

## Special Issue Invited Review

# The Photosensitizer Temoporfin (*m*THPC) – Chemical, Pre-clinical and Clinical Developments in the Last Decade<sup>†‡</sup>

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## ABSTRACT

This review follows the research, development and clinical applications of the photosensitizer 5,10,15,20-tetra(*m*-hydroxyphenyl)chlorin (*m*THPC, temoporfin) in photodynamic (cancer) therapy (PDT) and other medical applications. Temoporfin is the active substance in the medicinal product Foscan® authorized in the EU for the palliative treatment of head and neck cancer. Chemistry, biochemistry and pharmacology, as well as clinical and other applications of temoporfin are addressed, including the extensive work that has been done on formulation development including liposomal formulations. The literature has been covered from 2009 to early 2022, thereby connecting it to the previous extensive review on this photosensitizer published in this journal [Senge, M. O. and J. C. Brandt (2011) *Photochem. Photobiol.* 87, 1240–1296] which followed its way from initial development to approval and clinical application.

## INTRODUCTION

Slowly but surely photodynamic therapy (PDT) is becoming a standard treatment modality in clinical practice. The basic principle of PDT was established over a century ago by O. Raab, H. v. Tappeiner *et al.* (1–3), later H. Kautsky, D. R. Snelling, C. S. Foote *et al.* contributed to the understanding of the underlying mechanism (4). Fundamentally, the light of a suitable wavelength is used to photoexcite a dye molecule (the photosensitizer). After intersystem crossing, a longer-lived triplet state of the dye molecule is formed allowing diffusion-controlled interaction with neighboring molecules. The triplet photosensitizer can then facilitate either an electron transfer

(Type I) or energy transfer to oxygen (Type II) generating reactive oxygen species (5,6). These result in various biological effects, such as destruction of biomolecules, cell death, vascular damage or vascular destruction and promotion of immune responses (7–9). In a clinical setting, the ultimate goal of PDT is to eradicate malignant cells and tissue (10), the promotion of wound healing (11), use in cosmetics and dermatology (12,13) and the treatment or prevention of bacterial and viral infections (14–16). Emerging efforts are also targeted at using photosensitizers in a more ‘materials oriented’ setting, e.g. for degradation of pollutants and remediation (17).

All these interests and efforts have resulted in an almost exponential growth in the available literature. Certainly, more than an individual researcher can follow. If we take a simple look at the occurrence of the term ‘photodynamic therapy’ in Clarivate’s Web of Science database, the first mention in 1972 (18) was followed by 100 publications in 1987, crossed the 1000 papers per year in 2004, and, since 2020, now exceeds 4000 publications per year. In part, this is driven by contemporary prolific publication practices but is also a result of continuous advances (19,20) in developing new clinical photosensitizers (21–23), advances in nanomedicine (24,25), new developments such as sonodynamic therapy and other deep tissue activation methods (26–28), increased awareness of PDT by clinical practitioners, and global issues. Here we note the worsening situation regarding antibacterial resistance (29), and the recent Covid-19 pandemic (30), both with a resultant focus on new antimicrobials. In a sense, the latter brought the PDT community back to its roots; after all, the very first large-scale clinical success of phototherapy by Finsen (31) is probably due to antimicrobial PDT (32).

One way of making sense of the advances in the field is to focus on a specific photosensitizer or ‘success story’ in PDT. Despite all the developments, the number of photosensitizers in clinical practice or trials is still limited (33,34). Historically, haematoporphyrin derivative (Porfimer sodium, Photofrin®), the first clinically approved PDT agent (5),  $\delta$ -aminolevulinic acid (ALA, e.g. Levulan®) as the biosynthetic precursor of protoporphyrin (35), Verteporfin (‘benzoporphyrin derivative’, Visudyne®) and its success in treating age-related macular degeneration (36), and the established photochemotherapeutic

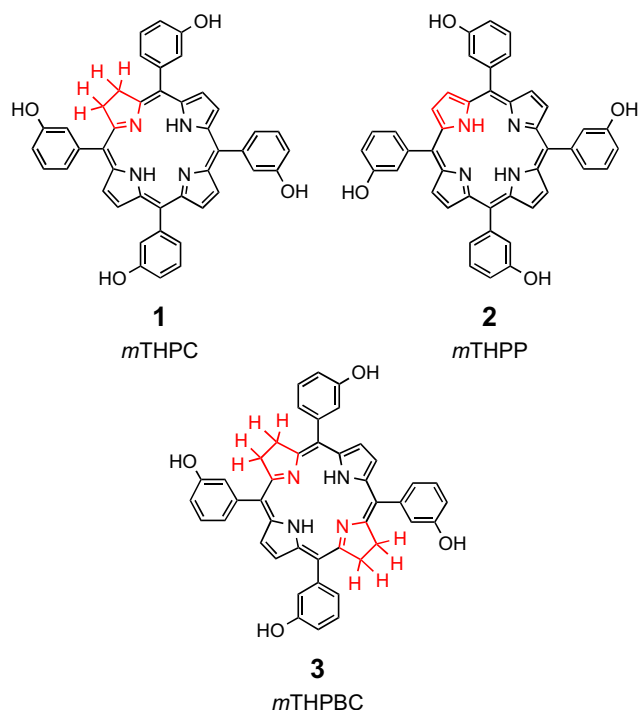
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†This publication is dedicated to Prof. Silvia E. Braslavsky, a pioneer in photobiology and photobiophysics, on the occasion of her 80th birthday.

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

PUVA therapy (37) are noted success stories. In terms of resulting from a more logical drug design development leading to clinical use, temoporfin (Fig. 1, *m*THPC, 5,10,15,20-tetrakis(3-hydroxyphenyl)chlorin, 1) still takes a unique position (38) and thus can serve as an example of the state-of-the-art, ongoing developments and limitations of PDT (39).

Developed by Bonnett almost 40 years ago (40) to create a second-generation photosensitizer, this compound has the advantage of being a well-characterized single molecule. Temoporfin is the active pharmaceutical ingredient in the medicinal product Foscan® which is on the market in the EU for the palliative treatment of head and neck cancer and since then is in continuous clinical use. In the intervening years, it has served as a test bed for many advances in third-generation photosensitizer design. This is reflected in – again using Web of Science (with the key words *m*THPC, *m*-THPC, temoporfin, Foscan) as a benchmark – about 900 publications since the early 1990s (41). The number of annual publications is about 40 per year, a more manageable body of work than of PDT as a whole.

The historical development of temoporfin and the related literature on its uses, developments and clinical applications up to 2010 was covered in a review by Senge and Brandt in this journal (42). In addition, a brief review covering the literature on developments in formulation, chemical modifications and targeting strategies was published at the same time (43). The main purpose of the present treatise is to survey the primary literature since 2010 and close the gap with these earlier reviews. For ease of access, we have maintained the structure and topical breakdown of the earlier paper (42).

## CHEMISTRY

The synthesis (40,44), standard modifications of the macrocycle and the 3-hydroxyphenyl groups, and degradation reactions of temoporfin were reviewed earlier (42,43). Here we focus on recent developments in the chemical modification of *m*THPC, synthesis of related structures, and methodological advances with related porphyrins. In chemical terms *m*THPC 1 is derived by the reduction of *m*THPP 2 and can be further reduced to the bacteriochlorin *m*THPBC 3 (Fig. 1). Overall, its synthesis involves the condensation of pyrrole with (protected) 3-hydroxybenzaldehyde to the respective porphyrinogen, oxidation to *m*THPP and then reduction.

In a new development, the use of MnO<sub>2</sub> under microwave conditions for the oxidation step gave a 2 in 30% yield (45). The same authors also prepared the bacteriochlorin 3 from 1 via Whitlock reduction using microwave conditions. This could then be oxidized with MnO<sub>2</sub> to the chlorin 1 in ~90% yield. This approach uses less solvents and is more facile; however, both the reduction and oxidation steps yielded the respective chlorin and porphyrin as by-products (10%). Mechanochemistry has also been applied to the diimide reduction of porphyrins giving *m*THPC in 55% of *m*THPP (46).

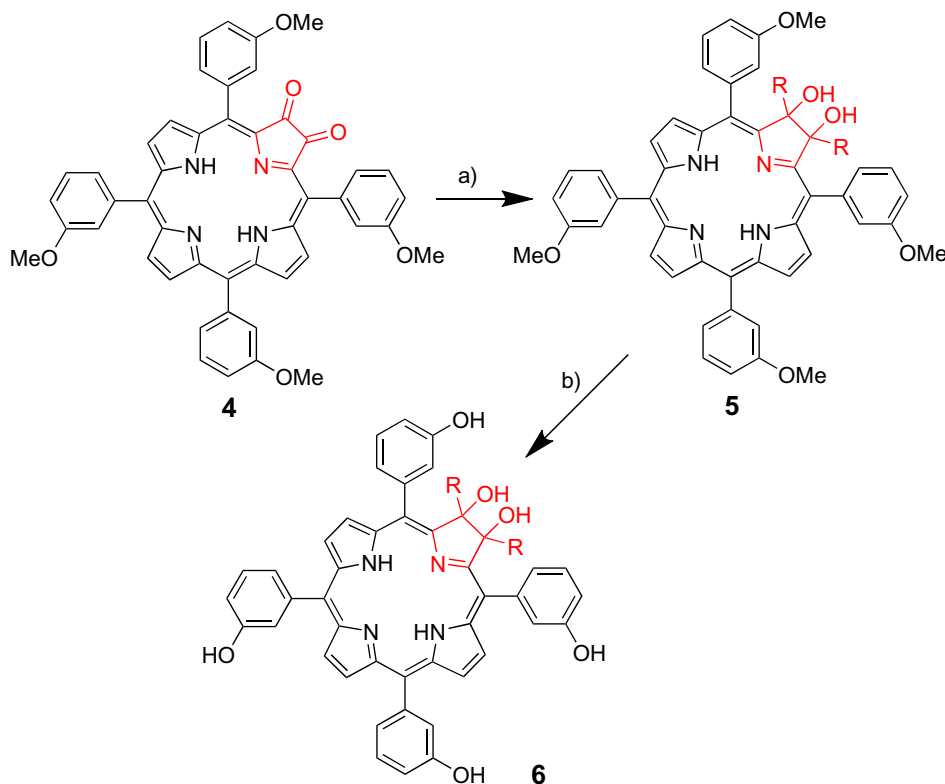
### Chemical modifications of *m*THPP and *m*THPC

Next to the central core for metallation, only the β-pyrrole positions and 3-hydroxyphenyl groups lend themselves to functionalization reactions. With regard to the former, Wiehe and coworkers presented an alternative approach towards 5,10,15,20-tetrakis(3-hydroxyphenyl)chlorins, albeit requiring a total synthesis approach (Fig. 2) (47). Using a method established by Crossley's group (48) they prepared the 3-methoxyphenyl diketone 4. This compound could be disubstituted to the *vic*-dihydroxy chlorin 5 using Grignard reagents. Demethylation with BBr<sub>3</sub> then yielded the respective *meso*-3-hydroxyphenyl chlorin 6 (47). The compounds exhibited absorption and photophysical properties similar to *m*THPC. While the compound with 3,5-di(trifluoromethyl)phenyl residues exhibited a 50% higher singlet oxygen quantum yield compared to temoporfin, its PDT activity against HT29 cells was lower than the other compounds.

Naturally, there is more scope for functionalization of the hydroxyphenyl groups. They can be used to attach targeting groups, link the chromophore to carrier systems, or create covalently linked nanomaterials. However, one has the problem of having four reactive groups in the molecule, which, in the case of monofunctionalization, gives rise to regioisomeric mixtures.

To give only some examples for using standard substitution reactions, Capobianco and coworkers reacted *m*THPC with 4-(bromomethyl)benzoic acid in the presence of NaH to yield a mixture with 1 to 3 linkers units attached to temoporfin, most likely via O-CH<sub>2</sub>-linkages (49). The modified chromophore was then used to functionalize LiYF<sub>4</sub>:Tm<sup>3+</sup>/Yb<sup>3+</sup> upconverting nanoparticles (UCNPs) via reaction with the carboxylic acid groups to yield nanoparticles capable of inducing cell death with 980 nm irradiation. In another study, temoporfin mono-anhydride conjugates, which additionally contained a disulfide linker, were prepared to generate folate-conjugates (50).

In terms of synthetic methods, Rogers *et al.* (51) used Steglich conditions to prepare mono- and tetrafunctionalized



**Figure 2.** Synthesis of *vic*-dihydroxychlorins related to Temoporfin (46). Reagents and conditions: (a) RMgBr or Me<sub>3</sub>SiCF<sub>3</sub>/TBAF, THF, -45°C, 44–53%; (b) BBr<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, -50°C, 57–87%. R = hexyl, 3,5-bis(trifluoromethyl) phenyl, CF<sub>3</sub>.

*m*THPC derivatives. The reaction of *m*THPC **1** with a range of non-steroidal anti-inflammatory drugs (NSAIDs) using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and hydroxybenzotriazole (HOBt) gave the respective ester conjugates as shown in Fig. 3. Depending on the number of equivalents used for the NSAIDs, either the mono- (2–4 equiv.) (**7**) or tetrafunctionalized (**8**) derivatives (10–20 equiv.) were obtained in 36–43% and 61–70% yield, respectively. While the compounds were all taken up by OE33 or SKGT-4 cells, no phototoxicity was observed at short illumination times (2 min). Similar reactions were used to link *m*THPC to nanodiamonds (**52**). Esterifications were also used in the generation of *m*THPC-Au-nanoparticles (AuNPs) (**53**). The reaction of the AuNPs with mercaptopropionic acid then allowed reaction of the carboxylic acid on the NPs with the hydroxy groups of temoporfin.

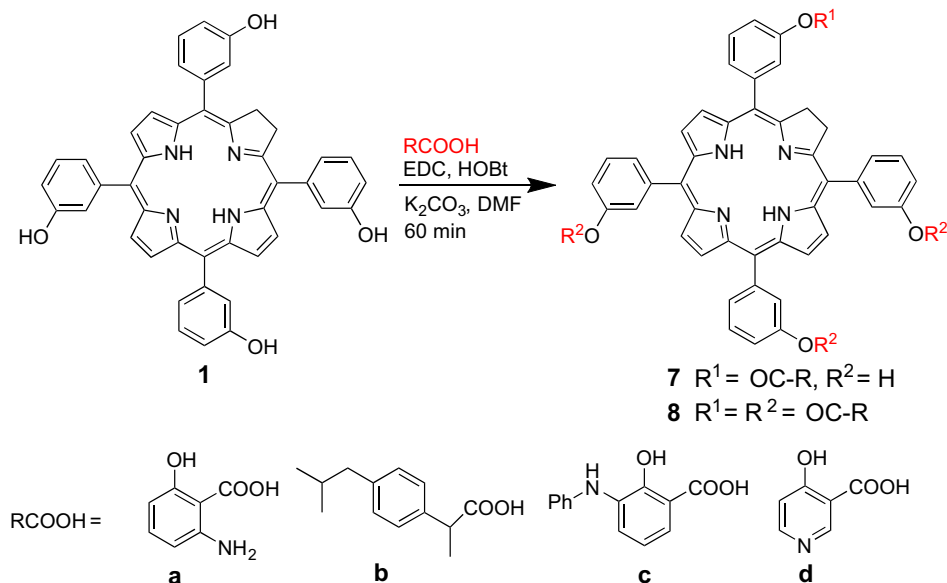
Rogers *et al.* (**54**) also developed methods for the selective mono- and tetrafunctionalization of *m*THPC and *m*THPP. The reaction of **1** or **2** with propargyl bromide in the presence of K<sub>2</sub>CO<sub>3</sub> could be controlled to yield, e.g. **10** in 48% (with 2 equiv.) or **11** in 98% (with 10 equiv.) yield (Fig. 4). Similar reactions of broad substrate scope were implemented for reaction with 1-iodopropane, benzyl chloride, 4-bromobenzyl bromide, 4-nitrobenzyl bromide, 2-bromobenzoic acid and benzoic anhydride. Using NaH as base reaction with 1 equiv. yielded the monosubstituted *m*THPP derivatives in 35–44% yield, while 10 equiv. gave the tetrasubstituted products often in quantitative yield (59–96%). The reaction also allowed the preparation of the respective triflate and tosylate derivatives. Related reactions were used for the preparation of picket-fence and cofacial bisporphyrin derivatives. The Zn(II) complexes of *m*THPP also underwent

Chan-Lam couplings with boronic acids yielding the respective monosubstituted derivatives in 35–39% yield.

Direct substitution of the hydroxyl groups can also be used to generate amphiphilic porphyrins. For example, the reaction of **2** with hexyl bromide gave the monofunctionalized compound in 30% yield. In turn, this could then be reacted with 2,3,4,6-tetra-O-acetyl-1-O-(3-chloropropyl)- $\alpha$ -D-mannopyranoside to yield compound **13** in 33% after deprotection of the carbohydrate units (Fig. 5) (**55**).

The propargylic group, e.g. in **9** or **10** is a useful synthetic handle for Cu(I) mediated 1,3-dipolar cycloaddition ('click') reactions. This allows for the facile preparation of bioconjugate derivatives of photosensitizers. For example, the reaction of the zinc(II) complex of **9** with an azido functionalized bile acid gave the bile acid conjugate **14** in 61% under microwave conditions (**55**). Such compounds were readily taken up in esophageal cancer cells, localized in the ER and Golgi apparatus, but showed no phototoxicity. In related works click reactions between ethynylphenyl residues on porphyrins with azido-functionalized carbohydrates generate amphiphilic photosensitizers (**56**). Similarly, *p*-azidophenylporphyrins can be reacted with propargylic derivatives of carbohydrates to generate water soluble and amphiphilic porphyrins (**57–59**). Click reactions are also a facile method for linking photosensitizers to nanomaterials. One such example was given by Chen *et al.* (**60**) who linked aliphatic azido residues with the hydroxyphenyl groups in *p*THPP and then performed click reactions with alkynyl residues on polyhedral oligomeric silsesquioxanes to yield nanoscale photosensitizing materials.

The propargylic groups in **9–12** can also be used in Pd-catalyzed coupling reactions. For example, a reaction of **12** with



**Figure 3.** Synthesis of NSAID conjugates of temoporfin.

4-iodobenzaldehyde under Sonogashira conditions gave the tetraaldehyde **15** in 45% yield which exhibited an enhanced two-photon absorption cross section compared to temoporfin indicating the possibility of using such derivatives in two-photon induced PDT (61).

An alternative method to achieve monofunctionalization is to use *m*THPC/*m*THPP derivatives carrying one functionality that can be addressed directly (62). This was exemplified by Staegemann *et al.* who prepared *m*THPP analogues, where one meso-hydroxyphenyl residue was replaced by a pentafluorophenyl group. The *p*-position in the latter can easily be substituted under conditions not affecting the hydroxyphenyl groups, e.g. via reaction with amines (63). The use of amines with terminal alkynyl groups then allowed an entry into click-chemistry, which was used to link the chromophore with stimuli-responsive hyperbranched polyglycerols.

### Related porphyrinoids

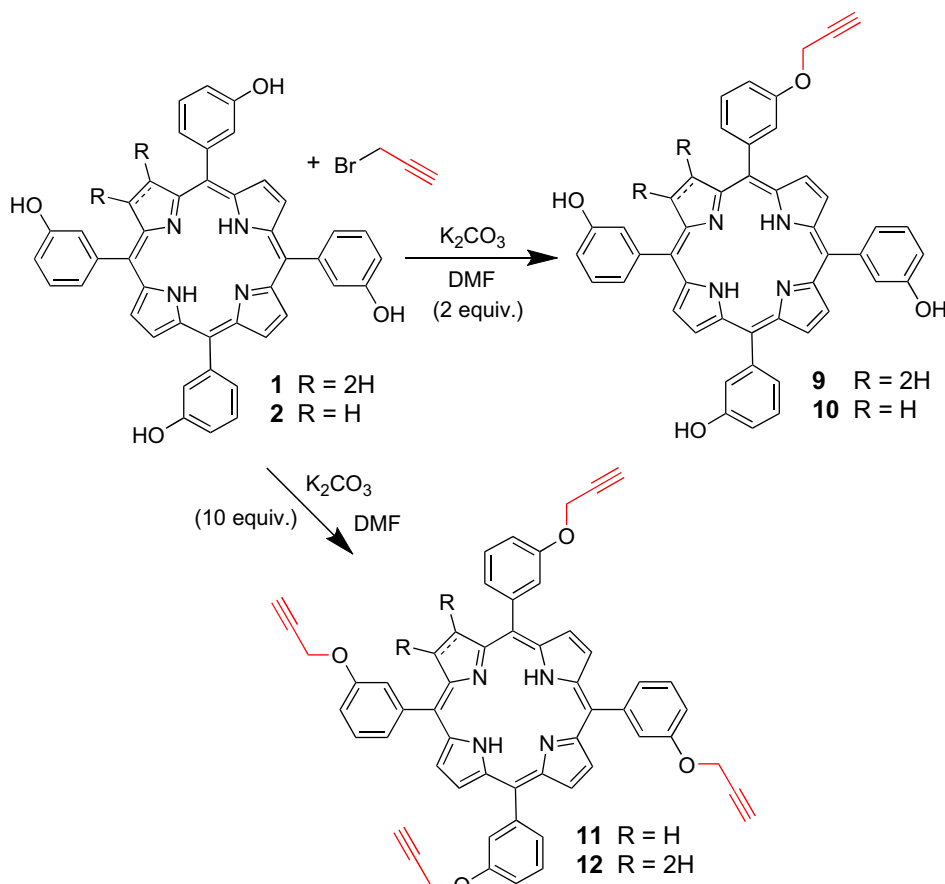
The range of related compounds, *i.e.*, with similar substituent patterns or functional groups, is ever expanding (64). In the following, we highlight selected examples that have been directly compared with temoporfin (see also below) or conceptual approaches which could also be applied to *m*THPP/*m*THPC systems.

An interesting approach, albeit realized only for the 5,10,15,20-tetraphenylporphyrin framework, was reported by Nykong *et al.* (65). Using a standard sequence via Michael addition of dimethyl malonate with 2-nitro-5,10,15,20-tetraphenylporphyrin, they prepared the ‘cyclopropanochlorins’ **16** and **17** (Fig. 6). The compounds showed high photostability and *in vitro* and *in vivo* studies indicate promise for the treatment of cholangiocarcinoma. Another chlorin of interest is disulfonated 5,10,15,20-tetraphenylchlorin **18**. Prepared by Berg *et al.* via diimide reduction of the precursor porphyrin, **18**, as a mixture of three isomers (TPCS<sub>2a</sub>), was used for photochemical

internalization (PCI) (66) of bleomycin (67). *In vivo* studies with a CT26.CL25 subcutaneously growing mouse carcinoma model showed that it effectively facilitated import and activation of the toxin, induced siRNA-based gene silencing, and resulted in tumor growth delay superior to *m*THPC PDT.

At the bacteriochlorin level, we note a broad study by Dabrowski *et al.* on photostable sulfonamides of halogenated bacteriochlorins (68). With either chlorine or fluorine atoms in the *o*-phenyl positions, these compounds (**19**) absorb in the near-IR, and show high cellular uptake, localization in the ER. Together with low cyto- and high phototoxicity these photosensitizers operate both Types I and II photochemical reactions. One of these compounds (Redaporfin, **20**) has progressed to the clinical trial stage, as preclinical data showed it to be a potent photoactivated antineoplastic that also facilitates indirect immune-dependent destruction of malignant tissue (69). Due to the presence of the sulfonamide groups, these compounds are formed as atropisomeric mixtures. Depending on the relative orientation of these groups four different atropisomers are possible ( $\alpha_4$ :  $\uparrow\uparrow\uparrow$ ,  $\alpha_3\beta$ :  $\uparrow\uparrow\downarrow$ ,  $\alpha_2\beta_2$ :  $\uparrow\downarrow\downarrow$ ,  $\alpha\beta\alpha\beta$ :  $\uparrow\downarrow\uparrow\downarrow$ ). Investigation of the individual redaporfin atropisomers showed the  $\alpha_4$  atropisomer, where the sulfonamide substituents are on the same side of the tetrapyrrole macrocycle, to exhibit the highest cellular uptake and phototoxicity as the most amphiphatic rotamer (70). Thus, atropisomerism as a drug design principle must be taken into account for *m*THPC derivatives with at least two *o*-phenyl residues (provided the rotation barrier is high enough).

Going back to the porphyrin level the range of compounds is much wider. An interesting study from 2009 investigated 5,15-diarylporphyrins with only one *m*-hydroxyphenyl group (**21**). The compounds induced apoptosis in HCT111 human colon carcinoma cell, generated ROS and NO<sup>•</sup>, and showed a higher photodynamic effect compared to *m*THPC, most likely due to higher uptake (71). Interestingly, related tetraarylporphyrins and -chlorins with 3,4-dihydroxyphenyl residues – in a sense a composite of *m*THPC and *p*THPC – were reported by Marydasan



**Figure 4.** Synthesis of propargylic derivatives of *m*THPP and *m*THPC.

*et al.* (72). The chlorin **22** was prepared via diimide reduction of the respective dimethoxyphenylporphyrin, followed by demethylation with  $\text{BBr}_3$ . Water solubility of **22** was increased 6-fold compared to *m*THPC, with excellent triplet excited state and singlet oxygen yields. *In vitro* studies with human ovarian cancer cells (SKOV-3) and *in vivo* studies in a mouse model confirmed high photodynamic activity. Di- and trisubstituted phenyl substituents were also employed by Rojkiewicz *et al.* (73). They prepared a range of 5,10,15,20-tetraarylporphyrins of the general type **23** using a combination of pyrrole condensation and hydroxy group substitution reactions to generate amphiphilic systems. All compounds exhibited singlet oxygen quantum yields in the range of 0.6–0.7.

Other compounds of interest are the hydrophobic 5,10,15,20-tetrakis(quinolin-2-yl)porphyrin (**74**), water-soluble phosphorous (V) 5,10,15,20-tetraalkylporphyrins (**75**), water-soluble 5,10,15,20-tetracarboxyporphyrins (**76**) and temocene, the porphyrane analogue of *m*THPP (**77**). The latter absorbs stronger in the red spectrum compared to *m*THPC, is more photostable, has lower dark toxicity, and, depending on the delivery means, localizes in mitochondria or lysosomes (**78**). Its singlet oxygen quantum yield is lower compared to *m*THPC, and the DFT study relates this to the slightly higher spin-orbit coupling matrix elements (SOCME) in the former (**79**).

Computational methods are increasingly used to suggest suitable photosensitizers or to explain features of existing ones. For example, DFT calculations of a range of synthetic, expanded

bacteriochlorins gave higher computed SOCME compared to temoporfin thus suggesting the PDT potential of such compounds (**80**). In one study DFT was used to design new candidate molecules with strong red-shifted absorption bands. The spectra were shown to depend on the substituent pattern and one chlorin with four propenoic acid groups ( $\lambda_{\text{max}} = 755 \text{ nm}$ ) was suggested as a lead compound (**81**), while time-dependent DFT using long-range corrected functionals accurately predicted the long-wavelength absorption of temoporfin (**82**). However, a theoretical quantitative structure property relationships (QSPR) study which aimed to correlate  $\lambda_{\text{max}}$  of the Q band with eight descriptors (*e.g.* aromaticity, electrostatics, reactivity) showed that the wavelength is a multidimensional parameter and cannot be correlated with a single descriptor. This approach gave good agreement between theoretical and experimental data (**83**).

A quantum chemical study investigated the *m,o,p*THPC isomers (**84**). While most features (chemical hardness, ionization potential, triplet energy level, and UV spectra) are similar for the isomers, differences in the dipole moment confirmed the greater lipophilic character of *m*THPC over the *o*- and *p*-isomers.

### Photochemistry

A study by Brault and coworkers used laser flash photolysis to investigate the reaction of the *m*THPC triplet state with antioxidants (**85**). Both the antioxidant Trolox and the anesthetic Propofol reacted with the photosensitizer triplet state in

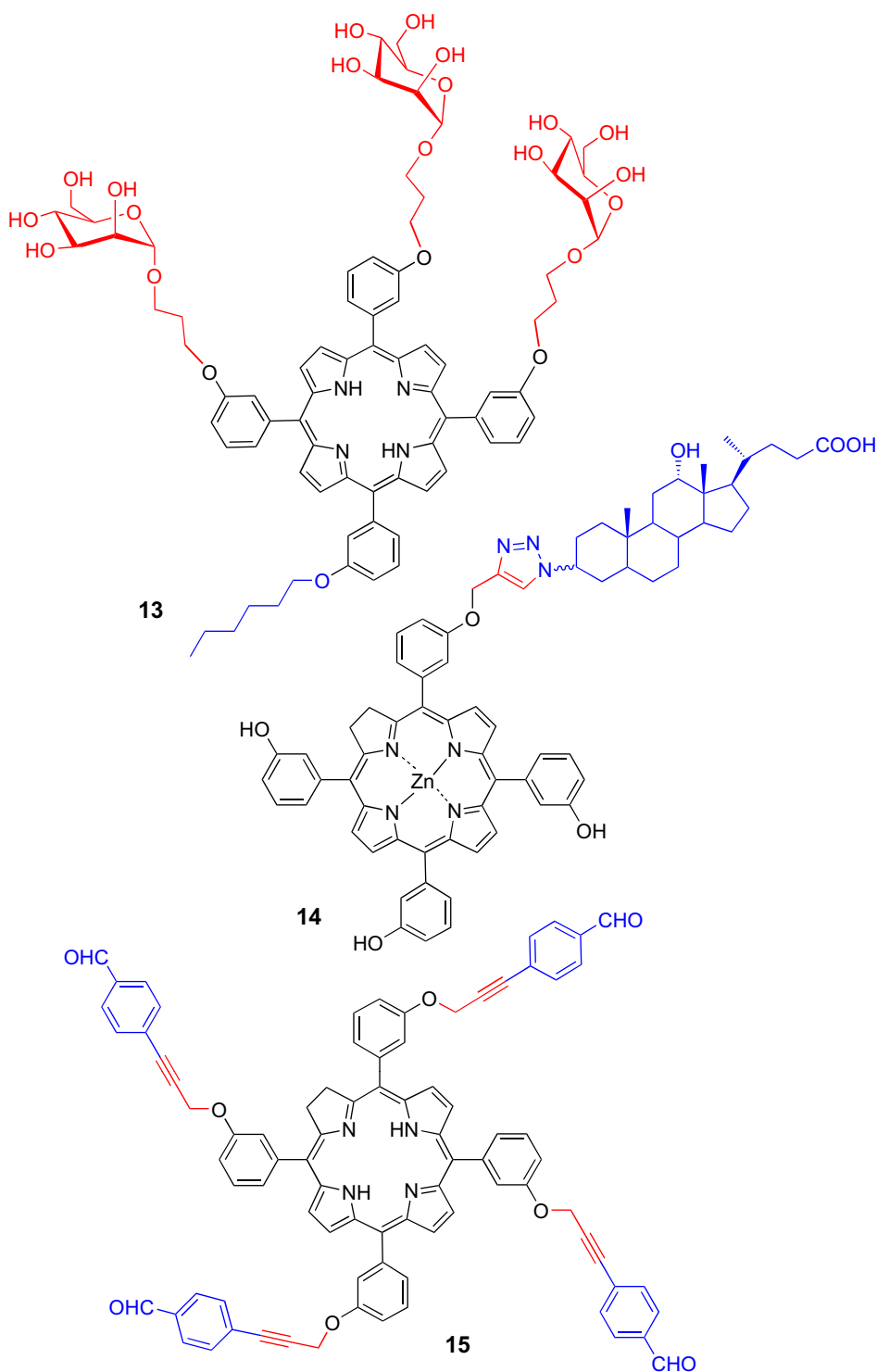


Figure 5. Selected bioconjugates and derivatives of *m*THPC.

solution regenerating the ground state. In the latter case, a unimolecular reaction was observed, while in the former a bimolecular reaction with intermediary detection of a Trolox radical and *m*THPC radical anion was observed. The reaction kinetics with Propofol indicated that quenching of triplet state *m*THPC could occur under clinical conditions when using this anesthetic.

## PHARMACOLOGY AND BIOCHEMISTRY

### *In vitro* tests

The first step in the evaluation of any drug candidate for its medicinal potential is characterization at the *in vitro* level. This serves to establish its uptake mechanism, intracellular localization, (dark)

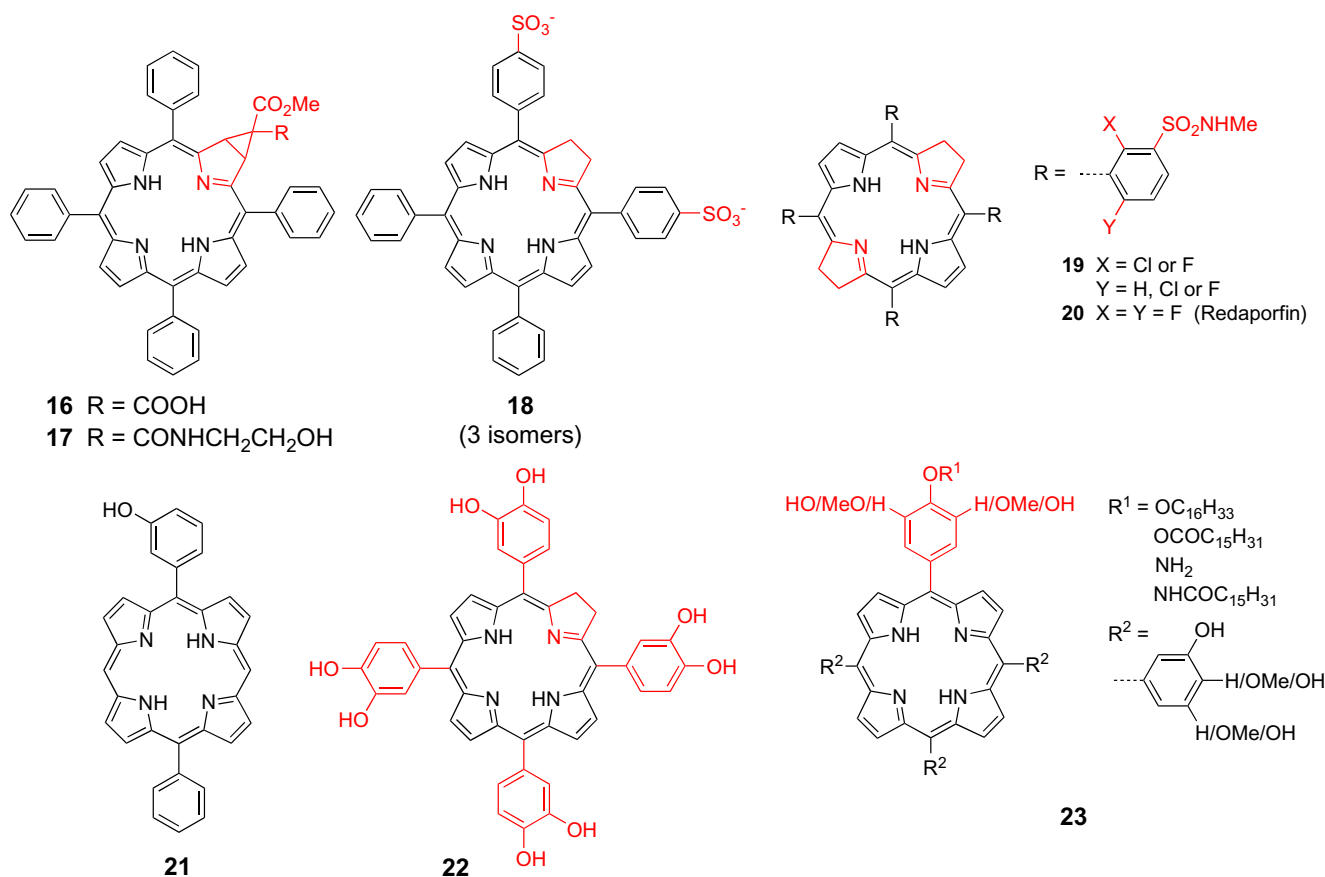


Figure 6. Representative examples of novel photosensitizers.

cytotoxicity, phototoxicity upon illumination, and modes of cell death. The relevant classic studies related to temoporfin have been discussed before (42). Still, the field does not stand still, and the decade of work covered in this review has seen numerous additional *in vitro* studies with temoporfin and its congeners. These were aimed at expanding clinical applications to other types of cancer or diseases, improving PDT protocols, assessing the impact of co-administration of other effectors, evaluating new targeting strategies, validating new formulations, and more.

While facile and cheap, simple 2D *in vitro* cell tests have their pitfalls (86). There may be changes in cell morphology, different interactions of the cellular and extracellular environments, altered polarity or cell division. 2D cell cultures do not reflect the 3D tissue environment, neglect interactions and influence with other cells, cannot mimic influences such as anti-angiogenesis, immune effects, etc. As a result, the predictive power of 2D *in vitro* studies within the translational pipeline, or even for transition to *in vivo* animal models, is very limited (87).

The same issues affect PDT studies, *i.e.* simple 2D systems cannot provide information on the migration and invasiveness of cancer cells (88) or the influence of the tumor vasculature (89). Different Human cancer cell lines can give drastically different responses to PDT (90). Another problem is the adherence of photosensitizers to the culture microplates used in the cell tests (91). Plaetzer and coworkers investigated a range of hydrophilic and hydrophobic photosensitizers with respect to their adherence to the surfaces of 96-well microplates (92). Lipophilic compounds (temoporfin, hypericin, Photofrin®) exhibited strong

adherence to the microplates. Using lysis and fluorescence measurements they showed that 50–90% of the fluorescence signal was caused by PS adherence. The composition of the medium can impact the PDT efficacy as well. Pretreatment of the medium (RPMI 1640 supplemented with fetal calf serum (FCS)) with Rose Bengal or temoporfin (and light) used for the growth of rat glioma cells reduced cell survival by 40% upon irradiation of the cell suspension (93). In the medium pre-treated with Rose Bengal and light the presence of oxidizing species was detected suggesting the formation of such long-lasting oxidizing species, *e.g.* peroxides, as the result of initial ROS formation in the medium (93).

By now 3D cell systems have become the standard of analysis and have been employed in a wider range of PDT studies (see Table 1) (236,237). Amongst others, they have been used to evaluate the use of extracellular vesicles for drug delivery (211,212). They have also been used to evaluate how the stroma-rich microenvironment affects the uptake and activity of lipid-based nanoformulations (219,220). Often such studies show that the PS (as indicated by fluorescence) is confined to the external cell layers of spheroids (205). A quantitative means to determine the penetration in tumor spheroids with the high spatial resolution is the use of laser ablation coupled to inductively coupled plasma mass spectrometry (238). This requires incubation with metal complexes and Pd-tagged *m*THPP was used in this context.

3D cell systems also offer the advantage of using different cell types within one system (236). For example, Philipps *et al.*

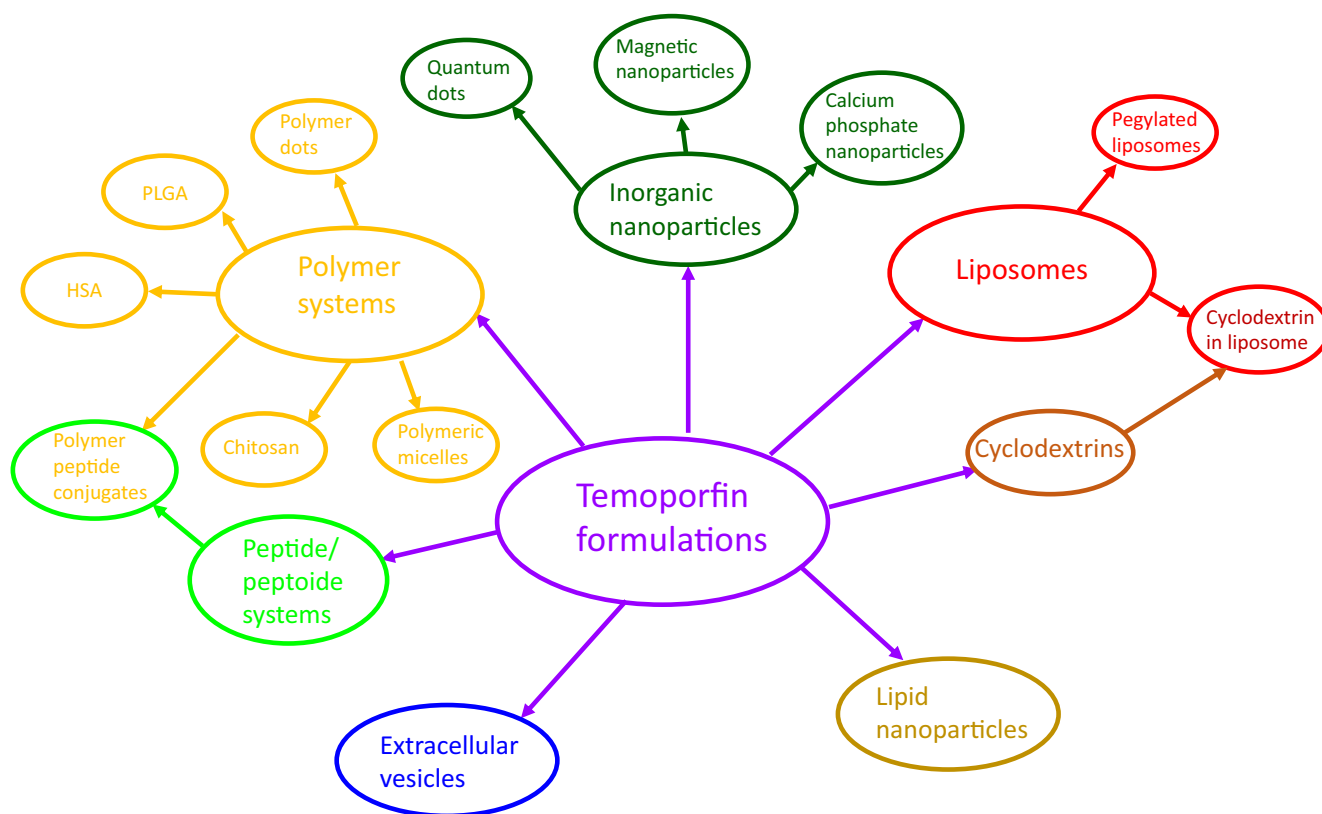


Figure 7. Graphical illustration of pharmaceutical formulation systems explored with temoporfin.

studied the peripheral neural cell sensitivity to temoporfin in a 3-dimensional collagen gel culture system and found MCF-7s and satellite glia to be more sensitive to PDT than neurons (207). A similar system comprised only of the glia cells showed that inhibition of antioxidant pathways increased the sensitivity of neurons to PDT (225).

In tandem with new developments in using 3D cell methods, screening methods are advancing as well. Thus, high content screening and analysis are slowly replacing standard assays (51,58,75,158,239). Such assays require the identification of cellular parameters (e.g. nuclear area, reciprocal form factor, cell area, cell number, PS integrated area, and PS area) which can be used to determine the phototoxic effects of porphyrin compounds. Using esophageal cell lines Vaz *et al.* evaluated parameters such as these in a screen of five photosensitizers, including temoporfin (240). This proof of principle study showed that the HCS assay offers significant advantages and correlated with MTT data.

### Temoporfin as a comparator

As an established clinical photosensitizer temoporfin and its various formulations often serve as a comparator, *i.e.* are used to evaluate the PDT potential of novel compounds and other drug candidates. Without going into the details or analyzing all other photosensitizer classes, compounds from almost all of them have been compared with temoporfin and related formulations (151). Some individual representative examples include BODIPYs (241), bacteriochlorins such as redaporfin (109), unsymmetrically

5,15-disubstituted porphyrins (119,242), picolyl-functionalized porphyrin zinc(II) complexes (243), new chlorin  $e_6$  derivatives (155,213), chlorin  $e_6$  derivative liposomal formulations (134), gallium(III) corroles (125), tetra(3,4-pyrido)porphyrazines embedded in cationic cages (100), and phthalocyanines and tetrapyrzino porphyrazines (244). The most relevant data relating to temoporfin are compiled in Table 1.

Such studies reveal differences in the PDT efficacy and mode of action and cell death between photosensitizers. For example, a comparison with redaporfin showed different susceptibilities of A549 and CT26 cells to the two photosensitizers (Table 1) indicating that e.g. A549 cells are more susceptible to PDT operating via Type I. Using redaporfin in combination with ascorbate and inhibition of antioxidant enzymes significantly improved its effect against such cells (109).

### Mechanistic aspects

The basic mechanism of action of photosensitizers is quite well understood. It relies on the formation of reactive oxygen species (ROS) generated by Type I or Type II photoreactions. In the case of temoporfin, the dominant mechanism is the Type II reaction leading to the formation of singlet oxygen (42,109,245). This singlet oxygen reacts, depending on the photosensitizer localization in the cell, with surrounding biomolecules, thereby damaging the cells and provoking cell death *via* apoptosis, autophagy or necrosis, though other cell death pathways like paraptosis also play a role (110,246–250). These direct effects on the tumor cells are important for PDT, however, they cannot



Table 1. Cell lines used for selected *in vitro* studies with *m*THPC or derivatives thereof in solution.

| Cell line   | Compound/Formulation  | Study  | Comments/Phototoxicity   | Year | References |
|---|---|--|--|------|------------|
| African green monkey kidney cells CCL-81                    | Styrene maleic acid copolymer (SMA) micelle encapsulating <i>m</i> THPC; SMA@ <i>m</i> THPC | PDT study  | Phototoxicity: IC <sub>50</sub> SMA@ <i>m</i> THPC = 0.15 µg mL <sup>-1</sup> ; dark toxicity: IC <sub>50</sub> SMA@ <i>m</i> THPC = 20 µg mL <sup>-1</sup>  | 2021 | (94)       |
| Head and neck squamous cell carcinoma SCC19                 | <i>m</i> THPC   | Cell death mechanism   | Enhanced apoptosis with fenretinide via de novo sphingolipid biosynthesis pathway  | 2016 | (95)       |
| Head and neck squamous cell carcinoma SCC19                 | <i>m</i> THPC (+C6-pyridinium ceramide or Fenretinide)                                      | Combination with anti-cancer agents C6-pyridinium ceramide/Fenretinide | Fumonisin B1 inhibits apoptosis after PDT  | 2017 | (96)       |
| Head and neck squamous cell carcinoma UMB-SCC 745 (and 969) | Foslipos  | Cell death mechanism; combined used with hypericin                     | Necrosis; upregulation of heat shock proteins  | 2013 | (97)       |
| Head and neck squamous cell carcinoma UMB-SCC 745 (and 969) | Foslipos+hypericin (1:1)  | Dark toxicity  | Combination has no dark toxicity, unlike individual PS   | 2012 | (98)       |
| HeLa  | <i>m</i> THPC   | Comparison with temocene   | Temocene is more stable, has lower dark toxicity, but is less active than <i>m</i> THPC  | 2011 | (77)       |
| HeLa  | <i>m</i> THPC   | Comparison with 5,10,15,20-tetracarboxyphorphyrin                      | IC <sub>50</sub> = 1.6 µM  | 2019 | (76)       |
| HeLa  | <i>m</i> THPC SOF1  | Temporin supramolecular framework (SOF) PDT [                          | IC <sub>50</sub> : 54.2 µg L <sup>-1</sup>   | 2022 | (99)       |
|   | <i>m</i> THPC SOF2  |  | IC <sub>50</sub> : 13.7 µg L <sup>-1</sup>   |      |            |
|   | <i>m</i> THPC SOF3  |  | IC <sub>50</sub> : 24.2 µg L <sup>-1</sup>   |      |            |
| HeLa  | <i>m</i> THPC   | PDT comparison with tetra(3,4-pyrido)porphyrines                       | IC <sub>50</sub> : 23.0 µg L <sup>-1</sup>   | 2016 | (100)      |
| HeLa  | <i>m</i> THPC-S-S-PEG-Folate conjugate  | Development of releasable conjugate systems                            | EC <sub>50</sub> = 0.045 µM (λ > 570 nm, 12.4 mW cm <sup>-2</sup> , 15 min, 11.2 J cm <sup>-2</sup> ); > comparator  | 2019 | (50)       |
| HeLa  | <i>m</i> THPC-PEG-Folate conjugate  |  | Redox-responsive folate targeting PS <i>m</i> THPC-S-S-PEG-Folate showed slightly higher phototoxicity than <i>m</i> THPC-PEG-Folate   |      |            |
| HeLa  | <i>m</i> THPC loaded folate-PEOz-PLA micelles   | PDT study  |  | 2012 | (94)       |
| HeLa  | <i>m</i> THPC loaded P(CL <sub>x</sub> -TMC-Bz <sub>y</sub> )-PEG micelles                  | PDT study  | Phototoxicity: EC <sub>50</sub> = 5–13 µg mL <sup>-1</sup> ; free <i>m</i> THPC = ~1.5 µg mL <sup>-1</sup>   | 2020 | (101)      |
| HeLa  | <i>m</i> THPC loaded PCLn-PEG micelles decorated with EGFR targeting nanobody (EGa1)        | EGFR targeting PS  | EGa1-conjugated micelles are internalized upon specific binding of the NP with the EGFR receptor overexpressed on the surfaces of A431 cells, resulting in enhanced cellular uptake and phototoxicity on A431 cells, as compared to EGFR low-expressing HeLa cells | 2020 | (102)      |
| HeLa  | <i>m</i> THPP loaded P(Glu-b-NADA), amphiphilic block-co-polypeptides                       | amphiphilic co-polypeptides as drug delivery systems                   | No dark toxicity, <i>m</i> THPP release in controlled manner   | 2012 | (103)      |
| HeLa  | <i>m</i> THPC + iron oxide magnetic NP + doxorubicin in liposomes                           | PDT/photothermal study   | Almost complete elimination of cancer cells; effect of magnetic hyperthermia = PDT > chemotherapy  | 2016 | (104)      |
| HeLa  | <i>m</i> THPC covalently linked to LiYF4; Tm3+/Yb3+ upconverting nanoparticles (UCNPs)      | PDT study  | 70% cell death under 980 nm illumination   | 2014 | (49)       |
| HeLa  | <i>m</i> THPC   | Test of LEDs as light source   | Improved efficacy with sequential administration   | 2016 | (105)      |
| Hep-2   | <i>m</i> THPC + cisplatin   | Synergistic effect of Pt(II) complexes                                 |  | 2019 | (106)      |
| Human acute myeloid leukemia AML                            | <i>m</i> THPC   | <i>in vitro</i> O <sub>2</sub> luminescence measurements               |  | 2011 | (107,108)  |
|   |   |  |  | 2012 |            |

(continues)

Table 1. (continued)

| Cell line   | Compound/Formulation   | Study  | Comments/Phototoxicity   | Year | References |
|---|--|--|--|------|------------|
| Human adenocarcinoma SKGT-4                               | <i>m</i> THPC derivative <b>13</b>   | Impact of bile acid conjugates                       | Good uptake in cells but no PDT effect   | 2013 | (55)       |
| Human adenocarcinoma SKGT-4                               | <i>m</i> THPC  | Comparison with P(V) 5,10,15,20-tetraalkylporphyrins | High content analysis, <i>m</i> THPC IC <sub>50</sub> = 5 μM   | 2014 | (75)       |
| Human adenocarcinoma SKGT-4                               | <i>m</i> THPC-NSAID conjugates   | PDT study  | Good uptake but no phototoxicity at 2 min illumination   | 2015 | (51)       |
| Human alveolar basal epithelial adenocarcinoma cells A549 | <i>m</i> THPC  | Comparison with 5,10,15,20-tetracarboxyporphyrin     | IC <sub>50</sub> = 0.5 μM  | 2019 | (76)       |
| Human alveolar basal epithelial adenocarcinoma cells A549 | <i>m</i> THPC  | Comparison with redaporfin                           | LLD <sub>50</sub> = 20 mJ cm <sup>-2</sup> at 0.5 μM ( <i>m</i> THPC)  | 2016 | (109)      |
| Human alveolar basal epithelial adenocarcinoma cells A549 | <i>m</i> THPC  | Cell death mechanism                                 | LLD <sub>50</sub> = 250 mJ cm <sup>-2</sup> at 5 μM (redaporfin)   | 2021 | (110)      |
| Human alveolar basal epithelial adenocarcinoma cells A549 | <i>m</i> THPC  | Temporin supramolecular framework (SOF) PDT [        | <i>m</i> THPC initiates paraptosis after ER photodamage  | 2022 | (99)       |
| Human alveolar basal epithelial adenocarcinoma cells A549 | <i>m</i> THPC SOF1   |  | IC <sub>50</sub> : 45.9 μg L <sup>-1</sup>   |      |            |
|   | <i>m</i> THPC SOF2   |  | IC <sub>50</sub> : 26.7 μg L <sup>-1</sup>   |      |            |
|   | <i>m</i> THPC SOF3   |  | IC <sub>50</sub> : 34.6 μg L <sup>-1</sup>   |      |            |
|   | <i>m</i> THPC-loaded PLGA nanoparticles  |  | IC <sub>50</sub> : 17.5 μg L <sup>-1</sup>   |      |            |
| Human alveolar basal epithelial adenocarcinoma cells A549 | <i>m</i> THPC in micelles + Remilla luciferase-immobilized quantum dots-655 (QD-RLuc8) | Nanoparticles versus free PS                         | No dark toxicity of temoporfin in nanoparticles; reduced cellular uptake of NP, but similar phototoxicity  | 2012 | (111)      |
| Human alveolar basal epithelial adenocarcinoma cells A549 | Fospeg®  | Bioluminescence activated PDT                        | Bioluminescent QD-RLuc8 conjugate exhibits self-illumination at 655 nm after coelenterazine addition which allows activation of the PS               | 2013 | (112)      |
| Human alveolar basal epithelial adenocarcinoma cells A549 | <i>m</i> THPC in folate-targeted liposomes   | PDT study  | Less dark toxicity of Fospeg compared to <i>m</i> THPC; internalization via endocytosis and localization in Golgi; 30–40% lower efficiency of uptake | 2011 | (113)      |
| Human alveolar basal epithelial adenocarcinoma cells A549 | <i>m</i> THPC  | Folate targeting                                     | Similar degree of internalization and phototoxicity for folate targeted and non-targeted liposomes   | 2013 | (114)      |
| Human alveolar basal epithelial adenocarcinoma cells A549 | <i>m</i> THPC derivative <b>13</b>   | Evaluation of Raman spectroscopy for diagnostics     | No interference of irradiation with 785 nm   | 2021 | (115)      |
| Human Barrett's esophagus adenocarcinoma OE33             | <i>m</i> THPC  | Impact of bile acid conjugates                       | Good uptake in cells but no PDT effect   | 2013 | (55)       |
| Human Barrett's esophagus adenocarcinoma OE33             | <i>m</i> THPC  | Comparison with P(V) 5,10,15,20-tetraalkylporphyrins | High content analysis, <i>m</i> THPC IC <sub>50</sub> = 5.1 μM   | 2014 | (75)       |
| Human Barrett's esophagus adenocarcinoma OE33             | <i>m</i> THPC-NSAID conjugates   | PDT study  | Good uptake but no phototoxicity at 2 min illumination   | 2015 | (51)       |
| Human breast adenocarcinoma MCF-7                         | <i>m</i> THPC  | Cell death mechanisms                                | Autophagy contributes to cell death  | 2011 | (116)      |
| Human breast adenocarcinoma MCF-7                         | <i>m</i> THPC  | Intracellular localization                           | ER and Golgi as primary sites of action  | 2003 | (117)      |
| Human breast adenocarcinoma MCF-7                         | <i>m</i> THPC  | ER targeting   | Development of luciferase reporting system   | 2014 | (118)      |
| Human breast adenocarcinoma MCF-7                         | <i>m</i> THPC  | Temporin supramolecular framework (SOF) PDT [        | IC <sub>50</sub> : 43.5 μg L <sup>-1</sup>   | 2022 | (99)       |
|   | <i>m</i> THPC SOF1   |  | IC <sub>50</sub> : 21.1 μg L <sup>-1</sup>   |      |            |
|   | <i>m</i> THPC SOF2   |  | IC <sub>50</sub> : 23.3 μg L <sup>-1</sup>   |      |            |
|   | <i>m</i> THPC SOF3   |  | IC <sub>50</sub> : 12.2 μg L <sup>-1</sup>   |      |            |
| Human breast adenocarcinoma MCF-7                         | <i>m</i> THPC  | Comparison with 5,15-diarylporphyrins                | IC <sub>50</sub> = 5.4 nM; > comparator  | 2019 | (119)      |
| Human breast adenocarcinoma MCF-7                         | <i>m</i> THPC loaded FH-PDots  | PDT study of polymer dots                            |  | 2014 | (120)      |

(continues)

Table 1. (continued)

| Cell line                                   | Compound/Formulation  | Study   | Comments/Phototoxicity   | Year         | References |
|---|---|---|--|--------------|------------|
| Human breast adenocarcinoma MCF-7           | <i>m</i> THPC loaded MEH-PPV and CN-PPV   | PDT study of polymer dots                                 | Active; cell viability increase with concentration; more uptake in cancer cells compared to normal ones due to folate receptor targeting   | 2016         | (121)      |
| Human breast adenocarcinoma MCF-7           | <i>m</i> THPC-LNP (lipid NP)  | PDT study   | Phototoxicity similar to free <i>m</i> THPC, some dark cytotoxicity of LNP   | 2011<br>2014 | (122,123)  |
| Human breast carcinoma MDA-MB-231           | <i>m</i> THPC   | Cell death mechanism                                      | c-PARP expression is dependent on treatment-induced apoptosis.   | 2009         | (124)      |
| Human breast carcinoma MDA-MB-231           | <i>m</i> THPC   | Comparison with 5,15-diary/porphyrins                     | IC <sub>50</sub> = 1.84 nM; ~ comparator   | 2019         | (119)      |
| Human breast carcinoma MDA-MB-231           | <i>m</i> THPC   | PDT comparison with corroles                              | IC <sub>50</sub> = 0.87 μM; > comparator   | 2020         | (125)      |
| Human breast carcinoma MDA-MB-231           | <i>m</i> THPC core shell nano drug: ~80 nm size poly(lactic-co-glycolic acid) (PLGA) nano-core encapsulating <i>m</i> THPC and ~20 nm size albumin nano-shell encapsulating tyrosine kinase inhibitor dasatinib | Combination therapy                                       | Blocking of cancer migration: disruption of Src kinase albumin-dasatinib nano-shell and generation of photoactivated oxidative stress by <i>m</i> THPC-PLGA nano-core; combinatorial photo-chemotherapy resulted in synergistic cytotoxicity in ~99% of motility-impaired metastatic cells | 2014         | (126)      |
| Human breast carcinoma MDA-MB-231           | <i>m</i> THPC-T-SLNP; temoporfin-loaded 1-tetradecanol-based thermoresponsive solid LNP/Foscan®   | PDT study   | Faster accumulation of LNP formulation   | 2016         | (127)      |
| Human breast carcinoma MDA-MB-231           | <i>m</i> THPC bound to Ce-doped-γ-Fe <sub>2</sub> O <sub>3</sub> maghemite nanoparticles (MNPs)   | Magnetic targeting of PDT agents                          | High uptake and phototoxicity; use of magnetic forces during incubation directed NPs to selected sites given 2× accumulation compared to no magnetic field   | 2019         | (128)      |
| Human breast carcinoma MDA-MB-231 (and 436) | <i>m</i> THPC   | Evaluation of Raman spectroscopy for diagnostics          | No interference of irradiation with 785 nm   | 2021         | (115)      |
| Human Burkitt's lymphoma Raji               | <i>m</i> THPC-β-cyclodextrins   | Effect of β-CD methylation                                |  | 2021         | (129)      |
| Human cervix adenocarcinoma SISO            | <i>m</i> THPC   | Combination of PDT with glutathione peroxidase inhibition |  | 2021         | (130)      |
| Human cervix adenocarcinoma SISO            | <i>m</i> THPC   | Cell death mechanism                                      | Apoptosis main mechanism, autophagy can occur as well. Wide variation in PDT response among 5 cell lines   | 2019         | (90)       |
| Human cervix adenocarcinoma SISO            | <i>m</i> THPC + carboplatin (CBDCA) or cisplatin (CDDP) or oxaliplatin (LOHP)   | Synergistic effect of Pt(II) complexes                    |  | 2018         | (131)      |
| Human cholangiocarcinoma QBC-939            | <i>m</i> THPC   | Comparison with cyclopropanochlorins                      | IC <sub>50</sub> = 1.96 μM [1 J cm <sup>-2</sup> ], slightly less effective than other chlorins  | 2020         | (65)       |
| Human cholangiocarcinoma TFK-1              | Nanoparticle albumin-bound <i>m</i> THPC  | Nanoparticles versus free PS                              | Reduced cytotoxicity of NP while retaining phototoxicity   | 2020         | (132)      |
| Human colon carcinoma HCT116                | <i>m</i> THPC   | Comparison with 5,15-diary/porphyrins                     | IC <sub>50</sub> = 7.6 nM; slightly less effective than other cmpds.   | 2009         | (71)       |
| Human colon carcinoma HCT116                | <i>m</i> THPC-loaded PLGA nanoparticles   | Nanoparticles versus free PS                              | No dark toxicity of temoporfin in nanoparticles; reduced cellular uptake of NP, but similar phototoxicity  | 2012         | (111)      |
| Human colon carcinoma HCT116                | <i>m</i> THPC   | Comparison with 5,15-diary/porphyrins                     | IC <sub>50</sub> = 3.6 nM; = comparator  | 2019         | (119)      |
| Human colon carcinoma HCT116                | <i>m</i> THPC multifunctional NPs (( <i>m</i> THPC@VeC/TRGD NPs)  | PDT and immunotherapy                                     |  | 2021         | (133)      |

(continues)

Table 1. (continued)

| Cell line                                  | Compound/Formulation  | Study   | Comments/Phototoxicity   | Year | References |
|--|---|---|--|------|------------|
| Human colon carcinoma HCT116               | <i>m</i> THPC   | PDT comparison with chlorin <i>e</i> <sub>6</sub> derivatives in liposomal formulations | induced apoptosis and/or necrosis and stimulated systemic immune response; sensitized tumors to PD-L1 blockade therapy<br>IC <sub>50</sub> = 0.2–1 μM (20 J cm <sup>-2</sup> ); ~ comparator | 2020 | (134)      |
| Human colon carcinoma HCT116 (and SW480)   | <i>m</i> THPC   | Cell death mechanism  | Autophagy via activation of the ROS/JNK pathway  | 2020 | (135)      |
| Human colon carcinoma HCT-15               | <i>m</i> THPC   | Evaluation of low light fluence rate  | Significant PDT activity with low light fluence 5–20 J cm <sup>-2</sup>  | 2019 | (136)      |
| Human colon carcinoma HT29                 | <i>m</i> THPC-loaded PLGA nanoparticles   | Nanoparticles versus free PS  | No dark toxicity of temoporfin in nanoparticles  | 2011 | (137)      |
| Human colon carcinoma HT29                 | <i>m</i> THPC-loaded PLGA nanoparticles   | Nanoparticles versus free PS  | Reduced cytotoxicity of NP while retaining phototoxicity   | 2015 | (138)      |
| Human colon carcinoma HT29                 | <i>m</i> THPC loaded PEGMA-co-DPA (NPs)   | pH sensitive NPs for PDT  | <i>in vitro</i> release rate of PS within 48 h at pH 5.0 was faster than at pH 7.0 (58% versus 10% released), no cytotoxicity of NP, good photocytotoxicity                                  | 2010 | (139)      |
| Human colon carcinoma HT29                 | <i>m</i> THPC- Nip-SLrPExx-PLGA 100-xx-NP (light sensitive)   | Light-responsive NPs for drug release   | Improved efficacy using a two-step illumination protocol   | 2021 | (140)      |
| Human colon carcinoma HT29                 | <i>m</i> THPC loaded micelles based on poly(2-ethyl-2-oxazoline)- <i>b</i> -poly(D,L-lactide) diblock copolymer | PDT study   | pH-sensitive delivery system   | 2010 | (141)      |
| Human colon carcinoma HT29                 | <i>m</i> THPC invasomes   | PDT study   | Good biocompatibility without irradiation, high PDT efficacy upon irradiation  | 2010 | (142)      |
| Human colon carcinoma HT29                 | <i>m</i> THPC loaded lipid-DNA micelles (UUI1mer)   | Lipid DNA as drug solubilizer   |  | 2018 | (143)      |
| Human colon carcinoma HT29                 | <i>m</i> THPC-β-cyclodextrins   | PDT study   | Improved uptake and phototoxicity compared to free <i>m</i> THPC   | 2016 | (144)      |
| Human colon carcinoma HT29                 | <i>m</i> THPC Foslip®   | Evaluation of delivery system   | <i>m</i> THPC-DCLs ~ Foslip®   | 2019 | (145)      |
| Human colon carcinoma HT29                 | <i>m</i> THPC-β-CD (methylated) complexes in liposomes ( <i>m</i> THPC-DCLs)                                    | PDT study   | LD <sub>50</sub> = 0.4 μg mL <sup>-1</sup> (3 J cm <sup>-2</sup> )   | 2015 | (146)      |
| Human colon carcinoma HT29-MTX             | Fospag®   | Light-responsive NPs for drug release   | Increased efficacy of light-responsive NPs; mucus-penetrating effect of PEGylated NP compared to free PS   | 2019 | (147)      |
| Human colon carcinoma HT29-MTX             | <i>m</i> THPC-(LrPC50%-PEG)-PLGA14 NP (light sensitive)   | Light responsive polymers   | Increased drug release after illumination when using light-responsive polymers   | 2019 | (148)      |
| Human colon carcinoma HT29-MTX             | <i>m</i> THPC-(PEG-PLA)-PLGA-NP (non light-sensitive)   | Evaluation of low light fluence rate  | Significant PDT activity with low light fluence 5–20 J cm <sup>-2</sup>  | 2019 | (136)      |
| Human colon carcinoma RKO                  | <i>m</i> THPC-(PEG-PLA)-PLGA-NP (non light-sensitive)   | Cell death mechanism  | c-PARP expression is dependent on treatment-induced apoptosis.   | 2009 | (124)      |
| Human colorectal adenocarcinoma HT29       | <i>m</i> THPC   | Comparison with 2,3-dihydroxychlorins   | PDT activity similar to temoporfin   | 2011 | (47)       |
| Human colorectal adenocarcinoma HT-29      | <i>m</i> THPC   | Cell death mechanism  | Cytoplasmic cytoskeleton destruction   | 2021 | (149)      |
| Human colorectal carcinoma SW480 (and 620) | <i>m</i> THPC   | PDT study   |  | 2018 | (150)      |
| Human colorectal carcinoma SW480 (and 620) | <i>m</i> THPC   | Comparison of different PSs   | Foscan®: LD <sub>50</sub> = 0.03 μM  | 2010 | (151)      |

(continues)

Table 1. (continued)

| Cell line  | Compound/Formulation   | Study   | Comments/Phototoxicity   | Year | References |
|--|--|---|--|------|------------|
| Human epidermoid carcinoma A431                                  | <i>m</i> THPC (Fospeg®)  | Cell death mechanism                                      | Fospeg®: LD <sub>50</sub> = 0.06 μM  | 2009 | (152)      |
| Human epidermoid carcinoma A431                                  | <i>m</i> THPC  | Combination PDT + chemotherapy                            | Autophagy and apoptotic features   | 2014 | (153)      |
| Human epidermoid carcinoma A431                                  | <i>m</i> THPC + doxorubicin  | PDT effect on cytokines                                   | Combination more cytotoxic than individual treatments; upregulation of IL-1alpha + VEGF in PDT-treated cells   | 2012 | (154)      |
| Human epidermoid carcinoma A431                                  | <i>m</i> THPC  | PDT study   | Up-regulation of VEGF and IL-1alpha  | 2020 | (101)      |
| Human epidermoid carcinoma A431                                  | <i>m</i> THPC loaded P(CL <sub>x</sub> -TMC-Bz <sub>y</sub> )-PEG micelles           |   | Photocytotoxicity: EC <sub>50</sub> = 4–11 μg mL <sup>-1</sup> ; free <i>m</i> THPC = ~1.5 μg mL <sup>-1</sup>   |      |            |
| Human epidermoid carcinoma A431                                  | <i>m</i> THPC  | PDT comparison with chlorin e <sub>6</sub> derivatives    | IC <sub>50</sub> = 0.7 μM at 2 J cm <sup>-2</sup> ; > comparator   | 2019 | (155)      |
| Human epidermoid carcinoma A431                                  | VHH <sup>[Fluo]</sup> -decorated <i>m</i> THPC loaded micelles                       | Targeted dual-labeled llama antibody-NP Conjugates        | Bioorthogonal VHH (variable heavy chain domains of heavy chain antibodies) modification and conjugation strategy   | 2017 | (156)      |
| Human epidermoid carcinoma A431                                  | <i>m</i> THPC loaded PCLn-PEG micelles decorated with EGFR targeting nanobody (EGa1) | EGFR targeting PS   | EGa1-conjugated micelles are internalized upon specific binding of the NP with the EGFR receptor overexpressed on the surfaces of A431 cells, resulting in enhanced cellular uptake and photocytotoxicity on A431 cells, as compared to EGFR low-expressing HeLa cells | 2020 | (102)      |
| Human epidermoid carcinoma A431                                  | <i>m</i> THPC loaded lipid-DNA micelles (UUI Imer)                                   | Lipid DNA as drug solubilizer                             | Good biocompatibility without irradiation, high PDT efficacy upon irradiation  | 2018 | (143)      |
| Human epidermoid carcinoma A431                                  | <i>m</i> THPC  | Investigation of adherence to microplates                 | Significant adherence to plastic material  | 2011 | (92)       |
| Human epidermoid carcinoma A431                                  | <i>m</i> THPC invasomes  | PDT study   |  | 2010 | (142)      |
| Human esophageal squamous cell carcinoma KYSE-70                 | <i>m</i> THPC  | Combination of PDT with glutathione peroxidase inhibition | Synergistic effect   | 2021 | (130)      |
| Human esophageal squamous cell carcinoma KYSE-70                 | <i>m</i> THPC  | Cell death mechanism                                      | Apoptosis main mechanism, autophagy can occur as well. Wide variation in PDT response among 5 cell lines   | 2019 | (90)       |
| Human esophageal squamous cell carcinoma KYSE-70                 | <i>m</i> THPC loaded silica NP   | PDT study   | 50% reduced uptake of <i>m</i> THPC with NP but same concentration-dependent PDT effect and intracellular localization   | 2009 | (157)      |
| Human esophageal squamous cell carcinoma KYSE-70                 | <i>m</i> THPC + carboplatin (CBDCA) or cisplatin (CDDP) or oxaliplatin (LOHP)        | Synergistic effect of Pt(II) complexes                    |  | 2018 | (131)      |
| Human esophageal squamous cell carcinoma OE21                    | <i>m</i> THPC in comparison to porphyrin-glycoconjugates                             | Carbohydrate bioconjugates                                | Slower uptake of carbohydrate bioconjugates and no phototoxicity   | 2012 | (58)       |
| Human esophageal squamous cell carcinoma OE21                    | <i>m</i> THPC in PEG-grafted, transferrin (TF)-conjugated liposomes                  | Targeting of transferrin receptor                         | Neither increased uptake nor increases PDT effect  | 2013 | (158)      |
| Human glioma E98   | VHH <sup>[Fluo]</sup> -decorated <i>m</i> THPC loaded micelles                       | Targeted dual-labeled llama antibody-NP Conjugates        | Bioorthogonal VHH (variable heavy chain domains of heavy chain antibodies) modification and conjugation strategy   | 2017 | (156)      |
| Human head and neck squamous carcinoma um-scc-U2 (and um-scc-U8) | Foscan®, Fospeg®, Foslip® + treatment with bleomycin                                 | PCI study   | Significantly increased bleomycin cytotoxicity when Foslip® or Fospeg® PDT was performed before BLM treatment  | 2014 | (159)      |
| Human hepatoma carcinoma Huh7                                    | Fospeg®  | PDT study   | Effective for killing 80-90% of HCC cells; PDT-induced apoptosis might be due to p53 upregulation  | 2013 | (160)      |
| Human hepatoma HepG2   | <i>m</i> THPC  | Comparison with 5,10,15,20-tetracarboxyporphyrin          | IC <sub>50</sub> = 1.3 μM  | 2019 | (76)       |
| Human immortalized keratinocytes HaCaT                           | <i>m</i> THPP loaded P(Glu-b-NADA), amphiphilic block-co-polypeptides                | amphiphilic co-polypeptides as drug delivery systems      | No dark toxicity, <i>m</i> THPP release in controlled manner   | 2012 | (103)      |

(continues)

Table 1. (continued)

| Cell line   | Compound/Formulation   | Study  | Comments/Phototoxicity   | Year | References |
|---|--|--|--|------|------------|
| Human lung carcinoma A-427  | <i>m</i> THPC  | Combination of PDT with glutathione peroxidase inhibition        |  | 2021 | (130)      |
| Human lung carcinoma A-427  | <i>m</i> THPC  | Cell death mechanism   | Apoptosis main mechanism, autophagy can occur as well. Wide variation in PDT response among 5 cell lines                                     | 2019 | (90)       |
| Human lung carcinoma A-427  | <i>m</i> THPC + carboplatin (CBDCA) or cisplatin (CDDP) or oxaliplatin (1-OHP) | Synergistic effect of Pt(II) complexes                           |  | 2018 | (131)      |
| Human mammary breast carcinoma EMT6 (transfected with plasmid pR70/GFP) | <i>m</i> THPC  | PDT effect on heat shock proteins                                | Extracellular release of HSP70   | 2011 | (161)      |
| Human mammary breast carcinoma HB4a-Ras                                 | <i>m</i> THPC  | PDT effect on cytoskeleton                                       | Reduction of the migratory and invasive ability in Ras transfected cells   | 2017 | (162)      |
| Human monocyte THP-1  | Foslip®<br>Nanoparticulate N2  | Effect on macrophages  | Temoporfin nanoparticles induces a shift to M1-like phenotype in M2-polarized macrophages  | 2018 | (163)      |
| Human monocyte THP-1  | <i>m</i> THPC  | Development of extracellular vesicles as theranostic materials   | Generation of <i>m</i> THPC loaded FVs   | 2012 | (164)      |
| Human nasopharyngeal carcinoma C666-1 (EBV-positive)                    | Fospeg®  | Multidrug resistant cancer                                       | PDT efficiency is independent of MDR1 gene and P-gp protein expression   | 2015 | (165)      |
| Human nasopharyngeal carcinoma C666-1 (EBV-positive)                    | Fospeg®  | Epstein-Barr virus infections                                    | Up-regulation of both LMP1 mRNA and protein  | 2013 | (166)      |
| Human nasopharyngeal carcinoma HK1 (EBV-negative)                       | Fospeg®  | Multidrug resistant cancer                                       | PDT efficiency is independent of MDR1 gene and P-gp protein expression   | 2015 | (165)      |
| Human nasopharyngeal carcinoma NPC/C666-1                               | Fospeg®  | Comparison 2D 3D cell system                                     | Down-regulation of LMP1 mRNA in MCL spheroids; PS uptake in 3D model 50% of 2D; different protein expression patterns in 2D versus 3D models | 2020 | (167)      |
| Human nasopharyngeal carcinoma NPC/HK1                                  | <i>m</i> THPC  | Cell death mechanism   | Induces early apoptotic responses  | 2009 | (168)      |
| Human nasopharyngeal undifferentiated carcinoma KJ-1 was used           | <i>m</i> THPC  | Test of cell migration in Matrigel invasion assay                | Decrease in migration distance of KJ-1 cells   | 2014 | (88)       |
| Human neuroblastoma SH-SY5Y   | <i>m</i> THPC-AuNP   | PDT study  | Better PDT effect of gold NPs; no dark toxicity  | 2018 | (53)       |
| Human non-tumorigenic lung epithelial cells BEAS-2G                     | <i>m</i> THPC  | Comparison with 5,10,15,20-tetracarboxyphorphyrin                | IC <sub>50</sub> = 1.9 μM  | 2019 | (76)       |
| Human oral adenocarcinoma CAL27   | <i>m</i> THPC  | Cell death mechanism; damage-associated molecular pattern (DAMP) | Presentation of ceramide and sphingosine-1-phosphate (S1P) on the cell surface after PDT   | 2014 | (169)      |
| Human oral squamous cell carcinoma BHY                                  | <i>m</i> THPC  | Combination of PDT with glutathione peroxidase inhibition        |  | 2021 | (130)      |
| Human oral squamous cell carcinoma BHY                                  | <i>m</i> THPC  | Cell death mechanism   | Apoptosis main mechanism, autophagy can occur as well. Wide variation in PDT response among 5 cell lines                                     | 2019 | (90)       |
| Human oral squamous cell carcinoma BHY                                  | <i>m</i> THPC + carboplatin (CBDCA) or cisplatin (CDDP) or oxaliplatin (1-OHP) | Synergistic effect of Pt(II) complexes                           |  | 2018 | (131)      |
| Human oral adenocarcinoma CAL27   | <i>m</i> THPC loaded lipid-DNA micelles (UU11mer)                              | Lipid DNA as drug solubilizer                                    | Good biocompatibility without irradiation, high PDT efficacy upon irradiation  | 2018 | (143)      |

(continues)

Table 1. (continued)

| Cell line   | Compound/Formulation  | Study  | Comments/Phototoxicity   | Year         | References |
|---|---|--|--|--------------|------------|
| Human oral adenosquamous carcinoma CAL27              | <i>m</i> THPC in calcium phosphate nanoparticles conjugated with RGDfK peptide and DY682-NHS  | PDT study  | Combined near-IR imaging and PDT   | 2015         | (170)      |
| Human oral adenosquamous carcinoma CAL27              | Foslip  | Uptake study   |  | 2013         | (171)      |
| Human ovarian carcinoma SKOV-3                        | <i>m</i> THPC   | Comparison with 5,15-diaryliporphyrins   | IC <sub>50</sub> = 2.9 nm; > comparator  | 2019         | (119)      |
| Human ovarian carcinoma SKOV-3                        | <i>m</i> THPC + magnetic NP loaded EVs  | Development of extracellular vesicles as theranostic materials                                 | Translation to <i>in vivo</i>  | 2012         | (164)      |
| Human ovarian carcinoma SKOV-3                        | <i>m</i> THPC + iron oxide magnetic NP in liposomes   | PDT/photothermal study   | Complete cancer cell death   | 2015         | (172)      |
| Human ovarian carcinoma SKOV-3                        | <i>m</i> THPC-MWCNT complex (multi-walled carbon nanotubes)   | PDT/photothermal study   | Different signaling pathways leading to cell apoptosis for PDT and PTT   | 2016         | (173)      |
| Human ovarian carcinoma SKOV-3                        | <i>m</i> THPC formulated in tetraether liposomes  | PDT study  |  | 2020         | (174)      |
| Human ovarian carcinoma SKOV-3                        | Lipid-enveloped PLGA NP with <i>m</i> THPC + pirarubicin  | Combination of PDT and chemotherapy  | THP nanoparticles coated with <i>m</i> THPC liposomes yielded lipid-coated nanoparticles (LCNPs) with good activity              | 2021         | (175)      |
| Human promonocytic cells U937                         | <i>m</i> THPC-loaded PLGA nanoparticles   | Nanoparticles versus free PS   | No dark toxicity of temopofin in nanoparticles; reduced cellular uptake of NP, but similar phototoxicity                         | 2012         | (111)      |
| Human prostate carcinoma LNCaP                        | <i>m</i> THPC (+ <i>P. halepensis</i> extracts)   | Effect of natural antioxidants on PDT  | <i>P. halepensis</i> bark extracts are cytotoxic and enhanced the PDT effect   | 2022         | (176)      |
| Human prostate carcinoma LNCaP                        | Fospeg®   | PDT study  | LD50 = 0.15 µg mL <sup>-1</sup> at 180 mJ cm <sup>-2</sup> ; improved compared to Foscan® (1.2 µg mL <sup>-1</sup> )             | 2009<br>2012 | (177,178)  |
| Human prostate carcinoma LNCaP                        | <i>m</i> THPC   | Time resolved sub-cellular singlet oxygen detection  |  | 2012         | (179)      |
| Human prostate carcinoma PC-3                         | <i>m</i> THPC   | Cell death mechanism   | Release of extracellular vesicles 1 h after illumination   | 2016         | (180)      |
| Human prostate carcinoma PC-3                         | <i>m</i> THPC   | PDT effect on intracellular trafficking  | Depolymerization of microtubule network; 100-fold lower endosomal trafficking  | 2013         | (181)      |
| Human prostate carcinoma PC-3                         | Foslipos  | Cell death mechanism   |  | 2011         | (182)      |
| Human prostate carcinoma PC-3                         | <i>m</i> THPC + magnetic NP loaded EVs  | Development of extracellular vesicles as theranostic materials                                 | Translation to <i>in vivo</i>  | 2012         | (164)      |
| Human retinoblastoma RB Y79                           | Liponanoparticles consisting of a poly (D,L)-lactide (PDLLA) NP coated with a phospholipid (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine/1,2-dioleoyl-3-trimethylammonium-propane) bilayer + loaded with anticancer drug beta-lapachone (β-Lap) and <i>m</i> THPC | Combined chemotherapy + PDT  | LNPs are cytotoxic at lower doses of the two encapsulated compounds as compared to the single therapies                          | 2019         | (183)      |
| Human squamous carcinoma 14C                          | <i>m</i> THPP loaded NOCCS/PMMA NP  | <i>In vitro</i> release study  | High loading capacity and stability  | 2011         | (184)      |
| Human tumor cells A-231 (submaxillary salivary gland) | <i>m</i> THPC encapsulated in Eudragit® RS 100 (NPs)  | Evaluation of upscaling of NP production for peroral PDT                                       | Successful process development   | 2015         | (185)      |
| Human umbilical vein endothelial cells HUVEC          | <i>m</i> THPC<br>Foscan®<br><i>m</i> THPC loaded magnetic nanoparticles   | Development of extracellular vesicles as theranostic materials<br>PDT comparison with corroles | EVs encapsulating iron oxide nanoparticles and PS as biocomouflaged agents for PDT, MRI, magnetic manipulation, and hyperthermia | 2017<br>2018 | (186,187)  |
|   | <i>m</i> THPC   |  |  | 2020         | (125)      |

(continues)

Table 1. (continued)

| Cell line  | Compound/Formulation   | Study  | Comments/Phototoxicity  | Year | References |
|--|--|--|---|------|------------|
| Human umbilical vein endothelial cells HUVEC           | <i>m</i> THPC  | Combination of PDT with glutathione peroxidase inhibition        | IC <sub>50</sub> = 1.50 μM; < comparator free bases; > comparator Ga complexes  | 2021 | (130)      |
| Human urinary bladder transitional cell carcinoma RT-4 | <i>m</i> THPC  | Cell death mechanism   | Synergistic effect  | 2019 | (90)       |
| Human urinary bladder transitional cell carcinoma RT-4 | <i>m</i> THPC + carboplatin (CBDCA) or cisplatin (CDDP) or oxaliplatin (LOHP)                        | Synergistic effect of Pt(II) complexes                           | Apoptosis main mechanism, autophagy can occur as well. Wide variation in PDT response among 5 cell lines  | 2018 | (131)      |
| Human-transformed breast cells MCF10A neoT             | <i>m</i> THPC-loaded PLGA nanoparticles  | Nanoparticles versus free PS                                     | No dark toxicity of temoporfin in nanoparticles; reduced cellular uptake of NP, but similar phototoxicity   | 2012 | (111)      |
| Jurkat cells (human T-cell lymphocytes)                | <i>m</i> THPC loaded HSA nanoparticles   | Photophysical study  |   | 2010 | (188)      |
| Jurkat cells (human T-cell lymphocytes)                | <i>m</i> THPC loaded HSA nanoparticles   | Drug release study   | <sup>1</sup> O <sub>2</sub> production in cells   | 2010 | (189)      |
| Jurkat cells (human T-cell lymphocytes)                | <i>m</i> THPP loaded HSA nanoparticles   | Drug release study   | <i>m</i> THPC-NP: high phototoxicity 5-24 h after incubation, 70-85% apoptosis  | 2011 | (190)      |
| Jurkat cells (human T-cell lymphocytes)                | <i>m</i> THPP loaded HSA nanoparticles   | Drug release study   | <i>m</i> THPP-NP: max. phototoxicity 1 h after incubation, then no further release of <i>m</i> THPP   | 2012 | (179)      |
| Jurkat cells (human T-cell lymphocytes)                | <i>m</i> THPC  | Time resolved sub-cellular singlet oxygen detection              | Better PDT effect with discoidal carriers compared to spherical ones  | 2019 | (191)      |
| KB Cells   | <i>m</i> THPC containing discoidal NP with folate/ <i>m</i> THPC containing vesicular NP with folate | Impact of delivery system morphology on PDT                      |   |      |            |
| KB cells   | <i>m</i> THPC in folate-targeted liposomes   | Folate targeting   | Low level internalization of folate-targeted liposomes via specific endocytosis; however, doubled <i>m</i> THPC and increased phototoxicity compared to untargeted liposomes                                | 2013 | (114)      |
| Mouse colon adenocarcinoma CT29                        | <i>m</i> THPC  | Comparison with redaporfin                                       | LLD <sub>50</sub> = 5 mJ cm <sup>-2</sup> at 0.5 μM ( <i>m</i> THPC)  | 2016 | (109)      |
| Mouse colon carcinoma C26                              | Styrene maleic acid copolymer (SMA) micelle encapsulating <i>m</i> THPC; SMA@ <i>m</i> THPC          | PDT study  | LLD <sub>50</sub> = 500 mJ cm <sup>-2</sup> at 5 μM (redaporfin)  | 2021 | (192)      |
| Mouse hepatoma 1c1c7                                   | <i>m</i> THPC  | Cell death mechanism   | pH-dependent release profile; lower dark and light toxicity than <i>m</i> THPC; phototoxicity: IC <sub>50</sub> SMA@ <i>m</i> THPC = 0.015 μg mL <sup>-1</sup> ; <i>m</i> THPC = 0.0005 μg mL <sup>-1</sup> | 2021 | (110)      |
| Mouse mammary carcinoma 4T1                            | <i>m</i> THPC  | PDT resistance   | <i>m</i> THPC initiates paraptosis after ER photodamage   | 2017 | (193)      |
| Mouse mammary carcinoma 4T1                            | <i>m</i> THPC  | PDT comparison with corrolors PDT study                          | Generation of PDT-resistant clones; resistance based on a mechanism based on sequestration of the drug to lysosomes; resistance can be overcome by using other PSS  | 2020 | (125)      |
| Mouse mammary carcinoma 4T1                            | <i>m</i> THPC-T-SLNP; temoporfin-loaded 1-tetradecanol-based thermoresponsive solid LNP/Foscan       | PDT study  | IC <sub>50</sub> = 0.05 μM; < comparator  | 2016 | (127)      |
| Mouse mammary carcinoma 4T1                            | <i>m</i> THPC loaded silica NP   | PDT study  | Faster accumulation of LNP formulation  |      |            |
| Mouse squamous cell carcinoma SCCVII                   | <i>m</i> THPC  | Cell death mechanism; damage-associated molecular pattern (DAMP) | Better PDT effect compared to <i>m</i> THPC   | 2018 | (194)      |
| Mouse squamous cell carcinoma SCCVII                   | <i>m</i> THPC (+acid ceramidase inhibitor LCL521)  | Cell death mechanism   | Presentation of ceramide and sphingosine-1-phosphate (SIP) on the cell surface after PDT  | 2014 | (169)      |
| Mouse squamous cell carcinoma TC-1                     | <i>m</i> THPC + magnetic NP loaded EVs   | Cell death mechanism   | LCL521-promoted PDT-mediated cell killing   | 2020 | (195)      |
| Murine cervical carcinoma TC-1                         | <i>m</i> THPC + magnetic NP loaded EVs   | Translation to <i>in vivo</i>                                    | Translation to <i>in vivo</i>   | 2012 | (164)      |

(continues)



Table 1. (continued)

| Cell line   | Compound/Formulation  | Study   | Comments/Phototoxicity   | Year | References |
|---|---|---|--|------|------------|
| Murine colon carcinoma CT26                         | <i>m</i> THPC multifunctional NPs (( <i>m</i> THPC@VeC/T-RGD NPs)   | Development of extracellular vesicles as theranostic materials<br>PDT and immunotherapy     | Induced apoptosis and/or necrosis and stimulated systemic immune response; sensitized tumors to PD-L1 blockade therapy                   | 2021 | (133)      |
| Murine colon carcinoma CT26                         | EVs fused with liposomes carrying <i>m</i> THPC (Foslip®)   | Development of extracellular vesicles as theranostic materials<br>PDT study of polymer dots | Fusion of EV with Foslip liposomes generates hybrid-EVs with 3-4 improved cellular delivery efficiency compared to Foscan or Foslip      | 2018 | (187)      |
| Murine embryonic fibroblasts                        | <i>m</i> THPC loaded FH-PDots   | PDT study of polymer dots   | Active; cell viability increase with concentration; more uptake in cancer cells compared to normal ones due to folate receptor targeting | 2014 | (120)      |
| Murine endothelial cells C166                       | <i>m</i> THPC loaded Ben-PCL- <i>m</i> PEG micelles   | Effect on macrophages   | RAW264.7 macrophages degrade micelles faster and activate the PS earlier than C166 cells, allowing for selective killing of macrophages  | 2017 | (196)      |
| Murine fibroblasts L929                             | <i>m</i> THPC loaded lipid-DNA micelles (UUI1mer)   | Lipid DNA as drug solubilizer   | Good biocompatibility without irradiation, high PDT efficacy upon irradiation  | 2018 | (143)      |
| Murine fibroblasts L929                             | <i>m</i> THPC organic crystalline NPs   | Preparation and testing of neat PS NPs<br>PDT study   | Preferential uptake in macrophages compared to fibroblasts   | 2018 | (197)      |
| Murine glioma C6                                    | <i>m</i> THPC loaded PLGA NPs   | PDT study of polymer dots   | Reduced cytotoxicity of <i>m</i> THPC-NP but higher phototoxicity compared to <i>m</i> THPC  | 2019 | (198)      |
| Murine glioma C6                                    | <i>m</i> THPC loaded FH-PDots   | PDT study of polymer dots   | Active; cell viability increase with concentration; more uptake in cancer cells compared to normal ones due to folate receptor targeting | 2014 | (120)      |
| Murine hematopoiesis monocytic macrophages J774A.1  | <i>m</i> THPC loaded lipid-DNA micelles (UUI1mer)   | Lipid DNA as drug solubilizer   | Good biocompatibility without irradiation, high PDT efficacy upon irradiation  | 2018 | (143)      |
| Murine hematopoiesis monocytic macrophages J774A.1  | <i>m</i> THPC organic crystalline NPs   | Preparation and testing of neat PS NPs  | Preferential uptake in macrophages compared to fibroblasts   | 2018 | (197)      |
| Murine hepatoma MH22                                | <i>m</i> THPC   | Cell death mechanism  | Apoptosis associated with caspase activation   | 2009 | (199)      |
| Murine Lewis lung carcinoma LLC1                    | <i>m</i> THPC   | PDT effect  | CDS0 at 60 mJ cm <sup>-2</sup> with 400 ng mL <sup>-1</sup> <i>m</i> THPC  | 2010 | (200)      |
| Murine melanoma cells B16-F10                       | <i>m</i> THPC   | Temoporfin supramolecular framework (SOF) PDT I   | IC <sub>50</sub> : 58.8 µg L <sup>-1</sup>   | 2022 | (99)       |
|   | <i>m</i> THPC SOF1  |   | IC <sub>50</sub> : 26.7 µg L <sup>-1</sup>   |      |            |
|   | <i>m</i> THPC SOF2  |   | IC <sub>50</sub> : 47.1 µg L <sup>-1</sup>   |      |            |
|   | <i>m</i> THPC SOF3  |   | IC <sub>50</sub> : 25.0 µg L <sup>-1</sup>   |      |            |
| Murine mesenchymal stem cell line C3H/10T1/2 (mMSC) | <i>m</i> THPC   | Development of extracellular vesicles as theranostic materials                              | Generation of <i>m</i> THPC loaded FVs   | 2021 | (201)      |
| Murine prostate TRAMP-C1                            | <i>m</i> THPC + IR780 loaded PFOB@IMHNP; tumor acidity-responsive lipid membrane-enclosed perfluorooctyl bromide oil droplet NPs surface modified with <i>N</i> -acetyl histidine-modified <i>D</i> - $\alpha$ -tocopheryl polyethylene glycol 1000 succinate | PDT/photothermal study  | Effective killing of hypoxic cancer cells <i>in vitro</i>  | 2020 | (202)      |
| Murine RAW264.7 macrophages                         | <i>m</i> THPC loaded Ben-PCL- <i>m</i> PEG micelles   | Effect on macrophages   | RAW264.7 macrophages degrade micelles faster and activate the PS earlier than C166 cells, allowing for selective killing of macrophages  | 2017 | (196)      |
| Rat glioma F98                                      | <i>m</i> THPC (+Rose Bengal)  | Pretreatment of medium  | Pretreatment reduced cell survival by 40%  | 2009 | (93)       |

(continues)

Table 1. (continued)

| Cell line  | Compound/Formulation   | Study  | Comments/Phototoxicity   | Year         | References |
|--|--|--|--|--------------|------------|
| 3D systems/spheroids<br>Biliary Tract Cancer Cell Lines                                      | Foscan®  | Comparison with Photofrin; influence of miRNA                | Identification of miRNAs which improve susceptibility to PDT. cell lines EGI-1, GBC, MzChA-1, SkChA-1, and TEK-1 showed relatively low phototoxic effects; CCSW-1, BDC, and MzChA-2 have significantly higher phototoxic efficiency  | 2014<br>2010 | (203,204)  |
| HeLa multicellular spheroids   | Foscan®<br>Foslip®<br>Fosp®g®<br>Foscan®   | PDT study  | cells with a low cytokeratin-19 (ductal differentiation), high vimentin (mesenchymal marker), and high proliferative phenotype show higher uptake of <i>m</i> THPC and phototoxicity   | 2016         | (205)      |
| HeLa multicellular spheroids   | <i>m</i> THPC  | Evaluation of LED illumination                               | Localization mainly in outer area of spheroids, PDT effect comparable for all three  | 2020         | (206)      |
| Human breast adenocarcinoma<br>MCF-7 + neural and satellite glial cells in a collagen matrix | <i>m</i> THPC  | PDT study  | PDT outcome is more efficient with violet light followed by red light  | 2009         | (207)      |
| Human colon carcinoma HCT116   | <i>m</i> THPC  | PDT study, effect on fluorouracil resistant cells            | MCF-7s and satellite glia are more sensitive to PDT than neurons   | 2017         | (208)      |
| Human colon carcinoma HT29   | <i>m</i> THPC  | Cell death mechanism   | PDT overcomes resistance   | 2009         | (124)      |
| Human colon carcinoma HT29   | Foslip®  | PDT study  | c-PARP expression is dependent on treatment-induced apoptosis.   | 2020         | (209)      |
| Human colon carcinoma HT29 – multicellular spheroids   | <i>m</i> THPC-β-CD complexes in liposomes  | Evaluation of drug-β-CD in liposomes concept                 | IC <sub>50</sub> = 2 ng mL <sup>-1</sup> with 2 J cm <sup>-2</sup> ; Foslip® PDT effect on HT29 cells was independent to 5-FU resistance   | 2017         | (210)      |
| Human colon carcinoma HT29 – multicellular spheroids   | <i>m</i> THPC<br>Foslip®<br><i>m</i> THPC-β-CD (methylated) complexes in liposomes ( <i>m</i> THPC-DCLs)   | Evaluation of delivery system                                | Homogenous accumulation across spheroid volume   | 2019         | (145)      |
| Human colon carcinoma HT29FU (fluorouracil resistant)  | Foslip®  | PDT study  | <i>m</i> THPC-DCLs > Foslip®   | 2020         | (209)      |
| Human colorectal adenocarcinoma HT29 – Multicellular spheroids                               | <i>m</i> THPC loaded in endothelial extracellular vesicles<br>Foslip®  | <i>m</i> THPC nanovectorization using extracellular vesicles | IC <sub>50</sub> = 2 ng mL <sup>-1</sup> with 2 J cm <sup>-2</sup> ; Foslip® PDT effect on HT29 cells was independent to 5-FU resistance   | 2018         | (211)      |
| Human colorectal adenocarcinoma HT29 – Multicellular spheroids                               | <i>m</i> THPC loaded in endothelial extracellular vesicles<br>Foslip®  | <i>m</i> THPC nanovectorization using extracellular vesicles | <i>m</i> THPC-EVs are stable in murine plasma; better <i>m</i> THPC accumulation and penetration in MCTS compared to Foslip®; light dose required for 50% cell death was 4 and 2.5-times lower than free and liposomal <i>m</i> THPC | 2020         | (212)      |
| Human esophageal carcinoma Eca 109   | <i>m</i> THPC  | PDT comparison with chlorin e <sub>6</sub> derivative        | Deeper penetration after 24h incubation compared to Foslip®  | 2018         | (213)      |
| Human glioblastoma U87MG spheroids   | <i>m</i> THPC)-loaded NP, based on vitamin-E-succinate-grafted chitosan oligosaccharide and cyclic (arginine-glycine-aspartic acid-D-phenylalanine-lysine) (c [RGDFK])-modified D-α-tocopheryl polyethylene glycol 1000 succinate, were prepared (RGD-NPs) | Targeting integrin-rich tumors                               | PDT efficacy < comparator  | 2017         | (214)      |

(continues)

Table 1. (continued)

| Cell line   | Compound/Formulation  | Study  | Comments/Phototoxicity   | Year | References |
|---|---|--|--|------|------------|
| Human glioblastoma LN229  | <i>m</i> THPC in liposomes formulated with DMPC and a cationic gemini surfactant  | Effect of surfactant   | Stereochemistry of the spacer of the gemini surfactant strongly affects uptake, interaction with cell membrane and intracellular distribution of PS            | 2010 | (215)      |
| Human monocyte THP-1  | <i>m</i> THPC + magnetic NP + doxorubicin + TPCS <sub>2a</sub> + tissue plasminogen activator (t-PA) in EV  | PS + magnetic NPs in cell-derived microvesicles                | Hybrid cell microvesicles were magnetically responsive + MRI-detectable; PS action could be modulated by magnetic targeting                                    | 2015 | (216)      |
| Human nasopharyngeal carcinoma NPC/C666-1/3D model  | Fospeg®   | Comparison 2D 3D cell system                                   | Down-regulation of LMPI mRNA in MCL spheroids; PS uptake in 3D model 50% of 2D; different protein expression patterns in 2D versus 3D models                   | 2020 | (167)      |
| Human osteosarcoma 143B (metastatic)  | Foscan®   | PDT study  | Time- and dose-dependent uptake, light dose-dependent phototoxicity associated with apoptosis  | 2017 | (217)      |
| Human osteosarcoma 143B (metastatic)  | Foslip®   | PDT study  | LD <sub>50</sub> = 0.012 to 0.047 µg mL <sup>-1</sup> with 652 nm laser light (2.5–10 J cm <sup>2</sup> )  | 2012 | (218)      |
| Human pharynx squamous cell carcinoma FaDu  | <i>m</i> THPC   | Influence of stromal microenvironment                          | Generation of stroma-rich 3D HNSCC spheroids; <i>m</i> THPC-EVs best uptake; PDT efficiency comparable to other NPs  | 2021 | (219)      |
| Human pharynx squamous cell carcinoma FaDu  | Foslip®   | Development of 3D spheroid pre-clinical <i>in vitro</i> system | Improved uptake and PDT efficiency of <i>m</i> THPC  | 2019 | (220)      |
| Human pharynx squamous cell carcinoma FaDu  | <i>m</i> THOC-EV  | Evaluation of cross-linked cyclodextrins as carrier system     | Improved penetration of spheroids with cross-linked CD system but lower cellular uptake of PS  | 2020 | (221)      |
| Human pharynx squamous cell carcinoma FaDu  | <i>m</i> THPC-β-cyclodextrins   | O <sub>2</sub> measurements during PDT                         | Simultaneous use of time resolved NIR luminescence and spectrally resolved UV/VIS fluorescence   | 2020 | (222)      |
| Human pharynx squamous cell carcinoma FaDu  | Foslip®   | PDT study  | Similar phototoxicity compared to <i>m</i> THPC but lower dark toxicity; necrosis and apoptosis with <i>m</i> THPC-PDT, apoptosis with 50 nm <i>m</i> THPC-LNP | 2016 | (223)      |
| Human squamous cell carcinomas of the tongue CAL-33   | <i>m</i> THPC-LNP   | Effect of surfactant   | Stereochemistry of the spacer of the gemini surfactant strongly affects uptake, interaction with cell membrane and intracellular distribution of PS            | 2010 | (215)      |
| Murine glioblastoma C6  | <i>m</i> THPC in liposomes formulated with DMPC and a cationic gemini surfactant  | PS-neutrophils for sonodynamic therapy                         | Selective accumulation in tumors, enhancement of tumor O <sub>2</sub> levels, triggering of anticancer sonodynamics upon ultrasound stimulation                | 2022 | (224)      |
| Murine melanoma cells B16-F10   | <i>m</i> THPC + oxygen-carrying perfluorocarbon encapsulated into cRGD peptide modified multilayer liposomes (C-ML/HPT/O <sub>2</sub> ), which are then loaded into live neutrophils (“Acous-cyte/O <sub>2</sub> ”) |  |  |      |            |
| Murine osteosarcoma K7M2  | Foscan®   | PDT study  | Time- and dose-dependent uptake, light dose-dependent phototoxicity associated with apoptosis  | 2017 | (217)      |
| Neural and satellite glial cells in a collagen matrix   | Foslip®   | PDT study  | Inhibition of anti-oxidant pathways increases sensitivity of neurons to PDT  | 2012 | (225)      |
| Other   | <i>m</i> THPC   |  |  |      |            |
| Hen's egg test on chorioallantoic membrane (HET-CAM) model with HuTu-80 or HT29-MTX-E12 tumor spheroids | <i>m</i> THPP loaded PLGA NPs + others  | Vascularized intestine tumor model                             | Assay is suitable link between standardized <i>in vitro</i> models and animal models   | 2022 | (226)      |
| Anti-microbial <i>Candida albicans</i>  |   | Anti-bacterial PDT   | 59% inhibition with 15 min illumination  | 2021 | (227)      |

(continues)

Table 1. (continued)

| Cell line                           | Compound/Formulation  | Study                                     | Comments/Phototoxicity   | Year | References |
|-------------------------------------|---|---|--|------|------------|
| <i>Clostridium difficile</i>        | <i>m</i> THPP-ethyl cellulose/chitosan nanocomposite                            | Anti-bacterial PDT                        | <3 log unit reduction  | 2015 | (228)      |
| <i>Enterococcus faecalis</i>        | <i>m</i> THPC   | Anti-bacterial PDT, endodontic infections | 50 mM PS, 100 J cm <sup>-2</sup> ; complete eradication; 10 mM PS: reduction in CFU by 5.8 log-units   | 2011 | (229)      |
| <i>Enterococcus faecalis</i>        | <i>m</i> THPC containing biomaterial for periodontal regeneration               | Anti-bacterial PDT                        | 3.1–3.7 log unit reduction   | 2018 | (230)      |
| <i>Escherichia coli</i>             | <i>m</i> THPP-nanodiamond conjugates  | Anti-bacterial PDT                        | 56% inhibition   | 2021 | (52)       |
| <i>Porphyromonas gingivalis</i>     | <i>m</i> THPC containing biomaterial for periodontal regeneration               | Anti-bacterial PDT                        | Total suppression  | 2018 | (230)      |
| <i>Pseudomonas aeruginosa</i>       | Liposomal formulation: <i>m</i> THPC + antimicrobial peptide WLBU2              | Anti-bacterial PDT                        | 3.3 log unit reduction   | 2011 | (231)      |
| <i>Pseudomonas aeruginosa</i>       | Liposomal formulation <i>m</i> THPC conjugated with wheat germ agglutinin (WGA) | Anti-bacterial PDT                        | 2 log unit reduction   | 2012 | (232)      |
| <i>Pseudomonas aeruginosa</i>       | Temporin  | Anti-bacterial PDT                        | Less than 1 log reduction after 20 min illumination  | 2021 | (233)      |
| <i>Pseudomonas aeruginosa</i>       | <i>m</i> THPP-ethyl cellulose/chitosan nanocomposite                            | Anti-bacterial PDT                        | 71% inhibition with 15 min illumination  | 2021 | (227)      |
| <i>Staphylococcus aureus</i>        | Fosp@   | Anti-bacterial PDT                        | 100 nM PS, 5 min incubation, 30 min illumination at 75 mW cm <sup>-2</sup> → 4–5 log unit reduction  | 2010 | (234)      |
| <i>Staphylococcus aureus</i>        | Temporin  | Anti-bacterial PDT                        | 12.5 μM <i>m</i> THPC resulted in complete eradication   | 2021 | (233)      |
| <i>Staphylococcus aureus</i>        | <i>m</i> THPP-ethyl cellulose/chitosan nanocomposite                            | Anti-bacterial PDT                        | 74% inhibition with 15 min illumination  | 2021 | (227)      |
| <i>Staphylococcus aureus</i> (MRSA) | Liposomal formulation: <i>m</i> THPC + antimicrobial peptide WLBU2              | Anti-bacterial PDT                        | Complete eradication   | 2011 | (231)      |
| <i>Staphylococcus aureus</i> (MRSA) | Liposomal formulation <i>m</i> THPC conjugated with wheat germ agglutinin (WGA) | Anti-bacterial PDT                        | Complete eradication   | 2012 | (232)      |
| <i>Streptococcus mutans</i>         | Foslipos  | Anti-bacterial PDT, dental caries         | Significant dark toxicity at 10–1.25 mg mL <sup>-1</sup> ; complete eradication with a mixture of Foslipos/hypericin (0.625 mg mL <sup>-1</sup> , each), 15 min incubation, 120 s illumination | 2009 | (235)      |
| <i>Streptococcus sobrinus</i>       | Foslipos  | Anti-bacterial PDT, dental caries         | Complete eradication with 5 mg mL <sup>-1</sup> , 15 min incubation, 120 s illumination  | 2009 | (235)      |

**Table 2.** Animal models used for *m*THPC and related compounds (sorted by animal model and by publication year).

| Animal model   | Compound/Study  | Comments  | Year | References |
|--|---|---|------|------------|
| Mouse  |   |   |      |            |
| Male C57 mice bearing B16-F10 tumors (melanoma)                              | <i>m</i> THPC loaded on supramolecular organic frameworks (nanoparticles): PDT efficacy   | Suppression of tumor growth   | 2022 | (99)       |
| Male C57BL/6 mice bearing B16F10 tumors                                      | Perfluorocarbon-oxygen carrier and <i>m</i> THPC in peptide-modified liposomes combined with neutrophils: characterization and sonodynamic application  | Apoptosis induced by ROS generated under ultrasound treatment, tumor growth inhibition  | 2022 | (224)      |
| Female BALB/c mice bearing CT26 tumors                                       | <i>m</i> THPC-loaded vitamin-E-succinate-grafted chitosan oligosaccharide/D- $\alpha$ -tocopheryl PEG succinate nanoparticles (RGDfK peptide-modified) combined with anti-PD-L1: PDT and immune effects | Combination of PDT and anti-PD-L1 checkpoint blockade inhibited growth of local and distant tumors  | 2021 | (133)      |
| Female BALB/c and C57BL/6 mice bearing CT26 and ID8-Luc tumors, respectively | <i>m</i> THPC vs. liposomal <i>m</i> THPC (Foslip®) and extracellular vesicles derived from mesenchymal stem/stromal cells loaded with <i>m</i> THPC: PDT efficacy                                      | Enhanced tumor selectivity and anti-cancer efficacy of the <i>m</i> THPC in extracellular vesicles, promotion of antitumor immune cell infiltration                             | 2021 | (201)      |
| Female albino (BALB/c) mice  | Combination of Pirarubicin PLGA nanoparticles and liposomal <i>m</i> THPC: Acute toxicity and particle characterization   | Cytotoxicity synergism, lower toxicity compared to the free drugs   | 2021 | (175)      |
| Male ddY mice bearing S180 mouse sarcoma                                     | <i>m</i> THPC in styrene maleic acid copolymer micelles: characterization, release profile, PDT efficacy  | Similar anti-tumor efficacy as free <i>m</i> THPC, less side effects  | 2021 | (192)      |
| Female NMRI <sup>nu/nu</sup> mice bearing HT-29 tumors                       | Extracellular vesicles loaded with <i>m</i> THPC vs. liposomal <i>m</i> THPC (Foslip®): PDT efficacy  | Efficient tumor volume reduction with <i>m</i> THPC in extracellular vesicles   | 2020 | (212)      |
| Female Balb/c nude mice bearing A431 tumors                                  | <i>m</i> THPC loaded polymeric micelles: Pharmacokinetics and tumor accumulation  | Long circulating micelles resulted in significantly higher tumor accumulation compared to short circulating micelles  | 2020 | (280)      |
| Female Balb/c nude mice bearing A431 tumors                                  | Polymeric micelles loaded with <i>m</i> THPC and EGFR targeting: Formulation and pharmacokinetics   | Prolonged blood circulation kinetics for the nanoparticles, independent of antibody presence  | 2020 | (102)      |
| Female Balb/c nude mice  | <i>m</i> THPC and <i>m</i> THPC loaded on polymeric micelles: Comparison of biodistribution   | $\pi$ - $\pi$ stacking interactions to improve cargo retention in polymeric micelles <i>in vitro</i> , differences to <i>in vivo</i> distribution                               | 2020 | (101)      |
| Male C57BL/6 mice bearing TRAMP-C1 tumors                                    | Combined photothermal and <i>m</i> THPC-PDT with oil droplet nanoparticles: Antitumor efficacy  | Inhibition of tumor growth  | 2020 | (202)      |
| Male BALB/c mice   | Effect of <i>m</i> THPC-PDT on tumor growth of HCT116 xenografts, role of autophagy   | Inhibition of tumor growth, importance of autophagy   | 2020 | (135)      |
| Female athymic nude mice   | <i>m</i> THPC encapsulated in (folate) nanodiscs vs. liposomal <i>m</i> THPC: antitumor efficacy  | Highest tumor reduction with the <i>m</i> THPC-loaded folate nanodiscs  | 2019 | (191)      |
| Female NMRI <sup>nu/nu</sup> mice bearing HT-29 tumors                       | <i>m</i> THPC/cyclodextrin inclusion complexes in liposomal <i>m</i> THPC vs. liposomal <i>m</i> THPC (Foslip®): PDT efficacy   | Similar PDT efficacy of liposomal <i>m</i> THPC (Foslip®) and double-loaded liposomes   | 2019 | (145)      |
| Male Hsd:Athymic Nude-Foxn1 mice bearing MDA-MB231 tumors                    | <i>m</i> THPC-loaded Ce-doped maghemite nanoparticles: PDT efficacy   | Faster accumulation compared to free <i>m</i> THPC, tumor regression  | 2019 | (128)      |
| Nu/Nu mice bearing MDA-MB-231 tumors (human breast carcinoma)                | <i>m</i> THPC (Foscan®) vs. silica nanoparticles loaded with <i>m</i> THPC: PDT efficacy  | Efficient intracellular delivery of silica nanoparticles, passage of blood brain-barrier, <i>m</i> THPC silica nanoparticles more effective, partial tumor relapse in all cases | 2018 | (194)      |
| Nude mice bearing HeLa tumors  | <i>p</i> THPP vs. <i>p</i> THPP polyhedral oligomeric silsesquioxanes with PEG: PDT efficacy  | Superiority (biocompatibility, tumor volume reduction) of the nanoparticle formulation  | 2018 | (60)       |
| Female BALB/c athymic nude mice bearing HT-29 tumors                         | Combination of <i>m</i> THPC-PDT and anti-VEGF monoclonal neutralizing antibody bevacizumab: Efficacy   | PDT treatment followed by bevacizumab more effective than opposite combination (bevacizumab treatment decreased <i>m</i> THPC tumor accumulation)                               | 2018 | (281)      |
| BALB/c mice bearing fibrosarcoma   | <i>m</i> THPC: pharmacokinetics   |   | 2018 | (282)      |

(continues)

Table 2. (continued)

| Animal model  | Compound/Study  | Comments  | Year | References |
|---|---|---|------|------------|
| Subcutaneous-U87MG tumor-bearing nude mice  | <i>m</i> THPC-loaded vitamin-E-succinate-grafted chitosan oligosaccharide/D- $\alpha$ -tocopheryl PEG succinate nanoparticles (RGDfK peptide-modified) vs. unmodified nanoparticles: PDT efficacy | Rebound effect (new local drug accumulation)<br>Higher anti-tumor efficacy, less systemic toxicity for the modified nanoparticles   | 2017 | (214)      |
| SCID (143B/SCID, human xenograft mouse model) and BALB/c mice (K7M2L2/BALB/c, syngeneic mouse model), intratibial mouse model     | <i>m</i> THPC (Foscan®) and liposomal <i>m</i> THPC (Foslip®): PDT efficacy, immune effects   | Uptake of both formulations higher in tumor than in healthy control tissue, significant tumor growth inhibition by PDT, significant immune system-dependent suppression of lung metastasis in the K7M2L2/BALB/c model | 2017 | (217)      |
| BALB/c syngeneic mice bearing 4T1 tumors and tumors derived from cells of PDT-resistant clones                                    | Ethylene glycol derivatives of tetraphenylporphyrin and <i>m</i> THPC: Investigation of resistance to PDT   | Different resistance mechanisms, for <i>m</i> THPC: sequestration to lysosomes  | 2017 | (193)      |
| Nu/Nu mice bearing MDA-MB-231 tumors  | <i>m</i> THPC (Foscan®) vs. <i>m</i> THPC in 1-tetradecanol thermoresponsive solid-lipid nanoparticles: PDT efficacy  | Improved anti-cancer efficacy of the nanoparticle formulation   | 2016 | (127)      |
| CD1-Foxn1 <sup>nu</sup> nude mice bearing CAL-33 tumors   | <i>m</i> THPC vs. liposomal <i>m</i> THPC (Foslip®) and <i>m</i> THPC lipid nanoparticles: PDT efficacy   | Better biocompatibility of both nanoparticle formulations, highest tumor volume reduction with liposomal <i>m</i> THPC  | 2016 | (283)      |
| Syngeneic C3H/HeN mice bearing murine SCC (SCCVII)  | Combination of anti-GR1 antibody and <i>m</i> THPC-PDT: Tumor response, immune effects  | Better anti-tumor effect with delayed anti-GR1 treatment  | 2016 | (284)      |
| NMRI male nude mice bearing PC-3 prostate carcinoma   | Effect of <i>m</i> THPC PDT and doxorubicin on release of extracellular vesicles  | Release of large amounts of extracellular vesicles (higher for PDT)   | 2016 | (180)      |
| Radiation-induced fibrosarcoma tumor mouse model  | Benzoporphyrin derivative monoacid ring A, Photofrin® and <i>m</i> THPC: PDT threshold dose   | <i>In vivo</i> <sup>1</sup> O <sub>2</sub> threshold doses for Photofrin®, BPD, and <i>m</i> THPC ~ 20 times smaller than those observed <i>in vitro</i>  | 2015 | (285, 286) |
| NMRI nude mice bearing A431 tumors  | Liposomal <i>m</i> THPC, with iron oxide nanoparticles in the aqueous core: combination of PDT and magnetic hyperthermia  | Synergistic effect, complete tumor ablation   | 2015 | (172)      |
| NMRI <sup>nu/nu</sup> mice bearing HT-29 tumors   | Pegylated liposomal <i>m</i> THPC (Fospag®): Pharmacokinetics and biodistribution <i>via</i> optical imaging  | Good correlation between <i>ex vivo</i> tissue fluorescence and reflectance imaging and chemical extraction   | 2015 | (287)      |
| Athymic Nude-Foxn1 <sup>nu</sup> mice bearing CAL-27 tumors   | <i>m</i> THPC-loaded calcium phosphate nanoparticles modified with RGDfK peptide and NIR fluorescence dye: PDT efficacy   | Decrease in tumor vascularization and tumor volume  | 2015 | (170)      |
| Female NCRNu mice, dorsal skin-fold window chamber tumor model (luciferase- and green fluorescent protein-transduced gliosarcoma) | <i>m</i> THPC (Foscan®): Correlation tumor response and singlet oxygen luminescence   | <sup>1</sup> O <sub>2</sub> luminescence as dosimetric technique for PDT in tumor tissue  | 2015 | (288)      |
| Female C57BL/6 mice with azoxymethane/dextran sulfate sodium-induced colitis and tumors   | Liposomal <i>m</i> THPC (Foslip®): Effect of low-dose-PDT on colitis and colitis-associated carcinogenesis  | Reduction of tumor growth, expression decrease of inflammatory mediators, lowering neutrophil influx  | 2015 | (289)      |
| Female NMRI <sup>nu/nu</sup> mice bearing HT-29 tumors  | Liposomal <i>m</i> THPC (Foslip®) and pegylated liposomal <i>m</i> THPC (Fospag®): Distribution and PDT efficacy  | Enhanced permeability and retention-based tumor accumulation and circulation stability for Fospag®  | 2013 | (290)      |
| Female athymic nude-Foxn1 <sup>nu</sup> mice bearing Cal-27 tumors  | Fluorescence optical imaging of liposomal <i>m</i> THPC (Foslip®) in combination with DY-734 annexin V  | Detection of apoptotic cells shortly after treatment, reduction of tumor vascularization at later time points   | 2013 | (171)      |
| Female nude NMRI mice bearing TC-1 tumors   | <i>m</i> THPC vs. extracellular vesicles loaded with <i>m</i> THPC and maghemite (iron oxide) nanoparticles: PDT efficacy and imaging   | Improved anti-cancer efficacy (reduction of tumor growth) of the nanoparticle formulation, dual imaging by fluorescence and MRI   | 2013 | (164)      |
| Female BALB/cAnN.Cg-Foxn1 <sup>nu</sup> /CrINarl nude mice bearing A549 tumors  | Luciferase-immobilized quantum dots combined with <i>m</i> THPC loaded micelles: PDT efficacy, ROS generation   | bioluminescence resonance energy transfer for PDT, delayed tumor growth   | 2013 | (112)      |

(continues)

Table 2. (continued)

| Animal model   | Compound/Study  | Comments  | Year | References |
|--|---|---|------|------------|
| Male Hsd:ATHymic Nude-Foxn1 mice bearing HCT-116-luc tumors                                | <i>m</i> THPC vs. <i>m</i> THPC in PLGA and pegylated PLGA nanoparticles: PDT efficacy  | Reduced dark toxicity for the nanoparticles, improved tissue distribution for pegylated PLGA nanoparticles  | 2012 | (111)      |
| SCID mice bearing LNCaP tumors   | Interstitial PDT with pegylated liposomal <i>m</i> THPC (Fospeg®): Effect of natural antioxidants   | Prevention of photosensitivity with the natural antioxidants  | 2012 | (291)      |
| Female BALB, cAnN.Cg-Foxn1 <sup>nu</sup> and CrINarl nude mice bearing KB and HT-29 tumors | <i>m</i> THPC vs. <i>m</i> THPC loaded on folate-conjugated polymeric micelles: PDT efficacy  | Folate-conjugated micelles have higher PDT efficacy than <i>m</i> THPC or <i>m</i> THPC polymeric micelles without folate   | 2012 | (94)       |
| Male Balb/C mice bearing SIRCC1.15 (kidney) tumors   | <i>m</i> THPC: PDT efficacy against kidney tumors   | Renal tumor destruction, <i>m</i> THPC uptake and PDT sensitivity increased in endothelial cells compared renal cell carcinoma and renal cells  | 2012 | (292)      |
| Female BALB/c mice bearing hsp70-GFP/EMT6 tumors   | <i>m</i> THPC: Investigation of tumor efficacy and heat shock protein levels  | Maximum heat shock protein levels at PDT doses corresponding to 30% cell survival   | 2011 | (161)      |
| Syngeneic C3H/HeN mice bearing murine SCC (SCCVII)   | <i>m</i> THPC in combination with ceramide analog (LCL29): Effect on PDT  | Increase of cancer cell apoptosis by combination of LCL29 and <i>m</i> THPC-PDT   | 2011 | (273)      |
| Female NMRI <sup>nu/nu</sup> mice bearing HT-29 tumors                                     | Pegylated liposomal <i>m</i> THPC (Fospeg®): Drug quantification  | Fluorescence imaging (time-resolved fluorescence white Monte Carlo simulations combined with the Beer-Lambert law)  | 2011 | (293)      |
| Female syngeneic C3H/HeN mice bearing SCCVII tumors  | <i>m</i> THPC in combination with ceramide analog (LCL29): Effect on PDT  | Combination improved long-term tumor cure, changes in sphingolipid profile  | 2011 | (275)      |
| Female SKH-1 mice  | Liposomal <i>m</i> THPC in collagen matrix: antibacterial photodynamic inactivation   | Faster wound healing (scab detachment) with <i>m</i> THPC loaded an illuminated collagen implant  | 2011 | (294)      |
| Female Swiss (nu/nu) mice bearing RB-102-FER, RB-109-LAK and RB-111-MIL xenograft tumors   | <i>m</i> THPC and Verteporfin: PDT efficacy, xenograft model for retinoblastoma   | Transient response to <i>m</i> THPC for RB102-FER and response with partial regression to <i>m</i> THPC for RB111-MIL   | 2010 | (295)      |
| Female BALB/c mice bearing EMT6 tumors   | <i>m</i> THPC: PDT efficacy using fractionation of drug administration  | Investigation <i>m</i> THPC biodistribution profile and regional distribution of apoptosis; improved tumor cure with fractionated drug administration                                 | 2010 | (296)      |
| Hybrid DBA/2 × BALB/c male mice bearing L1210 ascitic tumors                               | Combination of <i>m</i> THPC PDT, adoptive immunotherapy, and chemotherapy: Efficacy study  | Synergistic anti-tumor effect   | 2010 | (297)      |
| Male C57BL/6 mice bearing Lewis lung carcinoma   | <i>m</i> THPC: PDT efficacy   | 0.25 mg kg <sup>-1</sup> <i>m</i> THPC, 24 h DLI: Inhibition of tumor growth, prolonged survival  | 2010 | (200)      |
| Female athymic Foxn1 <sup>nu</sup> /Foxn1 <sup>nu</sup> mice bearing HT-29 tumors          | <i>m</i> THPC treatment: Apoptosis and caspase activity   | Different expression pattern of caspase-3 and caspase-7 in <i>m</i> THPC treated tumors   | 2009 | (124)      |
| Female Foxn1 <sup>nu/nu</sup> mice bearing EMT6 tumors                                     | <i>m</i> THPC vs. liposomal <i>m</i> THPC (Foslip®): Pharmacokinetics and PDT efficacy  | Highest tumour to muscle ratios at 6 and 15 h post-administration, best tumor response for 6 h DLI  | 2009 | (298)      |
| Rat  |   |   |      |            |
| Male Wistar rats (HsdCpb:W) bearing 4NQO-induced tumors                                    | <i>m</i> THPC (Foscan®), liposomal <i>m</i> THPC (Foslip®) and pegylated liposomal <i>m</i> THPC (Fospeg®): Tumor accumulation vs. normal tissue    | Higher <i>m</i> THPC fluorescence of Fospeg® in normal and tumor tissue compared to Foscan® and Foslip®, significant differences between tumor and normal tissue for all formulations | 2013 | (299)      |
| Male Wistar rats bearing Walker-256 tumors   | <i>m</i> THPC: pharmacokinetics, distribution, and elimination in orthotropic liver cancer model  | Highest tumor/normal tissue-ratio at 24 h   | 2013 | (300)      |
| Female Wistar rats and Female HL rats bearing MC28 tumors                                  | Pegylated liposomal <i>m</i> THPC (Fospeg®): distribution, effect of different degrees of pegylation  | Longer elimination half-life for higher degree of pegylation  | 2012 | (301)      |
| Female Fisher-344 rats bearing transplanted R3230AC tumors                                 | <i>m</i> THPC (Foscan®), liposomal <i>m</i> THPC (Foslip®) and pegylated liposomal <i>m</i> THPC (Fospeg®): Tumor accumulation vasculature kinetics | Skin-fold observation chamber model, maximum tumor fluorescence 8 h for Fospeg®, 24 h for Foslip® and Foscan®, higher bioavailability of liposomal formulations                       | 2011 | (302)      |
| Female Fischer-344 rats bearing transplanted R3230AC tumors                                |   |   | 2011 | (303)      |

(continues)

Table 2. (continued)

| Animal model                   | Compound/Study   | Comments   | Year | References |
|--------------------------------|--|--|------|------------|
| Fisher-344 rats bearing tumors | <i>m</i> THPC: <i>in vivo</i> quantification/<br>pharmacokinetics <i>via</i> fluorescence,<br>method development           | Skin fold observation chamber model,<br>dual wavelength excitation, NIR<br>imaging   | 2009 | (304)      |
| Male Wistar rats               | <i>m</i> THPC: <i>in vivo</i> quantification/<br>pharmacokinetics by fluorescence<br>measurement                           | Skin-fold observation chamber model,<br>correction for autofluorescence  | 2009 | (305)      |
| Male Wistar rats               | <i>m</i> THPC: <i>in vivo</i> quantification/<br>pharmacokinetics by fluorescence<br>differential path length spectroscopy | Good correlation with chemical extraction  | 2009 | (306)      |
| Male Wistar rats               | <i>m</i> THPC: Fluorescence and reflectance<br>spectroscopic monitoring of interstitial<br>PDT                             | Decrease in fluence rate during PDT,<br>observation of differences in vascular<br>response even between animals of the<br>same treatment group | 2009 | (307)      |
| Male Wistar rats               | <i>m</i> THPC and other PS: Determination of<br>PDT threshold dose depth of necrosis<br>and superficial necrosis           | Determination of PDT threshold dose by<br>measurement of necrosis depth and<br>superficial necrotic area                                       | 2009 | (307)      |
| Dog                            |  |  |      |            |
| Male beagles                   | <i>m</i> THPC: Dosimetry for interstitial PDT in<br>the canine prostate  | Light-dose escalation plan, less damage to<br>surrounding tissue with dosimetry<br>planning, light energy dose-response<br>relationship        | 2016 | (308)      |
| Sheep                          |  |  |      |            |
| Swiss white alpine sheep       | <i>m</i> THPC: Pharmacokinetics by blood<br>sampling and fluorescence,<br>optimization of oesophagus PDT                   | Similar pharmacokinetics and sensitivity<br>to <i>m</i> THPC-PDT compared to humans  | 2009 | (309)      |
| Cat                            |  |  |      |            |
| Cats bearing SCC (animal PDT)  | <i>m</i> THPC: PDT treatment of feline SCC<br>(63 lesions in 38 cats)  | Complete remission 61%, partial<br>remission 22%, mean progression-free<br>interval of 35 months, median overall<br>survival time 40 months    | 2018 | (310)      |
| Horse                          |  |  |      |            |
| Horse sarcoid (animal PDT)     | Pegylated liposomal <i>m</i> THPC (Fospeg®):<br>PDT and surgical treatment of equine<br>sarcoid (case report)              | Local treatment, tumor remission or tumor<br>growth stagnation   | 2012 | (311)      |

account for the extent of tumor eradication observed *in vivo*. Here indirect effects, mainly on the tumor vasculature (42,251), are critical for long-term tumor control (42,245). In addition, PDT effects on the immune system contribute to tumor eradication (42,245,252–254). PDT can provoke the formation of antitumor inflammatory cells and can result in a persistent antitumor immune response (8,42,252–257). A significant body of research effort has been done addressing these effects and processes related to PDT at the molecular and cellular level as well as at the systemic level of the organism.

Photosensitizers rely on the ability to generate singlet oxygen and other ROS when irradiated with light in the presence of oxygen. The photophysics and basic photochemistry of temoporfin have therefore been investigated in various surroundings and under various conditions (42). In recent years, de Vetta *et al.* (258,259) and de Oliveria *et al.* (84) studied the photophysics of temoporfin using quantum chemical methods. Whereas the former analyzed temoporfin in a polar solvent (258), and later also in liposomes (see below) (259), the latter compared temoporfin to its corresponding *ortho*- and *para*-isomers concluding that the high efficacy of temoporfin – the *meta*-isomer – is not due to photophysical parameters but the better localization in tumor cells and tissues (84). Quantum chemistry (*vide supra*) has also been used for photophysics optimization approaches of porphyrins and chlorins for PDT involving temoporfin (81–83). Quantum chemical methods were

also used to satisfactorily explain the higher photodynamic activity of temoporfin compared to its porphycene analogue, temocene (79). Also, the two-photon absorption properties of temoporfin have been analyzed (see below under ‘Dosimetry and Detection’) (61,260–262).

Only a limited number of investigations studied different frequently used photosensitizers under the same conditions. In one of those, Berlanda *et al.* (151) tested Foscan®, Fospeg® (a liposomal formulation of temoporfin containing pegylated lipids, see below), hypericin, aluminium(III) phthalocyanine tetrasulfonate chloride (AlPcS<sub>4</sub>), δ-aminolevulinic acid (ALA) and Photofrin® in the A431 cell line with wavelength-specific LED-based illumination, finding distinct differences between them. They confirmed that temoporfin in both formulations is a very effective photosensitizer that induced high phototoxicity already at very low concentrations giving the lowest LD<sub>50</sub> value under PDT of all tested photosensitizers (151). The efficiency of several photosensitizers including temoporfin, measured as the depth of necrosis was hypothesized to be correlated with their photostability in solution; however, a clear correlation could not be established (263). Already in 2005 Mitra and Foster compared the PDT efficacy of Photofrin® and temoporfin in EMT6 tumor cell spheroids and found that the higher efficacy of temoporfin is due to its photophysical properties (enhanced redshift absorption compared to Photofrin®) but also due to its ability to sequester tightly in (tumor) cells (264).



The photophysical properties of temoporfin in different carrier systems have been investigated as well (see also 'Formulation Development'). In a comparative investigation of three chlorin systems (temoporfin, disulfonated tetraphenylchlorin (TPCS<sub>2a</sub>), chlorin e<sub>6</sub>) in solution and in liposomes, the order of efficacy followed the sequence given above. This was attributed to the embedding of the different photosensitizers in the liposomal membrane (265). In human serum albumin (HSA) nanoparticles the ability of temoporfin to generate singlet oxygen depended on the loading ratio with only particles of lower loading able to generate singlet oxygen (188). However, after incorporation in Jurkat cells, all particles generated singlet oxygen based on the release of temoporfin. Also, the influence of antioxidants on the photophysics of temoporfin has been investigated (85). The phototoxicity in cells and photophysics of halogenated bacteriochlorins carrying sulfonic acids and sulfonamide moieties have been investigated in comparison with temoporfin. Whereas the PDT effect of temoporfin was associated primarily with a Type II reaction, the PDT effect of investigated bacteriochlorins was concluded to rest on Type I and Type II photochemical reactions (68). In a series of publications, the photosensitizing mechanism of temoporfin – serving as a model photosensitizer – has been analyzed with field-induced droplet ionization mass spectrometry at the air-water interface using lipids and on-purpose designed lipid-like molecules (266–268). The authors observed the formation of allyl hydroperoxides by oxidation of unsaturated lipid chains rationalized by the Type II mechanism (266). Later, evidence for the involvement of Type I photoreaction was found as well (267). This oxidation could be hindered by host-guest complexation of such amphiphilic molecules (268).

At the cellular level, PDT elicits a multitude of effects and affects numerous intracellular pathways (245). In addition, such effects observed on the cellular level depend on the light dose and the time after illumination (42,116). Therefore, sometimes seemingly contradictory results originate from different cell testing parameters and different observation windows. For PDT with temoporfin, the ER and the Golgi apparatus have been identified as primary sites of action in the cell (116,117,247,269,270). This cellular targeting of the ER has been studied in detail for three different photosensitizers (hypericin, temoporfin and methylene blue) using multifunctional luciferase reporter systems, confirming the ER targeting for hypericin and temoporfin (118). Mitochondrial oxidative stress followed by cytochrome c release and caspase activation resulting in an apoptotic cell response was determined for PDT with temoporfin (42,152,168,271), though this is not always observed in cellular assays (110). The change from apoptotic events to autophagy has been investigated in MCF-7 cells subjected to temoporfin and light (116). The importance of autophagy for temoporfin PDT has also been shown in colorectal cancer cells and *in vivo* in mice (135). The ROS generated by PDT were found to activate the signaling pathway *via* the c-Jun N-terminal kinases (135). PDT-induced cell damage results in the release of large numbers of extracellular vesicles, an effect that has been observed *in vitro* as well as *in vivo* (180). These extracellular vesicles can be exploited themselves as carriers for photosensitizers (see below under 'Formulation development').

PDT is also associated with the increased expression of heat shock proteins (97,272). Mitra *et al.* (161) found a correlation between long-term tumor control *in vivo* in mice and extracellular release of heat shock protein 70. This up-regulation of

selected heat shock proteins has also been observed in two head and neck cancer cell lines (UMB-SCC 745 and 969) and the increased expression of heat shock proteins was used for specific targeting with verteporfin as a photosensitizer (272). PDT with the liposomal temoporfin formulation Fospeg® increased the P-glycoprotein expression – which is an important protein with respect to multidrug resistance in cancer cells – in human nasopharyngeal carcinoma cells (165). However, as temoporfin was no substrate to this transporter protein the PDT efficacy was not affected. The same authors also investigated the effect of Fospeg® on Epstein-Barr virus positive nasopharyngeal carcinoma cells (166). Temoporfin PDT led to an up-regulation of the cytokines VEGF and IL-1alpha in A431 carcinoma cells (154). Apart from such effects on specific proteins temoporfin has been observed to have a more general impact, e.g. on microtubules and cell endosomal transport (181). Other recent publications found phenotype shifts in macrophages elicited by temoporfin nanoparticles (163) and in Ras-transfected mammary cells, where a reduction of the migratory and invasive ability was observed for cells treated with PDT using different photosensitizers including temoporfin (162). Sphingolipids and their ceramide subgroup are building blocks of membranes, but also fulfil numerous other functions in the cell and have been shown to be involved in PDT (95,169). In a series of publications, Korbek and Separovic (95,96,169,195,273–275) analyzed the relevance of sphingolipids for PDT with temoporfin and investigated the synergistic effect of combination therapies, e.g. with the ceramide analogue LCL29, the synthetic retinoid derivative fenretinide and a ceramidase inhibitor.

Cell death mechanisms and pathways have been and are still a matter of debate in PDT (8,110,248–250). Whereas for a long time the modes of unregulated cell death *via* necrosis or the programmed cell death *via* apoptosis in combination with autophagy were the main focus, recently, other modes of cell death have found increased attention with respect to PDT, specifically cell death triggered by the bodies' immune response (249). Induction of apoptosis by temoporfin PDT has been shown in many cellular assays (42,90,116,124,135,149,150,152,168,199). This cell death by apoptosis is often found to be accompanied by autophagy (90,116,135,152). In a comparative investigation, Lange *et al.* (90) investigated cellular death pathways after temoporfin PDT in five cancer cell lines (A-427, BHY, KYSE-70, RT-4, and SISO cells). In this case, cells were treated with equitoxic concentrations of temoporfin. Mitochondrial photodamage and ROS formation were observed in all cases. Apoptosis was identified as the dominant cell death mechanism, accompanied by autophagy. Lipid peroxidation and cell death due to loss of membrane integrity were found to be less important. However, there were considerable differences between the cell lines and the times when certain effects were found, and the authors suggest an interplay of different cell death mechanisms. That other cell death mechanisms also play a role for temoporfin has also recently been shown by Kessel who reported on the induction of paraptosis following temoporfin PDT-induced ER photodamage (110).

As mentioned above, the effects of PDT on the immune system contribute to the tumor control effect observed *in vivo*, and this has been discussed in numerous reviews (8,249,252,253,255–257,276,277). By activation of the T-cell adaptive immune response PDT can trigger immunogenic cell death (249). In the treatment of actinic keratosis and squamous

cell carcinoma, PDT has been found to activate the innate and adaptive immune system, following the local inflammatory response (277). Theodoraki *et al.* (254) analyzed blood samples from nine head and neck cancer patients treated with PDT for changes in different immune-cell subsets. Samples were taken before, during and after PDT and were compared to samples from age-matched healthy donors. They observed a systemic inflammatory immune response and found altered profiles of immune cell populations and cytokine concentrations (increased number of Treg and NK cells). The authors suggest that a combination of PDT with immune checkpoint modulators could lead to an improved anti-tumor response. Looking at the systemic effects of PDT resistance to PDT comes into focus as well, which was discussed in a recent review (278). Possible resistance mechanisms to PDT with temoporfin were investigated by Kralova *et al.* in cells and in mice bearing tumors grown from PDT-resistant cancer cells (193). Apart from temoporfin, ethylene glycol derivatives of 5,10,15,20-tetraphenylporphyrin were used as well. Interestingly, different resistance mechanisms were found; the more polar porphyrin ethylene glycol derivatives showed increased drug efflux through ABCB1 P-glycoprotein, whereas for the more lipophilic temoporfin a sequestration to lysosomes was observed.

### Animal testing

Animal testing is still an indispensable measure in the pre-clinical development of pharmaceuticals though there is of course intense research going on to reduce, refine and replace such experiments according to the three R's principle (279). Quite a number of animal tests have been performed in recent years with temoporfin and its formulations (see Table 2). Of the many investigations listed in the review by Senge and Brandt from 2011 (42), many focused on elucidating the basic pharmacokinetic and pharmacodynamic properties of temoporfin in precedence of the marketing authorization application. As can be seen from the studies listed in Table 2 the newer *in vivo* investigations are to a large extent focused on the development of new formulations, new (tumor) indications and new treatment regimens. More than half of the investigations cited in Table 2 studied the *in vivo* behavior of new pharmaceutical formulations of temoporfin. Consequently, those publications are reviewed in the chapter about formulation development.

Animal experiments with temoporfin have almost exclusively been performed in rodents, predominantly in mice and also in rats. Single studies have been done in beagle dogs (308) and sheep (309), the two animal species chosen due to the specific requirements of the tumors to be treated, prostate and esophagus, respectively. Special cases are the treatment of cats (310) and horses (311). In these cases, a curative tumor treatment was intended, showing that PDT is also a treatment option for certain tumors in animals (*vide infra*).

A number of publications investigated the pharmacokinetics and the PDT effect of temoporfin with respect to specific tumors. Etcheverry *et al.* looked at the pharmacokinetics of the PS in mice bearing fibrosarcoma *via* fluorescence measurements (282). They observed a 'rebound effect', *i.e.* a fluorescence increase in certain parts of the tumor compared to that measured immediately after illumination. Fluorescence detection has also been used in other cases to investigate pharmacokinetics *in vivo*

(303–306). In an investigation of rats, Kruijt *et al.* found a good correlation between quantification by fluorescence and chemical extraction (305). Also, singlet oxygen luminescence has been used as a dosimetric technique *in vivo* showing a good correlation between tumor response and singlet oxygen luminescence (288). Ferraz *et al.* determined the PDT threshold dose for different photosensitizers including porfimer sodium, bremachlorin (Radachlorin®) and temoporfin using a model employing the depth and width of necrosis in rat liver. They determined a value of  $5.3 (\pm 2.0) \text{ J cm}^{-2}$  at a temoporfin dose of  $0.3 \text{ mg kg}^{-1}$ , considerably smaller than for porfimer sodium ( $28.0 (\pm 2.0) \text{ J cm}^{-2}$ , dose  $2.0 \text{ mg kg}^{-1}$ ) (307). In a similar approach, *in vivo* necrosis experiments in mice combined with modelling were used to determine singlet oxygen threshold doses for PDT for different photosensitizers including temoporfin (285,286,312). Garrier *et al.* investigated the effectiveness of a compartmental targeting in xenografted tumors in mice, finding that fractionated double-injection of temoporfin with 24-h and 3-h drug-light intervals (DLI) yielded 100% tumor cure (296).

In a study with cellular assays and in mice the effect of temoporfin PDT on the induction and release of heat shock protein 70 was analyzed, showing that there was a clear correlation between temoporfin PDT doses achieving long-term tumor cure and those that effect high levels of surface exposed or extracellularly released HSP70 (161). In a mechanistic investigation, the apoptosis and activity of different caspases has been studied in cell cultures and in mice (124). The authors observed that photosensitized tumors had a higher number of cells with active caspase-3 and poly-ADP-ribose polymerase 1, suggesting that polymerase expression was mediated by treatment-induced apoptosis. A similar mechanistic investigation has been addressing the importance of autophagy in cells and in mice (135). As mentioned above (cf. 'mechanistic aspects'), resistance to PDT was investigated by Kralova *et al.* (193) in mice bearing tumors grown from PDT-resistant cancer cells and found to differ depending on the photosensitizer structure.

Temoporfin in a liposomal formulation was combined with natural antioxidants derived from *Pinus halepensis* in an investigation in mice to assess the use of such antioxidants to prevent photosensitizer-induced (skin) photosensitivity (291). The combination allowed to eliminate the tumor in this murine prostate cancer model while at the same time reducing photosensitivity. Similar protective effects of *P. halepensis* extracts were observed in *in vitro* experiments in the LNCaP prostate cancer cell line (176). Korbek *et al.* (273,275) investigated the combination of temoporfin and a ceramide (LCL29) for PDT in a murine tumor model with squamous cell carcinoma. They found that this combination increased cancer cell apoptosis and long-term tumor cure. In addition, specific changes in the sphingolipid profile were observed during and after PDT (275). The authors also addressed the role of ceramides and sphingosines in PDT in several *in vitro* investigations (95,96,169,195,273,274).

In addition, the combination of PDT with antibody treatments has been tested in murine models. Korbek *et al.* (284) analyzed whether an immunodepletion of granulocytic myeloid regulatory cells by administration of the anti-GR1 antibody would improve the effect of PDT and PDT-generated anti-cancer vaccines. They found that this strongly depended on the timeframe, immediately after PDT the administration of the anti-GR1 antibody eliminated the curative PDT effect in the mice, whereas after 1 h an

increased tumor response was observed. Temoporfin PDT has also been combined with chemotherapy and immune lymphocytes collected from mice pre-treated with PDT to cure advanced L1210 tumors in mice, showing a synergistic anti-tumor effect (297). A similar synergistic effect was observed for the combination of temoporfin PDT with the anti-VEGF antibody bevacizumab; however, only if PDT preceded the administration of bevacizumab (281).

One of the fields, where the clinical use of PDT is explored is liver cancer (see below). Wang *et al.* looked at temoporfin pharmacokinetics, distribution and elimination in an orthotopic liver cancer model in the rat in preparation for clinical investigations (300). The plasma concentration over time could be fitted with a two-compartment model. Tissue distributions showed the highest accumulation in the tumor with a subsequent decrease to liver, heart, spleen, muscle, and skin, the tumor-to-normal tissue localization ratio being highest at 24 h. PDT with temoporfin was studied in cell cultures and in mice bearing Lewis lung carcinoma and effectively inhibited tumor growth and prolonged survival. However, tumors regained their growth potential after nine days (200). PDT with temoporfin (0.6 mg kg<sup>-1</sup>) and verteporfin (1 mg kg<sup>-1</sup>) was tested in a mouse xenograft model for retinoblastoma, using an irradiation wavelength of 514 nm for temoporfin and 689 nm for verteporfin. The retinoblastoma xenografts derived from different patient cell lines reacted differently to the PDT treatment, with temoporfin showing a significant but transient response and verteporfin PDT being effective in xenografts where temoporfin was not (295). Temoporfin has also been investigated in mice with kidney tumors, suggesting it may be suitable as a nephron-sparing therapeutic option for small tumors (292).

While the majority of pre-clinical animal testing is done in rodents, sometimes specific indications require different animal models. *e.g.* Glanzmann *et al.* (309) assessed a sheep animal model for a PDT treatment of esophagus lesions with temoporfin. Sheep and human esophagus are closely comparable histologically and the pharmacokinetics of temoporfin were investigated by blood sampling and fluorescence *in vivo* measurements using the clinically approved dosage of 0.15 mg kg<sup>-1</sup>. The maximum temoporfin concentration in plasma was observed after 10 h, and the maximum temoporfin fluorescence was observed in the oral cavity between 30 and 50 h. As the sheep and human tissue sensitivity to temoporfin PDT was found to be similar as well, the model was deemed suitable for optimizing esophagus PDT with temoporfin (309). In a similar way, the canine prostate was used as a model to perform light dosimetry during interstitial PDT with temoporfin (308). Again, temoporfin was used in a dose of 0.15 mg kg<sup>-1</sup> and administered 72 h prior to illumination. The PDT effect was assessed by magnetic resonance imaging, pathology and histopathology of excised tissue. The results allowed to determine a threshold dose of 20 to 30 J cm<sup>-2</sup> to induce prostate tissue necrosis.

### Formulation development

Temoporfin is a quite lipophilic substance with an estimated octanol-water partition coefficient (logP) of >9, rendering it practically water-insoluble (313). The combination of the tetrapyrrole skeleton with NH-groups, together with the polar hydroxyphenyl groups gives the molecule a high affinity to membranes, proteins

and lipoproteins, which is desirable with respect to the mode of action – the photodynamic damage to tumor cell structures. The affinity of temoporfin and other photosensitizers to membranes, proteins, and lipoproteins is also important with respect to their transport to the tumor tissue after intravenous administration (42,314). However, this poses a challenge for their pharmaceutical formulation (315,316). Of course, the problem of the pharmaceutical formulation of highly lipophilic, water-insoluble drug molecules is not unique to photosensitizers but a general issue for many new drug substances (317,318).

The presently approved drug formulation of temoporfin (Foscan®) is a solution of temoporfin in a mixture of ethanol and propylene glycol (319). This formulation shows a delayed pharmacokinetics profile, which is why the PDT treatment is performed 96 hrs after injection (42,319). Hence, numerous publications deal with possible new formulations of temoporfin (see below) (320,321). As for many other anti-cancer drugs, nanoparticles and nanoparticle formulations play an important role in this respect (322), often with reference to the EPR (enhanced permeability and retention) effect (323,324). Hence, nanoparticles have been specifically exploited as carriers for photosensitizers (24,315,316,321,323,325–331). Apart from serving as carriers, the nanoparticulate formulation of photosensitizers can additionally be used to enhance the singlet oxygen generation (332) or may be used to switch the operating photochemical mechanism (333).

The studies related to the delivery of temoporfin *via* nanoparticles have quite recently been reviewed expertly by Yakavets *et al.* (320). Investigations on the incorporation of temoporfin in nanoparticles cover a broad range of materials comprising systems such as extracellular vesicles (186,211,212), supramolecular organic frameworks (99), or polymer-*block*-peptides (334,335), hydrogels (336–338), as well as carrier systems for which there is already considerable pre-clinical and clinical experience. Examples for the latter are PLGA [poly(lactic-co-glycolic acid)] (111,137,138) and HSA nanoparticles (132,189,190) and liposomes (42,298,320) (Fig. 7).

### Chitosan nanoparticles

*m*THPP has been used as a model substance for temoporfin in the incorporation into polymethacrylic acid (PMAA)-modified chitosan nanoparticles finding a high loading capacity for the substance and uptake into 14C cells (184). Temoporfin itself has been incorporated into polyplex nanoparticles consisting of sodium alginate and a chitosan polymer or oligomer (339). The nanoparticle size distribution could be modified by the concentration of the solutions, order of addition, the ratio of addition and the pH. Chitosan oligosaccharides were used in the formation of core-shell nanoparticles loaded with temoporfin and targeted with an RGD peptide to address integrin-rich tumors (214). The targeted core-shell nanoparticles showed a deeper penetration into U87MG tumor spheroids and had a higher anti-tumor efficacy than non-modified nanoparticles in U87MG tumor-bearing mice. Recently, these nanoparticles were combined with checkpoint inhibitors (anti-PD-1/PD-L1 antibodies) for PDT and immunotherapy for colorectal cancer inhibiting primary and distant tumor growth in mice (133). *m*THPP-ethylcellulose/chitosan nanoparticles have also been studied for their antibacterial action (227).

### Human serum albumin nanoparticles

Wacker *et al.* (189) reported on the preparation of temoporfin-loaded HSA nanoparticles, the photophysical properties of which were later investigated in more detail (188). For these particles, a freeze-drying method was developed which is important with respect to the storage of a future pharmaceutical formulation (189). In photophysical investigations in D<sub>2</sub>O only particles with low temoporfin loading generated singlet oxygen; however, in Jurkat cells all formulations generated singlet oxygen suggesting that the particles were successfully taken up by the cells releasing temoporfin (188,189). The release of temoporfin from the HSA nanoparticles was found to occur via lysosomal decomposition of the nanoparticles with the HSA nanoparticles exhibiting a higher phototoxicity than free temoporfin (190). Quite recently, an optimized protocol for albumin nanoparticles loaded with temoporfin was published making use of the nanoparticle albumin-bound (*nab*)-technology which is also applied for the medicinal product Abraxane®. The nanoparticles were effectively taken up in the TFK-1 cholangiocarcinoma cell line and showed high phototoxicity (132). Recently, pulsed dipolar electron paramagnetic resonance (EPR) has been used to investigate the binding of *m*THPP to BSA (bovine serum albumin) and identified the proton-rich pocket of HSA subdomain IIIA and the main binding site (340).

### PLGA nanoparticles

The incorporation of photosensitizers in nanoparticles often results in a reduced dark (cyto)toxicity compared to the free photosensitizer. The same was also observed for the incorporation of temoporfin in PLGA nanoparticles (137). The lysosomal decomposition and release of the temoporfin were faster for PLGA than for HSA nanoparticles (341). Rojnik *et al.* (111) compared pegylated and non-pegylated PLGA nanoparticles in cells and in mice. Both kinds of nanoparticles were found to be distributed in the cytoplasm in MCF10AneoT cells shortly after incubation with a preference for endoplasmic reticulum and Golgi apparatus, whereas at later time points temoporfin fluorescence was observed in lysosomal-endosomal compartments. Differences were observed for *in vivo* distribution, e.g. in colon tissue the temoporfin delivery was highest for the pegylated PLGA nanoparticles whereas in lung tissue it was highest for the non-pegylated nanoparticles (111). The rational design and scale-up manufacture of temoporfin-loaded pegylated PLGA nanoparticles has been investigated in more detail, again revealing reduced dark toxicity but efficient phototoxicity of the final formulation (138). The nanoparticles also exhibited an altered pharmacokinetic behavior: With Foscan®, precipitation of the compound at the injection site (the tail vein) and a delayed distribution was observed. This was not the case for the nanoparticle formulation (138). Temoporfin-loaded PLGA nanoparticles were employed for the fabrication of core-shell nanoparticles with a temoporfin-PLGA-core and an albumin shell (126). Similar systems were prepared with *m*THPP (342). Boeuf-Muraille *et al.* (198) found in *in vitro* release studies in a buffer of temoporfin-loaded PLGA nanoparticles that approx. 50% of temoporfin was retained in the nanoparticles after 5 days. Cell testing in the murine C6 glioma cell line revealed apoptosis as the main cell death mechanism. Elberskirch *et al.* (226) used the HET-CAM model with the duodenum adenocarcinoma cell line HuTu-80 employing

temoporfin-loaded PLGA nanoparticles as well as nanoparticles with two porphyrin-based photosensitizers, finding that the model was suitable to simulate *in vivo* endoscopic irradiation. PLGA nanoparticles coated with a phospholipid bilayer have also been tested for co-encapsulation of temoporfin and a chemotherapeutic, exhibiting an additive effect against a retinoblastoma cell line (183).

### Polymer systems

Different polymer systems (343) have been used for the encapsulation of *m*THPP (344) and temoporfin and for studying the PDT effect of the resulting nanoparticle formulations. Temoporfin has been loaded on Eudragit® nanoparticles – which is an approved pharmaceutical ingredient – and up-scaling for this formulation has been investigated (185). In addition, the drug release from these particles was studied using mathematical models (345). One of the advantages of polymeric carriers is the broad variation of functionalities allowing the inclusion of specific linkers that enable the release of – sometimes covalently bound – photosensitizers based on specific triggers (346). pH-sensitive nanoparticles based on hyperbranched polyglycerol and 2-(diisopropylamino)ethyl methacrylate loaded with temoporfin have been prepared (63,139) while polyglycerol-based nanogels with the temoporfin congener *m*THPP have also been specifically tested for dermal delivery (347). Such systems are of interest for the local administration of photosensitizers specifically in skin cancer (348). Systems with a disulfide linker have been prepared as well, which make use of the difference in the redox potentials between the cellular membrane and the bloodstream (50,63). It is known that the bloodstream has a global potential that is mildly oxidative whereas the intracellular potential is mildly reductive (63).

Recently, the concept of light-responsive polymers (349) as delivery systems for temoporfin has come into focus (148). Light-sensitive polycarbonates were combined with PLGA to encapsulate temoporfin. Light-induced nanoparticle degradation then led to the local release of the photosensitizer. Upon irradiation, with UV light a decreasing particle count rate and an increased release of temoporfin compared to standard PLGA nanoparticles were found (350). Pegylation was used to obtain particles with mucus-penetrating properties (351,352) for intestinal PDT (147). Later this concept was extended to other polymers (140).

Temoporfin has also been incorporated in polymeric micelles based on different polymers. For example, Fang *et al.* (192) prepared styrene maleic acid copolymer micelles loaded with temoporfin and tested their in cell cultures and in mice. They observed a lower dark toxicity *in vitro* and reduced side effects *in vivo*; concomitantly, the polymeric micelles maintained their antitumor PDT effect longer as compared to free temoporfin. Similar results were obtained for poly(2-ethyl-2-oxazoline)-*b*-poly(*D,L*-lactide) diblock copolymer micelles loaded with temoporfin (141). For folate-conjugated polymeric micelles loaded with temoporfin, an enhanced PDT efficacy compared to free photosensitizer or polymeric micelles without folate conjugation was observed in *in vitro* and in a murine model (94). Polymeric micelles based on  $\epsilon$ -caprolactone have been described in several publications. Wennink *et al.* (196) prepared specific temoporfin-loaded  $\epsilon$ -caprolactone micelles which were degraded faster by macrophages than by endothelial cells thereby releasing

temoporfin and exhibiting higher phototoxicity for this cell type – in the context of a selective macrophage elimination by PDT. However, as the temoporfin was found to be rapidly released from the micelles in plasma this selectivity could not be exploited *in vivo*.  $\epsilon$ -Caprolactone micelles loaded with temoporfin were also tested in two other *in vivo* investigations in mice investigating the effect of aromatic groups on the polymer (101) as well as that of dithiolane-crosslinking and pegylation (280). The presence of aromatic groups in the polymer and non-crosslinked micelles resulted in higher temoporfin retention. This polymeric micelle approach has also been employed to produce antibody-nanoparticle conjugates with a Llama or an EGFR-targeted antibody (102,156).

### Peptides

Peptides have been used for a long time as targeting units for photosensitizers (353). However, the combination of peptides and polymers has also been exploited as drug transporters for temoporfin. Ahmadi *et al.* (103) used amphiphilic block-copolyptides as carriers for dermal drug delivery, again taking the temoporfin congener *m*THPP as a model drug. In another approach polymer-block-peptides were exploited for drug delivery. Combinatorial chemistry methods were used to select small peptide sequences to specifically bind small drug molecules, using temoporfin as an example (334,335,354–357).

As in many other cases, temoporfin proved to be a very suitable compound for investigating the encapsulation of hydrophobic drug molecules in nanoformulations, as its encapsulation and release can easily be followed by means of optical spectroscopy (355). The loading capacity of the formulation as well as release kinetics were found to be strongly influenced by the specific peptide sequence, and transfer from the nanoformulation to BSA, as a model for HSA, could be observed (355). Switchable release profiles with these systems could be realized by introducing disulfide linkers into the peptide sequence (334) and the pharmacokinetics could be further modified by PEGylation (335,354). The concept has also been applied to the preparation of nanogels suitable for dermal drug delivery (356). The utility of the concept was further tested by comparing the solubilization of three related tetrapyrrole-based photosensitizers, temoporfin, chlorin *e*<sub>6</sub>, and pheophorbide *a*, finding significant binding capacity differences (357). Apart from peptides, the concept was additionally investigated, using temoporfin as a model drug, with lipid-DNA (143), thiolactone polymers (358), peptoid sequences (359), and alternating co-polymers (360).

Resonance energy transfer can be used to excite photosensitizer molecules. This has been exploited in *m*THPP containing quantum dots (361,362) and the combination of semiconducting polymer dots (Pdots) with temoporfin. Zhang *et al.* (120) prepared Pdots functionalized with folic acid and horseradish peroxidase. The fluorescent polymer backbone served as a light antenna and as a hydrophobic carrier for temoporfin. In this system, after luminol-H<sub>2</sub>O<sub>2</sub> activation, indications for chemoluminescence resonance energy transfer were found. In another system, Pdots with coatings of different pegylated lipids were synthesized, the polymer backbone serving again as the light antenna and the lipid coating ensuring close contact to the photosensitizer temoporfin, enabling efficient fluorescence energy transfer (121,363). Both compositions were effectively taken up in cancer cells (120,121,363).

### Extracellular vesicles

Extracellular vesicles (EVs) have recently found considerable interest as new carrier systems (364). Aubertin *et al.* (180) showed that PDT with temoporfin as well as chemotherapeutic treatment with doxorubicin resulted in a high production and release of extracellular vesicles from cancer cells, an effect that was also observed *in vivo* in mice. The effect was more pronounced for PDT than for chemotherapy (180). Such cell-released vesicles could be loaded simultaneously with temoporfin and magnetic nanoparticles (164). Piffoux *et al.* (186) prepared extracellular vesicles loaded with temoporfin and iron oxide nanoparticles as theranostic agents for PDT, MRI and hyperthermia and compared different methods for their production. They identified starvation of cells in a serum-free medium followed by ultracentrifugation as a suitable method for production and purification of the vesicles. The same authors later modified the extracellular vesicles by fusion with liposomes (187). Loading with hydrophobic or hydrophilic molecules was possible without leakage of the intrinsic load of the vesicles. Temoporfin-loaded extracellular vesicles have been compared to liposomal temoporfin in multicellular tumor spheroids and *in vivo* in HT29 xenografted mice (211,212). The authors observed deeper penetration of temoporfin into the tumor spheroids and an improved biodistribution and PDT efficacy *in vivo* (212). In the context of a PDT-immunotherapy combination extracellular vesicles have been prepared from mesenchymal stem/stromal cells targeting peritoneal metastasis in mice, where the authors observed a promotion of antitumor immune cell infiltration in addition to the primary PDT effect (201). Temoporfin-loaded extracellular vesicles have also been tested in comparison to a liposomal temoporfin formulation and a liposomal formulation additionally containing cyclodextrin-bound temoporfin (see also below) (219). In this investigation of stroma-rich head and neck cancer tumor spheroids (220) the extracellular vesicles demonstrated the highest loading capacity and deep penetration into the tumor spheroids (219).

### Cyclodextrins

Pronounced differences in pharmacokinetics in mice were observed between free temoporfin and temoporfin inclusion complexes with cyclodextrins (CDs) (144). The photosensitizer forms 1:2 inclusion complexes with  $\beta$ -cyclodextrins in a 2-step-mechanism (365). With temoporfin- $\beta$ -cyclodextrin complexes, a faster and increased accumulation in the tumor of HT29 tumor-bearing mice were found, together with a decreased level of temoporfin accumulation in skin and muscles (144). The release of temoporfin from the CD inclusion complexes and transfer to serum components could be studied making use of the Soret band shape which was found to be sensitive to changes in the immediate surroundings of the molecule (366). However, other optical methods amended by computational modelling can be used for such analyses (367,368). Similar to the above-mentioned extracellular vesicles,  $\beta$ -cyclodextrin complexes led to a higher accumulation and deeper penetration in HT29 multicellular tumor spheroids compared to free temoporfin (210). However, the temoporfin- $\beta$ -CD complexes are sensitive to dilution; therefore, hyper-crosslinked cyclodextrin monomers were prepared, capable of forming inclusion and non-inclusion complexes (221). With *m*THPP it was shown that covalently linked PS- $\beta$ -

cyclodextrin compounds had better PDT activity *in vitro* against glioblastoma cells compared to inclusions complexes (369). The affinity of temoporfin to cyclodextrin could also be influenced by methylation of the cyclodextrin carrier (129,365). Temoporfin and  $\beta$ -cyclodextrins have also been combined with liposomes as a second carrier to obtain 'drug-in-cyclodextrin-in-liposome' nanoparticle systems. In these, most of the temoporfin was bound to cyclodextrins in the inner aqueous liposome core (370). Later this system was modified to obtain lipid vesicles containing temoporfin  $\beta$ -cyclodextrin inclusion complexes in the inner aqueous core as well as lipid compartments of the vesicles (145). However, in an *in vivo* test in mice with xenografted HT29 tumors, the PDT efficacy was similar to that of a liposomal temoporfin formulation (145).

### Lipid nanoparticles

*Lipid nanoparticles* (371) have been used as carrier systems for temoporfin as well. Navarro *et al.* (122,123) reported on the preparation, characterization and cell testing of solid lipid nanoparticles encapsulating temoporfin. The lipid nanoparticles were composed of a lipid core, stabilized by phospholipids and pegylated surfactants. The temoporfin-loaded lipid nanoparticles exhibited photocytotoxicity in MCF7 cells; however, there was also some toxicity induced by the empty lipid nanoparticles. Hinger *et al.* (223) evaluated these lipid nanoparticles in two different sizes (50 and 120 nm) in 3D CAL-33 cancer cell spheroids, where the 50 nm lipid nanoparticles exhibited the same PDT efficacy as free temoporfin. Later, the same authors compared the temoporfin-loaded lipid nanoparticles with the approved drug formulation (Foscan®) and a liposomal formulation (Foslip®, see below) in CAL-33 tumor-bearing mice (283). With respect to tumor response, Foslip® gave the best results, whereas the temoporfin-loaded lipid nanoparticles were better tolerated and gave less side effects. Brezaniouva *et al.* (127) compared the *in vitro* and *in vivo* efficacy of 1-tetradecanol-based and copolymer stabilized solid lipid nanoparticles loaded with temoporfin with Foscan®. In the experiments in mice, certain formulation variants performed considerably better than the approved drug formulation. However, partial tumor relapse was observed, mostly at the periphery of the original tumor (127). Rad *et al.* (191) compared temoporfin-loaded lipid vesicular (liposomal) and discoidal nanoparticles with and without folate targeting for their PDT efficacy in cellular assays and in mice. The authors found a clear morphology effect with the folate-decorated discoidal nanoparticles being more effective than the folate-decorated vesicular nanoparticles.

In a combined photothermal and PDT approach oil droplet nanoparticles were loaded with temoporfin and the photothermal agent IR780 (202). The combined photothermal/PDT efficacy was investigated *in vitro* and *in vivo*. Irradiation at 808 nm (for IR780) and 660 nm (for temoporfin) effectively suppressed tumor growth in TRAMP-C1 tumor-bearing mice. Lipid nanoparticles have also been used in release studies (372,373). Trimyristin lipid nanoparticles or phospholipid liposomes were loaded with substances of different polarity, including temoporfin, and transfer from these donor nanoparticles to oil-in-water emulsions was studied by flow cytometry and separation of donor and acceptor by ultracentrifugation (372). Flow cytometry proved to be a suitable method to investigate dye transfer.

### Inorganic nanoparticles

*Inorganic nanoparticles* have been used for loading with or encapsulation of temoporfin, *e.g.* temoporfin PDT and fluorescence imaging have been combined with RGDfK peptide targeting in CAL-27 cells and in mice with calcium phosphate nanoparticles (170). Haimov *et al.* (53) covalently conjugated temoporfin to gold nanoparticles and tested their PDT efficacy in SH-SY5Y human retinoblastoma cells. Temoporfin has been loaded on maghemite nanoparticles and magnetic targeting followed by PDT was investigated in mice (128). Silva *et al.* (164) prepared cell-derived vesicles loaded with temoporfin and magnetic nanoparticles and tested them *in vivo* in a murine tumor model. The distribution in the animals could be followed by fluorescence as well as MRI. Temoporfin and iron oxide nanoparticles were also incorporated in liposomes – temoporfin in the lipid bilayer and the iron oxide particles in the aqueous core – and these liposomes were successfully tested on mice for a combined hyperthermia-PDT application (172). Shah *et al.* (104) used a similar approach of liposomes incorporating temoporfin and magnetite nanoparticles but additionally encapsulated the chemotherapeutic doxorubicin. They investigated the separate and combined effect in HeLa cells finding the combination to be more effective than the individual treatments. Silva *et al.* (216) employed cell-derived vesicles to load them with doxorubicin, a tissue-plasminogen activator and the two photosensitizers temoporfin and disulfonated tetraphenylchlorin (Fimaporfin, TPCS<sub>2a</sub>). They could show *in vitro*, using photosensitizer fluorescence, that the microvesicles are taken up by cancer cells (SKOV-3, TC1 and PC-3 cells) and that the vesicles can be magnetically manipulated. The two photosensitizers localize in different cellular compartments; temoporfin was found in the cytoplasm whereas TPCS<sub>2a</sub> accumulated in endosomes (181). Silica nanoparticles have successfully been tested as well *in vitro* (157,194) and *in vivo* (194) as carriers for temoporfin. In the *in vivo* tests in mice, it was found that the temoporfin-loaded silica nanoparticles were able to cross the blood-brain barrier (194). Silica nanoparticles containing carbon dots have also been simultaneously loaded with temoporfin and a nitric oxide photodonor (374). Hsu *et al.* (112) prepared luciferase-immobilized quantum dots which were activated by addition of coelenterazine. These bioluminescent quantum dots served as an internal light source for temoporfin PDT. The PDT effect was studied in cancer cell cultures and in mice. *In vivo*, a delayed tumor growth due to tumor cell apoptosis was observed (112). Yu *et al.* (49) performed a chemical modification of temoporfin with 4-(bromomethyl)benzoic acid to connect it to the surface of LiYF<sub>4</sub>:Tm<sup>3+</sup>/Yb<sup>3+</sup> upconverting nanoparticles. Upon irradiation, with light of 980 nm the particles generated singlet oxygen and photocytotoxicity in HeLa cells was detected. Timor *et al.* (375) reported on the solubilization of CdSe/CdS quantum rods by decoration with lecithin and pegylated phospholipids. This solubilizing layer was capable of carrying temoporfin allowing close contact with the quantum rods and enabling efficient fluorescence resonance energy transfer and singlet oxygen generation.

### Liposomes

*Liposomes* are by far the carrier system employed most widely for the pharmaceutical formulation of temoporfin (320). This is not unexpected as liposomes are long established as carrier

systems for pharmaceuticals, including anti-cancer drugs (376–378), and are of specific interest as carriers for photosensitizers (379–384). Moreover, liposomes carry the option of additional functionalization for drug targeting (378,379,385,386). Liposomes are unilamellar vesicles consisting of an aqueous core surrounded by a lipid bilayer. This structure allows hydrophilic compounds to be included in the aqueous core or lipophilic compounds to be incorporated in the lipid bilayer (377,386). Of course, both functions can be combined to simultaneously transport hydrophilic compounds in the aqueous core and lipophilic compounds in the lipid bilayer (see also the examples mentioned above). Temoporfin as a lipophilic membrane-affine compound is transported in the lipid compartment of the liposomes (145,368). The interaction of temoporfin with the liposomal membrane has also been studied by computational methods (259).

From a practical point of view, liposomes have the additional advantage that they can be freeze-dried – with the addition of suitable cryo-protectants – and then re-constituted prior to use, which is beneficial with respect to transport and stability. Visudyne®, which contains the photosensitizer verteporfin as the active substance and is used in the treatment of the age-related macular degeneration, is such a freeze-dried liposomal formulation to be re-constituted before administration to the patient (387). Various liposomal formulations of temoporfin have been prepared and related photosensitizers have been incorporated into liposomes as well (226), e.g. the temoporfin analogue temocene (78). There is also a special interest in liposomal formulations with respect to applications in dermatology and topical delivery (371,384,388,389).

Two liposomal temoporfin formulations are often mentioned in the publications under their tradenames, one is Foslip®, the other is Fospeg®. Foslip® are unilamellar vesicles (liposomes) consisting of dipalmitoylphosphatidylcholine (DPPC) with the addition of 10% dipalmitoylphosphatidylglycerol (DPPG) and temoporfin at a concentration of 1.5 mg mL<sup>-1</sup>. The molar drug:lipid ratio is approximately 1:12 (290,320,390). Fospeg® is similar to Foslip®, but additionally contains pegylated lipids (290). Incorporation of temoporfin in liposomes (or other carrier systems) changes the optical properties of temoporfin (265,368) and can alter the cellular response compared to simple administration of the PS (182). On the other hand, the properties of the phospholipid membrane are also affected by temoporfin (390,391).

Temoporfin-loaded liposomes based on dipalmitoylphosphatidylcholine/glycerol have a phase transition temperature near body temperature (390). Computational studies suggested that temoporfin in the lipid membrane acts as a hydrogen donor in hydrogen-bonding interactions with the polar groups of the phospholipids (259). The generation of singlet oxygen of the photosensitizers temoporfin, fimaporfin (TPCS<sub>2a</sub>) and chlorin e<sub>6</sub> – which was similar in ethanolic solution – is different when incorporated into liposomes (265). As liposomes serve as carrier system for the photosensitizer the release and transfer of temoporfin from liposomes to acceptor compartments, to membranes, or to plasma components, has been extensively studied. Different methods have been employed to study this transfer, e.g. optical methods (392,393), radioactively labelled temoporfin or lipids (391,394–396) or by flow-field-flow fractionation (391,394,397,398). The latter method has also been used for related tetrapyrrole systems (399–401). Flow field-flow fractionation was found to reliably recover liposomes loaded with temoporfin (drug recovery ~ 80%); however, drug loss was much

higher for drugs with a lower octanol-water partition coefficient (397).

Methods to rapidly screen and characterize different liposomal formulations, e.g. for size and incorporation efficiency, have been developed as well (402). Chen *et al.* investigated the transfer of a hydrophilic model drug (carboxyfluorescein) and temoporfin from vesicular systems to skin *in vitro*. They found the vesicular systems capable to improve the transfer of carboxyfluorescein into the skin, but not for temoporfin (403). Hefesha *et al.* (404) analyzed the transfer mechanism of temoporfin between liposomal membranes of different compositions. Finding apparent first-order kinetics, the transfer rates strongly depended on temperature, with positively charged donor liposomes exhibiting faster transfer than negatively charged ones. The maximum amount of temoporfin transferred was nearly the same in both cases and a model describing the release kinetics was developed (405). A strong influence of the membrane composition on temoporfin release was observed in other investigations as well (394).

The rigidity of the liposomal membrane can be influenced by membrane additives. A comparative investigation of cholesterol and temoporfin as membrane additives revealed that cholesterol increases the rigidity of the membrane whereas temoporfin lowers it (391,396,398). Reshetov *et al.* compared the transfer of temoporfin from pegylated and non-pegylated liposomes to proteins and model membranes (406,407). Using fluorescence quenching and fluorescence polarization they determined that in Fospeg® a part of the temoporfin is also in the PEG shell and not only in the lipid bilayer. They stated that at short incubation time redistribution from Foslip® and Fospeg® occurred via drug release and liposome destruction whereas at longer periods drug release is dominant (407). In a similar investigation on the transfer of temoporfin from pegylated liposomes to human plasma proteins, the authors additionally evaluated phosphatidyl oligoglycerols. These alternatives to pegylated lipids resulted in a lower transfer rate compared to pegylated liposomes (396). Using <sup>14</sup>C-labelled temoporfin in transfer experiments with human plasma, Kaess and Fahr could show that approximately 15% of temoporfin is retained in the liposomes after 48 h (395). The main acceptors in plasma were HDL and LDL, whereas albumin played only a minor role (395,397). Recently, a dispersion releaser technology combined with a four-step-model has recently been described for such release experiments using temoporfin and temoporfin-loaded liposomes as the test systems (408).

The pharmacokinetics of liposomal temoporfin were investigated in mice (298) and in rats (299,302). A study in mice bearing EMT6 xenografted tumors with i.v. administration of 0.3 mg kg<sup>-1</sup> temoporfin (as Foslip®) found that plasma pharmacokinetics and biodistribution could be described with a three-compartment model. The highest tumor-to-muscle ratios for temoporfin were observed at 6 and 15 h post administration with the best tumor response for 6 h DLI (298). In a pharmacokinetic investigation in rats using fluorescence detection with the window-chamber tumor model at a temoporfin dose of 0.15 mg kg<sup>-1</sup> de Visscher *et al.* (302) compared Foscan®, Foslip® and Fospeg®. They observed maximum tumor fluorescence at 8 h for Fospeg® and at 24 h for Foscan® and Foslip® with higher fluorescence from the liposomal formulations, suggesting a higher bioavailability for these formulations. Similar results were obtained by de Visscher *et al.* (299) with the 4NQO carcinogenesis model in rats. Fospeg® showed higher fluorescence differences in tumor vs. normal tissue at earlier time points. Decker

*et al.* (409) studied the pharmacokinetics of temoporfin and the lipid carrier in rats using radioactively labelled compounds. The pharmacokinetic data showed that part of the temoporfin is released from the liposomes before leaving the bloodstream. This release increased with decreasing bilayer fluidity. Liposomes modified with pegylated lipids or oligo-glycerols showed an increased temoporfin loss from the liposomal carrier (409,410).

Other pharmacokinetic studies of Foslip® have been reviewed earlier (42). Additionally, predictive models for pharmacokinetics and biopharmaceutics have been developed using Foslip® and Fospeg® as test formulations (411,412). Comparing the simulation to the plasma-concentration-time profiles from a phase I clinical trial with Foslip®, the authors showed that their model was able to adequately describe the pharmacokinetic profile in humans (411). Xie *et al.* (293) developed a method to quantify temoporfin from Fospeg® in HT29 tumor-bearing mice tissue samples using fluorescence imaging and correlated this with HPLC determination after tissue extraction. For Fospeg®, a higher plasma peak concentration, a longer circulation time, and a better tumor-to-skin ratio compared to data for Foslip® were found (287). Reshetov *et al.* compared Foslip® and Fospeg® with respect to, e.g. pharmacokinetics, tumor uptake and PDT efficacy *in vitro* and *in vivo* in HT29 tumor-bearing mice (290). Temoporfin release from Foslip® was found to be faster than from Fospeg®. The highest temoporfin levels were found in the spleen and liver for both formulations. The highest concentration in the tumor was reached earlier for Fospeg® (6 h) than for Foslip® (15 h).

The PDT efficacy testing of Foscan® and Fospeg® in the LNCaP prostate cancer cell line revealed a higher temoporfin fluorescence and a higher photocytotoxicity after incubation with Fospeg® (177,178). Foslip® was found to be effective in 5-fluorouracil-resistant HT29 cancer cells (209), similar to results with temoporfin in HCT116 cells (208). An increased tumoricidal effect for Fospeg® compared to Foslip® has been observed in the EMT6 CAM model (413). In comparative *in vivo* tests in MC28 tumor bearing rats with Foscan® and temoporfin-loaded liposomes with different degrees of pegylation, the latter reached the maximal tumor-to-skin ratio at earlier time points and also resulted in higher tumor necrosis (301). A higher PDT efficacy of liposomal temoporfin was also found in tests in CAL-33 tumor bearing mice (283). Related studies in osteosarcoma cells and osteosarcoma mouse models (217,218) revealed an immune-system dependent suppression of lung metastasis in the K7M2L2 mouse tumor model (217). Liposomal temoporfin and hypericin were tested in combination on head and neck SCC, finding synergistic effects of the combination (97,98). An investigation in HeLa tumor cell spheroids found temoporfin fluorescence from all three formulations (Foscan®, Foslip®, Fospeg®) only in the outer cell layer of the spheroids, with a little higher accumulation for the liposomal formulations (205). The liposomal delivery of temoporfin reduced dark toxicity effects (205). A lower dark toxicity for temoporfin in pegylated liposomes was also observed in cellular assays in CCD-34Lu fibroblasts and in A549 lung cancer cells (113). Temoporfin PDT with Fospeg® effectively triggered apoptosis in HT29 tumor cells (146). It has also been used against nasopharyngeal carcinoma cells and tumor spheroids (165–167) and Huh7 hepatocellular carcinoma (160). A photochemical internalization approach in um-scc-U2 and um-scc-U8 head and neck cancer cells showed the cytotoxicity of bleomycin to be considerably increased when PDT with Foslip® and Fospeg® was performed prior to the treatment with the drug

(159). Low-dose PDT with liposomal temoporfin reduced tumor growth in a colitis-associated murine carcinogenesis model (289). This low-dose PDT had a strong effect on inflammatory markers and was able to effectively decrease inflammation.

Apart from pegylation, other modifications have been used in connection with liposomal temoporfin formulations. *e.g.* temoporfin has been studied in DPPC monolayers investigating the effect of folate conjugation (414). A comparison of pegylated liposomes loaded with temoporfin with and without folate-targeting showed that folate-modified liposomes are taken up more in folate receptor-positive cells (114). In a similar study transferrin-conjugated liposomes loaded with temoporfin were tested with transferrin receptor-positive OE21 esophageal cancer cells. In this case, an increased uptake or increased photocytotoxicity could not be observed (158). ROS generated by the photosensitizer can in principle also attack the carrier system, *e.g.* the lipids of liposomal carriers. This has been used as an option for photo-triggered liposome membrane alteration with several photosensitizers, including *m*THPP (415).

The addition of gemini surfactants (dimeric surfactants) was tested as a measure to improve the delivery efficiency of temoporfin-loaded liposomes (215). Recently, tetraether liposomes were proposed as a new carrier system for temoporfin (174). Another option for modifying the liposomes is the combination of two carriers, like in the abovementioned cyclodextrin-liposome systems, where temoporfin was complexed by cyclodextrin within the aqueous core of the liposome (370) or additionally included in the lipid compartment of the liposomal carrier (145). Of course, liposomes can be used to combine two therapeutic or diagnostic principles as well. For example, PLGA nanoparticles containing the chemotherapeutic pirarubicin have been coated with a lipid bilayer loaded with temoporfin (175). These dual function particles were then tested in SKOV3 ovarian tumor cells and in mice. The *in vitro* tests suggested a synergistic effect (175). As already discussed above with the inorganic nanoparticles, liposomes have been loaded with temoporfin in the lipid bilayer and iron oxide particles in the aqueous core for combined hyperthermia-PDT application (172). In a similar approach, liposomes incorporating temoporfin and magnetite nanoparticles were prepared encapsulating additionally the chemotherapeutic doxorubicin, thus combining PDT, chemotherapy and hyperthermia (104).

Temoporfin has also been incorporated into liposomes with additives to enhance the penetration properties, *e.g.* to allow *i.a.* better skin penetration (384). Skin penetration has been investigated with neutral, anionic and cationic flexible liposomes based on phosphatidylcholine (416). Of these, cationic liposomes, containing additional stearylamine showed the highest penetration efficacy. As an alternative temoporfin-containing liposomal gels were prepared using carbomer as the gelling agent (417,418). So-called invasomes (419) containing ethanol and terpenes as penetration enhancers have been investigated in a series of publications (142,388,420,421). The effect of the different enhancers was analyzed, *e.g.* with respect to ethanol as a penetration enhancer the formulation with the highest ethanol content (20%) had the highest penetration efficacy, and the PDT efficacy was evaluated in cellular assays against HT29 and A431 tumor cells (142,388,421).

Liposomal formulations of temoporfin have also been tested for photodynamic antimicrobial chemotherapy (see also ‘Antimicrobial PDT’). Garrier *et al.* (294) tested collagen and collagen incubated with Foslip® as a means for wound healing in a



Table 3. Selected clinical investigations (sorted by cancer type/clinical target and by publication year).

| Cancer type/Clinical target                                   | Treatment                                | Conditions   | Sample size*       | Result/Tumor response   | Side effects/comments   | Year | References |
|---|--|--|--------------------|---|---|------|------------|
| Gastrointestinal<br>Anal intraepithelial neoplasia, grade III | <i>m</i> THPC (i.v.)                     | 0.03, 0.075, or 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 48 h, $\lambda = 652$ nm or $\lambda = 532$ nm, starting at 50 mW cm <sup>-2</sup> , 20 J cm <sup>-2</sup> , later 105 mW cm <sup>-2</sup> , 105 J cm <sup>-2</sup> , finally 105 mW cm <sup>-2</sup> , 340 J cm <sup>-2</sup> | 15 (25 treatments) | Initial response 28%, initial partial response 16%, 16% persistent complete response (at 532 nm)  | Red light illuminations caused more significant side effects combined with no persistent complete response  | 2012 | (460)      |
| Anal intraepithelial neoplasia, grade III                     | <i>m</i> THPC (i.v.)                     | 0.03 or 0.075 mg kg <sup>-1</sup> <i>m</i> THPC, 48 h, $\lambda = 652$ nm, 45–50 mW cm <sup>-2</sup> , 10–17 J cm <sup>-2</sup>  | 4                  | Development of a light applicator, monitoring blood saturation, blood volume, fluorescence and fluence (rate) in situ                                   | No influence of the applicator on the fluence rate profile of the light treatment fiber   | 2010 | (461)      |
| Anal intraepithelial neoplasia, grade III                     | Fosgel (topical)                         | 0.75 mg mL <sup>-1</sup> (3 mm) <i>m</i> THPC, 8 h, $\lambda = 652$ nm, 50 mW cm <sup>-2</sup> , 20 J cm <sup>-2</sup> , 2 × treatment after 7 d   | 9                  | No response   | No effect attributed to limited penetration in AIN III  | 2009 | (462)      |
| Cholangiocarcinoma  | <i>m</i> THPC (i.v.)                     | 0.032–0.063 mg kg <sup>-1</sup> <i>m</i> THPC, 20 h and 67–72 h, $\lambda = 652$ nm, 200 mW cm <sup>-1</sup> , 50 J cm <sup>-1</sup>   | 13                 | Median survival time 13 months  | Add. fluorescence measurements; <i>m</i> THPC fluorescence at tumor site clearly detectable, no significant fluorescence contrast of tumor to adjacent healthy tissue | 2013 | (463,464)  |
| Biliary tract cancer  | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm   | 20                 | Primary endpoint: feasibility and safety, combination PDT + chemotherapy + stenting feasible, progression free survival numerically longer in PDT group | Randomized controlled trial (10 patients per group), chemotherapy + stenting vs. PDT, number of cholangitis equal in both groups, two abscesses in PDT group          | 2016 | (465)      |
| Biliary tract cancer  | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 28.2 J cm <sup>-2</sup>  | 29                 | PDT with porfimer sodium: prolonged time to local tumor progression, fewer PDT treatments needed, higher 6-month survival rate.                         | Adverse events: 4 cholangitis, 2 liver abscesses, 2 cholecystitis, 5 phototoxic skin and 7 injection site reactions   | 2015 | (466, 467) |
| Hilar bile duct cancer  | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 72–96 h, $\lambda = 652$ nm, 28.2 J cm <sup>-2</sup>   | 11                 | Overall median survival time 18 months after 1 <sup>st</sup> PDT; doubled depth of local tumor ablation compared to porfimer sodium                     | Adverse events: 4 phototoxic skin reaction, 3 cholangitis, and 3 liver abscesses  | 2013 | (468)      |
| Bone<br>Hyoid chondrosarcoma                                  | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial treatment output per fiber 0.5 W, 20 J cm <sup>-2</sup> per site  | 1 (case study)     | Tumor shrinkage, 2 <sup>nd</sup> PDT treatment after 11 months  | Ultrasound guidance for needle insertion  | 2009 | (469)      |
| Brain<br>Primary brain tumors                                 | <i>m</i> THPC (i.v.) and porfimer sodium | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 20 J cm <sup>-2</sup>  | 41                 | Median progression-free survival: 10 months for glioblastoma, 26 months for anaplastic astrocytoma, and 43 months for oligodendroglioma                 | Administration of PS, craniotomy, surgical resection and laser illumination of the surgical bed combined with temozolomide-based chemotherapy and                     | 2015 | (470)      |

(continues)

Table 3. (continued)

| Cancer type/Clinical target                          | Treatment            | Conditions  | Sample size*    | Result/Tumor response   | Side effects/comments  | Year           | References |
|--|----------------------|---|-----------------|---|--|----------------|------------|
| Prostate<br>Prostate cancer (dosimetry)              | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, λ = 652 nm, 5 J cm <sup>-2</sup>  | 4               | Incomplete treatment  | radiotherapy; side effects: pain, neurologic post-operative deficits in 5 patients   | 2010           | (471)      |
| Prostate cancer (dosimetry)                          | <i>m</i> THPC (i.v.) | 652 nm, interstitial treatment  | not spec.       | Reconstruction of spatial distribution of fluorescent photosensitizer ( <i>m</i> THPC)  | Threshold dose too low, fiber output power attenuated by heterogeneities   | 2009           | (472)      |
| Skin<br>Non-melanoma skin cancers (SCC and BCC)      | <i>m</i> THPC (i.v.) | BCC: 0.05 mg kg <sup>-1</sup> <i>m</i> THPC, 48 h, λ = 652 nm, 20 J cm <sup>-2</sup> ,<br>SCC: 0.1 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, λ = 652 nm, 20 J cm <sup>-2</sup> | 18              | SCC patients: complete response, BCC patients: 12 complete response, 2 complete response after 2 <sup>nd</sup> PDT                | 4 Hypopigmentation, 1 hyperpigmentation  | 2020           | (473)      |
| Non-melanoma skin cancer (SCC)                       | <i>m</i> THPC (i.v.) | 0.05 mg kg <sup>-1</sup> <i>m</i> THPC, 48 h, λ = 652 nm  | 22              | 3-Year follow-up: complete response in 20/22 patients (1 PDT treatment), recurrence in 2 patients (treated by surgical resection) | Side effects: local paresthesia (1), local hyperesthesia (1), full recovery of both within 3 months post-PDT, hypopigmentation of treatment site (3)   | 2019           | (474)      |
| Non-melanoma skin cancers (BCC)                      | <i>m</i> THPC (i.v.) | 0.05 mg kg <sup>-1</sup> <i>m</i> THPC, 48 h, λ = 652 nm, 20 J cm <sup>-2</sup>   | 62              | 116 Lesions, 60 patients complete response, after 5 years 92% complete response rate  | Both superficial and nodular types responded better than invasive type   | 2017           | (475)      |
| Non-melanoma skin cancers (SCC and BCC)              | <i>m</i> THPC (i.v.) | 0.05-0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 24 h, λ = 652 nm and λ = 514 nm (1 case), 10-40 J cm <sup>-2</sup> (for 652 nm)  | 4               | Complete remission for 105 out of 106 lesions, no response for treatment at 514 nm  | Blisters, lipodystrophy, urinary tract infection, leg infection, severe itching  | 2015           | (476)      |
| Non-melanoma skin cancers (BCC)                      | <i>m</i> THPC (i.v.) | 0.06-0.15, 0.05, 0.04, and 0.03 mg kg <sup>-1</sup> <i>m</i> THPC, 1-6, 24, 48, and 72-96 h, λ = 652 nm, 20, 40, 50, 60, and ≥100 J cm <sup>-2</sup>                          | 117             | 460 Lesions, sustained clearance rate 93.7%, overall treatment success rate 90.7%   | Favorable long-term outcome for 'difficult to treat' BCCs with high-dose (0.06-0.15 mg kg <sup>-1</sup> ) and reduced-dose (0.05 mg kg <sup>-1</sup> ); optimized low-dose parameters: 0.05 mg kg <sup>-1</sup> , 24 h, ≥40 J cm <sup>-2</sup> | 2008/2009/2012 | (477-479)  |
| Non-melanoma skin cancer (SCC)                       | <i>m</i> THPC (i.v.) | 0.05 mg kg <sup>-1</sup> <i>m</i> THPC, 48 h, λ = 652 nm  | 1 (case report) | Complete response, no recurrence  | Optical coherence tomographic guided PDT (assessment of tumor extent and margins)  | 2011           | (480)      |
| Non-melanoma skin cancers (SCC and BCC)              | <i>m</i> THPC (i.v.) | 0.05 mg kg <sup>-1</sup> <i>m</i> THPC, 24 h, λ = 652 nm, 40 J cm <sup>-2</sup>   | 9               | 152 Lesions, 117 complete response, 35 partial response   | No adverse clinical and serological effects  | 2011           | (481)      |
| Head and neck<br>Different head and neck pathologies | <i>m</i> THPC (i.v.) | Different doses of <i>m</i> THPC  | 38              | Study on the quality of life of patients after PDT with <i>m</i> THPC   | Improvement of quality of life (visual symptoms, breathing, speaking and swallowing), main side effect: pain   | 2022           | (482)      |
| Oral and oropharyngeal carcinoma                     | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 72-120 h, λ = 652 nm, interstitial and superficial illumination,  | 26              | 76% Complete response, local control in 42.3% median overall survival 24 months   | Retrospective cohort analysis, multiple but transient adverse events, mostly PDT specific  | 2021           | (483)      |

(continues)

Table 3. (continued)

| Cancer type/Clinical target                | Treatment                                | Conditions   | Sample size*    | Result/Tumor response   | Side effects/comments  | Year | References |
|--|--|--|-----------------|---|--|------|------------|
| Head and neck cancer                       | <i>m</i> THPC (i.v.)                     | mean total energy 0.66 W per spot<br>0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 100 mW cm <sup>-2</sup> , 20 J cm <sup>-1</sup> | 1 (case report) | Follow-up at 6 weeks: tissue healing and regeneration with no clinical evidence of recurrence, tumor-free 3 months after treatment  | PDT treatment ( <i>m</i> THPC) as additional treatment after chemotherapy (Bleomycin) combined with photochemical internalization with disulfonated tetraphenyl chlorin TPCs <sub>2a</sub>                   | 2020 | (484)      |
| Paranasal sinuses tumors (dosimetry)       | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 100 mW cm <sup>-2</sup> , 20 J cm <sup>-2</sup> (target fluence and fluence rate)    | 11              | Major temporal and spatial variations in fluence rate and fluence   |  | 2020 | (485)      |
| Head and neck cancer                       | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 100 mW cm <sup>-2</sup> , 20 J cm <sup>-2</sup> (range: 10–30 J cm <sup>-2</sup> )   | 54              | Primary endpoint: Progression-free survival; 2-year progression-free survival rate 30%; 2-year overall survival was 51%. Disease-free survival was significantly better for time interval between surgery and PDT was $\geq 6$ weeks. | Retrospective cohort analysis, PDT as adjuvant therapy for tumor positive resection margins, no adverse events in 78% of patients, most common adverse events localized skin burn (grade I–II) and dysphagia | 2018 | (505)      |
| Head and neck carcinoma                    | <i>m</i> THPC (i.v.)                     | <i>m</i> THPC, 96 h,   | 9               | Investigation on the effect of PDT treatment on immune cell subsets, systemic inflammatory immune response with altered peripheral immune cell populations and cytokine concentrations  |  | 2017 | (254)      |
| Nasopharyngeal carcinoma                   | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 20 J cm <sup>-2</sup>  | 21              | 20 Patients (95%): Complete response 10 weeks post-treatment; 2-year local control rate 75%, progression free survival 49%, overall survival 65%.   | Phase II study, local recurrent or residual nasopharyngeal carcinoma after curative intent (chemo-)radiation, no serious adverse events  | 2015 | (486)      |
| Oral carcinoma (base of tongue)            | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 100 mW cm <sup>-2</sup> , 20 J cm <sup>-2</sup>                                      | 2               | Disease free at 42 and 24 months of follow-up   | Adjuvant treatment after salvage surgery to treat the remaining microscopic disease at resection margins   | 2015 | (487)      |
| Paranasal sinuses tumors                   | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 100 mW cm <sup>-2</sup> , 20 J cm <sup>-2</sup>                                      | 15              | Complete response in 5 patients, 6 patients with short progression-free intervals   | PDT as additional tool for the treatment of recurrent tumors of the paranasal sinuses and the anterior skull base, main side effects: pain and edema   | 2015 | (488)      |
| Early laryngeal cancer and porfimer sodium | <i>m</i> THPC (i.v.) and porfimer sodium | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial treatment, 30 J cm (fiber), 20 J cm <sup>-2</sup>                       | 10              | 7 Patients tumor-free on PDT alone (6 <i>m</i> THPC, 1 porfimer sodium followed by <i>m</i> THPC), 2 after add. radiotherapy  | Descriptive, retrospective study, no unexpected side effects or airway problems; most common symptom hoarseness  | 2014 | (489)      |
| Head and neck cancer                       | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial treatment, 20 J cm <sup>-2</sup>  | 1 (case report) | Use of endoluminal tracheal stenting prior to PDT   | Counter measure against possible post operative swelling following PDT   | 2014 | (490)      |

(continues)

Table 3. (continued)

| Cancer type/Clinical target       | Treatment            | Conditions  | Sample size*                          | Result/Tumor response   | Side effects/comments  | Year | References |
|-----------------------------------|----------------------|---|---------------------------------------|---|--|------|------------|
| Nasopharyngeal carcinoma          | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial and superficial illumination, 100 mW cm <sup>-2</sup> , 20 J cm <sup>-2</sup>              | 6                                     | Patients receiving transnasal direct illumination postero-superior recurrence disease-free for 38 and 71 months   | Coupling with ear, nose, throat (ENT) magnetic navigation system   | 2014 | (491)      |
| Head and neck cancer              | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial treatment  | 1                                     | Treatment stimulation of MR- and CT-assisted interstitial PDT   | Method development   | 2013 | (492)      |
| Head and neck cancer              | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial and superficial illumination, 100 mW cm <sup>-2</sup> , 20 J cm <sup>-2</sup>              | 88                                    | Airway management vital for planning PDT treatment for tongue-based tumors, specifically for location at higher risk site; Floor of mouth/posterior tongue/tongue base or multiple oral sites being treated | Retrospective clinical study on the role of airway management in PDT for head and neck cancer  | 2013 | (493)      |
| Oral SCC                          | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 100 mW cm <sup>-2</sup> , 20 J cm <sup>-2</sup>   | 214 (treatments; 156 PDT, 58 surgery) | T1 tumors: similar results for PDT or transoral surgery, T2 tumors: PDT seemed less effective; PDT and surgery: similar overall survival rates for both T1 and T2 tumors                                    | Comparison PDT vs. surgical treatment  | 2013 | (494)      |
| Oral and oropharyngeal carcinoma  | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 100 mW cm <sup>-2</sup> , 20 J cm <sup>-2</sup>   | 15                                    | 14 Complete responses, 1 partial response, overall survival 72% at 1 year and 36% at 5 years, disease-specific survival 82% at 1 year was 82% and 45% at 5 years.   | Side effects: pain in all cases, 2 burns, 1 edema (tongue base)  | 2013 | (495)      |
| Early oral cavity carcinoma       | <i>m</i> THPC (i.v.) | Not spec.   | 98 (55 PDT, 43 surgery)               | Primary endpoint: local disease-free survival, PDT comparable to trans-oral resection in terms of disease control and survival  | Matched cohort comparison  | 2013 | (496)      |
| Kimura disease                    | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial treatment, 20 J cm <sup>-2</sup>   | 1 (case report)                       | PDT 14 months later similar reduction of disease volume   | Ultrasound-guided PDT  | 2012 | (497)      |
| Nasopharyngeal carcinoma          | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, and 0.075 mg kg <sup>-1</sup> <i>m</i> THPC, 48 h, 24 h $\lambda = 652$ nm, 100 mW cm <sup>-1</sup> , 20 J cm <sup>-1</sup> | 22                                    | Biopsy in 17 of the 22 patients after 40 weeks showed no tumor in all 17 biopsies, highest clinical efficiency in group 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h DLI                                    | Feasibility study, most common side effect: headache (33%), no skin burn or other skin adverse reactions caused by the photosensitizer | 2012 | (498)      |
| Nasopharyngeal carcinoma (T4N0M0) | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm  | 1 (case report)                       | Treatment of residual T4 nasopharyngeal tumor, no tumor recurrence for five years after PDT   | Special nasopharyngeal applicator  | 2012 | (499)      |

(continues)

Table 3. (continued)

| Cancer type/Clinical target               | Treatment            | Conditions   | Sample size*    | Result/Tumor response  | Side effects/comments   | Year | References              |
|---|----------------------|--|-----------------|--|---|------|-------------------------|
| Solitary neurofibroma (neck)              | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 20 J cm <sup>-2</sup>  | 1 (case report) | Reduction of large neurofibroma, reduction of pain, dysphagia and shortness of breath issues   | Ultrasound-guided light delivery  | 2012 | (500)                   |
| Nasopharyngeal carcinoma                  | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 20 J cm <sup>-2</sup>  | 7               | Palliative treatment of recurrent advanced carcinoma, moderate to significant response, symptom reduction, control of disease progression  | Case series, naso-endoscopic guidance   | 2012 | (501)                   |
| Base of tongue cancer                     | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial treatment, 100 mW cm <sup>-1</sup> , 30 J cm <sup>-1</sup>    | 20              | 9 Patients complete response (6 months), with 4 patients free of disease after 46–80 months  | No short-term complications, pharyngo-cutaneous fistula (6 patients), bleeding (1 patient, cutaneous metastasis (2 patients)) | 2012 | (502)                   |
| Proliferative verrucous leukoplakia       | <i>m</i> THPC (i.v.) | not spec.  | 1 (case report) | not spec.  | Bisphosphonate-related osteonecrosis (parallel treatment with bisphosphonates)  | 2011 | (503)                   |
| Vascular anomalies of the head and neck   | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial treatment, 100 mW cm <sup>-2</sup> , 10–20 J cm <sup>-2</sup> | 43              | Clinical assessment: 50% 'good treatment response', moderate clinical response 30.2%, radiological assessment (6-week post-PDT) moderate response 25.6%, significant response 34.9%  | Ultrasound guidance, post-operative infection, peripheral oedema, skin ulceration, skin burn, fistula, hemorrhage             | 2011 | (504)                   |
| Base of tongue cancer                     | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial treatment, 20 J cm <sup>-2</sup>                              | 33              | Moderate to significant tumor response (> 50% reduction) in 20/33 patients, improvement of breathing, swallowing, and speech in the majority of patients   | End-stage base of tongue cancer, ultrasound guidance, side effect: skin burns   | 2011 | (505) cf. f. also (506) |
| Head and neck cancer, vascular anomalies  | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial treatment, 100 mW cm <sup>-2</sup> , 20 J cm <sup>-2</sup>    | 110             | App. 50% of patients showed 'good response' to treatment, 5 patients became disease free: improvement of breathing (27/32), improvement of swallowing (30/37), speech improvement (22/29) reduction of disfigurement caused by their pathology (43/52) | Prospective evaluation, ultrasound guidance   | 2011 | (507)                   |
| Extramedullary plasmacytoma (nasopharynx) | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 100 mW cm <sup>-1</sup> , 20 J cm <sup>-1</sup>                            | 1 (case report) | Surgical debulking combined with PDT, patient disease free since 6 years   | Dedicated PDT nasopharynx × applicator  | 2011 | (508)                   |
| Oral carcinoma (OSCC, T1/T2)              | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 100 mW cm <sup>-2</sup> , 10–20 J cm <sup>-2</sup>                         | 38              | Most recent clinical review post PDT: in 26/38 patients normal clinical appearance of oral   | Multiple PDT treatments on patients   | 2011 | (509)                   |

(continues)

Table 3. (continued)

| Cancer type/Clinical target       | Treatment                                | Conditions   | Sample size*               | Result/Tumor response   | Side effects/comments  | Year | References           |
|-----------------------------------|--|--|----------------------------|---|--|------|----------------------|
| Base of tongue cancer             | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial treatment, 20 J cm <sup>-2</sup>                            | 21                         | mucosa, overall recurrence rate 15.8%, 5-year survival 84.2%<br>>50% 'good response' and ~ 1/3 'moderate response' to treatment, improvement of breathing (9/11), improvement of swallowing (19/21), speech improvement (11/13) | Multiple PDT treatments on patients, 85% of patients: no problems post PDT, side effects: skin burns (3), skin necrosis (1), airway treatment (2), fistula (1) | 2011 | (506) cf. also (505) |
| Oral dysplasia                    | <i>m</i> THPC (i.v.) and 5-ALA (topical) | 0.1 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 20 J cm <sup>-2</sup>   | 147                        | Complete response in 119/147 patients (81%), partial response in 12 (8.2%), stable disease in 5 (3.4%), and progressive disease in 11 (7.5%) patients   | Prospective study, side effects: mild-to-moderate pain in 126 (85.7%) patients, mild-to-moderate skin photosensitivity for 22 (15%) patients                   | 2011 | (510)                |
| Base of tongue cancer             | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial treatment, 20 J cm <sup>-2</sup> and 100 J cm <sup>-2</sup> | 1 (case report)            | Improvement of dysphagia and dysphonia  | Ultrasound guidance, 2 <sup>nd</sup> treatment with increased light dose   | 2011 | (511)                |
| Oral and oropharyngeal carcinoma  | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 100 mW cm <sup>-2</sup> , 20 J cm <sup>-2</sup>                          | 170                        | Complete response rate of 70.8%, median local disease-free interval (for complete response cases) 102.0 months, 2- and 5-year local disease-free survival 74 and 61%, respectively  | Retrospective analysis   | 2011 | (512)                |
| Oral carcinoma                    | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 100 mW cm <sup>-2</sup> , 20 J cm <sup>-2</sup> (for the patients)       | 3 (+ 8 healthy volunteers) | Monitoring PDT by fluorescence differential path length spectroscopy  | Monitoring of PDT by fluorescence differential path length spectroscopy is feasible  | 2011 | (519)                |
| Oropharyngeal/laryngeal carcinoma | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial treatment, 20 J cm <sup>-2</sup>                            | 1 (case report)            | Palliative treatment, 6 months follow-up stable disease with no progression   | Radiologically inserted endoluminal carotid stenting prior to PDT for vessel protection  | 2010 | (513)                |
| Head and neck cancer              | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial treatment 100 mW cm <sup>-1</sup> , 30 J cm <sup>-1</sup>   | Not spec.                  | Fluorescence differential path length spectroscopy for monitoring <i>m</i> THPC-PDT for interstitial treatment of head and neck cancer  |  | 2010 | (514)                |
| Subglottic carcinoma              | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 20 J cm <sup>-2</sup>  | 1 (case report)            | Surgery with adjuvant PDT, patient disease free 18 months post PDT  | Elective tracheostomy performed due to the airway risk as a result of the postoperative swelling, 3 consecutive PDT treatments                                 | 2010 | (515)                |
| Head and neck cancer              | <i>m</i> THPC (i.v.)                     | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 100 mW cm <sup>-2</sup> , 20 J cm <sup>-2</sup>                          | 39                         | Response rate 54%, median survival significantly longer for responders (37 months) than for non-responders (7.4 months), 9 patients were alive at 3.7 to 6.5 years (median, 4.8 years) post-treatment, 7 free of disease        | Multicenter study, most frequent adverse events: cancer pain (21%), pain in general (13%), dysphagia (13%), and photosensitivity reactions (10%)               | 2010 | (516)                |

(continues)

Table 3. (continued)

| Cancer type/Clinical target  | Treatment            | Conditions  | Sample size*    | Result/Tumor response   | Side effects/comments   | Year | References |
|--|----------------------|---|-----------------|---|---|------|------------|
| Cystic hygroma   | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial treatment, 20 J cm <sup>-2</sup>   | 1 (case report) | 50% Size reduction of cystic hygroma  | Ultrasound-guided PDT   | 2010 | (517)      |
| Head and neck cancer   | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, 100 mW cm <sup>-2</sup> , 20 J cm <sup>-2</sup> | 35              | Local control in 21 patients (60%), partial remission in 10 patients (28.5%), 4 patients were non-responders, 1-year survival rate was 53.4% for all patients, 62.4% for patients with complete local control, and 30% for patients with partial remission                      | Side effects: pain at injection site 83%, pain associated with necrosis in the illuminated area 46% | 2009 | (518)      |
| Tumors and malformations (mainly oropharyngeal region, face and limbs) | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial treatment, 20 J cm <sup>-2</sup>   | 68              | App. 50% of patients showed 'good response' to treatment, 1/3 'moderate response', 2 patients became disease free; improvement of breathing (14/17), improvement of swallowing (25/30), speech improvement (16/22) reduction of disfigurement caused by their pathology (33/40) | Prospective study, ultrasound guidance  | 2009 | (519)      |
| Hemangiomas and other malformations                                    | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial treatment, 20 J cm <sup>-2</sup>   | 1 (case report) | Size reduction of hemangioma, increased function of the leg with the angioma  | Ultrasound-guided PDT, skin burn on left arm following inadvertent sun exposure                     | 2010 | (520)      |
| Non-tumorous malformations   | <i>m</i> THPC (i.v.) | 0.15 mg kg <sup>-1</sup> <i>m</i> THPC, 96 h, $\lambda = 652$ nm, interstitial treatment, 20 J cm <sup>-2</sup>   | 21              | At the 6-week follow-up review, significant shrinkage (50–80%) detected in all patients with hemangiomas, lymphangiomas, arteriovenous malformations and neurofibromas  | Ultrasound guidance, up to 10 treatments per patient  | 2009 | (521)      |

\*Number of protocol compliant patients.

murine model. The mice implanted with temoporfin-treated collagen and subsequent illumination showed earlier scab detachment and elastin neosynthesis. PDT with liposomal temoporfin in combination with hypericin has been used against cariogenic bacteria the *Streptococcus mutans* and *Streptococcus sobrinus* (235), and PDT with temoporfin in the liposomal formulation was tested on *Enterococcus faecalis* which is often found in endodontic infections (229,422). With 10  $\mu\text{M}$  of temoporfin a bacterial reduction (measured in CFU, colony forming units) of *E. faecalis* of 5.8 log units was achieved (229). Engelhardt *et al.* (234) tested water-soluble formulations of hypericin and temoporfin (Fospeg®) as aqueous sprays for the inactivation of *Staphylococcus aureus*. With 100 nm of photosensitizer, a bacterial count reduction of 4-5 log units was observed. In terms of a better bacterial targeting Yang *et al.* (231,232) modified temoporfin-loaded liposomes either with an antimicrobial peptide or with a lectin (wheat germ agglutinin) and tested them on *S. aureus* and *Pseudomonas aeruginosa*. Photodynamic antimicrobial chemotherapy with these modified liposomes was highly effective against *S. aureus* and partly effective against *P. aeruginosa*.

### Other nanoparticulate formulations

The title compound has been used with some other nanoparticulate formulations. For example, it has been combined with carbon nanotubes for a synergistic PDT and photothermal approach (173). In this investigation in SKOV3 ovarian cancer cells a synergistic effect was detected based on the induction of different apoptosis signaling pathways by PDT and the photothermal treatment. Nanoparticles of different sizes have also been prepared from neat temoporfin (197). The nanoparticles were produced either by dissolution in an organic solvent (ethanol, DMSO) followed by precipitation with distilled water and ultrasound treatment or by direct ultrasound treatment of temoporfin powder (197). These crystalline temoporfin nanoparticles were more efficiently taken up in macrophages (J774A.1 cells) than in L929 fibroblast cells. Later it was found that PDT with these temoporfin nanoparticles can induce a phenotypic shift in macrophages (163). Dissolution from temoporfin nanocrystals has been analyzed using mathematical models (423). In another combinatorial approach, temoporfin and a nitric oxide photodonor have been incorporated into calix[4]arene nanoassemblies (424). Upon visible light excitation, the nanoconstruct was able to independently release nitric oxide and singlet oxygen. Recently, nanoparticles consisting of supramolecular organic frameworks and temoporfin have been prepared and tested in cell cultures and *in vivo* in mice (99). The nanoparticles showed photocytotoxicity in several cancer cell lines and were able to suppress tumor growth in mice. In a theranostic approach, Sun *et al.* prepared cRGD-modified liposomes encapsulating oxygen-carrying perfluorocarbon and temoporfin, these liposomes then being loaded into living neutrophils (224). These modified neutrophils served as sonosensitizers and effectively suppressed tumor growth in mice. Imaging of the tumors was realized by temoporfin fluorescence detection and perfluorocarbon-microbubble enhanced ultrasound imaging.

## CLINICAL EXPERIENCE

Temoporfin is the active substance in the medicinal product Foscan® which is authorized in 2001 in the European Union for

the palliative treatment of head and neck cancer. The standard dosage in this treatment is 0.15 mg per kg body weight. Illumination is performed with laser light at 652 nm (425). Temoporfin is one of the few photosensitizers authorized for clinical use in the European Union (245,426,427). Others are padeliporfin (Tookad®) (428), verteporfin (Visudyne®) (429) or ALA (5-aminolevulinic acid hydrochloride, Ameluz®) (430), all of those being authorized via the centralized procedure with the European Medicines Agency (EMA) (431). Padeliporfin is authorized for the treatment of low-risk prostate cancer, verteporfin for the treatment of the so-called 'wet' form of age-related macular degeneration, and ALA for the treatment of actinic keratoses and basal cell carcinoma. A different medicinal product, also containing ALA as the active substance, Gliolan®, is authorized in the European Union for intraoperative fluorescence diagnosis of tumorous tissue in the surgery of malignant glioma (432). The photosensitizer fimaporfin (Amphinex®) has received an orphan drug designation by the EMA (433) for the treatment of cholangiocarcinoma in combination with the chemotherapeutic gemcitabine (434). Fimaporfin (disulfonated tetraphenylchlorin, TPCS<sub>2a</sub>) enhances the effect of gemcitabine *via* photochemical internalization (PCI) (67,435). The well-known photosensitizer porfimer sodium is authorized in some European countries for the treatment of cholangiocarcinoma. Worldwide, numerous other photosensitizers are authorized for clinical use or are under clinical development. Examples are tin ethyl etiopurpurin (Purlytin®), motexafin lutetium (Antrin®), radachlorin, talaporfin (Laserphyrin®) or redaporfin (426,436–438).

During its development temoporfin, like other photosensitizers, has been investigated for the treatment of numerous indications, mainly certain forms of cancer (42,43). This development continues after the marketing authorization. Based on the established level of quality and safety shown in the original marketing authorization new indications can be explored in clinical studies. For example, verteporfin which - being authorized for the treatment of the age-related macular degeneration - has been investigated in the palliative treatment of pancreatic cancer (439). In recent years a number of comprehensive reviews on the role of temoporfin and other photosensitizers in the therapy against cancer and other diseases have appeared paving the way through the ever-expanding field of PDT research (15,19,39,426,427,436,437,440–448). Algorri *et al.* (449) recently undertook the effort to compile all the latest reviews in the area of PDT. This is further amended by reviews on PDT of specific forms of cancer, encompassing the use of temoporfin in the area (42) and among other therapies for brain cancer (450,451), lung cancer (452), prostate cancer (453–455), esophageal cancer (456,457), peritoneal metastasis (458), and recurrent respiratory papillomatosis (459). Table 3 gives an overview of the main fields of clinical applications of temoporfin in roughly the last ten years. These include clinical trials, as well as single-patient treatments (case reports). Most clinical experience has been collected in the field of head and neck cancer and other head and neck malformations, which is not surprising given the authorized indication. Apart from these investigations, considerable clinical experience has also been acquired with gastrointestinal diseases and certain forms of skin cancer.

In addition, recent years have seen single reports on the use of temoporfin for bone cancer (469), primary brain tumors (470), prostate cancer (308,471,472), and *ex-vivo* studies with human retinoblastoma (522).



### Skin cancer

Temoporfin has been investigated for its use against non-melanoma skin cancers and a number of clinical studies and case reports have been published (see Table 3) (42,473–481). These non-melanoma skin cancers – the most common malignancy among the Caucasian population – comprise *i.a.* actinic keratoses (pre-cancerous lesions), Bowen's disease (carcinoma *in situ*) and basal cell (BCC) carcinoma as well as squamous cell carcinoma (SCC) (523). Specifically for actinic keratoses, Bowen's disease and BCC PDT has been established as an alternative treatment to surgery, with ALA or MAL (methyl aminolevulinate) being the most widely applied photosensitizer (prodrug) (523,524). The largest study with temoporfin PDT on BCC comprised 117 patients with a total of 460 lesions treated (for these and all other clinical investigations compare Table 3) (477–479). In 2008 Betz *et al.* published the initial study (478,479), which was later followed by the evaluation of the long-term outcomes in 2012 (477). The mean follow-up period was 42 months with a sustained clearance rate of 93.7% and an overall success rate of 90.7%. The authors compared four different dose groups ranging from 0.06 to 0.15 mg kg<sup>-1</sup> (highest dose group) to 0.03 mg kg<sup>-1</sup> (lowest dose). They also specifically looked at the effect on 'high-risk lesions' (recurrent lesions, lesions of >3 mm thickness). Overall long-term outcomes for BCC were best for the high-dose (0.06–0.15 mg kg<sup>-1</sup>) and the reduced dose (0.05 mg kg<sup>-1</sup>). The authors name the optimized treatment parameters for this reduced dose as 0.05 mg kg<sup>-1</sup> temoporfin, 24 h DLI, and a light fluence of  $\geq 40$  J cm<sup>-2</sup>. For the high-risk lesions, the authors state that the results are favorable compared to conventional treatment or ALA and MAL PDT. The observed side effects of the treatment were more common in patients of the high drug dose group and comprised mostly of pain and phototoxic reactions (479).

In 2017 Jerjes *et al.* (475) published the retrospective evaluation of the treatment of 148 patients with BCC who were treated with MAL PDT or temoporfin PDT, the MAL PDT group comprising 86 patients with 127 thin BCCs and the temoporfin PDT group with 62 patients with 116 thick BCCs. The attribution to the respective patient (and photosensitizer) group was done on the basis of the thickness of the lesions, as local treatment with MAL due to the low treatment depth is only possible for superficial lesions (~1 mm), whereas thicker lesions required systemic administration of the photosensitizer (temoporfin). Treatment with temoporfin was done with a reduced dose (0.05 mg kg<sup>-1</sup>) and a DLI of 48h. Of the 62 patients treated with temoporfin 60 had a complete response after one PDT treatment. In the temoporfin, PDT group both superficial and nodular types of BCC responded significantly better than invasive BCCs (475). This is in line with other clinical observations (523). The most common side effect for both patient groups (MAL and temoporfin PDT) was hypopigmentation at the treatment site (5 of 148 patients) (475). In 2019 Jerjes *et al.* (474) published a retrospective study on the use of temoporfin PDT for non-metastatic SCC (T1/T2N0), again using a dose of 0.05 mg kg<sup>-1</sup>, and a DLI of 48 h. Of the 22 patients treated 20 showed complete response after one round of treatment (3-year follow-up). The two patients experiencing recurrence then underwent surgical resection. The same team investigated temoporfin PDT for the treatment of peri-orbital skin cancers, 14 patients with BCC and four patients with T1N0 SCC (473). Of the BCC group, 12 out of 14 patients

showed a complete response after one PDT treatment, the two remaining patients underwent a second PDT treatment which led to a complete response. Complete response was also observed for the four treated patients with SCC. For the treatment of SCC, a higher dose (0.1 mg kg<sup>-1</sup>) and a longer DLI, 96 h, was employed (473). In an earlier case report, the authors showed that this treatment of SCC can be combined with optical coherence tomography to better assess tumor extent and tumor margins (480).

Motta *et al.* (481) reported on the treatment of different non-melanoma skin cancers in 9 patients with recurrent multiple lesions. In total, 152 lesions were treated achieving complete response for 117 and partial response in 35 cases. In a review combined with case reports Horlings *et al.* (476) summarized the results of temoporfin PDT for non-melanoma skin cancers until 2015. Their clinical experience supports the high response rates found in earlier studies, however, they observed slow healing when applying the PDT to the lower leg area, the facial and head and neck area had fewer problems in this respect and showed good cosmesis, which corresponds to the recent results by Hamdoon *et al.* (473).

### Gastrointestinal cancer

Temoporfin has been tested for the treatment of anal cancer (460–462). Anal cancer has a low incidence, with one of the main risk factors being a persistent high-risk human papillomavirus infection (462,525). Originally, the anal intraepithelial neoplasia, grade III, was treated topically with temoporfin in a thermosetting gel formulation. However, this proved to be ineffective, presumably due to the low penetration depth of temoporfin from this formulation into the neoplasia, exemplarily underlining the importance of pharmaceutical formulation development (462). In a second study, the anal neoplasia was treated after intravenous administration of temoporfin (460). For this, a specific light applicator was developed (461). Treatment was performed with different doses of temoporfin (0.075–0.15 mg mL<sup>-1</sup>) with 48 h DLI and with red and green light illumination. With intravenously injected temoporfin the treatment was partially effective; 4 of 25 patients showed persistent complete response, all of these receiving green light illumination (460). Red light illumination was found to cause more severe side effects (intense pain, bleeding) than green light illumination (460).

Cholangiocarcinoma is a rare form of cancer – <2% of all cancers – of the bile duct with a poor prognosis; hence, there is a high need for additional treatment options (526–531). In 2013, Kniebühler *et al.* (463) published an exploratory study on the treatment of nonresectable cholangiocarcinoma with stenting and PDT. The 13 patients were treated with different doses of temoporfin (0.032–0.063 mg per kg body weight, a lower dose compared to the dose used for head and neck cancer), different DLIs, starting with 20 h later extended to 67–72 h, and different light doses. With these low doses, side effects such as skin phototoxicity and perforations were not observed. The median survival time was 13 months. The temoporfin fluorescence from the tumor tissue could clearly be observed; however, a distinct fluorescence contrast between the tumor and adjacent healthy tissue was not observed (463,464).

In non-resectable bile duct cancer, PDT is an established option for palliative treatment improving cholestasis and survival

thereby improving quality of life and median survival time (532,533). Most often porfimer sodium is used as a photosensitizer (533). However, the lower light absorption at longer wavelengths limits the tumoricidal effect of porfimer sodium compared to chlorin or bacteriochlorin photosensitizers (42,534). In 2013 Wagner *et al.* (468) published the results of the first stage of a phase II clinical trial on temoporfin PDT for the treatment of hilar bile duct cancer in a small group of patients. Looking at the local response and tumoricidal penetration depth, they observed complete local response in 1 out of 10 patients and partial response in 8 patients. In four patients a tumoricidal effect was observed up to a depth of  $\geq 7.5$  mm which is approximately a doubled tumor-ablative depth compared to porfimer sodium. The overall median survival time was 18 months. The results of the second stage of this trial were published in 2015 this time analyzing the data from 29 patients (466,467). In the patients with occlusion of biliary segments at the start of the study (29), 16 local response was observed, 11 patients stable disease was noted and one case of progressive disease. The overall results of the study were compared to a historical cohort of patients treated with porfimer sodium (466,467). The effect of PDT on biliary cancer has also been studied by the authors at the cellular level. They detected pronounced differences between cell lines and identified possible markers for the prediction of PDT efficiency (203,204). In this comparison the authors note a prolonged time to local tumor progression, the need for fewer PDT treatments and a higher 6-months survival rate. The observed trend for longer overall median survival; however, was not significant compared to treatment with porfimer sodium (466). In 2016, Hauge *et al.* (465) published a report on a randomized trial in patients with nonresectable biliary tract carcinoma about the combination of chemotherapy and stenting with and without temoporfin PDT treatment, with ten patients in each group. The study was primarily concerned with the question of feasibility and safety of this combination. Looking at these endpoints no serious, procedure-related complications due to PDT or the treatment combination were observed. The number of cholangitis was equal in the two treatment groups; however, the progression-free survival was longer in the PDT group.

### Head and neck

As mentioned above, temoporfin is authorized in the EU for the palliative treatment of head and neck cancer therefore most of the clinical investigations with temoporfin PDT have been done in this area. A number of reviews have appeared giving of overview on the use of PDT in this indication (535–539). Though the majority of clinical applications of temoporfin PDT in the head and neck area are focused on cancer, it has also been successfully employed for other malformations in this region of the body (Table 3) (497,500,503,504,507,508,515,517,519–521). The predominant number of clinical investigations were performed with a dose of 0.15 mg temoporfin per kg of body weight, a DLI of 96 h, and illumination at 652 nm with 20 J per  $\text{cm}^2$ ; however, there are reports where a different dosage and other DLIs as well as other light dosages have been used (483,498,504,509,510). Head and neck cancer comprises cancers usually starting from the squamous cells that line the mucosal surfaces of the head and neck region, like the oral cavity, the pharynx, the larynx and the nasal cavity. These cancers are referred to as squamous cell carcinomas (SCCs). Head and neck

cancers can also originate from the paranasal sinuses as well as the salivary glands. For all of these, temoporfin PDT has been applied (cf. Table 3).

The anatomy of the head and neck area poses specific challenges for light delivery, so for some of these applications, *e.g.* in the nasopharyngeal area specific light applicators needed to be developed (486,499,508). This specific issue with light administration in the nasopharyngeal area was recently investigated in more detail with eleven patients where the fluence and fluence rates at the target location – paranasal sinuses – were detected using *in vivo* light dosimetry during PDT. As the result, major temporal and spatial variations in fluence rate and light exposure time were found (485). The highest measured fluence rate was 328  $\text{mW cm}^{-2}$ , in other cases it was below 20  $\text{mW cm}^{-2}$ , illustrating how difficult light dosage in certain anatomic areas can be.

Several temoporfin PDT studies with larger patient cohorts have been published, *e.g.* studies by de Visscher *et al.* (494) on temoporfin PDT for oral SCCs (214 patients), by Karakullukcu *et al.* (496) on early oral cavity carcinoma (98 patients), and on oral and oropharyngeal carcinoma (170 patients) (512), by van Doeveren *et al.* (540) on head and neck cancer (54 patients), and by Jerjes *et al.* on head and neck cancer including vascular anomalies (110 patients) (507), on oral dysplasia (147 patients) (510), and on tumors and malformations in the oropharyngeal region but also including face and limbs (68 patients) (519). Clinical endpoints were mainly local response, progression-free and overall survival (cf. Table 3).

De Visscher *et al.* (494) and Karakullukcu *et al.* (496) compared PDT treatment to surgery for oral SCC and early oral cavity carcinoma. They found similar results for disease control and overall survival, though in the study with oral SCC PDT seemed less effective for larger T2 tumors (tumor size  $\sim 2\text{--}5$  cm) whereas for smaller T1 tumors (tumor size  $< 2$  cm) a similar effectivity was found (494). Visible light of 652 nm has only limited penetration depth into the tissue, which in turn limits the PDT effect specifically for larger and thicker tumors. One way to overcome this problem is the use of an interstitial treatment, whereby multiple fibers are introduced into the tumor tissue allowing to treat of the complete tumor mass and improving local tumor control (541). This treatment variant has been applied for many cases in the head and neck area using temoporfin PDT (469,483,489–493,497,501,502,504–507,511,513,514,517,519–521). To achieve complete illumination of the tumor a correct positioning of the fibers is important; this treatment is usually supported using imaging methods like MR, CT or mostly ultrasound (469,491,492,497,500,504–507,511,517,520,521). In addition, fluorescence-based methods like fluorescence differential path length spectroscopy have been used (514,542). Prior to its application in patients, this method was previously developed in animal studies (305,306,543).

Very recently, a study in 38 patients was published analyzing the effect of PDT with temoporfin in the head and neck area on the quality of life of patients (482). In this study, all patients reported an improved quality of life after PDT. The main problem reported by the patients was pain after the PDT treatment. In the 4 weeks after treatment improvement was reported for visual symptoms, breathing, speaking and swallowing and for the following weeks an improvement in daily life activities, social life, mood and anxiety were reported (482).

PDT is primarily a local treatment - though exerting systemic immunological effects which find increasing interest

(253,276,277) – PDT with temoporfin in the head and neck area is usually combined with chemotherapy. Based on the marketing authorization it is specifically used for recurrent tumors which are resistant to other treatments like chemo- or radiotherapy or cannot be treated by surgery (425,486). One application is the adjuvant therapy after chemotherapy or salvage surgery, allowing to treatment of remaining microscopic diseases after tumor resection (484,487,488,540). In this respect, Jerjes *et al.* published a case report, where cutaneous SCCs in the face were treated by PCI with fimaporfin/bleomycin followed by interstitial PDT with temoporfin for deeper tumor areas (484). With PDT, often effective local control of tumor growth can be achieved (483,486,487,489,495,498,499,509,510,512) with extended periods of progression-free disease (487,499,502,508,509). Effective local tumor control was found to correlate positively with survival times (516,518).

### Side effects

Concomitant with their desired pharmacological effect all medicinal products can elicit side effects. These side effects differ from substance class but of course also from drug to drug (544). The side effects of temoporfin and the medicinal product derived therefrom, Foscan®, are reflected in the patient information and the official EMA documents (319). As for many other drug products, the side effects of temoporfin and Foscan® are associated with their intended photodynamic action mechanism. Therefore, the most common side effects apart from pain are phototoxic reactions, e.g. skin photosensitivity, skin burns or phototoxic reactions at the injection site (see also Table 3) (466,467,495,506,520,540). Most of these may be avoided or limited by strictly adhering to the light-protection given in the patient information.

Severe side effects like cholangitis have been observed in biliary tract cancer treatment though those are also observed in the standard chemotherapeutic treatment for this disease (465–467). When the tumor to be treated is near a large blood vessel this poses a risk for PDT treatment. In a publication by Hamdoon *et al.* (513) endoluminal carotid stenting has been reported as a precautionary measure. In PDT treatments in the head and neck area swelling after PDT can lead to complications therefore airway management is important specifically for tongue-based tumors (490,493). An important issue is also the unintended interaction with other drugs. For temoporfin, an interaction with other drugs with photosensitizing potential is a cause for concern (319,545). In a single case in the literature, the authors report on bisphosphonate-related osteonecrosis of the jaw which occurred associated with PDT for the treatment of oral proliferative verrucous leukoplakia (503). An interaction of a drug molecule with others may also be beneficial. In an interesting publication in this respect, Lange and Bednarski investigated the synergistic effects of three platinum-based chemotherapeutics, carboplatin, cisplatin and oxaliplatin in combination with PDT with temoporfin in five cancer cell lines. Depending on the type of cancer cell line pre-treatment with PDT sensitized the cells for the treatment with the platinum complexes (131). A similar effect was shown in Hep-2 cells (106).

### Veterinary applications

In contrast to clinical use for humans, PDT is much less developed in the veterinary field and progress has been slow. It is

mainly used to treat early skin tumors in cats and some studies have reported treating urinary tract neoplasia in dogs and equine sarcoids (546). With regard to temoporfin, a study by Flickinger *et al.* (310) investigated the long-term outcome of using Foslip for systemic treatment of feline squamous cell carcinomas. The study (0.15 mg drug kg<sup>-1</sup> body weight i.v., 6 h postinjection, illumination with 652 nm diode laser, 0.5 W cm<sup>-2</sup>) showed a very good response rate of 84% and a mean progression-free interval of 35 months. However, the results were unsatisfactory for invasive tumors and larger lesions.

Using Fospeg®, a case study reported the successful surgical and photodynamic treatment of an equine sarcoid (311). Treatment conditions were similar to those used for cats. The main tumor mass and some lateral tumors showed complete remission while remaining tumors decreased in size or stopped growing.

## DOSIMETRY AND DETECTION

PDT faces the issue of dose not only a drug (like in chemotherapy) or radiation (as in radiotherapy) but both. Hence, drug and light dosage, and especially light dosimetry are a constant challenge in PDT (446,547–551). For drug dosage of temoporfin in clinical practice there is a quite clear picture in the literature: In most cases a dosage of 0.15 mg per kg body weight and a DLI of 96 h has been used, corresponding to the marketing authorization (cf. Table 3) (425). In exploratory clinical investigations for anal intraepithelial neoplasia, cholangiocarcinoma and non-melanoma skin cancers lower dosages (down to 0.03 mg kg<sup>-1</sup>) have been used (cf. Table 3). Treatment dosage variations, e.g. compartmental targeting using a fractionated double-injection protocol have been investigated in animals (296).

Dosing of light is a challenge in PDT which is reflected in the general PDT review literature (449,548,550,551) but becomes also apparent from Table 3 listing temoporfin clinical trials which shows a broader variation compared to the drug dosage, though in many cases a fluence of 20 J cm<sup>-2</sup> has been used. In some cases, lower (461,471) but also much higher fluences have been employed (460,477,478,481). The use of temoporfin with low light doses has been discussed as an option for PDT (136,552). Light dosage in PDT is influenced by the optical properties of the tissue to be treated (549); anatomical factors can influence the light dose that can be administered as well, and sometimes specific light applicators are required (461,485,486,499,508). As mentioned above, in interstitial PDT imaging methods, mostly ultrasound, are employed to assure correct positioning of the light-guiding fibers (469,491,492,497,500,504–507,511,517,519–521). Nevertheless, in some cases pronounced differences in fluence and fluence rate were detected (485).

A laser is the common light source for PDT generally (548), for temoporfin PDT in the clinical environment (cf. Table 3) and in most pre-clinical investigations in animal species. However, with the development of high-power LEDs, irradiation with LEDs has become an alternative that is investigated and has been used with temoporfin (105,206,282). Etcheverry *et al.* (105) performed a comparative *in vitro* investigation on the use of a 637 nm LED and a 654 nm laser light source in PDT with temoporfin on HeLa cells and found the photodynamic efficiency to be similar. Apart from this, other light sources are investigated as well for PDT, e.g. implantable ones (446,553).

Due to temoporfin's intense fluorescence, fluorescence methods, e.g. the aforementioned fluorescence differential path length spectroscopy, can be employed to measure its concentration *in vivo* aiding the optimization of PDT treatment parameters (171,293,305,306,463,464,480,514,542,543). Optical methods like optical coherence tomography can also be used to more precisely determine tumor extension margins, e.g. in skin cancer (480). Recently, the combination of Raman spectroscopy and PDT (with ALA, protoporphyrin IX and temoporfin) has been proposed for theranostics (115). Efforts to optimize treatment parameters for PDT with temoporfin are also supported by pre-clinical testing in animals and modelling approaches (288,293,302–304,307–309,471,472,554,555), e.g. for prostate cancer (308,471,472), liver cancer (300,307,555), esophagus (309), transplanted mammary carcinoma (302,303), and gliosarcoma (288). Axelsson *et al.* (472) described an experimental approach to reconstruct the spatial distribution of temoporfin inside the human prostate. In some of these animal investigations with temoporfin detection *via* fluorescence, the window-chamber tumor model has successfully been used (288,302–304). The detection of temoporfin by fluorescence spectroscopy methods as well as absorption spectroscopy has also proven to be a valuable or perhaps even the standard tool in pharmaceutical formulation development, allowing to follow the active substance molecule when the pharmaceutical formulation interacts with body fluids and is distributed to body compartments (129,171,221,293,365,367,392). Moreover, changes in fluorescence behavior of temoporfin and changes in its absorption spectrum can be used to characterize the immediate surroundings of the photosensitizer (366,368,393,554). Analysis of temoporfin fluorescence in PDT investigations is closely associated with the photobleaching of the photosensitizer, where the photosensitizer is destroyed by photochemical reactions under illumination (42,556–558). Photobleaching of temoporfin has been investigated in detail by Bonnett *et al.* (559,560) and has been reviewed before (42). In a series of publications, Atif reported on photobleaching studies in keratinocytes treated with PDT with temoporfin (554,557,558,561). These investigations found that photobleaching of temoporfin is increasing with the higher local concentrations of the photosensitizer (558).

In general, PDT relies on the local availability of oxygen to achieve its desired effect on the tumor cells (6,245). Thus, the question of the supply of oxygen at the tissue and cellular level is critical for PDT and hence intensely discussed in the PDT literature (6,245,440,442,449). Tumorous tissue is often hypoxic, *i.a.* due to fast tissue growth and insufficiently developed vasculature (6,245,440,442). This is an important medical issue as hypoxic zones in tumors can be more resistant to chemo- and radiotherapy (562). In addition, photosensitization by itself reduces cellular oxygen levels quickly, especially at higher light fluences, not only by oxygen consumption through the PDT process but also by occluding tumor vasculature (6,245,440,442,449). This self-limiting effect of PDT can partly be overcome by optimizing light dose and light fluence, e.g. by fractionated light administration or by using hyperbaric oxygen conditions or oxygen carriers (6,438,442,449,563).

Therefore, the detection, measurement and modelling of singlet oxygen luminescence is an important approach to PDT dosimetry (6,564–568). Singlet oxygen monitoring for dose–response studies in PDT is challenging because, as mentioned above, there is oxygen depletion during treatment; additionally, the oxygen

concentrations in the tumor differ between different areas, e.g. cellular tissue and vasculature (569,570). However, considerable progress has been made with respect to singlet oxygen measurements (222,568,571), direct and time-resolved measurements of singlet oxygen in living cells and tumor models like the CAM model have been performed (179,222,571). Using the CAM model and a liposomal temoporfin formulation it was possible to deduce the oxygen content during PDT from the singlet oxygen kinetics (222,571). The importance of singlet oxygen kinetics and time-resolved luminescence measurements has already been pointed out by Jarvi *et al.* (107,108) these are critical in using singlet oxygen signal as a dose metric when employing temoporfin as photosensitizer. Wilson *et al.* (288) investigated singlet oxygen luminescence in comparison with bioluminescence from luciferase- and green fluorescent protein-transduced gliosarcoma grown in a dorsal window chamber in mice, finding that tumor response (measured via the bioluminescence signal) correlated well with singlet oxygen luminescence, underlining that singlet oxygen can serve as a dose metric. Zhu *et al.* (285,286,312) looked at determining singlet oxygen threshold doses for PDT for different photosensitizers including temoporfin, also including a comparison to literature data. Not unexpectedly, they found large differences, observing that the experimental *in vivo* singlet oxygen threshold doses for the photosensitizers investigated (porfimer sodium, verteporfin, and temoporfin) were about 20 times lower than those found *in vitro* – underlining, that additional factors to singlet oxygen mediated cell death contribute to the PDT effect observed *in vivo* (286). These PDT threshold investigations should illuminate clinical experiences, where a too low light threshold dose can result in incomplete tumor response (308,471).

The *in vitro* separation and detection of temoporfin, e.g. in biological samples relies on chromatographic methods, with detection *via* absorption or fluorescence (572). These classical chromatographic methods are now amended by newer separation methods, specifically, if particles are involved, like the asymmetrical flow field-flow fractionation which has become a routine technique in nanoparticle separation and characterization. This technique has successfully been applied in the characterization of temoporfin-loaded liposomes and in the investigation of the drug transfer from such liposomes (394,397,398).

With respect to the photophysical properties of temoporfin, it should be noted that also the two-photon cross-section of temoporfin has been investigated experimentally and theoretically (260–262) in the context of two-photon PDT (573). The values found were lower than those for specifically designed two-photon sensitizers (61,573) but higher than expected from theoretical calculations (256).

## OTHER APPLICATIONS

### Combination of PDT with other therapies

The interest in the combination of PDT with other therapies and treatment modalities mainly comes from two directions: finding synergistic effects of combined treatments could increase effectiveness (446) and, as PDT in nearly all of its medical applications is not a first-line treatment – except, perhaps, for certain forms of skin cancer like actinic keratosis – in investigating compatible pathways for patients already under treatment. In addition, PDT is principally a local treatment thus requiring systemic tumor treatment to prevent metastasis. Tumor therapy with PDT

is therefore in clinical practice mostly combined with other tumor treatments such as chemo- or radiotherapy (574). This is also the case of temoporfin which is authorized for the palliative treatment of head and neck cancer meaning that patients are concomitantly treated with, *e.g.* chemotherapy. Therefore, knowledge about interactions and possible synergism of such combinations is of high medical importance.

### Combination with chemotherapeutics

This dual motivation is apparent in the investigations on the interaction of temoporfin PDT with chemotherapeutics. A synergistic effect of temoporfin PDT followed by cisplatin was reported for Hep-2 cells, the combination resulting in increased apoptosis, necrosis, and mitochondrial destruction and reduced autophagy (106). Lange and Bednarski (131) investigated the effect of a combination of temoporfin PDT with carboplatin, cisplatin, or oxaliplatin in five cancer cell lines (A-427, BHY, KYSE-70, RT-4, and SISO cells). The authors identified some synergistic combinations, *e.g.* for oxaliplatin in three of the cell lines (BHY, RT-4, and SISO cells), however, in some cases even an antagonistic effect was found. In cases of synergy elevated ROS levels were found but these did not necessarily lead to increased apoptosis (131). The same authors analyzed the synergism of a combination of temoporfin PDT with two glutathione peroxidase inhibitors in the five above-mentioned cell lines (130). Again, a synergistic effect was found for both peroxidase inhibitors but not in all cell lines (130). Also, the combination of PDT with the chemotherapeutics taxotere and doxorubicin has been investigated *in vitro* in A-431 cells (153). Cells were exposed to the taxotere followed by temoporfin PDT or cells were incubated with temoporfin followed by the PDT-treatment and exposure to doxorubicin. The combination treatment was found to increase cytotoxicity at the same time differences in the expression of VEGF and IL-1alpha were detected (153). Doxorubicin has also been combined in a liposomal formulation with temoporfin PDT and magnetic nanoparticles and tested on HeLa cells, the combined treatment being more effective than the separate treatments (104). In another approach temoporfin was combined with doxorubicin, magnetic nanoparticles and second photosensitizer (TPCS<sub>2a</sub>) in extracellular vesicles as the carrier system (180,216). Combinatorial approaches have also been described for temoporfin with the DNA topoisomerase inhibitor  $\beta$ -lapachone (183), Navelbine® (297), and fenretinide (96).

An important issue in cancer therapy is the resistance of certain cancer cell types to common chemotherapeutics (575,576). In this context temoporfin PDT has been tested on 5-fluorouracil-resistant HCT116 (208) and HT29 (209) cancer cells. In both investigations, PDT proved to be effective against these cancer cell lines (208,209).

### Antibodies

The efficacy of the combination of the anti-VEGF monoclonal neutralizing antibody bevacizumab and PDT with temoporfin has been evaluated in a mouse colon cancer model (HT29 tumors) (281). PDT followed by administration of the antibody increased the anti-tumor effect. Administration of bevacizumab prior to temoporfin, however, led to a decreased accumulation of

temoporfin in tumor tissue at 24 h after photosensitizer administration (281). In another approach involving antibodies, micellar nanoparticles containing temoporfin labelled with llama single-domain antibody fragments for targeting have been prepared (156). In this case, temoporfin served also as a fluorescence label for the carrier systems. On the other hand, bispecific antibody-redirected T lymphocytes have been used as carrier vehicles for *m*THPP (577).

### Other photosensitizers

In a few cases, temoporfin has also been combined with other photosensitizers and photoactive compounds, so for example with TPCS<sub>2a</sub>, used for photochemical internalization (216), a nitric oxide photodonor (424), and hypericin (97,98). The latter combination showed a reduced dark toxicity in head and neck squamous carcinoma cells (UMB-SCC 745 and 969) and a combination of apoptotic and necrotic cell death (97,98). See also Table 1.

### Quantum and polymer dots

Temoporfin has been incorporated into quantum dots and polymer dots to exploit the resonant energy transfer for and an increased and optimized PDT effect (112,120,121,363,375,578). The respective examples are discussed above under 'Formulation development'.

### Hperthermia

*Hyperthermia* combined with temoporfin PDT was investigated with liposomes and extracellular vesicles carrying the photosensitizer as well as magnetic iron oxide nanoparticles *in vitro* and *in vivo* in mice (see above under 'Formulation development') (164,172,216).

### Photothermal treatment

*Photothermal treatment* was used in a combination of temoporfin and multi-walled carbon nanotube PDT (173). The two treatments were found to induce apoptosis by different cell signaling pathways.

### Photoprotection

PDT is associated with side effects like skin photosensitization. In this respect the photoprotective properties of plant extracts have been investigated (176,291,579). In investigations with a plant extract from *P. halepensis* the authors reported that the extract prevented photosensitivity after temoporfin PDT in mice (291) and even increased intracellular ROS levels (176).

### PDT-immunotherapy combinations

The immune response to the PDT treatment contributes to the PDT effect *in vivo* as mentioned above. Hence, there are a number of publications investigating these effects for PDT with temoporfin and utilizing this for PDT-immunotherapy combinations. Korbelik *et al.* thoroughly evaluated the role of ceramides and sphingosines in PDT immune response in several *in vitro*

(95,96,169,195,274) and *in vivo* investigations, also aiming at anti-cancer vaccines generated by PDT (273,275,284). They, as others, emphasize the role of neutrophils in the immediate reaction to PDT (201,224,284). It has been reported for the nasopharyngeal KJ-1 cell line that treatment with temoporfin and light is able to inhibit migration and invasion in this cancer cell line (88). In a mouse model, a synergistic antitumor effect could be demonstrated for a combination of PDT with temoporfin, chemotherapy and immune lymphocytes (297). Extracellular vesicles from the mesenchymal stem and stromal cells, respectively, loaded with temoporfin showed increased necrosis and a decrease in intratumoral proliferation in a mouse model of peritoneal metastasis (201). Recently, PDT with temoporfin in a formulation, containing RGD-modified nanoparticles has been combined with a PD-L1 checkpoint inhibitor (PD-L1 blockade antibody) in a mouse model (CT26 tumors) (133). This combination was found to inhibit distant tumor growth and also stimulate immune memory response. In addition, even without the antibody treatment, the RGD-modified nanoparticles were able to inhibit cell proliferation and stimulate an immune response. This is attributed to promotion of dendritic cell maturation (133).

### Antimicrobial PDT

Following the general trend in PDT in recent years, which has seen a renewed focus on antimicrobial applications, temoporfin and its formulations are featured in related studies. Target bacterial species were primarily those related to wound healing and dental hygiene. In the former, a study using Foslip® showed that low doses of photosensitizer embedded in collagen matrixes after implantation and illumination did significantly advance wound healing in mice (294). *Staphylococcus* infections are often a complicating factor in wound and skin diseases. Here, both PVP-hypericin and Fospeg® resulted in a 4-5 log reduction in bacterial count (100 nM PS, 5 min incubation, 30 min illumination 75 mW cm<sup>-2</sup>) (234). This effect could be significantly enhanced by using antimicrobial peptide (AMP) modified liposomal formulations. The use of the AMP WLBU2 for conjugation to temoporfin liposomes resulted in the complete eradication of methicillin-resistant *Staphylococcus aureus* (MRSA). Together with a 3.3 log reduction of *Pseudomonas aeruginosa* this indicates the utility of bacteria-targeted PS delivery to combat both Gram-positive and -negative bacteria (231,233). Likewise, wheat germ agglutinin (WGA) modified liposomes containing temoporfin proved effective with similar results in a related study (232).

*Clostridium difficile*, the main cause of antibiotic-associated diarrhea, was the target of a comparative investigation using 13 standard photosensitizers (228). In contrast to PSs such as talaporfin or chlorin e<sub>6</sub>, which achieved up to 3 log reductions, temoporfin proved to be inactive, most likely due to the formation of turbid suspensions in the medium used.

In dentistry, cariogenic bacteria such as *Streptococcus mutans* and *Streptococcus sobrinus* are potential targets for aPDT. Studies with hypericin and Fospeg® showed that both species could be completely eradicated using short PDT protocols with either PS or a combination of both (235). Another target, esp. in endodontic infections, is *Enterococcus faecalis*. The use of Foslipos (50 nM mTHPC, illumination with 100 J cm<sup>-2</sup>) completely eradicated the bacterium, while 10 nM mTHPC still gave a CFU

reduction by 5.8 log units (229). A comparative study of temoporfin incorporated into liposomes and invasomes using freshly extracted human wisdom teeth and inoculated with *E. faecalis* showed a 3.6 log unit reduction directly at the root canal wall with the latter. This was a significant improvement compared to a standard treatment with 1% chlorhexidine gel (reduction by 1.2 log units) (422). Ultrasonic activation can be used to improve the aPDT as it facilitates deeper diffusion into dentinal tubules and biofilms (580).

Eradication of bacteria alone is not enough. In periodontitis treatment often grafting materials are required to support the regeneration of injured periodontium. In an innovative approach, Kranz *et al.* (230) developed antimicrobial photodynamic active biomaterials for periodontal regeneration. For this, curable biomaterials (either urethane dimethacrylate or oligoester urethane methacrylate which contained a mixture of tricalcium phosphate microparticles and temoporfin were prepared). Both materials exhibited suitable mechanical and biocompatible properties and were capable of completely suppressing *P. gingivalis* and significantly reduce *E. faecalis* after illumination.

### Antiviral PDT

The Coronavirus pandemic has elicited an increased awareness of the potential of PDT to combat viruses (15,581). In a natural product screening study pheophorbide a (a chlorophyll derivative) showed a light-dependent antiviral activity on SARS-CoV-2 and inhibited virus-cell fusion. A similar mechanism was shown for temoporfin; however, it exhibited only moderate antiviral activity (582). Note, temoporfin has also been suggested in a pre-print as a potential blocker of SARS-CoV-2 E channel based on a docking study (583). A computational study by Absalan *et al.* identified temoporfin as the best candidate for docking with the COVID-19 major protease (6LU7) (584).

A promising development is the identification of temoporfin as a potential drug to inhibit the replication of the Zika virus (585). Initially endemic to Africa Zika infections have now spread and are a global health concern. A broad screening of >2800 existing drugs by Li *et al.* (586) in 2017 identified three lead compounds as flavivirus NS2B-NS3 interaction inhibitors with nanomolar potencies. Among them, the most promising is temoporfin as a cytotoxic compound. Zika virus infection was inhibited in human placental and neural progenitor cells and the virus-induced viremia and mortality could be prevented in mouse models. Docking studies indicate that the chlorin binds to NS3 pockets resulting in non-competitive inhibition of flaviviral polyprotein processing.

### Other infectious disease pathogens

A study by Preuß *et al.* (587) investigated the use of temoporfin, a cationic and anionic photosensitizer for Mosquito larvae control. Using *Chaoborus* sp. as a model they showed that all three PSs accumulated in the intestinal tract of larvae, not the tissue. Only the cationic PS (5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin toluene sulfonate) which is known to be active against Gram-positive bacteria resulted in the photodynamic killing of the larvae, possibly by inactivation of the intestinal flora.

### Non-medical applications

Although developed as a drug candidate temoporfin-related compounds are occasionally used for other purposes. One such example relies on the capability of (free base) porphyrins to act as sensors (588) or in CBRN defense and remediation (589). Rout and coworkers showed that changes in the Q-band absorption pattern of temoporfin upon binding of metal ions allow temoporfin to be used as a ‘miniaturized unimolecular analytic system’ to detect metal ions. Furthermore, considering the resultant absorption intensities at different wavelengths as output signals allowed us to use of temoporfin a logic 4-to-2 encoders and 2-to-3 decoders in logical computing (590,591).

Temoporfin has also been used to modify protonated graphitic carbon nitride (pCN) (592). Compared with unmodified pCN the new material showed stronger absorption in the visible and near-IR regions and higher photocatalytic activity in hydrogen evolution.

### CONCLUSIONS

This overview of the literature underlines the continuous scientific and medical interest in the photosensitizer temoporfin. Temoporfin – being on the market since 2001 – is one of the few clinically established photosensitizers. The advances and continuing research outlined herein illustrate the increasing interest in PDT in general, visible from the continuously high number of publications in the field of PDT. Clearly, the field is not standing still. Currently, several clinical studies with different photosensitizers including temoporfin are under way (426,437).

With respect to clinical development, there are diverse fields of application, from human to emerging uses in animal PDT. Clearly, the main clinical application remains in head and neck cancer and malformations, but PDT with temoporfin is now also used in other fields such as skin cancer and gastrointestinal applications like cholangiocarcinoma. Apart from this, temoporfin and its formulations have also found interest for use in antibacterial and antiviral photodynamic treatments. Together with approved photosensitizers such as verteporfin, ALA, or porfimer sodium, temoporfin is one of the gold standards in contemporary PDT. Temoporfin is also – due to the extensive scientific knowledge about this photosensitizer and the fact that it is a well-characterized single small molecule – often used as a comparator to test the PDT efficacy of other new photosensitizers and their formulations.

In the last decade, most medical developments for temoporfin have been in the development of advanced pharmaceutical formulations with a focus and extensive work on liposomal formulations. Here, temoporfin is often used as a model photosensitizer to be incorporated in new formulations as a test case. Increasingly, the available body of information facilitates its use as a general model compound for highly lipophilic drugs benefitting from its easy detectability *via* absorption or fluorescence spectroscopy.

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### CONFLICT OF INTEREST

A. Wiehe is an employee of the biolitec research GmbH which belongs to the biolitec group. The biolitec Pharma Ltd., which is also part of the biolitec group, is the marketing authorization holder for the medicinal product Foscan® which contains the photosensitizer temoporfin as the active substance.

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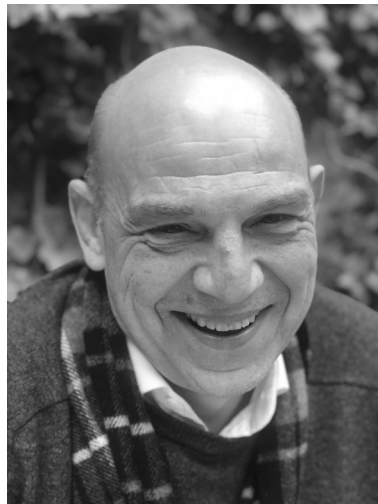
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