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## Special Issue Invited Review

# Photodynamic Therapy and Immunity: An Update†

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## ABSTRACT

**Dr. Thomas Dougherty and his Oncology Foundation of Buffalo were the first to support my (S.O.G.) research into the effects of photodynamic therapy (PDT) on the host immune system. The small grant I was awarded in 2002 launched my career as an independent researcher; at the time, there were few studies on the importance of the immune response on the efficacy of PDT and no studies demonstrating the ability of PDT to enhance antitumor immunity. Over the last decades, the interest in PDT as an enhancer of antitumor immunity and our understanding of the mechanisms by which PDT enhances antitumor immunity have dramatically increased. In this review article, we look back on the studies that laid the foundation for our understanding and provide an update on current advances and therapies that take advantage of PDT enhancement of immunity.**

## INTRODUCTION

Photodynamic therapy (PDT) is approved by both the Food and Drug Administration and by the European Medicine Agency as curative therapy for precancer lesions and solid tumors and as palliative therapy for advanced malignancies. PDT is minimally invasive with high specificity of action on tumor tissue. PDT involves topical or systemic administration of a photosensitive drug (photosensitizer; PS) followed by illumination of the tumor with light of appropriate wavelength that excites the PS. Energy from excited PS converts molecular oxygen available in tumor tissue to reactive oxygen species (ROS) (1–4). The generation of ROS causes direct cytotoxicity of cells in the tumor microenvironment resulting in tumor cell death and destruction of tumor vasculature. Loss of vasculature depletes the tumor microenvironment of essential survival components: oxygen and nutrition (2,3,5–7). Preclinical studies in mouse models and clinical studies have shown that PDT efficacy depends on the presence of an intact immune system (1). PDT-induced traumatic insult and oxidative stress to the tumor tissue activate an acute inflammatory process required for removal of tissue debris and for restoration of homeostasis. In addition, immunogenic cell death (ICD) caused by PDT releases damage-associated molecular patterns (DAMPs) that activate innate immunity, which leads to activation of adaptive immunity (8,9). Henderson *et al*. have shown that PDT regimens can be developed to activate antitumor immunity (5). Multiple studies have linked PDT-induced acute inflammation to enhancement of systemic antitumor immunity (3,10–12).

In this review, we discuss PDT induction of ICD and the resultant inflammation and subsequent activation of antitumor immunity, thus highlighting the potential of PDT to act as adjuvant immunotherapy.

## COMPONENTS OF PDT: PHOTOSENSITIZER, LIGHT AND MOLECULAR OXYGEN

PSs typically contain a tetrapyrrole structure as found in porphyrins such as protoporphyrin. The first photosensitizing material used in preclinical studies was hematoporphyrin derivative (HPD), a collection of monomeric and oligomeric porphyrin ethers and esters (13). Dr. Thomas Dougherty and colleagues at Roswell Park Comprehensive Cancer Center developed Photofrin® or porfimer sodium, a purified version of HPD that lacks the monomers. Photofrin® (absorption peak of 630 nm) was the first PS to be approved by FDA for clinical PDT in the Unites States (3,14). Although Photofrin has a preference for tumor cells, it also has an extended period of retention in normal tissues resulting in photodamage to skin upon exposure to sunlight (15). In addition, 630 nm, the wavelength of light that excites Photofrin, has low tissue penetration capacity making the development of next-generation PS necessary for PDT. PSs with increased tumor specificity and stronger absorbance (>650 nm) are currently under various stages of clinical trial with some already receiving approval for clinical use (16).

Photochemical reactions during PDT generate singlet-state oxygen ($^1O_2$) which requires energy transfer from PS to molecular oxygen. Hence, tissue oxygenation is critical during PDT efficacy. Several studies have demonstrated that light delivery at low rates (i.e. low fluence rates) results in oxygen conservation (17–20). PDT induction of acute inflammation is regulated by fluence rate (5). Henderson *et al*. showed that low fluence rates result in high levels of inflammation, which are characterized by increases in inflammatory cytokines and neutrophils infiltration into the tumor bed. Subsequent studies have demonstrated that induction of acute inflammation, in particular mobilization of neutrophils, is required for PDT enhancement of antitumor immunity.

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## Immunological consequences of cell death by PDT

The mechanisms leading to cell death upon PDT have been widely studied. Depending on PS localization and extent of photodamage, PDT can induce cell death by necrosis, apoptosis, autophagy or paraptosis (3,21–32). Each of these cell death programs can result in exposure or release of intracellular components known as damage-associated molecular patterns (DAMPs) or alarmins. DAMPs are recognized by pattern recognition receptors (PRRs) expressed on immune cells. DAMPs binding to PRRs result in immune cell activation; thus, cell death that triggers activation of immune cells is referred to as immunogenic cell death (ICD) (33–36). PDT-induced cell death programs that have been associated with ICD are discussed below:

*Apoptosis.* Apoptosis is the most widely studied form of ICD in the context of PDT. PDT can cause rapid apoptosis or programmed cell death characterized by chromatin condensation and cellular fragmentation (21). PS localization dictates the mechanism of apoptosis. Localization of PS to mitochondria and subsequent mitochondrial photodamage results in release of cytochrome c to cytosol, thereby initiating apoptosis (23,31). Additionally, both mitochondrial and ER photodamage result in destruction of the anti-apoptotic proteins Bcl2 and Bcl-xL without affecting pro-apoptotic protein Bax (29,32,37) and thus further promoting apoptotic cell death. Photosensitizer localization to lysosomes results in release of lysosomal proteases to cytosol, thereby cleaving pro-apoptotic protein Bid to its active form tBid. This truncated form of Bid binds to mitochondrial membrane and initiates apoptosis (38–40). PDT can also induce death receptor-mediated apoptosis when PSs localizing to plasma membrane are used. Photodamage to plasma membrane results in multimerization of death receptors like Fas which belongs to TNF receptor superfamily. Initiation of Fas signaling results in cleavage of procaspase 8 to the active caspase 8, triggering apoptotic program (7).

Apoptosis is generally a tolerogenic process and functions to maintain homeostatic cellular balance. However, apoptotic tumor cells are reported to be highly immunogenic (41–44). Indeed, multiple studies have demonstrated that Photofrin–PDT results in surface expression of HSPs, CRT and release of HMGB1 (45,46), all of which are characteristic of ICD. Garg *et al*. have demonstrated surface expression of CRT and secretion of ATP upon immunogenic apoptosis induced by hypericin-based PDT (47). Jalili *et al*. (48) have reported both apoptosis and necrosis of EMT6 murine mammary tumor cell line upon Photofrin–PDT and increased expression of HSPs.

*Autophagy.* PDT can induce autophagy in which bulk of the cytoplasm is sequestered in double membrane-bound vacuoles called autophagosomes. Conditions of cellular starvation and mitochondrial toxicity trigger formation of autophagosomes, which carry cellular components to lysosomes for degradation (49,50). Generation of ROS during PDT is a powerful trigger for formation of autophagosomes (22,24,30). Cancer cells use autophagy in a cytoprotective capacity to remove sources of ROS following PDT. However, cancer cell death by autophagy becomes prominent when apoptotic machinery is impaired or when photodamaged components are incapable of recycling (24,26,49,51,52).

To determine the effect of PDT-induced autophagy on antitumor immune activation, Korbelik *et al*. (53) injected SCCVII tumor-bearing mice with *in vitro* PDT-treated SCCVII cells that were incubated with or without lethal autophagy inhibitors. Incubation of PDT-treated SCCVII cells with autophagy inhibitor resulted in increased rate of tumor growth. This suggests PDT-induced cell death by autophagy might play role in activating antitumor immunity. Chemotherapy-induced autophagy has been demonstrated to release DAMPs such as CRT, HMGB-1 and ATP, thus strengthening the argument for potential of autophagy as a mode of immunogenic cell death during cancer treatment involving cellular toxicity (54).

*ICD.* PRRs that bind to DAMPs are either soluble (pentraxins and complement proteins) or membrane bound (Toll-like receptors; TLRs); PRRs are expressed on innate cells, and DAMP binding to PRRs initiates activation of these cells. PDT results in accumulation of the pentraxins, such as serum amyloid P component (SAP) and C-reactive protein (CRP) (55,56). Korbelik *et al*. have demonstrated association of complement receptors and TLRs with PDT-generated DAMPs (45,55). PDT-induced ICD, along with PDT-induced acute inflammation, is considered to be the initiating step toward PDT enhancement of antitumor immunity. This process is discussed in detail below.

## Inflammation and activation of innate immunity

Destruction of tumor tissue by PDT elicits an immediate localized inflammatory response aimed at containing and clearing the debris, restoring normal tissue function and homeostasis. Damage to tumor tissue results in release of lipid membrane derived arachidonic acid metabolites (prostaglandin, leukotriene and thromboxanes), rapid upregulation of inflammatory cytokines such as MIP2 (CXCL2), IL6, IL-1β, TNFα and activation of complement (10,57–60). Together, these factors facilitate the influx of innate immune cells into the tumor for attack and removal of dying tumor cells (10–12). Multiple studies have revealed the importance of PDT-induced inflammation in enhancing antitumor immunity (5,61,62). The involvement of innate immune cells in PDT-induced inflammation and subsequent antitumor immunity is discussed below.

*Involvement of neutrophils in PDT efficacy and activation of antitumor immunity.* There are multiple reports of local and systemic neutrophilia upon PDT (5,10–11,61,62). Photodamage to tumor vasculature causes contraction of endothelial cells allowing neutrophil adhesion to subendothelial matrix via the β2 integrin receptors (63,64). PDT results in increased expression of adhesion molecules. E-selectin and ICAM1 are critical for neutrophil adhesion on tumor microvessels and entry into tumor tissue (11,65). PDT also induces local expression of the chemokine MIP2 that facilitates neutrophil migration to tumor bed (11). In addition, PDT induces local and systemic activation of complement, which is critical for neutrophil infiltration into the tumor (12). Complement activation releases anaphylatoxins such as C3a and C5a from tumor tissue, which promote vascular permeability (66,67). Thus, structural changes in vascular endothelial walls and regulatory factors expressed upon PDT cause accumulation of neutrophils at the vascular interface of photodamaged tumor tissue, generate chemotactic gradient across the vascular

membrane and facilitate infiltration of neutrophils to the tumor (10–12). The recruitment of neutrophils to PDT-treated tumor tissue is supported by the accompanying strong acute-phase response, which is characterized by increased serum level of acute-phase proteins (APPs) such as CRP, mannose-binding lectins (MBLs) and SAP (68). APPs facilitate the systemic mobilization of neutrophils from storage pools and increased maturation of neutrophil progenitors in bone marrow and egress from bone marrow (10,69). Following PDT, neutrophils also enter the tumor-draining lymph nodes (TDLNs) via high endothelial venules (HEVs) in an IL-17- and IL-1β-driven MIP2-mediated pathway (61). This infiltration of neutrophils to TDLNs is short-lived and resolves within 24 h of PDT. PDT-induced local and systemic neutrophilia contributes to PDT efficacy by destroying tumor tissue and contributing to the activation of antitumor $CD8^+$ T cells. Several studies have demonstrated poor PDT efficacy and reduction in number of activated antitumor $CD8^+$ T cells when neutrophil entry into tumors and TDLNs is blocked (5,61,62,65). Although neutrophils are considered to be critical to induction of antitumor immunity following PDT, cell markers used to define neutrophils (CD11b and Gr1) are also expressed on myeloid-derived suppressor cells (MDSCs) (70) thus calling into question the nature of the cells induced by PDT. However, Brackett *et al*. performed extensive characterization of the induced cells, concluding that they could be classified as neutrophils based on histology and lack of suppressive activity (61).

*Involvement of macrophages in immune activation and effector function after PDT.* Macrophages are phagocytic cells that differentiate from monocytes and express PRRs, including TLRs. PDT-induced release of Hsp70, a TLR2/4 binding DAMP, from tumor cells results in TLR2/4 activation of macrophages and release of TNFα (45). TNFα is a cytolytic cytokine which may mediate indirect cytotoxicity of tumor cells following PDT. Macrophages also express complement receptors, which enable them to phagocytose tumor cells opsonized with C3 and MBLs, thus facilitating the clearance of photodamaged tumor tissue (55).

*Involvement of NK cells in PDT efficacy.* Studies by Gollnick *et al*. have revealed role of natural killer (NK) cells in PDT. In vitro studies using human and murine colon carcinoma cell lines reveal increased expression of MHC class I-like molecule MICA and NKG2D ligand on PDT-treated tumor cells (71). Park *et al*. (72) also reported similar results. These molecules are ligands for activation receptors on NK cells, thus indicating a possible role of NK cells in augmentation of antitumor immunity following PDT. Gollnick *et al.* (73) reported that control of distant disease by $CD8^+$ T cells, following PDT of the primary tumor, is improved in the presence of NK cells. Thus, PDT of primary tumors may enhance NK cell-mediated immunity to metastatic tumors. Systemic depletion of NK cells reduced PDT efficacy in EMT6 tumor model. However, splenic NK cells isolated from PDT-treated mice were noncytotoxic to EMT6 cells *in vitro* (74). This suggests an indirect mechanism of action of NK cells in PDT-induced antitumor immunity.

*Activation of dendritic cells following PDT.* Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that activate adaptive immune cells by presenting antigens on their surface in the context of major histocompatibility complexes. Antigen presentation by immature DCs in the absence of costimulatory molecules generates tolerogenic T-cell environment. However, activation/maturation of DCs in an inflammatory setting allows increased expression of MHC class II and costimulatory molecules. Co-incubation of DCs with PDT-treated tumor cell lysates induces phenotypic and functional maturation of DCs (75,76). DCs express PRRs that recognize DAMPS. Since tumor cells release or expose DAMPs during PDT-induced ICD, it is likely that signal transduction through ligand binding of PRRs facilitates DC activation after PDT. Indeed, Wang *et al*. reported that incubating DCs with ALA-PDT-treated SCCVII cells in the presence of blocking agents of DAMPs abrogated phenotypic and functional maturation of the DCs (77).

**Activation of adaptive immunity by PDT**

The need for an intact adaptive immune system for PDT efficacy is supported by multiple studies. PDT efficacy in *scid* mice-bearing EMT6 tumors was improved by reconstitution with splenocytes derived from PDT-cured immunocompetent mice (74,78). Transfer of T lymphocytes from immunocompetent naïve mice to EMT6 tumor-bearing *scid* mice improved PDT efficacy in the immunocompromised mice, suggesting specific role of T cells in antitumor immunity after PDT. In addition, EMT6 tumor-bearing *nude* mice, that lack only T lymphocytes, respond significantly poorer to PDT than BALB/c mice (79,80). Clinical studies have also shown that an intact immune system supports PDT efficacy (81).

The induction of adaptive immunity or antigen-specific immune response upon PDT was first demonstrated in elegant studies performed by Canti *et al*. (82). This group demonstrated that immunocompromised mice (*scid*) that were cured of MS-2 fibrosarcoma by PDT failed to resist rechallenge with the original tumor cells. On the contrary, immunocompetent tumor-bearing mice cured by PDT were resistant to rechallenge with the original tumor cells but not to challenge with unrelated tumor cells. These results demonstrate that PDT activates adaptive antitumor immunity and specific antitumor immune memory.

Adaptive immunity is broadly classified into type 1 immunity and type 2 immunity. Type 1 immunity is facilitated by $CD4^+$ T cells that express cytokines such as IL-12 and IFN-γ and leads to activation of $CD8^+$ T cells which have cytotoxic functions. Type 2 immunity, also considered to be tissue protective immunity, is skewed toward $CD4^+$ T cells of the Th2 phenotype which express cytokines such as IL-4 and facilitate antibody production by B cells (83). Korbelik *et al*. performed studies by depleting specific T-cell populations from splenocytes of PDT-cured immunocompetent mice. Engraftment of these specific T-cell-depleted splenocytes in *scid* mice and subsequent PDT revealed critical role of $CD8^+$ cytotoxic T lymphocytes (CTLs) in induction of antitumor immunity following PDT and a supportive role of $CD4^+$ helper T lymphocytes (78). $CD8^+$ T-cell depletion followed by PDT resulted in reduced PDT efficacy, confirming the role of CTLs in PDT-induced antitumor immunity (74). Several studies have shown that PDT enhances the activation of tumor-specific antitumor $CD8^+$ T cells (62,84,85). Interestingly, depletion of $CD4^+$ T cells inhibited tumor growth in both untreated and PDT-treated tumor-bearing mice, which may be due to elimination of simultaneous depletion of regulatory T (Treg) cells, thus making it difficult to interpret the role of $CD4^+$

T cells in adaptive immune response following PDT. To further dissect the involvement of T cells in antitumor immunity following PDT, Kabingu *et al*. used a two-tumor model where BALB/c mice were inoculated with EMT6 cells on both shoulders. PDT of one tumor led to increased infiltration of $CD8^+$ T cells but not $CD4^+$ T cells into the untreated contralateral tumor. Using an experimental model of lung metastasis, Kabingu *et al*. also showed that depletion of $CD4^+$ T cells prior to PDT of the primary tumor had no effect on growth of existing lung tumors, while depletion of $CD8^+$ T cells prior to PDT of the primary tumor significantly increased the number of lung tumor nodules (73). These studies confirm the critical role of $CD8^+$ T cells in the antitumor immune response following PDT and demonstrate a limited role of $CD4^+$ T cells. In contrast, Preise *et al*. (86) reported delayed or reduced tumor growth in naïve mice when CD4 T cells were adoptively transferred from PDT-cured mice. These $CD4^+$ T cells had increased expression of IFNγ upon restimulation, thereby confirming Th1 phenotype. Th17 cells, a subset of $CD4^+$ T cells that produce the cytokine IL-17, have recently being categorized as components of type 3 immunity; Th17 cells play important role in neutrophil activation (83). Brackett *et al*. identified an increased number of Th17 cells in the TDLN after PDT and a critical role of IL-17 on the efficient recruitment of neutrophils to TDLNs after PDT (61). As already discussed, neutrophils facilitate accumulation of activated $CD8^+$ T cells in the tumor tissue after PDT along with improved PDT efficacy (62). These results indicate PDT-mediated activation of a type 3 immunity that leads to type 1 immunity via inflammation characterized by neutrophilia. The mechanism of this switch is unclear but may be related to the inherent plasticity of Th17 cells (87).

### Immunosuppressive role of PDT

Several studies have reported that in addition to immune activation, PDT can also promote immune suppression, which was first shown as reduced immune response to application of the hapten dinitrofluorobenzene (DFNB) (88,89). A subsequent study reported a transient increase in immunosuppressive Tregs in spleens and TDLNs of tumor-bearing mice following PDT. This transient increase in Tregs might be a component of the homeostatic machinery needed to regulate immune activation following PDT (90).

Recently, Korbelik *et al*. have demonstrated increase in granulocytic myeloid-derived suppressor cells (MDSCs) or granulocytic myeloid regulatory cells (Mregs) following vaccination of tumor-bearing mice with PDT-treated tumor cells (91,92). Since systemic neutrophilia immediately following PDT plays beneficial role in PDT efficacy, Korbelik *et al*. studied the effect of immediate and delayed inhibition of Gr1 + cells by administering Gr1-depleting antibody immediately following PDT or 1 h after PDT. Depletion of Gr1 + cells immediately after PDT reduced cure rate of SCCVII tumor-bearing mice, in line with the established role of neutrophils in PDT-induced antitumor immunity. However, delayed depletion of Gr1 + cells also improved PDT efficacy, suggesting a delayed accumulation of MDSCs or Mregs replacing the initial neutrophilia.

Thus, combination of PDT with inhibitors of immune suppressive cells might improve PDT efficacy. Indeed, studies have demonstrated improved cure rate when all transretinoic acid (ATRA) that facilitates conversion of immune suppressive MDSCs to a nonsuppressive mature phenotype is administered in the context of PDT (91). Similar effects were obtained with inhibitors of Tregs such as cyclophosphamide and CD25-depleting antibody (90,91,93,94).

PDT increases expression of the inflammatory mediator prostaglandin E2 by tumor cells and immune cells along with increased expression of cyclooxygenase 2 (COX2), an enzyme that catalyzes rate-limiting step of PGE2 pathway (95–98). It is well established that COX2/PGE2 expression by tumor cells facilitates tumor progression by affecting angiogenesis, proliferation of tumor cells and suppression of antitumor immunity (99–103). PGE2 reduces the effect of necrotic cells upon macrophage production of the antitumor cytokines such as TNFα and IFNγ. Thus, PGE2 is considered as an "inhibitory DAMP" (104). Studies have shown improved PDT efficacy upon prolonged blocking of PGE2 synthesis pathway following PDT (97,105).

### Clinical evidence of PDT-enhanced antitumor immunity

The first indication for a role of antitumor immunity on clinical outcome of PDT was reported by Abdel-Hady *et al*. when they showed that vulval intraepithelial neoplasia (VIN) patients who were nonresponsive to ALA-PDT had higher likelihood of having MHC-I-negative tumors and reduced $CD8^+$ T-cell accumulation in the treated tumor (81). Kabingu *et al*. reported enhancement of antitumor immunity when PBMCs of BCC patients treated with PDT displayed increased tumor antigen recognition and cytokine production (106). Thong *et al*. published a case study of 64-year-old patient with multifocal angiosarcoma of the head and neck whose tumors regressed upon high-dose brachytherapy but recurred within 1 year. Fotolon-based PDT of the recurrent tumors resulted in spontaneous remission of the untreated tumors. Biopsy of these untreated tumors exhibited increased infiltration of CD8 T cells (107). In another report of a phase I clinical trial involving patients with breast cancer progression following mastectomy and electron-beam radiation therapy, treatment with continuous low irradiance PDT (CLIPT) resulted in complete or partial response in 67% of patients (6 out of 9); 2 out of 9 patients demonstrated regression of distant tumors (108). This clinical effect of low-dose PDT on antitumor immunity is in line with preclinical studies showing that low-dose PDT enhanced antitumor immunity more effectively than high-dose PDT (5,62). PDT was also shown to reduce immunosuppression, through reduction in Tregs, in invasive esophageal squamous cell carcinoma (ESCC) patients (8).

### Potential of PDT as adjuvant for checkpoint blockade therapy

Immune checkpoint blockade has established itself as a promising cancer therapy in the recent years. One of the conditions for the success of this therapy is activation of antitumor immunity prior to checkpoint blockade. PDT has the potential to be an ideal immune adjuvant to checkpoint blockade therapy for two reasons: Firstly, as stated in the previous sections, PDT regimens can be designed to activate antitumor immunity; secondly, PDT has limited off-target effects due to preferential retention of PS in tumor cells and illumination within the tumor boundary.

Several preclinical and clinical studies have reported improved PDT efficacy upon combination with immune checkpoint inhibitors. In an orthotopic model of murine renal carcinoma,

O'Shaghnessy *et al*. demonstrated increased regression of primary tumors treated with vascular targeted PDT combined with anti-PD1 and anti-PD-L1 treatment. The combination treatment also prevented lung metastasis. Neither treatment alone was efficacious. Efficacy of the combination therapy was attributed to an increase in the ratio of $CD8^+$ and $CD4^+$ T cells to Tregs (109). Santos *et al*. reported a case study of 62-year-old patient with locally advanced SCC of mouth floor that was progressing after surgery, radiation therapy and cisplatin (RT/CT) but regressed following redaporfin-PDT and anti-PD1 therapy (110). The success of PDT and checkpoint blockade combination therapy depends on the immune enhancement by PDT and not on the tumor ablative capacity of PDT. This is evident from study by Muchowicz *et al*. where blocking of lymphatic regeneration after PDT by administration of inhibitory molecules impaired DC migration to TDLNs and intratumoral accumulation of tumor-specific CD8 + T cells (111).

### Potential for PDT-induced ICD as cancer vaccines

Cancer vaccines involve introduction of lethally damaged cancer cells to tumor-bearing hosts with the aim of activating tumor-specific host immune response. Traditional methods of cancer vaccine generation relied on radiation-induced lethal damage to cancer cells. However, these vaccines are poorly immunogenic and require adjuvants for robust immune response. PDT induction of ICD (discussed above) suggests that cancer cells treated by PDT may be excellent cancer vaccines. Gollnick *et al*. generated cancer vaccines from Photofrin–PDT-treated lysates of the murine mammary EMT6 and mastocytoma P815 cells. Administration of these lysates to syngeneic mice once a week for four weeks followed by inoculation of the EMT6 or P815 tumor cells revealed protective role of the cancer vaccines as evident by delayed tumor growth or abrogation of tumor establishment in these mice. Vaccines generated from PDT-treated tumor cells also imparted greater protection against tumor challenge than vaccines generated UV or IR-treated tumor cells. PDT-generated cancer vaccines activated antitumor immunity by facilitating phenotypic and functional maturation of DCs and cytolytic activity of splenocytes (112). Korbelik *et al*. generated SCCVII cancer vaccines by BPD-PDT followed by lethal irradiation. Introduction of these vaccines to SCCVII tumor-bearing mice significantly delayed tumor growth and even resulted in cures. Vaccine-treated mice had increased DCs, T cells, B cells in TDLNs along with higher numbers of memory T cells. Examination of PDT-treated tumor cells isolated after vaccination showed that they were coated with complement protein C3. The importance of complement in effectiveness of the cancer vaccine-induced immune response was demonstrated by reduced efficacy upon complement blocking (55).

Recently, Garg *et al*. have described the success of DC vaccine generated upon coculture of bone marrow-derived DCs (BMDCs) with hypericin-PDT-treated murine glioma cell line GL261; hypericin-PDT-induced ICD in GL261 cells.

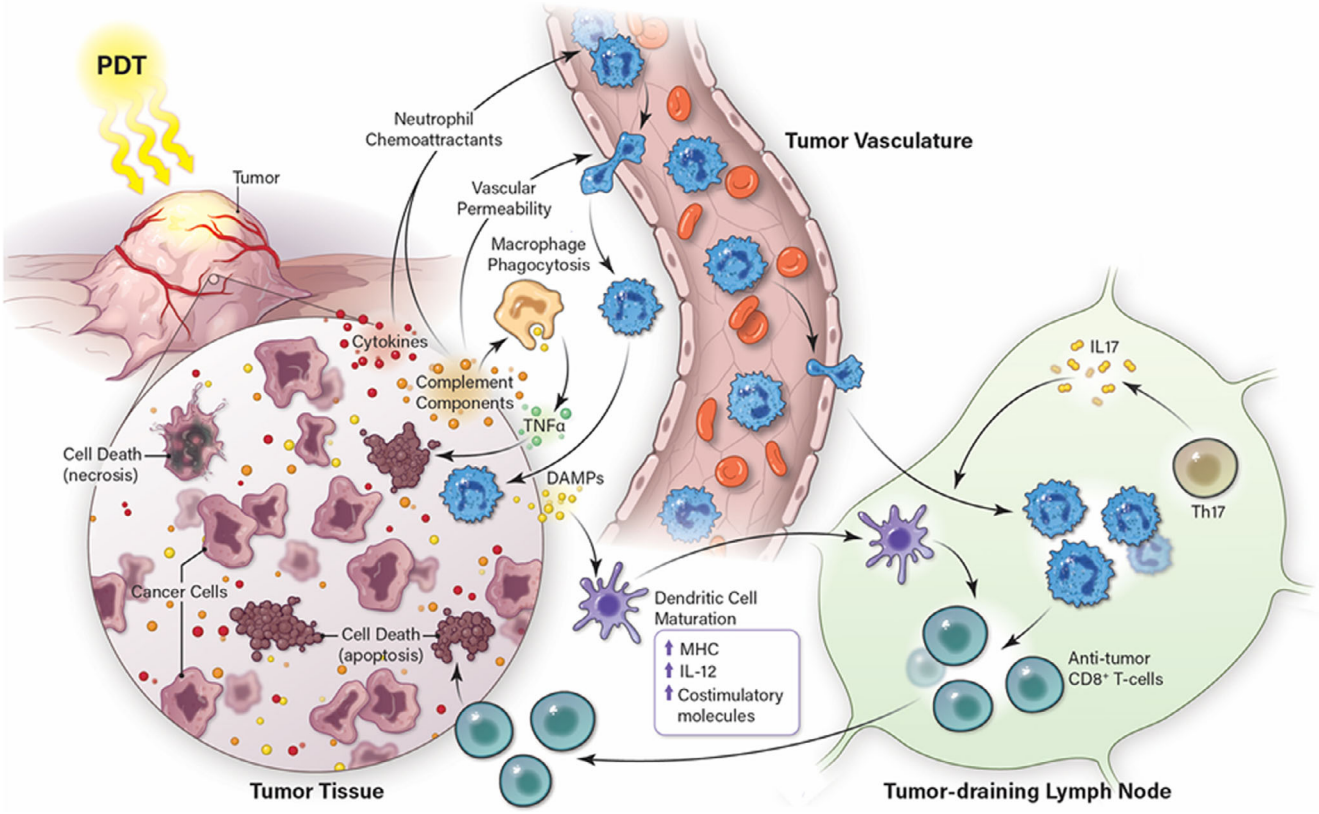


**Figure 1.** Induction of antitumor Immunity by PDT. PDT of tumors causes immunogenic cell death that is characterized by the release of immune activating DAMPs and is accompanied by induction of acute inflammation that leads to IL-17-dependent neutrophil infiltration into the treated tumor bed and tumor-draining lymph node. DAMPs stimulate dendritic cell maturation; mature dendritic cells work in concert with neutrophils to enhance antitumor-specific $CD8^+$ T-cell activation and increased tumor cell death.

Administration of DCs cocultured with PDT-treated tumor cells provided protective immunity against orthotopic implantation of parental cells in syngeneic mice. This protective immunity conferred by DC vaccine relied on $CD8^+$ T cells, generation of ROS during ICD of the glioma cells and expression of DAMPs upon ICD. Vaccination also improved infiltration of Th1 and Th17 cells and reduction of Tregs in the brain. Th1 and Th17 are associated with improved prognosis in human glioma, thus bringing to light the clinical relevance of PDT vaccines in glioma treatment (113). Zheng *et al*. reported similar results when BMDCs were cocultured with hypericin-PDT-treated Lewis lung carcinoma cells (LLC). They also demonstrated improved activation of tumor-specific T cells along with a reduction in Tregs (76).

### Improving the future of PDT as immune therapy by superior targeting of PS to tumor

In recent years, several strategies for controlled and targeted delivery of PS to tumor have emerged, thus increasing specificity of photodamage to tumor cells. Notable among these are near-infrared photoimmunotherapy (NIR-PIT) (114–117) and nanoparticles (118–120).

NIR-PIT targets the PS IRDye700 to tumor tissue by conjugating it with monoclonal antibody to antigens that are widely expressed on tumor cells. Excitation of the PS is achieved by illumination with NIR light, which has higher tissue penetration capacity than most wavelengths used for PDT. NIR-PIT induces ICD with exposure of HSPs, ATP and HMGB1 that results in maturation of dendritic cells (121). Bao *et al*. targeted IRDye700 to subcutaneous murine 4T1 tumor by utilizing a Fab fragment of antibody that binds to CD276, an antigen preferentially expressed on tumor cells. Although targeted NIR therapy reduced tumor regrowth, it significantly increased PD-L1 expression on tumor cells. Combination of CD276-targeted NIR-PIT and anti-PD-L1 treatment suppressed regrowth of the subcutaneous tumor and prevented lung metastasis by increasing intratumoral accumulation of $CD8^+$ T cells (114). Similar results were reported in another study in which IRDye700 was targeted to the integrin $\alpha v\beta 6$, which is widely expressed on cancer cells (122). In a combination study with CD44-targeted NIR-PIT and anti-PD-1 administration in subcutaneous model of MC38 murine colon carcinoma, Nagaya *et al*. demonstrated rejection of both treated primary tumor and untreated contralateral tumor, antigen-specific T-cell response and resistance to tumor establishment upon rechallenge (116).

PS specificity for tumors can also be increased using nanoparticles. Nanoparticles accumulate in tumor tissues due to the enhanced penetration and retention effect (EPR) that results from tumor vessel leakiness (118–120,123). Song *et al*. have reported development of nanoparticles containing PS conjugated to immune checkpoint inhibitors. These nanoparticles accumulate in tumors due to the EPR effect. Targeted delivery of PS and checkpoint inhibitor delayed of tumor regrowth, prevented lung metastasis and caused systemic increase of $CD8^+$ T cells (124). Hybrid nanoparticles that release PS and glucocorticoid-induced TNF receptor family-related protein/poly(lactic-coglycolic acid) (GITR-PLGA) take advantage of the immune activating role of PDT and GITR-PLGA-mediated inhibition of immunosuppression to enhance the number of antitumor $CD8^+$ T cells in the tumor (109).

## CONCLUSION

PDT is gaining popularity across USA, Europe, Japan, China and other Asian nations due to its selectivity to tumors and multiple mechanisms of action including enhancement of protective antitumor immunity. Mechanisms by which PDT augments antitumor immunity are becoming increasingly clear as roles of immunogenic cell death, complement, inflammation and adaptive immunity are being delineated (Fig. 1). Combination therapies that take advantage of immunostimulant role of PDT will pave way for successful implementation of PDT as curative therapy in clinical setting. Successful targeting of PS and immunostimulatory agents to tumor tissue by employing nanoparticle delivery methods is a great stride forward in improving efficacy of PDT.

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