Targeted Photodynamic Therapy

Nicolas Solban, PHD, Imran Rizvi, MS, and Tayyaba Hasan, PhD*
Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts

Background and Objectives: Photodynamic therapy (PDT) is an emerging modality for the treatment of various neoplastic and non-neoplastic pathologies.
Study Design/Materials and Methods: PDT usually occurs when reactive oxygen species (ROS) generated from light-activated chemicals (photosensitizer, PS) destroy the target. For non-dermatologic applications the PS are delivered systemically and accumulate, at different concentrations, in most organs.
Results and Conclusion: Typically there is a modest enhanced accumulation of the PS in tumor tissues, providing a first level of selectivity. Additional selectivity is provided by the confined illumination of the target area with the appropriate wavelength of light. For the treatment of pathologies in complex anatomical sites, such as in the peritoneal cavity, where restricted illumination is difficult; improved targeting of the PS is necessary to prevent damage to the surrounding healthy tissue. This article will focus on targeted PDT. Lasers Surg. Med. 38:522–531, 2006. © 2006 Wiley-Liss, Inc.

Key words: photodynamic therapy; targeted delivery; photosensitizer

INTRODUCTION

Photodynamic therapy (PDT) consists of the systemic or local administration of a photosensitizer (PS), its preferential accumulation in malignant tissues, and its subsequent activation by visible light. In the presence of oxygen, this activated PS can generate reactive oxygen species (ROS) that are toxic to the tumor [1–3]. For most PS in clinical and preclinical use, three primary mechanisms of PDT-mediated tumor destruction in vivo have been proposed: cellular, vascular, and immunologic. The relative contribution of each depends (1) on the nature of the PS and its localization within the tumor tissue, (2) on the tumor type (vascularity and macrophage content), and (3) on the time delay of irradiation after PS administration (which is one determinant of site of localization, for example, vascular vs. parenchymal). Vascular damage is the dominant mechanism of tumor death when irradiation is performed when the PS is in the vasculature, while direct tumor cell destruction is expected to dominate when the PS content is high within the tumor cells at the time of light activation. For intratumoral PS the mechanism of direct tumor cell destruction is dependent on the subcellular localization of the PS. In a series of studies, Kessel et al. [4,5] have shown that PS that localize in mitochondria are very rapid inducers of apoptosis, in contrast to PS localized in lysosomes and plasma membranes [4–6], which can induce necrosis, possibly because of the release of lysosomal enzymes and other toxic moieties. There is, however, a possibility of lysosomally localized PS relocating to mitochondria within the first few seconds of illumination, where they may be considerably more phototoxic [7].

Several thousand patients have already been treated with PDT for a variety of advanced neoplasms, and have shown an improvement in their quality of life and a lengthened survival [2,8]. With the use of modern fiberoptic systems and various types of endoscopy, light can be targeted accurately to almost any part of the body for the treatment of tumors. Selectivity of PDT is achieved from the somewhat preferential localization of the PS in target tissue and from the irradiation of a specified volume. Provided that the PS is not toxic, only the irradiated areas will be affected, even if the PS does bind to normal tissues. This rational works well if light can be directed specifically to the lesion as in dermatologic and ophthalmologic applications. However, for applications of PDT in complex anatomical sites, such as the abdominal or thoracic cavities, confined irradiation is not possible, and targeted PS delivery becomes necessary. We discuss three strategies for achieving enhanced PS concentration in target tissue.

PASSIVE TARGETING OF PS

Some systemically administered PS tends to accumulate preferentially in tumors compared to normal tissue [9]. The reason for this preferential accumulation is not clearly understood, but factors relating to tumor architecture as well as properties of the PS may play a role. This passive PS targeting may result from greater proliferative rates of neoplastic cells, poorer lymphatic drainage, leaky vasculature, or more specific interaction between the PS and neoplastic cells due to the charge, size, and structure of the PS.

One property of an injected molecule that determines its site of action is its degree of hydrophilicity. As a general

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*Correspondence to: Tayyaba Hasan, PhD, Wellman Center for Photomedicine, Massachusetts General Hospital, 40 Blossom Street, Boston, MA 02114. E-mail: thasan@partners.org
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rule, hydrophilic molecules usually remain in circulation until excreted, while hydrophobic compounds leak out of the vessels and are retained in tumor tissue [10]. This dynamic process results in a preferential accumulation of PS in tumors, and early clearance from the blood after injection. Therefore, based on two therapeutically relevant compartments of malignant tumors, PS are often classified as cellular or vascular [11], a reference to the primary site of action under the conditions in which they were studied. However, it is important to remember that the transport of a PS in vivo is a dynamic process and the timing of the light exposure after administration is important. The appropriate choice of delay time after PS administration may allow for targeting of different compartments within the tissue (e.g., vascular and extravascular) using the same PS.

The most commonly used strategy to improve the delivery of PS to target tissue involves their encapsulation in colloidal carriers, such as liposomes, oil-dispersions, polymeric particles, and polymers to facilitate drug delivery [12,13]. It was noted that accumulation of PS in tumors increases upon increasing the degree of hydrophobicity [14], however since hydrophobic PS are not very water-soluble, intravenous treatment is difficult. Therefore, most of the PS currently in use are encapsulated hydrophobic molecules. Furthermore, these formulations were shown to increase PS delivery to tumors. It has been shown that systemically administered PS bind to low density lipoprotein (LDL) [15], therefore it has been suggested that LDL receptors on the surface of tumor cells and on tumor vascular endothelial cell [16,17] are important for the uptake of PS. For example, the liposomal formulation of the PS benzoporphyrin derivative (BPD) enters tissues more rapidly and has a higher accumulation in tumor than non-liposomal BPD [18]. It was shown that 1 hour after administration of liposomal BPD, 91% is associated with lipoprotein, compared with only 50% of the non-liposomal BPD [18]. Other factors may also contribute to the retention of PS in tumors, such as the high amount of collagen in tumor stroma which was also shown to bind porphyrins [19], or to the high amount of lipid in tumors, which has a high affinity for lipophilic dyes [10].

TARGET MODULATION TO INCREASE PS ACCUMULATION

One strategy to selectively enhance PS levels in a disease site is to modulate the target tissue to produce more PS than the surrounding normal areas. δ-aminolevulinic acid (ALA)-based PDT uses a precursor (ALA) to stimulate the in situ synthesis of a PS, protoporphyrin IX (PpIX). There are two possible ways to increase PpIX levels in target tissues: (1) increase delivery of ALA or (2) increase the synthesis of PpIX by the target tissue. There are various formulations of ALA designed to increase its uptake, and the readers are referred to recent reviews for more details [20,21]. In this section we will describe ways to increase the production of PpIX by the target tissue.

ALA is a naturally occurring precursor in the biosynthetic pathway for heme production. The last step in this biosynthetic route involves conversion of PpIX, a photosensitizing species, to heme (a non-photosensitizing agent). Under physiologic conditions, heme synthesis is regulated in a negative feedback control of the enzyme ALA synthase by free heme; therefore, ALA synthesis is the rate-limiting step. However, when exogenous ALA is added, the control mechanism is bypassed, and downstream metabolites are synthesized in excess. Under these conditions, ferrochelatase, which catalyzes iron insertion into PpIX, becomes the rate-limiting enzyme. Following the addition of exogenous ALA, the low physiologic rate of iron insertion by ferrochelatase is unable to compensate for the excess PpIX that is formed. PpIX, therefore, accumulates in cells and renders them photosensitive.

A method to increase the accumulation of PpIX currently under investigation involves the co-administration of PpIX together with an iron chelator. Since iron is required for the formation of heme from PpIX, removal of iron should increase PpIX levels. The iron chelator desferrioxamine (DFO) has been shown to increase PpIX levels in various cancer cell lines [22,23]. However, in a recent clinical study, DFO was shown to increase PpIX levels after exposure to a low ALA dose only in healthy skin and not in skin with superficial skin tumors [24], therefore potentially limiting its clinical usage. Another iron chelator, 1,2-diethyl-3-hydroxypyridin-4-one (CP94) has been used in preclinical studies in normal rat colon [25]. CP94 administered with ALA produced double the PpIX fluorescence, while PDT treatment produced necrotic area three times larger than with ALA alone. CP94 could potentially be used for systemic ALA-PDT where lower (better tolerated) dose of ALA is required.

In another strategy to increase production of PpIX by the target tissue, our group looked at the effect of cellular differentiation. It is generally accepted that proliferating tissues and malignant cells are more efficient in ALA-PpIX formation [26] than normal tissue. However, our group has documented an inverse relationship, in which cellular differentiation was associated with an increase in PpIX formation [27]. For example, in differentiated (growth-arrested) primary keratinocytes, an increased production of ALA-PpIX was measured when compared with their non-differentiated (proliferating) counterparts [28]. This increased cellular PpIX content enhanced PDT efficacy. The same increase of ALA-PpIX formation with cellular differentiation was found in other cell lines including a human prostate cancer cell line (LNCaP) [28,29]. In the LNCaP cells, differentiation was induced with a synthetic androgen receptor ligand, and Figure 1 shows a dramatic increase in the PpIX content in differentiated LNCaP cells by confocal scanning fluorescence microscopy after 4 hours of ALA exposure. Quantification showed a more than tenfold enhancement in the PpIX content of the differentiated LNCaP cells over the undifferentiated ones, along with an increase in PDT responsiveness. To have a more clinically relevant model, since androgen could never be administered to treat prostate cancer, we tested methotrexate (MTX) in combination with ALA-PDT. MTX is a widely used anticancer agent, shown to induce cellular

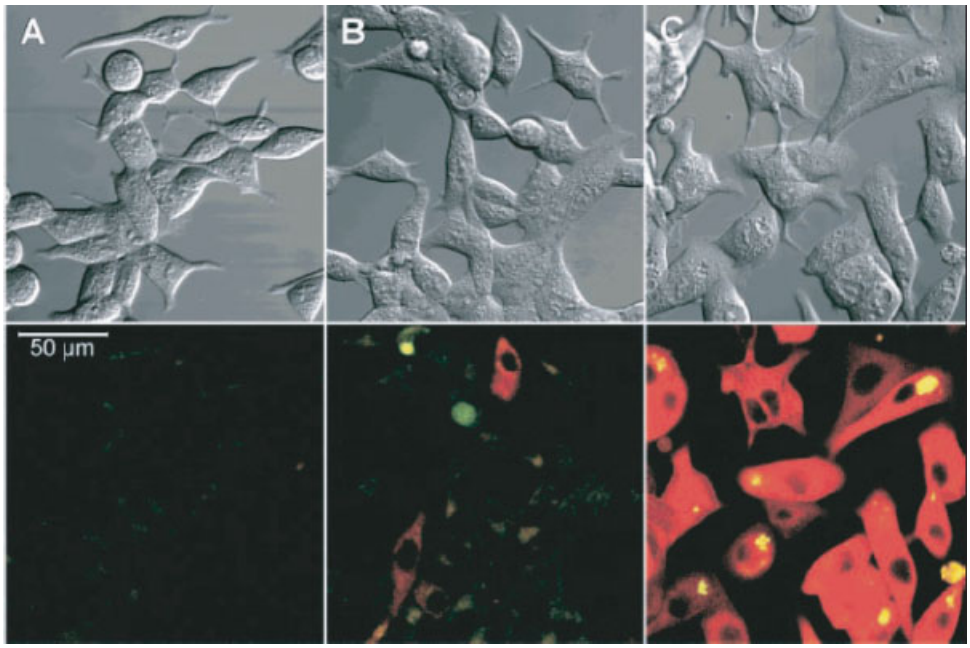


Fig. 1. PpIX fluorescence in living cells. LNCaP cells were pretreated with vehicle (**A**, **B**) or with 10^{-7} M R1881 for 72 hours (**C**). Microscopy transmission and fluorescence images of untreated LNCaP cells (A) and cells exposed to 0.3 mm ALA (B, C) without (B) or with (C) pretreatment with R1881 [27].

differentiation [30,31]. MTX pretreatment of prostate cancer cells followed by ALA administration resulted in a 3∼10-fold increase in PpIX levels and enhancement of cell killing. These findings suggest a potential for a new combined therapeutic regimen, where induction of differentiation precedes ALA-based PDT and makes tumors more susceptible to photosensitization. We are currently evaluating the combination of ALA with MTX in preclinical studies of orthotopic prostate cancer.

TARGETED PS DELIVERY

A third strategy for selective PS delivery utilizes targeting moieties, such as monoclonal antibodies (MAbs), directed against antigens or ligands that are specifically overexpressed on cancer cells. An important determinant of successful PDT is the localization of the PS at the time of irradiation. More precise drug targeting is therefore desirable in order to ensure success but also to reduce toxicity to uninvolved tissues and organs in complex sites, such as in the abdominal cavity. It is important to note that contrary to conventional targeted chemotherapy where the drug has to be released from the carrier moiety to elicit a response, this is not a prerequisite when carrier molecules are used for delivery of PS in PDT. Furthermore, the requirements for specificity of the delivery molecule are less stringent in PDT, due to the dual selectivity of the treatment. As long as the delivery agent has preferential (not necessarily exclusive) affinity for the target tissue, improved selective phototoxicity is expected. Thus, carrier-mediated PDT offers the possibility of using non-tumor specific targeting molecules, providing a greater repertoire of usable compounds. However, the problems associated with the use of large molecules, such as complicated synthesis, transport barriers, and potential systemic toxicity, are similar for PS conjugates and for other conjugates. For example, work by Savellano et al., compared cellular uptake and phototoxicity of free BPD to BPD conjugated to an epidermal growth factor receptor (EGFR) MAb. BPD is a hydrophobic and lipophilic small molecule that rapidly diffuses into the cell, whereas the conjugate is a macromolecule that enters the target cells via receptor-mediated endocytosis. They found that the amount of BPD/cell was comparable between free BPD and the conjugate after 14 hours incubation. However, to obtain similar phototoxicity, a 40 hours incubation period was required [32]. They subsequently showed that this longer incubation time was necessary for lysosomal degradation of the conjugate and the release of the highly phototoxic free BPD [33]. In this section we will further describe some macromolecular carriers used to specifically target PS to tumor tissues.

Photoimmunotargeting (PIT)

Tumor targeting with antibodies is based on the concept that molecular markers are present on tumor cells, and the ability to obtain specific MAbs that recognize these markers. It is thought that neoplastic transformation generates new and specific antigens not present on normal cells. However, this is not the case with all tumors, and MAb with a high level of specificity for tumor markers are extremely rare. Photoimmunotargeting (PIT) has therefore many advantages over standard

immunotherapies because it combines two therapeutic principles. The molecule recognized by the MAb does not have to be expressed exclusively on neoplastic tissues and the MAb does not need to have an intrinsic effector function. This means, the MAb does not need to initiate on its own a reaction that would lead to tumor destruction. However, PIT requires conjugates with high PS to MAb ratios, which makes the synthesis complicated. PS can be linked chemically to the MAbs directly or they can be linked via polymers [34,35]. The chemical reactions that are necessary for the synthesis and the resulting structural alterations of the molecules' environments may lead to a loss of activity of the MAb (reduced affinity) or of the PS (reduced singlet oxygen yield upon irradiation). In an ideal scenario the PS and antibody activities will be preserved, while at the same time allowing maximal PS incorporation [36].

Targeting of hydrophilic PS. Most PS in use are able to leak out of circulation and accumulate in tumors because they are hydrophobic molecules. By the same token, these molecules tend to aggregate readily in aqueous solutions and therefore require complex lipid-based formulation for systemic delivery. On the other hand, hydrophilic PS do not require lipid-based formulation for delivery but they remain in circulation and do not accumulate in tumors. Tumor targeting MAb can leak out of circulation and accumulate in tumors; therefore, conjugating hydrophilic PS to MAb would increase tumor retention of the PS and therefore increase the repertoire of available PS for tumor treatment. A study [37] tested this hypothesis. Using the hydrophilic PS TrisMPyP-Phico(2)H conjugated to the internalizing antibodies cMAb U36 or mMAb 425, it was shown that these conjugates were phototoxic to A431 cells, while non-internalizing Ab conjugates and free PS were harmless. The same group also showed that this method was effective in targeting aluminum (III) phthalocyanine tetrasulfonate, another hydrophilic PS, to tumors using these internalizing antibodies [38].

Targeting the epidermal growth factor receptor (EGFR). Many cancers, including head and neck cancers (oral cancer and precancerous lesions) overexpress EGFR. These findings suggested that the overexpression of EGFR might be used as a marker for early diagnosis and treatment of oral precancer. With this as a focus, we have tested an anti-EGFR MAb coupled to either the near infrared fluorescent dye Cy5.5 for detection or to the photochemically active dye, chlorin (ce_6) for therapy of premalignancy in the hamster cheek pouch carcinogenesis model [39]. In this model, malignant transformation is chemically induced, and EGFR overexpression is also detected [40], therefore adequately mimicking clinical pathologies. Targeting the EGFR with antibody-delivered photoactive molecules may destroy the EGFR overexpressing cells, while the normal, low expression levels of EGFR in the healthy tissue would not lead to enough PS accumulating in the normal mucosa. Targeted PDT would therefore cause only the premalignant lesion to regress, and the MAb bound fluorescent dye would allow the monitoring of the progress of treatment. Figure 2 shows a representative image from an animal with a papillary tumor measuring 5 mm in diameter. The normal cheek pouch has no visible fluorescence (Fig. 2a), whereas the tumor-bearing pouch has a clearly delineated tumor in the fluorescence image (Fig. 2b) after systemic injection of anti-EGFR-Cy5.5 MAb conjugate [39]. Cy5.5 has several advantages over fluorescein, a dye commonly used for diagnosis in animals and humans: it has good solubility, high fluorescence quantum yield, and a longer emission wavelength (emits at 702 nm after 675 nm excitation). This should lead to increased sensitivity of detection of premalignant lesions because fluorescence from molecules localized deeper into tissue can be detected, and background autofluorescence is minimized because the endogenous fluorophores present in tissue do not absorb at 675 nm. We have also conjugated EGFR-MAb to the PS Ce_6. Following PDT with this conjugate we found that the overexpression of EGFR in carcinogen-treated hamsters was significantly reduced to background levels compared to non-illuminated areas. The difference between illuminated and dark areas was not seen in the normal cheek pouch. These results demonstrate the potential for development of

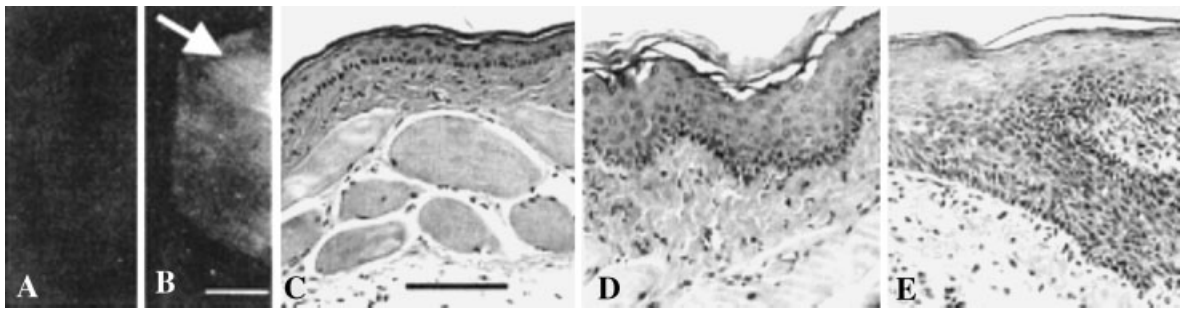


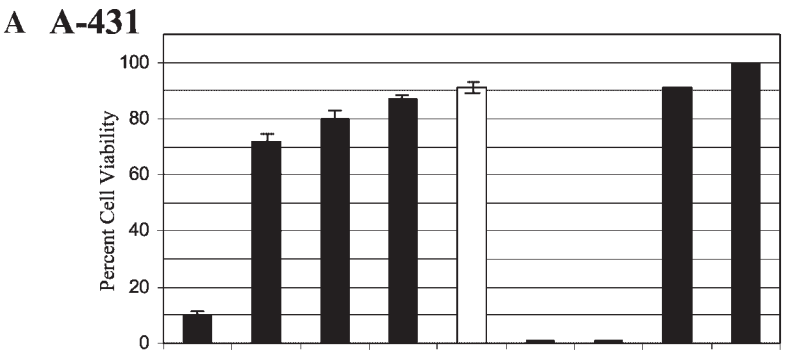
Fig. 2. Fluorescence images of normal (**A**) and carcinogen-treated (**B**) hamster cheek pouches, 6 days after the injection of the EGFR-Cy5.5 conjugate (bar, 5 mm). An area of increased fluorescence can be seen within the carcinogen-treated cheek pouch (arrow). H&E histology shows normal mucosal epithelium for the carcinogen-untreated cheek pouch (**C**) (bar, 100 μm), mild dysplasia for the carcinogen-treated cheek pouch (**D**), and moderate dysplasia for the hot spot (**E**) [39].

immunotargeted photodiagnosis as a diagnostic tool and as a method of monitoring response to therapy. Therefore, the real utility of PIT may lie in its use as a detection modality and therapeutic response monitoring methodology. Clearly, similar approaches can be used for many other intra- and extracellular molecular targets. Savellano et al. have conjugated the PS BPD to this EGFR MAb. Figure 3, which shows specific phototoxicity using this photoimmunoconjugate on A-431, an EGFR-expressing cell line (Fig. 3A), while no phototoxicity is observed in NR6, an EGFR-negative cell line (Fig. 3B) [32]. In another study [41] they have used two anti-HER2 MAb that recognizes different epitopes and conjugated them to the PS pyropheophorbide-a. Targeting HER2 with two MAbs was significantly more effective at killing HER2 positive cells than free PS or single epitope targeting, and did not have any effect on HER2 negative cells. This study demonstrates a way to increase PS delivery to cancer cells.

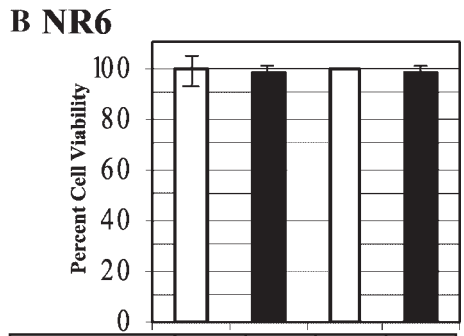
Another targeting strategy using antibodies involves a dual-step method wherein each modality is administered separately. Here, increased PS delivery is not necessarily the goal; instead the strategy is to enhance treatment response based on a mechanistic understanding of the pathology. With the goal of developing more effective treatment strategies for ovarian cancer, we tested a novel combination regimen using BPD-based PDT in conjunction with anti-EGFR Mab C225 [42]. We reasoned that by using mechanistically distinct treatment modalities directed against non-overlapping cellular targets, we could improve the efficiency of the individual therapies and limit treatment-related toxicity, which is particularly important in a complex site such as the peritoneal cavity.

Advanced epithelial ovarian cancer remains a difficult illness to treat, with less than a third of patients surviving to 5 years, mainly due to the development of chemoresistant disease [43]. EGFR overexpression is seen in 20%–30% of advanced ovarian cancers and is associated with a poor prognosis and an aggressive and invasive phenotype [44,45]. We used a xenograft mouse model for human ovarian carcinomatosis previously developed in our laboratory [46], which closely mimics the clinical disease pattern for advanced stage ovarian cancer. These mice were treated with BPD-PDT, using a diffusing tip fiber delivered via a catheter into the peritoneal cavity and four doses of C225 were administered between the two PDT treatments. In acute studies, the mice were sacrificed on day 21 and tumor burden was evaluated (Fig. 4). Compared to mice treated with PDT only or C225 only, there was a synergistic reduction in tumor burden in mice treated with PDT+C225. Relative to no treatment controls, there was a tenfold reduction in tumor burden in mice treated with the combination regimen.

Similarly, the combination treatment, PDT+C225, synergistically enhanced survival compared to PDT only



C225 PIC	1x	1x	1x	1x	1x	-	-	-	-
C225	-	1x	2x	4x	4x	-	5x	5x	-
Free BPD	-	-	-	-	-	+	+	-	-
Light 20 J/cm^2	+	+	+	+	-	+	+	+	+



C225 PIC	1x	1x	-	-
Light 20 J/cm^2	-	+	-	+

Fig. 3. Phototoxicity experiments. The molar loading ratio of the PIC was ~7 BPD/antibody. Light doses were either 0 (□) or 20 J/cm^2 (■). **A**: EGFR-overexpressing A-431 cells were incubated at 37°C for 40 hours with C225 PIC (approximately 20 nM antibody content or, equivalently, 140 nM BPD content), either in the absence or in the presence of 1×, 2×, or 4× fold equivalents of unmodified C225 antibody (approximately 20, 40, and 80 nM antibody content, respectively). Control experiments included 140 nM free BPD, 140 nM free BPD+5× equivalents (100 nM antibody content) of unmodified C225, 5× equivalents of unmodified C225 and media only. **B**: EGFR-negative NR6 cells were incubated at 37°C for 40 hours with C225 PIC (approximately 40 nM antibody content or, equivalently, 280 nM BPD content). Percent viability is relative to media only [32].

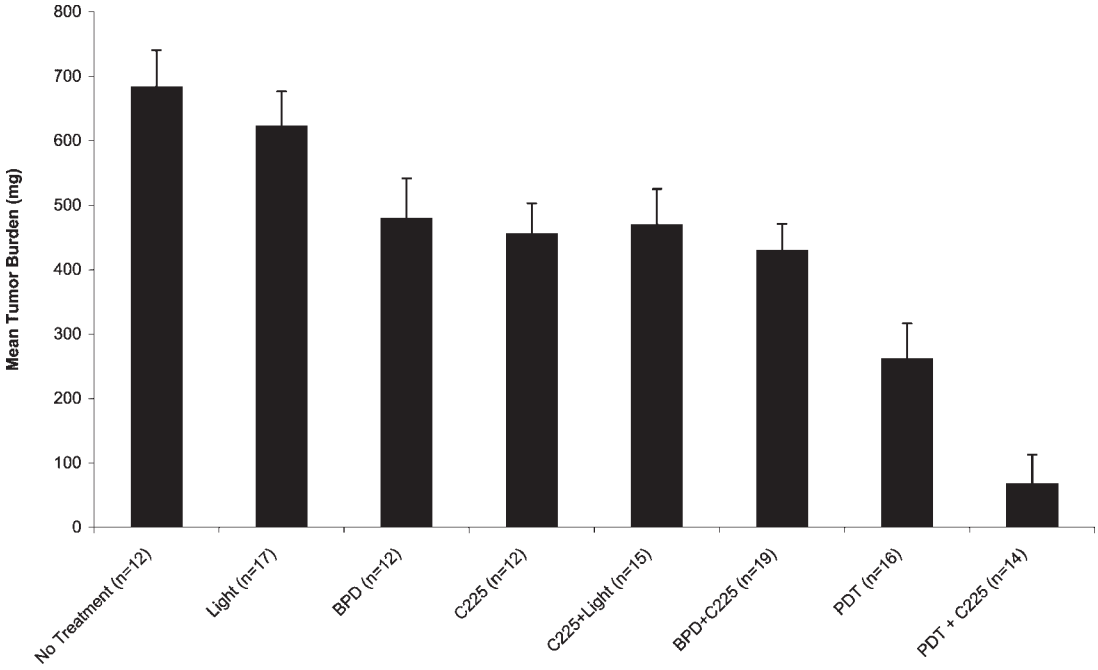
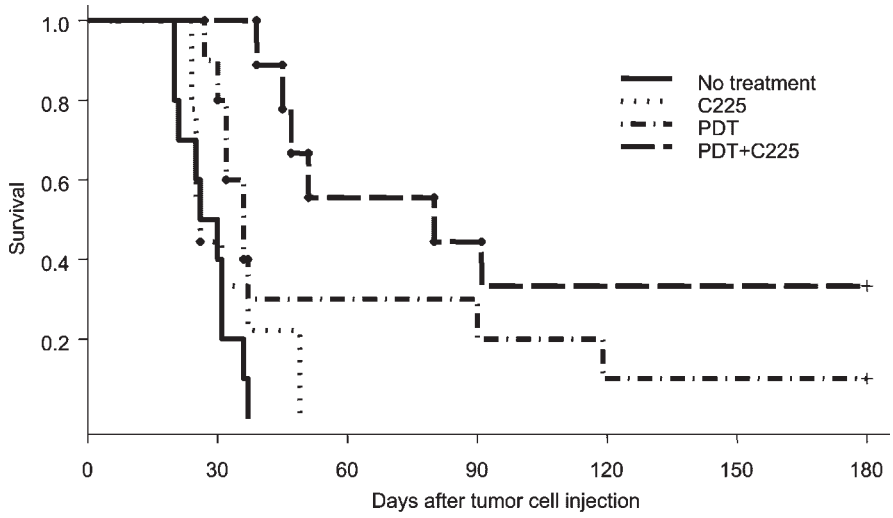


Fig. 4. Tumor burden for control and treated groups in acute treatment response studies. The combination treatment, PDT + C225, resulted in a synergistic reduction in mean tumor burden relative to the individual monotherapies ($P < 0.001$, ANOVA). Error bars represent 95% confidence intervals. Reproduce from Reference [42] with permission of Oxford University Press.

or C225 only (Fig. 5), and significantly improved survival relative to no treatment controls. Notably, one animal out of ten was alive in the PDT only group at the end of the survival study (day 180), as compared to three out of nine in the PDT+C225-treated mice. Upon examination of these four surviving mice for the presence of cancer, it was found that none of the combination treated animals showed gross evidence of disease. Conversely, residual carcinomatosis was found in the lone surviving animal treated with PDT only. Our data demonstrate that BPD-PDT in combination with C225-based immunotherapy is a synergistic and well-tolerated treatment for ovarian cancer.

Peptide Mediated Targeting

Growth factor receptors are often overexpressed in cancers; therefore they are attractive candidates for the specific targeting of therapeutic agents. These receptors can be targeted using specific MAbs; we have described in the previous section the use of an anti-EGFR MAb to specifically target PS to tumors- or they can be targeted with peptides that mimick the natural ligands of these receptors. For example, Gijsens et al. conjugated the epidermal growth factor (EGF) to the PS Ce_6 to target EGFR positive tumors. It was shown that this conjugate is highly phototoxic and highly specific since the co-incubation with a competing concentration of unconjugated EGF reduced phototoxicity [47]. Similarly, Akhlynina et al., conjugated Ce_6 to insulin for targeting liver tumors. This study demonstrated specific receptor mediated internalization and phototoxicity 100× higher than obtained with free Ce_6. Furthermore, specific inhibition of endocytosis of the conjugate abrogated phototoxicity. Despite these interesting results, the use of insulin conjugates in cancer therapy is limited since, unlike EGFR which is overexpressed in many cancers, many hepatoma cell lines have a low number of insulin receptors [48]. More interestingly, the transferrin receptor is often overexpressed in hyperproliferative cells while it is usually present at low levels in normal tissue. For this reason the PS Ce_6 was conjugated to transferrin, and was efficient in killing mammary adenocarcinoma cells in culture [49]. Alternatively, the group of de Witte, instead of conjugating the PS directly to transferrin, first conjugated transferrin to liposomes then encapsulated the PS in the transferrin-conjugated liposomes. They initially demonstrated efficient targeting of HeLa cells in vitro [50], then showed efficient targeting of rat bladder tumors. Furthermore, since superficial bladder tumors overexpress the transferrin receptor, this conjugate is a promising tool for treatment. Choroidal neovascularization (CNV), the abnormal growth of new blood vessels in the choroid, is commonly associated with macular degeneration. Endothelial cells forming new blood vessel express the vascular endothelial growth factor (VEGF) receptors. In a recent study Renno et al., conjugated the PS verteporfin, first to a polyvinyl alcohol (PVA) polymer, then to a peptide known to bind VEGFR. The use of this conjugate efficiently caused CNV closure and was more selective than unconjugated verteporfin [51]. To improve the selectivity of PpIX toward



		# of mice at risk of dying		
No treatment	n=10	5	-	-
C225	n=9	4	-	-
PDT	n=10	9	3	1
PDT+C225	n=9	9	4	3
Days after tumor cell injection		30	90	180

Fig. 5. Kaplan–Meier curves for survival. Median survival was 28 days (interquartile range [IQR] = 21–31 days) for untreated mice, 36 days (IQR = 32–90 days) for mice treated with photodynamic therapy only (PDT), 26 days (IQR = 25–37 days) for mice treated with C225 only, and 80 days (IQR = 47 days–upper range not calculable secondary to censored data) for mice treated with PDT+C225. The combination treatment, PDT+C225, resulted in a synergistic enhancement of survival compared to the individual monotherapies, as analyzed by the Wilcoxon test ($P = 0.027$). Reproduced from Reference [42] with permission of Oxford University Press.

cancerous cells Rahimipour et al., conjugated PpIX to a gonadotropin-releasing hormone (GnRH) agonist and to a GnRH antagonist. These conjugates would specifically target cancerous cells that express gonadotropin-releasing hormone receptors. This study showed that the hormone conjugate had lower binding affinity to the receptor, but proved to be long acting in vivo. Furthermore, both conjugates were more phototoxic toward pituitary gonadotrope alphaT3-1 cell line than unconjugated PpIX. In a primary pituitary cell culture the specificity of the antagonist conjugate was approximately ten times higher than unconjugated PpIX [52].

Other Approaches to Photosensitizer Targeting

Alternative ways of targeting PS to a specific cell population need to take advantage of certain properties of these cells, which either distinguish them from other cell or tissue types, or differentiate malignant from normal cells. An approach that was used by Zhang et al., [53] is based on the altered sugar metabolism of cancer cells. Rapidly growing tumors are able to maintain high glucose catabolic rate by upregulation of the enzyme hexokinase. This enzyme phosphorylates glucose to glucose-6-phosphate, which is then retained in the cell [54]. They demonstrated that the PS pyropheophorbide 2-deoxyglucosamide (pyro-2DG) selectively accumulated in tumors. Pyro-2DG is taken up by cells and becomes a substrate for hexokinase, the chemically altered PS is then retained in the cells. Since cancer cells upregulate this enzyme, more of the PS is retained in tumor cells than in normal cells. Another approach exploits the biological changes associated with T-cell activation. Activated T-cells play an important role in many inflammatory diseases and malignancies of lymphocytes, such as lymphomas. The P-glycoprotein, product of the *MDR1* gene is involved in multidrug resistance of cancer cells by extruding cytotoxic chemicals [55], and was shown to be downregulated upon T cells activation with certain mitogens [56]. A study by Guimond et al., used this characteristic to specifically target-activated T cells with the PS TH9402. This study showed that only activated T cells retained the PS and that photoactivation resulted in their selective depletion [57]. This targeting strategy could be potentially used to monitor and treat graft-versus-host disease or other immune diseases where only activated T cells need to be destroyed. In another study, using Ce_6 conjugated to maleylated albumin, Demidova et al. were able to specifically target the scavenger receptors of macrophages in the vulnerable plaques of a rabbit model of atherosclerosis [58]. Macrophage targeting is currently being developed for the detection and treatment of vulnerable plaque, but could also be used for destruction of tumor-associated macrophages.

CONCLUSIONS

PDT is an evolving technology that is approved as a first line treatment for age-related macular degeneration and for a variety of cancers. For early or localized neoplasms PDT has been shown to be a selective and curative therapy. For these reasons Porfimer sodium has been approved for use in advanced and early-stage lung cancers, superficial gastric cancer, esophageal adenocarcinoma, cervical cancer, and bladder cancer. Temoporfin, another PS, is also approved in Europe for the palliative treatment of head and neck cancer. No other systemically administered PS are currently approved for the treatment of neoplasms, however topically applied PS are approved for the treatment of actinic keratosis and basal cell carcinomas. Today PDT is not only considered as palliative therapy but also as a treatment option for early lung cancer, actinic keratosis, and basal cell carcinoma. Currently, the use of PDT for disseminated tumors, localized disease and precancerous lesions is under investigation for intraperitoneal carcinomatosis [42, 43], Barrett's oesophagus [59], bladder cancer [60], pituitary tumors [61], and glioblastoma [62]. As PDT becomes more widely used, the necessity of increasing specificity becomes important especially when treating tumors in complex anatomical sites such as in the peritoneal cavities. This review outlined only limited strategies for enhancing PDT effects selectively with some focus on increasing the concentration of PS in target tissues. This field is constantly evolving with new approaches to targeted PDT being tested almost every day. A popular method, and one used in our laboratory, was the conjugation of PS to MAb to increase PS delivery. We are currently investigating the use of a PS encapsulated in a nanoparticle using aptamers to bind to specific molecular targets in ovarian cancer cells. Aptamers have several advantages over MAb including higher specificity, smaller size, ease of synthesis, and absence of immunogenicity. Therefore the use of aptamers could replace MAb for targeting molecules to tissues. There will, in the future, be other molecular strategies to make PDT more efficient and selective.

There has been some emphasis, in this article, on PS delivery as a method of increasing PDT efficacy but it is important to note that this approach mostly enhances selectivity and that improving PDT efficacy may depend not only on increasing PS delivery to tissue, but also on understanding the mechanistic processes underlying PDT. This was the case in the combination treatment blocking EGFR signaling followed by PDT [42] or the example where cellular differentiation mechanisms were used for enhancing ALA-PDT outcomes [27,28]. Other approaches, focusing on understanding processes that impede PDT and devising approaches to overcome these impediments appear to be promising [63–65].

ABBREVIATIONS

PS: Photosensitizer
PDT: Photodynamic therapy
BPD: Benzoporphyrin derivative
ALA: δ-aminolevulinic acid
PpIX: protoporphyrin IX
LDL: Low density lipoprotein
PpIX: Protophophyrin IX
PIT: Photoimmunotargeting
MAb: Monoclonal antibody
EGFR: Epidermal growth factor receptor
ROS: Reactive oxygen species
Ce_6: Chlorin e_6

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