Using 5-Aminolevulinic Acid and Pulsed Dye Laser for Photodynamic Treatment of Oral Leukoplakia

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Objective: To determine the safety and efficacy of photodynamic therapy in the treatment of oral leukoplakia with 5-aminolevulinic acid and pulsed dye laser.

Design: Nonrandomized, single-arm, single-site phase 1/2 pilot study.

Setting: Academic referral center.

Patients: A total of 23 patients, aged 37 to 79 years, having a confirmed diagnosis of leukoplakia with or without dysplasia measuring at least 10 mm in diameter.

Interventions: Application of 5-aminolevulinic acid to lesions followed by activation with high-power 585-nm pulsed dye laser.

Main Outcome Measures: Maximum tolerated dose of laser, postprocedure complications, objective response to treatment, and immunohistochemical changes in treated tissue.

Results: No significant adverse events occurred; minor local adverse effects were observed during and following photodynamic therapy in the safety phase of the study. The maximum tolerated dose was 8 J/cm². Of 17 patients, 7 (41%) had more than 75% regression (significant response) and 9 (53%) had more than 25% regression (partial response), for an overall response rate of 94% at 90 days. This response rate was far higher than the null-hypothesis 20% rate ($P < 10^{-10}$) and the alternative-hypothesis 50% rate ($P = .0001$) for which the study was powered. When compared with baseline levels immunohistochemically, p53 expression was increased in 8 of 11 available samples (73%) and Ki-67 expression was decreased in 7 of 12 available samples (58%).

Conclusions: Photodynamic therapy with 5-aminolevulinic acid and pulsed dye laser could be used to achieve regression of oral leukoplakia. The treatment is safe and well tolerated. An application time of 1.5 hours and laser radiant exposure of 8 J/cm² with 1.5-ms pulse time were found to be the optimal settings in this study. The high-power laser used in this study allows completion of laser therapy within 1 to 3 minutes. Further studies are necessary to determine the optimal laser radiant exposure and drug application to maximize the response rate.

Trial Registration: clinicaltrials.gov Identifier: NCT00571974


Oral leukoplakia (OL) can be a precursor lesion to oral cavity cancer. Currently, the conventional care options for premalignant lesions in the oral cavity and oropharynx are observation, laser ablation, or aggressive surgical resection. Topical treatments have been attempted to achieve regression of the lesions, including vitamin A (isotretinoin), bleomycin sulfate, and fenretinide. These agents have varying degrees of success, but none has led to a satisfactory treatment for OL. Surgical resection has been shown to be curative, but it is an aggressive intervention associated with significant morbidity when used for large lesions.

Recent clinical pilot studies in Asia and Europe have shown that photodynamic therapy (PDT) with topical application of 5-aminolevulinic acid (5-ALA) is an effective treatment modality to achieve regression of OL and erythroplakia. 5-Aminolevulinic acid is a metabolite of the heme biosynthesis pathway, which is taken up actively and to a greater extent in tumor cells than in normal cells. The agent can be applied topically, and it penetrates well into the skin within 1 to 3 hours. The application of 5-ALA to the tissue leads to an accumulation of protoporphyrin IX (PPIX) in the cells. Protoporphyrin IX absorbs light in the visible range (400-700 nm) with distinct 10-nm-wide absorption peaks at wavelengths of 410, 500, 532, 585, and 635 nm. When illuminated with high-energy external light (ie, >4 J/cm²) with a wavelength at either of the absorption peaks, PPIX undergoes a chemical reaction that produces singlet oxygen and other free radicals that are cytotoxic to cells and vessels.
Chen et al7 used a twice-weekly PDT regimen with 5-ALA and a 635-nm light-emitting diode to treat OL in 24 patients. Before administration of PDT, 5-ALA, 20%, was topically applied for 1.5 hours. The optimal application time was determined via fluorescence diagnosis (FD). The maximal fluorescence intensity of the PPIX excited at 410 nm correlates to the maximum PPIX level in the lesional cells. Each lesion was treated repeatedly in 8 sessions separated by 6 to 8 weeks. In each treatment session, the lesions were irradiated with a power density of 100 mW/cm² for 1000 seconds to a total dose of 100 J/cm² per treatment. The investigators achieved complete response in 8 patients (33%) and partial response in 16 patients (67%) by the eighth treatment session. Sieroné et al8 treated 24 OL lesions in 12 patients using 6 to 8 treatment sessions separated by 2 weeks with PDT at 635 nm delivered by argon-pumped dye laser with a power density of 150 mW/cm² for 11 minutes to a total dose of 100 J/cm². They achieved complete response in 10 patients vs no response in the remaining 2 patients.

In the United States, 5-ALA (Levulan Kerastick; DUSA Pharmaceuticals, Inc, Valhalla, New York) is approved by the Food and Drug Administration to be used in conjunction with a blue light illumination (BLU-U) system for the treatment of actinic keratoses.11 The illumination system consists of a large concave lamp designed to illuminate large areas, such as the face and scalp. The lamp is placed over the patient’s head and illuminates 0.01 W/cm² to the targeted region for 1000 seconds (16 minutes 40 seconds) to provide a 10-J/cm² light dose. During this light treatment, the patients and medical personnel are provided with blue-blocking protective eyewear. The illumination system cannot be used as an intraoral light source.

This study, a clinical pulsed dye laser (PDL) (Scleroph Plus; Candela Corp, Wayland, Massachusetts) was used for lesional illumination. This laser can deliver up to 12 000 W/cm² of 585-nm light wavelength (fourth absorption peak of the PPIX) through an optical fiber, with radiant exposures that can vary from 6 to 18 J/cm² in 1.5-ms pulse time. Photodynamic therapy using 5-ALA with a 585-nm PDL has been used to treat skin lesions12-15 as well as Bowen disease15 and laryngeal keratosis with atypia16 in pilot studies; it has never been used to treat OL.

In this article, we report the results of a combined phase 1/2 nonrandomized prospective study that was designed to determine the safety and assess the clinical efficacy of PDT in the treatment of OL with 5-ALA and 585-nm PDL with a 1.5-ms pulse time. In the first part of the study, we determined the maximum tolerated dose (MTD) of the PDL radiant exposure in combination with 5-ALA. In the second phase of the study, this dose was used to treat patients with the MTD to determine the efficacy of the treatment by documenting the regression of the treated lesions.

**METHODS**

**PATIENTS**

This prospective, nonrandomized, single-arm, single-site phase 1/2 pilot study was conducted at the Department of Otolaryngology Head and Neck Surgery at the University of Arkansas for Medical Sciences under investigational new drug number 73269. The study was approved by the University of Arkansas for Medical Sciences institutional review board. Candidates were identified in the head and neck oncology clinic. Patients with at least 1 grossly visible leukoplakia lesion, with or without dysplasia, measuring 10 mm or more in diameter were included in this study. The exclusion criteria included sensitivity to porphyrins or photoactive medications or invasive carcinoma of the lesion as demonstrated by biopsy. Twenty-eight patients were enrolled in the study between January 1, 2007, and November 18, 2009. During the study, 4 patients did not meet inclusion criteria or were disqualified secondary to meeting exclusion criteria, and 1 patient was removed from the study because of nonadherence. In phase 1, 3 cohorts, each including 3 patients, were enrolled. Five participants were women and 4 were men, aged 50 to 79 years (mean, 61.8 years). In phase 2 of the study, 11 men and 6 women aged 37 to 79 years (mean, 62.6 years) were enrolled. Included in phase 2 were the 3 patients from phase 1 who received the MTD. Photodynamic therapy was performed in the outpatient head and neck cancer clinic at the University of Arkansas for Medical Sciences. The patients were seen at 30, 90, and 365 days after therapy.

**PROCEDURE**

At screening, or 60 days before therapy, 3-mm punch biopsy specimens were obtained from the center of the lesion with the patient under local anesthesia. Lesions determined by pathologic examination to be invasive carcinoma or carcinoma in situ were excluded from the study and treated with surgical intervention. Baseline documentation of the area of leukoplakia was performed by standardized digital photography immediately before PDT.

**DRUG APPLICATION**

Initially, 5-ALA was applied to the lesions topically. Before each treatment, the 5-ALA product containing 345 mg of active 5-ALA in 1.5 mL of solution was used to create a 5-ALA, 20%, solution. The 5-ALA solution then was dissolved onto gauze, and the saturated gauze was placed against the lesion. If the lesion was on the palate, a customized temporary dental prosthesis was used (Figure 2). The 5-ALA solution was topically applied for 1.5 hours. Application of 5-ALA in some patients proved to be difficult secondary to dilution by saliva as well as difficulty maintaining positioning. Hence, in the last 6 patients, an equivalent dose of 5-ALA was administered by intraleseional injection to optimize the bioavailability of the drug; the remaining methods were identical in these participants.

**FLUORESCENCE DIAGNOSIS**

Fluorescence diagnosis was used to confirm that the 5-ALA was absorbed and converted into PPIX after application (Figure 2). The lesion was photographed before and after drug application with an FD camera (Dyaderm; Biocam GmbH, Regensburg, Germany). This imaging technique has been described and validated.18 Briefly, the photographic system is a portable passive device consisting of a highly sensitive digital camera and light-emitting diode array that emits low-power (0.85-mW/cm²) light with a 405-nm wavelength. The camera system superimposes side-by-side display of fluorescent and color images in real time under normal room light conditions.

**PHOTODYNAMIC THERAPY**

A PDL that emits light with a 585-nm wavelength in pulses of 1.5 ms was used. Initially, a single laser pulse was delivered to...
as those provided by Storer. The highest radiant light dose to toxic effects was observed. Dose escalation rules were the same of 6, 7, or 8 J/cm². In each cohort, the number of dose-limiting rolled and treated with escalating radiant exposures (laser doses) decreased.

staining of the lesions would increase and Ki-67 staining would from a null-hypothesis 20% response rate. power at 5% was 7 or more of 17 responses overall. This design had 80% fewer of 9 responses in the first stage, and its success criterion mined at 90 days. This design's early termination rule was 3 or was objective response (significant sign, with 9 patients in the first stage and 8 in the second stage, was treated at the MTD were considered a part of phase 2. This part of the study used a Simon 2-stage minimax design. Three cohorts, each with 3 patients, were enrolled and treated with escalating radiant exposures (laser doses) of 6, 7, or 8 J/cm². In each cohort, the number of dose-limiting toxic effects was observed. Dose escalation rules were the same as those provided by Storer. The highest radiant light dose attained that did not produce 2 or more dose-limiting toxic effects was declared to be the MTD. Patients from phase 1 who were treated at the MTD were considered a part of phase 2.

In phase 2 of the study, all patients were treated with the MTD. This part of the study used a Simon 2-stage minimax design, with 9 patients in the first stage and 8 in the second stage, yielding 17 participants overall. The primary efficacy end point was objective response (significant + partial response) determined at 90 days. This design's early termination rule was 3 or fewer of 9 responses in the first stage, and its success criterion was 7 or more of 17 responses overall. This design had 80% power at 5% α to distinguish an efficacious 30% response rate from a null-hypothesis 20% response rate.

EVALUATION

Phototoxic effects and adverse events were evaluated in the clinic within 48 hours after treatment. Additional clinical evaluations were conducted 30 and 90 days after treatments. At the 90-day follow up visit, a 3-mm punch biopsy specimen was taken from the center of the lesion and a sample of healthy mucosa was taken from a distant normal-appearing site within the oral cavity. The response was determined by examination by an experienced head and neck surgeon (E.V., B.C.S., or J.Y.S.) and classified as follows: significant response, 75% or more resolution of the lesion; partial response, reduction of at least 25%; and no response, reduction by less than 25%.

PATHOLOGIC EXAMINATION

All biopsy specimens taken before and after 5-ALA and PDT treatment from both the lesion and adjacent grossly normal mu cosa were evaluated for morphologic evidence of tissue damage. The biopsied tissues were submitted for routine pathologic diagnosis. The tissue was processed, embedded in paraffin, sectioned, and stained with hematoxylin-eosin for routine examination. Additional slides were evaluated immunohistochemically using monoclonal antibodies against p53 and Ki-67 (Zymed Laboratory, Inc, San Francisco, California). An experienced head and neck surgical pathologist (C.-Y.F.) examined all the slides. Expression in p53 and Ki-67 markers was evaluated by pathologic scoring, using a scale of 0 (negative) to 3 (strongly positive).Slides of samples taken before and after treatment were stained at the same time. Breast carcinoma was used as a positive control for p53 and Ki-67 markers. As demonstrated in previous studies, p53 is a marker for the apoptosis pathway and Ki-67 is a marker for cellular proliferation. Hence, it would be expected that, after the PDT, the p53 staining of the lesions would increase and Ki-67 staining would decrease.

STUDY DESIGN

The objective of this study was to determine the safety and efficacy of PDT in the treatment of OL with 5-ALA and pulsed PDL. Phase 1 of the study used the traditional 3 + 3 dose escalation design. Three cohorts, each with 3 patients, were enrolled and treated with escalating radiant exposures (laser doses) of 6, 7, or 8 J/cm². In each cohort, the number of dose-limiting toxic effects was observed. Dose escalation rules were the same as those provided by Storer. The highest radiant light dose attained that did not produce 2 or more dose-limiting toxic effects was declared to be the MTD. Patients from phase 1 who were treated at the MTD were considered a part of phase 2.

In phase 2 of the study, all patients were treated with the MTD. This part of the study used a Simon 2-stage minimax design, with 9 patients in the first stage and 8 in the second stage, yielding 17 participants overall. The primary efficacy end point was objective response (significant + partial response) determined at 90 days. This design's early termination rule was 3 or fewer of 9 responses in the first stage, and its success criterion was 7 or more of 17 responses overall. This design had 80% power at 5% α to distinguish an efficacious 30% response rate from a null-hypothesis 20% response rate.

We originally planned to use the 1-sided exact binomial test to compare the observed response rate with the 20% rate envisioned under the original null hypothesis. Because all but 1 patient responded to treatment within 90 days, a second comparison was added in which the 1-sided exact binomial test was used to determine whether the observed response rate was significantly higher than the 50% rate originally envisioned under the alternative hypothesis. The Fisher exact test was used to determine whether the proportion of patients specifically attaining significant response differed between the first 11 patients who received topical 5-ALA and the last 6 patients who received injected 5-ALA.

STATISTICAL ANALYSIS

RESULTS

SAFETY

The 5-ALA and 585-nm PDL therapy was safe in treating OL. No major adverse events occurred during the
study; minor adverse effects encountered during treatment included sensitivity, pain, swelling, burning sensation, taste alteration, ulceration, and loss of sensation. These all occurred at the local area of treatment. Most had resolved by the 90-day follow-up, and all had resolved by the 365-day follow-up. The highest laser radiant exposure dose of 8 J/cm² produced acceptable levels of phototoxic effects and adverse events. This radiant exposure was declared to be the MTD, and all patients in phase 2 of the study were treated with this radiant exposure. Results of phase 1 of the study are given in Table 1.

**EFFICACY**

Of the 17 patients with OL treated with 5-ALA plus PDT, 7 patients (41%) achieved significant response (Figure 3), 9 patients (53%) showed partial response (Figure 4), and 1 patient (6%) showed no response (Table 2). The objective response rate was 16 of 17 patients (94%), which not only was far higher than the study’s null-hypothesis rate of 20% (exact binomial \( P < 10^{-10} \)) but also was significantly higher than the study’s alternative-hypothesis rate of 50% (exact binomial \( P = .0001 \)). A comparison was drawn to look specifically for an imbalance in significant response between patients who had topical application vs injection of 5-ALA. Five of 11 patients (45%) who received 5-ALA had a significant response compared with 3 of 6 patients (50%) who received injected 5-ALA (Fisher exact test, \( P = 1.00 \)). Immunohistochemically, when compared with baseline levels, p53 expression increased in 8 of 11 patients (73%) and Ki-67 expression decreased in 7 of 12 patients (58%) with available samples (Table 3). A correlation could not be found between response of the lesion and degree of dysplasia.

**COMMENT**

Our findings support the notion that PDT with 5-ALA and 585-nm PDL is safe for treatment of OL with radiant exposures of 6 to 8 J/cm² delivered in 1.5-ms pulses and 7-mm-diameter spot size. These results are in agreement with those of Karrer et al, who used a similar PDL system (ScleroLaser; Candela Corp) to deliver 585-nm light in 1.5-ms pulse and radiant exposure of 18 J/cm² for the treatment of actinic keratoses. The short laser illumination time (1.5 ms) may have also contributed to the relatively moderate pain that was reported in the present study and that was also reported by Karrer et al. The mean (SD) pain level on a 10-point scale in their study was 3.2 (1) during 5-ALA PDT with PDL in comparison with 7.7 (2.3) during the time exposure of incoherent light. In our study, the mean (SD) pain level was 2.7 (1.1) with low laser doses. However, 2 patients reported severe pain (level 8 on a 10-point scale) with the MTD, and thereafter local anesthesia was used by injection of lidocaine, 1%, which was sufficient to alleviate treatment-related pain. Notably, we did not observe photosensitivity in our study, although it is a common adverse effect of PDT. We attribute this outcome to the topical and local application of 5-ALA.

The use of a relatively small beam size (7 mm in diameter) required the physicians to constantly reposition the laser beam across the lesion to treat the entire target region. Although this manual scan was quick, it could have resulted in incomplete treatment of the lesion. To overcome this problem in future studies, we plan to attach the laser hand piece to a robotic arm. Of note, we assert that the manual scanning did not cause overtreatment. This assertion is supported by the minimal ad-
verse effects that we observed and by the fact that the laser pulses and cytotoxic action time intervals (1.5 × 10⁻³ and 0.04 × 10⁻⁶ sec) are much shorter than the time between pulses (1-3 seconds).

Photodynamic therapy with 5-ALA and 585-nm PDL was effective in achieving regression of OL, seeing that 8 of the 18 lesions (44%) had a significant response and an additional 9 (50%) had a partial response (Table 2). Only 1 eligible participant had no response to this treatment. These outcomes are comparable to the results reported in other pilot studies7,8 in which topical application of 5-ALA was activated with low-power diode laser. Conversely, in those studies, 6 to 8 sessions and treatment times up to 17 minutes were required to obtain an overall objective response of more than 90%. In our study, we achieved an overall objective response of 94% with 1 or 2 sessions and a laser treatment time of 1 to 3 minutes, thus making PDT with 5-ALA and 585-nm PDL an alternative to the long exposure times and multiple treatments required with the diode laser.

All immunostains for p53 and Ki-67 were performed on OL with or without dysplasia (smoke-related preneoplastic lesions) and adjacent normal-appearing squamous mucosa (nonneoplastic and nondysplastic control) before and after 5-ALA–PDT treatment. The conclusion of increased expression of p53 and decreased expression of Ki-67 in most posttreatment lesions in most samples was made by comparison between leukoplakia lesions and matched nondysplastic control samples from normal-appearing mucosa adjacent to the leukoplakia lesion before and after PDT treatment. Comparison was also made between samples ob-

Table 2. Phase 2 Results

<table>
<thead>
<tr>
<th>Lesion No. a</th>
<th>Sex/Age, y</th>
<th>Location</th>
<th>Application</th>
<th>Change in Staining</th>
<th>Response at Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/70</td>
<td>Buccal mucosa</td>
<td>Topical</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>M/79</td>
<td>Retromolar trigone</td>
<td>Topical</td>
<td>Increase</td>
<td>Decrease</td>
</tr>
<tr>
<td>3</td>
<td>M/58</td>
<td>Tongue</td>
<td>Topical</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>F/64</td>
<td>Alveolar ridge</td>
<td>Topical</td>
<td>Increase</td>
<td>Decrease</td>
</tr>
<tr>
<td>5</td>
<td>M/61</td>
<td>Buccal mucosa</td>
<td>Topical</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>6</td>
<td>M/63</td>
<td>Tongue</td>
<td>Topical</td>
<td>NA</td>
<td>Decrease</td>
</tr>
<tr>
<td>7</td>
<td>F/69</td>
<td>Tongue</td>
<td>Topical</td>
<td>Increase</td>
<td>Decrease</td>
</tr>
<tr>
<td>8</td>
<td>F/66</td>
<td>Tongue</td>
<td>Topical</td>
<td>Increase</td>
<td>Decrease</td>
</tr>
<tr>
<td>9</td>
<td>M/66</td>
<td>Tongue</td>
<td>Topical</td>
<td>Increase</td>
<td>Decrease</td>
</tr>
<tr>
<td>10</td>
<td>M/64</td>
<td>Buccal mucosa</td>
<td>Topical</td>
<td>No change</td>
<td>Decrease</td>
</tr>
<tr>
<td>11</td>
<td>M/62</td>
<td>Buccal mucosa</td>
<td>Topical</td>
<td>Decrease</td>
<td>Increase</td>
</tr>
<tr>
<td>12</td>
<td>F/65</td>
<td>Alveolar ridge</td>
<td>Topical</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>13</td>
<td>F/58</td>
<td>Buccal mucosa</td>
<td>Injected</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>14</td>
<td>M/61</td>
<td>Buccal mucosa</td>
<td>Injected</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>15</td>
<td>M/65</td>
<td>Tongue</td>
<td>Injected</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>16</td>
<td>M/65</td>
<td>Palate</td>
<td>Injected</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>17</td>
<td>M/61</td>
<td>Tongue</td>
<td>Injected</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>18</td>
<td>M/37</td>
<td>Alveolar ridge</td>
<td>Injected</td>
<td>Decrease</td>
<td>Increase</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not available; NR, no response; PR, partial response; SR, significant response.

aLesions 15 and 16 are from the same patient.

Figure 4. Pretreatment (A) and posttreatment (B) pictures of oral leukoplakia of the buccal mucosa in a patient (lesion 5) who experienced a partial response to photodynamic therapy with 5-aminolevulinic acid and pulsed dye laser.
cal 5-ALA and PDL may be beneficial in the treatment of acne inversa. Orringer et al. showed that PDT with topical PDL as a light source is not effective in the treatment of OL lesions. After PDT treatment, most OL lesions showed significantly increased p53 compared with the same lesions before the treatment as well as with the nondysplastic control (normal-appearing mucosa) taken at the same time after PDT treatment. We speculated that the increased p53 protein expression in these OL lesions is most likely the result of overexpression of wild-type p53 protein, a common cellular response to DNA damage. It is also possible that PDT treatment has induced mutation in the p53 gene, with subsequent overexpression of this mutant protein, even though this scenario is much less likely. In summary, we performed protein expression analysis for p53 and Ki-67 primarily to document the molecular changes in association with clinical responses of these lesions to PDT treatment. Small sample size precludes any meaningful statistical analysis; however, we observed increased p53 expression and decreased Ki-67 expression, which may be directly linked to DNA damage induced by PDT treatment.

An observation made but not measured in our study was that many of the lesions that showed significant regression were thin and superficial. The larger and more hyperkeratotic the lesion, the less of a response obtained. Our hypothesis is that this is likely secondary to the shallow penetration of the PDL (1-1.5 mm). These lesions may require treatment with PDT with a diode laser emitting 635 nm that has deeper penetration than the PDL.

Midway through phase 2 of the study, injection of 5-ALA into the lesion was undertaken to improve the mode of application of the drug. No significant difference was observed between injection and topical application. The use of FD was of some help to determine drug uptake (as shown in Figure 2). However, FD was not useful in determining the response of the lesion to PDT. Immediately after laser therapy, the FD intensity did not change significantly (data not shown).

The efficacy of PDT with short-pulse high-power lasers, such as PDLs, is the subject of an ongoing debate in the literature. In a study including 4 consecutive patients, Passeron et al. found that PDT with 5-ALA using PDL as a light source is not effective in the treatment of acne inversa. Orringer et al. showed that PDT with topical 5-ALA and PDL may be beneficial in the treatment of inflammatory acne in a subgroup of patients in a randomized, controlled, split-face clinical study. Karreter et al. demonstrated that, although PDL alone had no therapeutic effects, 5-ALA in conjunction with PDL caused significant regression of actinic keratoses, a result that is in agreement with a study by Alexiades-Armenakas. It is safe to assume that data concerning skin lesions are similar to those concerning mucosal lesions, since the absorption of these has been proven to be similar. Therefore, although a laser control was not used in our study, it is reasonable to assume that our PDT, which combined 5-ALA with a 585-nm PDL, induced the regression of OL rather than the laser alone. Other pilot studies have shown that 5-ALA in conjunction with PDL is effective in the treatment of laryngeal keratosis with atypia16 and Bowen disease. The mechanism that dictates the clinical response of superficial lesions to PDT with PDL is not clear.

Studies have confirmed the usefulness of 5-ALA PDT in the management of a multitude of possibly precancerous lesions, including those in the oral cavity. Our results substantiate the effectiveness of 5-ALA PDT in the management of OL. During phase 2 of the study, all but 1 lesion showed at least partial response after only 1 treatment. This therapy is safe, noninvasive, and less disfiguring than other treatments commonly used for OL. Further studies should assess the optimal laser radiant exposure and drug application to increase the likelihood of complete regression of OL lesions.

**Table 3. Tumor Marker Expression**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Complete Response</th>
<th>Partial Response</th>
<th>No Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>Increased 3</td>
<td>Decreased 1</td>
<td>Unchanged 4</td>
</tr>
<tr>
<td></td>
<td>Decreased 2</td>
<td>Unchanged 2</td>
<td>Decreased 1</td>
</tr>
<tr>
<td>Ki-67</td>
<td>Increased 4</td>
<td>Unchanged 0</td>
<td>Decreased 0</td>
</tr>
<tr>
<td></td>
<td>Decreased 3</td>
<td>Unchanged 0</td>
<td>Decreased 0</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

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**Author Contributions:** Drs Shafirstein and Suen are considered the principal investigators of this study. Dr Shafirstein had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Shafirstein, Baemler, Vural, Stack, and Suen. Acquisition of data: Shafirstein, Fan, Morehead, Vural, and Suen.
Analysis and interpretation of data: Shafirstein, Friedman, Siegel, Moreno, and Suen. Drafting of the manuscript: Shafirstein and Suen. Critical revision of the manuscript: Shafirstein and Suen. Financial Disclosure: None reported.

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Previous Presentation: This study was presented at the American Head and Neck Society 2011 Annual Meeting: April 28, 2011; Chicago, Illinois.

Additional Contributions: Linda Wood, RN, BSN, CCRP, and Karen DuVall, BS, CCRP, assisted with the study coordination. Scott Ferguson assisted with the laser setup. We thank all the personnel at the University of Arkansas for Medical Sciences research support center for their outstanding help in obtaining the approval for the investigational new drug No. 73269 and monitoring the study. We acknowledge the support of the Otolaryngology Department through the McGill Family Division of Clinical Research.

REFERENCES