-fold illumination scheme was significantly greater than that following a single light fraction ($p = 0.0002$, log-rank test). Five years after therapy, the relative CR in the 2-fold illumination group was 88%, whereas the corresponding CR in the single illumination group was

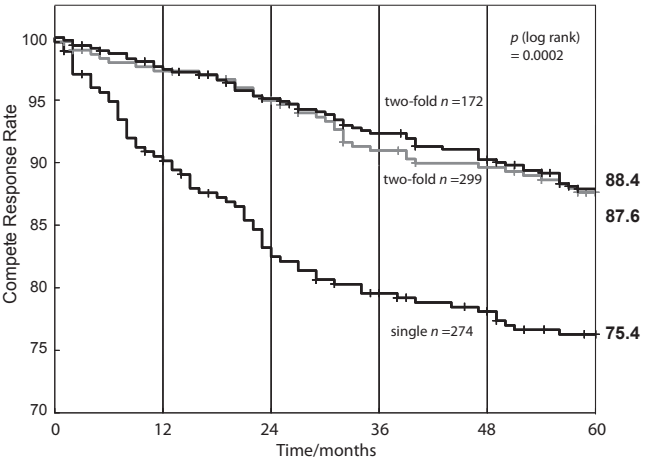

Fig. 2. Kaplan–Meier curves for complete response (CR) rate of superficial basal cell carcinoma lesions using a single light fraction of 75 J/cm², 4 h after the application of aminolaevulinic acid (ALA) (*bottom black curve*, $n = 274$) and a 2-fold illumination scheme of the first group of 20+80 J/cm², (*grey curve*; $n = 299$) 4 and 6 h after the application of ALA. The *top black curve* shows a second subsequent group of lesions treated with a 2-fold illumination scheme; $n = 172$. Note: the ordinate, CR rate, is plotted from 70% to 100% to aid visualization.

75% ($p = 0.0002$, Fisher's exact test). The relative CR in the second fractionated group was also 88%, which confirmed the result of the first fractionated group.

Of the 274 lesions in the single illumination group 15 failed to respond and 52 recurred during the follow-up period. Of the 299 lesions in the 2-fold illumination group, 6 failed to respond and 31 recurred during the follow-up period. Of the 172 lesions in the second 2-fold illumination group, no lesions failed to respond and 20 recurred during the follow-up period (Table II).

A secondary Kaplan–Meier analysis, which assumed lesions lost to follow-up had recurred at the time of their last observation also showed a very significant difference in CR between the single and 2-fold illumination scheme ($p = 0.0002$, log-rank test). In this analysis the relative CR 5 years after therapy in the 2-fold illumination group was 79%, whereas the corresponding CR in the single illumination group was 60% ($p = 0.0002$, Fisher's exact test).

Subgroup analysis

A subgroup analysis for high-risk patients (Gorlin-Goltz syndrome (nevoid basal cell carcinoma syndrome (OMIM #109400)), immunocompromised, prior radiotherapy, high sun exposure, and arsenic injection exposure) was performed for each treatment group. Patients in each of these high-risk groups did not influence the relative CR rates of the group receiving single illumination or the groups receiving 2-fold illumination.

A subgroup analysis for the different light sources (630 nm diode laser, broadband light source, spectral output 590–650 nm; and LED light source, spectral output 613–633 nm), did not influence the relative CR observed between treatment schemes.

A subgroup analysis for body site showed that lesions defined as treated within the hairy scalp showed significantly more recurrences compared with other body sites in both illumination groups ($p < 0.01$, log-rank test, in each case). In the single illumination group 71% (5 lesions of a total of 7) at this location showed recurrence, whereas in the combined fractionated group 55% (5 lesions of a total of 9) recurred. There was no statistically significant difference in the response rate between lesions treated within the hairy scalp using the single or 2-fold illumination.

Table II. *Outcome of 745 lesions; complete response (CR), recurrence, partial response, lost to follow-up at 5 years*

Patient group	75 J/cm^2 $n = 274$ n (%)	20+80 J/cm^2 (A) $n = 299$ n (%)	20+80 J/cm^2 (B) $n = 172$ n (%)
Complete response	165 (60.2)	237 (79.3)	139 (80.8)
Recurrence	52 (19.0)	31 (10.4)	20 (11.6)
Partial response	15 (5.5)	6 (2)	0 (0)
Died	27 (9.9)	17 (5.7)	2 (1.2)
Lost to follow-up	15 (5.5)	8 (2.7)	11 (6.4)

A: prospective randomized lesions; B: subsequently treated lesions.

DISCUSSION

The aim of this study was to determine whether our attempts to enhance ALA-PDT *in vivo* using light fractionation results in a clinically significant increase in the long-term response of sBCC. We have previously shown that performing light-fractionated PDT is significantly more effective in pre-clinical models (26, 27, 29–31). Based on these results, a large randomized comparative prospective open clinical study was performed in the treatment of 573 superficial BCC lesions, comparing ALA-PDT using one single fluence of 75 J/cm^2 and a 2-fold illumination of 20+80 J/cm^2 (28). An interim analysis at 12 months demonstrated a statistically significant improvement in CR rate for light-fractionated PDT (97% vs. 89% CR, $p < 0.002$, log-rank test). The data we present in the present study demonstrate that this statistically significant improvement in CR rate is maintained after 5 years follow-up (88% vs 75% CR, $p = 0.0002$, log-rank test). This study is one of the few large-scale long-term randomized clinical studies investigating PDT response and the first to show that efforts to optimize PDT can lead to a significant increase in long-term clinical response. This increase in clinical response is not simply a statistical difference; it is a significant result for patients. Light-fractionated ALA-PDT requires approximately 1 in 10 patients to be retreated after 5 years compared with 1 in 4 using the traditional approach to performing ALA-PDT.

Comparing our current results with those of small-scale short-term studies in the literature using ALA-PDT for sBCC (11, 15), we conclude that the CR rates for light-fractionated PDT are much higher at all time-points, while the CR rates of our group treated with single illumination correspond well with those reported by others using ALA-PDT (11, 32). For MAL-PDT, longer and more extensive follow-up data are available; for example, Basset-Seguin et al. (33) performed a randomized clinical trial comparing MAL-PDT (1–2 treatments) with cryotherapy (1–2 treatments) with a follow-up of 5 years in 219 superficial BCC where 114 lesions received MAL-PDT. A recurrence rate of 22% was reported at 5 years with 1 or 2 PDT treatment sessions. It is difficult to compare recurrence rates and CR exactly, since the rates are based on the total number of patients with recurrent BCC divided by the number of patients with initial tumours treated. This method ignores the patients who are unavailable for follow-up and artificially lowers the recurrence rates reported. In CR rates calculated by the Kaplan–Meier survival response all information about survival and lost to follow-up are included. However, keeping these caveats in mind, recurrence rates of ~11% in our study appear to be sig-

nificantly better than the 22% recurrence rates reported after conventional MAL-PDT. Therefore, in addition to the increase in CR rate using our light-fractionated approach patients do not require a second treatment day as recommended by the registered protocol for MAL-PDT using Metvix®. In healthcare decision-making, economic arguments need to be considered alongside clinical efficacy. Fractionated ALA-PDT appears to be significantly more cost-effective than MAL-PDT. ALA in Instillagel® is less expensive than Metvix® and our approach requires only a single treatment day. This reduces both direct and indirect costs and the (psychological) burden for the patient. Interestingly, the CR rate of our group treated with a single illumination is similar to that reported for MAL-PDT that is repeated twice.

Surgical excision is generally considered as the treatment of first choice for sBCC. Cumulative recurrence rates after 5 years follow-up reported for surgery vary from 92% to 96.8%, regardless of the histological subtype of BCC (33). Although lower, a 5-year CR of 88% observed in our group treated with fractionated ALA-PDT approaches the CR rate reported for surgery. Importantly, a generally better cosmetic outcome is reported after PDT in the treatment of sBBC compared with other treatment modalities, such as surgery and cryotherapy (34). It is encouraging that our study showed that the cosmetic outcome after 1-year follow-up was good to excellent for both single and light-fractionated groups (27). In addition to good cosmesis, PDT is associated with minimal skin deterioration, which is a drawback of invasive surgery. The risk of disfigurement, loss of function, or delayed wound healing at sites with poor vasculature and the risk of post-surgical bleeding and scarring is relatively low with PDT. A disadvantage of PDT is pain during illumination. A minority of our patients (14%) required additional pain relief (2% lidocaine subcutaneously). After the illumination, pain resolved quickly and there was no statistical difference in pain experience between the non-fractionated and fractionated groups.

We performed a subgroup analysis of patients with a higher risk of developing skin cancer (Goltz-Gorlin syndrome, immunocompromised, prior radiotherapy, high sun exposure, and arsenic exposure) and found that these patients did not show significantly different response rates in either illumination group. No difference in increased efficacy was found in patients with multiple lesions or those with sporadic sBCC. This result supports data that show that the efficacy of MAL-PDT in patients with Goltz-Gorlin syndrome or after radiotherapy are comparable with sporadic BCCs (35).

There are several factors known to affect the recurrence rate of basal cell carcinoma. The scalp is regarded as a relatively high risk localization for recurrences (36, 37). A subgroup analysis in the present study confirmed that lesions in the hairy scalp showed relatively more recurrences compared with other body sites in all 3 treatment groups: a CR 45% vs. 88% in the fractionated group and 29% vs. 75% in the non-fractionated group. Based on these results, our conclusion is that ALA-PDT is a suboptimal treatment for superficial BCC on the hairy scalp.

PDT using porphyrin pre-cursors has been applied as an experimental therapy for the treatment of tumour types outside the skin, and its effectiveness well documented. It has been applied in the oral cavity (12), the genitourinary tract (38) and the gastrointestinal tract (13). In the latter case the treatment of high-grade dysplasia in Barrett's oesophagus has been extensively investigated (39). A light-fractionated approach using ALA-PDT could be applied in other organs, although practical and logistical barriers may be more significant than in skin.

It is important to note that the cumulative fluence in each of our treatment groups is not equal. A fluence of 75 J/cm^2 was delivered in a single light fraction, compared with 100 J/cm^2 in the 2-fold illumination scheme. This is a direct consequence of our intention to deliver a large fluence in the second light fraction (26). The PDT dose delivered in a single light fraction is not directly related to fluence, particularly when the photosensitizer photobleaches rapidly. While the relationship between response to ALA-PDT and fluence has not been systematically investigated in the clinic, most investigators have applied both lower light fluence (rate) and light fluences both below 75 J/cm^2 and above 100 J/cm^2, with little evidence for a correlation between fluence and response. In retrospect, given our most recent pre-clinical results (31), we could have chosen a fluence for the second light fraction of more than 80 J/cm^2. We note, however, that this choice increases the overall treatment time.

Light fractionation is not the only method that has been studied to enhance the efficacy of PDT using porphyrin precursors. However, modulating the delivery of light is a relatively simple practical approach that is easily achievable in a clinical setting. Some investigators have suggested that there may be differences in response to PDT with light sources that deliver a different effective fluence rate by virtue of the overlap of their spectral output with the absorption spectrum of PpIX (40) and light sources that additionally excite the fluorescent photoproducts of PpIX (41). The fact that we did not see differences in response between light sources suggests that these effects are small and do not impact significantly on the effective dose of the first light fraction. This means that current fluence and dark interval used in the present study are also applicable for the large proportion of investigators that use non-laser light sources.

It is interesting to consider the mechanism behind the increase in efficacy that we have observed in a range

of models, and to speculate whether this mechanism can be utilized to further enhance efficacy. We initially believed that the increased effect was simply a consequence of the additional utilization of PpIX during 2 light fractions. We have shown in animal models that the effect is somewhat more complex: approximately the same overall amount of PpIX is utilized in treatment schemes that result in significantly different efficacies (25, 26). We have shown, using the surrogate metric of monitoring of PpIX photobleaching during therapy, that oxygen recovery during the dark interval between light fractions and its utilization during illumination are not significant factors in the increase in response. We have shown that a local immune response is significantly enhanced using light-fractionated ALA-PDT, but that it is not involved in the mechanism underlying the increase in response (42). Work is underway to investigate light fractionation effects in cells *in vitro*. We have found that while it is difficult to replicate effects that are observed *in vivo* (43), preliminary data suggest that efficacy may be enhanced, particularly in cells that are sensitized with low levels of PpIX (44). Also, interestingly, we have shown that light fractionation using MAL-PDT does not result in an increase in efficacy in the same way that it does using ALA. In pre-clinical models light-fractionated ALA-PDT is associated with significantly more oedema than MAL-PDT (45). We believe that the microscopic localization of PpIX after application of ALA plays a central role in the increase in response, and that vasculature of normal and lesional skin may be a target for the effect that leads to the increase in efficacy. When these mechanisms are fully understood, it may be possible to further enhance clinical efficacy and long-term response rates of PDT using ALA and other porphyrin pre-cursors. Encouragingly, a recent randomized study suggests that our approach to using light-fractionated PDT results in a significantly enhanced clinical response in actinic keratoses (46).

In conclusion, we have demonstrated a significant increase in the CR rate of sBBC, following ALA-PDT using an illumination scheme in which 2 light fractions of 20 and 80 J/cm^2 are delivered 4 and 6 h after the application of ALA, compared with traditional illumination.

The authors declare no conflicts of interest.

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