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# Photodynamic Therapy

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## Abstract

Photodynamic therapy involves administration of a tumor-localizing photosensitizing agent, which may require metabolic synthesis (i.e., a prodrug), followed by activation of the agent by light of a specific wavelength. This therapy results in a sequence of photochemical and photobiologic processes that cause irreversible photodamage to tumor tissues. Results from preclinical and clinical studies conducted worldwide over a 25-year period have established photodynamic therapy as a useful treatment approach for some cancers. Since 1993, regulatory approval for photodynamic therapy involving use of a partially purified, commercially available hematoporphyrin derivative compound (Photofrin®) in patients with early and advanced stage cancer of the lung, digestive tract, and genitourinary tract has been obtained in Canada, The Netherlands, France, Germany, Japan, and the United States. We have attempted to conduct and present a comprehensive review of this rapidly expanding field. Mechanisms of subcellular and tumor localization of photosensitizing agents, as well as of molecular, cellular, and tumor responses associated with photodynamic therapy, are discussed. Technical issues regarding light dosimetry are also considered.

## Background

Photochemotherapy of cancer is often called “photodynamic therapy (PDT).” The term “photodynamic action” (1) is used to distinguish photosensitized reactions in biology from the physicochemical processes occurring in the emulsions of photographic films. Blum (2) suggested that this definition should be applied only to photochemical reactions in which oxygen was consumed. Such reactions are also called photosensitized processes of type I and type II depending on the nature of the primary steps, namely, the initial involvement of radical intermediates that are subsequently scavenged by oxygen or the generation of the highly cytotoxic singlet oxygen ($^1O_2$) by energy transfer from the photoexcited

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sensitizer. $^1O_2$ has a short lifetime in biologic systems (<0.04 microsecond) and, therefore, was also shown to have a short radius of action (<0.02 μm) (3).

The current era of PDT began with studies by R. L. Lipson and S. Schwartz at the Mayo Clinic in 1960 who observed that injection of crude preparations of hematoporphyrin led to fluorescence of neoplastic lesions visualized during surgery. To gain an optimal tumor localizing preparation, Schwartz treated hematoporphyrin with acetic acid and sulfuric acid and obtained a porphyrin mixture that he termed “hematoporphyrin derivative” (HPD), which was used by Lipson et al. (4) for tumor detection. HPD contains several porphyrins, monomers as well as dimers and oligomers [reviewed in (5)]. HPD has been partially purified, with the less-active porphyrins’ monomers removed, to form Photofrin® (6), the most widely used photosensitizer in clinical PDT. Because of the long-lasting skin phototoxicity of Photofrin, several new photosensitizers have recently been introduced in clinical trials (7,8). Photofrin absorbs light only up to about 640 nm; light at longer wavelengths penetrates farther into tissue, and most of the new sensitizers have stronger absorbance at 650–850 nm.

## Localization of Photosensitizers

Why are the tissue/cellular sites of photosensitizer localization and photodamage important? To facilitate drug development, it is often necessary to identify a target. A systematic study of structure-activity relationships can then aid in improving the therapeutic procedure. As new sensitizers are prepared, studies on localization, both at a tissue and a subcellular level, can be carried out. A recent summary of current information relating to localization sites has now been provided (9). Since the second-generation sensitizers tend to be pure compounds, not mixtures, loci of localization can often be identified. Mitochondria, lysosomes, plasma membrane, and nuclei of tumor cells have been evaluated as potential PDT targets, along with the tumor vasculature. Vascular shutdown is clearly an important aspect of PDT (10), but since both vasculature and tumor are composed of individual cells, the identification of an optimal subcellular target remains relevant. At this point, clinical efficacy has been described for only a small group of agents. While studies to date suggest a hypothesis relating to localization and efficacy, it remains to be seen whether a single target will prove advantageous in all instances.

Since most photosensitizing agents are fluorescent, drug localization can be determined by fluorescence microscopy (9). A sensitive system is needed, since photobleaching can affect image acquisition and use of high fluences can cause photodamage and dye relocalization. The fluorescence yield can vary with the binding site, so that sites of photodamage may not be accurately indicated by fluorescence. Since the cytotoxic product, $^1O_2$, can migrate less than 0.02 μm after its formation (3), sites of photodamage will reflect the localization of sensitizer at the time of irradiation, and many workers have thus chosen to examine subcellular sites of PDT-induced alterations rather than to search for sites of sensitizer binding.

In spite of the heterogeneity of Photofrin, a series of reports (11,12) indicated that the mitochondria were among the targets of photodamage. Consistent with these observations, a

cell subline selected for PDT resistance showed marked mitochondrial alterations (13). In one of the first systematic studies, Henderson et al. (14) examined structure-activity relationships in a pheophorbide series. While hydrophobicity was an important factor, a related study (15) showed that a more important factor was the affinity of these agents for a plasma binding site that also binds benzodiazepines. Since there is a corresponding mitochondrial binding site, this report is consistent with the concept of mitochondrial target being optimal for effective PDT.

## Damage to Subcellular Targets

Because of the limited migration of $^1O_2$ from the site of its formation (3), sites of initial cell and tissue damage of PDT are closely related to the localization of the sensitizer (9). The most highly selective sensitizers currently known are the lysyl chlorin p6 for lysosomes (16), the monocationic porphyrin for membranes (17), and the porphycene monomer for mitochondria (18). Sensitizers that are not taken up by cells, e.g., uroporphyrin, are extremely inefficient even though some of them give a high photochemical yield of $^1O_2$. Moreover, since most PDT sensitizers do not accumulate in cell nuclei, PDT has generally a low potential of causing DNA damage, mutations, and carcinogenesis (5). Sensitizers that localize in mitochondria, like Photofrin, or are produced in mitochondria, like 5-aminolevulinic acid (ALA)-induced protoporphyrin IX, are likely to induce apoptosis, while sensitizers localized in the plasma membrane are likely to cause necrosis during light exposure (*see below*). Aggregated as well as hydrophilic sensitizers are likely to be taken up by pinocytosis and/or endocytosis and therefore become localized in lysosomes or endosomes. Light exposure will then permeabilize the lysosomes so that sensitizers and hydrolytic enzymes are released into the cytosol. Dyes that are present in the cytosol can sensitize tubulin to photodamage (19). This leads to accumulation of cells in mitosis, in some cases followed by cell death (20). The probability of cell inactivation per quantum of absorbed light is widely different among PDT sensitizers (20). Generally, this probability is lower for hydrophilic than for lipophilic sensitizers, indicating that membrane structures are notably vulnerable (21).

PDT damage to the plasma membrane can be observed within minutes after light exposure. This type of damage is manifested as swelling (22), bleb formation (22,23), shedding of vesicles containing plasma membrane marker enzymes, cytosolic and lysosomal enzymes (23), reduction of active transport (24), depolarization of the plasma membrane (25), increased uptake of a photosensitizer (26), increased permeability to chromate (24) and even to cytosolic enzymes like lactate dehydrogenase (27), inhibition of the activities of plasma membrane enzymes such as $Na^+K^+$-adenosine triphosphatase (ATPase) and $Mg^{2+}$-ATPase (28), a rise in $Ca^{2+}$ (29), up- and down-regulation of surface antigens (30), lipid peroxidation (31) that may lead to protein crosslinking (32), and damage to multidrug transporters (17).

## Apoptosis *In Vitro*

The discovery that PDT can lead to an apoptotic response in malignant cells has provided a rationale for the widespread efficacy observed. While apoptosis was first described in 1972

(33), it was not until 1991 that Agarwal et al. (34) reported an apoptotic response to PDT. Reports that PDT could rapidly induce apoptosis, both *in vitro* (34,35) and *in vivo* (36,37), have provided an insight into the nature of photokilling. Apoptosis is a mechanism whereby organisms initiate cellular death *via* a process that is normally part of the genetic apparatus (38,39). The end result is fragmentation of nuclear DNA and dissociation of the cell into membrane-bound particles that are engulfed by adjoining cells, minimizing release of inflammatory products, e.g., lysosomal enzymes. Malignant cell types often exhibit an impaired ability to undergo apoptosis, an effect associated with the ability to survive chemotherapy (38,40). Since a broad spectrum of clinical PDT responses is observed (10), PDT is effective against otherwise drug-resistant cell types. Although an apoptotic response to PDT is not always observed (35,41), this might be related to differences in intracellular site(s) of photodamage or use of suboptimal detection systems.

The time required for initiation of apoptosis varies widely. Most cells, in response to inducing agents, go through a latency period, variable in duration, which usually results in the death of greater than 80% of a cell population in 1–3 days. A novel feature of apoptosis after PDT is the rapidity of execution, as judged by the appearance of DNA ladders as early as 30 minutes after photodamage. It appears that neither DNA, RNA, nor protein synthesis is needed over such a short time frame. Although there are unique aspects to PDT, other examples of apoptotic responses to oxidative stress have been reported (39,42).

Signal transduction pathways are generally involved in the initiation of an apoptotic program (43–45). Xue and Oleinick's group (46) reported that among the early effects of PDT was enhanced phosphorylation of tyrosine residues. It was suggested that the latter may serve to protect cells from the effects of photodamage and may therefore not be involved in the apoptotic process. A recent report (47) confirms this proposal: protein tyrosine phosphorylation was inhibited by the drug staurosporin, although this agent promotes PDT-induced apoptosis (48).

The mechanism of apoptosis after PDT has perhaps been explained by recent reports that indicate an association between mitochondrial photodamage and apoptotic responses, while concurrent membrane photodamage can delay the apoptosis (18,49,50). It is known that release of cytochrome c and other mitochondrial factors into the cytoplasm can trigger an apoptotic response (51,52), effects that can also be produced by enhanced mitochondrial permeability (53–56). Mitochondrial permeability is known to be involved in a pore transition that can be triggered by protoporphyrin (in the dark) (54), and it is interesting to note that some other photosensitizing agents have a similar effect (57).

Marchetti et al. (53) pointed out that the protoporphyrin is a ligand for the mitochondrial peripheral benzodiazepine receptor, a site known for its ability to trigger the pore transition. Tsuchida et al. (15) found a relationship between PDT efficacy and binding to an albumin site that mimics specificity of the benzodiazepine receptor, suggesting that the more effective sensitizers bind to the mitochondrial benzodiazepine receptor. This may represent the binding site for mitochondrial photosensitizers. It is tempting to speculate that irradiation of sensitizers bound to the benzodiazepine receptor can initiate an opening of the

mitochondrial pore, followed by release of apoptosis-initiating factors. Such a mechanism could account for the previously reported structure-activity correlations (15).

Information summarized above is consistent with the proposal that PDT can directly initiate an apoptotic response, without the need for intermediate signal transduction pathways that may be missing in certain drug-resistant neoplastic cells. The prompt apoptotic cell death after PDT is not expected to depend on the state of the cell cycle or the status of genetic factors, e.g., p53, that can otherwise affect drug responsiveness. These considerations are consistent with experimental findings indicating a very broad spectrum of responses to PDT in the clinic.

## Effects of PDT as Revealed by Techniques of Molecular Biology

Positive clinical results involving PDT have led to an expanded desire to identify cellular and molecular responses associated with this treatment (58). Biochemical studies (7) performed over the past 15 years have provided a plethora of information on subcellular targets involving PDT-mediated cytotoxicity. Molecular biology procedures are playing an integral role in current research designed to examine the relevance of cell-signaling events induced by PDT-mediated oxidative stress. The downstream effector molecules of signal transduction pathways are often proteins encoded for by early response genes. These proteins function as transcription factors and act by regulating the expression of a variety of genes via specific regulatory domains.

PDT-mediated oxidative stress induces a transient increase in the downstream early response genes c-fos, c-jun, c-myc, and egr-1 (59). Assays of kinase activity have provided clues regarding the upstream molecules expressed and/or activated in cells following PDT (46,60). PDT, using a benzoporphyrin, induces a strong dose and time-dependent activation of stress-activated protein kinase and a high osmolarity glycerol (HOG-1) protein kinase in keratinocytes (60). Activation of these messenger proteins is implicated in the transcription of early response genes as well as the induction of cellular responses such as apoptosis. Tyrosine phosphorylation of a non-receptor-type protein (HSl) has also been observed in PDT-treated mouse lymphoma cells and concomitantly shown to correlate with protection of cells from PDT lethality (46). Future studies involving these molecules should expand our understanding of mechanisms of PDT cytotoxicity.

Biochemical and morphologic studies (7) have identified a variety of PDT cellular targets, and molecular studies (58) have further advanced our knowledge of sublethal responses to PDT by identifying an expanding number of genes activated by PDT-induced oxidative stress. Multiple genes encoding for stress-induced proteins can be activated following PDT. Porphyrin-mediated PDT enhances the transcription and translation of heme oxygenase (61). Likewise, PDT induces increased expression of glucose-regulated proteins and the translation of these proteins appears to play a role in modulating the cytotoxic effects of oxidative stress (62,63). Heat shock proteins are also overexpressed following PDT when examined at either the *in vitro* or *in vivo* level (64,65). Interestingly, PDT-induced expression of heat shock proteins appears to be dependent on the specific subcellular targets associated with each photosensitizer. The observation of strong transcriptional activation of

heat shock proteins following PDT has been instrumental in initiating new studies in which PDT oxidative stress is used for the temporal and spatial activation of heterologous genes ligated to the heat shock protein promoter (58).

Procedures designed to alter the expression of selected genes are also proving useful in understanding the molecular mechanisms of PDT cytotoxicity. Hamster fibroblasts transfected with a human Bcl-2 protooncogene expression vector exhibited a decreased incidence of PDT-induced apoptosis and decreased cytotoxicity when compared with parental cells (66). Photosensitivity has also been compared with HL-60 human promyelocytic leukemia cells genetically engineered to constitutively express either wild-type p53 or mutated p53 versus parental p53 null cells. HL-60 cells expressing wild-type p53 were more sensitive to porphyrin- and purpurin-mediated photosensitization when compared directly with HL-60 cells with deleted or mutated p53 (67). Moreover, porphyrin PDT photosensitivity results have recently been observed in human colon carcinoma cells exhibiting either a wild-type or mutated p53 phenotype (68). A different molecular approach was used to examine mechanisms involved in PDT sensitivity. Cell lines with a stable PDT-resistant phenotype were isolated and evaluated using messenger RNA (mRNA) differential display methodology to identify unique transcripts (69). A transcript encoding for alpha-2 macroglobulin receptor/low density lipoprotein receptor-related protein was consistently found expressed in parental cells but absent in the PDT-resistant clones.

The *in vivo* tumoricidal reaction after PDT is accompanied by a complex immune response. A variety of molecular protocols, including reverse transcription–polymerase chain reaction, have provided an opportunity for examining the underlying mechanisms responsible for these host effects. It has recently been demonstrated that PDT can modulate the expression of interleukin 6 (IL-6) and IL-10 in tumor and normal tissues *in vivo* (70). These results agree with an earlier study reporting that the transcription factor AP-1 is involved in the *in vitro* expression of IL-6 following PDT (71). Gel mobility shift assays have also demonstrated that PDT can activate the transcription factor, nuclear factor kappa B, which is also involved in regulating the expression of numerous immunologically important genes (72). It will be interesting to see how the molecular modulation of cytokines affects *in vivo* PDT tumoricidal action.

## Mechanisms of Selective Tumor Uptake and Localization of Photosensitizers

The mechanisms involved in the preferential distribution of sensitizers in tumors are not fully understood. Properties of tumor tissue may contribute such selective distribution. These include elevated numbers of low-density protein receptors, the presence of macrophages, and a decreased pH value. The abnormal structure of tumor stroma characterized by a large interstitial space, a leaky vasculature, compromised lymphatic drainage, a high amount of newly synthesized collagen (that binds porphyrins) (73–75), and a high amount of lipid (that has a high affinity for lipophilic dyes) (76) also favors a preferential distribution of sensitizers.

The use of delivery vehicles for formulation of porphyrin-type photosensitizers was prompted by the observation (77,78) that the affinity of such photosensitizers for neoplastic tissues increases upon increasing their degree of hydrophobicity. The selective biodistribution of these sensitizers is enhanced by their incorporation into amphiphilic systems, e.g., phospholipid vesicles or oil emulsions, which are stable in an aqueous milieu yet possess apolar compartments where hydrophobic substrates are embedded (79). This theory was reinforced by early reports (80) that liposome-associated photosensitizers exhibited greater efficiency and selectivity of tumor targeting as compared with the same photosensitizers administered in a homogeneous aqueous solution (81). Some second-generation photosensitizers, e.g., Sn-etiopurpurin, benzoporphyrin derivative, and Zn-phthalocyanine, are formulated in lipid-based delivery systems.

It is now apparent that the delivery vehicle can influence the serum distribution of a photosensitizer, hence the mechanism and kinetics of its transport to tissues, as well as subcellular biodistribution. Drug delivery via lipid-type carriers clearly enhances the tendency of porphyrins to bind with lipoproteins where they are almost completely partitioned in the lipid moiety. In general, the use of liposomal vesicles that are in a fluid state at the body temperature of 37°C appears to orient the photosensitizer toward low-density lipoprotein. This is exemplified by the data obtained with dimiristoyl-phosphatidylcholine vesicles (81). A marked low-density lipoprotein-orientating action is also shown by Cremophor EL emulsions (82). In any case, a certain degree of interlipoprotein exchange of the porphyrin occurs in the serum, although the rate of such process is dependent on its physicochemical properties of the porphyrin (83,84).

The association of the photosensitizer to lipoproteins, in particular to low-density lipoprotein, could result in selective or preferential release to neoplastic cells (85–87). Many types of tumor cells express a high number of membrane receptors for low-density protein, which promote the internalization of the low-density protein-bound photosensitizer by endocytotic pathways (82). The endocytosed porphyrinoids largely localize in membranous domains, including the plasma and mitochondrial membrane, the Golgi apparatus, and the rough endoplasmic reticulum (82). This distribution pattern has obvious implications for the mechanisms by which PDT induces tumor damage: thus, albumin-transported photosensitizers cause an extensive impairment of the vascular system promoting tumor ischemia and hypoxia, whereas low-density protein-delivered photosensitizers induce an early important damage of malignant cells through both random necrotic and apoptotic processes (36). However, several excellent tumor localizers (e.g., meso-tetraphenylporphin tetra-sulfonate, aluminum phthalocyanine tetrasulfonate, etc.) do not bind to low-density protein while some poor tumor localizers, like hematoporphyrin, bind to low-density protein (88). Thus, the significance of low-density protein-binding for selective tumor uptake has been debated (89,90).

Both microspheres and monoclonal antibodies directed against antigens located at the surface of neoplastic cells have been used as carriers of tumor-photosensitizing agents (91,92). The covalent photosensitizer-antibody complexes give excellent results with regard to the extent and selectivity of accumulation by cell cultures, however, the efficiency of tumor targeting *in vivo* appears to undergo serious limitations. At present, this approach can

be most usefully adopted for photodiagnostic purposes taking advantage of the fluorescence emission properties typical of several porphyrin derivatives.

It has been shown that tumor-associated macrophages in animal tumors take up large amounts of HPD (93) and Photofrin (94,95). Thus, tumor-associated macrophages play a role for the tumor-selective uptake of aggregated sensitizers.

The interstitial fluid is the fluid surrounding the cells and localized between their plasma membranes and the vascular walls. The pH value of interstitial fluid is lower and the content of lactic acid is higher in tumors than in most normal tissues (96–102). The intracellular pH, however, is identical or slightly higher in tumors than in normal tissue (102,103). The acidic pH in tumors offers several therapeutic possibilities (101). The equilibrium between different ionic species of porphyrins is complex (104,105), but generally the lipophilicity as well as the cell uptake increases with decreasing pH (105–107). Thus, the low tumor pH is probably one of the reasons for the selective uptake of Photofrin by some tumors that takes place in tumor-bearing animals (108,109).

## Mechanisms of Tumor Destruction

The targets of PDT include tumor cells, the microvasculature of the tumor bed as well as normal microvasculature, and the inflammatory and immune host system. PDT effects on all these targets may influence each other, producing a plethora of responses, the relative importance of each for the overall tumor response has yet to be fully defined. It seems clear, however, that the combination of all these components is required for long-term tumor control.

Exposure of tumors to PDT *in vivo* can reduce the number of clonogenic tumor cells through direct photodamage; this is insufficient for tumor cure. Studies (10,110–115) in rodent tumor systems employing curative procedures with several photosensitizers showed direct photodynamic tumor cell kill to be less than 2 logs and in most cases less than 1 log, i.e., far short of the 6–8-log reduction required for tumor cure. The *in vitro* irradiation of tumor cells isolated from photosensitized tumors *in vivo* predicts that total eradication is feasible with a sufficiently high light dose with some photosensitizers (10,110,116). But limitations appear to exist that do not allow the eradication to be realized for *in vivo* PDT treatment. Inhomogenous photosensitizer distribution within the tumor might be one of these limitations. Korbelik and Krosl (117) have also shown that both photosensitizer accumulation and tumor cell kill decrease with the distance of tumor cells from the vascular supply.

Another parameter that can limit direct tumor cell kill is the availability of oxygen within the tissue undergoing PDT treatment. Two mechanisms can produce such limitations: the photochemical consumption of oxygen during the photodynamic process and the effects of PDT on the tissue microvasculature. Since $^1O_2$ arises from ground state oxygen, it follows that this process can consume oxygen in the tissue environment. Rapid and substantial reductions in tissue oxygen tensions on illumination of photosensitized tissue were reported (115,118,119). Mathematical modeling supports these findings and demonstrates that the rate of oxygen consumption during Photofrin– PDT can be enough to move a fraction of the

tumor into very low levels of oxygenation, outpacing the rate of oxygen diffusion from the capillaries, and shrinking the radius of oxygenated tissue volume around them (120). The rates of $^1O_2$ generation and therefore tissue oxygen consumption/depletion are high when both tissue photosensitizer levels and the fluence rate of light are high (115,119,121).

The fluence rate can be adjusted downward to slow oxygen consumption sufficiently to facilitate the maintenance of (tumor) tissue $pO_2$ levels during treatment. An important parameter influencing the rate of tissue oxygen consumption is photobleaching of the sensitizer because the reduction of sensitizer levels also reduces the rate of photochemical oxygen consumption (122). Another approach toward maintenance of tissue oxygenation during PDT is the fractionation of light delivery (120,123). This consists of very short (in the order of 20–50 seconds) light and dark intervals, allowing reoxygenation during the dark periods (119). Generally, treatment regimens using a low fluence rate or intermittent light, show superior effectiveness in delaying tumor regrowth (115,120,123–126).

Preliminary clinical studies at the Roswell Park Cancer Institute show oxygen depletion also occurring during PDT in patients. The kinetics for this depletion varied from very rapid (within seconds of light exposure) to slow (>10 minutes of light exposure) and to no effect at all in basal cell carcinoma lesions in patients undergoing Photofrin (1 mg/kg)–PDT at a light dose rate of 150 $mW/cm^2$. No oxygen depletion was observed in cutaneous lymphoma lesions during ALA–PDT (20% topical ALA), possibly because the effects were too superficial to be detected by the interstitial oxygen probe used.

The oxygen supply in the tissue can also be diminished by the damaging effects of PDT on the microvasculature. With high doses of certain photosensitizers, e.g., Photofrin, these effects can be sufficient to limit the oxygen supply to the tumor during PDT (127). With lower photosensitizer doses and certain second-generation sensitizers, many of which exert diminished effects on the vasculature, this mechanism becomes less important.

Vascular damage, occurring after completion of the PDT tumor treatment, contributes to long-term tumor control. Microvascular collapse can be readily observed following PDT (112,127–130) and can lead to severe and persistent post-PDT tumor hypoxia/anoxia (131,132). The mechanisms underlying the vascular effects of PDT differ greatly with different photosensitizers. Photofrin–PDT leads to vessel constriction, macro-molecular vessel leakage, leukocyte adhesion and thrombus formation, all apparently linked to platelet activation and release of thromboxane (133,134). PDT with certain phthalocyanine derivatives causes primarily vascular leakage (135), and PDT with mono-L-aspartyl chlorin $e_6$ results in blood flow stasis primarily because of platelet aggregation (136). All of these effects may include components related to damage of the vascular endothelium. PDT may also lead to vessel constriction via inhibition of the production or release of nitric oxide by the endothelium (137). In preclinical experiments, the microvascular PDT responses can be partially or completely inhibited by the administration of agents that affect eicosanoid generation, such as indomethacin (138), various other thromboxane inhibitors (133), and aspirin (139,140), and this inhibition can markedly diminish the tumor response. On the other hand, administration of agents inhibiting nitric oxide synthase or scavenging nitric

oxide appears to enhance tumor cure, apparently by enhancing the PDT-induced disruption of vascular perfusion (141).

Much of the above information was obtained from studies on normal microvasculature. Damage to the tumor-supplying normal vasculature may greatly affect tumor curability by PDT as demonstrated by the lack of tumor cures when the normal tissue surrounding the tumor was shielded from PDT light (142). This is also supported by reports on the effects of fluence rate on vascular responses. A low fluence rate treatment can lead to shutdown of normal microvascular perfusion following PDT, while a high rate can protect microvascular patency (143). In contrast, there were no differences in effects on either tumor perfusion or oxygenation when treatment was delivered at low or high fluence rates (143). In all cases, these response parameters were equally and greatly reduced in tumors following PDT exposure. A high fluence rate treatment inhibited tumor curability, implying that the protection of the tumor-surrounding normal vasculature by high fluence rate PDT adversely affected long-term tumor control. It had been suggested earlier that PDT effects on normal and tumor vessels may be qualitatively and quantitatively similar (144). However, the above recent studies seem to reveal important differences between PDT effects on normal and tumor vasculature.

Mathematical models have predicted and experimental measurements have demonstrated that dynamic, dose-rate-dependent changes in tissue oxygenation can occur during PDT light delivery. Large interlesion and interpatient variations make predictions of these effects impossible. The need for further development of instrumentation allowing real-time monitoring of the parameters that influence these changes (and thus PDT dose), i.e., photosensitizer tissue concentration, photobleaching rates, blood flow, $pO_2$ etc., is as great as ever. Further insight into the mechanisms of vascular damage by PDT might uncover ways by which the differences between tumor and normal vasculature might be exploited to enhance treatment effectiveness and selectivity.

## Immunologic Effects of PDT

### PDT-Induced Tumor Inflammation

The curative properties of PDT arise from the death of cancer cells spared from the direct cytotoxic effect by a combination of oxidative stress-initiated secondary tumoricidal activities (145). Contrary to the contemporary prevailing conception, these secondary effects are by no means limited to the ischemic death caused by the occlusion of tumor vasculature. Other events that are increasingly coming into focus are as follows: 1) antitumor activity of inflammatory cells and 2) tumor-sensitized immune reaction. They all can be elicited by phototoxic damage that is not necessarily lethal and bears an inflammatory impact.

Photodynamically induced changes in the plasma membrane and membranes of cellular organelles, which represent the most abundant damage with a majority of photosensitizers used for PDT, can trigger events with far-reaching consequences. One process initiated at the membrane level involves signal transduction pathways. These include enhanced expression of stress proteins and early response genes (58), activation of genes regulating the process of apoptotic cell death (45), and possibly the up-regulation of some cytokine

genes. Due to their role in cell adhesion and antigen presentation, some of the PDT-induced stress proteins may participate in the development of inflammatory/immune responses manifested by this therapy (146).

Another PDT-induced membrane alteration involves inflammatory cellular damage. Photooxidative lesions of membrane lipids prompt a rapid activation of membranous phospholipases (45) leading to accelerated phospholipid degradation with a massive release of lipid fragments and metabolites of arachidonic acid (145,147). These products are powerful inflammatory mediators. Another source of such signals relates to the tumor vasculature. After even minor phototoxic lesions, the endothelial cells will contract and expose the basement membrane in the vessel wall (148). This rapidly attracts the attachment of circulating neutrophils and platelets, leading to a progressive impairment of vascular function accompanied with a massive release of various inflammatory mediators.

A strong inflammatory reaction is a central event in the mechanism of PDT-mediated tumor destruction. Differences in the nature and intensity of the inflammatory reaction between normal and cancerous tissues may contribute to the selectivity of PDT-induced tissue damage (149). A major hallmark of the inflammatory process is the release of a wide variety of potent mediators, including vasoactive substances, components of the complement and clotting cascades, acute phase proteins, proteinases, peroxidases, radicals, leukocyte chemoattractants, cytokines, growth factors, and other immunoregulators (147,148). Among cytokines, IL-6 mRNA and protein were found to be strongly enhanced in PDT treated mouse tumors, as well as in exposed spleen and skin (70). There is also evidence for PDT induced or up-regulated IL-1β, IL-2, tumor necrosis factor-α (TNF-α) and granulocyte colony-stimulating factor (G-CSF) (150–153). The observed inconsistencies in the detection of these mediators in different PDT-treated tumors and difficulties to detect other such substances are caused by the following: 1) differences in the up-regulation control for the respective genes in different tumors and 2) very short lifetime of these proteins due to extremely high levels of proteinase and RNase activity in tumor tissue after PDT. Some photosensitizers, shown to stimulate the hematopoiesis in treated mice (153,154), may induce cytokines or growth factors independently of light treatment.

### Antitumor Activity of Nonlymphoid Inflammatory Cells

The inflammatory signaling after PDT initiates and supports the recruitment of leukocytes from the blood and amplifies their activity. A massive regulated invasion of neutrophils, mast cells, and monocytes/macrophages during and after photodynamic light treatment has been documented in studies using rodent tumor models (70,155). These newly arrived nonspecific immune effector cells will outnumber resident cancer cells. Most notable is a rapid accumulation of large numbers of neutrophils. There is increasing evidence that these cells have a profound impact on PDT-mediated destruction of cancerous tissue. Neutrophils can remain within tumor blood vessels and be a key contributor to the infliction of endothelial damage or engage in the destruction of tumor parenchyma on extravasation. Degranulation of errant neutrophils liberates toxic oxygen radicals, myeloperoxidase, and lysosomal enzymes acting as a potent system for the breakdown of proteins and causing considerable damage to the affected tumor tissue (156). In turn, neutrophils also sustain

lethal damage during these events, releasing chemotactic substances that will attract a new wave of invasion of immune cells.

Depletion of neutrophils in tumor-bearing mice using the anti-GR1 monoclonal antibody, or blocking functions of the common chain of β integrins by anti-CD18 antibody, was found to decrease the PDT-mediated tumor cure rate (145,149). The response of rat tumors to PDT was improved by increasing the number of circulating neutrophils in the hosts by treatment with G-CSF; the opposite effect was achieved in rats treated with anti-rat neutrophil serum that reduced neutrophil levels in these animals (157). PDT aroused a selective increase of neutrophils in the peripheral blood of treated rats peaking around 8 hours after light exposure (150). This was preceded by the elevation in IL-1β serum levels and an increase in the number of circulating band neutrophils. Treatment with anti-G-CSF polyclonal antibody impaired not only the increase in neutrophil numbers but also the response of tumors to PDT. In another study, tumor localized treatment with GM-CSF was shown to enhance the PDT-mediated cures of mouse squamous cell carcinomas (158).

Another class of nonspecific immune effector cells whose activation substantially contributes to the antitumor effects of PDT is monocytes/macrophages. The tumoricidal activity of these cells was found to be potentiated by PDT *in vivo* and *in vitro* (155,159,160). Macrophages were reported to release TNF-α following PDT treatment (152) and to preferentially recognize PDT treated cancer cells as their targets (161). Adjuvant treatment with a selective vitamin D3-binding protein macrophage activating factor (DBPMAF) was shown to potentiate the cures of PDT-treated tumors (162).

## The Immune Reaction

There have been substantial advances in the understanding of the PDT-induced tumor-specific immune reaction. This effect may not be relevant to the initial tumor ablation, but may be decisive in attaining long-term tumor control. Tumor sensitized lymphocytes can, under reduced tumor burden, eliminate small foci of viable cancer cells that have escaped other PDT mediated antitumor effects.

Cancer immunity elicited by PDT has the attributes of an inflammation primed immune development process (145) and bears similarities to the immune reaction induced by tumor inflammation caused by bacterial vaccines or some cytokines (149). The initial critical step of tumor-specific immune development is likely mediated by tumor-associated macrophages and/or dendritic cells serving as antigen presenting cells (145). These cells are prompted to phagocytize large numbers of cancer cells killed or damaged by the antitumor effects of PDT. Directed by powerful inflammation-associated signaling, the antigen presenting cells will process tumor-specific peptides and present them on their membranes in the context of major histocompatibility class II molecules. Presentation of tumor peptides, accompanied by intense accessory signals, creates conditions for the recognition of tumor antigens by helper T lymphocytes. These lymphocytes become activated and in turn sensitize cytotoxic T cells to tumor specific epitopes. The generation of CD4 and CD8 T cell clones that recognize tumor cells as their targets is followed by their rapid expansion and activation leading to fully developed tumor immunity. There are indications that B lymphocytes and natural killer cells also become activated and may contribute to PDT-elicited immune responses, but the

role of these cells remains to be fully elucidated. The activity of tumor sensitized lymphocytes is not limited to the original PDT-treated site but can include disseminated and metastatic lesions of the same cancer. Thus, although the PDT treatment is localized to the tumor site, its effect can have systemic attributes due to the induction of an immune reaction. PDT generated tumor-sensitized lymphocytes can be recovered from distant lymphoid tissues (spleen, lymph nodes) at protracted times after light treatment (145). Therefore, these lymphocyte populations consist of immune memory cells. In contrast to most other cancer therapies, PDT can induce immunity, even against less immunogenic tumors (145,163).

The demonstration that lymphoid populations are essential for preventing the recurrence of PDT-treated tumors was provided by using a mouse sarcoma model growing in either immunocompetent or immunodeficient syngeneic hosts (164). Photofrin-based PDT treatment that was fully curative for EMT6 tumors growing in immunocompetent BALB/c mice resulted in initial ablation but not permanent cures with EMT6 tumors growing in severe combined immune deficiency (scid) or nude mice. If the bone marrow of scid mice was reconstituted with BALB/c bone marrow, the EMT6 tumors in these hosts (which acquired functionally active lymphocytes) were cured by PDT. Similar results were recently reported for PDT based on a benzophenothiazine analogue as a photosensitizer using the same experimental model as above (165). The induction of immunity against a weekly immunogenic murine fibrosarcoma MS-2 by aluminum phthalocyanine-based PDT was also described (163). In this case, the mice that remained tumor free 100 days after PDT were shown to resist a rechallenge with the same tumor.

In one series of studies, BALB/c mice, which had EMT6 tumors treated by a curative dose of Photofrin-based PDT 5 weeks earlier, served as donors of spleen cells adoptively transferred to scid mice. This fully restored PDT-mediated curability of EMT6 tumors growing in recipient scid mice (145). No cures were obtained with the host scid mice that received virgin BALB/c splenocytes or splenocytes from BALB/c mice previously cured by PDT from a different tumor (145). An improved, but not fully curative PDT response was observed with x rays used to eradicate EMT6 tumors in future splenocyte donors. These results demonstrate the generation of immune memory cells sensitized to PDT treated tumor and suggest that PDT may be particularly suitable for a combined application with adoptive immunotherapy.

Inflammation is frequently accompanied by immunosuppressive effects, as is the case with PDT. The PDT-induced immunosuppression was detected primarily as a transient reduction in the delayed-type contact hypersensitivity response, which appears to be mediated by antigen nonspecific suppressor cells (166). The immunosuppression in mice bearing tumors exposed to PDT was greatly reduced by treatment with DBPMAF (162), underlying the role of macrophages in this phenomenon. The severity of immunosuppression is much greater after the PDT treatment of the exposed musculoperitoneal layer than after treatment of subcutaneous tumors (160,164). Mouse skin graft rejection in allogenic recipients is diminished after low-dose PDT of skin grafts, and the mechanism appears to involve impaired function of antigen presenting cells (167). The cytokine IL-10, shown to be induced in PDT exposed skin of mice (but not in the tumor), appears to play a role in PDT

elicited immunosuppression (70). Blocking the induction of immunosuppression by agents like DBPMAF may augment the efficacy of PDT in cancer treatment (162).

Due to its inflammatory/immune character, PDT can be successfully combined with various immunotherapy protocols for achieving substantial gains in long-term tumor controls. A common strategy to such combination is to sustain and amplify the PDT-induced immunity against the treated cancerous lesion. Its effectiveness was demonstrated in a number of different rodent tumor models (including poorly immunogenic tumors) using a wide variety of nonspecific or specific immunotherapy agents (145).

## Current Status of Clinical PDT

### Regulatory Status—Photofrin

The first health agency approval for PDT (with Photofrin) was obtained in 1993 in Canada for the prophylactic treatment of bladder cancer. Subsequently, approvals for Photofrin were obtained in The Netherlands and France for treatment of advanced esophageal and lung cancers; Germany for treatment of early stage lung cancer; Japan for early stage lung, esophageal, gastric, and cervical cancers as well as cervical dysplasia; and in the United States for advanced esophageal cancer. In 1998, QLT PhotoTherapeutics (Vancouver, Canada) received U.S. Food and Drug Administration (FDA) approval for use of Photofrin for early stage lung cancer. Approvals are currently being sought in 11 additional countries in Europe.

### Approved Indications for Photofrin—PDT

**Advanced Stage Esophageal Tumors—**The results of the phase III clinical trial completed in the United States that led to U.S. FDA approval in December 1995 have been published (168). This was a multicenter, randomized, comparative trial against thermal ablation using a Nd-YAG laser for treatment of partially obstructing esophageal cancer. The results of this trial with 236 patients indicated similar relief of dysphagia in both arms, a longer lasting tumor response for PDT (32% at 1 month versus 20% for Nd-YAG), and more complete responses (negative endoscopic biopsies) for PDT than for Nd-YAG (9 versus 2).

In certain subgroups, objective responses were higher for PDT than for Nd-YAG in the upper and lower third of the esophagus as well as for tumors larger than 10 cm, but the number of patients in these groups was too small for statistical significance. Fewer procedures were required for PDT (mean 1.5) than for Nd-YAG (2.4). Overall, median survival was the same for both groups. There were more adverse reactions in the PDT group (92%) than in the Nd-YAG group (82%) but the withdrawal from the study because of adverse reaction was similar in the two arms. There were significantly more esophageal perforations in the Nd-YAG group (7%) than in the PDT arm (1%). Sunburn reactions were confined to the PDT group (19%) and were all mild in nature. Efficacy of the two therapies was equivalent; severe adverse reactions occurred at the same rate in both treatments except for the more frequent occurrence of perforation in the Nd-YAG treatment. PDT was considered more comfortable for the patient, was easier to perform than Nd-YAG ablation,

and was especially advantageous in situations where Nd-YAG is difficult to carry out due to morphology or tumor location.

## Bladder Cancer

**Prophylactic treatment for papillary tumors:** The trial resulting in approval for Photofrin-PDT in Canada in 1993 involved a prophylactic PDT treatment following transurethral resection of papillary bladder tumors in patients at high risk for recurrence. While final results of this trial have not been published, a preliminary report was given in 1991 (169). A 1-year follow-up of 34 patients indicated recurrence in 81% of patients in the control group (no PDT following resection) and 39% in the PDT arm. Median time to recurrence was 91 and 394 days for the control and PDT group, respectively. Photosensitivity occurred in one third of patients and urinary symptoms in 93% of patients receiving PDT.

Because of severe and long-lasting side effects, Nseyo et al. (170) suggested multiple treatments at lower drug dose to reduce the incidence and severity of symptoms following PDT for superficial bladder cancer (*see below*).

## Lung Cancer

**(a) Advanced non-small-cell lung cancer:** A prospective, randomized trial of PDT versus Nd-YAG ablation for partially obstructive lung cancer has been reported. This included data from 15 centers in Europe (141 patients) and 20 centers in the United States and Canada (70 patients). In the European trial, 40% of patients had prior therapy while all patients in the U.S./Canada trial had prior therapy. Tumor response was similar for both therapies at 1 week, but at 1 month, 61% and 42% of PDT patients were still responding in the European and U.S./Canada trial, respectively, whereas for the Nd-YAG, 36% and 19% were responding in the two trials. There were 12% and 6% of PDT patients versus 3% and 5% of Nd-YAG patients who achieved a complete biopsy-proven response in the European and U.S./Canada trials respectively. Improvement in dyspnea and cough were superior for PDT over Nd-YAG in the European group but similar in the U.S./Canada group.

It was concluded that PDT is superior to Nd-YAG for relief of dyspnea, cough, and hemoptysis. Overall, adverse reactions were similar for PDT and Nd-YAG (73% PDT, 64% Nd-YAG) and 20% of patients in the PDT group experienced a photosensitivity reaction due to lack of compliance with precautions.

There was a prospective trial of PDT plus radiotherapy versus radiotherapy alone (171) with 41 randomized patients. The obstructed airway in only 10% of patients was completely opened by radiation therapy alone, whereas 70% of patients achieved complete reopening when PDT was added to the radiation therapy.

**(b) Early stage lung cancer:** PDT appears to be particularly applicable to treatment of early stage lung cancer, since it preserves lung function, can be repeated as additional tumors appear (such patients are at high risk for developing new tumors), and does not preclude ultimate surgical intervention if deemed necessary. In patients with early stage lung tumors less than 2 cm, the incidence of lymph node metastasis was low to nonexistent, indicating the appropriateness of conservative treatments (172).

Edell and Cortese (173) have reported a group of 13 patients with 14 early stage lung cancers. These patients received 200– 400 J/cm$^2$ of 630 nm irradiation 2–4 days following injection of 2.5 mg/kg HPD. Eleven tumors showed a complete response after a single treatment and the remaining three after a second treatment; 77% of the tumors showed no recurrence after 7–49 months. No substantial complications were observed in the patients. Three patients had a mild sunburn reaction. The authors conclude that PDT may be an alternative to surgery for patients with early squamous cell carcinoma.

Furuse et al. (174) reported on 54 patients with 64 early stage lung cancers using Photofrin (2.0 mg/kg) and 630-nm illumination of 100–200 J/cm$^2$. Of 59 tumors assessable, 50 were considered a complete response, six were partial responders, and three had no response. Five of the complete responders had recurrence at 6–18 months after treatment. A predictor of response was the length of the tumor with those less than or equal to 1 cm obtaining a 97.8% complete response and only 42.9% of tumors greater than 1 cm achieving this response. The overall survival of patients was 50% after approximately 3 years.

Kato et al. (175) described a study involving use of Photofrin–PDT on 95 lesions in 75 patients with early lung cancer treated. The complete response rate was related to the tumor size, with complete response rate of 96.8% for lesions less than 0.5 cm, but only 37.5% for greater than 2 cm. The overall 5-year survival rate for all 75 patients predicted according to Kaplan– Meier analysis was 68.4%.

## Pending Photofrin Trials for Regulatory Approval

**Early Stage Esophageal Cancer—**This disease often occurs in conjunction with Barrett’s esophagus, a condition of replacement of the esophageal squamous epithelium by stomach glandular epithelium as a result of acid reflux. Patients with Barrett’s esophagus are at risk for development of esophageal cancer for which the usual procedure is an esophagectomy, a surgical procedure with high mortality and morbidity.

The largest PDT study was carried out on 55 patients with superficial esophageal cancer (176). A 6-month follow-up after PDT indicated 24 of 36 patients with initial high-grade dysplasia and Barrett’s esophagus had no dysplasia, and seven had no residual Barrett’s esophagus. Three of 36 patients with high-grade dysplasia showed no response to treatment and nine were converted to low-grade dysplasia. Eleven of 12 patients presenting with low grade dysplasia had no dysplasia and six had no residual Barrett’s esophagus after treatment, six of six patients with a $T_1$ cancer had complete response and three had no residual Barrett’s esophagus. One patient with a $T_2$ cancer also had no remaining disease; one with low-grade dysplasia showed recurring low-grade dysplasia after 6 months. The technique involves injection of 2.0 mg/kg Photofrin with light delivery 48 hours later. In some patients, a balloon catheter was used 3, 5, or 7 cm in length in which the light delivery fiber with a diffuser of appropriate length was centered. The balloon allows proper distention of the esophagus and assures uniform light delivery to the affected areas. Complications included stricture in 29 patients that required dilation to resolve, although the frequency of this complication appears to be less using larger balloons than with the bare diffuser or shorter balloons. Photosensitivity was of low frequency. For PDT versus surgery, mortality was 0% and 6%–14%, respectively. Moreover, PDT is an outpatient procedure versus 1.5–3

weeks in hospital for surgery and a recovery time of 3 weeks for PDT versus 2–4 months for surgery. The estimated costs are approximately $20 000 for PDT versus $35 000–95 000 for surgery.

**Head and Neck Cancers—**Biel (177) has reported excellent results in treatment of early stage head and neck cancers. In this study, there were 29 patients with cancer of the larynx (22 superficial), 32 patients with cancer of the nasal cavity and pharynx, one of the nasal cavity, two patients with Kaposi's sarcoma of the palate, three patients with cancer of the nasopharynx, and five with papilloma of the larynx-trachea. Patients received 2.0 mg/kg Photofrin, 48 hours prior to 630 nm light delivery via a microlens fiber at 50–75 J/cm$^2$. For tumors greater than 3 cm, diffuser fibers were implanted and a dose of 100 J/cm fiber delivered interstitially. All 22 patients with superficial cancer of the larynx achieved a complete response, with follow-up to 67 months (mean, 30 months), as did all patients with oral, intranasal, or nasopharyngeal cancer, who were followed up for a maximum of 61 months (mean, 33 months). Five patients with recurrent laryngeal/tracheal papillomatosis exhibited an initial response to PDT at 1 month, but had evidence of disease recurrence by 6 months after PDT. Two patients required oral steroids for 5 days because of sunburn. Pain varied from mild to severe and was adequately controlled with oral analgesics.

**Superficial Bladder Cancer—**Although not yet approved for general use, there are several successful reports on use of PDT for treatment of recurrent or drug-resistant superficial bladder cancer, a group at high risk for muscle invasion often requiring radical cystectomy with its attendant complications [reviewed in (178)]. Some investigators have concluded that in most trials of bladder cancer, the PDT treatment is overly aggressive (2.0 mg/kg Photofrin, 15 J/cm$^2$ whole bladder) and results in long lasting and severe urinary symptoms. Recently Nseyo et al. (170) have suggested that three less aggressive treatments be given every 6 months based on their results (12 of 14 treated patients had complete responses) obtained in patients receiving 1.5 mg/kg Photofrin with 15 J/cm$^2$ where bladder contracture has been avoided and symptoms have been minimized and reduced to a period of approximately 2 weeks.

### Adjuvant Therapy Procedures

**(a) Brain tumors:** Both groups of Muller (179) and Kaye (180) have had long-standing programs to combine PDT with resection of brain tumors (mainly glioblastoma or astrocytoma). These tumors are difficult to control by surgery alone, since some tumor cells exist beyond the operative bed; PDT after resection may destroy these cells. In Muller's study of 56 patients with recurrent tumors, all of whom had failed radiation therapy, the mean survival time for patients receiving PDT for glioblastoma, malignant astrocytoma, and mixed astrocytoma– oligodendroglioma was 30, 44, and greater than 61 weeks, respectively. For patients undergoing surgery alone, survival was only 20 weeks. The survival of patients with malignant astrocytoma was related to light dose, with those receiving a total of greater than 1800 J (2.0 mg/kg Photofrin) surviving longer (64 weeks median, 50% 1-year survival) than patients who received <1800 J (27 weeks survival, 33% 1-year survival).

In Kaye's study (using HPD), there were 120 patients in total, 38 with primary glioblastoma, 40 with recurrent glioblastoma, 24 with anaplastic astrocytoma, and 11 with recurrent anaplastic astrocytoma. The median survival was 24 and 9 months after treatment for primary glioblastoma and recurrent glioblastoma, respectively. Fifty percent of the patients with glioblastoma survived beyond 2 years. The median survival times have not been reached for the other groups (follow-up to 8 years for anaplastic astrocytoma). Survival appears to be longer when PDT is used in conjunction with surgery plus radiation therapy; a confirmatory prospective trial is underway in the United States and Canada.

**(b) Head and neck cancers:** In the study of Biel (177) a group of 10 patients with large head and neck tumors recurrent after surgery, radiation therapy, and chemotherapy received intraoperative PDT to the tumor following resection. With follow-up to 50 months, three patients demonstrated recurrent disease, two of which were outside the PDT field. It was noted that PDT did not appear to interfere with wound healing.

**(c) Intrathoracic tumors:** Tochner et al. (181) and Pass and Donington (182) at the National Institute of Health pioneered the use of PDT as adjunct to surgery for pleural cancers, especially malignant mesothelioma. Following resection of as much tumor as possible, the entire involved thoracic cavity is exposed to 630 nm light delivered 2 days following injection of 2.0 mg/kg Photofrin using intralipid as a diffusing medium. As a follow-up to these studies, Takita and Dougherty (183) have reported preliminary results for applying PDT as adjuvant to resection of malignant pleural mesothelioma. Forty-one patients underwent pleurectomy or pleural pneumonectomy followed by PDT to the thoracic cavity (15–35 J/cm$^2$) 2 days following 2.0 mg/kg Photofrin. The overall estimated median survival of all patients was 12 months, although patients with stage I and II diseases had a median survival of 37 months.

**(d) Intraperitoneal tumors:** Delaney et al. (184) have reported a phase I trial of PDT following debulking surgery for intraperitoneal tumors. The majority of patients had ovarian cancer (22 of 54), peritoneal studding from sarcoma (13 of 54), or gastrointestinal carcinomatosis (eight of 54). Doses of Photofrin were increased from 1.5 to 2.5 mg/kg, and light doses ranged from 2.8–3.0 J/cm$^2$ delivered 48–72 hours after injection. In some patients, a boost of 15 J/cm$^2$ of red light or 5–7.5 J/cm$^2$ of green light was used. The green light appeared to reduce small bowel complications. Dose-limiting toxic effects (pleural effusions and gastric perforation) occurred in two of three patients at the highest dose of 5.0 J/cm$^2$ green light with boost. At a median follow up of 22 months, 30 of 39 patients were alive and nine are disease free. Similarly, Glatstein and Hahn at the University of Pennsylvania have recently initiated a phase II trial of intraoperative PDT for disseminated intraperitoneal cancers.

## New Photosensitizers in Clinical Trials

**Tin Etiopurpurin, SnET2 (Purlytin)—**SnET2, a chlorin photosensitizer developed by Miravant Inc. (formerly PDT Inc., Santa Barbara, CA) currently is in phase II trials aimed at the U.S. FDA approval for cutaneous metastatic breast cancer and Kaposi's sarcoma in patients with acquired immunodeficiency syndrome. In a preliminary trial (185), which

included basal cell carcinoma as well as metastatic breast cancer, treated at 1.2 mg/kg SnET2 followed 24 hours later by 200 J/cm$^2$ (660 nm, dye laser or 664 nm, diode laser), 95%–100% of basal cell carcinoma lesions had responded 12 weeks post-treatment. All metastatic breast carcinoma lesions responded in which 96% of the lesions had complete responses and 4% were partial responses. In the trial of Kaposi's sarcoma, 60% of the lesions were complete responses and 40% were partial responses. The number of patients in each of these optimized trials was not reported; 10%–15% of patients experienced photosensitivity reactions at one or more months after treatment and one patient experienced a mild hypersensitivity to the vehicle (a lipid emulsion).

**Lutetium Texaphyrin (Lu-tex)—**A phase II/III trial using Lu-tex is about to begin for treatment of certain skin lesions. A preliminary report (186) has described some results from phase I trials involving various skin lesions (15 breast metastases, seven malignant melanomas, five Kaposi's sarcomas, and two invasive basal cell and two squamous cell carcinomas). Drug doses ranged from 0.6 to 7.2 mg/kg infused 3 hours prior to light treatment at 732 nm and 150 J/cm$^2$ from a dye laser or LED source. Of the 163 evaluated lesions at all doses, 48 (29%) were complete responses and 28 (17%) were partial responses. Severe pain was reported at the higher dose range (7.2 mg/kg). Unlike most other photosensitizers, Lu-tex appears to be highly selective for tumors versus normal skin with subcutaneous melanoma lesions undergoing complete response with minimal damage to overlying skin.

**Benzoporphyrin Derivative-Monoacid Ring A (BPD-MA)—**BPD-MA has been in phase I/II trials for treatment of skin cancers (187) but perhaps the most interesting application is the treatment of age-related macular degeneration, the commonest cause of blindness in people over the age of 50 years. In one form, it is characterized by leaky neovascularization near the macula that impairs vision. Current treatment involves the use of thermal lasers which can result in damage of the overlying retina with further loss of sight. With PDT, BPD-MA is infused and shortly thereafter, when the drug is confined to the vessels as much as possible, the drug is activated at 690 nm through an ophthalmoscope generally using a diode laser. This allows selective closure of the leaky vessels without damage to overlying retinal tissue. In a preliminary report of 107 patients (188) given a single treatment, 44% of the patients experienced improved vision although reappearance of leakage was frequently found after 4–12 weeks. With the use of multiple treatments, it appears that this recurrence may be reduced (189). Phase II trials for health agency approvals have been completely in the United States and Europe with more than 500 patients. Filing for approval in the United States is expected in 1999 (a 1-year follow-up of patients is required).

**Tetra(m-hydroxyphenyl)chlorin, mTHPC (Foscan)—**This chlorin photosensitizer (190) is undergoing clinical trials for head and neck cancer in Europe and the United States under the sponsorship of Scotia Pharmaceutical (Great Britain). This material appears to be the most active of all photosensitizers studied to date, requiring only very low drug doses (as little as 0.1 mg/kg) and light doses (as low as 10 J/cm$^2$) for efficacy. Grosjean et al. (191) reported 27 patients, most with one or more early stage squamous cell carcinoma of the

upper aerodigestive tract, three patients with $T_1$ or $T_2$ tumors, and one with Barrett's esophagus with superficial adenocarcinoma. Most patients received a bolus injection of 0.3 mg/kg and 652 nm irradiation at 8–12 J/cm$^2$ generally 4 days after injection. All patients with bronchial and esophageal tumors were treated under general anesthesia. Treatment of patients with 36 early tumors resulted in no recurrences after a follow-up of 3–35 months. Disease in only one of the four patients with advanced tumors was controlled. Major complications included bronchial stenosis (one patient), esophagotracheal fistula (one patient), and occult perforation of the esophagus (two patients). The authors suggested that the use of green light will reduce the complications without sacrificing efficacy. Twelve patients experienced phototoxic reactions within the first week after drug administration.

This sensitizer currently is undergoing early clinical trials for head and neck cancers in the United States and Europe based on results involving a trial of six primary cases and seven patients treated with palliative intent. All showed excellent responses with follow-up ranging to 28 months (192).

**N-Aspartyl Chlorin e6 (NPe6)—**NPe6 is undergoing clinical trials in Japan under the sponsorship of Nippon Petrochemicals for treatment of endobronchial lung cancer. Results of this trial are not available at this time. Previous reports using NPe6 in skin cancers have shown it to be an effective photosensitizer with little or no long term cutaneous photosensitivity (193).

## ALA-Based PDT and Diagnosis

ALA-induced endogenous photosensitization is a novel approach to both PDT and tumor detection that utilizes the heme biosynthetic pathway to produce endogenous porphyrins, particularly protoporphyrin IX, an effective photosensitizer (194– 196). Heme is synthesized from glycine and succinyl CoA. The rate-limiting step in the pathway is the conversion of glycine and succinyl CoA to ALA, which is under negative feedback control by heme (197). Excess exogenous ALA, however, can bypass this control point and produce porphyrins that, when photoactivated, generate the photosensitizing effect for PDT and porphyrin fluorescence for photodiagnosis (194–199).

There is a great variation of ALA-induced porphyrins in normal tissues. Such tissue selectivity may be due to various capacities of heme production or to different feedback control mechanisms. Rapidly proliferating cells may produce more ALA-derived porphyrins, probably owing to a low activity or/and a limited capacity of ferrochelatase (195–200). This differential provides a biologic rationale for clinical use of ALA-based PDT. In 1990 Kennedy et al. (195) first successfully treated skin disorders with topically ALA–PDT. Since then, this new approach has arisen a great interest and is now being studied intensively for its potential use for the treatment and/or detection of a large variety of superficial lesions (198,199).

In a total of 826 superficial skin basal cell carcinomas treated with topically ALA–PDT in nine hospitals in Europe and Canada the weighted average rates of complete response, partial response, and no response were 87%, 5%, and 8% respectively (199). In addition,

promising clinical results have been obtained for a variety of skin superficial malignant and nonmalignant lesions such as squamous cell carcinoma, Bowen's disease, mycosis fungoides, psoriasis, etc. (198,199). For example, DUSA Pharmaceuticals Inc. (Toronto, Canada) recently reported the results of two parallel phase III clinical trials using Levulan (ALA) for PDT of patients with actinic keratoses (a superficial benign lesion that can go on to squamous cell carcinoma) of the face and scalp. Two hundred forty patients, in total, received topically either 20% ALA or a placebo for overnight followed by irradiation with blue light at 10 J/cm$^2$. In the two trials, 86% and 81% of the treated lesions cleared after a single treatment, with 94% and 91% clearing after a second treatment, respectively. This compared with 32% and 20% clearance with the placebo, respectively. Each trial was statistically significant ($P$<.001).

The current protocols of the topical modality are, however, far from ideal for the treatment of nodular skin lesions. In a total of 208 nodular basal cell carcinomas treated in six hospitals the average rates of complete response, partial response, and no response were 53%, 35%, and 12%, respectively (199). PDT with topical application of P-1202 (ALA methylester), a product currently developed by PhotoCure AS (Oslo, Norway), has in recent clinical studies shown promising results for the treatment of skin lesions, particularly for the thick lesions (with prior simple debulking procedure). Among 506 lesions treated the rates of complete response of actinic keratosis (52 lesions), superficial basal cell carcinomas (217 lesions) and nodular basal cell carcinomas (237 lesions) were 89%, 86%, and 84%, respectively (Warloe T: unpublished data). The high complete response rates may be related to a high production of P-1202-induced protoporphyrin IX in the lesions. Furthermore, P-1202 produces much less protoporphyrin IX in normal skin than in lesions, so that it leads to a high selectivity using this compound (Peng Q: unpublished data). Generally, PDT with topical application of ALA or its methylester has several potential advantages over conventional treatments. It is noninvasive, convenient and well tolerated by patients; can be applied repeatedly; and produces excellent cosmetic results regardless of lesion size.

It is not yet fully understood whether there are side effects associated with systemic ALA administration. It appears that oral administration of ALA (<60 mg/kg) or intravenous infusion (<30 mg/kg) does not lead to any neurotoxic symptoms, although some patients may have mild, transient nausea and/or temporary abnormalities of liver functions (198,200). Treatment of patients with oral cavity squamous cell carcinomas was reported; there were few complete remissions, but the treated areas of all other 12 patients with dysplasia lesions were healed without scarring. No patients had cutaneous photosensitivity after 48 hours (201). Barr et al. (202) obtained promising results in the treatment of five patients with high-grade esophageal dysplasia in Barrett's esophagus. These results suggest that systemically ALA–PDT may have potential for the treatment of superficial mucosal precancerous and cancerous lesions of the aerodigestive tract without the risk of prolonged skin phototoxicity (203).

A preferential accumulation of ALA-induced porphyrins in neoplastic cells provides the possibility of photodetection of the porphyrin fluorescence in tumor cells. Such a procedure can be performed by means of fiberoptic point monitoring systems or of fluorescence imaging systems after topical, local internal or systemic administrations of ALA or its esters.

By using the fluorescence cystoscopy Kriegmair et al. (204) have observed a sharply marked red fluorescence induced by ALA in the urothelial carcinoma after intravesical instillation of 3% ALA solution. In a group of 104 patients with bladder carcinoma examined, the detection sensitivity of the ALA-based porphyrin fluorescence cystoscopy was 96.9%, substantially higher than that (72.7%) of conventional white light cystoscopy (205). ALA-induced porphyrin fluorescence may also be used for photodetection of early-stage lung carcinoma and malignant glioma (199).

## Light

Typically, fluences of 50–500 J/cm$^2$ of red light are needed in clinical PDT with Photofrin (206). New sensitizers, e.g., mTHPC, are usually more efficient, mainly due to larger extinction coefficients in the red. Consequently, smaller fluences are required, typically 10 J/cm$^2$ (207). If the surface irradiance exceeds 200 mW/cm$^2$, hyperthermia may contribute to the PDT effect (208–212). For interstitial treatment with diffusing fibers inserted into the tumor, the hyperthermia limit is below 400 mW/cm diffusing fiber (212). Hyperthermia and PDT may act synergistically when hyperthermia is given after PDT (213,214). Applying a nonhyperthermic surface irradiance of 100 mW/cm$^2$ for 30 minutes, which is about a maximum for practical applications, requires 0.27–2.7 W to provide 50–500 J/cm$^2$ to a tumor area of 10 cm$^2$. A metal halide lamp of 250 W filtered carefully to eliminate heat can provide up to 5 W of a 40-nm red light by use of an elliptic reflector and an all-dielectric bandpass filter (215). Such a lamp can be coupled to a 0.5-cm diameter light guide out of the distal end of which one can get up to 450-mW red light (5). A 300-W short arc plasma discharge (216), or a xenon arc lamp of similar power, is expected to give a similar fluence rate of red light to the metal halide lamp. This would hardly be enough for bladder PDT nor for treatment of a few cm of the oesophagus. Diode lasers giving a few watts of red light down to 630 nm (217) are now commercially available and are probably the light sources of choice if only one sensitizer is to be used. For surface irradiation light-emitting diode arrays may be applied. However, for investigational purposes one needs to vary the wavelength. Until recently, dye lasers, which can give up to a few watts of light in the red and near infrared region, have been the most widely used as light sources in PDT. For pumping the dye lasers, either argon ion, copper vapors, or frequency-doubled Nd-YAG lasers are being used. The sophisticated state-of-the-art light sources are pulsed lasers based on nonlinear crystal oscillators, so-called optical parametric oscillators, which can be tuned in a very wide wavelength region (220–2200 nm) (218,219). Their power is probably still not large enough for general clinical use in PDT. It has been claimed that lasers giving short pulses penetrate deeper into tissue than CW lasers, because of transient bleaching of tissue chromophores (220). This needs verification. Under normal conditions it has been shown that copper vapor-dye lasers, diode arrays and filtered arc lamps give similar depths of PDT necrosis (215).

Under some circumstances (e.g., to avoid perforation) it may be desirable to have a shallow penetration of the light. Then light sources emitting shorter wavelengths should be used. For porphyrins, 410 nm light is expected to give better results than 630 nm light down to about 1.5 mm in normal human skin and muscular tissue (221).

## Future Directions of PDT

Appearance of new photosensitizers being developed by various pharmaceutical companies will not only extend the number of choices for treating those cancers already treated with Photofrin but extend the indications as well. An example is the application of PDT with BPD-MA for treatment of age-related macular degeneration and perhaps for rheumatoid arthritis, the possible use of SnET2 or mTHPC for prostatic diseases, the topical use of ALA or its methylester for dermatologic superficial lesions, and perhaps the application of PDT for treatment of coronary artery diseases. However, the real challenge in the future is gaining physician acceptance of PDT as a viable treatment modality. This need is being met by the appearance of a variety of PDT courses which are organized by PDT centers, notably the University of Louisville, KY, Grant Medical Center in Columbus, OH, and the Royal London Hospital (U.K.). There is a relatively long learning curve in learning how to apply PDT. This relates, in part, to the potential for adverse reactions if light reaches normal tissues which have accumulated a photosensitizer. Since relatively expensive light sources are required, it is hoped that the advent of diode lasers, not only for the new photosensitizers, but now also for Photofrin, will mitigate the problem. It should be emphasized that substantial aid in protocol development is being provided by both the pharmaceutical companies involved in drug development as well as the device designers. Thus, development of new drugs with limited skin photosensitization, along with improved light sources, should aid in convincing physicians that there is a compelling reason for them to learn and use what to most of them is still an unknown entity. This will only come with time as those who are considered to be objective in their assessments indicate its utility to others.

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