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Lesion Oxygenation Associates with Clinical Outcomes in Premalignant and Early Stage Head and Neck Tumors Treated on a Phase 1 Trial of Photodynamic Therapy

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Abstract

Background—We report on a Phase 1 trial of photodynamic therapy (PDT) for superficial head and neck (H&N) lesions. Due to known oxygen dependencies of PDT, translational measurements of lesion hemoglobin oxygen saturation (S_tO_2) and blood volume (tHb) were studied for associations with patient outcomes.

Methods—PDT with aminolevulinic acid (ALA) and escalating light doses was evaluated for high-grade dysplasia, carcinoma-in-situ, and microinvasive carcinomas of the H&N. Among 29 evaluable patients, most (18) had lesions of the tongue or floor of mouth (FOM). Disease was

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intact in 18 patients and present at surgical margins in 11 patients. In 26 patients, lesion S_tO_2 and tHb was measured.

Results—Local control (LC) at 24 months was 57.5% among all patients. In patients with tongue/FOM lesions LC was 42.7%, and it was 50.1% for those with intact lesions. Lesion tHb was not associated with 3-month complete response (CR), but S_tO_2 was higher in patients with CR. In tongue/FOM lesions, baseline S_tO_2 [mean(SE)] was 54(4)% in patients (n=12) with CR versus 23(8)% in patients (n=6) with local recurrence/persistence (p=0.01). Similarly, for intact disease, baseline S_tO_2 was 54(3)% in patients (n=10) with CR versus 28(8)% in patients (n=5) without CR (p=0.03). In patients with intact disease, higher baseline S_tO_2 associated with 24-month local control (p=0.02).

Conclusions—Measurement of the physiologic properties of target lesions may allow for identification of patients with the highest probability of benefiting from PDT. This provides opportunity for optimizing light delivery based on lesion characteristics and/or informing ongoing clinical decision-making in patients who would most benefit from PDT.

Graphical abstract

Keywords

tissue hemoglobin oxygen saturation; aminolevulinic acid; head and neck; dysplasia; carcinoma-in-situ; microinvasive squamous cell carcinoma

Introduction

Photodynamic therapy (PDT) involves delivery of a photosensitizer or photosensitizer precursor followed by illumination at a specific wavelength of light to an identified target. In applications to head and neck malignancies, PDT has been studied with photosensitizers/precursors that include Photofrin, aminolevulinic acid (ALA), and Foscan. As reviewed by several investigators, [1, 2], PDT can be used for curative intent in the treatment of early stage primary or recurrent disease, such as that of the oral cavity, larynx, pharynx, and nasopharynx. It can also be used for palliation of later stage refractory disease. However, many benefits of PDT are particularly pertinent to the treatment of early stage oral malignancies, contributing to clinical success with several photosensitizers [3–5]. Lesions of the oral cavity are easily accessible to PDT light sources and the limited toxicity profile of PDT allows for repeated application [6]. This can be contrasted to functional limitations that can result from surgery, such as impairments in speech or difficulty in swallowing, or morbidities of external beam radiation therapy, such as changes in swallowing, taste, and salivation. When compared to resection of early stage lesions, PDT can achieve similar

efficacy to this more invasive modality, making it relevant to consider the quality of life benefit that may accompany PDT [7].

We previously reported our experience of toxicities and early outcomes in this Phase 1 trial of patients with superficial head and neck lesions [8]. ALA-PDT was found to be tolerable among 29 evaluable patients who at that time had been followed for a minimum of 6 months. The primary toxicity was mucositis, occurring in 97% of patients, but in approximately half of the cases it did not exceed grade 1 in severity, and all cases resolved within 30 days after PDT. Transient elevations in liver enzymes, a known side effect of ALA [9], were noted in 59% of patients, with all but one being grade two or less.

Among patients treated with two-part (fractionated) illumination on this trial, we have also previously studied lesion physiology during and after PDT [10]. The present report is based, in part, on our previous observation that lesion oxygenation was low in two patients who had a poor clinical response to PDT. The significance of this observation lies in the known importance of tissue oxygen to the effectiveness of PDT with many photosensitizers. PDT cytotoxicity is reduced by a factor of two at an oxygen tension of 7.6 mmHg, corresponding to a blood oxyhemoglobin saturation of ~5% based on the oxyhemoglobin dissociation curve. Full photodynamic effect is achieved at normal tissue oxygenation of ~40 mmHg, corresponding to a blood oxyhemoglobin saturation of ~75% [11, 12]. Thus, the presence of tissue hypoxia can impede PDT photochemistry and reduce treatment effect [13, 14].

Although preclinical studies have documented correlations between tumor hypoxia and tumor control after PDT [15, 16], few investigations have evaluated this association in clinical trials. Our results uniquely provide clinical evidence of better PDT response in patients with more highly oxygenated lesions. These data suggest the potential for personalizing administration of PDT based on clinical measurement of lesion physiologic properties.

Materials and Methods

Clinical Trial

Patients were enrolled if they had high-grade dysplasia, carcinoma-in-situ, or early microinvasive (< 1.5 mm depth of invasion) squamous cell carcinoma of the head and neck. Patients with intact disease or who underwent resection and had residual disease present at the resection margin were eligible, as long as the PDT procedure was within 4 months of pathologic diagnosis. All subjects were treated in accordance with protocols approved by the Institutional Review Board at the Hospital of the University of Pennsylvania.

As previously described [8], 29 evaluable patients were enrolled and treated on this trial between November 2009 and October 2014. One patient was not evaluable due to grade 5 toxicity secondary to pneumonia, sepsis and respiratory failure under circumstances of immunosuppression not allowing for a post-treatment follow-up interval to evaluate for response assessment. On autopsy, cause of death in relation to ALA and PDT could not be conclusively determined.

ALA was administered as an oral dose of 60 mg/kg. Vital signs were assessed immediately before and after ALA administration, every 15 min for the first two hours, and then hourly until the procedure. Illumination was delivered ~4-6 hours after ALA administration, with activating light (629-635nm) generated using a Ceralas Series GaAlAs diode laser (Biolitec Inc, Jena, Germany). Light delivery used either a microlens (MedLight SA, Ecublens, Switzerland) or a balloon-diffusing fiber (MedLight SA, Ecublens, Switzerland). Most patients presented with lesions accessible to microlens delivery, which provided a collimated laser beam covering a superficial circular area. For 9 patients, a microlens could not adequately cover a concave lesion. In these cases, a cylindrical diffusing fiber (active length of 2, 3, 4, or 5 cm) was placed within a balloon catheter. The balloon was inflated by saline to keep it in contact with the treated area. A fluence rate of 100 mW/cm² was delivered to the lesion surface, measured by a calibrated isotropic detector that was placed on the target surface. Escalating doses of total fluence at levels of 50, 100, 150 and 200 J/cm² were delivered to cohorts of 3–6 patients each. At each light dose, separate cohorts of patients were treated with continuous (unfractionated) illumination or fractionated light (two-part illumination). Fractionated illumination incorporated a 90–180 sec break in illumination when 20% of the fluence had been delivered. After this short dark interval, illumination resumed to the full treatment fluence. With the exception of one patient with an easily assessable lesion on the lower lip mucosa, PDT was delivered to patients under general anesthesia.

Light precautions were initiated at the time of ALA administration and included the optical filtering of operating room lights. As needed, normal tissue was shielded using blue surgical towels or by painting with a solution of methylene blue during the intraoperative period, and drapes or clothes were used to protect the patient's skin. Patients were instructed to avoid sunlight for 3 days following ALA administration.

Patients were followed for response at week 1, weeks 2-3, day 30, day 90, every 3 months until 24 months after PDT administration, and then annually. A complete response (CR) was defined as complete ablation or absence of the index lesion in the area treated with light. In the absence of a CR, evaluation was performed for the presence of local recurrence. Patients were taken off protocol if they experienced a recurrence but followed off-protocol for further in- and out-of-field progression.

Fluorescence/reflectance spectroscopy system

Prior to delivery of the PDT light, the oxygenation, blood volume, and photosensitizer fluorescence of the target was measured using an integrated contact fluorescence and reflectance spectroscopy (CFRS) system. Immediately after completion of PDT light delivery, repeat measurements in the same areas were taken. Measurements were performed using a custom-built fiber-based optical probe that was placed in contact with the tissue surface. This probe consists of two source optical fibers and a series of detection fibers spaced between 0.14 and 0.88 cm away from the first source fiber, but for the present study the signal from only the closest four fibers (source-detector distances 0.34 cm) was evaluated because of the size and curvature of the lesions. This probe allowed collection of diffuse reflectance using a white light source (Avalight; Avantes, Broomfield, CO) coupled

to the first source fiber for assessment of tissue physiologic properties by diffuse reflectance spectroscopy (DRS), while fluorescence spectra to assess relative photosensitizer levels were measured using a 403 nm excitation laser (Power Technologies, Inc., Little Rock, AK) coupled to the second source fiber. Both measurements use the same set of detection fibers, which are imaged by an integrated imaging spectrometer/multichannel CCD system (Inspectum; Roper Scientific, Trenton, NJ). Data acquisition was sequential, such that repeated diffuse reflectance, background and fluorescence spectra were acquired at each of multiple sample locations.

Spectroscopy data analysis

Data were analyzed using an iterative fitting algorithm (fminsearch) in Matlab (The Mathworks, Natick, MA) that is based on modeling of the absorption and scattering spectrum, as has been previously described [17–19]. The absorption spectrum of tissue was assumed to be a linear combination of the absorption spectra of oxy- and deoxyhemoglobin, weighted by their concentrations. Total hemoglobin concentration ([tHb]) was calculated as the sum of oxyhemoglobin concentration ([HbO₂]) and deoxyhemoglobin concentration ([Hb]), and from these quantities tissue hemoglobin oxygen saturation (S_tO₂) was calculated according to $S_tO_2 = [HbO_2]/[tHb]$. Fluorescence spectra were analyzed as previously described [10] and are reported as PpIX fluorescence relative to autofluorescence.

Statistical analyses

Patients were divided into groups receiving low (50 and 100 J/cm²) and high (150 and 200 J/cm²) fluence. Local recurrence-free survival was estimated by the Kaplan-Meier method and comparisons were performed using a log rank test (Stata version 13.1; Stata Corp, College Station, TX). Time-to-local failure was censored at last follow-up or at marginal recurrence because salvage therapy of the adjoining marginal area (potentially overlapping the PDT treatment field) could confound analysis of local recurrence. Paired t-tests were used to test for PDT-induced changes in lesion physiologic properties. T-tests of unequal variance were performed for association of 3-month CR with translational measures of oxygenation and blood volume. Hypothesis tests were two-sided with a type I error rate of 0.05.

Results

Clinical Outcome

We had previously reported on the tumor characteristics of our patients, including primary site and extent of disease [8], and these characteristics are reiterated in Supplemental Table 1. Three patients presented with invasive carcinoma, and the remainder were diagnosed with high grade dysplasia or carcinoma-in-situ. This analysis now reports on a prospective cohort with a median follow-up of 52.3 months in patients with local control (LC, n=9) and 25.7 months among all patients by the method of Schemper and Smith [20] based on data that censored time-to-local failure at last follow-up or marginal recurrence. All but one local recurrence occurred within 24 months of completing PDT, with a median time-to-local recurrence of 3.0 months (range 1.3 – 69.9 months). All marginal recurrences occurred within 36 months of PDT, with a median time-to-marginal recurrence of 2.7 months (range

1.25 – 27.28 month). Table 1 summarizes LC rates at 24 months as a function of low (50 or 100 J/cm²) or high (150 or 200 J/cm²) fluence. Although the number of patients in each group were too small to lend statistical significance, there was a pattern in which higher fluence appeared to achieve higher rates of LC, with rates of 42.9% (n=7), 62.5% (n=6), 66.7% (n=8), and 71.4% (n=8) at fluences of 50, 100, 150, and 200 J/cm² respectively, at 24 months. The group that received the higher fluence (150 and 200 J/cm²) had a 24-month LC rate of 67.7%, while the cohorts treated with the lower fluence (50 and 100 J/cm²) had a 24-month LC rate of 50.4% (Supplemental Figure 1, Table 1). Time-to-local recurrence was not different between these groups (p=0.97). There was also no difference in recurrence rates between patients who were or were not treated with fractionated PDT, with a 24-month LC rate of 57.5% (n=15) for non-fractionated versus 58.7% (n=14) for fractionated PDT (p=0.91).

The patient population for this Phase 1 study consisted of a heterogeneous mix of head and neck sites. The majority of patients (18) were treated for malignant or premalignant tongue/floor of mouth (FOM) lesions, 4 patients each were treated for disease of the buccal mucosa or glottic larynx, and 1 patient each had disease of the alveolar ridge, lower lip, or nasal cavity. Among patients with lesions of the tongue/FOM, lesion histology included 12 with high-grade dysplasia, 4 with carcinoma-in-situ and 2 with invasive disease. For the 18 patients with lesions of the tongue/FOM, the LC rate at 24 months for the lower fluence was 38.1% (n=9), compared to 57.1% (n=9) for the higher fluence (p=0.90).

Treatment history also varied significantly, with some patients receiving PDT subsequent to surgery. Lesions were deemed non-intact if PDT was administered on an adjuvant basis due to involved surgical margins (in all cases, residual disease was microscopic, and no gross disease was visible). Conversely, disease was intact if no prior treatment for a head and neck lesion had been received or in cases that visible recurrent or second-primary disease was confirmed by biopsy and treated definitively with PDT. Across all patients, LC was similar between intact (50.1%; n=18) and non-intact (68.6%; n=11) disease (p=0.47). As a function of fluence, LC with intact disease was 66.7% at high (n=8) and 45.0% with low (n=10) fluence (p=0.96). Similarly, in the cohort treated with resection and adjuvant PDT, no large numerical differences in LC were detected by dose (high fluence, 71.4%, n=8; low fluence, 66.7%, n=3, p=0.94).

Physiologic properties and PDT-induced changes

In 26 of the 30 measureable patients enrolled on the trial, lesion S_tO₂, tHb, and photosensitizer levels were assessed (Table 2). S_tO₂ had a mean(SE) value of 40(4.0)% at baseline and 46(4)% after PDT. tHb was 72(6.0) μM pre-PDT and 86(9.0) μM post-PDT. Responses were aggregated among those who did and did not receive fractionation because no effect of fractionation on either physiologic parameters or treatment outcome was detected. Specifically, in the 11 patients who received fractionation, S_tO₂ was 41(7)% at baseline and 48(6)% after PDT, whereas in the 15 patients who received continuous illumination, baseline and post-PDT values were 40(4)% and 44(7)%, respectively.

S_tO₂ values were also aggregated across tumor type. Patients with S_tO₂ measurements included those with high grade dysplasia (n=18), carcinoma in-situ (n=6), and invasive

carcinoma (n=2). Pre-PDT S_tO_2 [mean(SE)] was similar between patients with high grade dysplasia [37(5)%] and those with carcinoma-in-situ [42(7)%]. Post-PDT S_tO_2 was also similar between these groups [41(5)% for high grade dysplasia and 51(8)% for carcinoma-in-situ]. Patients with invasive carcinoma had pre-PDT S_tO_2 values of 59% and 76%, but there were notably only two patients in this group. Therefore, S_tO_2 values were similar across invasiveness characteristics.

Figure 1 summarizes PDT-induced changes in physiologic parameters by fluence. Patients treated at the highest fluence of 200 J/cm² had the lowest mean oxygenation at baseline, but variability was found among patient groups: S_tO_2 was 25(5)% (n=7), 44(8)% (n=9), 38(6)% (n=4), and 54(6)% (n=6) in patients subsequently treated at fluences of 200, 150, 100, and 50 J/cm², respectively. At none of the fluences was a PDT effect on lesion physiologic properties discerned by a change (post-/pre-PDT) in S_tO_2 (relative- S_tO_2). At fluences of 200, 150, 100, and 50 J/cm², mean relative- S_tO_2 (SE) was 1.5(0.4), 4.5(3.1), 1.5(0.3), and 0.9(0.1), respectively (non-significant, $p>0.12$ by paired t-test). Here we note that the mean relative- S_tO_2 of 4.5 at a fluence of 150 J/cm² was driven by changes in a single patient who demonstrated extremely low S_tO_2 prior to PDT (<1%). Although lesion oxygenation in this patient remained low after PDT at a value of ~11%, this represented a large relative increase. This patient was unique among those studied—all other patients demonstrated relative- S_tO_2 values that more tightly clustered around a value of 1. Relative-tHb was also evaluated at fluences of 200, 150, 100, and 50 J/cm² with respective values of 1.5 (0.5), 1.1 (0.2), 1.5 (0.3), and 1.1 (0.2) (non-significant, $p>0.28$ by paired t-test). Thus, comparison of pre- and post-values reveals PDT effect on physiologic parameters to be small and indistinguishable within each of the fluence cohorts. Furthermore, among the fluence cohorts, the PDT-induced changes in S_tO_2 and tHb were very similar.

Given the variety of treatment sites among all patients on this trial, it was also of interest to consider physiologic values at a consistent primary site. All patients with tongue/FOM lesions could be assessed by DRS, allowing for physiologic measurements among these 19 patients. However, one of the 19 patients was not evaluable for outcome and was, therefore, not included in subsequent outcome-based analyses. Similar to the entire group of head and neck (pre)malignancies, lesions of the tongue/FOM had similar oxygenation values at baseline [44(5)%] and post-PDT [46(6)%] (Table 2). Among patients with lesions of the tongue/FOM, PDT did not affect physiologic parameters ($p>0.38$) as reported by relative- S_tO_2 [2.61(1.48)] and relative-tHb [1.13 (0.12)].

Lesion physiologic properties could also be altered by the biological effects introduced by recent surgery. Although not statistically significant, S_tO_2 trended toward higher values before PDT in disease that was intact [45(4)%; n=16] versus not intact [33(7)%; n=10] (Table 2). Given the similarity of tHb in intact and non-intact disease, it does not appear that any differences in vascular volume were present as function of whether or not the lesions were intact. In intact disease, relative- S_tO_2 was 1.17(0.16) and relative-tHb was 1.38(0.25). In non-intact disease, relative- S_tO_2 was 4.38(2.77) and relative-tHb was 1.12(0.11). Thus, physiologic parameters in these groups did not meaningfully change after PDT ($p>0.12$ by paired t-tests).

Lesion photosensitizer level was also considered for each of the above groups of tongue/FOM, intact, and non-intact disease (Table 2). As with S_tO_2 , photosensitizer levels were highly variable among lesions. Average values were similar between all measured head and neck (pre)malignancies and lesions of the tongue/FOM. Also, photosensitizer levels did not differ as a function of the status of disease as intact or non-intact.

Tumor oxygenation associates with 3-month complete response

Since no appreciable differences in PDT effect on S_tO_2 and tHB were discernible by light dose, the entire patient cohort that did or did not demonstrate CR at 3 months was compared with lesion physiological properties across all light doses (Figure 2A). Among all patients, S_tO_2 prior to illumination was 29(7)% (n=8) in patients with recurrent or persistent lesions at 3 month-biopsy versus 46(5)% (n=17) in patients with CR at 3 months (p=0.07). Thus, those patients with better-oxygenated lesions trended toward a better response to PDT. Conversely, tHB was similar between responders and nonresponders (Supplemental Figure 2).

We next considered the subgroup of patients with the consistent primary disease site of tongue/FOM. In these patients, the relationship between higher lesion oxygenation and 3-month CR achieved statistical significance (Figure 2B), with a pre-PDT oxygenation of 23(8)% (n=6) in nonresponders compared to 54(4)% (n=12) in responders (p = 0.01). Importantly, pre-PDT lesion PpIX levels were similar between tongue/FOM lesions that demonstrated CR [26(5) a.u.] vs. those that persisted/recurred [32(8) a.u.].

Because of the previous observation that intact disease trended toward higher S_tO_2 pre-PDT, we further considered whether local recurrence in resected or intact disease was associated with local oxygenation. In lesions that had been recently resected, baseline oxygenation in the local tumor bed did not associate with 3-month CR (Figure 3A): S_tO_2 values were 31(16)% (n=3) vs. 33(9)% (n=7) respective to local persistence/recurrence vs. complete response, albeit patient numbers were small in these groups. Among patients with intact disease definitively treated with PDT (Figure 3B), 3-month CR was associated with higher S_tO_2 at baseline compared to lesions that persisted/recurred [54(3)% (n=10) vs. 28(8)% (n=5), p=0.03]. PpIX levels at baseline were similar between both groups of patients with intact disease [29(9) a.u. in complete responders vs. 31(19) a.u. in nonresponders], which suggests that outcome was not a function of differences in PpIX uptake.

In contrast to findings that relate poor lesion oxygenation to local recurrences, we noted no association between lesion oxygenation and marginal recurrence. In lesions of the tongue/FOM, baseline S_tO_2 was 31(8)% in those that recurred locally over the entire time course of observation (n=8) versus 51(8)% and 57(6)% in patients with no evidence of disease (NED) (n=5) and marginal recurrence (n=5), respectively. Thus, in the presence of marginal recurrence, a local CR was associated with high lesion oxygenation. This result further validates our findings relating lesion oxygenation to local (but not marginal) recurrence.

More highly oxygenated lesions have better 24-month local control

Given the strong association between lesion oxygenation and clinical outcome at 3 month-biopsy, we next assessed whether lesion oxygenation could stratify patients based on their

24-month response. Figure 4A depicts 24-month LC in patients with tongue/FOM lesions as a function of whether lesion oxygenation at baseline was above (high oxygenation) or below (low oxygenation) the median value of 42%. Figure 4B plots LC in patients with intact disease as a function of high or low baseline oxygenation. In patients with tongue/FOM lesions, oxygenation status was modestly able to predict long-term outcomes [LC rates of 58.3% (n=10) versus 29.2% (n=8) in high versus low oxygenation groups, respectively, $p=0.05$]. In patients with intact disease, oxygenation status was significantly ($p=0.02$) associated with LC. In these patients, a 24-month LC rate of 51.4% was found in highly oxygenated lesions (n=9), while 24-month LC was not achieved in the low oxygenation group (n=6).

Discussion

In this study, we report our experience in a phase 1 series of patients treated with ALA-PDT for high-risk premalignant and microinvasive carcinomas of the head and neck. In an attempt to understand the relationship between lesion physiology and PDT effectiveness, the trial incorporated noninvasive optical technology for assessment of tissue photosensitizer and physiologic properties. Levels of ALA-induced PpIX production varied greatly among patients, but without any association with outcome. Furthermore, while we did not find an association between tHb and 3-month CR, significantly higher levels of oxygenation were associated with higher rates of response to PDT in patients with lesions of the tongue/FOM, as well as those with intact disease at the time of PDT. This effect was independent of marginal, out-of field recurrence, suggesting that marginal recurrences were associated with insufficient light fields and not with the intrinsic physiologic properties of the treated lesion and site. We note that Biel et al. [1] similarly reported a high rate of recurrences along the margins of the PDT light field.

Our local control rate of 57.5% among the patients of this trial is based on Kaplan Meier estimates that censored all patients with marginal recurrences and subsequent retreatment. We felt this to be the most conservative approach due the introduction of salvage therapy at the margin of the target lesion, yet it also may underestimate the true effectiveness of PDT in controlling local disease. This experience leads us to suggest that future trials should incorporate larger treatment margins. It speaks to the nature of field cancerization for this disease and suggests that both larger margins and repeated treatment could improve outcomes.

Overall, our local control rates are similar to that reported by others using systemic or topical administration of ALA for PDT of oral disease. Fan et al. [21] studied systemically-administered ALA for treatment of moderate or severe dysplasia, reporting that among 12 patients treated, 7 had no clinical and/or histological evidence of disease at short interval follow-ups that ranged from 5 - 12 weeks. Topical administration of ALA for treatment of oral lesions has also met with varying degrees of success. Sieron et al. [22] reported that 10 of 12 patients had a complete response after PDT (100 J/cm²) for leukoplakia. Among these ten patients, one developed a recurrence within 6 months of follow-up. For ALA-PDT of leukoplakia, Kawczyk-Krupa et al. [23] reported a complete response rate of 72.9% over 6 months of follow-up. In both of these studies, PDT was repeated over multiple sessions in an

attempt to resolve all leukoplakia. Jerjes et al. [24] reported the results of treating carcinoma-in-situ or mild, moderate or severe dysplasia with either topically-applied ALA or systemically-administered mTHPC. ALA was generally used for patients with thin, mild-moderate dysplasia, while mTHPC was used for thicker lesions of dysplasia (all differentiation states) and carcinoma-in-situ. Results were combined for the photosensitizers, reported as a 5-year complete response rate of 79%.

Other studies have shown an association between tumor oxygenation and PDT outcome. In a recent clinical study of superficial oral squamous cell carcinoma, Rohrbach et al. [25] reported that PDT-induced changes in S_tO_2 improved the sensitivity of outcome prediction by the treatment-induced change in photosensitizer concentration. However, most evidence for a role of tumor oxygenation in predicting for in-field treatment response comes from animal models. Several preclinical studies have identified correlations between PDT response and tumor hypoxia, generally detected as the PDT-induced change in oxygenation ([15, 26]. While tumor hypoxia can be pre-existing and, therefore, intrinsic to the lesion, PDT itself can cause hypoxia by damaging the tumor vasculature and/or consuming oxygen in the photochemical process [27, 28]. Conversely, short-term reoxygenation of tumors can result from PDT-mediated acute cytotoxicity when cell death decreases the metabolic need for oxygen [29]. Collectively, animal studies find PDT-induced hypoxia to be associated with less treatment-induced cytotoxicity, measured as shorter tumor regrowth times or less necrosis [15, 26]. Under the conditions studied in this trial, we did not find PDT to produce consistent changes in S_tO_2 over the course of illumination, as assessed by post-PDT measurements made at several minutes after the completion of illumination. This may indicate that PDT did not lead to appreciable ischemia-induced hypoxia under the treatment conditions that were employed. However, it does not rule out the possibility that PDT produced hypoxia through photochemical oxygen consumption because the hypoxia produced by photochemical oxygen consumption can rapidly reverse upon termination of illumination and would not necessarily be detected even minutes after treatment [30]. Nevertheless, the absence of a consistent PDT effect on S_tO_2 likely accounts for the absence of an association between PDT-induced change in oxygenation (relative- S_tO_2) and outcomes. In contrast, the present study provides novel evidence in a clinical setting that poorer outcomes following PDT are associated with lower pretreatment lesion S_tO_2 .

We found the oxygenation of tongue/FOM and intact lesions to be associated with response across different illumination conditions. Fluence effects on LC and physiologic parameters are included among our presented data, but it is important to note that only 4 to 9 patients were assessed for S_tO_2 per fluence cohort. Such small numbers limited the further breakdown by fluence of patients into groups of consistent histology (i.e., those with tongue/FOM or intact disease) or by PDT delivery (unfractionated or fractionated illumination). Overall, results suggest that biological heterogeneities introduced by histology dominated any effect of PDT fluence or delivery. This is not surprising because hypoxia-associated limitations in PDT outcome would be expected to develop acutely at the initiation of illumination and, therefore, have potential to similarly impact all fluences and deliveries. It also speaks to the possibility that poorly oxygenated lesions may have a suboptimal response to other therapies as well. Indeed, in clinical trials of head and neck squamous cell carcinoma, the presence of pre-treatment hypoxia was associated with poorer tumor

response after treatments that incorporated radiotherapy [31, 32]. Based on the present study, we suggest that knowledge of lesion oxygenation prior to treatment can be used to better design PDT as definitive therapy, for example by employing oxygen-conserving fluence rates [33, 34] or by incorporating multiple treatment sessions [35]. Hyperoxygenation during anesthesia could also be considered, as has been studied in preclinical models [36]. This would enable personalization of therapy, at least in those treated with ALA-PDT, with a goal of improving clinical outcomes for patients.

We found baseline levels of lesion S_tO_2 and tHb to vary substantially between patients. Interpatient heterogeneities in S_tO_2 of oral lesions have also been measured by others. Lee et al. [37] report ranges of ~300 mM in tHB and up to 40% in S_tO_2 among patients with squamous cell carcinoma, verrucous hyperplasia, or hyperkeratosis/parakeratosis. These ranges are similar to those in the present study. However, higher oxygenation was reported by Lee et al. compared to that found in our trial. We detect baseline S_tO_2 to be ~40%. Some have reported average values in the range of 81-93% [37-39] or ~65-75% [40]. These differences can potentially be explained by the depth of tissue that was probed. Our instrument probed to tissue depths of ~1.7 mm, but less than half of this distance was probed by the above-mentioned studies [37]. We have previously examined the effect of probing depth, finding that superficial measurements detected higher values of S_tO_2 in oral lesions [10]. The ~1.7mm measurement depth correlates with the target in our study based on entry criteria.

Findings that poor outcomes associated with low baseline S_tO_2 in patients with intact disease or tongue/FOM lesions suggest that pre-existing hypoxia was of clinical relevance. Indeed, all patients who failed to achieve a local CR had S_tO_2 values of less than 75%, the blood oxygen saturation at which a full photodynamic effect would be achieved [11]. Two patients even had values that were similar or less than the half value for PDT cytotoxicity (~5%). We expect that lesion S_tO_2 prior to PDT could be of value in treatment planning itself, allowing for individualization of light delivery based on models of predicted efficacy. This is of particular importance since PDT is an alternative to resection, which in some cases can be disfiguring and/or functionally morbid. Additionally, patients deemed to be at higher risk of recurrence based on oxygenation parameters may merit closer follow-up and earlier interventions such as retreatment with PDT or resection.

In conclusion, in this Phase 1 study, we find translational measures of lesion oxygenation can associate with CR to PDT, especially in patients with tongue/FOM lesions, as well as those treated definitively for intact disease. This association of lesion oxygenation with durable LC is one of the first clinical studies to confirm animal investigations associating tumor hypoxia with higher rates of recurrence after PDT. Future studies may be considered in which patients are stratified based on lesion oxygenation to receive individualized PDT regimens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- In preclinical models, tumor oxygenation predicts for long-term outcomes to photodynamic therapy (PDT).
- Lesion oxygenation was measured in patients on a Phase I trial of PDT for superficial head and neck lesions.
- Higher pre-PDT lesion oxygenation associated with positive outcomes in lesions of the tongue and floor of mouth and lesions that were intact at the time of PDT.
- Physiologic monitoring may enable personalization of PDT by informing choice of light delivery parameters.

Figure 1.

PDT-induced changes in lesion S_tO₂ and tHb did not differ by fluence. Plots depict S_tO₂ (A) and tHb (B) at measured timepoints before PDT (pre) and after PDT (post) in each patient, categorized as a function of treatment fluence. Open symbols indicate continuous illumination and closed symbols indicate twopart (fractionated) illumination.

Figure 2.

Complete response at 3 months associates with better pre-PDT oxygenation in lesions of the tongue/floor of mouth (FOM). Treatment response is characterized by the absence (no) or presence (yes) of a complete response. Plots depict baseline S_tO_2 in all patient lesions (A) and in tongue/FOM lesions (B). p values are for the comparisons between patients with and without a complete response.

Figure 3.

Patients without intact disease at the time of PDT (A) did not demonstrate clear differentials in lesion oxygenation as a function of whether or not they achieved a complete response at 3 months. Conversely, in patients with intact disease (B) a complete response at 3 months was associated with better baseline lesion oxygenation. Intact status was based on whether lesions existed at surgical margins and required PDT on an adjuvant basis (non-intact) or were second-primary or recurrent disease from prior treatment (if given) that received PDT for definitive treatment (intact). p values are for the comparisons of patients with versus without a complete response.

Figure 4.

High baseline lesion oxygenation associates with 24-month complete response in patients with tongue/FOM lesions (A) and those with intact lesions (B). Patients are separated into high (n=12) versus low (n=13) oxygenation based on the median baseline S_tO_2 value of 42%. Kaplan Meier plots depict estimates of local control with a minimum 24-month follow-up. $p=0.05$ for lesions of the tongue/FOM and $p=0.02$ for intact lesions.

Local control (LC) for all patients and by PDT fluence for Low Fluence PDT (50 and 100 J/cm²) and High Fluence PDT (150 and 200 J/cm²) at 24 months.

Table 1

Category	N	All patients %LC (CI) ¹	Low Fluence %LC (CI)	High Fluence %LC (CI)	p-value ²
All	29	57.5% (35.1, 74.6)	50.4% (21.1, 73.9)	67.7% (34.9, 86.5)	0.97
Tongue/FOM ³	18	42.7% (16.8, 66.6)	38.1% (8.9, 68.0)	57.1% (17.2, 83.7)	0.90
Intact	18	50.1% (22.1, 72.8)	45.0% (13.9, 72.4)	66.7% (19.5, 90.4)	0.96
Not Intact	11	68.6% (30.5, 88.7)	66.7% (5.4, 94.5)	71.4% (25.8, 92.0)	0.94

¹Values based on Kaplan Meier estimates of local control (LC) at 24 months after PDT with 95% confidence intervals (CI)

²p-value for comparison of high and low fluence PDT

³Floor of mouth (FOM)

Summary of physiologic measurements pre- and post-PDT in patients with head and neck lesions

Table 2

	N	S _t O ₂ pre mean(SE)	S _t O ₂ post mean(SE)	tHb pre mean(SE)	tHb post mean(SE)	PpIX Fluorescence (a.u.) mean(SE)
All ¹	26 ²	40(4)%	46(4)%	72(6) μ M	86(9) μ M	32(7) ³
Tongue/FOM ⁴	19 ²	44(5)%	46(6)%	71(7) μ M	78(10) μ M	27(6) ³
Intact	16 ²	45(4)%	49(6)%	69(8) μ M	88(14) μ M	29(7)
Not Intact	10	33(7)%	41(6)%	78(10) μ M	81(8) μ M	37(12) ³

¹ All patients with lesions accessible to optical spectroscopy were measured for tissue hemoglobin oxygen saturation (S_tO₂) and total hemoglobin concentration (tHb) within ~5 min before and after the delivery of illumination for ALA-PDT.

² Includes one patient who was not evaluable for outcome.

³ PpIX fluorescence data were not available in one patient with non-intact disease of the tongue/FOM.

⁴ Floor of mouth (FOM)