

# Local Physiological Changes During Photodynamic Therapy

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**Background and Objective:** Herein an overview is provided of the causes, consequences, and significance of photodynamic therapy (PDT)-mediated effects on tumor oxygenation and blood flow during illumination.

**Study Design/Materials and Methods:** Techniques particularly valuable to this research have included tissue oxygen tension measurement by the Eppendorf pO<sub>2</sub> Histogram; spatial quantification of hypoxia by EF3 and EF5; and tissue oxygenation/blood flow monitoring by diffuse reflectance/correlation spectroscopy.

**Results:** Severe hypoxia was measured in vivo during PDT and is shown to be a consequence of photochemical oxygen consumption and/or compromised vascular perfusion. Oxygen depletion can be controlled by treatment regimen, occurs in a spatially-definable pattern, and is therapy-limiting. PDT-induced changes in tumor oxygenation during illumination are correlated with outcome. In PDT-treated tissues, blood flow also is determined by treatment regimen and correlates with treatment response.

**Conclusions:** Photodynamic therapy creates distinct, measurable changes in tumor oxygen and blood flow during illumination. These physiological changes may ultimately affect treatment efficacy. *Lasers Surg. Med.* 38:494–499, 2006. © 2006 Wiley-Liss, Inc.

**Key words:** blood flow; EF3; EF5; hypoxia; oxygen; pO<sub>2</sub>; SO<sub>2</sub>; tumor

## INTRODUCTION

Photodynamic therapy (PDT) can initiate acute, severe changes in local tumor physiology within literally a minute of beginning illumination. Such changes include effects on tumor oxygenation and blood flow, which may subsequently determine treatment outcome. For example, tumor hypoxia secondary to PDT-created vascular damage has long been known as a mechanism of PDT effect [1]. Accumulation of perfusion-limiting, vascular damage accompanied by the development of hypoxia *after* PDT favors treatment response, but the consequences of vascular damage and hypoxia *during* PDT are less straightforward. Damage to the tumor vascular network during PDT can compromise the supply of oxygen to the tumor. Oxygen is necessary for the creation of tumor-damaging reactive oxygen species by PDT. Furthermore, in typical PDT photoreactions, the production of reactive oxygen species is associated with the consumption of ground state oxygen.

This process, termed photochemical oxygen consumption, is another mechanism by which PDT can create hypoxia during treatment [2]. Acting independently or in cooperation, vascular damage and photochemical oxygen consumption can result in the development of outcome-limiting hypoxia during PDT.

## PDT-CREATED TUMOR HYPOXIA DURING ILLUMINATION

In early reports, the biological consequences of photochemical oxygen consumption were presented as mathematical models, which plotted the radial distribution of oxygen concentration relative to distance from a capillary. These models predicted that PDT could create severe distance-dependent depletion of oxygenation [3,4]. Model inputs, that is, variables affecting oxygen concentration during PDT, included the treatment regimen (e.g., fluence rate, photosensitizer concentration), photosensitizer photophysical properties (e.g., extinction coefficient, quantum yield of singlet oxygen), and microenvironmental influences (e.g., inter-capillary distance, pre-existing oxygen concentration). The process of photochemical oxygen consumption was experimentally demonstrated in spheroids, as was the potential to control the rate of oxygen depletion through choice of PDT fluence rate [3,5].

We undertook investigations to directly measure in vivo fluence rate effects on oxygen depletion, using murine tumors as a model system [6]. In vivo measurement of tumor oxygen partial pressure was performed using the Eppendorf pO<sub>2</sub> Histogram. The data showed that Photofrin®-PDT (5 mg/kg) created rapid and severe decreases in the oxygenation of radiation induced fibrosarcoma (RIF) murine tumors within minutes of initiating illumination [6]. Low tumor pO<sub>2</sub> was maintained throughout treatment at high fluence rate (150 mW/cm<sup>2</sup>), whereas low fluence rate (30 mW/cm<sup>2</sup>) allowed for recovery of pO<sub>2</sub>, sometimes to even slightly above baseline levels (Fig. 1). In other tumor

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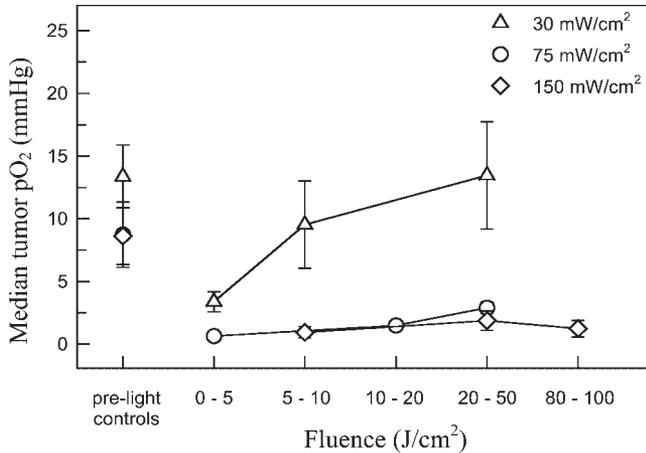


Fig. 1. Median  $pO_2$  of RIF tumors during treatment with Photofrin<sup>®</sup> (5 mg/kg) PDT at three fluence rates. Pre-light controls contained Photofrin<sup>®</sup>, but received no light. During PDT at each fluence rate tumor  $pO_2$  was measured within the indicated fluence ranges; these ranges are approximate and were dictated by the fluence rate and the time needed for instrument set-up (Eppendorf  $pO_2$  Histogram) and data collection. Two groups of mice were used for each fluence rate, each group was studied pre-light then at two fluences; each point is the mean  $\pm$  SE from at least eight mice.

models, low fluence rate has similarly been shown to preserve tumor oxygenation during PDT [7].

The investigation of intratumoral oxygen gradients during PDT became possible with the use of an in situ hypoxia-labeling drug, EF3 ([2-(2-nitroimidazol-1[H]-yl)-N-(3,3,3-trifluoropropyl)acetamide]. Validating investigations demonstrated that EF3 bound to hypoxic tumor cells during PDT (Fig. 2A), and that tumor-averaged oxygenation, approximated from EF3 binding levels, was comparable to published values of tissue  $pO_2$  measured by the Eppendorf  $pO_2$  Histogram in the same tumor line under the same treatment conditions [8]. Quantitative spatial analysis of intratumoral hypoxia distributions [9] showed, for the first time in vivo, PDT-created gradients in tumor oxygenation relative to blood vessels (Fig. 2B) [10]. Compared to controls, Photofrin-PDT (5 mg/kg) at 75 mW/cm<sup>2</sup> produced a significant increase in hypoxia along the radial distance between perfused blood vessels and tumor cells. This finding was suggestive of PDT-created oxygen consumption, but PDT-associated reductions in tumor perfusion were also evidenced by a significant decrease in the percentage of perfused blood vessels and a significant increase in the median distance of a cell to the nearest perfused blood vessel. In contrast, PDT at a lower fluence rate of 38 mW/cm<sup>2</sup> did not cause substantial changes in tumor perfusion during PDT. Consequently, at 38 mW/cm<sup>2</sup>, PDT-created gradients in hypoxia along the radial distance between perfused blood vessels and tumor cells could primarily be attributed to photochemical oxygen consumption.

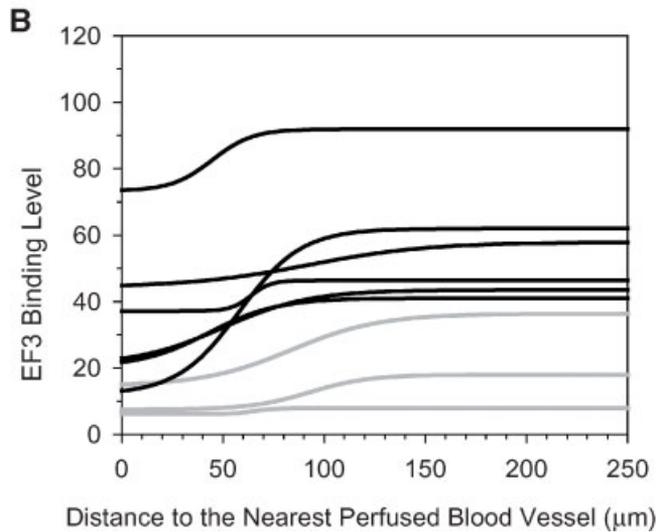
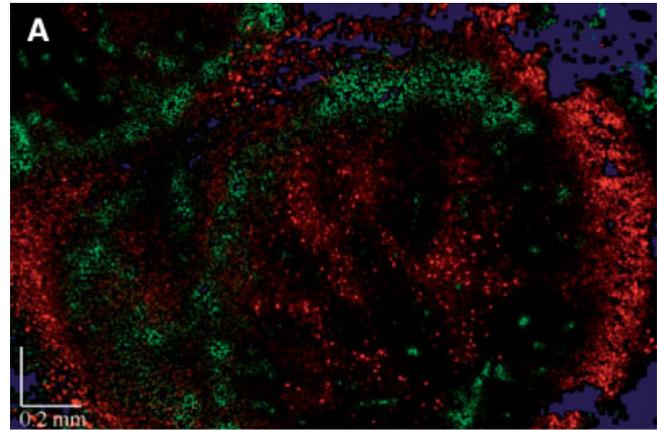


Fig. 2. Representative photomicrograph of EF3 binding (red) and vascular perfusion (green) in a section of PDT-treated RIF tumor (A). Tissue-containing areas were labeled by flooding the section with a solution of Hoechst 33342 following other photography; blue areas indicate the inverse of the Hoechst fluorescence; that is, nontissue areas. The spatial distribution of EF3 binding relative to cell distance to the nearest perfused blood vessel (B). Plots represent PDT-treated (black) and control (gray) tumors; curves are a four parameter sigmoid fit to the data points (not shown for clarity). EF3 incubation took place during PDT and perfusion was labeled by animal injection with saline-dissolved Hoechst 33342 at 1.5 minutes before tumor excision. PDT conditions were Photofrin at 5 mg/kg and illumination at 75 mW/cm<sup>2</sup>, 135 J/cm<sup>2</sup>. Panel B was previously published in Busch et al., *Cancer Res* 2002; 62(24):7273–7279.

Another finding of micro-regional study of tumor hypoxia during PDT is that the base of intradermal RIF tumors exposed to PDT at 75 mW/cm<sup>2</sup> demonstrates substantially more hypoxia than the surface of the same tumors. This result seems counterintuitive since fluence rate is attenuated in tissues distant from the light source, and low fluence rates are less oxygen consumptive. Accordingly, the

primary cause of this hypoxia appears not to be photochemical oxygen consumption, but rather PDT effect on blood flow in the tumor base. Others have also noted intratumoral heterogeneity in oxygen responses during PDT [11]. An association between pre-PDT oxygen level and the degree of PDT-induced oxygen depletion has been reported [11], perhaps implicating a role for pre-existing tumor microenvironment in determining the distribution of PDT-created hypoxia.

Finally, spatial investigations of hypoxia during PDT have demonstrated that oxygen depletion during illumination can extend to tumor cells immediately adjacent to the vasculature [10]. This was a finding not previously captured by mathematical models of photochemical oxygen consumption. Both a high ( $75 \text{ mW/cm}^2$ ) and a low ( $38 \text{ mW/cm}^2$ ) fluence rate were capable of creating hypoxia at the tumor boundary with vascular endothelium, and the PDT-induced increase in hypoxia was greater at high compared to low fluence rate. The presence of vascular-adjacent hypoxia during PDT suggests that blood oxygen concentration or hemoglobin oxygen saturation may be adversely affected by PDT, which in turn supports the use of local oxyhemoglobin ( $\text{HbO}_2$ ) monitoring as a means of following treatment progression [12].

#### **THERAPEUTIC CONSEQUENCES OF PDT-CREATED HYPOXIA**

Control of oxygen depletion or enhancement of oxygen levels during PDT can improve treatment outcome. Compared to oxygen-favoring low fluence rate treatment regimens, the induction of hypoxia during high fluence rate PDT is associated with poorer tumor response, characterized by fewer tumor cures and a shorter time to tumor regrowth [6,7,13]. Both decreases in direct tumor cytotoxicity [14] and a failure to sustain reductions in the perfusion of tumor-adjacent vasculature have been identified as causes of poor treatment efficacy at high fluence rate [6]; however, the vascular component of this response overwhelmingly dominated long-term outcome. This was demonstrated through administration of a nitric oxide inhibitor immediately after PDT, which led to inhibition of post-illumination reperfusion in the treatment field and corresponding improvements in response [15]. The enhanced efficacy of low fluence rate PDT extends to normal as well as tumor tissues, making it necessary to reduce the treatment dose at low compared to high fluence rate in order to avoid increases in normal tissue toxicity [16].

In addition to fluence rate adjustment, other means of modulating tumor oxygen concentration during PDT have also had a favorable impact on treatment outcome. Hyperfractionation of the illuminating light into brief on/off periods facilitates the recovery of tumor oxygen concentration in between illumination intervals; it is associated with significant improvement in the long-term response of multiple tumor models to PDT with various photosensitizers [17,18]. Augmentation of tissue oxygen concentration through normoxic or hyperbaric oxygen breathing during PDT has been shown to improve response in preclinical

[19,20] and clinical [21] disease. Also, for certain photosensitizers, the choice of drug-light interval can affect tissue oxygen status during illumination. For example, in verteporfin-mediated PDT a long drug-light interval facilitated increases in tumor oxygenation during PDT, whereas a short drug-light interval led to decreases in oxygenation [22]. The treatment regimen creating decreases in oxygenation was associated with decreases in perfusion during illumination [23]. Conversely, oxygen-enhancing treatment was hypothesized to be a result of photochemical-related increases in blood flow or a decrease in cellular metabolism and oxygen consumption due to PDT-created cell death [22].

Despite the fact that oxygen-conserving treatment regimens produce more favorable outcome as measured by the average response in *groups* of PDT-treated animals, the importance of oxygenation on PDT response at the level of *individual* animals has been little evaluated. Recently, optical technology has been applied toward the goal of noninvasively monitoring tumor oxygenation in PDT-treated animals [12,24–26]. In studies by Wang et al., diffuse reflectance spectroscopy (DRS) was used to measure tumor hemoglobin oxygen saturation ( $\text{SO}_2$  or  $\text{S}_t\text{O}_2$ ) immediately before and after Photofrin-PDT ( $5 \text{ mg/kg}$ ,  $38 \text{ mW/cm}^2$ ,  $135 \text{ J/cm}^2$ ). The PDT-induced change in  $\text{SO}_2$  was calculated for each animal, that is,  $\text{SO}_2$  after PDT was normalized to  $\text{SO}_2$  before PDT in the same animal. The normalized change in tumor  $\text{SO}_2$  (relative- $\text{SO}_2$ ) ranged from 0.58 to 4.99 among animals, in other words some animals demonstrated a decrease in oxygenation during PDT, whereas others demonstrated an increase. The value of relative- $\text{SO}_2$  in an individual animal was predictive of treatment durability for that animal. This was demonstrated by a highly significant positive correlation between relative- $\text{SO}_2$  and time of tumor growth to a volume of  $400 \text{ mm}^3$ . Furthermore, a highly significant association was found between increasing relative- $\text{SO}_2$  and increasing probability of survival, defined as an absence of tumor recurrence in 90 days.

Others have similarly reported that knowledge of PDT-associated oxygen consumption or reactive oxygen species deposition is a valuable indicator of therapy effectiveness at the level of the individual animal. In subcutaneous rat tumors the PDT-induced difference in tumor  $\text{SO}_2$  was correlated with histological damage (area of necrosis) at 3 days after PDT [24]. Also, luminescence-based monitoring of singlet oxygen production in mouse skin during PDT demonstrates a strong correlation between cumulative singlet oxygen count and response to treatment [27]. All told, these findings strongly point toward prognostic or dosimetric value in measurement of oxygen species during PDT.

#### **TUMOR HEMODYNAMICS DURING PDT**

The benefits of damage to the vasculature of tumor and adjacent tissue have long been known in PDT. In some applications, PDT is purposefully performed at a time when blood levels of the photosensitizer are high, leading to

localized shutdown of tissue blood flow. However, in the absence of sustained reductions in blood flow, short-term compromise of tumor perfusion and oxygen delivery during PDT could prove therapy-limiting. Decreasing blood flow during illumination [11] is accepted to be a result of acute damage to the vascular endothelium, which causes vessel constriction or obstructive thrombi formation [28]. However, blood flow can also increase during PDT [23], perhaps as a consequence of a heating effect or physiological response to oxygen depletion. The averaged direction of PDT effect on blood flow notwithstanding, it is misconceiving to perceive of this change as unidirectional, that is, steadily increasing or decreasing as treatment progresses.

Continuous monitoring of hemodynamic properties during PDT by ourselves [29] and others [30] demonstrates pronounced fluctuations in blood flow or vessel luminal diameter during illumination. The overall pattern of change in blood flow during PDT can vary with photosensitizer and treatment regimen. For example, Photofrin-PDT can cause both vessel constriction and dilation during illumination [28], whereas PDT with mono-L-aspartyl chlorin  $e_6$  (NPe6) causes steady reductions in vessel luminal diameter due to accumulating platelet aggregation [31,32]. Tumor hemodynamic response during PDT with verteporfin is highly dependent on the drug-light interval [30,33,34]. At drug-light intervals of 5–30 minutes, verteporfin-PDT causes a rapid and durable reduction in tumor blood flow. In contrast, during PDT with a 3 hour drug-light interval, blood flow experiences small decreases that may reverse after treatment completion.

Employing diffuse correlation spectroscopy, Yu et al. [29] have carried out noninvasive continuous monitoring of tumor hemodynamic response during Photofrin-PDT (5 mg/kg, 75 mW/cm<sup>2</sup>, 135 J/cm<sup>2</sup>) of RIF tumors. Blood flow increased within the first ~10 minutes of illumination, followed by a decrease and frequently, but not always, subsequent peaks and declines (Fig. 3A). A similar trend has also been found in normal tissue, including murine leg muscle (unpublished data). In RIF tumors the slope and duration of the decrease (interval time) in blood flow following the first PDT-induced peak was directly related to treatment efficacy. Animals with a rapid decrease in blood flow responded poorly to treatment, that is, they demonstrated short times to tumor growth (400 mm<sup>3</sup>) after PDT, whereas animals with a slow decrease in blood flow exhibited a more durable treatment response (Fig. 3B). Furthermore, PDT-induced changes in blood flow in the hours after PDT were also predictive of response, confirming the commonly accepted result that shutdown of tumor vasculature after PDT leads to more efficacious response. The correlation between blood flow parameters and PDT outcome was highly significant ( $r^2 = 0.79$  and  $0.80$ ,  $P = 1.0e-4$  and  $5.63e-05$ , during and after PDT, respectively), suggesting promise for hemodynamic monitoring as a rapid, real time indicator of treatment progression. However, unlike PDT effects on oxygenation, for which mechanisms are well studied, the mechanisms governing fluctuations in blood flow during PDT are not well under-

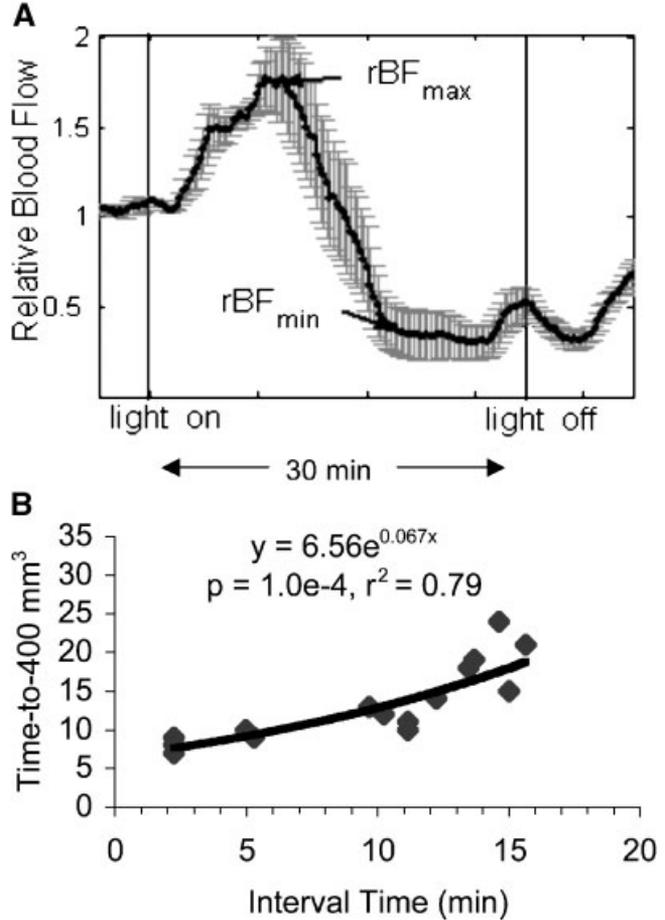


Fig. 3. Trace of relative blood flow (rBF; average  $\pm$  SD) in a representative RIF tumor during Photofrin (5 mg/kg) PDT (75 mW/cm<sup>2</sup>, 135 J/cm<sup>2</sup>); max and min points used to define the slope and interval time are indicated (A). The duration (interval time) of the decrease between rBF<sub>max</sub> and rBF<sub>min</sub> was significantly associated with treatment durability, that is, number of days after PDT for tumor growth to 400 mm<sup>3</sup> (B). Graphs were previously published in Yu et al., Clin Cancer Res 2005; 11(9):3543–3552.

stood and require further investigation to define their contribution to outcome.

### TUMOR PHYSIOLOGY IN CLINICAL PDT

Many solid tumors are characterized by regions of hypoxia. This includes findings of mild to severe tumor hypoxia in patients with intraperitoneal carcinomatosis or sarcomatosis who are scheduled to undergo PDT [35] (Fig. 4A). Yet, even the most hypoxic and smallest of tumors exhibited vascular structure [35], and metabolic-created gradients in hypoxia as a function of distance to a blood vessel were readily visible (Fig. 4B). Furthermore, Photofrin uptake was evident in small nodules [35,36], suggesting the presence of a functional vascular network amidst

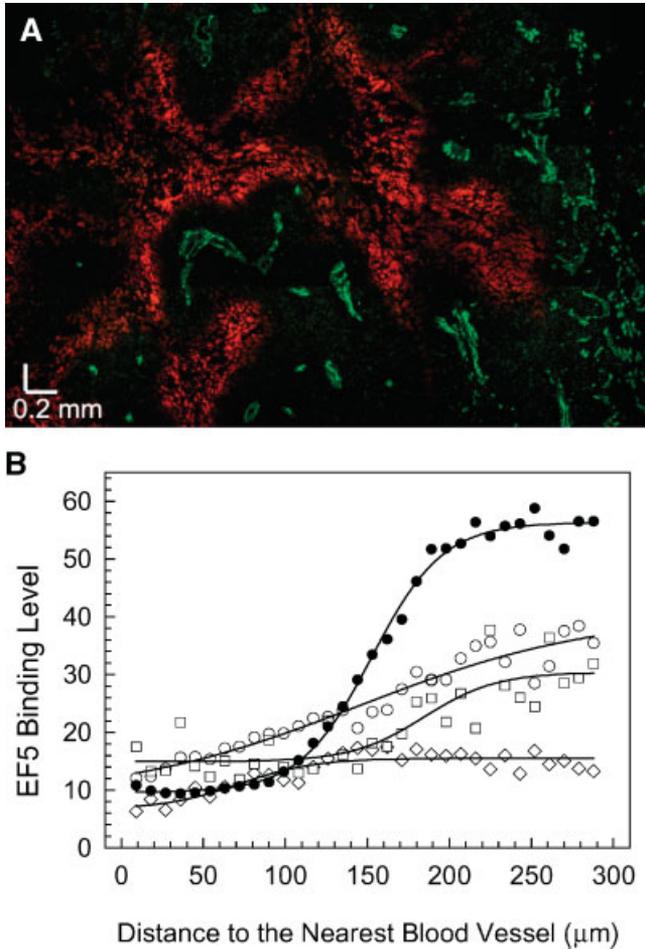


Fig. 4. Photomicrograph of EF5 binding (red) and CD31-labeled vascular structure (green) in colon carcinoma (A). The spatial distribution of EF5 binding relative to cell distance to the nearest blood vessel in specimens of colon carcinoma (B). Plots represent three separate specimens collected from the same patient, and one specimen evaluated at two different levels (○ and ●) separated by > 1 mm. EF5 labeling took place over 24–48 hours prior to specimen collection. The image of Panel A, albeit with a slightly smaller field of view, was previously published in Busch et al., *Clin Cancer Res* 2004; 10(14):4630–4638.

diseased tissue. Clinically-acceptable systems to measure tissue oxygenation and blood flow during PDT are available and have been employed in the collection of limited data [37–39]. Modest to substantial changes in tumor oxygenation and blood flow were detected during PDT of cancerous prostate [39], while fluence rate-dependent depletion of tumor oxygenation has been reported in basal carcinomas [40]. The importance of tumor oxygenation in a clinical setting is indicated by findings of more favorable outcome in PDT of actinic keratosis when low fluence rate was used [41] and better local response in PDT of esophageal cancer when treatment was combined with hyperbaric oxygen [21]. Although these data are encouraging, the need exists

for more definitive correlative studies between tumor physiological parameters and patient outcome to PDT.

## CONCLUSIONS

Photodynamic therapy can significantly affect local tumor physiology, including outcome-altering changes to tissue oxygenation and blood flow. Causes of oxygen depletion during PDT include photochemical oxygen consumption and reductions in tumor perfusion. PDT-created tumor hypoxia can be controlled through choice of fluence rate, demonstrated by macro- and micro-regional investigations of tumor oxygenation during PDT [7,10,16]. Yet, there remains much to be understood about the significance of heterogeneity in vascular and oxygenation responses to PDT, as well as mechanistic pathways governing blood flow fluctuations during PDT. Research continues in this field with the penultimate goal of outlining pre-clinically proven, clinically-relevant guidelines for individualized optimization of PDT based on local physiological changes during treatment.

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