Published as:

Senge, M. O.; Brand, J. C. (2011):

Lead structures for applications in photodynamic therapy. 3. Temoporfin (Foscan®, 5,10,15,20-Tetra(*m*-hydroxyphenyl)chlorin)–A Second-generation Photosensitizer.

Photochemistry & Photobiology **87**, 1240–1296.

Temoporfin (Foscan®, 5,10,15,20-Tetra(*m*-hydroxyphenyl)chlorin), a Second Generation Photosensitizer[†,‡]

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[†] Lead Structures for Applications in Photodynamic Therapy. Part 3

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ABSTRACT
This review traces the development and study of the second generation photosensitizer 5,10,15,20-tetra(*m*-hydroxyphenyl)chlorin through to its acceptance and clinical use in modern photodynamic (cancer) therapy (PDT). The literature has been covered up to early 2011.

INTRODUCTION

The field of photodynamic therapy (PDT) (1) has seen continuous advances in the past decades. Modern studies began with investigations on haematoporphyrin (Hp) (2). To some extent the progress made can be traced along the lines of the different types of photosensitizers (PS) which have been developed. Typically, the various photosensitizers (PS) used in PDT are termed as first, second, and third generation PS (3). The first generation is basically haematoporphyrin derivative (HpD) which was approved as Photofrin for clinical use in 1993 (4), the second generation are simple, chemically pure and defined PS (e.g., Foscan) (5) and the third generation are systems currently in development which contain specific targeting groups or have been developed based on clear medical, photophysical and chemical design principles (6).

Broadly speaking, the properties for an ideal photosensitizer are: chemical purity, high quantum yield of singlet oxygen production, significant absorption in the long wavelength region (700-800 nm), preferential tumor localization, minimal dark toxicity and delayed phototoxicity, stability and easy to dissolve in the injectable solvents (formulation). While Photofrin is an effective photosensitizer, its shortcomings could not be neglected. This gave rise to the development of chemically pure PS with optimized photophysical properties. This so-called *second generation* photosensitizers have entered a lively competition during the last decade to the benefit of both science and the patients (7,8,9). This is related not only to the use as a photosensitizing drug for treatment but for the use in photodiagnostics (PD) and imaging as well (10). Within this 2nd generation, porphyrins are the most widely investigated class of compounds and it is generally assumed that these macrocycles and their reduced derivatives such as chlorins and bacteriochlorins are the most suitable candidates for PS drug development (11,12,13,14,15,16,17).

5,10,15,20-Tetra(*m*-hydroxyphenyl)chlorin (*m*THPC, **1**) with the generic name 'Temoporfin' and the proprietary name 'Foscan®' is one of these promising reduced porphyrins which has been the subject of investigations by a number of research groups for almost two decades. Now, Temoporfin has reached an exciting state of development as it has gone through the requisite clinical testing and is regarded as an established cancer drug on the market. It can thus serve as an illuminating show and test case for the potentials and limitations of current PDT drugs and might give an insight into the trends of development for the new generation of photosensitizers. This review intends to discuss the published works of *m*THPC with the intention of: Summarizing the available facts and discussing *m*THPC's potential continuation; to compare known properties with the required attributes for an ideally designed photosensitizer; if possible, delineate novel design and application principles from the biochemical and clinical results obtained with *m*THPC. For ease of argument, unless expressively stated otherwise, in the following "PS", "drug", etc. relates to *m*THPC.

Many general PDT reviews have appeared over the years. They are too numerous to list them all here and often their content overlaps. Likewise, a multitude of brief reviews on individual cancer types or PDT applications can be found; those that add to the context of this treatise are listed in the individual subsections. Next to historical reviews (18,19,20,21), Bonnett's book on *Chemical Aspects of Photodynamic Therapy* (3) is still a good entry into the field for medicinal chemistry students, as are classic reviews by Dougherty and Kessel (4,18,22,23,24) and more chemically oriented treatises (25). A more recent three part series on the basics of PDT by Castano *et al.* from 2004 is recommended for an entry into the field especially for students (26,27,28).

CHEMISTRY AND DEVELOPMENT OF TEMOPORFIN

Synthesis, characterization, and detection

In the mid eighties Bonnett *et al*. undertook a significant screening procedure (biophysically, biochemically and pharmacologically) of a library of porphyrins for the purpose of discovering an effective 2nd generation photosensitizer (29). They decided on the tetra(hydroxyphenyl)porphyrins as the most promising compounds after initial tests with their porphyrin analogues revealed a 25-30 times enhancement in photosensitization compared to HpD in tumor models (30) and gave superior tumor localization in C3H mice (31). After thoroughly comparing the essential properties of the *ortho*, *meta* and *para* phenyl isomers (32) a similar necrosis depth was noted for the *m,p* compounds, while the *ortho* isomer resulted in higher skin sensitivity. Overall, the *meta* isomer was the best in terms of cost and benefits (Fig. 1). The only significant drawback of these porphyrins was their weak absorption in the area of 630 nm which meant nothing was gained in this regard compared to Photofrin®. Nevertheless, a number of *in vitro* and *in vivo* tests established the basic PDT properties and advantage of the *m*THPP porphyrin (33,34,35,36,37,38,39,40,41).

To improve the absorption properties, chlorin analogues of these hydroxyphenylporphyrins were synthesized *via* a diimide reduction (42). This reduction step was introduced to gain a more intense and more red-shifted absorption band to allow deeper penetration into tissue. The chlorin 5,10,15,20-tetra(*m*-hydroxyphenyl)chlorin (*m*THPC, **1**) was derived from the parent porphyrin (5,10,15,20-tetra(*m*-hydroxyphenyl)porphyrin (*m*THPP, **2**) and showed effective induction of necrosis in tumor tissue in PC6 tumor inoculated mice with less muscular edema than in other PDT compounds. This project was the first successful attempt at a methodical approach to synthetically optimize the absorption characteristics of tetrapyrrolic photosensitizers for PDT. Formally, the synthesis of *m*THPC involves the acid-catalyzed condensation of pyrrole with *m*-hydroxy benzaldehyde to yield the porphyrinogen, followed by oxidation to *m*THPP and then reduction to *m*THPC.

<Figure 1>

In many regards *m*THPC fits many of the requirements specified above for an ideal photosensitizer, and thus may be regarded to be "better" than Photofrin (43,44). It can be prepared as a chemically pure compound, it shows enrichment in tumor versus normal tissue and has an absorption maximum at 650 nm, red-shifted compared to HpD which means deeper light-penetration. In addition, *m*THPC requires smaller quantities for administration, shorter treatment times and a lower light dose to achieve the desired PDT response (45).

Briefly, its industrial development proceeded as follows. Initially, an UK biopharmaceutical company (Scotia Pharmaceuticals Ltd., UK) developed the drug, first in collaboration with Boehringer Ingelheim and in 1999 was granted orphan drug status by the US Food and Drug Administration (FDA) and was accepted for marketing review by the European Medicines Evaluation Agency (EMEA). In 2000, it was granted fast track review status by the FDA. Nevertheless, approval by the FDA was declined in September 2000, and then in January 2001 by the EMEA. As a result, Scotia Holdings plc. had to go into administration. Scotia was one of Britain's oldest biotech companies and at its height in 1997 had a market value of £500 million. Nevertheless, an appeal to the EMEA was successful in June 2001 and later in 2001 marketing authorization was given as a local therapy for the palliative treatment of patients with advanced head and neck cancer who have failed prior therapies and are unsuitable for radiotherapy, surgery or systemic chemotherapy. Financially,

this did not save Scotia, which, renamed as Quantanova, was sold to the German Biolitec AG in April 2002. Since then, Biolitec Pharma distributes the drug and investigates further uses.

After *m*THPC was recognized as a potential PS analytical investigations were undertaken to obtain detailed knowledge of the compounds properties. The fine structures in the ^{1}H-, ^{13}C- and ^{15}N-NMR spectra have been clarified (46) and mass spectrometric studies undertaken (47). To enable research into kinetic phenomena of the radical type I mechanism, ESR experiments observing *m*THPC with and without stable free radicals (e.g., di-*tert*-butyl nitroxide) have been undertaken (48). A suite of photophysical measurements were performed on the *o*-, *m*- and *p*-tetra(hydroxyphenyl)porphyrins in 1988 by Bonnett *et al*. including fluorescence, flash photolysis and pulse radiolysis studies in order to characterize the singlet and triplet excited states of these macrocycles (49). A comparative study including the reduced derivatives *m*THPC and the bacteriochlorin analogue (*m*THPBC, **3**) was performed in 1999 (50). The absorption maximum of the long wavelength absorption band is shifted from 644 in the porphyrin to 650 nm in the chlorin and 735 nm in the bacteriochlorin. Photophysical properties of the first excited state were similar in all three compounds and the quantum yield for singlet oxygen formation was 0.43-0.46 in aerated methanol.

A study of the absorption and emission dependence upon pH was also done for *m*THPC, *m*THPP, and other photosensitizers (51). Within the physiological pH range 6.5-7.2 no pH dependence of the fluorescence intensity was found. Only below pH 6 a significant loss of intensity was observed (52). The dynamics of the excited states of *m*THPC have been investigated in time-resolved studies on the fs to μs time scales (53). The lifetime of the singlet state was found to be longer for *m*THPC compared to other photosensitizers, which might be one of the reasons for its high efficacy as a PS. Experiments within the cytoplasm of cells later revealed a decrease of this lifetime from 7.5 ns to 5.5 ns which was attributed to aggregation in the biological environment (54). Resonance light scattering experiments showed the formation of J-aggregates for *m*THPC in aqueous solution (55,56).

The acid-base properties of *m*THPC were analyzed by Bonnett *et al*. (57). Spectrophotometric titration of the photosensitizer in a methanol/buffer mixture gave pK_3 and pK_4 values of 3.45 and 1.45 respectively, while the phenolic groups exhibited a pK_a of 10. They also investigated the singlet oxygen production from *m*THPC through the photobleaching of bilirubin, which was accelerated five-fold in the presence of a catalytic amount (5 mol%) of the PS, but hindered in the presence of the singlet oxygen quenchers, *β*-carotene and 2,5-dimethylfuran. A comparative analysis of the pH-dependent properties showed that only *m*THPP but not *m*THPC changed lipophilicity in the pH range 4-8 (58).

The analytical detection of PS in biological material is a challenging topic. Detection of *m*THPC in human plasma following infusion was carried out successfully *via* reverse-phase HPLC-UV detection with a sensitivity of 15 ng.mL^{-1} (59). This method was then extended to mouse, rat and human tissue (60). Using coloumetric detection, Barberi-Heyob *et al*. improved the sensitivity for detecting *m*THPC in plasma to as low as 5 ng.mL^{-1} (61). A similar detection could also be achieved using an improved HPLC fluorescence detection method (62). Formation of a 1:2 cyclodextrin:*m*THPC complex resulted in an up to 300times enhanced fluorescence signal (63) and could be used for detection in human plasma (64). Similar results were later reported for 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin (65). Two-step laser mass spectrometry (MS) was used as a qualitative analytical tool to study PS purity (66). There is also significant variability in the fluorescence properties depending on the tissue and PS type. Tissue relevant fluorescence excitation-emission matrices have been collected to aid

in the analysis (67). Dynamic capillaroscopy has been used as a means to evaluate pharmacokinetics *in vivo* (68,69).

Photobleaching

One of the most important questions regarding the use of a PS relates to its photostability. Porphyrins typically undergo slow photodegradation with time. The importance of the phenomenon of photobleaching, i.e. the irreversible photodestruction of the pigment, for PDT has been recognized as a potential means to control both the pharmacokinetics of the drug and the occurrence of undesired side-effects. However, the balance of photobleaching *versus* photoaction is delicate, for if the sensitizer bleaches too rapidly, tumor destruction will not be complete. However, with appropriate knowledge of bleaching rates and drug dosage, light damage to normal tissue at the treated site may be reduced due to controlled minimized sensitizer concentrations in these areas. Another plausible application of photobleaching is the elimination of the drug, consequently reducing postoperative skin photosensitivity, which is the main adverse effect patients experience when undergoing PDT.

Initially, Bonnett *et al*. examined in a comparative study the photobleaching properties of methanolic solutions of *m*THPC, *m*THPP, and *m*THPBC (70). *m*THPC and *m*THPBC were subject to pronounced photodegradation following laser irradiation at 514 nm while *m*THPP resulted in the formation of quinoid porphyrins (70,71). The photophysical impact on *m*THPP is less pronounced than for either reduced analogue, but the rapid degradation of mTHPBC precludes a practical use in PDT. An analysis of the degradation of these sensitizers via NMR, UV and mass spectrometry showed the photoproducts largely to consist of benzoquinone isomers (**4**), dihydroxyphenyl derivatives (**5**) and ring-opened chains followed by further breakdown to methyl 3-hydroxybenzoate, succinimide and maleimide (Fig. 2).

<Figure 2>

Recent results indicate the rate of photobleaching of *m*THPBC to be strongly dependent on the degree of aggregation of the PS (72). A combined use of HPLC and electrospray ionization tandem MS (ESI-MS) identified *m*THPP and several hydroxylated derivatives of both *m*THPC and *m*THPP as initial photobleaching products (73). Angotti *et al*. found evidence for both Type I and Type II reactions being involved in the photobleaching of *m*THPC (74) and identified *m*THPP as the primary photoproduct (75). Similar results were obtained using the HPLC separation of *m*THPC photoproducts after irradiation with 514 nm laser light in combination with MALDI-TOF MS and UV/vis spectroscopy. Again, the formation of mono-, di- and trihydroxylated derivatives of *m*THPC and an unidentified degradation product was reported (76). Interestingly, pulse laser irradiation of *m*THPP at 647.5 nm resulted in the formation of benzoquinoid porphyrins followed by formation of dimeric (**6**) and oligomeric photoproducts (77).

ESR experiments of organic solutions of *m*THPC were performed to elucidate the mechanism of photobleaching in more detail (78,79). *m*THPC produces superoxide anion radicals more efficiently than Photofrin and while both singlet oxygen and superoxide anion radicals contribute to the photobleaching the latter is primarily involved in the conversion to *m*THPP. Initial *in vitro* photobleaching experiments were undertaken with Chinese hamster lung fibroblasts and showed that the rate of photobleaching was much higher for *m*THPC than *m*THPP or Photofrin (80). A study of *m*THPC in solution with fetal calf serum (81) showed that this process depends on oxygen level and alterations in the microenvironment of the sensitizer. A clear relationship between $^1O_2$ and bleaching rate was found (82) and the singlet

oxygen concentration is inversely related to cell survival (83). Likewise, there is a correlation between drug concentration and rate of photobleaching for a fixed laser fluence rate (84).

Overall, the light-induced chemical changes of *m*THPC described in the various works covering *ex vivo* experiments are similar in terms of identified photoproducts. However, the quantitative findings differ strongly depending on the experimental conditions and the microenvironment (83,85,86,87,88,89). This limits the utility of the *in vivo* photobleaching models. For example, the intracellular photobleaching in a murine macrophage cell line was found to exhibit a complex dependence on oxygen level and an inverse dependence on fluence rate (90). For rat skin the photobleaching kinetics were biphasic and were different from those for tissue oxygen consumption (91). Monitoring photobleaching *in vivo* is especially difficult in an interstitial environment (55).

Few studies have investigated the relative photostability of various PS. E.g., a study investigating the stability of hypericin in various model systems noted significant differences in stability, with *m*THPC being one of the least stable PS compared to phthalocyanine derivatives and hypericin (92). Likewise, a recent study compared Photoditazine, Radachlorin and Foscan and found Foscan to be the most stable of the three PS (93). This can be attributed to the higher degree of aggregation for *m*THPC in solution, which inhibits singlet oxygen formation.

PHARMACOLOGY AND BIOCHEMISTRY

Uptake, localization and metabolism

Uptake. The uptake of the compound into cells appears to be pH independent (94). A comparative study of the uptake of some PS into human breast cancer cells showed a pH dependent uptake for haematoporphyrin IX and similar results were obtained with other human cancer cell lines (95). However, in contrast to this, its activity once taken up decreased with extracellular pH (better at 6.8 than 7.4). Likewise, low temperature (4 ºC versus 37 ºC) resulted in increased photosensitivity of human colon carcinoma cells (95,96). The uptake is determined to some extent by the deaggregation process of the drug and the drug appears to bind to lipoproteins (97). This was further indicated by experiments with [^{14}C]-*m*THPC which was shown to bind to several lipoprotein fractions of the human serum. The approximate distribution was: VLDL 6 %, LDL 22 %, Lp(a) 17 %, HDL 39 % and soluble fraction of *m*THPC 16 % (98). The exact physicochemical type of binding to these species is still unknown. However, a binding study of 5,10,15,20-tetra(4-hydroxyphenyl)porphyrin with human serum albumin showed that hydrophobic interactions appear to be the main driving force (99). Interestingly, this study indicated that chromophore binding reduces the α-helix structure of the protein.

The uptake of Foscan is affected by many factors. For example, *in vitro* studies indicated that the PDT activity is five times lower in the presence of protein as compared to serum-free medium (100). It appears that the drug is taken up in aggregated form and that subsequent monomerization is a slow process which may explain the observed time delay in maximum PDT activity. However, the presence of serum during the illumination period had no effect indicating that the drug may be sequestered upon entering the cell (101). There is also evidence for the existence of aggregated forms of Foscan in the presence of protein which are not protein bound, indicating the possibility for similar states of matter *in vivo* (102). Aggregation effects were also noted in HeLa cells (103). A model study investigated the release and redistribution of Foscan from various plasma proteins. The rate of redistribution

was low and HDL-mediated endocytosis was proposed as the main mode of drug transport in cells (104). Nevertheless, the uptake of LDL and thereby *m*THPC is far more efficient and may reflect the real uptake pathway for the drug. Administration of glucose resulted in increased PDT effect in animal models, most likely due to changes in tumor physiology or transport processes (96). Metabolic inhibitors were reported to have no effect (105). In a mouse model the plasma *m*THPC level correlated with the PDT effect, while the tumor uptake did not, indicating that shorter drug-light intervals with low drug doses might be better for treatment (106).

A detailed study compared the pharmacokinetics in mice and humans and found that the drug distribution over the lipoproteins and the metabolism of the lipoproteins had no impact on the plasma pharmacokinetics. Thus, the longer time needed for high drug concentrations in human plasma compared to that in mice could indicate the formation of a drug depot. The different pharmacokinetics between rodents and human illustrate the problems associated with using animal models for predicting drug behavior in humans (107).

Localization. The exact localization of the PS in the cell determines its impact on the molecular machinery and thus the efficacy of the PDT effect. This is mainly a result of the short life time and diffusion constant of the reactive oxygen species. In practical terms the identification of the localization sites has become much easier since the advent of confocal laser scanning microscopy. Administration of the drugs with organelle specific probes which have fluorescence maxima different from the PS now allows the identification of the intracellular sites where PS accumulate and the damaged sites after photoactivation.

Generally speaking the localization of PS depends on their charge, their hydrophobic or hydrophilic properties, their aggregation state, and the molecular substituent pattern. As a rough approximation hydrophobic PS with ≤ 2 negative charges pass the plasma membrane easily and typically show good uptake. More polar PS with >2 negative charges cannot pass the membrane via diffusion and are taken up by endocytosis. Thus, the various PS exhibit quite different intracellular localizations (26). This can be modulated significantly through use of different delivery vehicles and strategies for the PS drugs (108).

In terms of organelles the most interesting ones for PDT are the lysosomes, mitochondria, plasma membrane, Golgi apparatus and endoplasmatic reticulum. Examples for PS localizing (predominantly) in these organelles include aluminum phthalocyanine disulfonate, benzoporphyrin derivative, deuteroporphyrin IX, and Foscan. However, it must be taken into account that intracellular redistribution and relocalization may occur as a result of light-induced relocalization (109). Even more specific intraorganellar localizations have been observed for PS related to the natural protoporphyrin IX system. For example, dicationic protoporphyrin IX PS were found to be highly effective inhibitors of the peripheral benzodiazepine receptor in the outer mitochondrial membrane, which is upregulated in some tumors (110). The binding to such specific receptor sites is the driving force for the development of third generation targeted PS bioconjugates. For an excellent review on the various physicochemical parameters affecting PS localization see Mojzisova *et al.* (111).

The subcellular localization of *m*THPC in human adenocarcinoma cells was studied by Melnikova *et al*. using fluorescence microscopy (112). Initial studies reported that *m*THPC localized generally within cellular organelles, but not the nucleus. After cell illumination, photosensitized damage was detected using a fluorescent probe. The mitochondria and the Golgi apparatus were found to exhibit extensive cytoplasm vacuolization and other alterations.

Kessel initially described the localization in mitochondria, where illumination resulted in a release of cytochrome c (97). Chinese hamster lung fibroblasts exhibited a diffuse localization in the cytoplasm (113), as did murine macrophages (114). A 2005 study of mouse sarcoma cells using fluorescence anisotropy imaging showed localization of Foscan in the nuclear envelope (115). No such localization was found for other PS (PPIX, LS11, Photofrin, Pc4, HPPH). Some studies reported localization of *m*THPC in the lysosomes (116). More details are given in Table 1 for individual cell lines.

A detailed confocal fluorescence microscopy study of a human adenocarcinoma by Guillemin and coworkers showed only weak localization in lysosomes and mitochondria. Instead, Foscan was found to be localized in the ER and the Golgi apparatus and the latter two were the sites of primary PDT damage (117,118). In this context it has been speculated that the Human ATP-binding cassette transporter isoform B6 (ABCB6), which is located in the Golgi and has elevated expression levels in breast cancer patients, might play a role in transporting porphyrin-related compounds (119).

The tissue localization has been studied in many experiments most of which are discussed below in the animal model section. An interesting effect noted in an early study was the difference between *m*THPP and *m*THPC (120). Chemically, both are similar in hydrophobicity. Nevertheless, the porphyrin localized mainly in the stroma of tumors (breast cancer implanted in mice) while the chlorin was distributed in the vascular interstitium and neoplastic cells of the tumor. Thus, PDT with the former resulted mainly in destruction of the microvasculature of the tumor while the latter affected both the vascular walls and the tumor cells. However, the PDT drug blood serum levels and vascular photosensitization appear to be decoupled (69) indicating a more indirect effect of the PS on the blood capillaries.

The time course of the uptake differs from organ to organ. A SCC hamster model showed initial uptake in the liver and kidney and then later in the blood vessels and smooth muscle (121). However, many of these studies report overall tissue distribution or bulk uptake and that tells little about the real extravascular distribution. This was clearly shown in a study by Mitra *et al.* who used two-color confocal spectroscopy and a mouse mammary tumor model and identified the intratumor distribution of the drug. It clearly varies significantly with time and spatially within the tumor (122). All these aspects clearly have clinical implications.

Metabolism. An important question in drug development is whether *m*THPC is metabolized by the body. However, only a small number of studies have addressed this question. Analytical work on samples of plasma, bile, feces, urine and liver using HPLC and electrospray MS confirmed that *m*THPC was not metabolized but excreted unchanged apart from fractions of *m*THPP and hydroxylated *m*THPC derivatives which were already known to be photochemical oxidation side products (123,124). Its plasma binding is quite distinct from other PS (125). A study with radiolabeled Temoporfin in colorectal cancer induced mice showed a rapid clearance from blood and the concentration in the tumor reached a maximum at 2 d. Nearly 40 % of the drug was excreted in the feces during the first day, almost none was recovered from the urine. The elimination half-life was estimated to be 10-12 d for this system (125).

Pharmacokinetics. The pharmacokinetics of any PS depend on a variety of factors. These range from aggregation-deaggregation equilibria in the blood, binding to serum components, binding to and penetration through the blood vessel wall, diffusion through the organ/tumor parenchyma, potential metabolization and finally excretion. In principle the quantification of

PS in tissue and blood is easy due to their fluorescence. For Foscan, *ex vivo* fluorescence spectroscopy is a relatively fast and reliable method (121) and can be accompanied by *in vivo* spectrofluorimetry and chemical analysis after extractions (127). However, quantification methods based on this are problematic and depend on the extraction method used and the type of PS. One example for this is the use of Solvable™ as a tissue solubilizer (128).

Other methods include HPLC analysis of tissue sample or administration of radiolabeled compounds followed by scintillation measurements. Likewise, noninvasive measurements based on tissue fluorescence are possible. In reality a comparison of pharmacokinetic data from different sources and laboratories is difficult. This relates to the use of different standards, sample preparation and the incompatibility of some animal models. Based on the different uptake and localization mechanisms the overall pharmacokinetics vary significantly from PS to PS and half-lives range from a few minutes to days (28). A good comparison of the pharmacokinetics of the various PS available up to the mid nineties was given by Moore *et al.* (129). Similar to the situation for uptake, the exact pharmacokinetics depend on the species and tumor model used (28).

For Foscan, the following data are based on a number of phase I studies (130,131,132). The pharmacokinetics of *m*THPC in humans was evaluated thoroughly in 1996 in a clinical study with concentration measurements of plasma, cancer- and normal tissue biopsies (130). After drug delivery blood was taken in periodic time intervals and biopsies were taken immediately prior to PDT from 25 patients who suffered from various cancers. The maximum concentration of *m*THPC in blood was reached by 10h - 24h which distinguishes Foscan® from other drugs when delivered directly into the blood cycle. Depending on the study a half-life of the drug of 30-45 h was established (130,131). Probably, it is initially accumulated in the liver followed by a slow release mechanism. The pharmacokinetics of *m*THPC compared to ALA was reviewed previously (133). Early studies in a murine model indicated that Temoporfin concentrations in heart and skeletal muscle decline much slower than in blood (134).

Clearly the interaction of Temoporfin with plasma is of relevance. A study of the distribution in human plasma and in a rat model *in vivo* concluded, that the drug quantitatively binds to plasma components (125). The site of binding appeared to be highly localized to a hitherto unidentified nonlipoprotein fraction of plasma. This behavior is unique compared to other photosensitizers and might be responsible for the specific pharmacokinetic behavior of *m*THPC. The importance of a careful study of the pharmacokinetics was shown again in a recent study on "compartmental targeting" of Foscan. A mouse model showed clearly that a fractionated double injection protocol was superior to a single dose administration of the drug (135).

Dosimetry

Many efforts have been made to develop effective PDT dosimetry methods (136). The photobleaching *m*THPC (see above) has been proposed as one method. However, while the photobleaching rate is oxygen dependent and the oxygen concentration correlates with the photobleaching rate, this dependency is related to the microenvironment. Thus, without knowledge about the tissue oxygenation photobleaching cannot be used for dosimetry (82). Photobleaching results can differ widely depending on the experimental conditions making clear conclusions about the utility of dosimetry difficult (83,85,86,87). An alternative dosimetric method might be the determination of the *m*THPC plasma level, as indicated by a comparative study of the mucosa of the oral cavity, the esophagus and the bronchi of 27

patients using light-induced fluorescence spectroscopy (131). This is supported by a study which compared dogs, rabbits, rats and humans (137).

Other methods included low-power remittance fluorescence (138), optic fiber spectrofluorimetry, reactive oxygen species assays using dichlorofluorescein determination (139) and soft tissue models based on agar gel (140), amongst others. Overall, it has to be concluded that an approximation of the appropriate drug dose from preclinical results is problematic. The biodistribution of the drug varies from tissue to tissue and from species to species. To use body weight and/or amount of surface area as a means of extrapolation from species to species is unreliable, and even variations between individuals are often huge (133,137). This is clearly a significant problem. A better approach might be taking into account the plasma concentration of drug and/or good fluorescence measurement and clinical experience (131). A study by Wang *et al.* criticized the correlation/prediction of the PDT response based on singlet oxygen production metrics (141). However, the model employed is rather specific and it is yet unclear how general these results are.

Another problem is how to effectively measure and control the various parameters that affect PDT during interstitial application. While this area is well developed for radiotherapy, phototherapeutical applications still require further developments (142). This is a fundamental problem irrespective of the tumor treated. A first animal study that attempted to address the problem of monitoring both explicit (fluence rate) and implicit parameters (photobleaching, oxygenation and blood volume) was performed recently and showed that indeed such a monitoring is possible, albeit difficult (55). PDT dosimetry is probably the most complex and difficult aspect of any PDT application. The exact methods used will always depend on the individual situation.

Formulation

Formulation is an important aspect of any drug development. For PS this is especially important as different treatment modalities (e.g., skin *versus* internal cancers) or chemically very distinct drugs (e.g., ALA *versus* porphyrin based drugs) require diverse application systems. Thus, significant potential still exists for the investigation of different formulations and drug delivery strategies for PS (143). For example, a possibility is the incorporation of the drugs into gel systems. The cubic phase (monoolein/water, 70:30, w/w) appears to be a suitable system as initial studies showed good stability of various PS in the gel formulations (144).

Due to the poor solubility *m*THPC can only be used with a formulation process prior to administration. Initial applications used formulations in PEG and ethanol or propylene glycol (97). Some results point towards problems with regard to optimum administration of the drug. For Human skin fibroblasts it was found that preincubation of *m*THPC at 37 ºC for a day prior to administration significantly improved the PDT effect (145). This indicates problems with solubility (or drug preparation) of the active PS. Considerable effort has been spent on the preparation of nanocarrier formulations for PS (146,147). For *m*THPC this is evidenced by the shift in developmental attention from Foscan® (a solution) to Foslip® (a liposomal formulation).

Light

As a photochemical reaction PDT requires investigations into the dependency on exciting wavelength, light intensity, fluence rate and type of light application, photophysical

characteristics of the PS and so on. It is also dependent on the clinical setting, i.e., the type of cancer to be treated. Nevertheless, some general considerations, for example those made for photobleaching above, can be made for Foscan.

Early studies with malignant mesothelioma cells indicated that the drug-light interval has a significant effect on the therapeutic action. Typically selectivity is improved with longer drug light intervals and Foscan showed better results with longer intervals than used for Photofrin (148). Higher light doses were found to be more advantageous than increasing the drug dose (149). Obviously, this also means that the light dose is important when considering the toxicity of the treatment for healthy tissue (150). The optimum drug-light interval varies with the tissue. Various studies addressed the effects of green (514 nm) *versus* red light (652 nm). Roughly speaking the tissue absorption is 2-3 times greater at 514 nm and 4-5 times as much light might be required to achieve the same phototoxic effect as 652 nm light (151). Even more importantly light penetration is shorter at 514 nm which reduces the chance of tissue perforations, e.g in the esophagus.

Fractionation of light can also improve the PDT effect. However, the effects are very dependent on the dark intervals between light administrations. Typically, short dark times appear to be better. For example, an animal study on Rifi tumors indicated dark times of 30 s to be best (152). An interesting study using MCF-7, an estrogen-dependent human breast carcinoma cell line, showed that fractionated light, i.e. giving the illumination in short pulses (0.05 sec light, 0.05 sec dark) instead of continuous illumination for a few minutes increased the photodynamic effect significantly (about 50 % for *m*THPC, somewhat less for *m*THPC-MD_{2000}) (153). A later study with on/off illumination delivering the same total light fluence, i.e. a 50 % reduction of the power density, doubled the cytotoxicity (154). A study using an SCC model showed a clear dependence of the photodynamic effect on the fluence rate. Lower fluence rates at both 514 and 652 nm induced higher tissue damage but less selectivity (155). Other animal studies gave similar results (156). A detailed study by Coutier *et al.* correlated the fluence rate, tumor oxygenation and PDT effect and found that lower fluence rates limit the extent of oxygen depletion in the tumor and thus result in better PDT outcomes (157).

Mechanism of Action

From a chemical viewpoint the mode of action of any porphyrin PS is based on simple photochemistry and *m*THPC primarily acts through the generation of singlet oxygen like other PS. Early studies on the photodynamic effect in the presence of singlet oxygen scavengers showed a reduction in the photoinactivating ability of *m*THPC (113). For example, sodium azide strongly suppressed PDT activity in human tumor cells, while superoxide anion radical, H_2O_2, or hydroxyl radical scavengers had no effect (158). However, optimization of current and future drugs requires a more detailed understanding of the intracellular chemical and biochemical reactions in molecular medicine terms. In conceptual cell biological terms this requires knowledge about the impact of PDT on cell signaling and cell metabolism (159). In clinical terms it is necessary to know the mechanism of tumor destruction at the individual tumor cell level, to understand vascular effects and unravel the related immune system effects (160). Unfortunately, single cell studies on the singlet oxygen distribution are thus far available only for other PS (161).

Cell signaling and metabolism. Photosensitizers can affect many different signaling pathways (162). For example, a number of PS induce a rise in intracellular Ca^{2+} and affect directly or

indirectly Ca^{2+}-binding chaperones, anti-apoptotic proteins, caspases, phospholipases, and the nuclear factor of activated T cells. The phospholipases link the calcium signaling to the lipid metabolism, e.g., through the release of acrachidonic acid derivatives. Likewise, cyclooxygenase-2 (COX 2) expression can be activated through PDT. Other affected second messengers are ceramide, cytokines and tryrosine kinases, especially mitogen-activated protein kinase and epidermal growth factor. PDT also acts on transcription factors, e.g. activator protein 1, NFκB and transcription factor E2F. Cell-cell interactions, e.g. cell adhesion can be influenced as well. Other regulatory factors such as cytokines and neutrophils or stress response proteins are important for the PDT effect as well. Other possible PDT effects include angiogenesis and tumor responses to changes in the oxygen level. The latter notably includes tumor hypoxia as PDT can rapidly deplete the tissue oxygen and affects the tumor blood supply. Note, that many of these responses are interconnected. For a detailed discussion of the effect of the various PS see Castano *et al.* (27). Likewise, NO might be an important target for PDT action. While data on the interplay of *m*THPC and NO action are sparse, the importance of nitric oxide has been discussed widely for other PS (163).

For Foscan the available data are as follows. A study by Kessel in 1999 showed that illumination of murine leukemia cells treated with *m*THPC resulted in the release of cytochrome c and activation of caspase-3 resulting in an apoptotic response (97). Mitochondrial damage and cyt c release was also described for other myeloid leukemia cells (164), human colon adenocarcinoma cells (165) and squamous cell carcinomas (166). Later Bezdetnaya and coworkers showed that the ER and Golgi are the sites of the primary effects (117,118). They clearly showed that enzymes in the Golgi, such as uridine 5'-diphosphate galactosyl transferase, or in the ER, such as NADH cyt c reductase, are inactivated through PDT treatment, while mitochondrial marker enzymes (cyt c oxidase and dehydrogenases) were unaffected (167). In order to correlate these data it must be noted that 24h post treatment the fluence dependency of the PDT effect was similar for changes in mitochondria and cell death (118). Thus, although mitochondria are not affected directly they will be affected significantly by late and indirect effects. This gives rise to a model wherein the ER/Golgi complex originates a death signal for the mitochondrial apoptotic processes. Thus, ER/Golgi localizing PS act in a different, more indirect manner compared to mitochondria localizing PS.

Reactions of the lipid peroxidation pathway appear to be not very important for the Foscan action. A study of human adenocarcinoma cells which incorporated α-tocopherol, a lipophilic phenolic antioxidant, did not elicit any photoprotection against PDT. Instead, the *m*THPC PDT effect was enhanced at concentrations of 0.33 mM and above (158,168). An interesting study investigated the effect of radio- and phototreatment on the level of serum α-*N*-acetylgalactosaminidase, an extracellular matrix degrading enzyme that appears to be exclusive for cancer cells (169). While radiodynamic treatment reduced the level of this enzyme significantly, PDT lowered it to background level within 2-3 d after PDT. The level of this enzyme appears to be a suitable diagnostic tool for dosimetric applications. Another notable enzyme activation involves poly(adenosine diphosphate-ribose) polymerase, a key regulator of cell death-survival transcriptional programs. It is activated significantly by Photofrin or Foscan PDT (170). This induction occurred 30 min after PDT, followed by further increases in enzyme levels by 2h.

Few biochemical studies of isolated cell organelles were performed to assess the intracellular Foscan PDT effects. Both type I and II effects were noted in isolated rat mitochondria. The PDT treated mitochondria had a reduced membrane potential and impaired

Ca^{2+} uptake (171). Similarly, only limited information is available for the impact of Foscan PDT on cell adhesion. Post PDT cell removal by trypsinization was found to depend on the PS type and the fluence rate (172).

Sometimes laser hypothermia is compared with PDT as both use the same application principles. However, their modes of action are different, especially with regard to collagen damage. A comparative study clearly showed no effect of *m*THPC PDT on collagen-related heat shock protein expression (173). Likewise HpD PDT had no effect; only riboflavin PDT gave an upregulation of the HSP47 heat-shock protein (174). This supports the notion that PDT "spares" the connective tissue. In this context Mitra *et al.* developed an interesting model system that utilized green fluorescent protein as an analytical tool of the PDT effect. They transfected EMT6 cells with a plasmid containing the gene for green fluorescent protein (GFP) driven by an hsp70 promoter (175). *m*THPC induced a concentration dependent GFP expression in cells and in a rat model.

Cell death. Ultimately the aim of PDT is the death of unwanted cells and tissues in the body. The type and degree of cell mortality depends on the various factors involved in PDT and typically two types of cell death are considered. Necrosis is a rapid form of cell death, whereby the cell organelles are destroyed and the plasma membrane ruptures. This results in inflammation and a release of the intracellular materials. In contrast, apoptosis is characterized by a shrinking of the cell and blebbing of the plasma membrane. The cell organelle and plasma membrane structure are maintained for a considerable time. Ultimately apoptosis results *in vitro* in fragmentation into vesicles while in the body the cell remnants are taken up by phagocytes without inflammation. Apoptosis is a highly controlled process and involves the transcriptional activation of specific genes, DNA degradation and caspase activation. By now, other forms of cell deaths, e.g. programmed necrosis, mitotic cell death, autophagy and lysosomal death pathways have been described. In most cases PDT related studies focus on the first two types. However, Foscan has been implicated in autophagy as well (176). Necrosis and apoptosis can often be induced at the same time and the exact mode of cell death then depends on the PS, cell type, oxygen supply and experimental conditions. For basic reviews on cell death in PDT see the following articles: (162,177,178). While evidence for the signaling role of reactive oxygen species is mounting (179) it remains an open question to what extent the various effects are directly related to PDT or if they are more general effects resulting from cell damage.

Both necrosis and apoptosis effects are associated with the action of Temoporfin. One indication is that low levels of post-treatment energy metabolism favor further cell decay (180). A lack of glucose in the incubating medium significantly increased the extent of necrosis (181). Bourré *et al.* undertook a first *in vitro* study to quantify the apoptotic effect of Foscan. ALA (proto IX) and, more so *m*THPC, were found to be strong apoptotic inducers. The maximal apoptosis enrichment factor, a measure of this effect, depended on the PS and cell type (182). Interestingly, apoptosis inhibitors had only slight effects on the PDT efficacy indicating that necrosis and apoptosis might be linked and share common initiation pathways. This requires active caspases and these results would indicate that apoptosis inhibition reorients cells to necrosis (183). A related study investigated the role of two genes, p53 a tumor suppressor gene important for cellular stress response and ATM, a gene mutated in patients suffering from ataxia telangiectasia. *m*THPC PDT did kill the cells with these mutations quite efficiently through necrosis. Apoptosis appeared to be more a long term

process that depends to some extent on the p53 and ATM function (184). A detailed assessment of the expression of caspase-3, caspase-7 and cleaved poly-ADPribose polymerase 1 in Foscan treated cells has been given by Bressenot *et al.* (185).

*m*THPC resulted in a much better apoptotic effect than merocyanine 540 in murine myeloid leukemia cells and cleaved the DNA to very small 150 base pair fragments (186). Induced apoptosis was observed in an intracranial tumor model (187) and in human colon adenocarcinomas (116). The action of some cationic PS can be counteracted to some extent through antiapoptotic protocols. A study of mouse fibrosarcoid L929 cells with an overexpressed protooncogene (bcl-2), which protects cells against apotogenic stress, showed no evidence for a positive or negative effect on the PDT results (188). However, for murine leukemia cells it was clearly shown that the first detectable effect of several PS, including *m*THPC, is the loss of activity of the antiapoptotic protein Bcl-2, followed by cyt c release and caspase-3 activation (189). A detailed study using MCF-7 cells addressed the question whether the post PDT apoptotic effects originated from the ER/Golgi or the mitochondria. The study clearly showed localization of Foscan in the ER and Golgi. After 3 h leakage from the Golgi was observed and prolonged incubation times showed photodamage to Bcl-2 in the cell extract, but not in the mitochondrial fraction. Likewise caspase-7 and -6 activation was observed indicating that localization in the ER enhances the photoactivation of the caspase-7 apoptotic pathway (190).

An intriguing treatment possibility is the co-administration of apoptosis inducing drugs. One such possibility to increase the tumor selectivity is the presence of the TAT-$RasGAP_{317-326}$ peptide which sensitizes tumor cells to cytostatic agents. A comparison of H-meso-1 and human fibroblast cell cultures showed that, indeed, the PDT effect is enhanced by peptide administration but only in the H-meso-1 cells. This effect occurs only at low Temoporfin concentrations (0.04 $\mu g.ml^{-1}$). No such effect was found with 0.10 $\mu g.ml^{-1}$ Foscan (191). Thus, this effect is dose dependent. Another important effect in tissues is the bystander effect, i.e. the degree to which a dead cell results in death of a neighbor (192). A model study using MDCK II cells indicated that this bystander effect was significantly lower for *m*THPP than for Photofrin or ALA-PDT and treatment with metabolic inhibitors (193).

Any phototreatment has the potential to affect DNA at the molecular level through photochemical reactions. This occurs either through direct absorption by nucleobases or through photosensitized reactions involving endogenous PS (natural porphyrins and flavins) or the drug themselves. DNA is highly susceptible to singlet oxygen, resulting in single strand breaks and free radicals. ALA and porphyrin based PDT clearly can result in DNA damage (194). However, while this probably does not give lethal effects the mutagenic potential depends on the cell type and the effectivity of the repair mechanisms (195). No evidence was found for Temoporfin genotoxicity using a simple *Drosophila melanogaster* model (196). Likewise no DNA damage was found in *in vitro* tests with Human leukemia cells (197). This study revealed DNA damage upon PDT with HpD or methylene blue, indicating quite different effects of various PS. Use of murine glioblastoma cells showed that *m*THPC PDT does induce DNA damage in a dose-dependent manner but that treated cells are able to repair this damage (198).

A different issue is the relative susceptibility of different tissues and cell types to PDT. A comparative study of microvascular endothelial cells, fibroblasts and tumor cells indicated that endothelial cells are not per se more sensitive than other cell types (199). Only continued drug uptake after 24 h resulted in increased photosensitivity under certain conditions. PDT

also does not induce any resistance to chemo- or radiotherapy or subsequent cycles of PDT in Human breast cancer cells (200). DNA mismatch repair-deficient cells are known to be resistant to many chemotherapeutic drugs and to radiotherapy, and have the potential of rapidly acquiring additional mutations leading to tumor progression. Using *in vitro* cell lines either proficient or deficient in DNA mismatch repair no differences were found between the cell lines upon Foscan PDT. Thus PDT appears to not induce loss of DNA mismatch repair nor does loss of mismatch repair result in resistance to PDT (201). But how do all these mechanisms lead to tumor destruction? Tumor eradication requires and relies not only on effects at the molecular level, i.e. individual cell death, but even more so involves more "indirect" effects such as vascular and immune system effects.

Direct tumor cell effects. This might be considered the first target of PDT. However, studies with several PS (mostly with Photofrin) showed that while PDT results in a decrease in the number of tumor cells, the overall effect is not enough for tumor eradication. This is a result of the often inhomogenous PS distribution, of photobleaching and the requirement for oxygen, which limits the utility of PDT in hypoxic tissue. The latter may be overcome by using lower light fluence rates or fractionated light delivery.

Vascular effects. Almost from the advent of PDT studies it was clear that vascular effects impact on long term tumor control. In general these effects are associated with vascular damage that occurs after the PDT treatment and result in tumor hypoxia. Depending on the PS various mechanisms are involved. These include vessel constriction and vascular leakage, mostly related to platelet activation and aggregation. Early studies with chlorins noted that the primary target of PDT is often the tumor vasculature (202,203). Although differences exist in the exact distribution of HP and *m*THPC the latter seems to act in a more indirect manner on the blood capillaries (69). Animal models showed that the exact nature of the PDT effect depends on the pharmacokinetics. Thus, often an "early" response can be differentiated from a later effect in tissue (204). While tumor and plasma drug levels often do not correlate with PDT efficacy the Foscan concentration in leukocytes does. This indicates that leukocytes might play a role in the vascular damage process (205). For example, a detailed study of the pharmacokinetics of human tumor xenografts in mice showed that plasma levels of *m*THPC decrease exponentially with time while the tumor drug levels remained at maximum for 48 h. At 3 h postadministration the drug was located in the blood vessels and only later distributed in the rest of the tumor. Illumination at 3 h resulted in a 100 % cure rate while illumination at 48 h resulted in tumor regrowth and only 10 % cure. Thus, the early vascular response appears to be responsible for optimum Foscan PDT response (206).

Immune system effects. The immune system plays a significant role in the success of any PDT treatment. Based on initial studies with Photofrin it is now accepted that PDT can result in the generation of anti-tumor inflammatory cells and can lead to a persistent anti tumor immune response. The advantage of this response is that it can be elicited by PDT even when not all cells are killed directly. There are even indications that Photofrin PDT can be used to generate anti tumor vaccines (207). The immune system effect involves complex signaling pathways and relies on cytokines, growth factors and neutrophils all of which are significantly affected by PDT (160,208).

Secondary effects of the oxidative stress imposed by PS include vascular damage,

ischaemia-reperfusion injury, cytolytic activity of inflammatory cells and tumor-sensitized immune reactions (209). The latter was investigated through adjuvant administration of mycobacterium cell-wall extract, a non-specific immunostimulant that gives a local inflammatory response associated with antitumor activity (210). Studies of a mammary sarcoma revealed that the PDT effect of various PS including *m*THPC could be increased by a single treatment of this extract directly after light treatment. Mouse models clearly showed that Photofrin or Foscan PDT of solid tumors results in a strong and lasting induction of systemic neutrophils mediated by complement activation (211). More detailed studies revealed an increase in neutrophilic myeloperoxidase and an expression of MHC class II molecules in PDT treated tumors. The most important regulatory inflammatory cytokine appears to be IL-1β, which inactivation diminished the PDT cure rate (212).

Similar to other PS *m*THPC PDT activates macrophage-like cells (213). A detailed study of a model system revealed a light-energy dependent production of tumor necrosis factor TNF-α and a fluence dependent release of NO in U937ø cells. Foscan treatment of rat liver tumors showed that while PDT effectively necrosed the tumor tissue it had no effect on the growth of nonilluminated tumor areas in the same liver. PDT did not result in an increase of T cell numbers, natural killer (NK) cells or macrophages in nonilluminated tumors. Thus, no PDT induced systemic immune response was observed in this system (214).

IN VITRO TESTS AND ANIMAL MODELS

In Vitro Tests

The photodynamic action of *m*THPC became clear quickly after its synthesis, for example in a cost benefit analysis comparing it with other PS (44,215). Naturally, the early tests included mostly *in vitro* tests of various cell lines. A survey of cell lines used for studies with Foscan, i.e. simple solutions of *m*THPC, is given in Table 1, which also gives a brief description of the main results.

<Table 1>

Initial tests with murine leukemic cells (216) or Chinese hamster lung fibroblasts (80,113) indicated that Temoporfin would be a better PS than Photofrin. This was substantiated by studies of human colorectal adenocarcinoma grafted subcutaneously in mice (216). Next were studies with human breast cancer cell cultures that indicated almost a total cell killing at non toxic drug concentrations (217). Other studies indicated that lower light doses might be better (100) and experiments addressed the role of reactive oxygen scavengers (158), the beneficial effect of lower fluence rates (218), activation of phagocytic capacity (213) or the biodistribution of the drug with time (219,220), to name only a few. Initial studies on the effect of PDT on cell adhesion have also been reported (172). A study of the intracellular aggregation processes in MCF-7 cells indicated progressive sensitizer aggregation with increasing incubation time. Fluorescence lifetime imaging measurements showed a substantial decrease in the lifetime of *m*THPC fluorescence at 24 h compared to 3 h. In addition, the intracellular localization changed from a diffuse pattern at short incubation times to a punctiform pattern at 24 h. Thus, the loss of photosensitizing efficiency at higher *m*THPC concentrations is probably due to self-quenching of the triplet states of the PS (221).

*m*THPC ticked more of the boxes of an optimum PS than other PS available in the early nineties. A comparison of its effect in human colon adenocarcinoma with that of BPD showed that it was more efficient by a factor of 20 (222). However, even the then available pegylated *m*THPC derivatives (see below) appeared to be better suited than the unpegylated

form (223). For an ovarian cancer cell line it was shown that the cytostatic cytotoxicity of *m*THPC is comparable to that of cisplatin and only one order of magnitude below that of taxol (224). Similarly, Temoporfin was superior to merocyanin 540 (225) and HpD (166) in nasopharyngeal cells. *In vitro* studies also indicated the utility of PDT to perhaps suppress haematogenous metastasis (229). Cell studies indicated a rapid onset of apoptosis in such cells (227). Naturally, Foscan is not always the best PS for everything. Studies on the photoinduced haemolysis of red blood cells showed that cationic and hydrophobic phthalocyanines have improved activity compared to *m*THPC and other PS (228).

Experiments with human breast cancer cells expressing a multidrug resistance phenotype showed that both the resistant and normal cancer cells showed a similar uptake profile for *m*THPC. However, the drug and light dose that resulted in 50 % cytotoxicity in the "normal" cell gave 85 % cytotoxicity in the drug resistant cell (229,230). Such an effect has been noted frequently with PDT. Thus far most studies indicate that this is the result of a different localization of the drugs. For the MCF-7 cell line a more pronounced localization in lysosomes in the drug resistant strain was discussed (229,230). *In vitro* tests have also been performed with freshly prepared human gynecological tumors (breast, ovary and ascites) and indicated that individual treatment protocols might be required (231). Cell tests also indicated the utility of Foscan (and Foslip) for perihilar cholangiocarcinoma. Treatment of two biliary cancer cell lines gave a high phototoxic potential (LD_{90} 600 ng ml^{-1} and irradiation with 1.5 J cm^{-2} (660 ± 10 nm). Both formulations gave similar localizations and similar PDT effects (232,233).

Other studies investigated the combined use of PDT and radiotherapy in human breast cancer cells (234), or effects on resistance against chemo- or radiotherapy, which is not induced by PDT (200). Two cell lines resistant against polyhaematoporphyrin or PPC were prepared through radiation. However, they showed no cross-resistance against *m*THPC-PDT (235). The results indicated that the mode of resistance depends to some extent on the nature of the PS.

For future applications it is also important to know the ability of neurons to survive. Here an interesting in vitro study compared the stability of neurons, satellite glia and human adenocarcinoma cells (236). The latter two were significantly more susceptible to Foscan PDT than the neurons. Even more importantly, Wright *et al.* identified conditions where no effect on the neurons was observed but the other two were killed. Thus, neurons in culture can survive *m*THPC-PDT under conditions sufficient to kill tumor cells and other nervous system cells. An intriguing model, based on the use of isolated nerve cells from a crayfish was developed by Uzdensky *et al.* (237). It allows the continuous recording of PDT-induced electrophysiological and biochemical properties. *m*THPC was found to be highly effective in the nM range to abolish neuronal activity. It was much more effective than many chlorin e_6 derivatives.

Individual cell phenotypes exhibit significantly divers drug uptake and phototoxicity. A comparison of nine different biliary tract cancer cell lines identified two groups of cell lines with either high or low susceptibility to Foscan PDT (238). The high susceptibility ones were characterized by low cytokeratin-19, high vimentin and a high proliferative phenotype. This indicates the potential to identify suitable markers for the optimization of clinical cholangiocarcinoma treatment. Other related cell line studies are discussed below in the related sections on animal and clinical studies.

Animal Models

Many animal models have been employed over the years. The first such report on hydroporphyrins was published by Bonnett *et al.* in 1989 (29). As in many other cases the use of animal models to predict effects in humans is problematic. Many studies have pointed out the varying biodistribution of Temoporfin in different animal species (137,239). Likewise, sometimes similar distribution but different photophysics are observed (240). In addition, such models are only of limited use for predicting the best conditions for clinical purposes. For example, a comparison of three different xenografts in mice showed different pharmacokinetics for the same drug/light dose (241). Thus, these systems can only serve to establish the basic conditions for further tests. Even with limited goals in mind the choice of the right animal model is critical for the utility of the *in vivo* tests. Here, orthotopic tumor models present the closest resemblance to the clinical situation with regard to PDT and a concise analysis of the PDT relevant models has been given by D'Hallewin *et al.* (242).

<Table 2>

Table 2 summarizes most of the models and techniques used with *m*THPC and related compounds. The following sections give only a brief overview on selected animal studies with Temoporfin.

Skin. Many studies on squamous cell carcinoma used a SCC hamster model (121,127). Although obvious from a chemical viewpoint, the same model was used to determine the range of λ = 647.5 to 652.5 nm as the most effective wavelength range for PDT (243). A determination of the threshold of muscle damage (1-3J) during interstitial PDT showed that 85 % of the SCC tumor could still be destroyed under these conditions (244). A comparison with BpD showed that this drug localizes to a smaller extent in the smooth muscle and is cleared more rapidly indicating some advantages for using BpD (245). However, a comparison of the pharmacodynamics of normal hamster and human mucosa indicated that this model is limited in its use for clinical predictions but has value for preclinical screenings (240). Earlier localization studies based on fluorescence spectroscopy were confirmed using ^{14}C-labelled *m*THPC (246). Skin cancer offers itself to topical applications. Thus, an initial study of a *m*THPC thermogel formulation after topical application on hairless SKH-1 mice bearing nonmelanoma skin carcinomas showed good tumor:tissue selectivity (247). The highest PS concentration in tumor was observed 6h after drug application while no drug was detected (248) in normal tissues or plasma after topical drug application. A study using a hairless mice skin papilloma model indicated that HpD can give a slightly higher tumor/normal skin tissue ratio than *m*THPC, but the latter reached its peak at a lower dose (249). The related carcinoma cells showed lower but similar tumor/normal skin ratio for both PS (250).

Head & Neck. Several animal model studies were performed for H&N cancers (251). Human oral SCC cells xenografted in mice gave a good response to *m*THPC (252). Analysis of a series of human keratinocyte cell lines derived from human SCC gave interesting results with regard to the regulation of invasion promoting tumor factors. Exposure to sublethal PDT doses showed that both active and latent MMP-2 and MMP-9 were down-regulated in some cell lines (UP, VB6) while H376 showed an increase in active MMP-2 (253). Thus while there is clearly potential for using this for predicting PDT outcomes the divergent results for different cell lines indicate the need for further studies. In terms of detection one study, with cat H&N SCC, showed that the vascular effects of PDT could be followed with power Doppler

ultrasonography (254).

Chest. Breast cancer carcinoma implanted in mice was effectively treated with *m*THPC and the chlorin was much more effective than the porphyrin *m*THPP (120). A comparison with Photofrin for treatment of a murine cancer clearly showed *m*THPC to be the better PDT agent and to have a more rapid skin clearance (255). The same PDT effect could be obtained with lower light doses when the drug was administered together with the bioreductive agent mitomycin C (202). This animal model also revealed the possibility to improve the PDT effect through light fractionation (152). Use of labeled *m*THPC showed that the drug concentration in the tumor was highest after 24-48 h (256). Surprisingly, the best tumor response was achieved 1-3 h after drug administration when the drug tumor concentration had not yet reached its highest value. BCG (Bacillus Calmette-Guérin) vaccine administration was found to enhance the cure rate for mouse mammary carcinoma. Interestingly, this effect was still observed when the BCG injection was given 7 d after the PDT treatment, i.e. it effects events involved in preventing tumor recurrence (257).

Early studies with human malignant mesothelioma implanted in nude mice established the basic therapeutic conditions (148,149) and also indicated better effects with *m*THPC-MD_{5000} (258). For example, these studies showed the importance of the drug-light interval and the light dose (149). Detailed studies of a human mesothelioma xenograft showed a marked difference between tumor or skin drug level and PDT effect and between tumor drug level and optimum drug light interval (259). Preclinical studies showed a strong dependence of the PDT effect on the drug-light interval but not the fluence rate (260). In addition, the PDT effect could be enhanced by an increase in tumor oxygenation through carbogen breathing or nicotinamide injection.

IPDT was shown to give zones of necrosis in the normal lung that heal safely, indicating potential for PDT treatment of lung cancers (261). An endobronchial study with pigs showed that PDT of the trachea results in atrophy and acute inflammation of the epithelium and the submucosal glands. Using fluences of 50 $J.cm^{-2}$ or less, full recovery occurred in 14 d (262). Initial pharmacokinetic data indicated that the drug level was the same in the trachea at day 6 and 20. Nevertheless, significant inter animal variation was found indicating the necessity of optimum dosimetry. A comparison of laser photocoagulation and IPDT of normal pig lung parenchyma showed that both techniques offer good potential, with the latter giving better healing (263). An attempt to use IOPDT of the chest cavity for malignant pleural mesothelioma failed. All rats treated with pneumonectomy followed by spherical PDT died within 48 h (264). A comparison of Foscan and Verteprofin intracavitary PDT on local malignant mesothelioma in the mediastinum of syngenic rats showed that both PS can be used. The PDT effect of verteporfin was better (265).

Similar results were obtained with papillomavirus-induced tumors in rabbits (299). The canine laryngeal edema was used as a model to define safety standards for drug use within the airways (266). Intraperitoneal PDT (IPPDT) of rats was used to establish the basic toxicity profile, which was similar for both *m*TPHC and Photofrin (150). A comparison of red and green light illumination indicates the latter might be better for IPPDT treatment, however, it is limited by the ineffective light distribution in the peritoneum (151). Nevertheless, IPPDT results were better than i.v. applications (267).

Induced adenocarcinoma was used to determine the tissue distribution in rats and indicated a significant difference between tumor and muscle tissue (268). This early study

indicated that abdominal or thoracic treatment might be better performed with longer drug light intervals. Lewis lung carcinoma was studied in a mouse model and gave initially good results, inhibited tumor growth and prolonged mice survival. However, tumor growth was regained after 9 d (269).

Gastrointestinal tract. A mouse model showed the utility of adjuvant intraoperative photodynamic therapy (AIOPDT) for colon cancer (270). Application of 0.3 $mg.kg^{-1}$ *m*THPC, 3 d incubation followed by total tumor resection and then followed by illumination (λ = 652 nm, 5 $J.cm^{-2}$) gave an increase of the time to tumor recurrence from 11.4 d to 33.3 d. Likewise, colon adenocarcinoma induced in the rat liver was used to study the potential for PDT on liver metastases. Different pharmacokinetics were found for the liver and tumor tissue with the former rapidly being cleared of the drug. PDT under standard conditions gave 87 % tumor free animals after 28 d indicating that this modality might be able to induce complete tumor remission of liver tumors (271). Fractionated light application or lower light fluences improved the PDT effect (156). In another study with murine colorectal cancer implanted mice it was shown that the highest tumor : tissue ratios after 4 d were found for the small intestine, liver and skeletal muscle (134). This animal model was used as a general test bed for PDT assessment (272) and for AIOPDT (45). A study of healthy hamsters showed highest drug levels in the gastric and duodenal mucosa and acinar pancreas after 2-4 d (273). Except for the duodenum all lesions healed safely. Similarly, intravesical PDT in normal mice resulted in moderate to severe functional bladder damage with full recovery within a few weeks (274). The utility of treating pancreatic cancer in animals was shown with a hamster model (275).

One of the first animal studies related to esophageal cancer used a sheep model (276). A test of mucosal ablation through PDT in normal animals revealed significant complications in the esophagus following illumination with green light (514 nm) especially at high doses (277). Blue light irradiation (412 nm) resulted in significantly less damage. Clearly treatment of the precursor lesions of esophagus adenocarcinoma would be an optimum treatment choice. However, here an appropriate animal model was lacking. A thorough study of sheep treated with *m*THPC showed that this might serve as such a model (278). The plasma pharmacokinetics were similar in sheep and humans, with Foscan reaching its maximum at 10 h post i.v. Two d after injection it was mainly distributed in the lamina propria, followed by a penetration into the epithelium. The sensitivity of both sheep and human tissue to *m*THPC PDT was similar. Use of rodents as models for esophageal cancer is problematic as they do not allow a study of stricture side effects. Thus far, pigs might be the best choice for related PDT studies (279).

Ovary. A rat model for the study of peritoneal cancer gave a promising tumor : tissue ratio of greater than four (280). Likewise, both superficial irradiation and IPDT of *m*THPC treated hypercalcemic ovary small cell carcinoma in a mouse xenograft model gave good results (281). A NuTu-19 ovarian cancer rat model was developed and used for testing debulking techniques. Use of *m*THPC-PEG gave a very high (40±12) tumor/tissue ratio after 8 d (282).

Brain. Most studies in this area have focused on ALA PDT and its effect on the blood brain barrier. Initial tests with Temoporfin in this area indicated less skin toxicity than other PS and effective cytotoxicity at a wavelength of 652 nm and light doses of up to 20 $J.cm^{-2}$. The tumor/normal brain tissue ratio was 20:1 (120,219). A first study of the pharmacokinetics of

^{14}C-labeled *m*THPC was performed using a rat glioma model (283). A rabbit model showed induced apoptosis as a result of several PS (187). A comparison of intratumoral administration and systemic one showed that both result in complete tumor localization in a C6 rat model. However, intratumoral administration gave an optimum drug dose already after 4 h post administration (284).

Other. A first rat study showed that the adrenal gland can take up PS and indicated the possibility for treating medullary neoplasia (285). Interestingly, only steroid synthesizing cells of the adrenal gland exhibited an intense photosensitizer-induced fluorescence after administration (286). Treatment of the prostate was evaluated in beagles and showed a good localization of the drug in the prostate within 2-3 d. Significant areas of glandular tissue could be necrosed with good healing (287) although regeneration of urethral epithelium could take up to 3-4 d (288). Various Temoporfin formulations were evaluated for the treatment of choroidal neovascularization associated with age-related macular degeneration (289). Possible side effects of IOPDT on blood vessels and nerves were assessed in rabbits (290). PDT at high drug concentrations (0.3 $mg.kg^{-1}$) and light doses (20 $J.cm^{-2}$) led to necrosis of all illuminated tissue. At lower concentrations the blood vessels showed severe edema, media hyperplasia or loosening of the endothelial layer, but no damage of the vessel wall or rupture was observed. Nerve cells underwent 75 % demyelization, however, without clinical symptoms. Thus IOPDT was suggested as a safe treatment option.

Veterinary Applications

Only few specific studies have addressed veterinary applications of PDT. This is surprising as the regulatory process is less involved and the related drug development cheaper. With the increasing willingness of individuals to spend significant amounts of money on pet health care this area offers potential for industry. One study investigated the biodistribution of *m*THPC in cats and found a situation similar to other species and no clinical or pathological changes attributable to the drug (291). Likewise, the time necessary for maximal accumulation in various feline tissues has been determined (292). Thus, Foscan PDT might be a good treatment protocol for feline neoplasm, e.g. SCC. Here, *m*THPC-LIP was found to be superior to standard *m*THPC (293). An *in vivo* study with 18 cats showed a complete remission (CR) of 75 % after one year (294). The only application in the equestrian area was the test of a novel light diffuser during the treatment of equine sarcoids (295).

CLINICAL EXPERIENCE

Apart from Photofrin, Foscan is the only other photosensitizer approved for use in systemic cancer therapy. Various derivatives of ALA (e.g., Levulan and Metvix) have also received approval as sensitizers for topical applications, meaning they are at present limited to skin cancers but with a high degree of success (296). Additionally, Verteporfin must be mentioned here. Developed as a photosensitizer for light-activated treatments it was successfully used for the treatment of age related macular degeneration (297,298). Excellent overviews of recent advances in the clinical field concerning these established photosensitizers were published in 2004 (299,300) and newer treatises are available as well (301,302). The clinical impact of *m*THPC and other PS in the treatment of gynecological diseases and head & neck cancer has been discussed (303,304,305). Reviews on skin cancer (basal cell carcinoma) (306) as well as gastrointestinal and esophageal (307,308,309,310), brain (311), prostate (9,312), pancreas

(313), breast (314), urological malignancies in general (315,316) and new treatment options thereof (317), and gynecological (318) malignancies show that these cancers can be treated with a reasonable degree of success with Temoporfin. A review by Konan *et al.* has surveyed the drug delivery and formulation aspects of drugs used for PDT (143). Table 3 summarizes details about the various clinical studies available for Foscan and related drugs and formulations.

A first comprehensive meta analysis of the available data on PDT for most applications has just been published by Fayter *et al.* (319). They analyzed 88 trials reported in 141 publications and clearly outlined the problems associated with the currently available data. Their analysis indicated good prospects for PDT for actinic keratosis and Bowen's disease. Likewise PDT of BCC might be superior to surgery or cryotherapy due to better cosmetic outcomes. Similar indications for Barrett's esophagus were promising but no conclusions could be made for esophageal cancer. Limited evidence was noted for applications in lung, brain and H&N cancer and cholangiocarcinoma. Overall, no significant adverse side effects were noted. The authors noted as problematic the absence of large scale randomized control trials and noted limitations in the quality of life and resource outcome reporting.

<Table 3>

Side Effects

Obviously photosensitivity is a major concern for light activated drugs. Several studies have indicated that *m*THPC results in less photosensitivity than Photofrin (132). Still, the major problem involving *m*THPC PDT is skin photosensitivity in the few weeks post drug administration. Injuries resulting from this light hypersensitivity are generally minimized by following medical advice. Thus, burn related incidents are rarely seen in PDT patients; those that are observed mostly occur when medical advice is ignored. However, in isolated cases, injuries have been received during treatment in the operating theater where devices such as the pulse oximeter have been a source of causing burns to the victim (320). To reduce the risk to patients several types of creams have been tested on patients and for example, dark cover cream promises acceptable protection (321). Other side effects are mild to moderate pain in the treated area. More specific, but also rarer, are side effects related to specific cancer treatment protocols, e.g., for esophageal cancer (*vide infra*).

In 2000 a report of post PDT partial sickness burn occurring in 6 out of 12 patients was challenged by the Foscan producers and created a lively debate in *BMJ*. Since then no similar reports have been made and it appears to have been the result of the drug application procedure. The furor created by this report and its subsequent discussion in the scientific literature and (financial) mass media is an illuminating example for the interrelationship of financial interests, research, scientific publication ethics and news spin (321).

Head & Neck

Foscan was first approved in 2001 in Europe for the treatment of advanced H&N cancer. There is a relatively high morbidity and cost associated with standard head and neck mucosal squamous cell cancer treatment. Typically, therapy involves a combination of radical surgery, radiotherapy and chemotherapy. Radiotherapy to the head and neck area commonly causes a wide range of debilitating side effects, such as xerostomia, mucositis, loss of taste and loss of smell (322). In severe cases, malignancies have sometimes been found unsuitable for any treatment because of the imminent loss of functionality. The treatment of squamous cell carcinoma (SCC) of the soft palate is an example (323); however, there are many more. While

multidisciplinary H&N cancer care is well established (324), PDT has been slow to gain an entry (304). In an overview from 1997 on multitreatment approaches it was mentioned only as an emerging technique (325). More recent overviews of this area are available (326,327,328).

Nevertheless, while it was initially only used for palliation in inoperative patients with advanced stage H&N SCC, PDT quickly was used with a curative intent for the treatment of small localized and recurrent tumors and premalignant lesions. Initial clinical studies were promising and showed that PDT is suitable for the treatment of early (CIS, T1) carcinomas and of small T2/T3 superficial carcinomas (<0.5 cm) (329). Likewise, fluorescence guided resection was quickly shown to be a feasible option (330). *In situ* dosimetry during oral cavity illumination is indicated and should be required as the basis for light dose prescription in H&N PDT (331).

The introduction of *m*THPC PDT, even as an adjuvant treatment, has offered a new possibility of tumor clearance and possesses the advantage over the related use of ALA or Photofrin, in that much lower drug and light doses are required (332,333). The length of time skin remains photosensitive is halved while deeper tissue penetration is achieved. The pharmacokinetics of *m*THPC were studied by Brachiotte *et al*. in early clinical tests on patients with SCC in the oral cavity using fluorescence spectroscopy and a non-invasive optical fiber (334) and later in a hamster model (240). The measurements were performed at 420 and 520 nm and showed encouraging results for oral SCC at both wavelengths. A more recent trial using standard conditions with longer mean follow-up times (37 months) of early SCC in the oral cavity resulted in a total clearance of 86%, which underlines the usefulness of *m*THPC for these and similar cancers (335). The complete response rates for *m*THPC PDT are similar to those of surgery and/or chemoradiotherapy but with the advantage of a lower morbidity and better cosmetic results (333,335,336).

The first studies on 17 H&N cancer patients (SCC, malignant melanoma and verrucous carcinoma) treated with different PS (15 Foscan, 1 ALA, 1 Photofrin) were published in 1995 and were encouraging (332). An early explicit trial including 20 cases of SCC and basal cell carcinoma (BCC) confirmed the potential use of low light dose (5-10 $J.cm^{-2}$), and found the outcome of treatments at low light dose preferable as selectivity of necrosis of normal to cancer tissue was often higher (337). However, this only was found to be true for minor lesions. All cases of SCC showed complete response with a follow up of 8-24 months. Lymph node metastasis was shown to be absent. Complete response of BCC was slightly lower (92%) with the remainder showing only partial reduction of the tumor. *m*THPC mediated PDT was found to be superior to that of Photofrin, ALA and benzoporphyrin derivative (BPD). Additional medication (e.g. antibiotics or pain reliever) after irradiation was unnecessary as side effects were minimal. Side effects only occurred because of oppositional behavior to medical advice (burns due to exposure to open daylight during the first weeks after treatment).

But do isolated tumors and “field change” tumors (multiple lesions at one anatomical site) respond equally well to irradiation? Clinical trials performed by Bown's group on various oral SCC's indicated that isolated lesions gave the best PDT response whilst cases of “field change” showed less photoreaction and often incomplete necrosis of cancer tissue (338). Repeated treatment at higher light doses sometimes led to an improved outcome. Still, evaluation of a variation of the light dose between 5 and the recommended 20 $J.cm^{-2}$ suggested that lower light doses might very well be sufficient for smaller isolated lesions.

In early 2001, the possibility of treating head and neck cancer with *m*THPC PDT came to be known more broadly as two complete phase II studies showed encouraging results

(339,340). Kübler *et al*. examined 25 patients with primary lip SCC and showed a 96% complete response after 12 weeks (339). A brief report on the treatment of lip cancer was also published in 1995 (341) and followed by a case report indicating good cosmetic results (342). About this time, Scotia sought regulatory approval in the USA and Europe for palliative use of Temoporfin in head and neck malignancies. Extensive clinical research in a phase III study had confirmed an improved quality-of-life benefit in 53 % of patients with incurable cases of recurrent/refractory SCC of the head and neck (343). Other clinical studies have been reported as well and indicated good results with patients for whom all other treatment options had been exhausted (344,345).

The efficacy of *m*THPC in the primary treatment of a wide range of head and neck mucosal SCC was reinforced in 2003 (346). Although this study encompassed a rather heterogenous group of patients with oral cavity, nasal, oropharyngeal, hypopharyngeal and laryngeal malignancies, some of which had already received radiotherapy, the results were promising. A CR to primary treatment was seen in 19/21 patients (90%) in tumors ranging in stage from T_1 to T_3. Treatment of laryngeal cancer, recurrent post radical radiotherapy, however, was less successful, with only 25% of patients showing CR. This study concluded that while the complete response rates are similar to those of surgery and chemoradiotherapy, PDT had a lower morbidity rate. There is also the ability to re-treat or treat uncompromised areas with standard techniques in the event of a recurrence. Prime sites for PDT when treating head and neck mucosal SCC were established to superficial looking (around 5 mm depth) floor of mouth, buccal pouch, lip, lateral pharyngeal wall/ tonsil, soft palate and posterior pharyngeal wall carcinomas. Larger studies of BCC skin cancer in the H&N region with Foscan indicated 97 % CR after eight weeks and a 100 % CR with low doses of Foscan (347). Thus, H&N PDT appears to be one area where PDT significantly improved on other treatment approaches (348). Foscan has been used clinically for the treatment of benign neoplasms in the H&N as well. All cases showed a significant reduction in the volume of abnormal tissue; better results were observed with lymphatic malformations compared to venous ones (349). Other studies involved use of a new light applicator for nasopharyngal cancer (350,351).

A contemporary Dutch study analyzed the results of Foscan treatment of early stage oral cavity and oropharynx neoplasms in 170 patients with 226 lesions (352). The very promising results gave an overall response rate of 91 % and a CR of 71 %. However, clearly the selection of the patients is of critical importance for the outcome of the treatment. A thorough subgroup analysis revealed that some sites (e.g., oral tongue and floor of mouth) are more suited to PDT treatment than others (e.g., alveolar process, soft palate). Unfortunately, the latter are the areas that are less suited for conventional surgical resection. Nevertheless, the success rate is promising, side effects were acceptable and the PDT effect was independent of the T stage for invasion depths <5mm. Overall, Foscan PDT appears to be a suitable first treatment for areas that would have functional problems after resection. A recent multicenter study of Foscan use for H&N SCC showed complete response in 19 of 39 treated patients with a significantly increased median survival (37 months compared to 7.4) (353). Mild to moderate side effects were observed, the most significant ones related to photosensitivity, pain and dysphagia. Foscan was the first PS to be investigated for its utility in H&N cancer in a multicenter study (354).

A second way to raise the effectiveness of treatment of large tumor sites was the advent of interstitial photodynamic therapy (IPDT) (355). IPDT involves the penetration of the cancer tissue with the optical fibers during irradiation to ensure complete irradiation of the

malignancy (356). Utilizing this modality on some recurring head and neck cancers, has delivered some impressive results. Several patients, previously regarded as otherwise unsuitable for any further treatment, showed a complete response (11% after 10 - 60 months) after *m*THPC PDT. Nevertheless, in some cases repeated PDT was necessary to reach this outcome. A recent, larger study with 68 patients with various deep seated pathologies showed that ultrasound-guided IPDT was well tolerated and that image-guided PDT gave improved results. Quality of life assessments were found to be a good criterion for the results, especially since the clinical results vary for patients with the same disease (357). Optical guidance methods for PDT in this area are currently under development. One study explored the use of fluorescence differential path length spectroscopy for monitoring Foscan IPDT (358).

Similarly, the use of surgical palliation using PDT is becoming more developed for the treatment of end-stage head and neck cancer. An excellent overview on the current best practice of this modality has been given by Jerjes *et al.* (359). A case study reported Foscan PDT use for the treatment of cystic hygroma in a 6 m old patient (360). They also presented a case study which showed that the occurrence of carotid artery rupture in the treatment of peri-carotid disease can be reduced via endoluminal carotid stenting prior to PDT (361). Other areas of potential use include the hyoid. Chrondrosarcoma of the hyoid has been reported to be radioresistant and can not effectively be treated with chemotherapy, which typically leaves hyoidectomy as the only option, resulting in disruption of speech, swallowing and respiration. A case study showed that IPDT can be used effectively for this treatment (362).

Practical applications also demand a cost-effectiveness analysis. An analytical model was developed for the UK market using a computerized cost-effectiveness model. It revealed that palliative *m*THPC PDT used against advanced head and neck cancers not only resulted in an increase of life-quality avoiding the common side-effects of extensive palliative surgery or palliative chemotherapy but was also much more cost-effective than the other optional treatments or even non-treatment (363). This study also showed significant health gains. Of the three treatment strategies PDT gave 129 extra days of life compared with no treatment and extensive palliative surgery and 48 extra days of life compared to four cycles of palliative chemotherapy. This model was also used for Germany using the local cost data in 2005 and confirmed the results of the British study. Financial costs for PDT were 8761 € compared to 11600 for four cycles of palliative chemotherapy (364).

Skin

Due to the accessibility by light skin cancer is a natural target for all PDT drugs (306). For a comprehensive clinical review on cutaneous malignancies see Allison *et al.* (365). Several *in vitro* and animal studies (249) were performed and established the principal effects of Temoporfin in this area. A pilot study using a gel formulation of *m*THPC for topical treatment of BCC and Bowen's disease showed some response but was found to be inferior to both, intravenous *m*THPC PDT and topical PDT with ALA (366). Still, initial studies of the treatment of basal cell carcinoma (306) were promising with about 86 % complete response (367). A slightly larger study optimized the drug-light interval and showed that light activation after 1 d gave the best results with 75 % CR after 6 m (368). Even better results were observed in a larger study with 117 patients where an overall CR of 96.7 % was reported (347). Overall, the clinical and cosmetic results are promising. Foscan has also been used in a case study to evaluate the utility of optical coherence tomography-guided photodynamic therapy. Administration of a 0.05 $mg.kg^{-1}$ dose 2 d prior to tumor mapping followed by light treatment

of the mapped area resulted in CR at 6 months (369). A recent review has addressed clinical treatment decisions for topical PDT in non-melanoma skin cancers (370).

GI Tract – Esophagus and Bronchi

The esophagus is often the site of second primary tumors occurring in patients suffering from SCC in the head and neck. PDT quickly became of interest for the relevant clinicians due to the possibility of endoscopic light delivery (307,308,309,310,371). Nevertheless, only a few groups were the driving force behind this obvious application in the early years (372). As *m*THPC PDT already showed success in these areas, and as esophageal cancer had previously been treated with Photofrin® PDT, it was logical to test the effect of *m*THPC PDT on esophageal SCC (373) or Barrett's esophagus (374). Preliminary studies (372) on the impact of treatment on patients with (second) primary SCC located in the esophagus or the bronchi showed success (375) and were confirmed in subsequent studies (376,377). For example, a total clearing rate of 84% in the treatment of early SCC in esophagus and bronchi was found after a mean follow-up of 15.3 months (378). With the assistance of fluorescence spectroscopy, an adjustment of the fluence rate to only 8-12 or 7-16 $J.cm^{-2}$ respectively was possible. An analysis of the early studies in this area using various PS in the GI and aerodigestive tract was given by Radu *et al*. (373,376) and they discussed their experiences with PDT and endoscopic mucosal resection in Switzerland in detail (276).

Experiments performed by Brachiotte *et al*. on oral SCC were extended into other treatment sites in patients such as the esophagus (379) and bronchi (380). The results showed that while there was a direct correlation of photosensitizer accumulation (as detected by fluorescence spectroscopy) to PDT response in the tumor, the efficacy between patients varied largely. This is largely due to each patient having an individual pharmacokinetic response and rate of metabolism for the same drug-time level. Thus, the efficacy of PDT in the esophageal and bronchial SCC can be improved by prior monitoring of the accumulation of *m*THPC in the tumor cells and before applying the correct light dosage. Zellweger *et al*. demonstrated that this is indeed possible in a study with five patients and avoids to a certain extent over- or under-irradiation, hence increasing total response rate and abating side-effects (381). However, due to the difficulty of endoscopic measurements, fluorescence measurement of the oral cavity was recommended as the ideal methodology. Indeed, it was shown that intrapatient fluctuations in oral measurements were two to three times smaller than in endoscopic procedures, thus being more reliable.

Light dosimetry is especially important in esophageal treatments (382). To facilitate endoscopic irradiation in esophageal PDT, a through-the-scope balloon applicator was proposed and tested on a small number of patients who underwent either *m*THPC or ALA PDT (383). In the bronchi, the complex and elaborate architecture of the tracheobronchial tree leads to less accurate delivery. Consequently, the risk of recurrences due to probable under-treatment is enhanced for this site. But at least in early non-invasive tumors slight over-illumination might help, as non-malign bronchial cartilage has a low uptake of *m*THPC and is hence relatively insensitive to photosensitization. But still, sufficient light-delivery in this part is a medical challenge.

Although early esophageal SCC was found to respond very well to *m*THPC PDT, irradiation at 652 nm also led to necrosis of a substantial amount of surrounding normal tissue and a deeper level so as to impair functionality. This often led to heavy side-effects due to perforations and the development of tracheo-esophageal fistula. To avoid this, therapy with

green light (514 nm) was introduced, allowing light penetration of only a few mm which subsequently diminished the undesired photosensitization of deeper tissues (384, see also 375). As one means of optimizing PDT for this particularly sensitive area, Blant *et al*. studied the time-dependent histological localization in the aero-digestive tract by comparative fluorescence spectroscopy of normal and malign tissues (385). However, for advanced invasive tumors in the esophagus it is likely that, due to the mentioned disadvantages, conservative treatment (surgery, radiotherapy) is the better choice at present.

Recent studies have shown success with *m*THPC PDT in patients with Barrett's esophagus (386,387) and/or adenocarcinoma (388). Both conditions are closely related to one another as patients who show a Barrett's associated dysplasia are 40-50 times more likely to develop an adenocarcinoma than the general population. Thus far, PDT is only used for high grade dysplasia or carcinoma. The initial results suggested that response rates with *m*THPC PDT are better compared to ALA or Photofrin, with a 100% clearance rate of early stage neoplastic lesions and a complete re-epithelization of the squamous within a mean of 34 months using green light. Interestingly though, a comparative study was performed by Lovat *et al*. recently in the treatment of dysplasia and early esophageal cancer using red and green light (389). In this study, the most effective light delivery technique was found to be the use of red light equipped with a diffuser. Note, that this study pointed out that complications (including death) can arise from taking biopsy species too early. It was recommended that biopsy specimens should not be taken for at least 2 months after treatment. A comparative analysis of the various PS available in 2004 concluded that depending on the presence or absence of macroscopic abnormalities Photofrin or ALA might be the best indication (390).

As adenocarcinoma is a very severe illness with a one-year survival rate of only around 20% the different light-delivery techniques recommended in the various studies complicate the issue of treating esophageal cancer using PDT. As a result, the general conservative solution remains esophasectomies as the primary solution. Despite this, *m*THPC PDT offers a potential second line treatment and a solution for patients who are deemed inadequate for surgery. For a recent review of this rapidly developing field see Gross and Wolfsen (391).

Chest

The very first published clinical work with *m*THPC described trials investigating the photodynamic effect in the treatment of diffuse malignant pleural mesothelioma, an asbestos-related cancer (392). This severe disease is characterized by its resistance to conventional modalities such as surgery, radiotherapy and chemotherapy (393). Even with radical resection combined with chemoradiotherapy, close to 1/3 of all patients develop a recurrence of the malignancy and a small number develop multiple recurrences (394). The tumor is fast growing and very aggressive in invading the surrounding vital structures and thus only cases in which early detection is achieved are classified as suitable for surgery. In these instances two procedures have been established depending upon the severity of the malignancy: extrapleural pneumonectomy, which involves the radical removal of one lung or pleurectomy, a procedure involving the debulking of the tumor. Extrapleural pneumonectomy is accompanied by heavy morbidity. While pleurectomies are less morbid for the patients there is an associated enhanced risk that the original tumor tissues remain in the chest cavity. The main drawback to both surgical procedures is that mesothelioma has the tendency for frequent local recurrence within a short time with associated high lethality. A recent review on the use of PDT in this area was given by Lindenmann *et al.* (395) while a comparison of the state-of-the-art

techniques was published by Yarmus *et al.* (396).

At present cryotherapy, brachytherapy and PDT are the three methods suitable for the endoluminal treatment of lung cancers. While no comparative studies have been published it appears as if a complementary use of these methods is indicated. Cryotherapy is cheap and is suitable for the treatment of superficial tumors (3 mm) similar to PDT, while brachytherapy allows a deeper invasion into the bronchial wall and thus allows the treatment of more aggressive tumors (397). Since *m*THPC PDT was known to be superficially effective against certain malignancies Ris *et al*. tried to combine *m*THPC PDT with the known surgical procedures (392). The idea was to illuminate the tumor surrounding surfaces in the regarded chest hemisphere post tumor removal to destroy remnants of malignant tissue, thus improving local tumor control. Investigations of the potential conditions for PDT on mesothelioma were carried out with some preliminary experiments using nude mice bearing human malignant mesothelioma xenografts (148,149). These studies also showed the utility of pegylated *m*THPC (258) and the importance of the drug-light interval. Likewise, low oxygenation levels either resulting from oxygen consumption or vascular occlusion may adversely affect the PDT effect (398). This can be counteracted by nicotinamide injection and carbogen breathing (260).

In a first phase I study on diffuse malignant mesothelioma of the chest, Ris *et al*. concluded that 0.3 $mg.kg^{-1}$ drug dose should be combined with a total fluence of 10 $J.cm^{-2}$ at a drug-light interval of 48 h. The study used surgical tumor resection followed by intraoperative photodynamic therapy with *m*THPC. These conditions led to tumor necrosis of up to 10 mm in depth (399). Such a high impact at such low light dose had not been reported with either HpD or Photofrin previously and was an early indication for the utility of *m*THPC in PDT. However, the treatment of more patients with longer follow-up times revealed that the chosen conditions were less than optimal. Longer drug-light intervals (72 h) showed a greater selectivity for tumor necrosis and less pronounced burning of normal tissue.

Intraoperative PDT (IOPDT) was initially faced with a number of difficulties. First of all, the equal illumination of the surface of the lungs takes a very long time, increasing operational theater times which made the already heavy impact of the procedure on the patient even more troublesome (400). The fact that the large areas to be treated were complex and delicate did not help to make the practical work and estimation of the correct fluence any easier on the physician. Due to the therapy taking place in the chest, the vicinity of the site being treated also houses many of the vital organs, thus over-illumination must be avoided. As a direct result of IOPDT, patients showed an additional morbidity beyond that of surgery alone, but the procedure related mortality rate was thought to be comparable to surgery without the adjuvant phototherapy. In a follow-up of 4-18 months, all patients developed either contralateral disease or distant metastasis and eventually died of their illness. Thus *m*THPC IOPDT of mesothelioma has so far not led to improved survival rates, but has given some conditions for better local tumor control if the pathways for cellular localization for this type of cancer can be understood better. Further PDT developments in this area will depend on improved sensitizer accumulation and a more detailed understanding of the synergistic effects between PDT and chemotherapeutic agents.

Several other clinical studies were performed in this area (401,402). In a preliminary study, Baas *et al*. introduced a light delivery system that allowed integral chest illumination and reduced the treatment time significantly. After patients had undergone extrapleural pneumonectomy the cavity was filled with a sterile plastic bag with 2.5-4 L saline solution. The bag contained a spherical bulb fiber as light source. Four isotropic detectors were

positioned at various places within the chest cavity therefore allowing real time light dosimetry. The fiber was repositioned in a way that all detectors reported comparable fluence rates. During a succeeding phase I/II study adjustments to the conditions were made for adjuvant PDT alongside EPP to 0.1 $mg.kg^{-1}$, 10 $J.cm^{-2}$ and 96 h drug–light interval, since previous treatments with 0.15 $mg.kg^{-1}$ led to several fatalities (403). However, the choice of quantities and light dosage for intraoperative PDT was difficult due to the small number of patients in the study (28 in this particular case). Twenty patients showed local or distant recurrences during follow-up; however, local control could be registered in about 50% of the patients. A median follow-up of 31 months showed a median survival time of 10 months.

Friedberg *et al*. recently carried out a phase I study combining surgical debulking (either by pleurectomy or extrapleural pneumonectomy) and adjuvant *m*THPC mediated PDT (402). The maximally tolerated dose of *m*THPC PDT was found to be 0.1 $mg.kg^{-1}$ with a fluence rate of 10 $J.cm^{-2}$ after a 6 day drug-light interval. Patients treated at higher doses showed several cases of acute capillary leak syndrome, which was assumed to be a dose limiting toxicity; the PDT treatment correlated with an increase in interleukin-6 level (404). It was concluded that, unlike most other surgery-based multimodal treatments for mesothelioma, *m*THPC PDT affords the option of accomplishing tumor debulking with a lung-sparing procedure rather than an extrapleural pneumonectomy. IOPDT has so far not been an answer to the highly metastatic nature of the disease (occurrence in > 50% of all patients) but in future optimized IOPDT-gained local tumor control might become part of a combined treatment strategy. Note, that the 6 d interval differs from the standard 4 d used by Schouwink *et al.* (403). Both studies taken together clearly indicate that compromises between minimizing toxicity, maximizing efficacy and ability of the patient to tolerate the procedures will always need to be made on a case by case basis (405,406). An excellent review of the practical aspects of clinical use of PDT and IOPDT for malignant pleural mesothelioma has been given by Friedberg (393). A few meta analyses have addressed the use of PDT for malignant pleural mesothelioma in general. Moghissi and Dixon compared data from 10 papers (up to 2004) with 230 patients (170 with Photofrin, 60 with Foscan) (407). They calculated an overall mortality of 4.9 % for Photofrin and 13.3 % for Foscan. Morbidity was 38 % and 70 %, respectively. Overall, the mortality and morbidity associated with IOPDT was not larger than that of the related surgical procedures.

Brest cancer offers another possible area for PDT (314,408). This was indicated by early *in vitro* tests (217). Breast cancer patients with chest wall recurrences after mastectomy were treated successfully with *m*THPC. In a study by Wyss *et al*. all 89 treated lesions showed complete responses (409). Two different conditions of treatment (0.1 $mg.kg^{-1}$, 5 $J.cm^{-2}$, 48 h or 0.15 $mg.kg^{-1}$, 10 $J.cm^{-2}$, 96 h) led to comparable results. The amount of pain during the first days after treatment differed considerably, depending on the size of illuminated area, but was controllable with analgesics. Still, normal tissue morbidity was relatively high.

Gynecological Malignancies

For general reviews on the use and utility of PDT in this area see Gannon and Brown (410), Allison *et al.* (303) and Hillemanns *et al.* (318). Relevant *in vitro* and *ex vivo* tests have been described above (231). The use of *m*THPC in gynecological cancers was initiated by Wierrani *et al.*, who reported success in a small group of women suffering from recurrent intraperitoneal carcinoma of the ovary (411). Post-operative observations showed total clearance and a significant improvement of life quality. A study of 8 patients with terminal disease and treated

with IOPDT also indicated the utility of this drug (412). Continued work on recurrent ovary-, cervix- and corpus-cancers showed responses in selected cases, but whether a significant life extension can be achieved remains unclear as the number of reported patients up to now is limited (413). Other investigators treated four advanced cases of SCC or adenocarcinoma located in the vulva, vagina or cervix with a rather unsuccessful outcome. The overall survival of the patient was between 3 and 4 months (414). In these instances, the patient suffered local recurrences despite an initial good PDT response, or developed systemic disease. One explanation offered was the advanced status of the tumor being beyond recovery.

*m*THPC PDT was also found to be an effective measure in the treatment of vulval intraepithelial neoplasia (415). Most of the patients exhibited total response with a follow-up of two years. Subsequent management, like minor excisions or multiple sessions of PDT, was required in most cases. However, good cosmetic healing and preservation of function was reported overall. A case study of vulval intraepithelial neoplasia III (severe atypia) with a lowered drug dose (0.05 $mg.kg^{-1}$) gave excellent results in a case study (416). For a recent review on PDT of human papillomavirus-related genital dysplasias see Soergel and Hillemanns (417).

Other Clinical Uses

Naturally many other potential applications exist. These include the treatment of brain (311,418,419), prostate (9,312), pancreas (313), and others.

Brain. Malignant brain tumors carry a lethal prognosis with a median survival of 15 months, despite surgery, radiotherapy and chemotherapy. Since it is difficult to differentiate between glioma brain tumor tissue and normal brain tissue by examination, intraoperative fluorescence diagnosis has become an important tool for neurological cancer surgery. Most work on this field was done with the ALA/PpIX and HpD, systems which afforded promising results due to the articulated concentration differences between malign and normal tissue (418,419). Temoporfin has been tested for this purpose as well. An accordant procedure has been introduced and initial encouraging experiences on patients have been made using a combination of IOPDT and fluorescence guided resection. The results showed a limited advantage compared to the first generation PS (420). One of the first larger studies was performed by Zimmerman *et al*. who conducted their experiments on 138 samples from 22 patients and were able to achieve 88% selectivity and 96% specificity from intraoperative PDT involving fluorescence guiding resection of brain tumors such as glioblastoma multiforme (340). Similar selectivities and specifities were observed in other studies. For example, intraoperative photodiagnosis and fluorescence guided resection for radical tumor removal in a group of 25 patients improved the tumor prediction and gave a radical resection in 75 % under fluorescence guided resection compared to 52% in the control group. The median survival time for the PDD/PDT group was 9 months compared to 3.5 months in the control group (421).

Stomach. A preliminary study of *m*THPC PDT of early cancers of the stomach resulted in 80% total response in cancers of the intestinal type at a mean follow-up of 12 months (422,423). The drug dose chosen was lower than usual (0.75 $mg.kg^{-1}$) to obviate possible perforations. It was concluded that if this encouraging outcome was to be verified in following studies with larger numbers of patients *m*THPC PDT could become a therapeutic option alternative to

radical gastrectomy (423).

Pancreas. An initial study of normal hamsters indicated that the normal pancreas is able to tolerate PDT well, indicating that PDT might be a good modality for treatment of pancreatic cancers (273,424). This was confirmed by a related transplanted pancreatic cancer model (275). In 2002, two phase I studies were reported on the application of percutaneously implemented *m*THPC on inoperable adenocarcinomas localized in the pancreas (425) and on locally recurring prostate cancer (426). Several needles were inserted into the tumor separated by approximately 1.5 cm to deliver light in both of these circumstances. Optical fibers matching these needles were subsequently inserted. During illumination, the needles were pulled back step-wise in approximately 1 cm steps to cover the entire tumor with the same light-dose. For the pancreatic cancer, substantial tumor necrosis was reported. The median survival rate was an encouraging 9.5 months and the quality of life was found to be improved after PDT in most patients. Recurrences at the site of PDT were not found, but often occurred at the periphery indicating that illumination should include more tumor surrounding tissue. However, the use of PDT was complicated if either major blood vessels or the duodenum wall were involved with the tumor. PDT on tumors located at these sites led to notable bleedings or perforation of the duodenum after PDT. The prostate adenocarcinomas showed significant tumor response but PDT was commonly accompanied by loss of sexual function and discomfort in urination. Still, conservative treatments of this disease have similar or worse side-effects and thus further research in this is warranted (313). Later *in vivo* studies with a hamster model showed that Verteporfin gave a profile similar to Foscan, however with the added benefit of shorter drug-light intervals and elimination times (427).

Prostate. A recent clinical review was given by Ahmed *et al.* (316) and a comparison of the state of the art use of lithotripsy versus PDT was published by Waidelich (428). *m*THPC was first used in studies with dogs and was shown that it can be safely used for treatment of the gland (287). Next PDT was described as a salvage treatment after failure of external beam radiotherapy (426). A phase I study showed that prostate cancer can indeed be treated; the level of prostate specific antigen fell by up to 67 % in the 6 patients tested (429). Dosimetry is of critical importance for an effective treatment in this area (430). In this context an interactive dosimetry by sequential evaluation module has been developed for clinical trials of primary prostate cancer with Foscan (431). Preliminary studies on the use of IPDT have been reported as well (432). Alternative minimally invasive techniques are cryotherapy and high intensity focused ultrasound.

Biliary system. PDT treatment of bile cancers is only slowly emerging (433,434). A significant problem for the palliation of patients with malignant bile duct obstruction using metal or plastic biliary stents is stent occlusion. In this context endoscopically performed Foscan PDT was investigated in 13 patients with malignant biliary carcinoma obstructions (9 biliary tract, 3 pancreas, 1 stomach). All patients had been initially palliated with stents, however, recurrent obstructive jaundice had occurred due to local tumor progression. After PDT tumor necrosis and/or metal stent recanalization was observed in all patients and the median patency of plastics stents increased from 3.5 m to 5 m. Median survival times increased from 8 m to 21 m. However, although PDT was tolerated well, significant complications were observed in this study (435). At present intraoperative radiotherapy appears to be more suitable. However,

preliminary data of PDT in bile duct cancer gave good reduction of cholestasis, improvement of life quality, and prolongation of survival time (436,437). Studies on cholangiocarcinoma are presently expanding (233,438).

Papillomatosis. Only one investigation has explored the efficacy of *m*THPC PDT in the treatment of recurrent respiratory papillomatosis (439). A reduction of the severity of laryngeal papillomas was seen during the first year after PDT. However, this was attributed to a probable improved immune response.

Retinoblastoma. An initial study with Foscan utilized three xenograft cell lines derived from surgical samples taken from children. A significant response upon Foscan treatment was observed for one of the cell lines (RB111-MIL) and partial regression was observed after 60 d. (440).

Anal cancer. One study with four patients studied the use of a light applicator for the treatment of anal intraepithelial neoplasia grade III. Here an applicator based on a standard anoscopy instrument was used and it was shown that blood saturation, volume and fluorescence and fluence rate can be monitored without changes in the light protocol (441). Nevertheless, a study with Fosgel administered to nine patients (8 HIV-positive males and 2 HIV-negative women) with anal intraepithelial neoplasia (III) showed no effect of the PDT treatment (442). This was attributed to the limited penetration of the liposomal formulation and the study of other topical PS applications is indicated.

OTHER APPLICATIONS

Many possibilities exist to use PS in areas other than cancer treatment. These include the well known treatment of age-related macular degeneration, use in dermatology (443), aesthetics (photodynamic photorejuvenation), periodontal uses (444) and more. Sterilization and antibacterial action are clearly suitable application areas as well (445). Antifungal PDT may also be used, but has not been used with *m*THPC derivatives yet (446). However, porphyrins, ALA, and phthalocyanines have all been shown to kill yeast and dermatocytes through photodynamic action, indicating the utility of this approach. Use of *m*THPC is ever expanding and will see many new applications in the near future. To give only one example, an interesting study by Hansch *et al.* showed that *m*THPC-based phototreatment may be used for the treatment of rheumatoid arthritis (447). They used a murine antigen induced arthritis model and found that *m*THPC-PEG was taken up best in arthritic joints. Light treatment resulted in lowered arthritic scores at lower drug doses compared to what is used in cancer PDT. Thus, local treatment of arthritis appears to be feasible.

Ophthalmology

Ophthalmological applications for Foscan PDT are only slowly emerging (448). Liposomal formulations of BPD-MA and Foscan were tested in the chick chorioallantoic membrane model with regard to the treatment of choroidal neovascularization which is secondary to age-related macular degeneration. Here, PDT treated areas were treated with a soluble anti vascular endothelial growth factor (recombinant human soluble VEGF R1 (sFlt-1)/Fc chimera). The topical application of this factor helped with occlusion and limited subsequent angiogenesis in a dose-dependent manner (449).

Antibacterial PDT

More and more studies with PS address their use for antibacterial action, including intracellular pathogens (327,445). A study from 1995 using isomers of THPP already indicated the potential antibacterial action of this type of compounds (450). An early study into the antibacterial action showed that Foscan has antibacterial effects on *Staphylococcus aureus* (wild type) in the dark while hematoporphyrin derivative showed suppressive growth effects only after white-light illumination (413). Many of the newer derivatives and formulations of Temoporfin (*vide infra*) are now tested for the treatment of infectious diseases.

Antibacterial nanoparticle formulations are especially interesting. For example, a test of different cationic liposomal formulations of Foscan for antibacterial action showed that subtle variations significantly affect the uptake. Only one of the formulations tested gave results similar to the free drug (451). Likewise, calcium phosphate nanoparticles were surface-functionalized with different polymers, and PS were incorporated into this layer (452). Here, methylene blue and *m*THPP showed good performance with HIG-82 synoviocytes. Moderate activity was found with HT29 epithelial cells while good photoxicity was found against *Staphylococcus aureus*, both with positively and negatively charged nanoparticles loaded with *m*THPP. The Gram-negative *Pseudomonas aeruginosa* could only be treated with positively charged nanoparticles containing *m*THPP (453).

The dental care field has also become interested in PDT. Mostly this involves antibacterial PDT for periodontal applications (444). Such an approach is warranted as there is increased antibiotic resistance, the number of immune-suppressed patients has risen, and diverse pathogens play a role in periodontal infections, mandating different drug approaches. Foscan fluorescence in the buccal mucosa can be used to determine its concentration (381). A recent study investigated the effect of PDT on cariogenic bacteria. A comparative analysis of *m*THPC and Hypericin with *Streptococcus mutans* and *Streptococcus sobrinus* showed that the latter could easily be killed with both PS (the light source used was a dental polymerization instrument). At similar concentrations the former exhibited dark toxicity to *m*THPC and no effect with Hypericin. However, coadministration of both PS eradicated the bacterium effectively. Thus potentially there is a role for PDT in oral cavity antibacterial action. However, while the study showed very good results for a single bacterial species it appears that the really multispecies environment of the oral cavity presents a significant treatment problem (454). The use of Fospeg was studied as well. It provides a means to deliver an aqueous solution via spray techniques, and gave a significant reduction in bacterial cell count (455).

PDT Combined with Other Methods

Naturally PDT is often used in conjunction with surgical treatments. However, some potential exists for the combined use with other cancer treatment protocols. This is a rapidly emerging field and includes the combined use of PDT with other chemotherapeutic drugs or immunostimulants and dual modality treatments.

PDT and chemotherapeutics. Foscan PDT could be improved by coadministration of mitomycin C in an animal model (202). This allowed the use of lower light and PS doses for the same therapeutic effect. This effect did not occur when using a bacteriochlorophyll a analogue. A water-soluble vitamin E analogue, Trolox, has been shown to enhance the PDT

effect. When administered together with the PDT drug it resulted in an increase in the tumor doubling time in a human tumor xenograft model (456). Possibly, this is the result of the Trolox-mediated radical pathway acting in conjunction with the singlet oxygen reactions; Trolox did not prevent photobleaching of the PS.

Immunostimulants have been shown to enhance the PDT effect in animal models (210). Adoptive immunotherapy using a human natural killer cell line (NK92MI) that was genetically altered to produce interleukin-2 has also been successfully applied to several human and murine cancer cell lines (457). BCG immunotherapy was found to be effective in a mouse mammary carcinoma model (257). Another possibility would be to impair the complement system to affect the antitumor response to PDT. Using mouse tumor models it was shown that for example Zymosan, an alternative complement pathway activator reduces the recurrence rate of tumors and increases the permanent cure rate (458). Likewise systemic complement activation with streptokinase and PDT treatment gave better results. Thus, complement activating agents can be used for adjuvant treatment. Another immunostimulant which has shown potential as an adjuvant for PDT is a glycated chitosan, described by Chen *et al.* (459). In a related study the ceramide analog LCL29 was coadministered with Foscan PDT and resulted in enhanced long term tumor cure (460). The effect was highly specific for certain sphingolipids and indicated that the sphingolipid profile distribution can serve as a biomarker for the PDT response.

Clearly the combined use of chemotherapy and PDT would be attractive. However, early studies gave mixed results. *In vitro* tests of the effect of fluoropyrimidines with PDT showed a significant dependence of the outcome on the treatment protocol and the cell lines (MCF-7 and LNCaP) (461). A human case study showed significant side effects and indicated the necessity for further studies (461). A validation of viability assays with two rodent tumor lines showed an increased PDT effect in the presence of the cytostatic drug doxorubicin (462). Similar results were obtained with studies on murine hepatoma (463). Interestingly, preliminary data indicate that the treatment of PDT + doxorubicin was better than first giving doxorubicin and then PDT. Thus, administration of doxorubicin directly after light treatment appears to be best (463). On the other hand a study of murine leukemia treated with a combination of Foscan and either cisplatin or navelbine as chemotherapeutics and administration of immuno lymphocytes from PDT pretreated cells showed a significant synergistic effect (464). A possible means to lower the PDT effects on healthy tissues was reported from a pig stomach study. Standard Foscan PDT gave 71.4 % full-thickness necrosis. However, with octreotide the PDT effect was only 28.5 % (465). Octreotide is a somatostatin analog and decreases the splanchnic blood flow and has multiple inhibitory effects on the peptic system. Thus, it might be possible to down regulate the PDT effect in normal gastric tissue in pigs.

Dual treatment modalities. A combination of PDT with other treatment modalities is indicated for several cancer types and has been discussed above. Typically this involves PDT in conjunction with surgery, chemotherapy or radiotherapy. For example, an early *in vitro* study of breast cancer cells already showed that PDT and radiotherapy effects are additive and independent of each other (234). Another possibility is the use of magnetic nanoparticles. This allows the combination of PDT with magnetic hypothermia therapy (466).

Nonmedical Applications

The physical properties of *m*THPC, although principally investigated for its potential as a PDT photosensitizer, have fortuitously resulted in its use in other applications. For example, *m*THPC has been tested successfully as matrix material for the use in MALDI-TOF mass spectrometry for low molecular weight compounds (47). Potential for application in a nonclinical area exists in the nutrition industry, where sterilization of surfaces is an important subject. The usefulness of non *m*THPC photosensitizers was successfully demonstrated to kill microorganisms fixed on solid surfaces (467). Water sterilization is another potential application for PDT. In the context of the topic of this review one example is the use of immobilized 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin for the disinfection of water (468). This indicates that in practical terms PS which failed in medical terms due to high toxicity may still be useful for technical applications.

RELATED SENSITIZERS AND DEVELOPMENTS

Clearly, *m*THPC has proven to be a successful and interesting photosensitizer to date. Thus, it is none too surprising that the development of new PS is partly resting upon the so far gained knowledge of its properties. In fact, a significant area of photosensitizer development relates to the modification of *m*THPC in an attempt to address some of its shortfalls. This is similar to the situation for any other second generation PS, where continuously advances are made with regard to chemical modification, different formulations or new targeting strategies. Many of these advances are related to new strategies for drug delivery (108). In addition, many PS drugs closely related to *m*THPC are now available. For reasons of space only the developments relating to direct use of the *m*THPC molecule will be described. An analysis of chemically related compounds will be given elsewhere. No real QSAR study involving the various derivatives has been performed for the latter and an assessment of their utility and benefits relies on individual studies and comparisons. Likewise, it is somewhat surprising that the *p*-isomer of THPP still often features in preclinical studies.

PEG Conjugates

General. Efforts to modify the basic *m*THPC properties through chemical manipulations began early in the Foscan "story". In an attempt to improve the localization of *m*THPC in tumor cells and its solubility, it was proposed that the construction of a photosensitizer-polymer conjugate would lead to an increased selective retention of the drug in tumor tissue resulting in an enhancement of selective tumor destruction by light in photodynamic therapy (469). Since then the development and use of *m*THPC-PEG (**7**) has developed almost into a separate research interest (223,251,252,258,282,293,470,471,472,473,474,475,476,477478,479,480,481,482,483,484). In chemical terms pegylation of Foscan is achieved through etherification reactions of the hydroxy groups. Depending on the exact type of side chain attached (mostly its length) or the type of linker group used for attachment different types of *m*THPC-PEG have been prepared. Examples are *m*THPC-MD_{2000} (sometimes listed as SC102), *m*THPC-MD_{5000}, and others. Often the tradename Fospeg® is used as well. Sometimes clinical publications are problematic in that the exact type of "PEG" is not specified.

<Figure 3>

Different materials require different detection methods. For the analytical detection of *m*THPC-PEG, HPLC is not sensitive enough and spectrofluorimetric methods are better suited (470). Mass spectrometric analysis requires HPLC ESI-MS to separate the oligomers for

molecular mass analysis (485). Its absorption spectrum is similar to that of unmodified *m*THPC and the major difference in solution is the absence of dye aggregation effects. Based on the fluorescence quantum yield of chlorophyll a and *m*THPC (0.22), *m*THPC-PEG exhibited a slightly lower quantum yield of 0.19 in aerated ethanol (472).

Cell and animal tests. Fospeg and analogues have tested in various *in vitro* and *in vivo* settings (Tables 2 and 3). Selected *in vitro* tests are compiled in Table 4. Representative cell studies included investigations on malignant mesothelioma (223,258,477,484). Chinese hamster lung fibroblasts (223), human colorectal carcinoma cells (472) and xenografts (469), and murine leukemic cells L1210 (472). Other examples were studies on ovarian cancer cells (476) with very high tumor/tissue ratios (282) and liver tumor models (480), which showed no significant advantage. For example, *m*THPC-MD_{2000} in a rat colon adenocarcinoma resulted in an increase of PS concentration in the liver with time, thus resulting in a loss of tumor selectivity (480). In human prostate cancer cells Fospeg exhibited a lower LD_{50} than Foscan (486). *In vitro* studies related to ophthalmology were reported as well (449).

<Table 4>

Many of the studies made comparisons of the PDT efficacy between free *m*THPC and *m*THPC-PEG. However, which one prevails as the more efficient PDT photosensitizer is dependent upon the site of the tumor, linker groups (251,471,475), and the conditions employed for the study (252). A study with SCC cells yielded similar PDT effects for *m*THPC and *m*THPC-PEG and at lower light intensities *m*THPC was shown to be 20times more effective. However, *m*THPC-PEG showed higher tumor accumulation rates (479). The photodynamic effect of *m*THPC-MD_{2000} is dependent on the light protocol, as was observed for *m*THPC. Similar to the situation for *m*THPC the photodynamic effect of *m*THPC-MD_{2000} is dependent on the light protocol used (153). For example, studies with interthoracic tissue of minipigs showed that while *m*THPC resulted in damage to almost all tissues except nerves at short drug-light intervals, *m*THPC-PEG showed no obvious damage to any tissues at any drug-light intervals (258,472) and was considered a safe drug for further applications in PDT (578). An endobronchial PDT study in minipigs showed that *m*THPC under standard conditions resulted in ulceration and bronchial mucosa necrosis while *m*THPC-PEG gave no such effect (473). Similarly, good results were obtained in testing the feasibility of extrapleural pneumonectomy followed by *m*THPC-MD_{2000} PDT. No adverse side effects were observed (484). Other animal tests included SCC (251,473,477) and adenocarcinoma xenografts (473,477), where *m*THPC-PEG led to larger tumor necrosis than *m*THPC in the former but not in the latter. Rabbits inoculated with cottontail rabbit papilloma virus served as another test case and gave a 58 % cure rate with high drug doses. Skin tolerance was excellent in the rabbit model and in a healthy dog larynx model (487). For human oral SCC xenografts *m*THPC was found to be superior to *m*THPC-PEG (252). The action of *m*THPC-MD_{5000} is delayed compared to *m*THPC, but significantly exceeds that of the latter at day 4 (477).

In human colon carcinoma xenografts the pegylated form gave on average a 2-fold higher tumor uptake than free *m*THPC and, at early times after injection, showed a 2-fold longer blood circulating half-life and a 4-fold lower liver uptake (474). *m*THPC-MD_{5000} was preferentially localized near the tumor vessels while *m*THPC was distributed more diffusely in the tumor tissue. Maximum fluorescence in this system was observed after 24 h with adequate tumor : skin (2.95) and tumor : muscle (6.61) ratios (472). Human malignant mesothelioma implanted in mice showed a similar PDT response for *m*THPC and *m*THPC-MD_{2000} but lower

skin phototoxicity for the latter (488). Other studies focused on side effects (478) or the preparation of (489) and incorporation into nanocapsules (483,490). Alternative developments for drug delivery are liposomal pegylated formulations (Fospeg) that have been used in studies on SCC (293) or *m*THPC incorporated into liposomes (Foslip) (289). All three were compared for their effect to occlude neovascularization and it showed that Fospeg was the superior of the three (289). Multiphoton excitation of pegylated derivatives has been studied (481,482) and the antibacterial action of Fospeg was shown (455). More detailed biochemical studies on the intracellular effects of pegylated compounds are slowly emerging (491).

Related Hydroporphyrins

In line with other PS further reduction of the *m*THPC (a chlorin) will yield the related bacteriochlorin (*m*THPBC) **3** with red-shifted absorption spectrum (735 nm) (29). Its photophysical properties were described by Bonnett *et al.* (50). This bacteriochlorin undergoes photobleaching at about 20times the rate of *m*THPC (492). It also suffers partial phototransformation to *m*THPC in buffer solution (493) and can form other photoproducts as well (72). Initial tests with a mouse colon cancer cell line indicated that *m*THPBC is prone to aggregation and oxidation in aqueous media (494). The cell tests showed a similar uptake profile for *m*THPBC and *m*THPC and identified several "forms" of *m*THPBC in the cells. About 30 % of the bacteriochlorins was oxidized to *m*THPC in the cell within 24 h; after that its oxidation level remained stable. Initial PDT studies showed that *m*THPBC has about 60-70 % of the activity of *m*THPC. Its pharmacokinetics were determined in a rat colon tumor model and showed that the PDT threshold depends mainly on the administered drug dose (492). Necrosis of normal rat liver tissue was enhanced in IPDT compared to Temoporfin or Photofrin (495). Overall, the increased lability of the compound and only minor advantages suggest that this dye might not be worth further development.

Nanomedical Advances

Nowadays "nano" is often considered the savior of everything that is wrong in medicine and science and PDT is no exception. In the early years of PS development this mainly meant the preparation of nanodelivery vehicles, i.e., liposomes. Naturally, there are many different means by which a "nano" approach can be used in PDT. Many reviews on the use of "nano PDT" have been published in recent years and give a good overview of the field (146,147) and about potential carrier systems (496). Others addressed more specific aspects such as quantum dots or nanoparticles (497), liposomal formulations (498), skin permeation (499) or dendritic micelles (500). There are many reasons for using nanosized materials in PDT. Perhaps the most basic is to improve the tumor selectivity through limiting the reticuloendothelial system uptake, i.e. letting "size" dictate the localization of the drug. Longer retention times of the drugs, although counter intuitively with regard to photosensitivity, also allows multiple illuminations and such can overcome the problem of oxygen depletion in the tissue.

Liposomal formulations. Similar to the development of Fospeg® in parallel to Foscan liposomal formulations of *m*THPC (some under the trademark Foslip®) have progressed well. Foslip® is a recently designed third generation photosensitizer based on unilamellar dipalmitoylphosphatidylcholine/dipalmitoylphosphatidylglycerol (DPPC/DPPG) liposomal formulations of *m*THPC. By now many applications of liposomal formulations have been tested and overall indicated reasonable utility. For example, studies with feline SCC indicated

a 2-4times better bioavailability, a shorter distribution half-life, and good selectivity compared to *m*THPC (293). Detailed studies on the exact physicochemical composition of such systems are still rare (501). Fluorescence lifetime measurements indicate that Foslip and Foscan are taken up in a similar manner, but might be located in different intracellular sites (103). Incorporation into liposomes clearly changes the properties of Temoporfin (502). For example, a comparison of three chlorins, including *m*THPC, in solution and incorporated in dioleoyl-sn-phosphatidylcholine (DOPC) liposomes showed that the three PS had a similar propensity to generate singlet oxygen in solution. However, incorporation into liposomes gave a higher efficacy for *m*THPC. This was attributed to the relative distance of the PS to the water-lipid interface (503). The spectral properties significantly depend on the liposomal composition. Fluorescence measurements in DPPC liposomes with different DPPC:*m*THPC ratios demonstrated a dramatic decrease in fluorescence anisotropy with increasing local drug concentration. This indicates significant interactions between the PS molecules in the lipid bulk medium. Illumination with small light doses resulted in a drop in fluorescence which could be restored through addition of Triton-X-100. This photoinduced fluorescence quenching depended on the DPPC/PS ratio. Likewise, addition of plasma protein to liposomal solutions resulted in a slow redistribution of the drug to proteins (504). Thus any Foslip biodistribution analysis must take this effect into account.

Tests with mouse mammary carcinoma showed that Foslip is taken up over the course of about 1 d. Optimum but partial cure rates were observed 24 h post injection. Fluorescence was weak and inhomogenous during the initial times and became maximal at 24 h. This might be an indication that during the initial times the fluorescence is quenched (as the drug is still in the liposomes) and that a slow release to membranes occurs. The total amount of drug present was lower compared to systemic administration, probably due to reabsorption into the blood (505). Biliary cancer cell lines reacted similarly to Foscan and Foslip, both gave excellent phototoxicity (232). Taking the water solubility and thus easier administration of Foslip into account this indicates the utility of the latter. Foslip was first tested in an animal model in 2007. Pharmacokinetic studies with a HT29 mice model showed rapid take up and good selectivity for tumor/muscle, slightly higher than that for Foscan (506). The tumor/muscle selectivity was not time dependent, however, the tumor/internal organ selectivity increased at later times. The plasma concentration was low and did not change much after PS injection. Thus Foslip undergoes a rapid biodistribution and clearance (507). Studies in EMT6 xenografted nude mice indicated the highest tumor to muscle ratios to be reached at 6 and 15 h post-administration. The best tumor response was obtained for a drug-light interval of 6 h. At that time the drug was present in both endothelial and parenchyma cells. Nevertheless, the tumor and plasma concentrations were much lower than the maximal values (508).

A detailed analysis of the preparation of liposomal *m*THPC formulations incorporated into liposomes indicated that Foslip shows promise for the treatment of age-related macular degeneration (289). Foslip has also undergone initial tests for veterinary applications in cat SCC (294). A liposomal preparation of Foscan has also been used for the treatment of cellulitis. It was applied using a mesotherapy gun followed illumination with visible laser light with a wave length of 652 nm and at a light dose of 10 $J.cm^{-2}$. Improvements of the skin thermographic pattern, of the superficial capillary net and of clinical aspects of the skin cutaneous surface were noted. However, the mechanism of action was attributed to a purely thermal effect (509). Another means to increase the uptake of Temoporfin formulations is the use of liposomes containing ethanol. Liposomes containing 20 % ethanol were shown to

exhibit increased skin penetration (510). Another study of liposomal systems was performed by Molinari *et al.* (511). Cationic liposomes with cationic Gemini 1 surfactant were found to transfer *m*THPC more effectively into glioblastoma cells (512), increased its photocytotoxic effect and appeared to work in an early phase of the interaction with cells. For a general review on the current use of Gemini surfactants see Bombelli *et al.* (513).

Topical applications became quickly a focus of attention as well (514). A topical application of liposomal Temoporfin formulations has been tested with nonpigmented skin malignancies in humans, too. It exhibited high tumor selectivity and gave indication of a photometabolization process with a delay time of about 30 min. No pain occurred and no swollen tissue or skin reddening (as often with ALA) was observed (515). Recently attention has focused on liposomal hydrogel formulations. A gel containing 0.75% (w/w) carbomer and lecithin with high phosphatidylcholine content was considered to be the optimal formulation. It delivered high amounts of *m*THPC to the stratum corneum and deeper skin layers, and possessed desirable rheological properties (516). Such hydrogels were shown to exhibit good stability over a period of six months (517).

Another new development is the use of so-called invasosomes (518). These are phospholipid vesicles which contain a mixture of terpenes (e.g. cineole, citral or *d*-limone) as penetration enhancers. A first study for the use of such systems for the topical treatment of HT29 tumors in mice showed a slowed tumor growth in the treated animals (519). Initial tests showed enhanced deposition in human skin with these formulations (520,521). Flexosomes is another name recently coined for a special type of liposomes. It refers to flexible liposomes which are constructed using phosphatidylcholine plus polysorbate 20 (neutral), or dicetyl phosphate (anionic) or stearylamine (cationic) (522). Human abdominal skin was mounted in a Franz diffusion cell as a model for percutaneous penetration and treated with the respective *m*THPC liposomal (or flexosomal) formulation. The anionic flexosomes were found to show insufficient long term stability. Both neutral and cationic were stable over nine months and the cationic flexosomes showed the highest penetration enhancing ability.

mTHPC nanoparticles. Other attempts to prepare *m*THPC containing nanoparticles focused mainly on biodegradable systems (523). A study of *m*THPC encapsulated in poly(D,L lactic acid) or grafted with polyethylene glycol showed a reduced cellular uptake in HT29 tumor cells. However, the PDT effect was affected much less and the localization pattern was different to standard use (490). Other modifications resulted in significant modifications of the biodistribution and tumor retention and reduced liver uptake (483). Similar studies have been performed for related porphyrin systems (524,525). For example, a study of *m*THPP encapsulated into polymeric biodegradable poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles enhanced its photodynamic activity against mammary tumor cells when compared to the free drug (526). A recent study investigated the effect of loading *m*THPP or *m*THPC onto human serum albumin nanoparticles (527). Both the singlet oxygen production and the phototoxicity of the nanoformulation were increased compared to the free drugs (528).

Other possibilities are the use of quantum dots to increasing the PDT efficiency through electron transfer (529). Silica nanoparticles have been employed as well. To highlight a possible way to circumvent its low water solubility and the problems of localization, Yan *et al*. chemisorbed *m*THPC onto nanoparticle platforms of silica as a potential drug delivery technique (530). According to singlet oxygen studies, the $^1O_2$ production of *m*THPC embedded on silica nanoparticles was higher than that of free *m*THPC. But "nano" is not

necessarily better. For example, *m*THPC entrapped in organic-modified silica nanoparticles showed 50 % less cellular uptake than the "free" drug (531). The nanoparticles underwent aggregation under high salt conditions, which could be prevented by BSA. Fluorescence resonance energy transfer (FRET) experiments in esophageal cancer cells showed that the drug is transferred from the NP to serum proteins and is then internalized by the cells as a protein complex.

Micellar systems have been employed as well. For example, the water solubility of the hydrophobic 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin could be increased by a factor of 200 through incorporation into micelles formed from hexyl-substituted polylactides in combination with PEG to give amphiphilic block copolymers PEG-hexPLA (532). Another study investigated the uptake of *m*THPC loaded micelles of mPEG750-b-oligo(ε-caprolactone)$_5$ (mPEG750-b-OCL$_5$) with a hydroxyl, benzoyl or naphthoyl end groups (533). Micelles with benzoyl and naphthoyl end groups had the highest loading capacity. However, while they were taken up no PDT effect was observed in H&N SCC cells. Cellular uptake and photocytotoxicity was observed only in the presence of lipases, which catalyze micelle degradation. This suggests that intact micelles are not taken up by the cells. Micelles formed from poly(2-ethyl-2-oxazoline)-*b*-poly(D,L-lactide) (PEOz-*b*-PLA) diblock copolymer and loaded with Temoporfin gave a similar PDT effect in HT29 mice but resulted in less skin phototoxicity (534). Similarly, *m*THPP filled PEG-PLA micelles were photoactive against H&N cells (535).

An example for a pH sensitive nanosystem was given by Peng *et al.* (536). They prepared Temoporfin loaded poly(ethylene glycol) methacrylate-co-2-(diisopropylamino)ethyl methacrylate (PEGMA-co-DPA) nanoparticles (89% encapsulation efficiency) and showed a faster release at pH 5 in HT29 cells compared to pH 7. Other applications used Temoporfin loaded calcium phosphate nanoparticles for antibacterial studies (537). Another possibility is the use of magnetic nanoparticles. This allows the combination of PDT with magnetic hypothermia therapy. The preparation of a magnetic nanoemulsion of Foscan showed improved skin permeability and longer skin retention times in pig ears compared to standard formulations (466). Gold nanoparticles have been loaded with three layers of *m*THPP indicating the high loading capacity of such systems (538). Nevertheless, the biological utility of such systems remains unclear. Despite the current interest in nanoparticles for PDT drug delivery it is often difficult to predict the drug release from the nanoparticles *in vitro* or *in vivo*. Here, *m*THPC was used to develop a flow cytometry method that allows an investigation of the transfer of dyes from donor particles to acceptor emulsion droplets (539).

CONCLUSIONS AND OUTLOOK

Comparison with Other PS

Surely the questions arises how *m*THPC and its formulations and developments relate to other PS. However, a simple, clear cut answer is not possible. Drug development is driven by individual groups and companies, each of which may focus on different disease types, cell lines, animal models and so forth. Even at the *in vitro* level only few studies compare all the currently used PS (540). One example of such a study was recently presented by Berlanda *et al.* (541). A comparison of the six most widely used PS clearly showed the utility of both Foscan and Fospeg. Both exhibited the lowest LD_{50} values; however the respective IC_{50} values were higher than those of the other PS. Overall, significant differences were found between the various PS. Clearly this indicates the problem involved in choosing the right (or best) PS for a

given clinical application and the need for more comparative analyses at all levels. Another problem is that the patient numbers in clinical trials are still very limited in PDT and that in most cases a clinical trial focuses on one drug and not a comparison of different PS (542). Probably, a critical meta analysis of the various PS is needed from the clinical community. Foscan's simple preparation and purity probably makes it a better choice than the first generation PS Photofrin. Within the range of second generation PS questions such as type of cancer, possibility of topical application, formulations, etc., need to be taken into account.

Future Developments

From a chemical viewpoint, the substitution pattern of hydroxyphenyl groups seems to be important for the exceptional photoactivity, but the molecule has an overall symmetric structure which is to some extent an antagonism to the known potential of amphilicity within photosensitizers. Unsymmetrically substituted drugs are believed to possess enhanced abilities towards the hydrophilic/hydrophobic interfaces of membranes and proteins and thus appear to be of greater photodynamic importance (543,544). Hence, a concept to screen for improved drugs is to synthetically achieve a clearly laid out library of related unsymmetrical tetrapyrroles substituted with the crucial hydroxyphenyl- or related hydrophilic groups *and* various hydrophobic groups, e.g., based on the ABCD-type derivatives, which are now synthetically possible (545,546). Clearly formulation studies will feature prominently in new advances and offer cheaper developmental pathways for the pharmaceutical industry. Thus, it might be worthwhile to study Temoporfin formulations with respect to delivery advances made for other PS. One example is the recent report on the use of micelle encapsulated haematoporphyrin as a pulmonary delivery platform (547).

Different excitation methods can also enhance the PS effectivity. An initial study has shown that it is possible to use two-photon absorption for *m*THPC PDT (548). Here significant potential exists for the construction of appropriately designed "Temoporfin-based" two-photon absorbers (549). Possibilities also exist to improve PDT in general through different light administration (136), e.g., through saturation of the PS triplet state (550). Likewise significant potential for cost savings and safety exist through novel light sources (551). Thus, light emitting diodes have been tested in conjunction with Foscan and showed similar PDT effects in cell tests and Rat liver necrosis (552,553). Optimized analysis for PDT requires multivariant analysis and not only checking on light dose, dark-light interval, and so on (554). Likewise, improvements in *in vivo* quantification are necessary (555). An integrated approach using real-time dosimetry and treatment planning is required and needs to be combined with multimodality imaging and PS development (556).

Many new concepts for PS delivery and action have been developed in recent years. One example is photochemical internalization (PCI) (557,558). This technique utilizes the accumulation of a drug in endocytotic vesicles which, upon light exposure, release the PS into the cytosol in active form. With regards to PS, the best candidates appear to be amphiphilic ones, and most of the initial studies focused on anionic PS. For example, the related *m*THPP did not show any PCI effect (559). Still, appropriate functionalization of the *m*THPC framework is chemically possible and might lead to new candidates. Not everything that is described as a new concept or has been given a catchy new name is really novel. Biological photoinactivation is one of the oldest concepts in photomedicine and thus has led the groundwork for many areas of research that currently attract interest. For example, theranostics has been coined as a term for the combined use of therapy and diagnostics in

personalized medicine (560). Clearly PDT and PDD are classic examples for this, especially when combined with the need for individualized dosimetry.

Many areas of PDT have only just begun to show its potential. For example, immunostimulation offers significant potential but lacks large scale studies (208). For example, it is yet unclear whether a PDT treatment protocol to elicit local tumor control is different from that for immune stimulation. Translation studies in the latter area are clearly needed to test the utility (or lack thereof) of PDT induced anti-tumor immunity. In the context of hydrophobic PS such as Foscan this also requires addressing the intracellular bioavailability of the PS (160). Still, many of the currently investigated "new" applications might look very old and go back to the beginning of photomedicine (561). Due to the high costs of drug development companies and researcher might focus more on infection, sterilization, where regulatory approval might be easier.

Several of these topics listed above are now actively investigated for various third generation photosensitizers (562). The future will show what this ongoing process is able to deliver for the patients. At present, despite the many advances made in research and preclinical studies the translational status of PDT remains disappointing. After 40 years of studies its full clinical potential as an alternative or adjuvant therapy next to chemo- and radiotherapy and surgery has not been realized. In 2006 only six PDT drugs were marketed for various indications, all with clear cost efficiency compared to classical procedures (563). The technique is easily handled, requires only minimum investments and can be performed in simple medical settings allowing use in developing countries. Many general practitioners remain unaware of this technique. Marketing appears to be especially poor and, outside of dedicated PDT centers, this modality often has the status of a niche development in oncology. In a sense its development is reminiscent of the early decades of radiotherapy development (564).

The one drawback of PDT, photosensitivity, is much relieved with newer drugs, yet, more approvals are not forthcoming. There also seems to be an overemphasis on photosensitivity. Compared to other techniques this is a small price to pay for a potential cancer cure. An obvious problem with Foscan is that, while approval was granted in the EU none was given by the FDA for the US which limits further research (565). Large scale follow-up studies remain few and most clinical uses still utilize Photofrin despite some of its drawbacks. To date the field is dominated by very few relatively small pharmaceutical companies and is driven forward mainly by dedicated clinicians and research scientists with limited financial resources. Within their hands lies the potential to use the lessons learned from second generation PS such as *m*THPC and to develop photoactive drugs that will find more general acceptance and use. While the future might not necessarily be in Foscan or its follow ups, the use of PDT and PD in oncology has to be considered as one of the more fundamentally new treatment options in past decades (296,566).

Acknowledgments-- This work was supported by grants from Science Foundation Ireland (SFI Research Professorship 04/RP1/B482, P.I. 09/IN.1/B2650) and the Health Research Board (HRB Translational Research Award 2007 TRA/2007/11).

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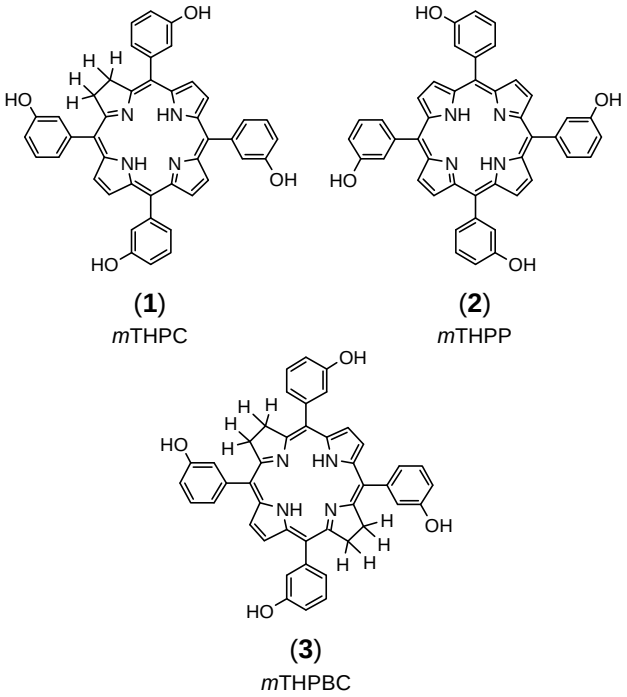
FIGURE CAPTIONS

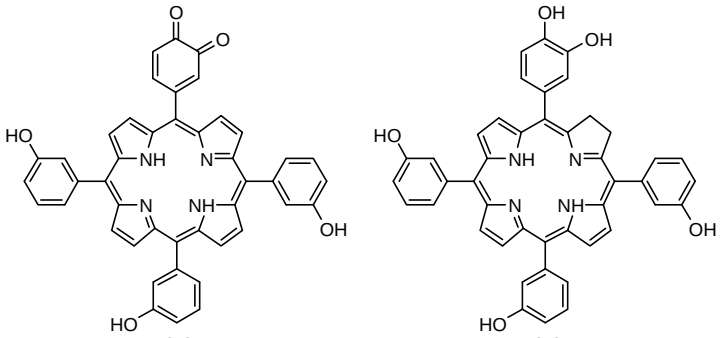
Figure 1. Chemical formulas of compounds of the 5,10,15,20-tetra(*m*-hydroxyphenyl)porphyrin series: **1**, the chlorin, a dihydroporphyrin, *m*THPC; **2**, the parent porphyrin, *m*THPP; **3**, the bacteriochlorin, a tetrahydroporphyrin, *m*THPBC.

Figure 2. Photobleaching products of *m*TPHC derivatives.

Figure 3. Chemical formula of pegylated *m*THPC.

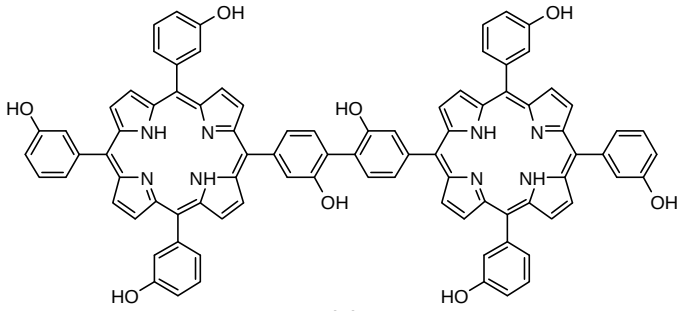


<Figure 1>



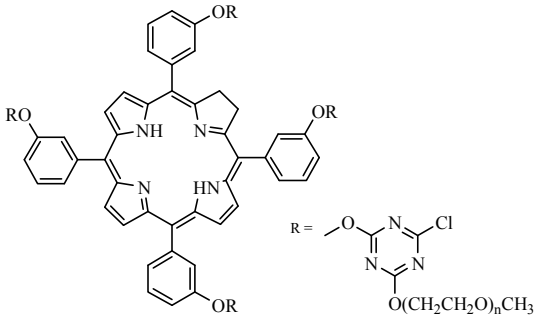
(**4**)

(**5**)



(**6**)

<Figure 2>



(**7**)

<Figure 3>

Table 1. Cell lines used for *in vitro* studies with *m*THPC in solution.

Cell line	Study	Comments	Year	Ref.
Crayfish mechanoreceptor neurons from *Astacus leptodactilus*	Effect on neurons	Irreversible loss of neuron activity at nM concentrations	2004	237
Drosophila melanogaster	Somatic genotoxicity	No effect	1997	196
Epithelial cell line	Two-photon absorption PDT	Possible with *m*THPC	2007	548
Hamster, baby; kidney cells BHK-21	Test of cell viability assays	Doxorubicin increases treatment effect	2004	462
Hamster, Chinese; lung fibroblasts V79	Photobleaching	Comparison with *m*THPP and Photofrin	1994	80
Hamster, Chinese; lung fibroblasts V79	PDT, comparison with *m*THPP and Photofrin	*m*THPC > *m*THPP > Photofrin	1994	113
Hamster, Chinese; lung fibroblasts V79	Phototoxicity and comparison with *m*THPC-MD_{2000}	*m*THPC-MD_{2000} has 100times less dark toxicity and is 10times less cytotoxic	1997	223
Human bile duct cancer, BDC	Comparison Foscan and Foslip	High potency for both, easier application with Foslip	2007	232
Human bile duct cancer, various cell lines	Effect of cell phenotype on *m*THPC PDT	Significant variation between individual cell lines	2010	238
Human breast carcinoma MCF-7	Combined use of PDT and radiotherapy	Effect of both treatments is additive	1995	234
Human breast carcinoma MCF-7	Cytotoxicity using ATP cell viability assay	LD_{50} 4.55 $\mu g.mL^{-1}$, λ = 650 nm, 2.5 $J.cm^{-2}$	1995	217
Human breast carcinoma MCF-7	Phototoxicity and comparison with *m*THPC-MD_{2000}	*m*THPC-MD_{2000} has 100times less dark toxicity and is 10times less cytotoxic	1997	233
Human breast carcinoma MCF-7	Resistance	No induced resistance to chemo- or radiotherapy or subsequent PDT	1998	200
Human breast carcinoma MCF-7	Fractionated light treatment	Fractionated light gives higher phototoxicity	1998	153
Human breast carcinoma MCF-7	Fractionated light treatment	Irradiation with on/off light gives doubling of cytotoxicity	2000	154
Human breast carcinoma MCF-7/DXR	Effect on multi-drug resistant cells	Good effect, more cell killing at same dose in drug resistant strain	2001	229, 230
Human breast carcinoma MCF-7	DNA mismatch repair	Repeated PDT does not result in loss of DNA mismatch repair	2002	201
Human breast carcinoma MCF-7	Localization and primary effect	ER and Golgi	2003 2003	117 118
Human breast carcinoma MCF-7	Effect of 5-fluoro-2'-deoxyuridine		2003	461
Human breast carcinoma MCF-7	Effect of aggregation states	Progressive sensitizer aggregation with increasing incubation time	2007	221
Human breast carcinoma MCF-7	Subcellular localization	Localized in ER and Golgi after 3h. After 24 h mainly damage to ER; activation of the caspase-7 apoptotic pathway	2007	190
Human breast carcinoma T47D	Effect of pH on uptake	No effect	2003 2005	95 567
Human colon adenocarcinoma Colo 201	PDT	LD_{50} 0.45 $\mu g.mL^{-1}$, 20h, λ = quartz lamp, 3 $J.cm^{-2}$, localization in lysosomes	2002	116
Human colon adenocarcinoma CX1	Test of haematogenous dissemination	Minimal tumor cell survival in blood, SF = 0.02 %	1998	226
Human colon adenocarcinoma HT29	PDT and comparison with HpD	*m*THPC: LD_{50} 0.2 $\mu g.mL^{-1}$, 1d, λ = 650 nm, 10 $J.cm^{-2}$; HpD: LD_{50} 4.2 $\mu g.mL^{-1}$	1996	222
Human colon adenocarcinoma HT29	Uptake	Uptake depends on presence of proteins in serum	1998	100
Human colon adenocarcinoma HT29	Photobleaching	Role of aggregation	1998	88
Human colon adenocarcinoma HT29	Localization study	Diffuse localization outside the nucleus	1999	112
Human colon adenocarcinoma HT29	Effect of α-tocopherol	No photoprotection from α-tocopherol, at concentration >0.33 mM enhanced PDT effect	1999	168, 158
Human colon adenocarcinoma HT29	Effect of NaN_3	Strong inhibition of PDT	1999	158
Human colon adenocarcinoma HT29	Effect of fluence rate	Correlation between fluence rate, oxygen depletion and PDT effect	2002	157
Human colon adenocarcinoma HT29	Apoptosis effects	Cyt c release and 12fold increase of caspase-3 activity within 24h	2004	165
Human colon adenocarcinoma HT29	Cell death	Mainly necrosis	2005	167
Human colon adenocarcinoma HT29	Apoptosis	Effect on apoptosis as measured by polyclonal antibodies to active caspase-3, active caspase-7, c-PARP Comparision with KB monolayer cells and HT29 spheroids and HT29 xenografts	2009	185
Human colon adenocarcinoma WiDr	Effect of pH on uptake	No effect	1999	94
Human colon adenocarcinoma WiDr	Effect of pH on uptake	No effect	2005	567

Human epidermoid carcinoma A431	Comparison of various PS	Foscan lowest LD_{50}, Photofrin lowest IC_{50}	2010	541
Human fibroblasts BCT-27	PDT		2004	199
Human gynecological tumors	PDT of *ex vivo* cells	Individual treatment conditions required	1999	233
Human keratinocytes	Photobleaching		2004	86
Human keratinocytes	Photobleaching	More rapid at fixed laser fluence-rate at higher drug concentration	2005	84
Human malignant glioma U87MG	Effect of pH on uptake	No effect	2005	567
Human mesothelioma H-meso-1	PDT		2004	199
Human mesothelioma H-meso-1	Effect of the TAT-$RasGAP_{317-326}$ peptide	Significantly higher apoptosis rate in the presence of TAT-$RasGAP_{317-326}$ at 0.04 μg/ml *m*THPC but not at 1.0 μg/ml *m*THPC	2007	191
Human microvascular endothelial cells hMVEC	PDT		2004	199
Human myeloid leukemia K562	DNA damage and repair	No DNA damage	1997	197
Human oral SCC H376, VB, UP	Invasion promoting tumor factors	Activity of MMP-2, MMP-9, MMP-13, uPA and VEGF downregulated in some cell lines	2006	253
Human ovarian cancer SK-OV3	Comparison with taxol and cisplatin	Effect on cell proliferation: *m*THPC: IC_{50} 0.9 μM, 1d, λ = 650 nm, 15 $J.cm^{-2}$; cisplatin: IC_{50} 4.6 μM; taxol: IC_{50} 78 nM	2000	224
Human prostate cancer LNCaP	Foscan *versus* Fospeg	no dark toxicity with Foscan or Fospeg Fospeg more effective: Fospeg: LD_{50} 0.15 $\mu g.mL^{-1}$, λ = 652 nm, 180$mJ.cm^{-2}$ Foscan: LD_{50} 1.2 $\mu g.mL^{-1}$, λ = 652 nm, 180$mJ.cm^{-2}$	2009	486
Human prostate carcinoma LNCaP	Effect of 5-fluoro-2'-deoxyuridine		2003	461
Human red blood cells	Haemolysis	Phthalocyanine PS > *m*THPC	1998	228
Human SCC A-431	Effect on mitochondria		2009	176
Human SCC CNE2 nasopharyngeal carcinoma	PDT and comparison with MC540		2000	225
Human SCC CNE2 nasopharyngeal carcinoma	PDT and comparison with BpD	Better uptake of *m*THPC and more mitochondrial damage	2000	166
Human SCC HNXOE	PDT		2004	199
Human SCC NPC/HK1 nasopharyngeal carcinoma	PDT and comparison with MC540		2000	225
Human SCC NPC/HK1 nasopharyngeal carcinoma	PDT and comparison with BpD	Better uptake of *m*THPC and more mitochondrial damage	2000	166
Human SCC NPC/HK1 nasopharyngeal carcinoma	Apoptosis	Cells apoptotic after 1 h, significant decline in Bcl-2 expression	2008	227
Human skin fibroblasts	Effect of pretreatment		1997	145
Human skin fibroblasts	Effect of pH on uptake	No effect	2005	567
Human skin fibroblasts GM00316B	Effect of the TAT-$RasGAP_{317-326}$ peptide	None	2007	191
Monocyte cell line U937 differentiated into macrophages	PDT activation of macrophage-like cells	Activation occurs	1999	213
Mouse colon carcinoma Colo26	Test of diode system as light source	Good effect	1996	552
Mouse colon carcinoma Colo26	Photobleaching: effect of fluence rate	Lower fluence rates increase efficacy	2001	218
Mouse embryonic fibroblast	Role of apoptosis-related genes	P53 and ATM play no role in PDT necrosis, but might be required for apoptosis	2003	184
Mouse friend erythroleukemic cells DP16	Photobleaching	Photobleaching as a measure of PDT damage *in vitro*	2002	85
Mouse mammary sarcoma cells EMT6	Localization study using fluorescence anisotropy	Localized in the nuclear envelope	2005	115
Murine fibrosacrcoma L929	Effect of antiapototics	No effect	2001	188
Murine fibrosacrcoma RIF-1	Uptake	Strong retention of *m*THPC compared to other PS	1999	101
Murine fibrosacrcoma RIF-1	Localization/comparison with phthalocyanine PS	Diffuse localization	1999	540
Murine fibrosacrcoma RIF-1	Effect of trypsinization	Depends on type of PS and fluence rate	2001	172
Murine fibrosacrcoma RIF-1, PDT resistant mutant strains	Radiation induced PDT resistant strains	No cross resistance	2001	235
Murine fibrosarcoma FsaR	Effect of poly(adenosine diphosphate-ribose) polymerase	Enzyme is induced by PDT	2003	170
Murine glioblastoma cells C6	Study of DNA repair after PDT	No significant effect on DNA repair within treatment regime	2000	198
Murine hepatoma cells MH22	Post-treatment conditions		1997	180
Murine hepatoma cells MH22	Energy metabolism	More necrosis under low glucose conditions	2003	181
Murine hepatoma cells MH22	Test of cell viability assays	Doxorubicin increases treatment effect	2004	507

Murine hepatoma cells MH22	Effect of doxorubicin	More effective in combination with PDT than either alone	2006	463
Murine leukaemic cells L1210	PDT	LD_{50} 0.88 $\mu g.mL^{-1}$, λ = 514 nm, 25 $J.cm^{-2}$, LD_{50} 6 $\mu g.mL^{-1}$ in darkness	1994	216
Murine leukaemic cells L1210	Uptake of PEG/EtOH formulation	Slow uptake, localization in cytosol; irradiation gave mitochondrial>lysosomal≫membrane photodamage, and a rapid apoptotic response	1999	568
Murine leukaemic cells L1210	Mode of action	Inactivates the antiapototic protein bcl-2	2001	189
Murine leukaemic cells P388	Test of formulations and localization		1999	97
Murine Lewis lung carcinoma, LLC1	PDT	LD_{50} with 60 $mJ.cm^{-2}$ and 400 ng/mL	2010	269
Murine macrophage J774A.1	Time-resolved imaging	Diffuse extranuclear localization	2001	114
Murine macrophage J774A.1	Photobleaching	Inverse dose-rate correlation, complex dependence on deoxygenation	2002	90
Murine melanoma B16A45	Apoptosis	Rate of apoptosis varies with PS and cell type, *m*THPC is a better apoptosis inducer than ALA	2002	182
		Apoptosis inhibitors modify PDT only slightly	2002	183
Murine myeloid leukemia cells M1	Comparison with merocyanine 540	*m*THPC much more potent and selective	1998	186
Murine myeloid leukemia cells M1	Study of aggregation state	*m*THPC localized at the mitochondria and perinuclear region	2000	164
Murine myeloid leukemia cells WHI 3B (JCS)	Comparison with merocyanine 540	*m*THPC much more potent and selective	1998	186
Murine myeloid leukemia cells WHI 3B (JCS)	Study of aggregation state	*m*THPC localized at the mitochondria and perinuclear region, pronounce release of cytochrome c	2000	164
Murine neurons	Effect on neurons	Stability of neurons towards PDT in comparison to satellite glia and MCF-7 cells. MCF-7 significantly more sensitive.	2009	236
Murine skin fibroblasts 3T6	Heat-shock protein expression	No effect of *m*THPC PDT	1997	173
Rat colon adenocarcinoma PRObb	Apoptosis	Rate of apoptosis varies with PS and cell type	2002	182
Rat pheochromocytoma PC 12 cells	Uptake		1997	285
Rat prostate adenocarcinoma MAT-LyLu	Dosimetry		2005	83
Staphylococcus aureus	Test of antibacterial toxicity	Antibacterial toxicity in the dark	1999	413
Streptococcus mutans and *Streptococcus sobrinus*	*m*THPC and hypericin for dental caries study with cariogentic bacteria	*Streptococcus sobrinus:* *m*THPC: LD_{100} 5 $\mu g.mL^{-1}$, λ = 400-505 nm Hypericin: LD_{100} 2.5 $\mu g.mL^{-1}$, λ = 400-505 nm *Streptococcus mutans:* *m*THPC: dark toxicity: 1.25-10 $\mu g.mL^{-1}$; CR required administration of both PS	2009	554

Table 2. Animal models used for *m*THPC and related compounds.

Animal Model	Compound/Study	Comments	Year	Ref.
Arthritis, antigen induced murine arthritis in C57Bl/6 mice	*m*THPC and other forms: rheumatoid arthritis	*m*THPC-PEG showed best accumulation in arthritic joints PDT reduced arthritic score, good results were obtained at lower doses (0.05 $mg.kg^{-1}$) compared to cancer PDT	2008	447
Canine laryngeal edema	*m*THPC: Safety standards		1994	266
Canine, (beagle)	*m*THPC: Prostate damage	Necrosis of the glandular tissue with good healing	1996	287
Canine, prostate	*m*THPC: Test of tolerance		1999	288
Carcinoma chemically induced in hairless mice (SKH-1)	*m*THPC: Tumor/normal skin ratio and comparison with HpD	Best tumor/normal tissue ratio HpD 3.2, *m*THPC 2.7	2000	250
Chick chorioallantoic membrane model	*p*THPP in PGLA nanoparticles	PS remains longer in vascular compartment and vascular effects are enhanced	2004	526
Chick chorioallantoic membrane model	Foscan, Foslip, Fospeg: Occlusion of neovascularization	Pharmacokinetics similar for all. Foslip less photothrombic than Fospeg	2006	289
Chick chorioallantoic membrane model	*m*THPC: Cotreatment with anti vascular endothelial growth factor drugs	Synergistic effect	2007	449
Colon adenocarcinoma induced in rats, Wistar/Furth	*m*THPC: Tissue localization study		1994	268
Colon adenocarcinoma (CC531) induced in rats, Wag/RijA	*m*THPC: Efficacy studies	Tumor : tissue ratio >4	1997	280
Colon adenocarcinoma (CC531) induced in rats, Wag/RijA	*m*THPC: Distribution after i.v. versus i.p.	IPPDT better than i.v.	1997	267
Colon adenocarcinoma (CC531) induced in rats, Wag/RijA	*m*THPC-MD_{2000}: utility for liver tumor	Loss of tumor selectivity with time, no advantage	2000	480
Colon adenocarcinoma (CC531) induced in mice, Swiss CD1 nu/nu	*m*THPC: IOPDT	3times longer recurrence free time in model	2000	270
Cottontail rabbit papillomavirus induced tumors in rabbits	*m*THPC: Nonrandomized control trial 0.3 $mg.kg^{-1}$, 6 d, λ = 652 nm	75 % cure rate of papillomas <100 mm^2	1994	266
Cottontail rabbit papillomavirus induced tumors in rabbits	*m*THPC-MD_{2000}: Pharmacokinetics	30 $mg.kg^{-1}$, 2× 75J.cm^{-2} after 6 and 10d gave 58 % cure rate; tumor/tissue = 4:1,	1999	487
Feline	Biodistribution	Similar to other species	2000 2002	392 391
Feline, H&N SCC	*m*THPC: Vascular effects	Study with power Doppler ultrasonography	2006	254
Feline, H&N SCC	*m*THPC liposomal formulation: Pharmacokinetics 18 cats	CR: 75 % at 1 a. Side effects: mild local toxicity such as erythema and edema in 15 % of the patients. Tumor recurrence rate: 20 % with a median time to recurrence of 172.25 (±87.1) days	2007	294
Feline, SCC, pet cats	*m*THPC liposomes: Pharmacokinetics	*m*THPC-LIP >> *m*THPC, no side effects	2005	293
Fibrosarcoma (FsaR) in female C3H/HeN mice	*m*THPC: effect on poly(adenosine diphosphate–ribose) polymerase	Strong induction of enzyme with *m*THPC and Photofrin	2003	170
Fibrosarcoma ($LSBD_1$) in BIX rats	*m*THPC: Pharmacokinetics	Two different phases of effect at 2 and 24 h	2003	204
H&N SCC (HNX-OE) xenografted in BALB/c mice	*m*THPC: Effect on vascular damage	PDT effect mainly determined by early vascular response	2005	206
Horse, equine sarcoids	*m*THPC: Test of textile light diffuser		2006	295
Human adenocarcinoma xenografted in BALB/c mice	*m*THPC and *m*THPC-PEG: Comparison	Effect similar for both PS	1998	473, 477
Human cervical SCC (SiHa) implanted in mice, NOD-*scid*	*m*THPC: Effect of adoptive immunotherapy	Improvement	2001	457
Human colon adenocarcinoma grafted onto Swiss nude mice	*m*THPC-PEG: Tissue distribution	Maximum tumor fluorescence after 24 h, tumor : skin ratio = 2.95, tumor : muscle ratio = 6.61	1997	472
Human colon adenocarcinoma xenografted in CH3 mice	*m*THPC: Uptake and histology	Different xenografts give different results at same drug/light dose	1998	241
Human colon adenocarcinoma (LS174T) injected in mice, Swiss nude	*m*THPC-MD_{5000}: Biodistribution	Better tumor localization of *m*THPC-MD_{5000} compared to *m*THPC	1998	474
Human colon adenocarcinoma (HT29) xenografted in Swiss mice, nu/nu	*m*THPC: PDT study	Best results 24 h after drug administration	1995	219
Human colon adenocarcinoma (HT29) injected in rats, Swiss, nu/nu	*m*THPC: PDT study	Higher activity with lower irradiance	1998	100
Human colon adenocarcinoma (HT29) xenografted in Swiss mice, nu/nu	*m*THPC: Effect of vitamin E analogue, Trolox	Enhance PDT effect and delayed tumor doubling time	2000	456
Human colon adenocarcinoma (HT29)	*m*THPC: Effect of adoptive	Significant improvement	2001	457

implanted in mice, NOD-*scid*	immunotherapy			
Human colon adenocarcinoma (HT29) injected in ICFW nu/nu mice	*m*THPC PLA nanocarriers:		2002	483
Human colon adenocarcinoma (HT29) xenografted in Swiss mice, nu/nu	*m*THPC: Modality of cell death		2004	165
Human colon adenocarcinoma (HT29) in NMRI nu/nu mice	Foslip:	High concentration in tumor tissue after 4-8 h Average tumor/muscle selectivity = 6.6 Tumor/skin selectivity: 2	2007	507
Human colon adenocarcinoma (HT29) injected in mice, NMRI, nu/nu	*m*THPC invasosomes:	Smaller tumor growth, pilot study	2008	519
Human colon adenocarcinoma (HT29) injected in female BALB/cAnN.Cg-*Foxn1*nu/CrlNarl mice	Micellar formulation of *m*THPC	Similar PDT effect to free drug but less skin phototoxicity	2010	534
Human colon adenocarcinoma (WiDr) injected BALB/c nude mice	*m*THPC: Effect of glucose and T	Cooling to 5 ºC and glucose administration enhance PDT	1999	96
Human hypercalcemic small cell carcinoma of the ovary xenografted in ICR nu/nu:Zur mice	*m*THPC: PDT study	Seven times more necrosis in IPDT treated tumors compared to control tumors	1999	281
Human malignant melanoma in athymic mice	*m*THPP: localization and PDT	Two effects: direct phototoxicity and vascular injury	1990	35
Human malignant mesothelioma implanted in BALB/c nude mice	*m*THPC: Test of drug-light interval	Best results with 3 d interval	1993	148
Human malignant mesothelioma implanted in BALB/c nude mice	*m*THPC: Test of therapeutic index	Best results with up to 5 d interval and increased doses of light	1993	149
Human malignant mesothelioma implanted in BALB/c nude mice	*m*THPC: PDT and comparison with *m*THPC-MD_{5000}	Tumor necrosis: *m*THPC-MD_{5000} >> *m*THPC after 4d	1997	258, 477
Human malignant mesothelioma (H-meso-1) xenografted in CH3 mice	*m*THPC: Uptake and histology	Different xenografts give different results at same drug/light dose	1998	241
Human malignant mesothelioma (H-meso-1) xenografted in nude mice	*m*THPC: PDT	Best response after 24h, strong dependence on drug-light interval but not on fluence rate	2001	260
Human malignant mesothelioma (H-meso-1) xenografted in nude mice	*m*THPC: Uptake and biodistribution		2003	259
Human malignant mesothelioma (H-meso-1) implanted in BALB/c nude mice	*m*THPC: Effect of PDT on hypoxia	Inconclusive results	2003	398
Human malignant mesothelioma (H-meso-1) xenografted in nude c1nu/nu mice	*m*THPC-MD_{2000}: PDT	Same PDT effect as *m*THPC but no skin or muscle damage	2003	484
Human malignant mesothelioma implanted in c1/nu/nu mice	*m*THPC-MD_{2000}:	PDT effect similar to *m*THPC but lower skin phototoxicity	2007	488
Human oral SCC xenografted in CH3 mice	Comparison of *m*THPC-PEG and *m*THPC	*m*THPC >> *m*THPC-PEG	2001	252
Human retinoblastoma, female swiss nu/nu mouse xenografts, RB102-FER, RB109-LAK, RB111-MIL	*m*THPC	Significant PDT effect in one cell line (RB111-MIL)	2010	440
Human SCC (xf-354) xenografted in rag-2 mice	*m*THPC: Uptake and histology	Different xenografts give different results at same drug/light dose	1998	241
Human SCC xenografted in BALB/c mice	*m*THPC; *m*THPC-PEG: comparison	Larger tumor necrosis with *m*THPC-PEG	1998	473, 477
Human SCC (xf-354) xenografted in rag-2 mice	*m*THPC; *m*THPC-PEG; Photofrin: comparison	*m*THPC and Photofrin effective, *m*THPC-PEG only effective with cyanurchloride linker	2001	251
Human tumor LOX in nude mice	*m*THPP: localization		1990 1991	34, 37
Intracranial carcinoma (VX2) implanted in rabbits, New Zealand white	*m*THPC: Effect on apoptosis	Apoptosis detected after 24h	2000	187
Mammary carcinoma (mouse CaD2) implanted in C_3D_2/F_1 mice	*m*THPC: Uptake, localization and PDT	*m*THPC >> *m*THPP	1995	120
Mammary carcinoma in male transgenic mice, strain FVB/NTgN(WapHRAS)69Lln YSJL	*m*THPC-MD_{2000}: Apoptosis effects	Mice express the human RAS gene Cytokeratin 18 cleavage is an early PDT response	2006	491
Mice mammary carcinoma (C3H)	*m*THPP: *o,m,p*-isomers effect on normal muscle	*m*THPP is most selective	1992	32
Mice, BALB/c	*m*THPP: Photobleaching		1996	89
Mice, BALB/c	*m*THPC: Photobleaching		1996	89
Mice, BALB/c	*m*THPC: Pharmacokinetics	neither lipoprotein levels nor cholesterol metabolism affects the pharmacokinetics of *m*THPC in plasma	2007	107

Mice, C3H/Hen Af-nu	*m*THPC: Evaluation of bladder damage	Moderate to severe bladder response with complete healing within a few weeks	1996	274
Minipigs	*m*THPC: Effect on intrathoraic tissue; comparison with *m*THPC-MD_{5000}	Severe damage with *m*THPC, none with *m*THPC-MD_{5000}	1997	258
Minipigs	*m*THPC; *m*THPC-PEG: Endobronchial PDT	Ulceration and necrosis of bronchial mucosa for *m*THPC but not for *m*THPC-PEG	1998	473
Minipigs	*m*THPC-MD_{2000}: Expleural pneumonectomy followed by IOPDT	Feasible method and well tolerated in animals	2003	484
Mouse (Line 1) lung adenocarcinoma in BALB/c mice	*m*THPC: Effect of immunostimulants	Glycated chitosan as immunoadjuvant raises cure rate form 0 → 37%	2005	459
Mouse glioma (VMDk)	*m*THPP	Basic PDT study	1991	36
Mouse Lewis lung carcinoma (LLC) in C57BL/6 mice	*m*THPC: Complement activation	Streptokinase increases PDT efficacy	2004	458
Mouse Lewis lung carcinoma (LLC1) in male C57BL/6 mice	*m*THPC	Initial reduction of tumor, but regrowth after 9d	2010	269
Mouse mammary sarcoma (EMT6) in BALB/c mice	*m*THPC: PDT effect on NaGalase activity	Enzyme level is reduced to background level	1998	169
Mouse mammary sarcoma (EMT6) in BALB/c mice	*m*THPC: Effect of adjuvant mycobacterium cell-wall treatment	Single treatment enhances PDT effect	1998	210
Mouse mammary sarcoma (EMT6) in BALB/c mice	*m*THPC: Effect of adoptive immunotherapy	Improvement	2001	457
Mouse mammary sarcoma (EMT6) in BALB/c mice	*m*THPC: Effect of BCG immunotherapy	Enhances cure rates	2001	257
Mouse mammary sarcoma (EMT6) in BALB/c mice	*m*THPC: Effect on neutrophile response	Pronounced neutrophila after PDT	2001	211
Mouse mammary sarcoma (EMT6) in BALB/c mice transfected with plasmid pR70/GFP	*m*THPC: Effect on heat shock proteins	PDT activated heat shock protein 70 promoter in a model system	2003	175
Mouse mammary sarcoma (EMT6) in BALB/c mice	*m*THPC: Tumor distribution	Temporally and spatially heterogenous	2005	122
Mouse mammary sarcoma (EMT6) in BALB/c mice	*m*THPC: Effect of immunostimulants	Glycated chitosan as immunoadjuvant raises cure rate form 38 → 75%	2005	459
Mouse mammary sarcoma (EMT6) in BALB/c mice	Foslip:	Partial cure 24 h post injection	2008	505
Mouse mammary sarcoma (EMT6) in BALB/c mice	*m*THPC: Dosimetry	PDT dosimetry does not correlate with singlet oxygen production	2008	141
Mouse mammary sarcoma (EMT6) in Foxn1 nu/nu mice	Foslip: pharmacokinetics and PDT effect	Highest tumor/muscle ratios after 6 and 15 h Best tumor response after 6 h when mTHPC was present in both endothelial and parenchyma cells	2009	508
Mouse mammary sarcoma (EMT6) in BALB/c mice	*m*THPC: Apoptosis and drug application	Fractionated double injection of *m*THPC results in 100 % cure	2010	135
Murine colorectal carcinoma (Colo 26) implanted in BALB/c mice	*m*THPC: Tissue localization study		1995	134
Murine colorectal carcinoma (Colo 26) implanted in BALB/c mice	*m*THPC: Distribution and excretion	[^{14}C]-labeled *m*THPC gives an elimination half-life of 10-12d	1996	126
Murine colorectal carcinoma (Colo 26) implanted in BALB/c mice	*m*THPC: PDT study	After 70 d: 25 % tumor recurrence in treated group compared to 80-90 % in control group	1997	45
Murine colorectal carcinoma (Colo 26) implanted in BALB/c mice	*m*THPC: Comparison of tumor and muscle damage	Development of an animal model for testing of PDT effects	1997	272
Murine colorectal carcinoma (Colo 26) implanted in BALB/c mice	*m*THPC-PEGs: PDT	Limited utility	1999	575
Murine colorectal carcinoma (Colo 26) implanted in BALB/c mice	*m*THPBC: PDT	Photodynamic threshold of 0.6 $mg.kg^{-1}$	2000	492
Murine colorectal carcinoma (Colo 26) implanted in Swiss nu/nu mice	*m*THPC: Pharmacokinetics	Accumulation in leukocytes fits PDT efficacy	2004	205
Murine glioblastoma (C6) injected in rats, Sprague-Dawley	*m*THPC: Uptake of ^{14}C-labeled PS	Suggests *m*THPC for PDT and ALA for PDD	1998	283, 420
Murine glioblastoma (C6) injected in rats, Wistar	*m*THPC: intratumoral administration	Intratumoral administration gave similar results to systemic administration. However, optimum concentration is already reached after 4 h	2008	284
Murine hepatoma (MH22A) in BDF_1 hybrid mice	*m*THPC: Effect of coadministration of doxorubicin	Coadministration better effect than either treatment alone	2006	463
Murine leukaemia in mice (L1210)	*m*THPC: + chemotherapy (navelbine or cisplatin) + immunotherapy (immune lymphocytes)	Significant synergistic antitumor effect	2010	464
Murine Rifi tumor induced in C3H/Km mice	*m*THPC: PDT in combination with mitomycin C	Lower light doses can be used for same PDT effect when using *m*THPC together with	1995	202

		mitomycin C		
Murine Rifi tumor induced in C3H/Km mice	*m*THPC; Photofrin: PDT, comparison	Good results with 0.15 $mg.kg^{-1}$, 1 d, λ = 652 nm, much better than Photofrin and more rapid skin clearance	1995	255
Murine Rifi tumor induced in C3H/Km mice	*m*THPC: Influence of light fractionation and fluence rate	Fractionation of light with short dark times can improve PDT	1996	152
Murine Rifi tumor induced in C3H/Km mice	*m*THPC: Uptake and pharmacokinetics	Stronger correlation between plasma level and effect than tumor uptake and effect	1997	256
Murine Rifi tumor induced in C3H/Km mice	*m*THPC: Pharmacokinetics	*m*THPC plasma level correlated with effect	1997	106
Murine Rifi tumor induced in C3H/Km mice	*m*THPC: Effect on hypoxia	PDT results in oxygen depletion and increase in relative hypoxi	2003	398
Murine SCC (SCCVII) in C3H/HeN mice	*m*THPC: Effect of complement activation	Zymosan increases PDT efficacy	2004	458
Murine SCC (SCCVII) in mice, C3H/HeN	*m*THPC: PDT effect on NaGalase activity	Enzyme level is reduced to background level	1998	169
Murine SCC (SCCVII) in mice, C3H/HeN	*m*THPC: Effect on neutrophile response	Pronounced neutrophila after PDT	2001	211
Murine SCC (SCCVII) in mice, C3H/HeN	*m*THPC: Effect on neutrophile response		2002	212
Murine SCC (SCCVII) in mice, C3H/HeN	*m*THPC: Effect of ceramide analogs	Sphingolipids can serve as biomarkers for PDT response	2011	460
Nonmelanoma skin carcinomas in SKH-1 mice	*m*THPC: Thermogel formulation	Good tumor:tissue selectivity	2006	247
Pancreatic carcinoma (PC-1) implanted in Syrian hamster	*m*THPC: PDT study of pancreatic cancer model	Larger volumes of tumor necrosis compared to other PS	1997	275
Papillomas chemically induced in hairless mice (SKH-1)	*m*THPC: Determination of tumor/normal skin ratio and comparison with HpD	Best tumor/normal tissue ratio |HpD 6.2, *m*THPC 5.1	1997	249
Pigs	*m*THPC: PDT effect on normal trachea	Damage confined to mucosa and submucosa, recovery within 14 d with 0.15 $mg.kg^{-1}$, 4 d, λ = 652 nm, <50 $J.cm^{-2}$	1999	262
Pigs	*m*THPC: IPDT normal lung parenchyma	Good potential	2001	263
Pigs	*m*THPC: Effect of octreotide	Suppresses severity of PDT effect. After PDT+octreotide: 28.5 % full-thickness necrosis, PDT alone: 71.4 %	2004	465
Plasma cell tumor (PC6) induced in BALB/c mice	*o,m,p*-THPC isomers and *m*THPBC: Comparison	First *in vivo* tests	1989	29
Plasma cell tumor (PC6) induced in BALB/c mice	*m*THPC: Cost benefit analysis	*m*THPC superior to other PS	1993	215
Rabbit	*m*THPC: Safety standards	Skin study	1994	266
Rabbit, white male New Zealand	*m*THPC: IOPDT effect on blood vessels and nerves	No clinical symptoms	2003	290
Rat colon adenocarcinoma (CC531) induced in WAG/RjjA rats	*m*THPC: Treatment of liver metastases	87 % tumor free after 28 d	1999	271
Rat colon adenocarcinoma (CC531) induced in WAG/RjjA rats	*m*THPC-MD_{2000}: Pharmacokinetics		2000	480
Rat colon adenocarcinoma (CC531) induced in WAG/RjjA rats	*m*THPC: Immunological effects of PDT	no antitumor effect of a systemic immune response	2003	214
Rat epithelial ovarian cancer (NuTu-19) in Fischer 344 rats	*m*THPC-PEG: Pharmacokinetics and selectivity	*m*THPC-PEG selectivity best after 8 d; pelvic debulking model	1999	282
Rat epithelial ovarian cancer (NuTu-19) in Fischer 344 rats	*m*THPC-PEG: IPDT	Suitable for debulking of pelvic cancer	1999	476
Rat fibrosarcoma (MC28) in Lister rats	*m*THPC: Optimization of light conditions	Fractionated or low power light better	2002	156
Rat malignant mesothelioma (IL-45) implanted in Fischer 344 rats	*m*THPC: IOPDT of the chest cavity	Pneumonectomy followed by PDT gave fatal complications	2005	264
Rat malignant mesothelioma (IL-45) implanted in Fischer 344 rats	*m*THPC; Verteporfin: Comparison	Verteporfin > Foscan	2006	265
Rat metastatic mammary carcinoma (DMBA-4) in Wistar-Furth rats	*m*THPC: Effect of immunostimulants	Glycated chitosan as immunoadjuvant raises cure rate form 0 → 9%	2005	459
Rats, WAG/RjjA	*m*THPC: Toxicity study	Similar toxicity profile as Photofrin	1994	150
Rats, WAG/RjjA	*m*THPC: Comparison of red and green light in IPPDT	Intestinal damage is dose limiting for IPPDT with red light	1997	151
Rats, Wistar	*m*THPC: Uptake and localization in adrenal gland	Only steroid synthesizing cells show PS-induced fluorescence	1998	286
Rats, Wistar	*m*THPC: Fine-needle IPD	Lesions heal safely	1999	261

Rats, Wistar	*m*THPBC	Liver necrosis shortly after administration, none after 72h, larger areas of necrosis compared to *m*THPC or Photofrin	2000	492, 495
Rats, Fischer 344	*m*THPC-PEG: PDT normal tissue	No adverse effects within the requires PDT dose	2000	478
Rats, Fischer	*m*THPC: Photobleaching in skin	Photobleaching kinetics different from O_2 changes	2002	91
Rats, Wistar	*m*THPC: Concentration in *ex vivo* samples	Protocol for determination with Solvable as a tissue solubilizer	2008	128
Rats	*m*THPC: *In vivo* quantification	Fluorescence differential path length spectroscopy showed good correlation with chemical extraction	2009	555
Rats, Wistar	*m*THPC: Monitoring institial PDT during treatment	Fluence (rate), fluorescence, hemoglobin oxygen saturation, and blood volume could be monitored during PDT without interruptions to the therapeutic illumination	2009	55
Sheep	*m*THPC: Test of mucosal ablation	Severe complications with green light in esophagus, suggests use of blue light	2003	277
Sheep, Swiss alpine white	*m*THPC: Pharmacokinetics; model for esophageal cancer	Model for precursor lesions of esophagus adenocarcinoma *m*THPC pharmacokinetics similar in humans and sheep	2009	278
SCC induced in Syrian hamster cheek pouches	*m*THPC: Uptake and clearance		1995	569
SCC induced in Syrian hamster cheek pouches	*m*THPC: Effect of fluence rate		1996	155
SCC induced in Syrian hamster cheek pouches	*m*THPC: Tissue localization study	Utilization of *ex vivo* fluorescence spectroscopy for pharmacokinetic study	1996	121
SCC induced in Syrian hamster cheek pouches	*m*THPC: Drug-light interval study	Light treatment 4-8 d after drug administration best	1997	127
SCC induced in Syrian hamster cheek pouches	*m*THPC: Wavelength dependency study	Optimum range is λ = 647.5 – 652.5 nm	1997	243
SCC induced in Syrian hamster cheek pouches	*m*THPC: Determination of threshold for muscle damage during interstitial PDT		1998	244
SCC induced in Syrian hamster cheek pouches	*m*THPC: Tissue localization study and comparison with BpD monoacid	BpD more selective	2000	245
SCC induced in Syrian hamster cheek pouches	*m*THPC: Pharmacokinetics	Limited utility of hamster model for clinical applications	2000	240
SCC induced in Syrian hamster cheek pouches	^{14}C-*m*THPC: Uptake and localization		2002	246
Syrian golden hamster	*m*THPC: Tissue localization study	Normal tissue in the pancreas region tolerate PDT	1996	327, 424

Table 3: Selected clinical studies.

Cancer type	Treatment	Conditions	Sample size[a)]	Result/Tumor response[b)]	Side effects / comments	Year	Ref.
Anal intraepithelial neoplasia, grade III	Fosgel	0.75 mg/kg *m*THPC, 8 h, λ = 652 nm, 50 mW.cm^{-2}, 20 J.cm^{-2}, 2 × treatment after 7 d	9	No response	No effect attributed to limited penetration in AIN III	2009	442
Bile duct: Malignant biliary strictures, irresectable	*m*THPC	0.15 mg/kg *m*THPC, 3 d	13	Median survival time 21 m (10-56) compared to 8 (1-43)	Complications: 2 cholangitis with 1 fatal liver abscess, 2 haemobilia with 1 death from gall bladder empyema	2007	435
Brain: intraoperative fluorescence guided resection, PD and PDT	intraoperative fluorescence guided resection, PD and PDT with *m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm, 20 J.cm^{-2} for superficial irrad., 90-140 J.cm^{-2} for interstitial irrad.	22	Test as diagnostic tool: 88 % sensitivity, 96 % sensitivity	138 tumor samples from 22 patients	2001	340
Brain: malignant brain tumor, intraoperative fluorescence guided resection, PD and PDT	intraoperative fluorescence guided resection, PD and PDT with *m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm, 20 J.cm^{-2}, intraoperative fluorescence λ = 370-440 nm	25	75 % radical resection with fluorescence guidance, 52 % in control Median survival 9 m *versus* 3.5 m in control	Side effects: 2 severe phototoxic reactions due to unintentional exposure to direct sunlight, 1 transitional brain swelling	2006	421
Breast: locoregional breast cancer	*m*THPC	0.10 mg/kg *m*THPC, 2 d, λ = 652 nm, 5 J.cm^{-2} 0.15 mg/kg *m*THPC, 4 d, λ = 652 nm, 10 J.cm^{-2}	3 4	Complete response in all patients with both protocols	Healing times 8-10 w 1 patient delayed healing time and severe pain due to large treatment area	2001	409
Chest: malignant mesothelioma	*m*THPC IOPDT	0.3 mg/kg *m*THPC, 2 d, λ = 650 nm, 10 J.cm^{-2}	4	10 mm deep tumor infarction	Severe chest pain, 1 death from aspiration pneumonia 2 patients had preliminary PDT	1991	392
Chest: malignant mesothelioma	*m*THPC IOPDT Phase I	0.3 mg/kg *m*THPC, 2 d, λ = 650 nm, 10 J.cm^{-2}	8	10 mm deep tumor infarction	PDT after thoracotomy and surgical tumor resection	1996	399
Chest: pleural mesothelioma	*m*THPC IOPDT Phase I	0.1 mg/kg *m*THPC, 4 d, λ = 652 nm, 10 J.cm^{-2}	5	80 % after 9-11 m	Maximal surgical resection followed by IOPDT	1997	401
Chest: pleural mesothelioma	*m*THPC IOPDT Phase I/II	0.075 → 0.15 mg/kg *m*THPC 4-6 d before complete surgical resection, then λ = 652 nm, 10 J.cm^{-2}	28	Median survival time: 10 m for all patients. Local tumor control: 50 % 9 m after IPDT		2001	403
Chest: pleural mesothelioma	*m*THPC IOPDT Phase I	0.1 mg/kg *m*THPC 4-6 d before complete surgical resection, λ = 652 nm, 5/10 J.cm^{-2}	26	Dose-limiting toxicity: 0.15 mg/kg *m*THPC. Three patients died in the perioperative period, one death directly related to PDT.	extrapleural pneumonectomy: 7 patients; lung-sparing pleurectomy-decortication: 19 patients followed by PDT Side effects: systemic capillary leak syndrome leading to death in 2 of 3 patients treated at the highest dose; wound burns and skin photosensitivity	2003	402
Chest: Malignant pleural mesothelioma	*m*THPC Phase I	0.1 mg/kg *m*THPC, 6 d, λ = 652 nm, 10 J.cm^{-2} IOPDT after thoractomy and pleurectomy or extrapleural	9	Variable, main interest was interleukin levels	Dose limiting toxicity: 0.1 mg/kg *m*THPC, 4 d, λ = 652 nm, 10 J.cm^{-2} (death in 2/3 patients through systemic capillary leach syndrome, hypertension, adult respiratory distress	2003	404

		pneumonectomy			syndrome)		
Chondrasarcoma of the hyoid	*m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm, 20 J.cm^{-2}	1	2 PDT treatments within 1 a, good results	Case report	2009	362
GI: aerodigestive tract: early SCC of the upper aerodigestive tract	*m*THPC Phase I/II	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm/514nm	27 4	83 % of early tumors in remission after 15.3 m 25 % complete response for T1 and T2 cancer	Side effects with red light: 1 bronchial stenosis, 1 esophagotracheal fistula, 2 probable occult perforations of the esophagus; none with green light Skin photosensitization in 12 patients for 1 w	1996	384
GI: early esophageal cancer	*m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm/514 nm	35 tumors	77 % disease free 3-38 m	Some side effects with 652 nm light, none with 514 nm light	1996	375
GI: early SCC	*m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm/514 nm, 100-150 mW.cm^{-2}, 7-16 J.cm^{-2}	25	85 % after 14 m	Patients previously treated for H&N cancer SCC from esophagus, bronchi and mouth	1997	377
GI: early esophageal cancer	*m*THPC	n/d	24 (8)	84 % cure rate after 2 a	Comparative study with Photofrin and HpD	1998	378
GI: early esophageal cancer	*m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm/514 nm, plus other variations	64 (59)	76 % CR for all PS	Comparative study with Photofrin and HpD, no significant difference between the PS. Complications: 3 esophagotracheal fistulae, w esophageal stenoses, 3 bronchial stenoses	1999	376
GI: Barrett's esophagus and high grade displasia	*m*THPC	0.15 mg/kg *m*THPC, 4d, λ = 652 nm, 8-20 J.cm^{-2}	7	Elimination of columnar-lined esophageal mucosa, reduction in length of Barrett's segment or downgrading of the dysplasia in all patients Patient with esophageal carcinoma CR at 27 m	Utility of Paterson lamp	2002	386
GI: Barrett's esophagus: early stage	*m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 514 nm (100 mW.cm^{-2}), 75 J.cm^{-2}	12	100 % after 12-68 m	Moderate severity: one stricture	2004	388
GI: Barrett's esophagus: early stage	*m*THPC Phase I	0.15 mg/kg *m*THPC, 3 d, λ = 652 nm/511 nm	19	3/4 4/6	7 Patients with high-grade dysplasia. 12 Patients with early esophageal cancer. Side effects: 1 death and 1 serious complication from taking biopsies too early; 2 esophageal strictures	2005	389
GI: rectal villous adenomas	*m*THPC	0.15 mg/kg *m*THPC, 2/4 d, λ = 650 nm, 10-15 J.cm^{-2}	2	60-80 % reduction in size	Comparison with ALA and Photofrin, photosensitivity for 5 w	1998	570
GI: superficial gastric cancer	*m*THPC Phase I/II	0.075 mg/kg *m*THPC, 4 d, λ = 652 nm, 20 J.cm^{-2}	22	73 % complete remission 12-20 m 80 % for intestinal type cancer 50 % for diffuse Lauren's carcinoma	Side effects: mild to moderate photosensitivity, local pain for 1-2 w.	1998	422, 423
Gynecological malignancies: ovary, recurrent carcinoma	*m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm, 5 J.cm^{-2}	3	No relapse after 2 a	2 patients PDT by laparoscopy, 1 patient additional palliative debulking of metastatic tumors	1997	411
Gynecological malignancies: ovary, terminal cancer	*m*THPC IOPDT Phase I	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm, 5 J.cm^{-2}	8	7/8 patients improved or no effect 6 patients free of relapse	All patients treated with all conventional methods before. 2 patients died due to other complications	1997	412
Gynecological malignancies:	*m*THPC	0.15 mg/kg *m*THPC, 4 d, then λ = 652 nm, 20 J.cm^{-2}	4	Survival: 3-4 m	Various pretreatments, wound healing delayed	1999	414
Gynecological malignancies:	*m*THPC	0.1 mg/kg *m*THPC, 4 d, λ =	6	Complete response after 2 a	Side effects: severe pain in 2 patients; all	2004	415

vulval intraepithelial neoplasia (VIN III)		652 nm, 10 J.cm^{-2}		(after 6 m a 2nd PDT or excision was necessary in 2 patients)	patients developed oedeme, 1 patient developed cellulitis		
Gynecological malignancies: vulval intraepithelial neoplasia (VIN III), severe atypia	*m*THPC	0.05 mg/kg *m*THPC, 1 d, λ = 652 nm, 40 J.cm^{-2}	1	CR after 6 m	Case report	2008	416
H&N: various	*m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm, 20 J.cm^{-2}	15	Good results	Preliminary studies with 1 a follow-up	1995	332
H&N: oral cancer, SCC of the soft palate	Case study	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm, 100 mW.cm^{-2}, 20 J.cm^{-2}	1	Healing complete after 2 m, CR at 16 months	Significant pain and edema. Case study.	1996	323
H&N: oral cancer (some with field change disease)	*m*THPC	0.15 mg/kg *m*THPC, 72-96 h, λ = 652 nm, 250 mW.cm^{-2}, 5-20 J.cm^{-2}	19	100 % single lesions up to stage T3 cleared after one treatment ~60 % patients with field change disease	Tongue tethering in a patient and necrosis in normal areas due to the light scattering	1997	338
H&N: oral cancer	*m*THPC	Study on dosimetry	18	-	*In situ* dosimetry is recommended	1999	331
H&N: SCC and BCC	*m*THPC	0.10/0.15 mg/kg *m*THPC, 4 d, λ = 652 nm, 5-20 J.cm^{-2}	18	92.7 % after 3-24 m (mean 15 m)	Good cosmetic outcome, high patient satisfaction	1999	337
H&N: oral cancer, primary lip cancer	*m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm, 100 mW.cm^{-2}, 20 J.cm^{-2}	25	96 % after 12 w 2 cases of tumor recurrence after 4 and 18 m 1 case lymph node metastasis after 7 m	1 patient with lymph node metastasis, 5 patients with photosensitivity, side effects: swelling and local pain Excellent functional results; cosmetic results better than after surgery.	2001	339
H&N: oral cancer; T1-T2 N0 tumors of the oral cavity and/or oropharynx	*m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm (100 mW.cm^{-2}), 20 J.cm^{-2}	25	86 % after 37 m (mean follow-up) CR for T1 lesions 95 %, for T2 57 %	For the 4 recurrences, salvage was achieved by conventional therapy Moderate pain for a few days	2003	335
H&N: SCC, T1 to T3 stages	*m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm (100mW.cm^{-2}), 20 J.cm^{-2}	21	90 % at 20.3 (mean follow-up)	Heterogenous group with oral cavity, nasal, oropharyngeal, hypopharyngeal and laryngeal malignancy 24 % required surgery for local recurrence or dysplasia after PDT	2003	346
H&N: oral cancer; SCC	*m*THPC Phase IIb	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm (100 mW.cm^{-2}), 20 J.cm^{-2}	114	85 % at year 1 77 % at year 2	Mild-to-moderate skin photosensitivity for 13 % of patients. Survival: 89 % at year 1; 75 % at year 2	2004	336
H&N: "last hope salvage treatment"	*m*THPC IPDT	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm (100mW per fiber), 20 J.cm^{-2}	45	20 % complete response after 1 m +53 % symptomatic relief	One carotoid blow out 2 weeks after PDT 5 patients disease free and alive 10-60 months after. Median survival: 16 m for 33 responders; 2 m for 12 nonresponders	2004	355
H&N: SCC, patients unsuitable for other treatment	*m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm (100 mW.cm^{-2}), 20 J.cm^{-2}	128	38 % overall tumor response 16 % complete tumor response	53 % of patients with significant clinical quality-of-life benefit	2004	343
H&N: oral cancer: nasopharyngal carcinoma	*m*THPC Phase I/II	λ = 652 nm	14	Effect of new light applicator	Large interpatient variations	2006	350
H&N: lymphatic and venous malformations	*m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm, 20 J per fiber	11	Better results with lymphatic malformations	Side effects: pain and swelling	2007	349
H&N: oral cavity and oropharynx neoplasm, SCC	*m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 653 nm (100 mW.cm^{-2}), 20 J.cm^{-2}	24	CR: 50 % after 1 a Partial response: 37.5 %	Mean duration of overall survival: 305.7 ±199.4 d Mean duration of recurrence-free survival:	2008	344

					302.7 ±144.9d for patients with complete remission		
	"	"	35		Mean duration of overall survival: 401.43 ±321.25 d Mean duration of recurrence-free survival: 327.7 ±131.1 d	2009	345
H&N: and limbs, various	*m*THPC Evaluation of ultrasound guided IPDT	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm (100 mW.cm^{-2}), 20 J.cm^{-2}	68	14/17 improved breathing 25/30 improved swallowing 16/22 improved speech 33/40 reduction in disfigurement	IPDT good modality for treatment with deep-seated pathologies	2009	357
H&N: SCC	*m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm (100 mW.cm^{-2}), 20 J.cm^{-2}	39	CR: 54 %	Median survival significantly longer for responders (37 months) than for nonresponders	2010	353
H&N: cystic hygroma	*m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm, 20 J.cm^{-2}	1	5 m old male patient: IPDT, reduction in size of lesion	Case report	2010	360
H&N: oral cavity and oropharynx neoplasms (Tis-T2)	*m*THPC	0.15 mg/kg *m*THPC, 4 d, λ = 652 nm (100mW.cm^{-2}), 20 J.cm^{-2}	170	90.7 overall response rate; CR 70.8 %	Detailed subgroup analysis identified oral tongue and floor of mouth sites as suitable subsites	2011	352
Lung: Recurrent respiratory papillomatosis	*m*THPC Phase I/II	0.15 mg/kg *m*THPC, 6 d, λ = 652 nm, 60→100 J.cm^{-2}	15	33 % in remission after 1 a	Recurrence after 3-5 a Tracheal disease was not responsive to PDT	2005	439
Pancreas: inoperable adenocarcinoma	*m*THPC Phase I	0.15 mg/kg *m*THPC, 3 d	16	Median survival time: 9.5 m 44 % patients alive after 1 a	Abdominal pain after procedure. 3 patients developed duodenal obstruction perhaps related to treatment	2002/ 2003	425 571
Pericarotid malignant disease	*m*THPC	Endoluminal carotid stenting prior to Foscan PDT; 0.15 mg/kg *m*THPC, 4 d, λ = 652 nm, 20 J.cm^{-2}	1	reduces carotid artery ruptures during the treatment of peri-carotid disease	Case report	2010	361
Prostate: recurrent prostate cancer after radiotherapy	*m*THPC Phase I	0.15 mg/kg *m*THPC, 3 d, λ = 652 nm, varying number of fibers	14	36 % no tumor after 2 m Necrosis in 91 % of cross sections	Side effects; stress incontinence (4 pat.), significant impairment of sexual potency No rectal complications	2002	426
Prostate	*m*THPC Phase I	0.15 mg/kg *m*THPC, 2-5 d, λ = 652 nm, 50→100 J per site	6	Prostate specific antigen fell up to 67 % after 8/10 sessions		2006	429
Skin: BCC and Bowen's disease	*m*THPC gel	Topical application, λ = 652 nm (60mW.cm^{-2}), 25-150 J.cm^{-2}, repeated treatments	28	64 % response 32 % pathological clearance		1999	366
Skin: BCC	*m*THPC	0.1 mg/kg *m*THPC, 4 consecutive d, λ = 652 nm (100 mW.cm^{-2}), 5-15 J.cm^{-2}	5	187 tumors: 86 % complete response after 18 m with 10/15 J.cm^{-2} d1 or d2	oedema and erythema around the treatment site more severe on day 1 than after longer intervals. Good cosmetic outcome	2001	367
Skin: BCC	*m*THPC	Applicationo of 5-FU cream (Efudix) for 3 w prior to PDT. 0.15 mg/kg *m*THPC, 3-4 consecutive d, λ = 645-655 nm (100 mW.cm^{-2}), 10-15 J.cm^{-2}	1	Case report: Effect of 5-fluoro-2'-deoxyuridine and PDT	Signficant side effects: oedema, ulceration, redness. Healing delayed by 2 m	2003	461
Skin: BCC	*m*THPC	0.1 mg/kg *m*THPC, 0.5/1/2/3/4 d, λ = 652 nm	13	Best results after 1 d when plasma level is high CR: 75 % (274 of 366 lesions)	Includes 5 patients from ref. 416 Good cosmetic results and tumor response was evaluated at 6 months	2006	368

		(100 mW.cm^{-2}), 10 J.cm^{-2}		after 6 m	and 1 year 2 cases of discolored veins at injection side after w to m		
Skin: BCC	*m*THPC	0.03–0.15 mg/kg *m*THPC, 1–96 h, λ = 652 nm, 20–120 J.cm^{-2}	117	460 BCCs After 8 weeks: Overall CR: 96.7 % CR 100 % with 0.05 mg/kg *m*THPC, 48 h, λ = 652 nm, 50 J.cm^{-2}	Very good cosmetic outcome Side effects: pain and phototoxicity Severe sunburns with scarring in 3 cases	2008	347
Skin: SCC	*m*THPC	0.05 mg/kg *m*THPC, 2 d, λ = 652 nm	1	Optical coherence tomography tumor mapping prior to PDT; CR after 6 m	Case report	2011	369

[a)] Number of protocol compliant patients. [b)] Unless specified otherwise to mean complete tumor response.

Table 4. Selected cell lines used for *in vitro* studies with derivatives and formulations of *m*THPC and related compounds.

Cell line	Study	Comments	Year	Ref.
Canine kidney cell; MDCK II	*m*THPP; bystander effect	Lower bystander effect compared to Photofrin	2001	193
Hamster, Chinese; lung fibroblasts V79	Test of phototoxicity and comparison with *m*THPC-MD_{2000}	*m*THPC-MD_{2000} has 100times less dark toxicity and is 10times less cytotoxic	1997	223
Human bile duct cancer cells; BDC	Comparison Foscan and Foslip	High potency for both, easier application with Foslip	2007	232
Human breast carcinoma; MCF-7	Test of phototoxicity and comparison with *m*THPC-MD_{2000}	*m*THPC-MD_{2000} has 100times less dark toxicity and is 10times less cytotoxic	1997	223
Human cervix carcinoma; NHIK 3025	*m*THPP: effect on mitosis	More effect with $AlPCS_4$	1992	39
Human colon adenocarcinoma; Colo 206	Test of liposomal formulations	Mixing of DMPC and gemini surfactants	2005	501
Human colon adenocarcinoma; HT29	*m*THPC-MD: pharmacokinetics	Max. fluorescence after 5h	1997	472
Human colon adenocarcinoma; HT29	*m*THPC encapsulated in nanocarriers:	Reduced uptake but only small effect on phototoxicity and more localized pattern in cells	2000	490
Human colon adenocarcinoma; HT29	*m*THPC invasosomes	Similar cytotoxicity for free *m*THPC and delivered in invasosomes	2010	521
Human colon adenocarcinoma; HT29	Micellar formulation of *m*THPC	Less phototoxic than free *m*THPC	2010	534
Human colon adenocarcinoma; HT29	pH sensitive PEGMA-co-DPA *m*THPC nanoparticles	Faster release at pH 5	2010	536
Human colon adenocarcinoma; HT29	Calcium phosphate nanoparticles with *m*THPC	No effect	2010	542
Human epidermoid cells, A431	*m*THPC invasosomes	Higher cytotoxicity for *m*THPC delivered in invasosomes compared to free *m*THPC	2010	521
Human epidermoid cells, A431	Comparison of various PS	Foscan and Fospeg lowest LD_{50}, Photofrin lowest IC_{50}	2010	541
Human gall bladder cells; GBC	Comparison Foscan and Foslip	High potency for both, easier application with Foslip	2007	232
Human glioblastoma; DBTRG, LN229, U118	*m*THPC liposomal formulations	Cationic liposomes increase uptake	2007	511, 512
Human glioblastoma; LN229,	*m*THPC liposomal formulation with Gemini surfactants	Stereochemistry of gemini spacer affects drug uptake efficiency	2010	572
Human H&N; SCC 14C	*m*THPC in micelles: photocytotoxicity and uptake	High loading capacity (30 %), uptake and PDT effect only in presence of lipases	2008	533
Human H&N; SCC, HSC-3	mTHPP in PEG-PLA micelles		2010	535
Human HeLa cervix cancer cells	Comparison Foscan and Foslip, uptake	Fluorescence lifetime measurements indicate initial aggregation	2008	103
Human acute T-cell leukemia: Jurkat Jurkat cells (clone E 6-1)	HSA nanoparticles with *m*THPP, *m*THPC	Nanoparticles more phototoxic than free drugs	2011	528
Human esophageal cancer; KYSE 510	*m*THPC entrapped in silica nanoparticles	50 % lower cellular uptake than standard *m*THPC Drug is rapidly transferred from nanoparticles to serum proteins and then internalized by the cells as a protein complex	2009	531
Human prostate cancer; LNCaP	Foscan/Fospeg	no dark toxicity with Foscan or Fospeg Fospeg more effective: Fospeg: LD_{50} 0.15 $\mu g.mL^{-1}$, λ = 652 nm, 180mJ.cm^{-2}	2009	486

		Foscan: LD_{50} 1.2 $\mu g.mL^{-1}$, λ = 652 nm, 180mJ.cm^{-2}		
Macrophage-like; J774 cells	*m*THPC encapsulated in nanocarriers	Reduced uptake but only small effect on phototoxicity and more localized pattern in cells	2000	490
Mouse colon carcinoma; Colo26	*m*THPBC: uptake and stability	Partial oxidation of *m*THPBC to *m*THPC	1997	494
Mouse mammary carcinoma, EMT-6	*m*THPP nanoparticles: uptake and localization	Higher intracellular accumulation of PS with nanoparticles	2003	524, 525
Murine glioblastoma cells; C6	*m*THPC liposomal formulations	Cationic liposomes increase uptake	2007	511
Murine leukaemic cells; L1210	Pharmcokinetics of *m*THPC-MD	Max. fluorescence after 3h	1997	472
Murine leukaemic cells; L1210	*m*THPC liposomal formulation with Gemini surfactants	Stereochemistry of gemini spacer affects drug uptake efficiency	2010	572
Murine macrophages J774.1	Calcium phosphate nanoparticles with *m*THPC	Good PDT effect	2010	452
Rabbit synoviocytes HIG-82	Calcium phosphate nanoparticles with *m*THPC	Good PDT effect	2010	452
Staphylococcus aureus; MRSA 110	Liposomal formulations, antibacterial PDT	Methicillin-resistant *Staphylococcus aureus* bacterial strain (MRSA similar to free drug	2008	451
Staphylococcus aureus	Fospeg, antibacterial PDT	4-5 log reduction in bacterial count	2010	455
Human abdominal skin; mounted in Franz cell	*m*THPC containing flexosomes	Cationic flexosomes give best skin penetration and long-term stability	2010	522
Synoviocytes; HIG-82	*m*THPP in calcium phosphate nanoparticles	Up to 87 % photocytotoxicity	2009	453
Macrophage cell line; J774A.1	*m*THPP in calcium phosphate nanoparticles	Dark toxicity	2009	453
Staphylococcus aureus	*m*THPP in calcium phosphate nanoparticles	Up to 87 % photocytotoxicity	2009	453
Pseudomonas aeruginosa	*m*THPP in calcium phosphate nanoparticles	Good photocytotoxicity only with positively charged nanoparticles	2009	453