Laser-assisted photodynamic therapy for superficial basal cell carcinoma and Bowen’s Disease: a randomised intra-patient comparison between a continuous and a fractional ablative CO₂ laser mode

Title page

Running head
Continuous vs fractional ablative laser-assisted PDT

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Names of authors as initials followed by surnames
E. Genouw¹, B. Verheire¹, K. Ongenae² MD PhD, S. De Schepper² MD PhD, D. Creytens³,⁴ MD PhD, E. Verhaeghe² MD PhD, B. Boone² MD PhD.

Names of the institutions at which the research was conducted, clearly linked to respective authors
¹ Faculty of Medicine and Health Sciences, Ghent University, Belgium
² Department of Dermatology, Ghent University Hospital, Belgium
³ Department of Pathology, Ghent University Hospital, Belgium
⁴ CRIG, Cancer Research Institute Ghent, Ghent University, Ghent, Belgium

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Abstract

Background: Laser-assisted photodynamic therapy is being explored as a method to enhance efficacy of photodynamic therapy (PDT).

Objective: To compare a continuous (CL) and a fractional (FL) ablative CO\textsubscript{2} laser-assisted methyl aminolevulinate PDT in the management of superficial basal cell carcinoma (sBCC) and Bowen’s Disease (BD).

Methods: Thirty treatment areas in fifteen patients with inoperable, histologically verified sBCC or BD, received CL or FL after intra-patient randomisation. Laser treatment was followed by MAL application and illumination occurred 3 hours later. This treatment was repeated after two weeks. An equivalence analysis was performed on the primary endpoint efficacy, while secondary endpoints pain, side effects and aesthetics were evaluated using paired samples tests. Patients were also asked for their preferred treatment.

Results: An excellent efficacy of 92,9\% (sBCC, 100\%; BD, 80\%) was found in both CL+PDT and FL+PDT after 12 months. Equivalence could not be established. Little pain was perceived in most patients during and immediately after PDT illumination. PDT treatment in FL+PDT was less painful, significantly during the second treatment (P=0,026). Side effects were mild to moderate with erythema being the most frequent immediate side effect, followed by oedema, crusting and burning sensation. Pigmentary changes occurred in 21\% (CL+PDT) to
29% (FL+PDT) and aesthetics were good to excellent in all patients. CL+PDT and FL+PDT did not significantly differ in side effects (P=0.219-1,000) or aesthetics (P=0.157-1,000).

Conclusions: Results in this pilot study support the promising role of laser-assisted PDT. Both treatment arms demonstrated the same efficacy as well as comparable side effects and aesthetics. PDT illumination was significantly less painful in the FL+PDT group, suggesting a preference for FL+PDT. The authors recommend further investigation with a larger sample size, a subgroup analysis between sBCC and BD and comparison of different treatment protocols before one technique could be preferred to another.

Introduction

Non-melanoma skin cancer (NMSC) is the most frequent skin tumour in the Caucasian population\(^1\), for which surgical excision is the golden therapeutic standard. Superficial basal cell carcinoma (sBCC) and squamous cell carcinoma in situ or Bowen’s Disease (BD) are two superficial types of NMSC whose diagnosis tends to be delayed because they present asymptomatically and often mimic benign skin conditions such as eczema. Frequently, these tumours present as large or multiple lesions on cosmetic sensitive areas. In this case, physicians may consider laser ablation, electrocardiography and curettage, cryotherapy, radiotherapy or topical non-invasive strategies such as photodynamic therapy (PDT) with methyl aminolevulinate (MAL), 5-fluorouracil (5-FU) or imiquimod as alternatives to surgery\(^1,2\). Cryotherapy and electrocardiography combined with curettage are simple and inexpensive treatments for low-risk tumours, but should be avoided on cosmetic sensitive areas\(^3,4\). Despite limited evidence in literature, tumour ablation with a carbon dioxide (CO\(_2\)) laser is a convenient treatment option for sBCC and BD in patients with multiple or recurrent tumours or with lesions on anatomical challenging treatment sites\(^5-7\). PDT, 5-FU and imiquimod generally result in good cosmetics\(^8-10\), however, recurrence following topical therapies is well known to be high compared to surgery\(^11,12\). While PDT is an office-based procedure, efficacy of 5-FU and imiquimod is dependent on patient compliance\(^3,4\).

The concept of laser-assisted PDT has increasingly been studied as an extension of the therapeutic arsenal for managing NMSC. Ablative laser pretreatment disrupts the stratum corneum, an important physical skin barrier. Additionally, the laser on its own adds therapeutic effect. Several preclinical studies showed facilitated accumulation of topically applied drugs\(^13-16\). Clinical studies demonstrated enhanced lesion response when CO\(_2\) or Erbium-doped Yttrium Aluminium Garnet (Er:YAG) laser preceded PDT compared to PDT or laser alone\(^2,17-25\). Ablative laser-assisted PDT is considered to offer good tolerability, satisfactory aesthetics and might provide other advantages such as reduced photosensitizer incubation time\(^18,25,26\).

Both continuous ablative and fractional ablative laser devices have been explored\(^13,15,27\). Whereas continuous ablative lasers (CL) reduce tumoural thickness by ablating the epidermis in a continuous manner, fractional ablative laser devices (FL) create microscopic vertical channels of ablation surrounded by a coagulated cuff, referred to as microscopic treatment zones. These zones facilitate penetration of topical molecules. The surrounding tissue is relatively spared, resulting in better
wound healing. An ex vivo study on porcine skin found both continuous and fractional ablative laser settings capable of enhancing PDT fluorescence intensities, with higher intensities observed after continuous ablation. Since CL is a treatment strategy on its own, it can be argued that CL pretreatment implies better efficacy compared to FL. The tissue-sparing effects of fractional laser technology, however, might possibly translate into less side effects and better aesthetics compared to CL+PDT. To the best of our knowledge, this randomised controlled trial is the first to make a direct comparison between MAL-PDT pretreatment with CL (CL+PDT) and FL (FL+PDT) for managing sBCC and BD. During a twelve-month follow-up, this study investigates if CL+PDT and FL+PDT provide an equivalent efficacy, and if differences in pain, side effects, aesthetics and patients’ preference can be observed.

Materials and methods

Inclusion, blinding and randomisation

This randomised controlled pilot study was approved by the Ethics Committee of Ghent University Hospital, Belgium, on September 1st 2014 and was performed in accordance with the Helsinki declaration. The trial was registered in the U.S. National Institutes of Health clinicaltrials.gov database (identifier: NCT03012009). All patients were recruited in the dermatological department of Ghent University between January 2015 and March 2016. An informed consent was signed prior to inclusion. Eligible patients had a histologically proven and inoperable sBCC or BD with a size larger than 5 cm² or had two smaller lesions. Inoperability was implied by characteristics of the tumour (size, localisation) and/or the patient (age, use of anticoagulants). Large lesions were subdivided into two treatment areas to make intra-patient comparison possible. Exclusion criteria were pregnancy or lactation, age below 18, history of an allergic reaction to local anaesthesia and history of side effects related to laser or PDT. Every patient received a personal number to guarantee anonymity. Prior to treatment, within-subject randomisation with sealed envelopes randomly assigned every treatment area per patient to receive CL+PDT or FL+PDT.

Treatment protocol

After local anaesthesia by means of a subcutaneous injection with 1 to 2.5 ml of lidocaine hydrochloride 2% with epinephrine (AstraZeneca, United Kingdom) per treatment area, half of the treatment areas was pretreated with CL and the other half with FL according to within-subject randomisation. The MiXto SX® Slim Evolution CO² laser (Lasering s.r.l., Modena, Italy) with 8 ms pulse duration treated clinical visible lesion with CL (12 W power, to the level of the papillary dermis) or FL (30 W power, 15% density, 180 μm spot size, 240 mJ energy, 943 J/cm² fluency). Ferric chloride 20% was used in case of bleeding. A 1 mm thick layer of MAL 16% (Metvix®, Galderma, Lausanne, Switzerland) was applied under occlusion and after 3 hours, lesions were illuminated with a red light-emitting diode at 37 J/cm² (Aktilite® CL128, Galderma, Lausanne, Switzerland). PDT pain management was administered according to patients’ wishes through optional intake of paracetamol 500 mg half an hour before illumination and by spraying cold water during illumination. Finally, wound care was executed using Fucidin® 2% ointment (Leo, Ballerup, Denmark) covered with...
Cosmopor E® (Hartmann, Heidenheim, Germany). Eau Thermale Avène Gel Anti-Brûlures (Pierre Fabre, Laboratoires Dermatologiques Avène, Boulogne, France) was used in case of burning sensation. This treatment modality was repeated after two weeks (Fig. 1).

Study protocol

The study protocol is summarized in Table 1. Histological assessment by means of punch biopsy occurred prior to treatment. Clinical visible tumour borders were marked on plastic templates in order to help the investigators identify the treatment areas during follow-up. A telephonic survey was held one week after every treatment session and follow-up visits were provided 3, 6 and 12 months after treatment. A blinded expert, who was always the same person and never the treating investigator, assessed clinically and in person the primary endpoint efficacy during the follow-up visits using a 3-point scale with complete regression (CR), partial regression (PR) and no regression (NR) defined as a regression percentage of respectively 100%, 25-99% and 0-24%. The clinical efficacy was verified histologically at the end of follow-up by means of a punch biopsy taken in the middle of each treatment area or at a location clinically suspicious for recurrence. Histological evaluation was performed blinded at the Pathology Department of Ghent University Hospital. Patients scored pain perceived during PDT illumination on a Visual Analogue Scale (VAS) of 100 mm. Side effects (erythema, oedema, crusting, burning sensation, pain, vesicles, hematoma, infection, pigmentary changes (hypo- or hyperpigmentation) and scarring) were assessed immediately after PDT, during the telephonic survey one week post-treatment, before the second treatment session and at every follow-up visit. Aesthetics were scored by both the blinded expert and patient using a four-point scale (excellent: no significant changes – good: minor changes – poor: serious hypo- or hyperpigmentation and/or visible scarring – very poor: important scarring). Patients were asked for their preferred treatment option during every follow-up visit and for factors influencing this preference (efficacy, pain, side effects or aesthetics).

Statistical analysis

On the assumption that both CL+PDT and FL+PDT imply an improvement of PDT in monotherapy, high efficacies in both treatment arms were expected. Therefore, the authors decided that an equivalence analysis on the primary endpoint efficacy would be the most meaningful choice. Equivalence analysis was performed with the ‘Tango’s score confidence interval for a difference of proportions with matched pairs’ ($\alpha=0.025$). The equivalence margin $\delta=16.1\%$ was based on existing literature by computing the difference in a clinical assessed efficacy of 97.1% found for CL+PDT$^{28}$ and of 81% for FL+PDT$^{19}$. The chosen value of $\delta$ is a rough estimate since these studies treated nodular BCC (nBCC) or the broad spectrum of BCC and a $\delta$ value of 16% is rather substantial from a clinical viewpoint. Paired samples tests determined if a difference in the secondary endpoints (pain, side effects, aesthetics and patients’ preference) existed. Side effects and patients’ preference were analysed by means of the McNemar’s test ($\alpha=0.05$). The Wilcoxon matched-pairs signed ranks test was used for the variable pain ($\alpha=0.05$). Data were analysed using IBM® SPSS® Statistics Version 22.0$^{29}$ and R version 3.3.2$^{30}$. We aimed to include at least 12 patients since a minimal sample size of 12 patients per group is recommended for pilot studies$^{31}$. 

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Results

Patient characteristics

Fifteen out of the 16 included patients with histologically proven sBCC (n=9) or BD (n=6) received study treatment. Two CL+PDT treatment areas, which were not sufficiently healed, received PDT only during the second treatment. One patient was loss to follow-up after 3 months because of health problems not related to the study treatment. Table 2 and Fig. 2 present an overview of the patients’ baseline characteristics and patient flow.

Efficacy

An overview of the efficacy during follow-up is summarized in Table 3. After 12 months, 92.9% (13/14) of patients in the intention-to-treat (ITT) group achieved clinical CR in both CL+PDT and FL+PDT, which was histologically confirmed. This equals a response of 100.0% (9/9) in the sBCC and 80.0% (4/5) in the BD subgroup. No equivalence in efficacy could be established 3, 6 and 12 months after treatment (δ = [-0.161;0.161], respective 95% confidence interval = [-0.298;0.151], [-0.315;0.159] and [-0.215;0.215]). Efficacy in the per protocol (PP) group equalled 91.7%. If a difference in clinical efficacy between CL+PDT and FL+PDT was noticed during follow-up, this was always in the advantage of FL+PDT. In one sBCC patient, a nodular component was detected on the skin biopsy. This patient was in CR after 12 months.

Pain during PDT illumination

In the FL+PDT group, less pain was observed during PDT illumination, which was not significant in the ITT analysis during the first treatment (P=0.144) but significant during the second treatment (P=0.026) (Fig. 3). The same trend can be noticed in the PP analysis (P=0.039). During the first treatment, a median VAS-score of 5 mm was observed in both treatment arms, ranging between [0-74] in CL+PDT and [0-60] in FL+PDT. During the second treatment session, a median VAS-score of 20 mm (range [0-85]) was observed in CL+PDT and a median VAS-score of 10 mm (range [0-60]) in FL+PDT in both ITT and PP analyses. In general, most patients experienced treatment as little painful. During the first and second treatment respectively 73% (11/15) and 80% (12/15) of the patients scored less than 20 mm on the VAS-scale in both treatment areas.

Side effects

Table 4 gives an overview of the side effects identified during hospital visits. Side effects are not significantly different between CL+PDT and FL+PDT (P = 0.219-1.000). Erythema was the most frequently observed side effect immediately after both treatments (CL+PDT, 93%; FL+PDT, 87%). Prevalence of erythema decreased while continuing follow-up but remained present in 21% after 12 months. Other frequently reported side effects were oedema, crusting, burning sensation, pain and pigmentary changes. Vesicles and infection were sporadically observed, hematoma never. During the telephonic survey one week after the first treatment session, crusting was reported equally as frequent in both treatment arms (36%), but was more pronounced in the FL+PDT group before the second treatment session was initiated (20% vs. 47%). Approximately one third of patients reported pain during the telephonic surveys one week post-treatment. No scars were present one year after treatment, but pigmentary changes were detected in 21% of the lesions treated with CL+PDT and...
29% of those treated with FL+PDT.

Aesthetic result

No significant difference in aesthetics was found 3, 6 or 12 months post-treatment according to the patients and blinded expert \( (P=0.075-1.000) \). After 3 months, 86% of the patients considered aesthetics as good or excellent. On the other hand, the blinded investigator considered aesthetics as good to excellent in only 67% (CL+PDT) to 53% (FL+PDT) of the patients. After 6 months, aesthetics in CL+PDT and FL+PDT were overall considered to be better, with good to excellent aesthetics in 84% respectively 92% according to the patients and 85% respectively 78% according to the blinded investigator. After 12 months, aesthetics scored good to excellent in all areas. Patients scored 60% of CL+PDT and 80% of FL+PDT as excellent, while the blinded investigator scored 50% of CL+PDT and FL+PDT as excellent (Fig. 4).

Technique of preference

After 3 months, 21% (3/14) of the patients preferred CL+PDT, 7% (1/14) preferred FL+PDT and 71% (10/14) had no therapy preference. After 6 months, 10% (1/10) of the patients preferred CL+PDT, 30% (3/10) preferred FL+PDT and 60% (6/10) had no preference. After 12 months, none of the 11 patients preferred one treatment to another. Of the 9 patients who indicated the most important factor determining their treatment preference, 67% (6/9) appointed efficacy, 22% (2/9) aesthetics, 11% (1/9) pain and 0% (0/9) side effects.

Discussion

Results in this study support the promising role of laser-assisted PDT. An excellent clinical and histological efficacy of 92.9% was seen in both CL+PDT and FL+PDT, but equivalence in efficacy could not be established. Mild to moderate side effects, outstanding aesthetics as well as little pain were observed, however, FL+PDT was considered less painful. This technique might be particularly useful for patients with surgical contraindications, in patients who refuse surgery, in whom surgery is challenging e.g. patients with large lesions on cosmetic sensitive areas or when self-application of topical therapy is difficult.

Previous clinical studies in the field of ablative laser-assisted PDT for BCC or BD applied variable CO\(_2\) or Er:YAG laser settings, varied in the number of treatment sessions and focussed on nBCC or a mix of BCC subtypes instead of sBCC. This has to be considered when comparing efficacy. Our study shows different efficacy in the 2 tumour subgroups. We found that all sBCC lesions responded to therapy. Previous studies found similarly high efficacy in CL+PDT for BCC, but results in FL+PDT are varying. Shokrollahi et al. observed 97.1% CR after one to three CL+PDT sessions in 177 BCC\(^2\). Smucler et al. found 99.0% CR in 194 recurring nBCC 12 months after CL+PDT and found good, however, lower efficacy after PDT (94.9% CR) and CO\(_2\) laser (91.8% CR)\(^6\). Haak et al. observed a low 12-month histological efficacy in FL+PDT (63% CR), which was not significantly different from the one in PDT (56% CR) in 32 high risk nBCC\(^9\). On the other hand, a study of Choi et al. found significantly better efficacy after one session of FL+PDT (78.9% CR) compared to two sessions of PDT (22.2% CR) during a follow-up period of 12 months in 42 thin nBCC\(^2\)\. A study of Lippert et al.
observed a better 18-month efficacy after two sessions of FL+PDT (92.9% CR) than after PDT (80.4% CR) in 56 nBCC pretreated with a diode laser. In BD, our study observes 80.0% CR, which is situated in between the results of previous studies. A RCT of Ko et al. found significantly higher efficacy after one session of FL+PDT (87.5% CR) compared to PDT (50.0% CR) in 58 BD. Although Cai et al. found a similar response after one to three sessions of CL+PDT (72.73% CR; 8/11 BD) and CL (63.33% CR; 7/11 BD), significantly higher recurrence was seen in the laser-only group after 6 months. Summarized, previous studies found laser-assisted PDT in BCC and BD more effective than PDT or laser alone, except for one study about FL+PDT in high risk nBCC.

Most patients experienced low pain levels during PDT illumination, which was performed 3 hours after subcutaneous injection of lidocaine hydrochloride 2% with epinephrine. Some of the patients probably still benefited from this anaesthesia during PDT illumination, regarding the fact that lidocaine 2% with epinephrine has an anaesthetic effect for 2 to 6 hours. This, however, is no explanation for FL+PDT being perceived as less painful as intra-patient comparison was made. Previous studies using lidocaine-prilocaine 5% anaesthetic cream considered FL+PDT as painful as PDT, but observed higher mean VAS-scores (42.2-48.6 mm) compared to ours (10.0-20.33 mm).

Observed side effects are similar to the known side effects of CO₂ laser and PDT in monotherapy. Previous studies about FL+PDT found a frequency of side effects comparable to those in this study, except for increased crusting (84-100%) and pigmentary changes (66.7-74%). Furthermore, the good to excellent aesthetics in this study were confirmed in previous studies and appear to be similar to PDT or laser ablation alone.

Strengths of this study consist of intra-patient comparison, histological confirmation of the efficacy after 12 months and evaluation of patient reported outcomes (aesthetics and treatment preference). A limitation is presented in the small sample size of this pilot study. According to sample size calculation with Sealed Envelope™, a total sample size of 125 patients is required (power of 80%, δ=16.1%; α=0.025). This study did not have enough power in order to demonstrate a possible equivalence in efficacy, which is, however, a condition sine qua non before one laser setting can be preferred to another based on secondary endpoints.

Larger trials with longer follow-up are necessary in order to gain better understanding of the added value of laser-assisted PDT compared to monotherapy of PDT or ablative laser treatment in sBCC and BD. Moreover, further trials should search for the optimal treatment protocol and laser settings, and should include a subgroup analysis between sBCC and BD. While cryotherapy and 5-FU are inexpensive treatment regimens for NMSC, higher costs are implied in laser ablation, imiquimod or PDT. Consequently, laser-assisted PDT is expected to be a relatively expensive treatment. Potentially higher efficacy might however justify these costs in large lesions on cosmetic sensitive areas where self-application of topical therapy is difficult. 5-FU in vitro showed enhanced skin permeation after CO₂ treatment. In a clinical setting, 5-FU application under occlusion for 7 days after FL pretreatment is reported by Nguyen et al. to reach histological clearance in 87% of 30 sBCC and BD lesions, with an overall treatment success of 79% after a mean of 15 months. The best results were seen in BD. It might be interesting to conduct further exploration of laser-assisted 5-FU delivery as a potential cost-saving alternative.
References


13. Forster B, Klein A, Szeimies R, Maisch T. Penetration enhancement of two topical 5-aminolaevulinic acid formulations for photodynamic therapy by erbium: YAG laser ablation of...


### Tables

#### Table 1: Study protocol

<table>
<thead>
<tr>
<th>Assessor</th>
<th>Inclusion</th>
<th>Treatment</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 1 and 14</td>
<td>Telephonic survey, day 7 and 21</td>
</tr>
<tr>
<td>Clinical efficacy</td>
<td>Blinded expert</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Histology(^a)</td>
<td>Pathologist</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Pain during PDT illumination</td>
<td>Patient(^c)</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Side effects(^d)</td>
<td>Investigator (follow-up) / Patient (telephonic survey)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Aesthetics</td>
<td>Blinded expert / Patient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients’ preference</td>
<td>Patient</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Punch biopsy

\(^b\) Only after 12 months of follow-up

\(^c\) Visual Analogue Scale (VAS)

\(^d\) Erythema, oedema, crusting, burning sensation, vesicles, hematoma, infection, pain, pigmentary changes (hypo- or hyperpigmentation), scarring
### Table 2: Baseline characteristics of the 15 treated patients

<table>
<thead>
<tr>
<th></th>
<th>Median [range]</th>
</tr>
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<tbody>
<tr>
<td><strong>Age in years</strong></td>
<td>73 [46-87]</td>
</tr>
<tr>
<td><strong>Sex, n(%)</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7 (47%)</td>
</tr>
<tr>
<td>Female</td>
<td>8 (53%)</td>
</tr>
<tr>
<td><strong>Tumour, n(%)</strong></td>
<td></td>
</tr>
<tr>
<td>sBCC</td>
<td>9 (60%)</td>
</tr>
<tr>
<td>BD</td>
<td>6 (40%)</td>
</tr>
<tr>
<td><strong>Localisation, n(%)</strong></td>
<td></td>
</tr>
<tr>
<td>Head- and neck area</td>
<td>4 (27%)</td>
</tr>
<tr>
<td>Thorax</td>
<td>5 (33%)</td>
</tr>
<tr>
<td>Arms</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Legs</td>
<td>4 (27%)</td>
</tr>
<tr>
<td><strong>Fitzpatrick skin type, n(%)</strong></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>II</td>
<td>12 (80%)</td>
</tr>
<tr>
<td>III</td>
<td>1 (7%)</td>
</tr>
</tbody>
</table>

sBCC, superficial basal cell carcinoma; BD, Bowen’s Disease
Table 3: Clinical efficacy in the intention-to-treat (ITT) analysis

<table>
<thead>
<tr>
<th>Tumour</th>
<th>No. of patients</th>
<th>Follow-up</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>3 months (n=15)</td>
</tr>
<tr>
<td></td>
<td>CL+PDT</td>
<td>FL+PDT</td>
</tr>
<tr>
<td>sBCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7°</td>
<td>CR</td>
<td>CR</td>
</tr>
<tr>
<td>1°</td>
<td>PR</td>
<td>CR</td>
</tr>
<tr>
<td>1</td>
<td>CR</td>
<td>CR</td>
</tr>
<tr>
<td>BD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>CR</td>
<td>CR</td>
</tr>
<tr>
<td>1°</td>
<td>PR</td>
<td>PR</td>
</tr>
<tr>
<td>1</td>
<td>PR</td>
<td>PR</td>
</tr>
<tr>
<td>1</td>
<td>CR</td>
<td>CR</td>
</tr>
<tr>
<td>% CR</td>
<td>80,0%</td>
<td>86,7%</td>
</tr>
<tr>
<td>95% CI</td>
<td>[-0.298;0.151]</td>
<td>[-0.315;0.159]</td>
</tr>
</tbody>
</table>

sBCC, superficial basal cell carcinoma; BD, Bowen’s Disease; CR – PR – NR, complete – partial – no regression; CL+PDT – FL+PDT, continuous – fractional ablative CO₂ laser-assisted PDT; 95% CI, 95% confidence interval

°, one patient received only PDT in the CL+PDT treatment area during the second PDT session

°, sBCC with nodular component

°, drop-out
Table 4: Side effects after treatment and during follow-up

<table>
<thead>
<tr>
<th>Side effects</th>
<th>Treatment</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average after treatment 1 and 2 (n=15)</td>
<td>Before treatment 2 (n=15)</td>
</tr>
<tr>
<td></td>
<td>CL+PDT</td>
<td>FL+PDT</td>
</tr>
<tr>
<td>Erythema</td>
<td>93%</td>
<td>87%</td>
</tr>
<tr>
<td>Oedema</td>
<td>44%</td>
<td>47%</td>
</tr>
<tr>
<td>Crusting</td>
<td>n.o.</td>
<td>10%</td>
</tr>
<tr>
<td>Burning sensation</td>
<td>33%</td>
<td>27%</td>
</tr>
<tr>
<td>Pain</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Vesicles</td>
<td>n.o.</td>
<td>n.o.</td>
</tr>
<tr>
<td>Hematoma</td>
<td>n.o.</td>
<td>n.o.</td>
</tr>
<tr>
<td>Infection</td>
<td>n.o.</td>
<td>n.o.</td>
</tr>
<tr>
<td>Pigmentary changes</td>
<td>n.o.</td>
<td>n.o.</td>
</tr>
<tr>
<td>(hypo- or hyperpigmentation)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scarring</td>
<td>4%</td>
<td>n.o.</td>
</tr>
</tbody>
</table>

CL+PDT – FL+PDT, continuous – fractional ablative CO\textsubscript{2} laser-assisted PDT.

n.o., no side effect observed

* not investigated
### Legends and figures

**1. Treatment protocol**

- **(a)** Photograph before laser treatment, intra-patient randomisation allocated one treatment area to CL+PDT (1=A) and the other to FL+PDT (2=F).
- **(b)** Dermoscopy before treatment in the area allocated to receive CL+PDT and (c) FL+PDT.
- **(e)** Fluorescence photograph 3 hours after MAL application, red light-emitting diode illumination took place immediately afterwards.
- **(f)** Clinical photograph on day 14, before initiation of the second treatment session.
- **(g)** Clinical photograph after a follow-up of 3 months.
- **(h)** 6 months and (i) 12 months.

**2. Histology**

- **(a)** Punch biopsy of the sBCC before treatment initiation.
- **(b)** Punch biopsy of the sBCC after 12 months follow-up in the area that received CL+PDT.
- **(c)** FL+PDT.

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**Fig. 1** Treatment protocol and histology illustrated with sBCC on the chest

sBCC, superficial basal cell carcinoma; CL+PDT = FL+PDT, continuous – fractional ablative CO₂ laser-assisted PDT; 95% CI, 95% confidence interval. **1. Treatment protocol.** (a) Photograph before laser treatment, intra-patient randomisation allocated one treatment area to CL+PDT (1=A) and the other to FL+PDT (2=F); (b) dermoscopy before treatment in the area allocated to receive CL+PDT and (c) FL+PDT; (d) clinical photograph immediately after laser treatment; (e) fluorescence photography 3 hours after MAL application, red light-emitting diode illumination took place immediately afterwards; (f) clinical photograph on day 14, before initiation of the second treatment session; (g) clinical photograph after a follow-up of 3 months, (h) 6 months and (i) 12 months. **2. Histology.** (a) Punch biopsy of the sBCC before treatment initiation; (b) punch biopsy of the sBCC after 12 months follow-up in the area that received CL+PDT and (c) FL+PDT.
Fig. 2 Patient flow

CL+PDT – FL+PDT, continuous – fractional ablative CO₂ laser-assisted PDT; ITT, intention-to-treat analysis; PP, per protocol analysis.
Fig. 3 Pain during PDT illumination in CL+PDT and FL+PDT on a Visual Analogue Scale (VAS)

(a) after the first treatment session; (b) after the second treatment session

CL+PDT – FL+PDT, continuous – fractional ablative CO₂ laser-assisted PDT
Fig. 4 Aesthetics after 3, 6 and 12 months follow-up in the intention-to-treat (ITT) analysis

(a) according to patients and; (b) according to the blinded expert

CL+PDT – FL+PDT, continuous – fractional ablative CO₂ laser-assisted PDT