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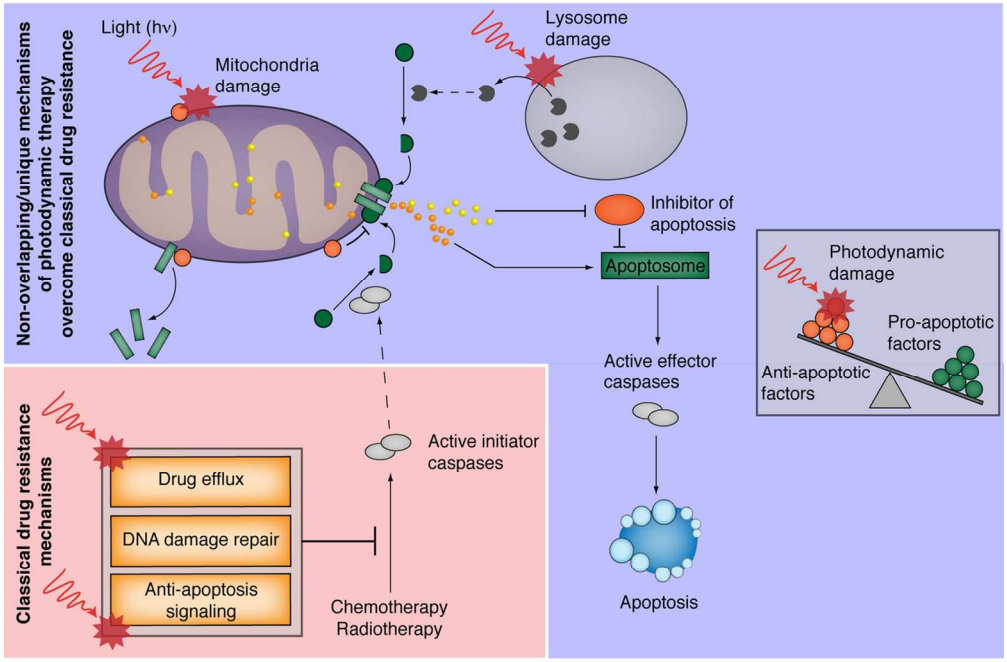

This Perspective highlights unique mechanisms of photodynamic therapy (PDT) that can be utilized to overcome classical drug resistance and to re-sensitize resistant cancer cells to standard therapies.
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Perspective

The role of photodynamic therapy in overcoming cancer drug resistance

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Many modalities of cancer therapy induce mechanisms of treatment resistance and escape during chronic treatments, including photodynamic therapy (PDT). It is conceivable that resistance induced by one treatment might be overcome by another treatment. Emerging evidence suggests that the unique mechanisms of tumor cell and microenvironment damage produced by PDT can be utilized to overcome cancer drug resistance, to mitigate compensatory induction of survival pathways and even to re-sensitize resistant cells to standard therapies. Approaches capturing the unique features of PDT, therefore, offer promise for increasing the efficacy of a broad range of therapeutic modalities. Here we highlight key preclinical findings that utilize PDT to overcome classical drug resistance or escape pathways and to enhance the efficacy of many pharmaceuticals possibly explaining clinical observations of PDT response to otherwise treatment resistant disease. With the development of nanotechnology, it is possible that light activation may be used not only to damage and sensitize tumors but also to enable controlled drug release to inhibit escape pathways that may lead to resistance or cell proliferation.

Major challenges in oncology include treatment toxicity and drug-resistance associated with advanced stage disease that cannot be completely removed by surgical resection. Because many patients present with local infiltrates and distant metastases, systemic chemotherapy has become an essential partner with surgery and radiotherapy for extending patient survival. Despite tremendous advances in each of these modes of cancer therapy, refractory disease and recurrence remain frequent. In fact, even patients who have a complete clinical response to the frontline therapies often suffer a relapse with the emergence of lethal, drug-resistant disease—stemming in part from microscopic deposits of surviving cancer cells that escape treatment by various mechanisms. For example, this is common for malignancies of the ovary[1] and the brain[2].

Drug-resistance stems from both intrinsic and acquired mechanisms. These mechanisms include alterations in the drug target, increased drug efflux and activation of signaling pathways that promote repair of damaged cellular components and that suppress cell death[3]. Many of these classical mechanisms of resistance influence both chemotherapy drugs and small-molecule inhibitors; thus, drug resistance has proven to be a tremendous challenge for gaining improvements using combinations of traditional agents. Compensatory signaling is also a common mode of resistance to molecular targeted therapeutics, where the cancer cell uses alternative pathways to compensate for the inhibition of a given pathway[3]. These adaptive processes are influenced by the tumor microenvironment[4], which can help to create a milieu conducive to resistance and escape. The epithelial-mesenchymal transition (EMT) program[4,5] as well as the cancer stem-like cell phenotype[6] are known to promote metastasis as well as resistance to cell death with decreased sensitivity to a variety of treatment modalities. For instance, cancer stem-like cells highly express drug transporters[6], are quiescent and therefore inherently less sensitive to DNA damage[6] while also possessing enhanced capacities for DNA damage repair[7]. The mesenchymal phenotype[5] can be induced by cellular, molecular or physical cues[5,8,9] in the microenvironment and promotes cell motility, survival and escape from localized stresses[4,10] as well as resistance to conventional agents[11-14]. The EMT is an important developmental program involved in cancer invasion and metastasis and can generate the cancer stem-like cell phenotype, suggesting plasticity amongst cancer cell subpopulations[15].

Therefore, an emerging concept in oncology is that many cancer therapies actually induce drug resistance as well as enhanced invasiveness and metastasis, which may explain why clinical trials of novel drugs all too often report gains in local tumor control without significant impact on overall survival (as postulated by Pàez-Ribes *et al.* in regards to antiangiogenic agents[16]). That is, increased local invasion and metastasis compensate for local tumor control. For example, this concept is now the subject of several thought provoking research and perspective articles regarding how best to inhibit tumor escape and progression in response to antiangiogenic therapy[16-19]. These findings point to the importance of utilizing mechanistically distinct, non-overlapping combination therapies to mop up mechanisms of treatment escape during each cycle of treatment. The combinations of therapeutic modalities should ideally also have non-overlapping toxicities. Dose-limiting toxicities

exist for all therapies such that combining agents with overlapping toxicities can be intolerable. If successful, rationally designed combination therapies offer great promise for reducing toxicity and for enabling the use of multiple treatment cycles to control local tumor growth whilst suppressing the emergence of drug resistance and invasion. This development may be key to achieving higher success rates in the clinic to impact patient survival.

In this Perspective, we begin by briefly introducing the principles of PDT. The following sections summarize the unique properties of PDT that overcome classical mechanisms of cancer drug resistance—including the reversal of chemoresistance and sensitization of tumors to molecular targeted agents—and how harnessing these distinctive features can make pharmaceuticals work better while also reducing toxicity. In many cases, provided the mechanistic interactions are appropriately matched, the pharmaceutical based therapy might in turn enhance PDT. The following discussion also introduces some concepts related to resistance to PDT itself but it is not meant to be a comprehensive review of these mechanisms, which we anticipate will be covered in other articles. Throughout we highlight several important examples of how the photodynamic effect induces mechanisms of physical damage to multiple cellular and tumor compartments—leading to distinct cell death signaling pathways, re-sensitization of drug-resistant cells and disruption of the tumor microenvironment. We also discuss the emergence of optically active nanomaterials and how light activation can be harnessed for both PDT and for stimulating tumor-confined, controlled drug release such that the drug is at the "right place at the right time". This development enables precise control of interactive combination therapies where PDT enhances the efficacy of a drug, and likewise, the drug mops up mechanisms of escape from PDT. Finally, we discuss prospects for broader clinical translation of PDT based on these unique advantages.

Principles of PDT

PDT is a photochemistry-based therapeutic modality in which a light-activatable chemical (photosensitizer, PS) is energized by light (600–800 nm) to produce cytotoxic molecular species via electron transfer to biological substrates (type I photosensitization), and potentially indirect excitation of molecular oxygen, or direct energy transfer to molecular oxygen (type II photosensitization)[20]. The principal feature of PDT is its intrinsic dual selectivity—both the PS and light must be present for photodamage—and, therefore, its ability for highly localized tissue damage and the absence of toxicity outside of the illumination field. Chromophores with absorption wavelengths beyond ~750 nm (<160 $kJ \cdot mol^{-1}$) lack sufficient energy for electronic coupling to facilitate the production of excited-state singlet oxygen species—a highly reactive oxygen species and key mediator of photodamage. For example, the S_1–T_1 electronic state energy gap must be at least 94 $kJ \cdot mol^{-1}$, and ideally more than 157 $kJ \cdot mol^{-1}$, to generate the major transitions of molecular oxygen[21]. Like radiation therapy, PDT requires a threshold concentration of these toxic species, which minimizes damage to surrounding tissues as long as there is a differential in PS concentration between the tumor and surrounding tissue. The differential tumor uptake of the PS can be accomplished by approaches for passive, targeted and target-activatable delivery[22-24]. A number of PSs are in clinical use or in clinical trials to treat cancer patients, including hematoporphyrin derivative, HpD (Photofrin), a first generation PS with prolonged skin phototoxicity approved by the US Food and Drug Administration for palliative treatment of obstructive lung and esophageal cancers. Second generation PSs, many with improved pharmacokinetics and reduced skin photosensitivity, include: aminolevulinic acid (ALA; a pro-PS that cancer cells assemble into protoporphyrin IX), benzoporphyrin derivative (BPD), 5-ethylamino-9-diethyl-aminobenzo[a]phenothiazinium chloride (EtNBS), silicon phthalocyanine (Pc4), m-tetrahydroxyphenylchlorin (mTHPC), mesochlorin e_6 (Mce_6), and mono-L-aspartyl chlorin e6 (NPe6). Many other PSs exist with unique properties and are in various stages of development as reviewed elsewhere[20,23].

Unique Mechanisms of PDT: Cell Death Pathways, Direct Damage to Proteins Responsible for Classical Drug Resistance and Enhanced Drug Delivery

Photodamage of antiapoptotic proteins. At the level of molecular cell biology, PDT induces mechanisms of cell death that depend on the subcellular localization of the particular PS as well as the photodynamic dose[25,26]. Most PSs localize to cellular organelle membranes due to the common core, hydrophobic aromatic ring structure with photodamage largely to these intracellular membranes, including their protein components. It is also possible to use molecular-targeted PDT to selectively damage specific proteins[27]. Cellular photodamage can lead to cell death via any of the normal modes—necrosis, autophagy or apoptosis. However, autophagy often plays a protective role in promoting cell survival after sub-lethal PDT[26]. On the other hand, protective autophagy may also play an important role in antigen presentation for PDT-stimulation of an anti-tumor immune response[26]. The two most well studied modes of PDT are photodamage of lysosomes (lyso-PDT) and mitochondria (mito-PDT). Lyso-PDT leads to spillage of proteases (e.g., cathepsins) into the cytosol, which in turn leads to cleavage (via the released lysosomal proteases rather than caspase-8) and activation of the proapoptotic factor BID (tBID)[28]. A

potential advantage of lyso-PDT is that lysosomal damage might circumvent autophagic protection[29]. PSs that localize to the mitochondria and endoplasmic reticulum selectively damage antiapoptotic proteins of the BCL-2 family, which are trafficked to the outer mitochondrial membrane[30], while the proapoptotic proteins are predominately cytosolic[31], such as BAX, and are left intact. Following the loss of antiapoptotic proteins, tBID helps insert BAX into the outer mitochondrial membrane to stimulate cytochrome c release, which in turn activates effector caspases that drive the cell along an irreversible path to apoptosis[32]. Thus, lysosomal and mitochondrial photodamage can tip the balance of the apoptosis pathway towards pro-apoptosis. This mechanism of inducing apoptosis bypasses many of the checkpoints that account for resistance to radio- and chemotherapy (Figure 1). In fact, overexpression of antiapoptotic BCL-2 family proteins, such as BCL-2 and BCL-X_L, is one of the major mechanisms of classical drug resistance[33].

Photosensitizer localization and impact on treatment outcome. Utilizing combinations of PSs that localize to different cellular compartments, Villanueva et al.[34,35] and others[36-38] have shown enhanced efficacy and synergistic induction of cell death in cancer cell cultures. In an elegant study, Kessel and colleagues demonstrated that sequential low dose lyso- plus low dose mito-PDT in cancer cell cultures achieves synergistic cancer cell photokilling via enhancement of proapoptotic signaling, whereas the reverse sequence is less effective[38] (Table 1). Cincotta *et al.* showed a synergistic enhancement *in vivo* in a subcutaneous mouse model of large fibrosarcoma tumors (~1 cm at the start-of-treatment) when EtNBS-PDT was combined with BPD-PDT to achieve a 95% tumor reduction and 76% cure rate, whereas each mode of PDT alone was ineffective[39]. The authors attributed this enhancement to different localizations of the two PSs within the tumor compartments (e.g., vascular versus tumor cell)[39]. It is also conceivable that a significant component of the robust response comes from the different subcellular localizations of the two PS—with EtNBS localizing to the lysosome (lyso-PDT) and BPD to the mitochondria (mito-PDT)[39]—such that the same low dose lyso- plus low dose mito-PDT sequence was used as proposed by Kessel.

Photodamage of drug-efflux pumps. ATP-binding cassette (ABC) transporter proteins, including ABCB1 (MDR1, P-glycoprotein), ABCC1 (MRP1) and ABCG2, constitute another class of proteins involved in classical drug resistance by increasing the cellular efflux and extracellular sequestration of many drugs[40], including some PS as a mechanism of PDT resistance (discussed below in *Mechanisms of Resistance to PDT*). In a pioneering study, Goler-Baron and Assaraf demonstrated photodestruction of ABCG2-rich extracellular vesicles associated with cancer cell drug efflux and sequestration[41]. This approach releases photosensitive drugs concentrated into these vesicles such that they can reach their intracellular targets[41]. Inspired by this report, and as another example of direct photodamage to proteins involved in drug resistance, our group has found that PDT can be applied to directly damage ABCG2 (Huang-Chiao Huang *et al.* unpublished data).

Stimulation of anti-tumor immunity. A tremendous advantage of PDT is that certain regimens stimulate anti-tumor immunity, either using PDT alone[42,43] or in combination with adjuvant immunostimulatory agents[44]. In contrast to the immunosuppressive effects of traditional therapies, low-dose PDT regimens can induce anti-tumor immunity and these regimens can be combined with high-dose PDT to achieve local tumor control with immune suppression of distant disease[43]. As reviewed in depth elsewhere[44], the mechanisms of PDT-enhanced anti-tumor immunity are under investigation. In brief, the potential mechanisms involve the acute inflammatory response following PDT, which might increase the presentation of tumor antigens to activate dendritic cells, and their homing to regional and peripheral lymph nodes—ultimately stimulating $CD8^+$ cytotoxic T cells and natural killer cells accompanied by immune memory and suppression of subsequent tumor growth upon rechallenge[43,44].

Photodamage of the tumor microenvironment. A unique property of PDT is that the PS–light interval (the time interval between PS administration and the start of photoirradiation) can be exploited to target various tumor compartments. It is possible to induce photodamage to the tumor microvasculature, the parenchyma and the stroma—or all of these compartments simultaneously—depending on the pharmacokinetics of the particular PS. For instance, the near infrared (NIR) photosensitizer BPD enables selective damage to microvessels at early time points (~15 minutes) and damage to both the cancer cells and microvasculature at later time points (60–90 minutes post-injection)[45-47]. The US Food and Drug Administration has approved BPD-PDT for the clinical treatment of macular degeneration, the major cause of blindness in older adults. For this application, BPD-PDT selectively destroys choroidal neovasculature associated with the disease while sparing the overlying neurosensory retina to preserve visual acuity[48].

Photodynamic enhancement of drug delivery. Finally, PDT can be utilized to enhance drug delivery. First, low-dose vascular PDT can be used to transiently enhance blood vessel permeability, enabling increased delivery of macromolecular and nanoparticle drug payloads to the tumor[49-51]. Although this phenomenon has been known for over a decade, more recently it was termed "super-enhanced permeability and retention (SUPR)" in reference to further boosting the enhanced permeability and retention (EPR) effect well known in tumor biology[52]. The mechanism of increased tumor vessel leakiness is attributed to the formation of endothelial intercellular gaps, which might be induced via endothelial cell microtubule depolymerization following vascular photosensitization[50]. An early demonstration of this concept by Henderson's group showed dramatic enhancements in delivery of fluorescent microspheres (0.1–2 μm) and of a liposome encapsulated formulation of doxorubicin (Doxil) to subcutaneous tumors[49]. This strategy has also been applied to enhance oncolytic virus accumulation in subcutaneous tumor models[51].

Secondly, PDT can be applied to facilitate cytosolic delivery of macromolecular drugs that normally cannot enter cells. Photochemical internalization (PCI) is a drug delivery method featuring endocytic escape—pioneered by Berg and colleagues based on the same principles as PDT for controlled delivery of novel therapeutic agents that normally cannot access their intracellular targets[53]. For PCI, the PS is used not only to elicit cytotoxic and vascular effects but also to photochemically rupture endocytic vesicles of the targeted cells to enable photoinduced release of endocytosed therapeutic agents. The therapeutic agent is then released to interact with intracellular targets rather than being subjected to lysosomal proteolysis and degradation. PCI has been demonstrated to facilitate intracellular delivery of a variety of macromolecules that do not otherwise readily enter cells, including type I ribosome-inactivating proteins (RIPs), RIP-based immunotoxins, genes and some chemotherapeutic agents[53]. PCI can be applied using PSs that are not ABCG2 substrates[54] and to kill cancer stem-like cells[55,56].

Mechanisms of Resistance to PDT

Multidrug resistance (MDR)—where cells with intrinsic or acquired resistance to a single drug also show cross-resistance to other structurally and mechanistically unrelated drugs—is often accompanied by increased expression of the ABC drug efflux transporters (introduced in *Unique Mechanisms of PDT*). Thus, many studies examining mechanisms of resistance to PDT have probed for changes in PS cellular uptake, efflux and localization. Hypoxia, stress responses and antioxidant enzymes are all possible mechanisms of resistance as reviewed previously[57]. Although hypoxia is a general limitation of PDT and many other therapies that depend on oxygen-mediated mechanisms (e.g., radiation), certain PSs can impart photodamage in environments of low molecular oxygen via type I photosensitization. For example, EtNBS-PDT has been applied to selectively kill hypoxic regions of tumor nodules grown as 3D cancer cell cultures[58]. PDT-induced tissue hypoxia as a result of vascular damage and photochemical oxygen consumption may also contribute to the appearance of resistant cells. Although mechanisms of PDT-resistance have yet to be fully elucidated, and nuances exist due to the diverse mechanisms of PDT and properties of the individual PSs, the evidence thus far points to involvement of a mix of novel mechanisms as well as some elements of classical MDR. Overall, PDT does not induce significant cross-resistance to other modes of therapy and in fact has been shown to reverse the MDR phenotype[57]. This may well be the case since PDT has not been used so far as a multiple administration modality and the problem of PDT-induced resistance may emerge only once it is administered several times as is done typically for chemotherapy and radiation therapy.

Novel resistance mechanisms. The seminal studies of PDT-resistance pointed to novel mechanisms involving changes in mitochondria structure, function and number[59,60]. In 1991, Luna and Gomer used chronic HpD-PDT to induce a PDT-resistant phenotype of mouse RIF-1 (radiation-induced fibrosarcoma) cells[59]. The RIF-1 cells were subjected to 10 cycles of HpD-PDT with each cycle administered at a dose that kills 99.9% of the wild-type cells using clonogenic assays[59]. The resulting cells are ~1.8-fold less sensitive to HpD-PDT comparing the dose needed for 90% killing of the parental versus the resistant cell line[59,60]. After this exhaustive selection protocol, alterations in the number, structure and function of the mitochondria were apparent whereas uptake, efflux and localization of the PS were not affected[60,61]. Changes in basal levels of antioxidant enzymes, reduced glutathione and stress responses were also minimal and ruled out as a contributing factor[59]. Interestingly, cross-resistance was observed towards exogenous PpIX-PDT, but not towards ALA-induced PpIX-PDT[60]. Casas *et al*. later developed ALA-induced PpIX-PDT resistant lines with 4- to 7-fold resistance but these cell lines are not cross-resistant to BPD-PDT[62]. Again, the mechanism was distinct from MDR and the resistant cells had increased numbers of mitochondria as well as increased protein content with reduced PpIX per protein, although, cellular PpIX production was similar for the parental and resistant cell lines[62]. These data highlight distinct mechanisms of

resistance to PDT, in contrast to classical MDR, and to a lack of cross-resistance amongst different PSs. This contrasts strongly with the classical MDR for which resistance to one drug leads to resistance to a broad spectrum of drugs.

About a third of PDT-resistant cell lines (resistant to PDT with a specific PS, but not necessarily to PDT with other PSs) have been found to be cross-resistant to standard chemotherapies[62]. For instance, the HpD-PDT resistant RIF-1 cells are ~1.6-fold cross-resistant to cisplatin chemotherapy[61]. However, these levels of PDT- and chemoresistance (~2–7-fold) resulting from mitochondrial alterations are both much lower than resistance levels commonly observed in drug resistant cell lines (~10–100-fold[63,64]). An interesting observation is that the HpD-PDT-resistant RIF-1 cells are several thousand-fold less efficient in tumor initiation than the parental line[59]. In contrast, drug-resistance is often associated with enrichment of the cancer stem-like cell population (often defined functionally as tumor-initiating cells based on their enhanced tumorigenic potential) that initiates tumors in mice with several orders-of-magnitude fewer cells than normally required[6]. It will be interesting to study the tumor initiation capacity of other PDT-resistant cell lines, as well as biomarkers of stemness and MDR.

Classical resistance mechanisms that impact PDT efficacy. A second class of PDT resistance does relate to classical MDR mediated by increased PS efflux[65,66]. Although HpD is not a substrate of ATP transporters (explaining why this mechanism of resistance was not apparent in prior studies with HpD), many second generation PS have been shown to be effluxed by ABCG2 (introduced above; but, not by other transporters)[65,67]. Resistance to photocytotoxicity via PS efflux was 4–30-fold depending on the PS and the treatment protocol[65]. This level of resistance approaches levels commonly found for classical MDR, in contrast to the resistant phenotype with mitochondrial changes discussed above. Thus, a concern is that PS uptake may be insufficient in certain tumor cell side populations (i.e., stem-like cells) that often express higher levels of ABCG2[67]. This effect is reversible using ABCG2 inhibitors[66], however, none are presently approved for clinical use due to adverse pharmacokinetic interactions with standard chemotherapies[40]. In addition, there are concerns about systemic use of these inhibitors, which could adversely affect transporter-mediated protection of the central nervous system (e.g., the blood brain barrier) and protection of the body broadly via the excretion of toxins (e.g., from the liver and kidneys). As an alternative to ABC transporter inhibitors, and as already mentioned, PDT can actually be used to directly damage the ABCG2 transporter (Huang-Chiao Huang *et al.* unpublished data), to downregulate cancer cell stemness (and ABCG2 expression)[68] and to rupture ABC transporter-rich extracellular vesicles, releasing high payloads of sequestered drugs[41]. These effects can be used for “chemosensitization” to enhance the effects of standard chemotherapy, as discussed more broadly in the next section.

Reversal of Chemoresistance and Potentiation of Chemotherapy

Dose-limiting toxicities and the development of treatment resistance limit the utility and efficacy of conventional chemotherapeutic agents. Despite efforts to combine different classes of chemotherapy agents with varying doses and schedules, clinical response is often not durable and produces only marginal survival benefit with poor quality of life[69]. For example, FOLFIRINOX, a combination of 4 chemotherapy drugs, generated excitement by extending patient survival of pancreatic cancer by ~4 months compared to standard gemcitabine chemotherapy, but only patients with a good performance status qualify for the treatment regimen due to its increased toxicity[70]. Broadly, the response to chemotherapy is often transient, and patients who develop chemoresistance have a dismal prognosis with little hope for effective treatment of their disease. Overcoming the resistance mechanisms that lead to treatment failure is of critical importance to improving cancer-related outcomes.

The distinct mechanisms of PDT synergize with chemotherapeutics and targeted biologics, and can reverse chemoresistance[23,71-74], and PDT has non-overlapping toxicities with these therapies. This section highlights key evidence of chemosensitization and examines the design of PDT-based combinations with chemotherapy. One crucial observation thus far is that successful implementation of PDT-enhanced chemotherapy requires a critical understanding of the biological targets, the specific chemotherapy agents and the PS being considered for the combination regimen. The following examples highlight sequence dependent effects amongst other complexities. Several combinations of antineoplastic drugs (e.g., cisplatin, carboplatin, doxorubicin, mitomycin C, and methotrexate) and PSs (e.g., HpD, indocyanine G, Mce_6, mTHPC, ALA, and BPD) have been tested with varying results[74]. The therapeutic interaction in these studies ranged from synergistic to antagonistic depending on the specific PS and chemotherapeutic combination that was evaluated, the treatment sequence and schedule as well as the tumor type[74].

Early results from preclinical models. Early investigations by Nahabedian *et al.* tested HpD-PDT in combination with cisplatin or doxorubicin in RIF-1 and EMT-6 tumor mouse xenograft models. EMT-6 tumors were only

moderately sensitive to cisplatin or doxorubicin alone at the doses evaluated in the study. HpD-PDT significantly enhanced the efficacy of doxorubicin in EMT-6 tumors, but showed no significant additional anti-tumor effect in combination with cisplatin[74]. Canti and co-workers evaluated the same chemotherapeutic agents followed by aluminum phthalocyanine-PDT in L1210 leukemia and P388 tumor-bearing mice and found an additive effect[74]. A study by Baas and colleagues highlighted the importance of the treatment schedule in designing PDT-based combinations. The authors evaluated the effect of combining HpD-PDT and mitomycin C in subcutaneous RIF-1 tumors. The mitomycin C was administered either 15 minutes before or immediately after illumination of the tumors. Mitomycin C given prior to HpD-PDT significantly increased the delay in tumor growth compared to the monotherapies, whereas there was no enhancement when mitomycin C was given after illumination[74]. A study by Kopecek et al. emphasized the importance of proper dosing to assess therapeutic interaction between two modalities. The authors evaluated a combination involving doxorubicin and Mce_6-PDT in OVCAR3 human ovarian epithelial carcinoma cells in vitro. Mce_6-PDT and doxorubicin acted additively when each treatment was administered above a dose that was 50% effective (ED_{50})[74]. The combination was synergistic when the treatments were given at 50% of their ED_{50} values[74]. Another class of chemotherapeutics includes agents such as lonidamine and levamisol, which are inhibitors of cellular energy metabolism and have a powerful inhibitory effect on oxygen consumption, aerobic glycolysis and lactate transport in neoplastic cells. These metabolic inhibitors showed a potent anti-tumor effect when combined with ALA-induced PpIX-PDT[74]. Nonaka and colleagues investigated a combination involving cisplatin and HpD-PDT in L5178 mouse lymphoma cells and found a synergistic enhancement of apoptotic cell death due in part to cooperative induction of caspases 3 activity by both PDT and cisplatin[74].

Evidence of re-sensitization and synergy in chemoresistant models. PDT is effective against a number of chemoresistant tumor cell lines: gemcitabine-resistant pancreatic cancer cell lines[75], platinum-resistant ovarian cancer cells[76], head and neck cancer stem-like cells[68] and temozolomide-resistant glioblastoma stem-like cells (Spring and Watanabe *et al.* unpublished data). PDT re-sensitizes chemoresistant ovarian cancer cell lines and patient-derived primary cultures to standard chemotherapy[73] as well as chemoresistant glioblastoma stem-like cells (Spring and Watanabe *et al.* unpublished data). The efficacy of PDT against gemcitabine-resistant pancreatic adenocarcinoma cells is instructive to consider as an example. The refractory nature of pancreatic adenocarcinoma to chemotherapeutics like gemcitabine—as well as its characteristic desmoplastic stroma—led to the hypothesis of insufficient drug delivery and stromal depletion (antistromal therapy) as an exciting avenue for enhancing drug delivery via penetration of the hypovascular stroma[77]. However, the pivotal clinical trial (NCT01130142) testing inhibition of the Hedgehog signaling pathway to elicit stromal depletion of pancreatic tumors was stopped due to a difference in survival favoring the placebo plus gemcitabine arm, which had a lower rate of progressive disease than the stomal depleting saridegib plus gemcitabine arm (Infinity Pharmaceuticals, Inc., press release). Although the stroma undoubtedly plays a major role in malignant progression, recent papers have elucidated roles of the stroma in restraining, rather than promoting, tumor progression[78,79]. PDT addresses perhaps a more fundamental challenge—pancreatic cancer cells are innately unresponsive to many drugs, even when barriers to delivery are not present. The study by Celli *et al.* showed that PDT is effective against a panel of gemcitabine-resistant cell lines by altering the balance of pro- and antiapoptotic factors towards a proapoptosis state, and this approach works even for cells that become even more resistant to gemcitabine treatment when grown in contact with an extracellular matrix (Figure 2). Therefore, PDT has the potential to be a valuable mechanism-based adjuvant to lower the doses and associated toxicities of gemcitabine treatment of pancreatic cancer.

Using patient tissue and cell lines, a study by Duska and colleagues demonstrated that photoimmunotherapy (PIT), which uses immunoconjugates to deliver the PS for enhanced PDT selectivity, reverses chemoresistance and synergistically enhances treatment efficacy[73] (Figure 3). The authors specifically investigated a combination of $chlorin_{e6}$ (c_{e6})-mediated PIT and cisplatin, a chemotherapeutic agent commonly used to manage many cancers including ovarian cancer. To increase PDT selectivity, c_{e6} was conjugated to the F(ab')2 fragment of OC-125, a murine monoclonal antibody that recognizes the cell surface antigen CA125, which is overexpressed in 85% of nonmucinous epithelial ovarian carcinomas. The potential of PIT in combination with cisplatin to potentiate toxicity in tumors was evaluated in five human ovarian and breast cancer cell lines, as well as in tumor samples obtained from 14 patients with ovarian cancer who underwent primary cytoreductive surgery. The combination produced a significant reduction in tumor viability, relative to the monotherapies and a synergistic enhancement of cisplatin efficacy was found in cisplatin-resistant samples[73]. In contrast, the effect was additive in cisplatin-sensitive samples[73].

Sequence-dependence and structural impacts on drug penetration. Results from our group revealed a sequence-dependent synergistic enhancement of carboplatin efficacy with BPD-PDT in a three-dimensional (3D) culture model of micrometastatic ovarian cancer. Treatment with BPD-PDT (1.25 μM × J·cm^{-2}) prior to low-dose carboplatin (40 mg·m^{-2}) produced a synergistic reduction in residual tumor volume, compared with PDT alone or carboplatin alone[72]. The reverse sequence, BPD-PDT after low-dose carboplatin, was not synergistic[72]. The explanation for this sequence-dependent synergism may lie in the mechanistic differences, and cooperation at the subcellular and tumor architecture levels, between BPD-PDT and carboplatin. BPD-PDT confers cytotoxicity in part by stimulating mitochondrial-induced apoptosis, which sensitizes the cells to subsequent nuclear damage and apoptotic signaling initiated by carboplatin (Figure 1). BPD-PDT also decreases the size of residual ovarian tumors and disrupts nodular architecture[72], which are key barriers to the efficacy of platinum based agents.

Emerging Concepts. An emerging consideration for the inclusion of PDT as part of the regular armamentarium for combination therapies is the potential for PDT to overcome resistance mechanisms conferred by cues from physical factors (such as flow-induced stress)[8] and communication with stromal partners. Endothelial cells, for example, are emerging as increasingly important drivers of tumor biology and response to treatment, including radiation therapy and chemotherapy[80,81]. EMT status is an additional critical determinant of response to chemotherapy and targeted inhibitors[11-14]. Early evidence suggests that PDT may overcome chemoresistance conferred by endothelial cells and may be agnostic to EMT status (Rizvi *et al.* unpublished data).

Sensitization to Molecular Targeted Therapies

While PDT successfully kills most tumor cells, like any therapy, it does also instigate molecular responses that provide growth and survival support to remaining cells. Like radiation therapy, PDT is a finite treatment that induces acute stress accompanied by bursts in molecular signaling transduction pathways in response to this damage[82] (L. Z. Zheng et al., 2009, AACR-NCI-EORTC International Conference, abstract). These signaling events support resistance to cell death via a variety of mechanisms and are mediated largely by secreted factors, cell-surface receptor tyrosine kinases (RTKs) and intracellular modulators of signaling networks. From another perspective, PDT sensitizes tumors to inhibition of these bursts in molecular signaling pathways. Combination therapies that mop up these signaling events can enhance local tumor control while also preventing increased invasion and metastasis. Here, we highlight a few molecular signaling pathways that have been exploited to enhance the outcome of PDT.

Sensitization to antiapoptotic complex inhibitors. Pioneering studies by Gomer and colleagues investigated the effects of PDT on molecular survival signaling cascades[83-85], ushering in a new area of research to understand survival signaling and optimal methods to mop up this signaling in the context of PDT. For instance, one early observation was that PDT induces the up-regulation of heat shock protein (HSP) expression[83] concomitant with increased survivin activity[85], a member of the inhibitor of apoptosis (IAP) family. The IAP family uniquely forms a final checkpoint that can impart chemo-, radio- and PDT-resistance to apoptosis by directly inhibiting the effector caspases (caspases 3 and 7). Up-regulation of HSP-90 assists survivin activity and thereby inhibition of apoptosis[85]. This effect was successfully suppressed by interfering with the HSP-survivin complex to enhance PDT cell killing using a derivative of the antibiotic geldanamycin[85].

Sensitization to kinase inhibitors. Further lines of investigation involved secreted factors and RTKs. The epidermal growth factor receptor (EGFR) is a RTK that regulates a number of critical cellular functions, including proliferation, differentiation, motility, and survival, via complex signaling cascades. Increased EGFR activity promotes cell cycle progression (G1 to S phase), causing disproportionate cell proliferation. EGFR overexpression is often associated with an increased aggressive or invasive phenotype and a poor prognosis in multiple cancers including ovarian cancer. Many approaches for targeted inhibition of the EGFR have been evaluated, but as with chemotherapy, the clinical response has shown limited durability, significant toxicities and, at best, modest improvements in patient survival. Cetuximab is a chimeric monoclonal antibody approved by the US Food and Drug Administration for the treatment of metastatic colorectal cancer and head and neck cancer. Cetuximab specifically recognizes the EGFR and competes with the native ligand, EGF, to interrupt normal cell proliferation pathways and to induce G1 arrest. To overcome the dose-limiting toxicities and frequent relapse associated with this approach, our group evaluated the effects BPD-PDT combined with cetuximab on acute tumor reduction and survival enhancement in a mouse model for advanced stage human epithelial ovarian cancer[71]. The combination treatment produced both a synergistic reduction in mean tumor burden and synergistic enhancement of median survival relative to the monotherapies, with no significant increase in toxicity[71]. A possible

explanation for this interaction involves cetuximab-mediated blockade of EGFR activity, which prevents cells that overexpress EGFR from aberrantly entering S phase, thereby inhibiting unregulated progression through the cell cycle leading to cytostatic inhibition of tumor growth. Upon inhibition of the EGFR, tumor cells that are highly dependent on these proliferation signals become particularly vulnerable to a secondary insult from a mechanistically non-overlapping treatment. Without this properly timed and rationally-selected insult, the tumor cells eventually develop compensatory pathways to overcome these inhibitory effects and escape cell-cycle arrest, which leads to treatment failure[86]. BPD-PDT complements the cytostatic effects of cetuximab by photochemically triggering apoptosis in part by inducing mitochondria-mediated apoptosis (Figure 1). Furthermore, a study by Gilaberte et al. [87] found that EGFR expression is correlated with resistance to PDT with methyl—aminolevulinic acid (MAL-PDT) in analyses of PDT-resistant squamous cell carcinoma (SCC) cells and tumor biopsies from patients with persistent SCC following MAL-PDT.

Sensitization to antiangiogenic therapy. Vascular endothelial growth factor (VEGF), is a well-studied secreted factor involved in tumor angiogenesis, growth and survival post-PDT[84,88-91]. VEGF and its RTKs (e.g., VEGFR2) represent key targets for antiangiogenic therapy, and up-regulation of VEGF signaling has been observed in response to radiotherapy[92], chemotherapy[93], cytoreductive surgery[94], and PDT[84,88]. Ferrario *et al.* introduced the concept of combining antiangiogenic therapy with PDT to improve therapeutic effectiveness motivated by their observation that HpD-PDT induces expression of hypoxia-inducible factor-1α (HIF-1α) and VEGF in mouse mammary carcinomas[84]. Our group found p38 mitogen-activated protein kinase (MAPK) induction of VEGF by BPD-PDT, without involvement of HIF-1α, in an orthotopic mouse model of prostate cancer[88]. Control of local tumor growth and reduced metastasis was observed when combining BPD-PDT with antiangiogenic agents in the same mouse model[95]. We also developed *in vivo* hyperspectral fluorescence molecular imaging to longitudinally monitor treatment-induced changes in VEGF expression, and found a burst in tumor VEGF secretion immediately post-BPD-PDT (peaking at ~6–24 hours) in subcutaneous pancreatic and prostate xenograft tumor models[82]. This latter finding points to the importance of timing molecular signaling inhibition after PDT.

Due to the bursts in tumor molecular signaling following PDT, the spatiotemporal dynamics of molecular targeted inhibitor delivery become critical. Thus, an attractive area of development is to create optically active nanoparticles that support light activated drug release in concert with PDT[96], such that the drug is present at the "right time and right place" to mop up dynamic survival signaling factors. A major contribution towards this goal are the porphyrin-liposome hybrids, termed porphyrosomes, developed by Zheng, Lovell and colleagues[97,98]. Porphyrin-phospholipd liposomes, for example, undergo reversible photopermeabilization under NIR irradiation and have been demonstrated to enable spatial control of drug release (e.g., doxorubicin)[98]. In addition, this concept has been developed for photo-induced gene transfer using optically active micelles[99].

Overcoming molecular signaling pathway co-activation, compensation and cross talk. Our group is investigating the use of light triggered release of multikinase inhibitors in combination with PDT using a single nanoconstruct. This work addresses a dilemma in oncology—many molecular signaling pathways cross talk, are co-activated in response to treatment and compensate for the loss of a given pathway[77,86,100]. VEGF, hepatocyte growth factor/scatter factor (HGF/SF) and their RTKs (VEGFR and MET, respectively) are prime examples of tumor signaling pathways that collaborate to promote treatment escape. Cancer cell MET signaling promotes the EMT, cancer cell stemness[101] as well as tumor growth, invasion and metastasis[18,102,103]. Moreover, MET signaling is upregulated in response to anti-VEGF therapy in a number of cell types (e.g., both cancer cells and vascular cells) and comprises a prominent escape mechanism from antiangiogenic treatments[18]. When the tumor vasculature is pruned by anti-VEGF therapy, the hypoxic tumor microenvironment stimulates MET expression[10,16-18]. Sennino et al. elegantly demonstrated that concurrent inhibition of the VEGF and MET signaling pathways results in enhanced antiangiogenic effects to control tumor growth while also mitigating cancer cell migration and invasive tumor growth along functional blood vessels or via lymphatic routes[10,18,104]. We recently found that pancreatic cancer cells transiently up-regulate MET signaling in response to PDT[105], which motivates a three-way interactive therapy that utilizes optically active nanoparticles for PDT with simultaneous drug release to enhance the efficacy of cancer cell death, prolonged local tumor control and suppression of metastatic escape (Spring, Sears and Zheng *et al.* unpublished data).

Prospects for Broader Clinical Translation of PDT

The complementarity of PDT with other standard therapies for cancer management suggests a broader use than is the case currently. This may be because, as for any therapeutic modality, there are concerns about the clinical

use of PDT. The most common concern is the limited penetration of far red and NIR light into tissue (attenuation depth of 1–4 mm in most tissues with photodamage reaching beyond the attenuation depth, up to ~1 cm[106]), whereas X-ray radiation penetrates much deeper (>10 cm) albeit with very high doses deposited near the tissue surface[106]. Thus, the reasoning is that PDT cannot be applied beyond the skin. However, in practice, fiber optic light conduits enable clinical applications of PDT using interstitial fiber placement directly in deep seeded tumors, and placement of multiple fibers is possible for treating large tumors. In our own clinical experience (a phase I/II clinical trial, VERTPAC), BPD-PDT produced a 1–4 cm zone of tumor necrosis (correlating with the administered light dose) with a 100% patient response rate for light delivered via optical fibers positioned percutaneously within locally advanced pancreatic adenocarcinoma tumors under computed tomography guidance[107] (Figure 4). PDT is also possible intraoperatively as an immediate follow-up to surgical debulking, as performed in the brain[108] as well as in the pleural[109] and peritoneal[110] cavities. The fact is that PDT of metastases and deep tumors is practical in the clinic with modern technology. Light transport is made efficient in lumens, surgical beds and cavities using diffusing tip fibers and scattering media (intralipid emulsion) to spread the light over large areas. For example, preclinical studies have demonstrated photodynamic tumor destruction in hepatic, pelvic, subgastric, diaphragmatic, spleen, and bowel sites with the peritoneal cavity[111], and that light delivery is feasible for PDT of cancer deposits in the following anatomical sites: the esophagus[20,23], bladder[20,23], oral cavity and larynx[112], brain[108], bone[113], lungs[20,23,109], pancreas[107], and those studding the peritoneal organs[110]. Finally, clinical trials have demonstrated feasibility, safety, and efficacy for photodynamic treatment of primary tumors in the pancreas[107], locally malignant glioblastoma multiforme in the brain[108], and disseminated, metastatic tumor deposits spread throughout the pleural (resulting from non–small-cell lung cancer)[109] and peritoneal (resulting from ovarian cancer as well as malignancies of the gastrointestinal tract) cavities[110].

A second common misconception is concern regarding heat generation during light irradiation. The light irradiances used for PDT (~0.1 $W \cdot cm^{-2}$) are an order-of-magnitude lower than those generally needed for laser coagulation and photothermal effects (~1 $W \cdot cm^{-2}$), although, a common mistake in the literature is to attribute photodynamic tumor destruction to photothermal effects under low irradiance continuous wave excitation. For example, photothermal cancer cell killing using IRDye700-antibody conjugates (and an irradiance of ~0.003 $W \cdot cm^{-2}$) has been reported as a new modality termed photoimmunotherapy (PIT)[114]; however, IRDye700 is a silicon phthalocyanine and phthalocyanines are well known to produce singlet oxygen[115]. In fact, silicon phthalocyanine Pc 4 is in clinical trials as a promising PDT agent[116]. Furthermore, phthalocyanine-antibody conjugates for cancer PIT were reported more than a decade ago as efficient PDT agents[117,118], with the general concept of PIT as a molecular-targeted approach to PDT being introduced over 30 years ago[119].

The major concern amongst those with clinical experience is the complex dosimetry of PDT. Heroic efforts have been made to ensure uniform light dosimetry by placing photodetectors into the patient during treatment for online monitoring (T. C. Zhu et al., 2013, Proceedings of SPIE, abstract). This enables the clinician to monitor photodeposition in real time. However, concerns still remain since additional factors, such as PS concentration and oxygen perfusion, influence the ultimate photodynamic dose. These are ongoing dilemmas, no different from radiation therapy, and are under intensive development. In the near term, utilizing PDT as an adjuvant alleviates many of these concerns surrounding incomplete treatment. Finally, improving the selectivity of PS accumulation within cancer cells is also key for overcoming toxicities when using PDT for wide-field treatments. As dosimetry and delivery technologies advance, we anticipate that PDT will be more widely adopted and become a core component of the armamentarium of cancer therapies.

Summary and Perspective

In conclusion, PDT has demonstrated promising clinical results for the treatment of cancer patients despite often being given as a last resort after all other options have failed. The use of PDT to address otherwise refractory disease has benefitted numerous patients worldwide—largely to provide palliation of advanced stage disease but also to obtain durable cures of some early stage cancers (e.g., >90% cure rate of oral and larynx cancers including tumors unresponsive to radiotherapy[112]). PDT has unique mechanisms of action that: (1) reverse chemoresistance and sensitize tumors to molecular inhibitors; (2) modulate vascular permeability for enhanced drug delivery and/or to induce vascular occlusion to starve tumors of nutrients; and, (3) stimulate anti-tumor immunity. Resistance to PDT itself is possible but minimal cross-resistance results with other PSs and other modes of therapy thus making it a legitimate partner, along with current conventional modalities for cancer treatment.

It is our view that, like other cancer therapies, PDT might best be utilized to potentiate a number of other modalities as part of novel frontline combination therapies. Rarely will a single treatment modality be curative. To date, there have been no clinical studies that have explored the use of PDT to potentiate chemotherapy,

radiotherapy or molecular inhibitors although there are several key publications in preclinical models to suggest the value of such a trial. Furthermore, multiple rationales motivate the integration of PDT into the clinical workflow. First, an abundance of preclinical evidence supports the use of PDT to overcome drug resistance. Second, the vast majority of PSs for PDT can be utilized for fluorescence-guided surgery[120] with follow-up PDT of the surgical bed. For instance, PS fluorescence-guided surgery with follow-up PDT doubled patient survival of glioblastoma multiforme versus standard surgery with follow-up radiotherapy (1 year versus 5.7 months) in a randomized, single center phase III trial. Third, for unresectable tumors, PDT can be applied using interstitial fibers to reach virtually any region in the body. As already mentioned, a phase I/II clinical trial demonstrated that PDT-induced tumor necrosis is feasible and safe in locally advanced, nonresectable pancreatic adenocarcinoma tumors[107]. Advances like these may pave the way forward to embark on more complex clinical trials incorporating combination regimens.

The major limitation of PDT, as for any cytotoxic modality, is the activation of tumor survival signaling pathways that promote treatment escape. That is, incomplete treatment carries risks for actually stimulating invasion and metastasis. Nanotechnology-based drug delivery vehicles are now emerging for spatiotemporally synchronized PDT and release of potent inhibitors of these molecular signaling events. These advanced drug delivery systems could be used to maximize efficacy per treatment cycle whilst thwarting survival mechanisms. The second major obstacle is alleviating the precision of light delivery needed to achieve effective PDT with minimal toxicity to off-target tissues. In contrast to traditional therapies, PDT carries an intrinsic dual selectivity for target lesions and, therefore, minimal toxicity. Nevertheless, dose-limiting toxicities to sensitive tissues has been observed in clinical trials of wide-field PDT of disseminated metastases[110]. To address this challenge, a recent advance in the field is to utilize tumor biochemistry for PS activation only in the tumor enabling fluorescence detection and tumor-confined PDT[22,24]. This approach, combined with targeting of cell surface molecules overexpressed by cancer cells, enables monitoring and selective destruction of disseminated, microscopic tumors[24] (Figure 5). These activatable PDT agents alleviate the need for precise light delivery in PDT, which should help clinicians use PDT more broadly in the clinic.

Presently, cancer patients endure toxicities associated with high intensity dose schedules needed for optimal chemotherapy, radiation and molecular inhibitor therapy. This use of high intensity dosimetry with poor efficacy per treatment cycle causes immense suffering, including: cardiovascular problems, hypertension, delayed wound healing, nausea, rash, diarrhea, hair loss, radiation scars, liver damage, gastrointestinal perforations, fatigue, immunosuppression, neurological damage, and more. PDT can be used to potentiate a number of molecular inhibitors and chemotherapy agents that have significant toxicities as single agents or additive toxicities when combined with other toxic drugs. On the other hand, chemotherapy and molecular inhibitor drugs potentiate PDT by enabling systemic effects to mop up residual tumor cells and survival signaling mechanisms. Combining these is mutually beneficial, and when done well, will enable the use of lower dosages of toxic drugs whilst maximizing impact per treatment cycle.

Acknowledgements
This work was supported by National Institutes of Health Grants R01-CA156177, R01-CA158415, R01-CA160998 and P01-CA084203 (to T.H.); and, K99-CA175292 (to I.R.).

Figures and Tables

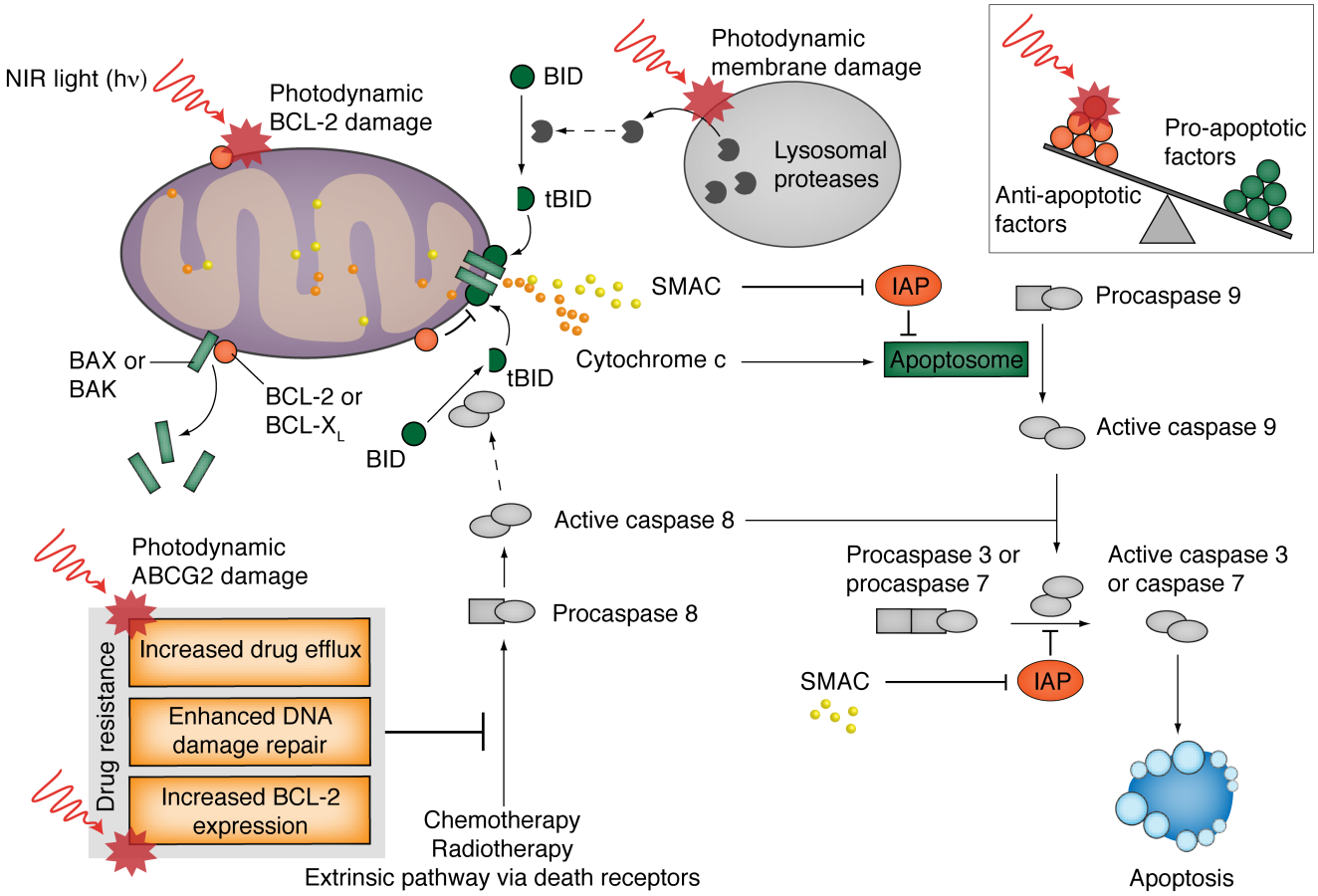

Figure 1. Overview of unique mechanisms of PDT-induced apoptosis. PDT directly damages antiapoptototic factors and drug efflux pumps involved in classical drug resistance. The antiapoptotic BCL-2 family proteins (e.g., BCL-2 and BCL-X_L) reside on the outer mitochondrial membrane and prevent mitochondria-mediated apoptosis by inhibiting the oligomerization and activation of the proapoptotic family members (e.g., BAX and BAK)[30]. Proapoptatoic proteins like BAX are in dynamic equilibrium between the cytosol and mitochondria outer membrane but largely within the cytosol of healthy cells[31]. Therefore, mito-PDT is observed to predominately destroy antiapoptotic factors. Lyso-PDT induces release of lysomomal proteases into the cytosol that can cleave BID (independent of capase 8) to form truncated BID (tBID), which tranlocates to the mitochondria to promote oligomerization of BAX and BAK. BAX and BAK oligomers form pore complexes that release cytochrome c and SMAC (second mitochondrion-derived activator of caspases) from the mitochondrial intermembrane space. Once released into the cytoplasm, cytochrome c forms a complex with apoptotic protease-activating factor 1 and procaspase 9, called the apoptosome, to activate caspase 9. SMAC, once it is released into the cytosol, promotes caspase activation through binding the IAPs (inhibitor of apoptosis proteins) and blocking their antiapoptotic activity. Once activated, the effector caspases (e.g., caspase 3 and caspase 7) carry out cellular degradation proceeds to execute the apoptotic program.

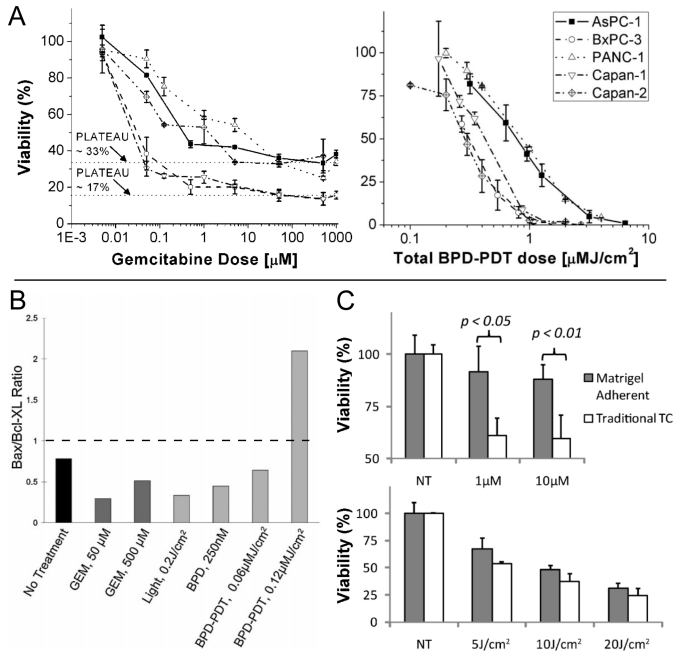

Figure 2. Cancer cells that are unresponsive to sustained gemcitabine chemotherapy are sensitive to BPD-PDT. (A) A panel of pancreatic adenocarcinoma cell lines contain gemcitabine unresponsive populations (17–33%) even at extreme gemcitabine doses (up to 1 mM) while moderate BPD-PDT doses (1–6 $J \cdot cm^{-2} \cdot mM$, where the units reflect the product of the light dose and the PS concentration; e.g., 10 $J \cdot cm^{-2}$ × 0.25 mM BPD = 2.5 $J \cdot cm^{-2} \cdot mM$) produce nearly complete cancer cell death. (B) BPD-PDT decreases BCL-X_L and increases the ratio of BAX-to-BCL-X_L toward a proapoptotic balance (data are results from quantification of western blots). (C) Insensitivity to gemcitabine (*top*), but not to BPD-PDT (*bottom*), is increased in cells that are adherent to Matrigel basement membrane relative to traditional tissue culture (TC) conditions (NT indicates the no treatment control). Collectively these results indicate the ability of PDT to bypass intracellular and extracellular cues leading to gemcitabine resistance and point to the emerging role of PDT for pancreatic cancer treatment. Adapted from Celli et al. (2011)[75].

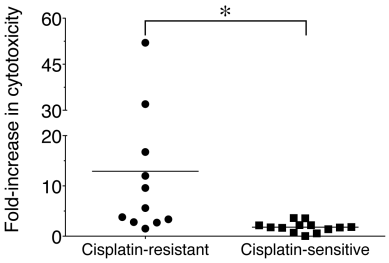

Figure 3. Photodynamic therapy reverses chemoresistance and synergizes with chemotherapy to destroy platinum-resistant disease. Fold increase of cytotoxicity following photoimmunotherapy (antibody-PS conjugates) in combination with cisplatin (platinum chemotherapy)—versus cisplatin alone—in cisplatin-resistant (•) and –sensitive (■) patient-derived samples and fcell line cultures. A total of 19 solid tumor and/or ascites samples were collected from 14 ovarian cancer patients (ages 37–80, stages 1C–4), and 5 cancer cell line cultures were also included. Cisplatin resistance versus sensitivity refers to whether the patient had disease progression or recurrence within 6 months of platinum chemotherapy. Photoimmunotherapy induces a 12.9× enhancement in cytotoxicity against platinum resistant primary cultures (ranging from 1.5–52×) versus 1.8× for platinum sensitive cells. The asterisk indicates $P<0.05$. Adapted from Duska et al. (1999)[73].

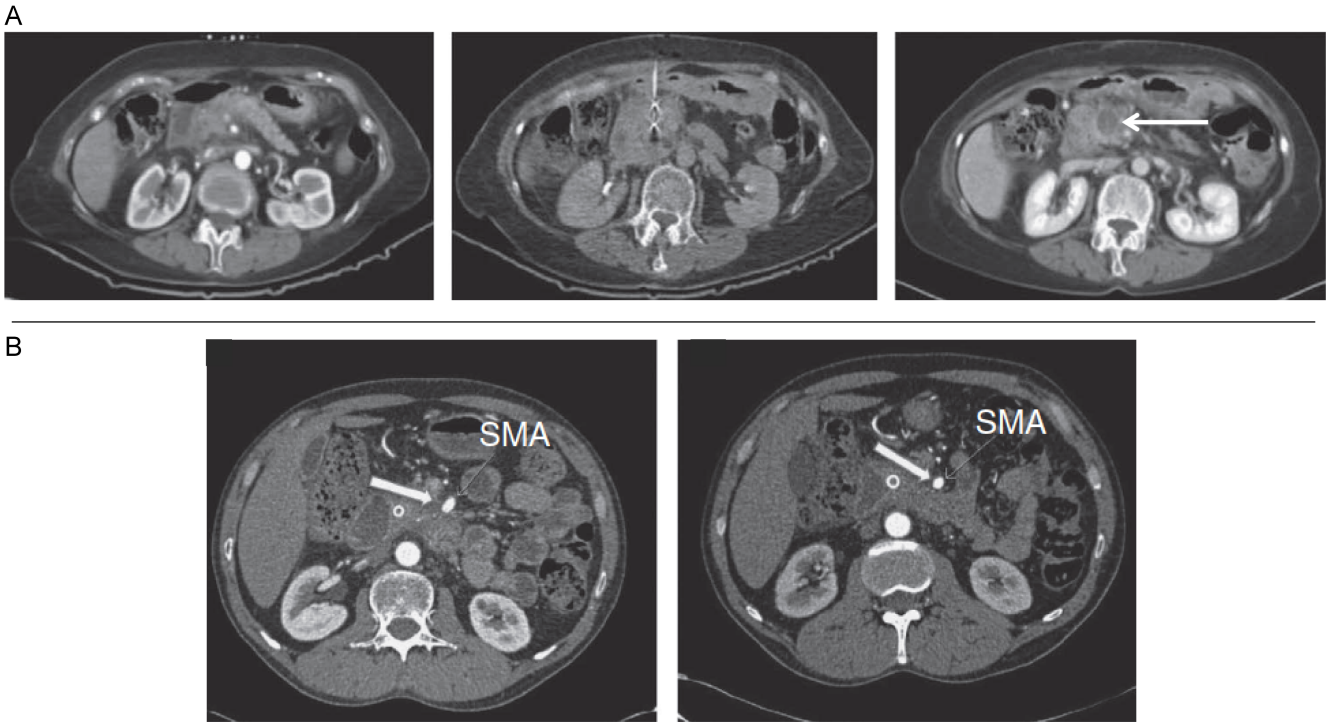

Figure 4. PDT of locally advanced, inoperable pancreatic adenocarcinoma in humans. (A) Contrast-enhanced computed tomography (CT) scans from a patient undergoing BPD-PDT. The images show (*left*) a low attenuation mass in the head of the pancreas prior to treatment, (*center*) placement of a percutaneous needle for fiber optic light delivery into the tumor, and (*right*) a 2.67 cm^3 zone of tumor necrosis 5 days post-PDT. (B) CT scans from a patient who qualified for and underwent a successful Whipple's tumor resection following PDT. The pre-PDT image (*left*) shows the tumor abutting the superior mesenteric artery (SMA; arrow); thus, this tumor was inoperable at presentation. Four weeks after PDT, the (*right*) follow-up CT scan for the same patient shows tumor reduction and minimal involvement with the SMA such that surgical resection could then be performed safely. Adapted from Huggett et al. (2014)[107].

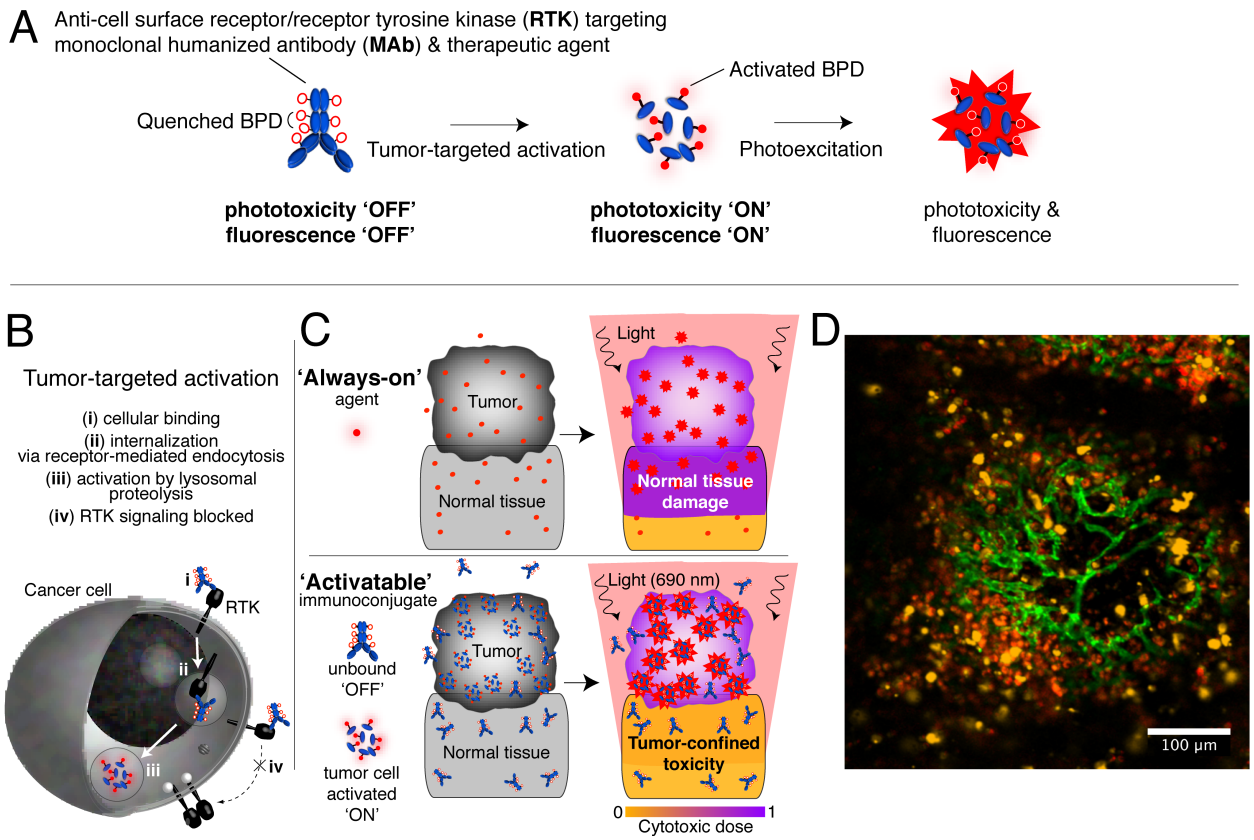

Figure 5. Concepts of tumor-targeted, activatable photoimmunotherapy (taPIT). (A) Activatable immunoconjugates for taPIT are comprised of multiple self-quenching, photocytotoxic chromophores conjugated to antibodies that target and neutralize key molecules involved in tumorigenesis (e.g., EGFR). (B) Cellular activation of the immunoconjugates via receptor-mediated endocytosis and lysosomal degradation. (C) taPIT concept in which the immunoconjugates accumulate selectively within the tumor nodules, are activated by cellular processing, inhibit molecular signaling and impart selective cytotoxicity to neoplasms upon irradiation while sparing neighboring vital tissues. (D) *Ex vivo* whole mount immunofluorescence image of a micrometastasis where an anti-human cytokeratin antibody has been applied to visualize the human epithelial cancer cells (orange), an anti-mouse CD31 antibody labels the endothelial cells (green) and immunoconjugates taken up and activated by tumor cells *in vivo* (red). taPIT enables safe use of 50× the photodynamic dose (PS × light dose) versus "always-on", unconjugated BPD, and 17× the photodynamic dose versus "always-on" PIT (using cetuximab-ce$_6$ conjugates) in a mouse model of peritoneal disseminated micrometastatic epithelial ovarian cancer[24]. A single cycle of taPIT plus chemotherapy reduces the micrometastatic burden by 97% versus 3% for chemotherapy alone in the same mouse model[24,121], using human chemoresistant OVCAR5 cells[64,122]. Wide-field taPIT was accomplished by administering scattering media (Intralipid) to the peritoneal cavity and NIR laser light via a cylindrically diffusing fiber optic tip. Adapted from Spring et al. (2014)[24].

Table 1. Cancer cell colony formation (murine hepatoma 1c1c7 cells) following lysosomal (NPe6, 660 nm)- plus mitochondrial (BPD, 690 nm)-PDT, versus the reverse sequence. Adapted from Kessel and Reiners Jr. (2014)[38].

PDT regimen	Clonogenicity (%)
No treatment control	100 ± 3
Low-dose mito-PDT	83 ± 5
Low-dose lyso-PDT	95 ± 2
Low-dose lyso-PDT ⇒ low-dose mito-PDT	17 ± 3*†
Low-dose mito-PDT ⇒ low-dose lyso-PDT	58 ± 4*

Data represent average ± SD. *Statistically different from controls; †statistically different from result obtained with reverse order of irradiation ($P < 0.05$).

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