Photodynamic therapeutics: basic principles and clinical applications

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Photodynamic therapy (PDT) is a promising new treatment for cancer that has been recently accepted in the clinic. PDT involves the localization of a light-sensitive drug (photosensitizer) in the target tissue prior to illumination using an appropriate wavelength. Cytotoxic agents generated upon illumination trigger a cascade of biochemical responses that inactivate cancer cells either directly or through the induction of vascular stasis. These treatments are better tolerated as they destroy diseased tissue while leaving normal tissue intact. The haematoporphyrin derivative, Photofrin®, has been approved in a number of European and Asian countries, as well as in North America. To enhance the potential of PDT and explore its application for other conditions, second-generation photosensitizers are being rigorously investigated.

Traditional cancer therapies such as surgery, radiation therapy and chemotherapy involve a delicate balance between removing or destroying diseased tissue and sparing surrounding normal healthy cells. These conventional treatments result in serious side-effects caused by the loss of normal cell function as a result of having relatively indiscriminate cytotoxic properties. Consequently, the development of new treatment protocols that display more selectivity for diseased tissue is very important.

Photodynamic therapy (PDT) is a promising new cancer treatment that involves the combination of visible light and a photosensitizer. Each factor is harmless by itself, but when combined with oxygen, can produce lethal cytotoxic agents that can inactivate tumour cells. This enables greater selectivity towards diseased tissue as only those cells that are simultaneously exposed to the photosensitizer, light and oxygen are exposed to the cytotoxic effect. The dual selectivity of PDT is produced by both a preferential uptake of the photosensitizer by the diseased tissue and the ability to confine activation of the photosensitizer to this diseased tissue by restricting the illumination to that specific region. Therefore, PDT allows for the selective destruction of tumours while leaving normal tissue intact.

Mechanism of action

The photochemical and photophysical principles of PDT have been extensively studied (Box 1)[1,2]. Briefly, upon illumination, the photosensitizer is excited from the ground state (S_0) to the first excited single state (S_1), followed by conversion to the triplet state (T_1) via intersystem crossing. The longer lifetime of the triplet state enables the interaction of the excited photosensitizer with the surrounding molecules, and it is generally accepted that the generation of the cytotoxic species produced during PDT occurs whilst in this state.

The excited triplet state can react in two ways, defined as Type I and Type II mechanisms[3]. A Type I mechanism involves hydrogen-atom abstraction or electron-transfer reactions between the excited state of the sensitizer and a substrate that is either biological, a solvent or another sensitizer, to yield free radicals and radical ions (see Box 1). These free radical species are generally highly reactive

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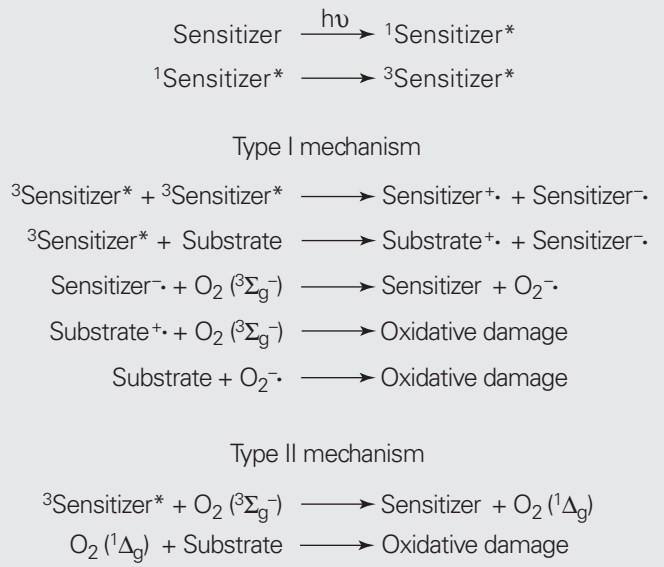
Box 1. Photochemical and photophysical principles of photodynamic therapy



The underlying mechanism involved in photodynamic therapy is governed by the ability of the photosensitizer to absorb light of a specific wavelength and jump to its first excited singlet state. From there, it can readily transform to the much longer lived triplet state via intersystem crossing. This excited state of the photosensitizer can effectively interact with its surroundings, either by Type I hydrogen-atom abstraction or electron-transfer reaction, or by a Type II energy transfer to ground-state molecular oxygen ($^3\Sigma_g^-$) to form singlet oxygen ($^1\Delta_g$). The reactive species generated (free radicals and reactive oxygen species) will ultimately lead to oxidative damage and cell death. Abbreviations: hν, light energy; *, excited state; •, radical.

and can readily interact with molecular oxygen to either generate reactive oxygen species such as superoxide anions or hydroxyl radicals or can cause irreparable biological damage. These reactions produce oxidative damage that is eventually expressed as biological lesions.

By contrast, a Type II mechanism results from an energy transfer between the excited triplet state of the sensitizer and the ground-state molecular oxygen, generating the first excited state of oxygen, singlet oxygen (see Box 1). This zwitterionic species is extremely reactive and can interact with a large number of biological substrates, inducing oxidative damage and ultimately cell death. While it is generally accepted that Type II processes predominate during PDT and that singlet oxygen is the primary cytotoxic agent responsible for the biological effects displayed[4–7], Type I reactions become more important at low oxygen concentrations or in more polar environments[1,8]. However, the initial reaction is of lesser importance as both Type I and Type II reactions lead to similar oxidative damage and comparable free radical chain-reactions in the presence of oxygen. The overall effect of either a Type I or Type II reaction pathway is the production of oxidative damage within the target cell that will ultimately lead to tumour destruction.

Biological response

While it is clear that PDT can induce the production of cytotoxic agents that readily destroy neoplastic cells, this response is affected *in vivo* by the complexity of biological systems. Any number of subcellular targets can be attacked during PDT, including mitochondria, lysosomes, plasma membranes and nuclei, and the exact target can affect whether cell death occurs by necrosis or apoptosis[9,10]. In addition, while it has been shown that the action of some amphiphilic sensitizers directly cause tumour cell death, most photosensitizers induce tumour necrosis via vascular shutdown[1,10–14]. Finally, it has been shown that PDT can induce inflammation and other tumour-specific immune reactions[1,9,10]. The exact method of PDT-induced tumour destruction depends on the photosensitizers used and varies greatly depending on the condition being treated and the light dose used. However, knowing the biological effects behind cell death can lead to the selection of an ideal photosensitizer to treat a given disease.

Photosensitizers

Photosensitizers are compounds that are capable of absorbing light of a specific wavelength and transforming it into useful energy. In the case of PDT, this would involve the production of lethal cytotoxic agents. There are hundreds of natural and synthetic dyes that can function as photosensitizers for PDT, ranging from plant abstracts to complex synthetic macrocycles. The key characteristic of any photosensitizer is its ability to preferentially accumulate in diseased tissue and to then generate cytotoxic agents to induce the desired biological effect. Table 1 provides an overview of the fundamental clinical characteristics of various photosensitizers currently in clinical or preclinical trials.

Photofrin®

The first generation photosensitizers are haematoporphyrin derivatives[15] such as Photofrin® (Fig. 1) and are the most

Table 1. Fundamental clinical characteristics of the photosensitizers presently in clinical or preclinical trials

Photosensitizer	Wavelength (nm)	Extinction coefficient (M^{-1} cm^{-1})	Mode of delivery	Delivery vehicle	Typical dose (mg kg^{-1})	Light dose (J cm^{-2})	Time post-injection	Duration of skin photosensitivity
Haematoporphyrin-derivative	630	3.0×10^3	IV or topical	5% Dextrose	2.0–5.0	100–200	24–48 h	2–3 months
Methylene blue	668	9.5×10^4	*Ex vivo*	Water-soluble	1 μM	50,000 lux	n/a	n/a
5-Aminolaevulinic acid (protoporphyrin IX)	635	$<5.0\times10^3$	Topical, oral or IV	Water-soluble	<60 (orally) <30 (IV)	100–200	–	1–2 days
Verteporfin	690	3.5×10^4	IV	Liposomal	0.1–2.0	100–200	30–150 min	3–5 days
Tin etiopurpurin	660	2.8×10^4	IV	Lipid emulsion	1.0–2.0	100–200	24 h	Up to 1 month
Temoporfin	652	3.0×10^4	IV	PEG/ ethanol/ water	0.1–0.3	8–12	24–48 h	Up to 6 weeks
Texaphyrins	732	4.2×10^4	IV	Water-soluble	0.6–7.2	150	3–5 h	Minimal
Phthalocyanines	670–680	2.5×10^5	IV	Liposomal or water-soluble	0.5–2.0	100	24–72 h	8–10 days
Naphthalocyanines	750–780	$>10^5$	IV	Liposomal	–	–	–	–
N-aspartyl chlorin e6	664	4.0×10^4	IV	Water-soluble	0.5–3.5	25–100	4 h	3–7 days
Rhodamines	511	2.0×10^4	*Ex vivo*	Water-soluble	25 μM	1–10	n/a	n/a
Porphycenes	630	5.2×10^4	Topical	Liposomal	1.0–3.0	–	n/a	–
Hypericin	590	4.4×10^4	Topical	Liposomal	–	–	–	–

Abbreviations: IV, intravenous; PEG, polyethylene glycol.

commonly used photosensitizers. Haematoporphyrin derivatives were originally synthesized[16] by combining haematoporphyrin with 5% sulphuric acid in acetic acid at room temperature. Subsequently, the mixture was treated with an aqueous base and then neutralized. This led to the formation of a complex mixture of dimers and oligomers primarily involving ester and ether linkages[17]. Partial purification of the most active of these oligomers by high-performance liquid chromatography (HPLC) or size-exclusion gel chromatography lead to Photofrin, 90–95% of which is the active component[18].

Photofrin is marketed by QLT PhotoTherapeutics (Vancouver, Canada) and has been accepted in the clinic in several countries for the treatment of early- and late-stage lung cancer, superficial and advanced oesophageal cancer, bladder cancer, superficial and early-stage gastric cancers, early stage cervical cancer and cervical dysplasia (a precancerous condition) (http://www.qlt-pdt.com; Ref. 19). In addition, Photofrin is being investigated as a possible therapy against Karposi's sarcoma, cancers of the head and neck, brain, intestine, lung, breast and skin (both primary and metastatic breast cancers), as well as urinary bladder, abdominal and thoracic cancers. Other conditions include Barrett's oesophagus, psoriasis and arterial restenosis[9,20], and all of these conditions are being investigated in clinical trials with mostly promising results.

Derivations of Photofrin are also being used. For example, Photoheme is produced in Russia and has been accepted by the Pharmacological Committee of Russia for a wide range of clinical uses including skin, breast, oropharingeal, lung, larynx and gastrointestinal cancers, as well as psoriasis and prophylaxis for corneal transplant opacity and recurrent blindness[21–23].

Second-generation photosensitizers

Despite its apparent successes, haematoporphyrin derivatives have two very important disadvantages[2,24]. First of all, these compounds are readily taken up and retained by

cutaneous tissue for up to ten weeks post-injection. This causes a marked skin photosensitivity that requires the patient to avoid bright sunlight, this being an obvious disadvantage especially for patients with late-stage malignancies. Secondly, while Photofrin has a number of absorption peaks between 400 and 650 nm, its weakest absorption band at 630 nm is used most often to excite the photosensitizer as the tissue penetration of light increases with increasing wavelength. While such disadvantages have not stopped Photofrin from becoming a useful tool against cancer and other conditions, the search for new photosensitizers remains an important goal.

An ideal photosensitizer for PDT would have the following characteristics[16,24–26]:

- Be chemically pure and of known and constant composition
- Have a minimal dark toxicity and only be cytotoxic in the presence of light
- Be preferentially retained by the target tissue
- Be rapidly excreted from the body, thus inducing a low systemic toxicity
- Have a high photochemical reactivity, with high triplet-state yields (ϕ_T) and long triplet-state lifetimes (τ_T) and be able to effectively produce singlet oxygen and other reactive oxygen species
- Have a strong absorbance with a high extinction coefficient (ε) at a longer wavelength (e.g. 600–800 nm) where tissue penetration of light is at a maximum while still being energetic enough to produce singlet oxygen. Furthermore, cheaper diode lasers can be used in this range, thus increasing the potential utility of PDT in a clinical setting.

While no photosensitizer can be deemed ideal for every possible application, a number of second-generation photosensitizers have been developed to overcome the shortcomings of Photofrin and to take advantage of their more ideal properties.

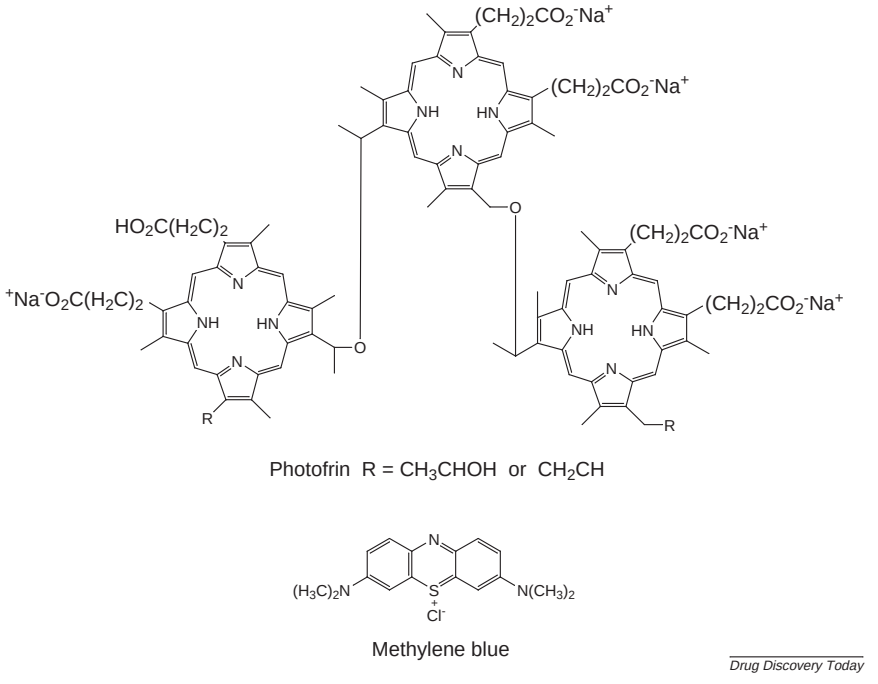


Figure 1. *Photosensitizers presently accepted in the clinic. Photofrin® is used to treat a variety of cancers in several countries and methylene blue is used by the Swiss and German Red Cross for the sterilization of freshly frozen plasma units. Photofrin® consists of a complex mixture of dimers and oligomers ranging from two to nine porphyrin units linked primarily by ether bonds.*

Methylene blue

The only photosensitizer currently used in the clinic other than the haematoporphyrin derivatives is methylene blue (Fig. 1), which is used by the Swiss and German Red Cross for the decontamination of freshly frozen plasma units[27,28]. This photosensitizer effectively inactivates extracellularly enveloped viruses[28] and is used in the clinic as a treatment for methaemoglobinaemia, thus showing its lack of toxicity in humans[29]. This phenothiazinium dye has been used extensively for over a century as a biological assay stain and can be used in the clinical diagnosis of a variety of diseases and as a tumour marker in surgery. However, its use as an *in vivo* photosensitizer is limited by its reduction by ubiquitous cellular enzymes to the colourless form, which is photodynamically inactive[30].

5-Aminolaevulinic acid

The use of 5-aminolaevulinic acid (ALA)-induced endogenous photosensitizers is a novel method currently being investigated for PDT (Ref. 31). The natural porphyrin, haem, is synthesized in every energy-producing cell and is the prosthetic group for haemoglobin, myoglobin and other haematoproteins. The rate-limiting step in the

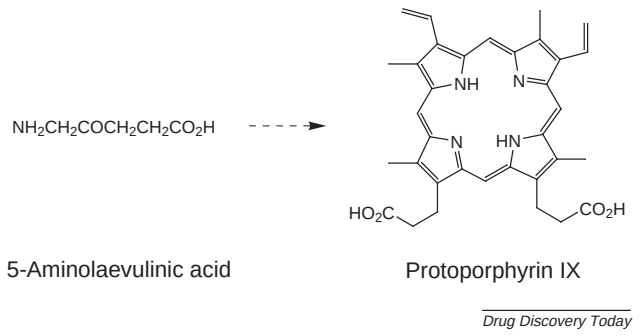


Figure 2. *5-Aminolaevulinic acid (ALA) and protoporphyrin IX. The stimulation of the production of endogenous photosensitizers such as protoporphyrin IX by ALA is a novel method being examined for photodynamic therapy with a new drug application submitted for the treatment of actinic keratoses.*

synthetic pathway for haem is the conversion of glycine and succinyl coenzyme A to ALA, this step being under a negative feedback control by haem. However, the addition of excess exogenous ALA can bypass this negative feedback, leading to a build-up of protoporphyrin IX, an effective photosensitizer for PDT (Ref. 32). As such, ALA has been extensively studied as a prodrug for the endogenous production and accumulation of protoporphyrin IX in diseased tissue, especially in malignancies (see Fig. 2). The tumour selectivity of ALA is influenced by a number of factors, including increased permeability of abnormal keratin, increased levels of porphobilinogen deaminase, decreased levels of iron and decreased activity of ferrochelatase in the tumour cells. These conditions result in an accumulation of protoporphyrin IX in diseased cells, resulting in selectivity for the target tissue[31,33].

Marketed by DUSA Pharmaceuticals (Toronto, Canada) under the name Levulan®, ALA is the closest compound to being accepted into the clinic for photodynamic applications, with its New Drug Application (NDA) being almost accepted by the Food and Drug Administration (FDA) for the treatment of actinic keratoses, a common sun-induced precancerous skin lesion (see http://www.dusapharm.com). The company has also announced Phase I/II clinical trials involving Levulan as a treatment for acne, for the removal of unwanted hair, and for the photodetection of bladder cancer. Other clinical trials are under way using ALA as a therapy for non-melanoma skin cancer[34], endometrial ablation[35], late-stage oesophageal cancer[36], gastrointestinal cancer[20], Barrett's oesophagus[37] and psoriasis[35]. Because of the low molecular weight and polar properties of ALA, it can also be used as a topical PDT agent against a number of dermatological conditions and has been shown to be effective against superficial basal cell carcinomas, Bowen's disease, erythroplasia of Queyrat, cutaneous T-cell lymphoma and hirsutism[35].

One of the problems associated with ALA is that it does not penetrate the skin very deeply when used as a topical agent and in an attempt to overcome this, ALA esters are now being examined. PhotoCure AS (Oslo, Norway) is marketing the methyl ALA ester, P1202, and is studying its potential against basal cell carcinomas and other skin lesions together with several conditions that have been shown to be treated effectively by ALA (Ref. 9).

Verteporfin

QLT PhotoTherapeutics has carried out extensive work on the second-generation photosensitizer verteporfin (benzoporphyrin-derivative monoacid ring A; Fig. 3) (see http://www.qlt-pdt.com, Ref. 38). In collaboration with CIBA Vision Corporation (Duluth, GA, USA), verteporfin (Visudyne™) is presently undergoing Phase III clinical trials for the treatment of wet age-related macular degeneration (AMD). Furthermore, an NDA has been submitted to the FDA for the use of Visudyne in AMD, as well as a marketing clearance application for the European Union through the European Medicines Evaluation Agency (EMEA). AMD is the leading cause of blindness in humans over the age of 50 and involves the rapid growth of abnormal blood vessels under the central retina. Leaking from these abnormal vessels causes scarring and an accelerated loss of visual acuity and there is no adequate treatment protocol for 80–90% of these patients[39]. As PDT is known to induce vascular shutdown, compounds such as verteporfin are ideal for treating this condition. Initial results have been very promising, showing a significant preservation of vision in a number of patients.

Verteporfin is also in Phase III clinical trials for cutaneous non-melanoma skin cancer and Phase I/II trials against other non-melanoma skin cancers (such as multiple non-melanoma skin cancer)[40], psoriasis[41], and psoriatic and rheumatoid arthritis. Extensive preclinical work has been carried out using verteporfin as a therapy for multiple sclerosis and Barrett's oesophagus and as an agent to achieve endometrial ablation and bone marrow purging[40]. Verteporfin has a much stronger absorbance at a longer wavelength (690 nm), where tissue penetration of light is 50% greater than that of Photofrin at 630 nm. In addition, verteporfin is rapidly absorbed by the tumour,

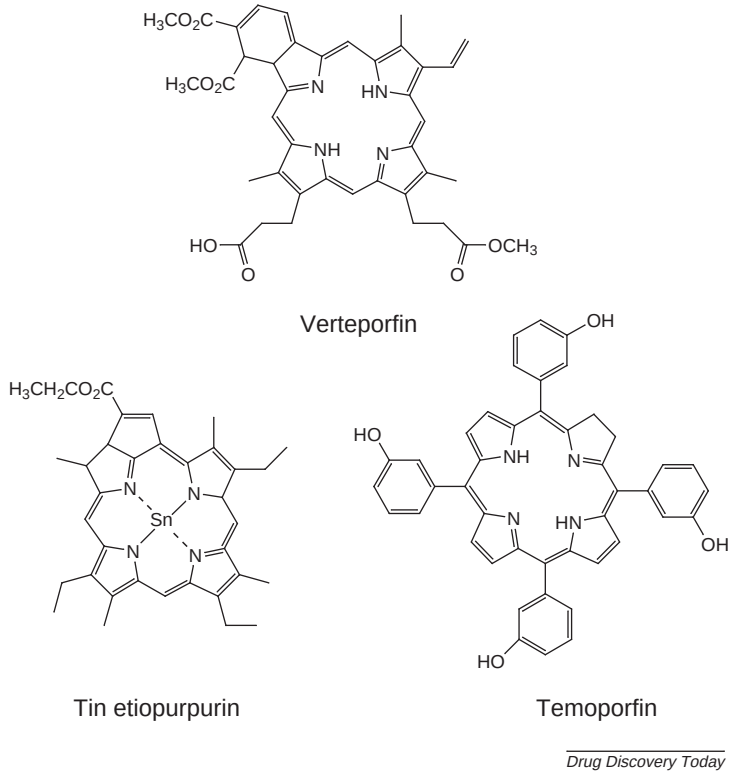


Figure 3. *Verteporfin and tin etiopurpurin are both in Phase III clinical trials for the treatment of macular degeneration, while Phase III trials are under way using temoporfin against head and neck cancers. Early stage clinical trials and preclinical work using these photosensitizers are also under way (see Table 2).*

reaching an optimal tumour–normal tissue ratio 30–150 minutes after intravenous injection, and is rapidly cleared from the body so that skin photosensitivity only lasts a few days[32].

Tin etiopurpurin

Miravant Medical Technologies (Santa Barbara, CA, USA) markets tin etiopurpurin (SnET2)[42] as Puryltin™ (Fig. 3) as part of their PhotoPoint™ procedure. The PhotoPoint procedure involves three components: a light-activated photosensitizer, a light-producing device and a light-delivery system and is described in details on their Internet site (http://www.miravant.com). Puryltin is among the most developed of Miravant's light-activated compounds, and is currently in Phase III clinical trials for the treatment of wet AMD (in collaboration with Pharmacia & Upjohn, Bridgewater, NJ, USA). The photosensitizer is also in Phase I clinical trials against prostatic cancer (that has not spread beyond the prostate itself)[43] and in Phase II trials for cutaneous metastatic breast cancer and Kaposi's sarcoma in patients with acquired immunodeficiency syndrome (AIDS)[9]. Preclinical work with SnET2 has included extensive examination of its effects on other malignancies such as brain, lung, skin, head and neck cancer. Non-malignant conditions such as psoriasis and restenosis are also effectively treated using SnET2.

Temoporfin

Temoporfin (Fig. 3) or tetra(*m*-hydroxyphenyl)chlorin (*m*THPC)[16,44], under the tradename Foscan™, is being marketed by Scotia Pharmaceuticals (Guildford, Surrey, UK) as a new second-generation photosensitizer for PDT (see http://www.quantanova.com). Phase III clinical trials have begun in Europe and the US using Foscan against head and neck cancers[45]. Trial work has concentrated on this area as the conventional treatments are difficult, ineffective and disfiguring. Recent press releases from the company state that Foscan has been given fast-track designation by the FDA for the palliative treatment of recurrent, refractory and second primary squamous cell carcinomas of the head and neck in patients considered to be incurable using surgery or radiotherapy, the file submission being expected soon. Foscan is also in clinical trials for late-stage oesophageal cancer and dysplasia in Barrett's oesophagus[36]. Future trials using this photosensitizer in Europe, the US and the Far East against malignant and non-malignant diseases are anticipated and will include trials against gastric and prostatic cancers, hyperplasia, field sterilization after cancer surgery and for the control of antibiotic-resistant bacteria[45]. In addition, topical formulations of temoporfin are being developed to compete with ALA against skin cancers and other dermatological conditions[45].

Temoporfin could be one of the most phototoxic of all the second-generation photosensitizers currently being investigated. It requires very low doses (as little as 0.1 mg kg^{-1}) as well as an unusually low light dose (as low as 10 J cm^{-2}), making it 100-fold more photoactive than Photofrin, which requires drug doses of 2–5 mg kg^{-1}

Table 2. Photosensitizers currently in clinical trials or late preclinical development

Company	Photosensitizer	Tradename	Clinical application	Clinical status
QLT PhotoTherapeutics	Haematoporphyrin-derivative	Photofrin®	Oesophageal, lung, bladder, gastric and cervical cancer, cervical dysplasia	Approved
QLT PhotoTherapeutics	Haematoporphyrin-derivative	Photofrin®	Head and neck, intestinal, lung, skin, bladder and metastatic breast cancer, Kaposi's sarcoma, Barrett's oesophagus, psoriasis, arterial restenosis	Phase I/II through Phase III
State Research Centre for Laser Medicine (Russia)	Haematoporphyrin-derivative	Photoheme	Skin, breast, oropharingeal, lung, larynx and gastrointestinal cancer, psoriasis, prophylaxis for corneal transplant opacity	Approved
German and Swiss Red Cross	Methylene blue	–	Sterilization of freshly frozen plasma	Approved
DUSA Pharmaceuticals	5-Aminolaevulinic acid (ALA)	Levulan®	Actinic keratoses	NDA submitted
			Hair removal, acne, non-melanoma skin, oesophageal and gastrointestinal cancer, endometrial ablation, psoriasis, Barrett's oesophagus	Phase I/II Preclinical
PhotoCure AS	5-Aminolaevulinic acid (ALA)	P1202	Basal cell carcinoma and other skin lesions	Preclinical
QLT PhotoTherapeutics	Verteporfin	Visudyne™	Macular degeneration	Phase III
		Verteporfin	Non-melanoma skin cancer, psoriasis	Phase I/II
			Psoriatic and rheumatoid arthritis, multiple sclerosis, Barrett's oesophagus, endometrial ablation, bone marrow purging	Preclinical
Miravant Medical Technologies	Tin etiopurpurin	Purlytin™	Macular degeneration	Phase III
			Metastatic breast cancer, Kaposi's sarcoma	Phase II
			Prostatic cancer	Phase I
			Brain, lung, skin and head and neck cancer, psoriasis, restenois	Preclinical
Scotia Pharmaceuticals	Temoporfin	Foscan®	Head and neck cancer	Phase III
			Oesophageal cancer, Barrett's oesophagus	Phase I/II
			Gastric and prostate cancer, hyperplasia, sterilization, antibiotic	Preclinical
Pharmacyclics	Texaphyrins	Lutrin™	Breast cancer	Phase II
		Antrin™	Angioplasty	Phase I
		Optrin™	Macular degeneration	Phase I
Ciba–Geigy	Phthalocyanine	CGP55847	Squamous cell carcinoma of upper aerodigestive tract, psoriasis	Phase I/II
State Research Centre NIOPIK (Russia)	Phthalocyanine	Photosense	Skin, breast, oropharingeal, lung, larynx and gastrointestinal cancer, psoriasis	Phase III
V.I. Technologies	Phthalocyanine	Pc4	Sterilization of blood products	Phase I/II (late 1999)
Nippon Petrochemical	N-aspartyl chlorin e6	NPe6	Endobronchial lung cancer and cutaneous malignancies	Phase I
Theratechnologies	Rhodamines	TH9402	Bone marrow purging	Phase I
Cytopharm and GlaxoWellcome	Porphycenes	ATMPn	Dermal applications (psoriasis, non-melanoma skin cancer)	Preclinical
Vim Rx Pharmaceuticals	Hypercin	–	Psoriasis, warts, skin cancer	Phase I

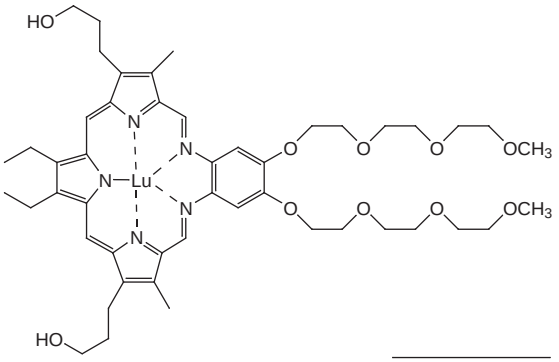


Figure 4. *Lutetium texaphyrin (Lutrin™).*

and light doses of 100–200 J cm^{-2} (Ref. 9). The reasons behind this exceptionally high activity are not fully known. While improved optical properties and singlet oxygen quantum yields can partially explain this increased phototoxicity, it appears the explanation resides in the subtumoral and subcellular localization of the compound. While on intravenous administration, lipophilic sensitizers bind to lipoproteins and hydrophilic compounds to serum albumin[46], temoporfin can bind to an unknown plasma protein presumably involving the polyethylene glycol (PEG) vehicle, which might produce differences in subcellular localization[47]. Furthermore, the interaction with a plasma protein other than albumin or lipoproteins could explain the novel pharmacokinetics. The immediate peak in plasma drug levels produced by intravenous administration is followed by a second plasma peak several hours later[48,49] and this could be a factor in the high phototoxicity of this compound.

Texaphyrins

Texaphyrins (Fig. 4) are 'Texas-sized' porphyrins[50,51] featuring a penta-aza core and are marketed by Pharmacyclics (Sunnyvale, CA, USA) as a photosensitizer (see http://www.pcyc.com). Under the trade name Lutrin™, lutetium texaphyrin is undergoing Phase II clinical trials as a possible therapy for breast cancer. The main advantage of using texaphyrins as PDT agents is their strong absorbency at a much longer wavelength (732 nm) so that treatment can be carried out effectively on a larger tumour or at a greater depth. The lutetium texaphyrin derivative, Antrin™, is also in Phase I clinical trials for angioplasty of atherosclerotic cardiovascular disease and the treatment and prevention of restenosis, while another derivative, Optrin™, is in Phase I trials for AMD. In addition, the company is also developing radiosensitizers and chemosensitizers based on the texaphyrin framework with the radiation sensitizer, Xcytrin™, currently in Phase III clinical trials for the treatment of brain metastases and Phase I trials for newly diagnosed primary brain tumours.

Phthalocyanines

Phthalocyanines are tetrapyrrolic macrocycles that, unlike the porphyrins, have nitrogen atoms linking the individual pyrrole units instead of methine bridges (Fig. 5). The periphery of the macrocycle is extended by benzene rings, which strengthens the absorption at longer wavelengths compared to porphyrins such as Photofrin. Phthalocyanines have long been used as dyes and colouring agents and have recently also been used as photoconducting agents in photocopying machines. They have been extensively studied as PDT agents because of their favourable photophysical properties and because their properties (such as solubility) can be altered through the addition of substituents to the periphery of the macrocycle[2,52].

Ciba–Geigy Ltd (Basel, Switzerland), in partnership with QLT PhotoTherapeutics, has developed a liposomal preparation of zinc phthalocyanine (CGP55847) that has been in Type I/II clinical trials in Switzerland for patients with squamous cell carcinomas of the upper aerodigestive tract[53]. Attempts have also been made to develop a topical formulation of this photosensitizer for use in treating psoriasis[20].

The oncological centre of the Russian Academy of Medical Sciences (Moscow, Russia) and the surgical clinic of the Moscow Medical Academy (Moscow, Russia) are currently carrying out trials using a mixture of sulphonated aluminium phthalocyanine derivatives (Photosense) against malignancies such as skin, breast, lung and gastrointestinal cancer[21,54,55]. The addition of the sulphonate

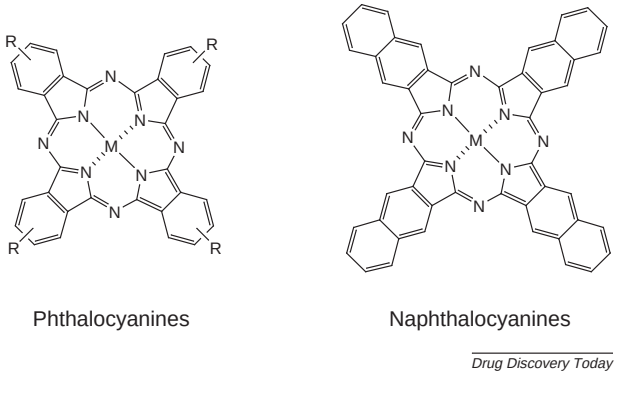


Figure 5. *Phthalocyanines and naphthalocyanines.*

groups to the periphery of the phthalocyanine greatly increases the solubility of these compounds, removing the need for liposomal delivery vehicles, and success with Photosense has been relatively promising.

A silicon-based phthalocyanine[56] (Pc4) is also being studied for the sterilization of blood components by V.I. Technologies (Vitex, Melville, NY, USA), who are based at the New York Blood Center. Preclinical results with this drug have been very promising and it is hoped that it will enter clinical trials by the end of 1999.

Addition of a second benzene ring to the periphery of the phthalocyanine produces naphthalocyanines[57] (Fig. 5). These compounds absorb at a higher wavelength than phthalocyanines (770 nm versus 680 nm), thus increasing the therapeutic depth that can be achieved and rendering them potential photosensitizers for highly pigmented tumours such as melanomas[58]. Significant work has been carried out evaluating these compounds as photosensitizers for PDT (Refs 58–60) and they are being pushed towards clinical trials in Bulgaria by the Bulgarian Academy of Sciences (Sofia, Bulgaria)[24].

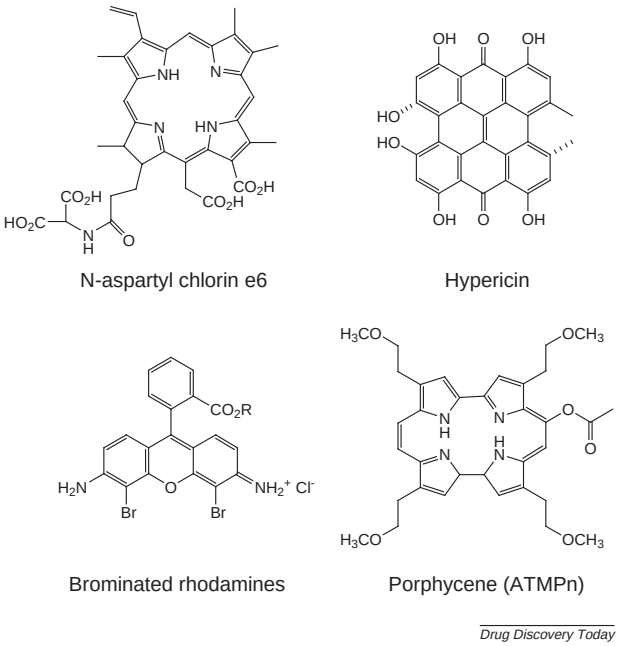


Figure 6. *Phase I/II clinical trials and preclinical work are being done using photosensitizers such as these for the treatment of different conditions (see Table 2).*

N-aspartyl chlorin e6

Under the supervision of Nippon Petrochemical (Osaka, Japan), N-aspartyl chlorin e6 (Npe6; Fig. 6)[61] is being studied as a possible photosensitizer for PDT (Refs 62,63). Phase I clinical trials are under way for the treatment of cutaneous malignancies[20] and it is also being investigated in Japan as a possible therapy for endobronchial lung cancer[9]. Npe6 has been shown to be an effective photosensitizer against skin cancers with little or no long-term cutaneous photosensitivity[9]. The photodynamic activity of Npe6 also involves a combination of vascular (indirect effect) and direct anti-tumour photodamage, which is another potential advantage of this photosensitizer[64].

Rhodamines

Because of their specific uptake by mitochondria and their known use as a fluorescent probe, rhodamines have been used extensively as photosensitizers[30]. This naturally led to their use as sensitizers in the treatment of malignant tumours. However, the readily available commercial dye, rhodamine 123, is a poor phototoxin because of its high fluorescence quantum yield[65]. This problem can be remedied by adding heavy atoms such as bromine or chlorine to the macrocycle (Fig. 6). Known as the heavy atom effect, the addition of these atoms to the chromophore increases intersystem crossing from the singlet to the triplet state by increasing spin-orbital coupling, allowing otherwise forbidden changes in the spin state ($S_1 \rightarrow T_1$). The addition of halogens to the chromophore also shifts the absorption maximum towards the red end of the spectrum. This is an important feature as rhodamines absorb light at approximately 500 nm, a wavelength where tissue penetration of light is minimal and every increase in wavelength represents an important increase in its tissue penetration[30]. Despite this, rhodamines have been shown to be very effective photosensitizers against malignant cells *in vitro* and Theratechnologies (Québec, Canada) has undertaken extensive preclinical studies to examine the ability of brominated rhodamine derivatives to eradicate of leukaemia cells from bone marrow extracts or mobilized peripheral blood stem cells for use in autologous transplantation[66]. Phase I clinical trials have begun using the brominated rhodamine analogue, TH9402, for the treatment of chronic myeloid leukaemia using the patented Photodynamic cell therapy process (PDP) as described on the company's Internet site (http://www.theratech.com). This *ex vivo* photodynamic therapy, used for purging autologous stem cell grafts, has been shown to destroy diseased cells while sparing normal healthy cells, an important prerequisite for such a treatment protocol.

Porphycenes

Glaxo Dermatology, a division of GlaxoWellcome (Research Triangle Park, NC, USA), along with Cytopharm

(Menlo Park, CA, USA) have done extensive preclinical work using the porphycene ATMPn [9-acetoxy-2,7,12,17-tetrakis-(β-methoxyethyl)-porphycene] (Fig. 6)[67–69]. The presence of its four β-methoxyethyl side-chains accelerates cellular uptake whilst the acetoxy-group increases the solubility and hydrophilicity of the molecule[67]. This compound can be applied topically, making it useful for dermal applications[20]. *In vitro* studies have shown that ATMPn has an unusually fast uptake into skin cells not seen for other second-generation photosensitizers and is undergoing preclinical testing against psoriasis vulgaris and superficial non-melanoma skin cancer[67].

Other photosensitizers

The success exhibited by Photofrin and the potential shown by a number of the second-generation photosensitizers has caused an explosion in photodynamic therapy, resulting in the unveiling of new photosensitizers along with an investigation into well-known naturally occurring chromophores. Hypericin (Fig. 6), for example, is well-documented as having photodynamic activity as it causes hypericism or photopoisoning in grazing animals that consume large quantities of plants containing this compound, often leading to skin irritation, fever and even death[70]. This multicyclic quinione, which absorbs light at around 590 nm (Ref. 65), is being investigated as a photosensitizer for PDT and is presently in Phase I clinical trials for the treatment of psoriasis, warts and skin cancer (see http://www.sante.univ-nantes.fr/med.laser/sensitizer.html). The naturally occurring perylenequinones such as hypocrellins, which are produced by fungi and insects[65], are also under evaluation as PDTs.

Several pharmaceutical companies are actively developing new synthetic photosensitizers. For example, Scotia Pharmaceuticals is interested in bacteriochlorins for photodynamic therapy (see http://www.quantanova.com) while Hamamatsu Phototonics is investigating ATXS10, a chlorin derivative[71]. In reality, any chromophore that can effectively produce photocytotoxicity upon illumination has the potential to be used in photodynamic therapy, leading to endless possibilities.

Conclusion

As the new millennium nears, the need for new protocols for the treatment of cancer and other diseases is becoming acute. With the population aging and established therapies operating close to optimal levels, new therapies that can effectively treat cancer and other conditions while being cost effective are at a premium. Photodynamic therapy is essentially a very simple concept that still offers the possibility of being an effective and specific method of destroying malignant, premalignant and benign tissues while sparing surrounding normal healthy cells. Initial clinical studies have shown that PDT is effective against cancer and a variety of other diseases (see Table 2) and offers a promising treatment option for patients whose conditions have no established or effective cure or that has become refractory to existing therapies.

As cancer encompasses a large family of diseases with widely different clinical patterns, it is very unlikely that a single photosensitizer will ever serve all the diseases in oncology. It is also desirable to extend PDT into the treatment of other conditions and hence, the need to develop new photosensitizers with optimal properties for treating a given condition becomes obvious. With the acceptance of the first-generation photosensitizer, Photofrin, into the clinic around the world, second-generation photosensitizers are being tested against numerous pathogenic states (Table 2). Photodynamic therapy could therefore be an important treatment of the future.

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In short…

Qiagen (Venlo, The Netherlands) and **Becton Dickinson** (BD, Franklin Lakes, NJ, USA) have formed an equally owned, worldwide joint venture for sample collection and molecular diagnostic testing processing. The purpose of the venture (named PreAnalytiX) is to develop, manufacture and market integrated systems for the collection, stabilization and purifications of nucleic acids for molecular diagnostic testing. The first product to be launched from this collaboration is expected late in the year 2000. The Worldwide President for BD Preanalytical Solutions said: 'As molecular diagnostic testing moves into the clinical laboratory environment, products used to collect and process samples will need to be standardized. Safe, easy-to-use products that are compatible with clinical laboratory practices must be developed to eliminate the complexity of DNA and RNA processing. These standardized products are expected to significantly improve specimen quality and enhance the accuracy of test results.'