Inhibition of photodynamic therapy induced-immunosuppression with aminolevulinic acid leads to enhanced outcomes of tumors and pre-cancerous lesions (Review)

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Abstract. Photodynamic therapy (PDT) is a treatment option for tumors and pre-cancerous lesions, but it has immunosuppressive side effects that limit its effectiveness. Recent studies suggest that PDT-mediated immunosuppression occurs through a cyclooxygenase type 2 (COX-2) mediated pathway that leads to increases in regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), which act as negative regulators of immune responses. Given this pathway, there are three main methods to block immunosuppression: i) Inhibiting the proliferation of Tregs, which can be achieved with the administration of cyclophosphamide or inhibitors of indoleamine 2,3-dioxygenase 1, an activator of Tregs; ii) inhibiting MDSCs by reducing hypoxia around the tumor to create an unfavorable environment or administering all-trans-retinoic acid, which converts MDSCs to a non-immunosuppressive state; and iii) inhibiting COX-2 through selective or non-selective COX-inhibitors. In the present review article, strategies that have shown increased efficacy of PDT in treating tumors and pre-cancerous lesions by blocking the immunosuppressive side effects are outlined and discussed.

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Abbreviations: PDT, photodynamic therapy; ROS, reactive oxygen species; ALA, 5-aminolevulinic acid; MAL, methyl aminolevulinate; PpIX, protoporphyrin IX; 5-FU, 5-fluorouracil; DNFB, dinitrofluorobenzene; MDSCs, myeloid-derived suppressor cells; Tregs, regulatory T cells; HSV, herpes simplex virus; TGF-β, transforming growth factor β; IL-10, interleukin-10; PAF, platelet-activating factor; COX-2, cyclooxygenase-2; PGE2, prostaglandin E2; IFNγ, interferon γ; IDO, indoleamine 2,3-dioxygenase; ATRA, all trans retinoic acid; ATP, adenosine triphosphate; PM-IR780-Met, platelet membranes as nano-carriers to co-encapsulate metformin and IR780; NSAID, nonsteroidal anti-inflammatory drugs; COX-1, cyclooxygenase-1

Key words: photodynamic therapy, immunosuppression, precancerous lesions, squamous cell carcinoma, actinic keratosis, pharmacologic therapy

Contents

1. Introduction

Photodynamic therapy (PDT) is a form of treatment for tumors and pre-cancerous lesions that works by inducing cell death. The treatment involves a photosensitizing agent which is topically applied or injected into the blood stream (1,2). It is absorbed by cells and after a pre-determined amount of time, the site is exposed to a light source such as a laser or light-emitting diode (1). When light is applied at a specific wavelength to the targeted area, the photosensitizing agent undergoes a reaction that forms reactive oxygen species (ROS) and kills the targeted cells (1,2) and/or induces vascular damage (3). To the host, PDT is usually minimally invasive and minimally toxic as the photosensitizers do not tend to accumulate in cell nuclei (2,4).

Photosensitizers are divided into families based on chemical structure including porphyrins, chlorins, and dyes (5). They are also grouped into first, second, and third generation photosensitizers (6). The first generation photosensitizer most commonly used is Photofrin, a mixture of porphyrin dimers and oligomers (6,7). First generation photosensitizers are used less frequently today due to side effects such as skin sensitivity and their weak absorption at 630 nm (8,9). Two examples of second generation photosensitizers commonly employed in dermatology practice are aminolevulinic acid (ALA) and the methyl ester form, methyl aminolevulinate (MAL) (10). Both

can be topically applied and have some selectivity for precancerous and cancerous cells, although MAL is more lipophilic and absorbed by deeper skin tissues than ALA (10). These agents are converted into the highly reactive protoporphyrin IX (PpIX) (11). Notably, rapidly proliferating cells, such as cancer cells, convert more ALA to PpIX in their mitochondria than their non-transformed counterparts in the epidermis. When neoplastic cells are exposed to light at various wavelengths (classically between 570 and 670 nm) (10), ROS are created which then damage and destroy target cells. In clinical practice, both red (most commonly 630 nm) and blue (410-420 nm) wavelength light sources are utilized. Moreover, 'daylight PDT' is also employed, using natural sunlight to activate these photosensitizing agents (10-12). Finally, third generation photosensitizers are antibody-directed and were developed to have a strong affinity for tumor cells, causing less damage to the surrounding tissues (5,6)

The clinical uses of PDT span from multiple neoplastic indications, skin disorders and ocular conditions. Considering the two-dose nature of the treatment, topical PDT is frequently favored over other field therapy options, even if reportedly less effective than other field therapy options such as 5-fluorouracil (5-FU) (13). It has been utilized for other dermatologic conditions, including psoriasis, basal cell carcinoma, verruca, and extramammary Paget's disease (14). Systemic PDT is also used to prevent severe vision loss in wet macular degeneration by targeting the vasculature that gives rise to the condition (15). Additionally, PDT has been used in patients with Barrett's esophagus, as well as cancers of the mouth and lungs (16).

However, there are multiple reports of side effects of PDT, including redness, pain and photosensitivity. Relevant to this review, PDT exerts immunomodulatory effects that could limit its effectiveness (17-23). This review paper highlights our current understanding of the immunosuppressive effects of PDT with ALA and will specifically focus on pharmacologic strategies to mitigate this unwanted effect, which has not specifically been reviewed in the current literature. The aim is to increase understanding of this process, which will be especially helpful in improving the effectiveness of topical PDT in treating tumors or pre-cancerous lesions.

2. Overview of PDT-induced immunosuppression

Despite the numerous indications that PDT is effectively used, there is strong evidence to suggest that PDT exerts local immunosuppressive effects (17-23). Those effects impact the overall success of the modality. Efforts to mitigate the resulting immunosuppressive effects could help improve efficacy or expand indications for PDT. For example, topical PDT has been associated with reactivation of orolabial herpes simplex virus (HSV) infections (24). Moreover, there is evidence from the literature, albeit anecdotal, that more aggressive melanomas and non-melanoma skin cancers can arise in PDT-treated skin (25,26). However, this is a controversial point due to confounding variables such as the fact that skin treated with PDT was more likely to develop skin cancer. Notably, more solid evidence has been provided by preclinical studies. PDT was initially discovered to be immunosuppressive in mice using dinitrofluorobenzene (DNFB), a sensitizing agent that induces a contact hypersensitivity response. In this study, mice treated with PDT showed a significant decrease in their cell-mediated immune response to DNFB applied to the PDT-treated area (20).

When PDT is applied to an area, there is a rapid invasion of neutrophils, mast cells, monocytes, and macrophages to destroy abnormal tumor cells (27), demonstrating a robust anti-tumor immune response in the host. However, in addition to its immuno-stimulatory effects, PDT causes an increase in regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) (28), which serve as counterregulatory mechanisms to impede the effector responses of the immune system (29). MDSCs secrete the immunosuppressive cytokines such as transforming growth factor-beta (TGF-β) and interleukin 10 (IL-10), which allows macrophages that migrate into a tumor to express inhibitor molecules (30) and converts Tregs to an active form, inhibiting effector T-cell proliferation (31). Tregs also suppress effector T cells directly, produce IL-10, and enhance MDSCs and regulate their differentiation through TGF-β signaling (31). Hence, MDSCs and Tregs effectively 'cross-talk' through the B7-H1 pathway which directly suppresses T-cell proliferation to form an immunosuppressive microenvironment within tumors (29,32) and thus favors tumor growth.

The exact mechanisms by which PDT exerts immunomodulatory effects is at present unclear. However, through its ability to generate ROS, PDT has been shown in cell lines and pre-clinical studies to be a potent generator of the lipid mediator, Platelet-activating factor (1-alkyl-2-acetyl-glycerophosphocholine, PAF) (33). Of note, exogenous PAF is known to induce systemic immunosuppression via its ability to generate Tregs (33). PAF upregulates IL-10, which inhibits the host immune response through the mechanism discussed earlier, but also leads to an increase in Tregs through a COX-2-mediated process (34,35). COX-2 generates prostaglandins such as PGE2 which promote blood vessel formation, allowing increased angiogenesis resulting in enhanced growth/proliferation of experimental tumor types, including melanoma (36,37). The activation of COX-2 is also connected to the induction and expansion of MDSCs (38) and increasing the levels of immunosuppressive Tregs (35), this mechanism was shown to exhibit its effects downstream of PAF in experimental models (34), with the overall pathway being PAF→COX-2→Tregs. Though demonstrated in mice, it has not yet been verified whether or not this pathway exists in humans.

Although PDT is an effective option in treating cancerous and precancerous cells, relapses can occur following treatment (39), likely due to the immunosuppressive side effects. This review is focused on studies, summarized in Table I, that have shown how blocking the immunosuppression can result in enhanced efficacy of PDT in decreasing tumors, pre-cancerous cells, and morbidity for patients. The relationship between MDSCs, Tregs, and COX-2 offers three targets for inhibiting PDT-induced immunosuppression, and illustrated in Fig. 1. These are the potentially targetable methods that will be explored in this review.

3. Inhibition of Tregs

Tregs are among the suppressive immunophenotypes which have been implicated in mediating immunosuppression or

Table I. Summary of studies detailing enhanced results by inhibiting PDT-induced immunosuppression.

First author, year	Category	Model	Medication	Results	(Refs.)
Castano *et al*, 2008	Inhibition of Tregs	Mouse	Cyclophosphamide	Low-dose cyclophosphamide + PDT led to permanent antitumor effects against a highly-metastatic reticulum cell sarcoma by decreasing the number of Tregs	(27)
Reginato *et al*, 2013	Inhibition of Tregs	Mouse	Cyclophosphamide	Levels of Tregs and TGF-β increased with PDT, but were attenuated to baseline levels with cyclophosphamide + PDT, which increased the effectiveness of PDT treatment	(21)
Oh *et al*, 2017	Inhibition of Tregs	Mouse	Anti-CD25 antibodies	Anti-CD25 therapy specifically decreased Treg populations in melanoma tumors, rendering PDT more effective. The systemic immune response was unaffected	(44)
Wachowska *et al*, 2020	Inhibition of Tregs	Mouse	Epacadostat	Tregs were decreased to control levels and neutrophil infiltration of tumors increased, but toxic systemic inflammation was also induced	(18)
Korbelik *et al*, 2015	Inhibition of MDSCs	Mouse	ATRA	Levels of MDSCs were reduced following injection of PDT-treated cells and ATRA This extended the time that PDT slowed the growth of the tumor	(17)
Mai *et al*, 2020	Inhibition of MDSCs	Mouse	PM-IR780-Met	PM-IR780-Met decreased oxygen consumption by inhibiting the mitochondrial respiratory chain. It reduced infiltration of MDSCs into tumors and increased the levels of effector T cells	(56)
Sun *et al*, 2020	Inhibition of MDSCs	Mouse	Sorafenib	When combined with PDT, sorafenib increased T cell infiltration and inhibited tumor growth more effectively than PDT alone, and in some cases decreased tumor size	(28)
Ferrario *et al*, 2002	Inhibition of COX-2	Mouse	NS-398	NS-398, a COX-2 inhibitor, was given in combination with PDT, and enhanced PDT response by resulting in an increased antitumor effect without causing an increased response in non-tumor cells	(60)
Makowski *et al*, 2003	Inhibition of COX-2	Mouse	COX-2 inhibitor	Tumor growth was significantly inhibited, mouse survival rates were increased and a higher complete cure rate was observed compared with PDT alone when the COX-2 inhibitor was given chronically after treatment with PDT	(39)
Van der Geer and Krekels, 2009	Inhibition of COX-2	Humans	Diclofenac	Improved efficacy of PDT + diclofenac in reducing actinic keratoses, but with increased side effects.	(64)
Rosenberg *et al*, 2019	Increasing $CD4^+$ T cells	Humans	Calcipotriol plus 5-FU	The combination had a pro-inflammatory effect, which may have potential as an adjunct to PDT	(68)
Thanos *et al*, 2012	Replenishment of intracellular ATP	Humans	Nicotinamide	Nicotinamide may have potential as a low-cost method of reducing PDT-induced immunosuppression and increasing its effectiveness	(72)
Frost *et al*, 2011	Decreasing oxygen consumption	Humans	Reducing radiation rate	Reducing the rate of radiation decreased oxygen consumption and was as effective as high-rate PDT at clearing tumors	(76)

PDT, photodynamic therapy; ROS, reactive oxygen species; ATRA, all trans retinoic acid; PM-IR780-Met, platelet membranes as nano-carriers to co-encapsulate metformin and IR780; ATP, adenosine triphosphate; COX-2, cyclooxygenase-2; Tregs, regulatory T cells; MDSCs, myeloid-derived suppressor cells.

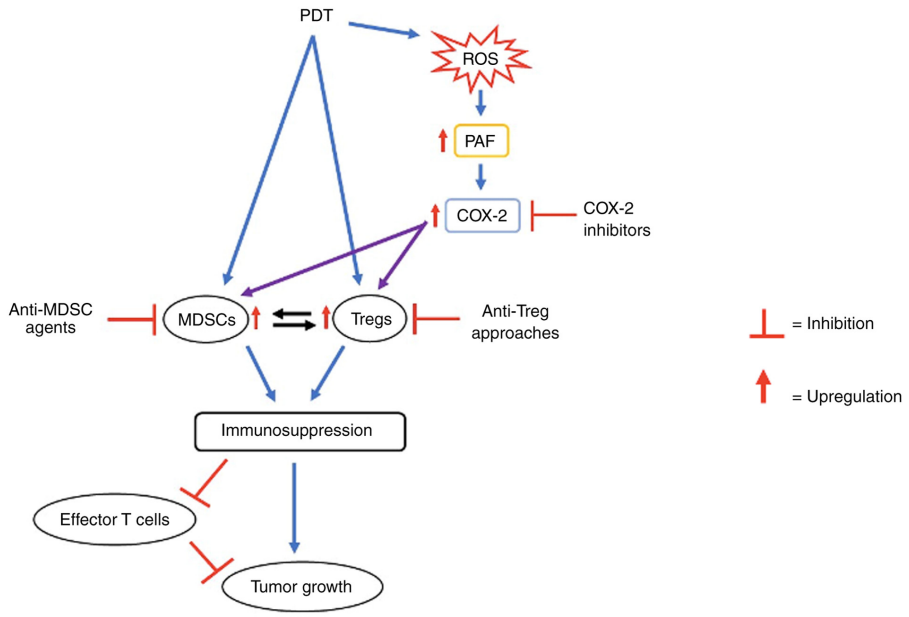

Figure 1. Schematic representation of the mechanisms involved in reducing the therapeutic efficacy of PDT. In this model, PDT, due to its prooxidant effects, generates ROS. As a consequence of this, PAF lipids are produced. The immunosuppressive cell types, Tregs and MDSCs, are upregulated via the downstream COX-2 pathway. This results in the immunosuppression and inhibition of effector T cells, leading to the reduced antitumor effect of PDT. Possible approaches to circumvent this PDT-induced immunosuppression and increase its efficacy include COX-2 inhibitors, anti-Treg approaches and anti-MDSC agents. PDT, photodynamic therapy; ROS, reactive oxygen species; PAF, platelet-activating factor; COX-2, cyclooxygenase-2; Tregs, regulatory T cells; MDSCs, myeloid-derived suppressor cells.

immune escape mechanisms (40). Multiple studies have demonstrated that Tregs proliferate at a larger extent in the tumor microenvironment, implicated in tumor progression and metastasis, as well as counterbalancing the anti-tumor immune responses of cancer therapies against malignancies including skin cancer (41,42). To that end, Treg reprogramming has been explored as a critical approach to circumvent immunosuppressive mechanisms to control tumor growth and enhance the efficacy of therapeutic regimens (42,43). As stated earlier, PDT activates the host's innate and adaptive immune systems, leading to a migration of inflammatory cells such as neutrophils and dendritic cells into the target area. However, a simultaneous immunosuppressive effect takes place in the tumor microenvironment, allowing it to evade the immune response. The first method of blocking this is to target the immunosuppressive Tregs.

One method is the administration of cyclophosphamide with PDT, the efficacy of which has been shown in multiple preclinical studies (17,21,27). In a mouse model of a highly-metastatic reticulum cell sarcoma, PDT plus cyclophosphamide administered at a low dose caused a decrease in Tregs and increased the immune system's response to tumor growth (21,27). PDT alone caused an increase in survival and tumor regression among mice, but no permanent cures. Cyclophosphamide alone also provided a survival advantage and reduced Tregs but led to no permanent cures. However, when PDT and cyclophosphamide were given together, the permanent cure rate was 70% (27). This synergistic effect was attributed to cyclophosphamide's ability to decrease the Treg population and prevent the immunosuppression induced by PDT (27).

In another study testing the effects of cyclophosphamide plus PDT, mice with colon carcinoma CT26 tumors were treated with either PDT alone or in combination with cyclophosphamide. The levels of Tregs were measured throughout treatment. Moreover, because TGF-β is an immunosuppressive cytokine that both promotes the development of Tregs and allows Tregs to regulate MDSCs (31), levels of TGF- β were also measured. The study showed that PDT alone leads to an increase in the Treg population, but that this effect is negated by the administration of cyclophosphamide before PDT, which brings down the Tregs to a level comparable to that of control mice (mice that were not inoculated with cancer) (21). Additionally, mice treated with PDT alone had an elevation in TGF-β, while mice treated with PDT and cyclophosphamide showed a significant decrease in TGF- β levels that was similar to control mice (21). Furthermore, untreated mice survived a median of 25 days after tumor inoculation, while the median survival for mice treated with PDT alone was 29 days (21). However, 9 out of 10 mice treated with both cyclophosphamide and PDT displayed tumor regression, and all 9 of those mice survived over 90 days (21).

Tregs were also selectively depleted in the tumor microenvironment of mice in a study by Oh *et al* (44). This was achieved by injecting anti-CD25 antibodies that were conjugated to a photosensitizer, which induces apoptosis in Tregs (44). The purpose of this study was to find a method that decreased tumor-associated Treg populations without inducing severe autoimmune or hyper-immune systemic responses. Overall, tumor growth was inhibited by PDT plus the CD25-targeted therapy (44). The local tumor-associated Treg population was depleted without systemic side effects and the combination caused significant anti-tumor immunity at the site of the melanoma (44). The mice did not exhibit significant hyper-immune responses and continued to have an adaptive immune response against the influenza virus, demonstrating that the systemic immune response was not significantly affected (44).

Another method to reduce the number of Tregs is to inhibit indoleamine 2,3-dioxygenase 1 (IDO). IDO is a heme-containing enzyme located in multiple tissues of the body that is expressed during inflammatory diseases and tumorigenesis (45). IDO is elevated after PDT and activates Tregs, preventing their conversion to effector T cells (46). Therefore, inhibiting IDO decreases Tregs and activates IL-6, which induces an acute inflammatory response (18). One study inoculated carcinoma tumor cells in murine models and showed that targeting IDO with inhibitors such epacadostat decreases Treg numbers to control levels and causes neutrophil infiltration of tumors, but also induces severe systemic inflammation at high doses of epacadostat through an IL-6 mechanism (18). The toxic reaction can be prevented with anti-IL-6 antibodies, but this negates the anti-tumor effect of the PDT/epacadostat combination, making its efficacy comparable to PDT alone (18). While this side effect is concerning, other studies have inhibited IDO through other methods without exhibiting the same toxicity. In one study, IDO was inhibited with a protoporphyrin IX and NLG919 conjugate in mouse models inoculated with breast cancer cells (47). This amplified PDT's immune response in tumor cells without significant toxicity of major organs (47). This study did not measure changes in Treg levels, but did report increased CT8+ T lymphocyte levels (47), implying that decreased Tregs likely played a role in augmented anti-tumor immunity. The role that IDO plays in the regulation of inflammation, both within the tumor and systemically, is poorly understood, and this method requires further investigation in preclinical and clinical trials to prevent toxic systemic side effects.

4. Inhibition of MDSCs

MDSCs are a heterogeneous population of immature myeloid cells which have been implicated to play important roles not only in pathological conditions, including cancer progression, but also in impacting the efficacy of anti-cancer agents (48-50). Importantly, these MDSC-induced effects are largely governed by their ability to induce immunosuppression, mediated via the orchestration of multiple signaling pathways as well as interactions with several immune cells and mediators (51-53). Therefore, strategies to target MDSCs have been hypothesized as one of the promising approaches to overcome immunosuppressive effects, restore anti-tumoral immunity response and/or enhance the efficacy of therapeutic agents. However, this is closely tied to the depletion of other immunophenotypes such as Tregs, thus, it is difficult, if not impossible, to affect one without affecting the other cell type(s).

One study by Korbelik *et al* revealed an improved cure rate in squamous cell carcinomas when all trans retinoic acid (ATRA) was administered with a vaccine made from tumor cells treated with PDT (17). In this study, squamous cell carcinoma cells were treated with PDT and injected into mice bearing the same squamous cell carcinoma tumors. Mice were also injected with ATRA, whose purpose was to facilitate the conversion of immunosuppressive MDSCs to a non-suppressive phenotype (54). In this study, ATRA reduced the number of MDSCs by causing their differentiation into mature myeloid cells, and overall made the PDT vaccine more effective by extending the time that PDT slowed the growth of the tumor (17). Thus, decreasing the MDSC population allows PDT to be effective against tumor cells for a longer period of time and reduce its overall size.

Reversing a hypoxic state in a tumor was also shown to impede the MDSC-regulated pathway (55,56). Hypoxia is produced by consuming oxygen after making ATP (adenosine triphosphate), thus, reversing it can be achieved by interfering with oxidative phosphorylation (57,58). This study used platelet membranes as nano-carriers called PM-IR780-Met, which included encapsulated metformin whose role was to decrease oxygen consumption by inhibiting the mitochondrial respiratory chain. This ultimately reduced the levels of MDSCs and their infiltration into tumor tissues. In turn, this reduced the number of Tregs being recruited by MDSCs and increased the infiltration of effector T cells into the tumor, lymph nodes, and spleen (56). Thus, it can be inferred that the nano-carrier increased both the anti-tumor and systemic immune responses. Furthermore, treatment with PDT without the nanocarrier decreased tumor growth from 7.5-fold to 4-fold, but the addition of metformin caused growth to decrease to 1.1-fold, showing a superior anti-tumor response (56). In addition to preventing the immunosuppressive effects of PDT, a constant supply of oxygen was supplied by the nano-carrier, and this allowed more ROS to be generated during PDT, rendering PDT more effective against tumors (56).

Sorafenib administered with low-dose PDT has been exploited as another method that enhances the T cell-mediated antitumor effects. Sorafenib has been shown to reduce MDSC and Treg populations (28,59), while recruiting more antigen-processing cells and cytotoxic T cells to the tumor (28). When combined with PDT, sorafenib increases T-cell infiltration and inhibits tumor growth more effectively than PDT alone, and in some cases decreases tumor size (28). This was attributed to Sorafenib's ability to limit the interaction between cytotoxic CD8+ T cells and immunosuppressive cells, inducing a stronger anti-tumor immune response.

5. Inhibition of COX-2

As discussed earlier, PDT has been shown in mice to generate systemic immunosuppression through the lipid mediator PAF in a pathway leading to increased COX-2 expression and levels of Tregs (33,60). However, COX-1, which is constitutively expressed in most cells, is not increased by PDT (60). Eicosanoids and COX-2-generated prostaglandins such as PGE2 have also been linked to local immunosuppression (61,62). This pathway has not yet been demonstrated in humans. Given the availability of COX inhibitors, this strategy could serve as an easy target to combat the PDT-induced immunosuppression and relapse of pre-cancerous cells. In particular, selective COX-2 inhibitors such as celecoxib are safe for short-term use and may also decrease the painful side effects of PDT, so this method merits additional investigation.

Although not yet tested in humans, combination therapies involving selective COX-2 inhibitors have been shown to improve the therapeutic effectiveness of PDT in treating solid tumors in mice (35,39,60,63). In one example, NS-398, a COX-2 inhibitor, was given in combination with PDT, and caused decreased levels of PGE2 and VEGF, which enhanced PDT's response in tumor cells of mouse carcinomas and

sarcomas and resulted in a significant increase in tumor cures (compared to PDT alone) (60). Furthermore, the combination did not cause an increased response in non-tumor cells; specifically, it did not affect skin sensitization nor did it cause increased skin damage in sites without tumor cells (60).

In a second example by Makowski *et al*, there was no increased PDT efficacy *in vitro* when tumor cells were incubated with COX-2 inhibitors (39). This result was unexpected, especially following the results of Ferrario *et al* that showed PDT's effect potentiated by the addition of a COX-2 inhibitor (60). This prompted the group to conduct two different *in vivo* experiments: For one set of mice the COX-2 inhibitor before PDT, and the other received it chronically after illumination with PDT. The former showed no increased anti-tumor response, but with the latter, there was a statistically significant retardation of a poorly differentiated colon adenocarcinoma C-26 tumor growth, increased mouse survival, and higher complete cure rate compared to PDT alone (39). The proposed mechanism by Makowski *et al* is that the COX-2 inhibitor decreases angiogenic factors-which is synergistic with PDT's ability to cause vascular damage-and triggers apoptosis in tumor cells (39). This would explain why the anti-tumor effects were only increased when COX-2 was administered after PDT, since the tumor would have more difficulty repairing blood vessels following the vascular damage caused by PDT. This study demonstrated that COX-2 inhibitors may improve the efficacy of PDT through methods other than inhibiting immunosuppression.

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) that functions as a cyclooxygenase-1 (COX-1) and COX-2 inhibitor. It has been shown in small clinical trials to improve the efficacy of PDT in reducing actinic keratoses when used as an adjuvant therapy, likely by targeting COX-2 receptors on actinic keratosis (64). Although not a COX-2 selective inhibitor, diclofenac shows promise because it is already used as a treatment on its own for actinic keratosis (33). Adjuvant therapy would not only make the treatment more effective, but also inhibit the immunosuppression caused by PDT. However, more patients reported pain, sometimes unbearable, and side effects such as pruritus, scaling, and crusting during PDT when used in conjunction with diclofenac (64). This might result in the diclofenac and PDT combination being reserved for small areas of skin.

6. Conclusions

With a better understanding of the mechanisms of immunosuppression of PDT with ALA, we can inhibit them and offer patients more effective treatments with potentially fewer side effects. This is the first review to specifically address methods of inhibiting immunosuppression for PDT with ALA. However, multiple options may not be considered practical due to the risk of side effects. For example, ATRA is only regularly used by oncologists and cyclophosphamide is only commonly used by rheumatologists, nephrologists, and dermatologists. Although diclofenac might not be a popular option due to side effects, the fact that it potentiated the effects of PDT merits exploration of other non-selective COX-inhibitors that could be used in conjunction with PDT. The approaches that use anti-CD25, sorafenib, abatacept, and COX-2 inhibitors are more realistic in most settings. There are likely other options, yet to be determined, that involve different pathways for blocking PDT-induced immunosuppression.

It should be noted that other promising strategies are being developed to augment tumor-specific production of PpIX to include the use of topical vitamin D analogues (65). One effect of vitamin D receptor activation involves its ability to increase Tregs (66,67), which could result in an ‘immunosuppressive phenotype’. However, combinations of topical vitamin D agonists (calcipotriol) with 5-FU chemotherapy appear to result in a more pro-inflammatory effect which has been reported to result in long-term remissions (68). Hence, the exact effects of vitamin D as an adjunct to PDT on the skin immune system is an area of future investigation. Additionally, topical and oral nicotinamide (vitamin B3) replenish cellular ATP after irradiation with UV light (69). Through this mechanism, it has been shown to reduce the immunosuppression associated with both high and low-dose PDT, making it more effective against actinic keratoses and nonmelanoma skin cancers (70-73). This is a useful discovery, as nicotinamide is low-cost, readily available, and has few, if any side effects (74). Finally, immunosuppression has been shown to decrease by simply reducing the rate of irradiation, perhaps because of the decreased oxygen consumption at lower rates (75,76). In pre-clinical trials, lower rates of PDT (15 or 45 $mWcm^{-2}$) were as effective as high-rate PDT (75 $mWcm^{-2}$) in clearing tumors (77).

Though the immunosuppressive pathways appear complex, there is considerable rationale for pharmacologic strategies to target this unwanted effect. Further investigations documenting the exact level and mechanisms of PDT-induced immunosuppression demonstrated in mice need to be pursued in humans. In addition, there is a need for studies testing both the ability of various strategies such as COX inhibitors to inhibit PDT-induced immunosuppression with a clinical benefit such as improved clearance of actinic keratosis.

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SB and CAR developed the concept and contributed to the majority of the manuscript writing. RPS and JBT made final revisions to the manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

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Not applicable.

Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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