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# Photodynamic Therapy: An Overview and Insights into a Prospective Mainstream Anticancer Therapy

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**Abstract:** Photodynamic therapy (PDT) procedure has minimum invasiveness in contrast to conventional anticancer surgical procedures. Although clinically approved a few decades ago, it is not commonly used due to its poor efficacy, mainly due to poor light penetration into deeper tissues. PDT uses a photosensitizer (PS), which is photoactivated on illumination by light of appropriate wavelength and oxygen in the tissue, leading to a series of photochemical reactions producing reactive oxygen species (ROS) triggering various mechanisms resulting in lethal effects on tumor cells. This review looks into the fundamental aspects of PDT, such as photochemistry, photobiological effects, and the current clinical applications in the light of improving PDT to become a mainstream therapeutic procedure against a broad spectrum of cancers and malignant lesions. The side effects of PDT, both early and late-onset, are elaborated on in detail to highlight the available options to minimize side effects without compromising therapeutic efficacy. This paper summarizes the benefits, drawbacks, and limitations of photodynamic therapy along with the recent attempts to achieve improved therapeutic efficacy via monitoring various cellular and molecular processes through fluorescent imagery aided by suitable biomarkers, prospective nanotechnology-based targeted delivery methods, the use of scintillating nanoparticles to deliver light to remote locations and also combining PDT with conventional anticancer therapies have opened up new dimensions for PDT in treating cancers. This review inquires and critically analyses prospective avenues in which a breakthrough would finally enable PDT to be integrated into mainstream anticancer therapy.

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## INTRODUCTION TO PHOTODYNAMIC THERAPY

PDT involves the use of electromagnetic radiation (emr) of a specific wavelength (light) and photosensitizer (PS) molecules to form in-situ singlet oxygen or other ROS (Reactive Oxygen Species) to eliminate malignant cells ((1). It is a clinically approved, but poorly utilized technique in treating cancers due to its poor efficacy and inability to reach deeper tissues. Compared to other oncology techniques, PDT is less invasive, and the known adverse effects are limited to prolonged residual photosensitivity (1).

Individual components of PDT, i.e., emr, photosensitizer, and molecular oxygen, are non-toxic. In PDT, topical or systemic administration of PS is followed by light activation through careful irradiation of the location by emr of appropriate wavelength, triggering a photochemical reaction generating other Reactive Oxygen Species (ROS) labeled as type I reaction or a photochemical reaction causing highly reactive singlet oxygen which is labeled as type II reaction (1). The accumulation of other ROS or singlet oxygen in cells results in direct cell killing by an intense oxidative burst or cell death via apoptosis or necrosis (1). In ideal conditions, it kills off the tumor cells while sparing the healthy ones.

### The Photochemical Aspect of PDT

In PDT, the PS administered is expected to concentrate on the cancer tissue selectively. The tissue is then exposed to suitable light in which

photons' absorption causes the excitation and subsequent de-excitation of photosensitizer molecules. De-excitation emits photons that are to be absorbed by the surrounding substrate molecules. Notably, a certain fraction of the excited singlet state molecules is transformed into an excited triplet state, relatively long-lived. The transformation occurs via intersystem crossing (1). The molecules that have attained the excited triplet state can either form free radicals or radical ions by electron transfer or hydrogen atom extraction to biochemical substrate molecules such as membrane lipids, oxygen, solvent molecules, etc.

These free radicals and the radical ions interact with ground-state molecular oxygen to produce ROS, such as superoxide anion radicals, hydrogen peroxides, and hydroxyl radicals through type I reaction (1). Highly reactive singlet oxygen is made from a type II reaction where energy from the excited triplet state molecule is directly transferred to ground state molecular oxygen. The above responses of types I and II coincide; the substrate's specific nature and the PS determine the ratio between the two reaction rates (2). ROS are usually considered toxicants that induce deleterious effects such as cell dysfunction, death, or malignant transformation (3). Under normal conditions, ROS are generated in the cells through enzymatic and non-enzymatic reactions (3). ROS may cause opposite cellular effects, such as promoting cell proliferation and tumor progression or cell death and tumor regression, which can be utilized in therapeutic techniques against cancer (1). In PDT, since the light activation of the PS triggers ROS generation, the selective accumulation of the PS in malignant tissue leads to improved therapeutic efficacy (3).

**Photosensitizers Used in PDT**

A photosensitizer suitable for PDT should display a strong absorption peak in the far visible region (650 - 800 nm) (4), in which the absorption by the body should be minimal (For example, haemoglobin absorbs radiation between 478 and 672 nm). Illumination by light having wavelengths longer than 800 nm does not provide sufficient energy to excite oxygen to its singlet state. It seems an ideal PS ought to have a strong absorption peak lower than 478 nm and a fluorescence peak within 478 nm to 672 nm. Most of the PSs used in PDT have a tetrapyrrole backbone and are relatively hydrophobic compounds. They rapidly diffuse through the plasma membrane into tumor cells and localize in organelles such as mitochondria and endoplasmic reticulum (ER) (4). An ideal PS's chemical nature facilitates its entry into the tumor cells without precipitation in aqueous environments and selectivity to target cells instead of healthy cells. It also enables appropriate extinction coefficients and accumulation rates in target tissues (5). It has been noted that many of the effective PSs are preferentially low-density lipoproteins (LDL) among various serum proteins. Hamblin *et al.* suggested that over-expressed LDL receptors found on tumor cells could be playing a role in tumor localization (4).

**PS Used in PDT**

Photosensitizers are generally categorized into porphyrins and non-porphyrins. Porphyrin-derived PSs are further classified into 1$^{st}$, 2$^{nd,}$ or 3$^{rd}$ generation PSs (1). Hematoporphyrin (Hp), its derivatives (HpDs), and Photofrin (Porfimer Sodium) are classified as 1st generation PSs.

Photofrin is a commercially available PS, employed mainly in PDT (1). At 500 nm, it absorbs light with maximum excitation along with two other peaks at 540 and 560 nm, while the maximum emission occurs at 615 nm, associated with a second peak at ~680 nm (5). The research work by Bechet (5) also sheds light upon the influence of electric pulses (EP) delivery on the Photofrin uptake and its localization in human breast cancer cell line (MCF-7) and Chinese Hamster Ovary (CHO) cells. With the aid of fluorescence image analysis, it was observed that epithelial cells (EP) of CHO cells significantly improved Photofrin uptake. Due to electropermeabilization, Photofrin entered the cell and accumulated in the entire cell (5). Photofrin is commonly employed as a PS in PDT to treat advanced-stage lung cancer, early-stage esophageal, gastric, and cervical cancer. Absences of intrinsic toxicity, the possibility of using in small doses, sufficient clearance from healthy tissue, and the possibility of repeated administrations without serious repercussions except for prolonged photosensitivity to the neoplastic patient are among the many benefits of using Photofrin as PS in PDT (1).

Benzoporphyrin derivatives, chlorins, phthalocyanines, texaphrins, naturally occurring hypericin and 5-aminolevulinic acid (5-ALA), and other related esters that promote the production of endogenous protoporphyrin IX (PpIX) are classified as 2$^{nd}$ generation PS(1). 5-aminolevulinic acid (5-ALA) is metabolically converted into photosensitizable protoporphyrin IX, and hence it acts as a pro-drug. During the biosynthesis of heme, both 5-ALA and PpIX are generated as intermediates, where heme inhibits the endogenous generation of excess 5-ALA. The presence of exogenous 5-ALA allows bypassing the abovementioned regulatory mechanism, resulting in the accumulation of PpIX in cells (1). 5-ALA induced PpIX is known to have good tumor selectivity, limited systemic toxicity, and low skin photosensitization (1).

Nyman *et al.* reported that PpIX displays a Soret peak (intense absorption peak in the blue wavelength region of the visible spectrum) at about 405 nm and additional absorption at 510, 545, 580, and 630 nm, which are referred to as Q bands. Its fluorescence peaks are observed at 635 nm and 705 nm (8).

Hypericin is a naturally occurring compound that can be a promising alternative to chemically synthesized photosensitizers (1), but it has a few unfavourable characteristics that limit its usage in PDT. Its absorption peaks are around 595 nm and it does not absorb light above 630 nm. This specific spectral region of light has limited penetration. The potential application of hypericin in PDT lies in treating superficial lesions. Recent clinical work has addressed its use as a PS in treating bladder cancer due to its specific accumulation in urothelial carcinoma lesions (8). Low selectivity, weak absorption in the red wavelength region, resulting in difficulty in treating deep tumors, and residual skin photosensitivity being the significant side effect are the drawbacks of 1st generation PS.

Meta-tetra(hydroxyphenyl)chlorine (m-THPC), commercially known as Foscan or Temoporfin, is a 2nd generation PS with a very high potential to be used in PDT for the treatment of neck and head cancers. The molecule has a hydrophobic nature and a short plasma half-life in humans. M-THPC can be photoactivated at about 652 nm, and the photosensitization results in a very high yield of singlet oxygen accumulating in tumor cells. In addition to the direct damage to tumor cells by oxidants, m-THPC also causes intense and sustained vascular damage owing to its pharmacokinetic behaviour (1). It has been observed that m-THPC has two major absorption peaks at 420 nm and 652 nm. In clinical use, light activation of m-THPC is done at 652 nm as light in the spectral region of 420 nm has limited penetration. Studies have been conducted to improve the efficacy of m-THPC in the treatment of neck and head cancers (1).

Mono-l-aspartyl chlorin e6 (Talaporfin sodium) is a 2ndgeneration PS with a core chlorin structure and a highly aromatic system, characterized by excellent solubility in aqueous media and short half-life. Preclinical studies involving Talaporfin sodium have revealed that on light activation, in addition to cytotoxicity, the simultaneous induction of systemic, tumor-specific immuno-modulation mediated by CD8+T cells aids in overcoming tumor resistance through micro-vessels closure and upregulation of both cytolysis and memory cells (1,2,9).

Attempts have been made to develop 3rd generation PSs by utilizing the Warburg effect, which relates to cancer cells' ability to absorb glucose in large quantities compared to healthy cells (9). 3rd generation PS ought to have higher tumor cell selectivity and specificity. G-chlorin is a probable candidate for such a PS displaying strong anti-tumor effects against gastric cancer and colon cancer. It was also recorded to be 20–50 times more cytotoxic than Talaporfin sodium (10). M-chlorin, which is mannose conjugate chlorin designed to target tumor-associated-macrophages, Katoaka *et al.* recorded similar cytotoxic effects as G-chlorin *in vitro* studies, but *in vivo* allograft model study, M-chlorin PDT showed the most substantial antitumor effects (10). Although results are inconclusive for G-chlorin and M-chlorin, this opens up a route to another line of work to develop PS conjugated with biological molecules to enhance their selectivity and specificity to become 3rd generation PS.

**Optimizing PDT Outcome**

To achieve a better therapeutic outcome, the three main pillars of PDT, namely light, PS, and tissue oxygen, are present in optimum proportions throughout treatment procedures. During clinical applications, light and PS dose is administered empirically without any inter or intra-treatment variations. A crude practice ignores fluctuations in the pharmacokinetics of PS, tissue optics, and tissue oxygenation, thus leading to poor efficacy. A strong, reliable, and more personalized dosimetric system has been suggested. The practical execution requires a system to measure PDT dose explicitly by extrapolating from measured PS and light amount or implicitly by measuring the photobleaching or directly measuring the concentration of singlet oxygen in the tissue under treatment. All the proposed dosimetric approaches to PDT aim at directly or indirectly quantifying the primary effector of PDT (singlet oxygen) (12).

Monitoring tissue oxygenation and a delivered dose of light in real-time is crucial so that the treatment plan can be manipulated to enhance the therapeutic efficacy. It is also beneficial to look into the cellular level mechanisms that affect PDT outcome to understand the possibilities available to influence overall tumor cell death for better therapeutic development.

*Accurate measurement of PDT dose*

In photobleaching, a dye or a fluorophore of a PS molecule in PDT undergoes a photochemical change resulting in its permanent inability to fluoresce, caused either by cleaving covalent bonds or non-specific reactions between the fluorophore and surrounding chemical species. Photo modification, where the loss of absorbance or fluorescence occurs at a particular wavelength, yet the chromophore is retained at an altered state, is also considered a photobleaching form (15).

As shown by Dysart *et al.,* the rate of change in the ground-state ($S_0$) photosensitizer concentration $\frac{d(S_0)}{dt}$ due to $^1O_2$-mediated photobleaching can be expressed by the differential equation given below (10). (Concentration of the PS is ($S_0$), and the concentration of the oxygen in the tissue is ($^1O_2$)).

$$\frac{d(S_0)}{dt} = -k_{os}[S_0] + \delta \times (^1O_2) \qquad \text{(Eq. 1)}$$

Thus, the reaction between $^1O_2$ and ground-state photosensitizer molecules is governed by the bimolecular reaction rate constant $k_{OS}$. The reaction leads to irreversible degradation of the photosensitizer and also oxygen consumption (11). The term $\delta$ accounts for the reaction of a $^1O_2$

molecule with the same photosensitizer molecule involved in its generation. The reactions of ground state photosensitizer molecules with $^1O_2$ are considered to be dominant reactions at the low concentrations usually found in cells and tissues (less than micromolar) since singlet oxygen has a higher probability of reacting with other targets before diffusing into another sensitizer molecule. δ is given by:

$$\delta = \frac{1}{d^3 N_A} \quad \text{(Eq. 2)}$$

Where $N_A$ is the Avogadro's number and $d$ is the mean $^1O_2$ diffusion distance, defined as $d = \sqrt{6 D\tau_D}$ where $D$is the diffusion coefficient of $^1O_2$ and $\tau_D$ is the $^1O_2$ lifetime. The instantaneous $^1O_2$ concentration can be determined by rearranging equation (1) to

$$\left(^1O_2\right) = \frac{d[S_0]}{k_{os}\left([S_0]+\delta\right)dt} \quad \text{(Eq. 3)}$$

As $^1O_2$ is the primary cytotoxic in PDT, the PDT dose is the cumulative of $^1O_2$ generated, thought to be equal to the amount of $^1O_2$ reacted, other deactivation pathways being less probable to occur. Hence, the PDT dose over a time T is (10)

$$Dose = \int_0^T [^1O_2](t)\frac{dt}{\tau_D}$$

$$Dose = \frac{1}{\tau D \times k_{os} \ln[S_0]_0 + \delta/[S_0](T) + \delta} \quad \text{(Eq. 4)}$$

The PDT dose is calculated from photobleaching of photosensitizer (decrease in ($S_0$)) if $1/(\tau_{D\,x} k_{OS})$ and $\delta$ are determined experimentally. In clinical practice, practitioners continue to face difficulties in determining the exact dose required to achieve complete healing from the condition. Such challenges often lead to inaccuracies that might result in under-treatment of the targeted lesions. Jarvi *et al.* report their findings employing SOL (singlet oxygen luminescence) to directly measure singlet oxygen dose compared to estimate made via measured photobleaching, displaying an excellent correlation between the two methods. The study also highlighted that photobleaching-based PDT dose analysis was unreliable at tissue oxygen concentrations below 5ℳ M due to rapid changes in fluorescence intensity, which weren't observed by Dysart *et al*. However, these results cannot be generalized to all PS as this study deployed m-Tetra(hydroxyphenyl)chlorin (m-THPC) (13).

Jarvi *et al.* further reported comparative polts between fluence rate, triplet state oxygen ($^3O_2$), and photobleaching, consistent with the findings of Dysart *et al.,* indicating a discontinuous and poorly correlated variation of light fluence and $^3O_2$ with singlet oxygen. Thus, it can be proposed that explicit measurement of PDT dose is a crude and unreliable method. The work done by Jarvi *et al.* provides additional evidence for singlet oxygen being the primary cytotoxin of PDT as proposed by photochemistry (13). SOL (singlet oxygen luminescence) based dosimetry analyses the luminescence signals emitted by singlet oxygen generated at the tissue under treatment, thus a direct measurement method was utilized by Jarvi et al. their investigation. Though SOL provides accurate dose measurements where difficulties in translating it to clinical use were encoutered due to cost, complexity, and weak signal strength, which constitutes an invaluable tool in evaluating alternative dosimetric techniques. Photobleaching-based dosimetry is an option to be used clinically, given that at tissue oxygen concentrations above 5 M (13). Sharwani *et al.* report a positive correlation between loss of fluorescence (photobleaching) and PDT outcome in a clinical study conducted using a fluorescence imaging system to estimate the photobleaching of PpIX (14). However, the findings of Sharwani *et al.* are yet to be validated with a more extensive range of test data.

*Effective delivery of light*

PDT requires a single dose of PS administered to the patient, followed by the photoactivation of the PS after a specific time interval by a single illumination using light of appropriate wavelength. Illumination is conventionally done by using broad-spectrum light sources such as arc lamps and filament lamps. Difficulty in coupling them with light delivery fibers without reducing the power output, difficulty in calculating the effectively delivered dose, limited maximum power output (1W), and the presence of UV and IR radiation are the limitations to their use over advantages of being cheap and easy to use. Modern lasers, being inexpensive, compact, and mobile compared to first-generation lasers, are widely used in PDT, and can be equipped with units capable of carrying out dosimetric calculations and programmed treatment plans (15). Diode lasers emit only one wavelength of light, limiting their versatility in contrast to light-emitting diodes (LEDs). Both varieties are used clinically. Fiber optic media is used to deliver the light dose locally. A successful PDT procedure requires providing an adequate amount of light from the source to the target and ensuring homogeneous distribution of light (15). A light delivery system must be used in conjunction with advanced dosimetric software for its efficient utilization, in which measured diagnostic signals are fed into a dose distribution calculation program (40-43). In the illumination, the accepted approach is to deliver a threshold dose, which is a minimum dose adequate to cause direct cell death, delivered to a target tissue volume (15).

$$D_{PDT} = \int_0^T \epsilon[PS]\phi\, dt \quad \text{(Eq. 5)}$$

$$D_{fluence} = \int_0^T \phi \, dt \qquad \text{(Eq. 6)}$$

Equation (5) refers to PDT dose, which is determined by the extinction coefficient of the PS (ε), the concentration of the PS [PS], fluence (number of photons arriving per unit cross-sectional area) rate (Φ), and the time interval of illumination (dt). Assuming a homogeneous distribution of PS in the target tissue volume, equation (6) relates the fluence dose to a simplified dose calculation. Fluence rate is estimated via calibrated optical probes; these point measurements are utilized to obtain representative readings of the light dose delivered to a location. The photon propagation is required to be theoretically calculated to spatially map the fluence rate throughout the entire target tissue volume (15). In addition to that, the optical properties of the target tissue are estimated.

$$\phi_{(r)} = \frac{P}{4\pi D r \exp\left(-\mu_{eff} r\right)} \qquad \text{(Eq. 7)}$$

The above equation, which illustrates a theoretical model for photon propagation, is obtained as an analytical solution for the diffusion equation, where P:source power in watts, D:diffusion coefficient (in cm), $\mu_{eff}$ :effective attenuation coefficient of light in the target tissue, r:radial distance from the point source (in cm) (15).

Davidson *et al.* devised a treatment-planning software package utilized in Phase II clinical trial of Tookad™-mediated I-PDT of persistent prostate carcinoma following radiation therapy (24). This software uses a patient-specific I-PDT treatment plan based on predicted light distributions in the prostate and surrounding tissue. The model used the diffusion equation and the finite elements method (FEM) numerical analysis, with the volume of interest discretized into a 4-noded tetrahedral mesh. Treatment plans were designed according to the pre-treatment MRI images. In tumors treated with a light dose greater than 23 J/cm$^2$, a complete pathological response was observed. No patient with a D(90) less than 23 J cm$^{-2}$ was reported to have a complete biopsy response, while 8/13 (62%) of patients with a D(90) greater than 23 J cm(-2) had negative biopsies six months post-treatment (23). Swartling *et al.* developed a treatment planning software utilizing Interactive Dosimetry by Sequential Evaluation (iDOSE) (23). The software enabled dose plans with optical fiber positions according to 3-D tissue models developed via ultrasound, calculated the best fiber positions, and provided an optimal treatment plan. The clinical study used Temoporfin-mediated photodynamic therapy (PDT) for low-grade (T1c) primary prostate cancer. Residual viable cancer cells were present in the prostrate tissue from a histopathological analysis of tissue biopsies taken six months post-PDT. The authors proposed that the low threshold dose of light, which was set to 5 J/cm$^2$ could be the possible cause of the incomplete treatment (23).

*Savenberg et al.* at Lund University reported a clinical trial using an 18-fiber interstitial PDT system on recurrent prostate cancer, yielding satisfactory results (15). The 18-fiber interstitial PDT system developed by the Lund University research group could carry out pre-treatment planning and dosimetric calculations during treatment. It is noteworthy to mention that the rationale of the research group at Lund University is to adopt bare-end optical fibers, which allow well-defined positions when used as sources or detectors, resulting in a well-defined source-detector distance in all measurements taken with the use of fibers (15). The Lund group has used the data from researchers and clinical practitioners over the past two decades, but the PDT system they developed was clinically tested with four prostate cancer patients, downplaying the reliability. The treatment planning software package from Davidson *et al.* comparatively has better footing in positive clinical test results and a larger sample size of 13 test subjects (24). Treatment planning software regimes should be tested clinically with larger samples to validate the findings and improve overall therapeutic outcomes. Better software can be developed to aid more complex mathematical models integrating variables such as PS concentration, tissue oxygen, distribution patterns of tumor cell death, etc. Another major limitation in treatment planning software is the assumption of light homogeneity and PS dispersion and the tissue structure when software and models are developed.

It was observed that collimated laser beams scatter forward when encountered by tissues. As a result, they have increased tissue penetration depth compared to non-coherent LED or arc lamps. In contrast, non-collimated light sources bear more divergent beam properties, leading to reduced forward scattering of light, making them unfit to treat deeper lesions (16). Lasers are a common source of illumination used in PDT nowadays. The first lasers ever used in PDT were gold (Au) vapor or copper (Cu) vapor lasers and argon-ion-pumped dye lasers, which emit light in the red wavelength region of the visible spectrum. Solid-state diode lasers came into clinical use in the late 1990s. The benefit of using lasers in therapeutic PDT is the possibility of transmitting the light through optical fibers to reach remote destinations. This procedure created an opportunity to treat tumors in hollow organs, such as the urinary bladder, the bronchus, the intestines, and the esophagus (15). In a clinical study, Jeries et al. reported image-guided optical fibers' placement in treating deep tumors in the head and neck. During the procedure, multiple fibers were positioned under ultrasound guidance into various deep-seated tumors, including head and neck tumors and vascular anomalies in the limbs. Over a hundred patients were treated with mTHPC-PDT, more than 50% of patients had a good response, while five patients became disease-free, and 80%

reported improvements in breathing, swallowing, and speech (17). Pulsed PDT regimes are proposed to increase the depth of necrotic cell death. Thus, it is thought to enhance PDT efficacy primarily on the hypothesis that the downtime between light irradiation will permit tissue re-oxygenation and re-accumulation of photosensitizer at the lesion, subsequently leading to a better therapeutic outcome (18).

The investigation conducted by Pogue *et al.* on the depth of necrosis of a 48 hrs post PDT resulting from continuous wave (CW), and pulsed irradiation displayed no significant difference statistically under the same average incident irradiance (19). A study by Grecco *et al.* yielded a contradictory result: a femtosecond laser irradiation produced a necrotic zone twice as deep in comparison to a CW laser at an equivalent dose (150 $J/cm^2$) using hematoporphyrin derivative (HpD) as PS (20). Pogue et al., by simulating the deposited amount, reported that the pulsed laser irradiation would help treat deep tissue tumors with PDT. These outcomes are modest and strongly dependent on the PS, the laser pulse width, the pulse energy, and the repetition rate (21). In a study conducted by Sterenborg et al., the simulations concluded that pulsed excitation in PDT was identical to continuous wave (CW) excitation for fluence rates below $4 \times 10^8$ $Wm^{-2}$. It was noted that at higher fluence rates, pulse PDT's effectiveness drops significantly (22). The available evidence is contradictory, so pulsed irradiation cannot be favoured against the use of CW irradiation to achieve an increased depth of tumor necrosis.

Despite the advancements in light sources, delivery, and dosimetric approaches, delivering therapeutic light to deep tumors is still a hurdle to be overcome as it vastly limits the use of PDT. The use of self-luminescent chemical or biological probes has been investigated. While Philip *et al.* were the first to report the use of chemiluminescent probes, Carpenter *et al.* reported the use of bioluminescent probes. The light dose produced by chemical or bio-luminescent probes is lower than that usually expected for PDT, but efficient induction of cytotoxicity is noteworthy. Due to the complexity and limited understanding of the processes involved, further investigations are required to validate their efficiency (either as free probes or in nanoparticle form) before clinical translations. NIR (Near Infra-Red) radiation is proposed as an ideal candidate to achieve increased tissue penetration in PDT. Although better than visible radiation, NIR radiation has a limited penetration depth of approximately 1 cm. Treatment of large superficial tumors may be possible with NIR light, but tumors residing in deeper tissues remain unreachable without a secondary light delivery strategy.To improve the penetration depth of photons, X-rays can be potentially employed in conjunction with PS and radiosensitizers (RS) with minimum tissue penetration limitations, even though ionizing radiation is known to cause intrinsic toxicities (27). Luksiene *et al.* investigated the RS *in vivo* properties of three different PS (HPde, Photofrin II (PII), and hematoporphyrin derivative (HPD)), and revealed RS effect of these PSs was cell line dependent (28). The low efficiency of PS that acts as RS under direct excitation and cell line dependence has contributed to diminishing the interest in any advancements in this area. Attention was given to approaches that can locally generate visible light using X-ray irradiation, such as Cerenkov radiation and nanoscintillators.

Cerenkov emission occurs when charged particles, such as electrons or positrons, travel faster than the phase velocity of light in a given medium. The works of Alexsson *et al.* and Kotagiri *et al.* have given solid proof of the concept and usability of Cerenkov radiation for deep tissue illumination in PDT. The number of studies conducted in this area is few until a wide range of investigations bears favorable conclusive evidence, clinical implementations will be afar (29,30). Implementation of Cerenkov radiation in PDT as per the investigations conducted so far was dependent on clinical linear accelerators or PET scanners, creating a barrier to the use of PDT as a low-cost alternative treatment approach to cancer. Chen and Zhang suggested the use of nanoscintillators in combination with PS. Scintillating nanoparticles are nanoparticles (NP) that convert ionizing radiation into visible light (31). With the advent of nanoscience, this area of research has been very dynamic in recent decades. Morgan *et al.* developed a model to predict the maximum amount of $^1O_2$ generated under X-ray irradiation via quantifying the amount of energy stored in nanoscintillators during the irradiation; this led to the conclusion that only X-rays with energy below 300 keV could cause sufficient cytotoxicity. These predictions were further refined using Monte Carlo simulations. A more accurate estimation of energy deposited in a nanoscintillator was found by Bulin *et al.* (32,33). Investigations have been conducted using nanoscintillators such as terbium oxide ($Tb_2O_3$@$SiO_2$ NPs), ($SrAl_2O_4$:$Eu^{2+}$), and ($LiYF_4$:$Ce^{3+}$) in conjunction with PS respectively, by Bulin *et al.*, Chen *et al.* and Zhang *et al.* providing insights into different mechanisms and successful tumor elimination (34-36). Their work is conclusive and reliable in terms of outcomes and sample size. We believe nanoscintillators hold the key to unlocking the reach of PDT into deeper tumors. We are hopeful that with more thorough *in vitro* and *in vivo* studies in the future, this technique's full clinical translation potential can be realized.

*Monitoring tissue oxygenation*

The current techniques available for monitoring tissue oxygenation during PDT are as follows. Point measurements are obtained via oxygen electrodes or luminescence-based optodes for direct tissue oxygen measurements or by employing optical spectroscopy for measuring the oxygen saturation of haemoglobin (123). Imaging is considerably more complicated but feasible with techniques like Blood Oxygen Level Dependent Magnetic Resonance Imaging (BOLD MRI), a functional MRI with a low

signal-to-noise ratio. Preclinical research has demonstrated dramatic changes in tissue oxygenation during PDT, which vary depending on the photosensitizer and light delivery methods. Better oxygenation throughout treatment can be achieved by keeping the light fluence rate low to maintain the rate of oxygen consumption at a low level. Real-time monitoring to ensure adequate oxygenation at strategic points in the targeted tissues during PDT is crucial for increased efficacy, particularly in the image-guided treatment of tumors in solid organs (123). In our evaluation, an image-based tissue oxygen monitoring mechanism coupled with an image-based dose measurement system would be a more feasible approach in clinical transformation.

## PHOTOBIOLOGICAL AND CYTOLOGICAL EFFECTS OF PDT

PDT-induced cytotoxicity, causing tumor cell death, is the primary biochemical phenomenon in anticancer PDT. In principle, it is believed that only the cells directly affected by the treatment undergo cell death due to the different toxic agents, and neighboring cells that are not affected by the treatment will live on (6). Recent evidence supports the idea called ‘By Stander Effect’, which states that cells die in clusters when treated with a photosensitizer and light, not as individuals (6). Dahle *et al.* reported that 'the propagated inactivation' model best describes the spread of dead cells mathematically by a study executed using Madin-Darby Canine Kidney (MDCK) II cells and some other cells (47-50). The available literature proposes no single pathway through which cell death occurs in photodynamic therapy (PDT). Instead, multiple cell death routes are activated due to PDT (1). The primary molecular targets of PDT should be located within a few nanometers from the intercellular site of photosensitizer localization since the singlet oxygen produced has a short-life span and spatially limited diffusion (1). PDT causes tumor cell death via direct pathways such as apoptosis, necrosis, and autophagy and indirectly through vascular shutdown and immune response. The balance between apoptosis and necrosis is considered as a major factor that determines the fate of tumor cells after PDT and also intracellular localization of PS, which is solely determined by the chemical nature of PS and the light fluence of delivered light (4).

### Apoptosis

PDT is capable of causing either apoptosis or necrosis, or a combination of both. In the majority of cases, PDT is highly effective in inducing apoptosis (6). Apoptosis, commonly addressed as programmed cell death, is a complex enzyme governed cell death program genetically inherited by all living cells (1). Apoptotic cells stand out due to their characteristic morphological appearance as shrunken cells with condensed nuclear chromatin retracted from surrounding cells (6). The apoptosis process eventually activates a specific protease family known as caspases (cysteine-dependent aspartate specific proteases) (1,6). In PDT, mainly oxidative stress leads to the initiation of apoptosis. Apoptosis is triggered either by the mitochondrial release of cytochrome c or by the activation of cell death receptors, triggering the activation of executioner caspases such as caspase 3, 6, and 7 (6). In cells, caspase activation occurs either by extrinsic or intrinsic apoptotic pathways, which refers to the location of origination of stimuli (1). The extrinsic pathway is activated upon the stimulation of death receptors of the TNFR (tumor necrosis factor receptor) family. The stimuli, such as DNA damage, cytotoxic insults, etc., activate the intrinsic apoptotic pathway, which acts through the mitochondria controlled by proteins in the Bcl-2 family (1). The Bcl-2 family is a set of proteins that has the potential to promote or inhibit apoptosis by adjusting the outer mitochondrial membrane (51).

The convergence of signals from death receptors at the cell surface or damaged sites on mitochondria results in the following changes: a) permeabilization of both inner and outer mitochondrial membranes; b) dissipation of the transmembrane potential of the inner mitochondrial membrane; c) release of cytochrome c and other apoptotic proteins, apoptosis-inducing factor (AIF), the second-mitochondria-derived activator of caspases (SMAC) and specific proteases from the mitochondrial intermembrane space. Although the above-stated mitochondrial changes have implications on apoptotic pathways, their respective order of execution remains in dispute, as reported by Kessel *et al.* and others (6, 52, 53). There are multiple viewpoints about how the discharge of apoptosis-related mitochondrial factors and the collapse of mitochondrial transmembrane potential occur. Permeability transition pore complex (PTPC) is one such model, which ascribes the loss of transmembrane potential and other changes that follows to an opening of a large conducting channel known as permeability transition pore complex (PTPC) formed at the contact sites of the outer and inner mitochondrial membranes (6), which is believed to be consisting of transmembrane proteins such as (a) 30kDa inner membrane adenine nucleotide translocator (ANT), (b) 32kDa outer membrane voltage-dependent anion channel(VDAC) or mitochondrial porin (c) 18kDa outer membrane peripheral benzo-diazepine receptor (PBR), etc. (6). Localization of the photosensitizer in mitochondria has been more efficient in inducing cell killing than PS localization at other cellular sites (6).

Bcl-2 family of proteins controls apoptosis induced by a variety of apoptotic stimuli. The pro-survival Bcl-2 family includes Bcl-2, BclXL, Bcl-w, A1, and Mc11 groups of proteins. It is believed that photodamage caused to pro-survival members of the Bcl-2 family triggers the activation of pro-apoptotic family members. Several *in-vivo* studies supported this idea, e.g., in cervical cancer cells during the apoptosis induced by 5-ALA mediated PDT, there was significant suppression of Bcl-2

mRNA level and an increase in Bax mRNA level. Similar results were observed with the oesophageal cancer cell line (62) and in hypericin mediated PDT with breast adenocarcinoma cell line where a down-regulation of Bcl-xl and up-regulation of Bax was observed (1,6,62). The Bax: Bcl-2 is known to play a pivotal role in PDT-induced apoptosis. A higher percentage is found to promote cell death via apoptosis, and researchers have recorded evidence to support this (63,64). He *et al.* reported this phenomenon in a study conducted using Chinese hamster ovary cell lines transfected with a Bcl-2 gene. The study results exhibited that in the presence of Bcl-2, the incidence of apoptosis following PDT was significantly lower, and Bcl-2 was capable of inhibiting overall cell killing (62). Genes that code for Bcl-2 family proteins differ between species, putting the validity of *in vivo* studies conducted using non-human cell lines into question. He *et al have* transfected the human Bcl-2 gene into Chinese hamster ovary cells to eliminate inter-specific variability.

Nuclear factor-kappa B (NF-κB) is a transcription factor that promotes gene expression of several proteins related to immune-regulatory and pro-inflammatory processes. NF-κB dimers are found in the cytoplasm in association with an inhibitory subunit; specific inhibitors are IκB factors (6,62). NF-κB is known as an inhibitor of programmed cell death "apoptosis." Granville *et al.* confirmed the idea that NF-κB generates an anti-apoptotic signal following PDT (65,66). Mitogen-activated protein kinases (MAPK) are a critical component of a complex signaling network in cells that regulate gene expression for various external stimuli. The MAPK signaling pathways modulate numerous cellular activities such as mitogen-induced cell cycle progression through the G1 phase, cell movement, and apoptosis (1,62). Apoptosis can be rightly called the most extensively studied form of cell death. Available literature provides a wholesome overview of the factors contributing to and pathways leading to apoptotic cell death, but the sequence of events remains in dispute. PDT-related apoptosis investigations are conclusive and sound, many of them have converged on the same primary factors, yet stimuli triggering apoptosis are poorly understood. Currently accepted mechanisms and signaling pathways have been integrated into computational models to simulate apoptotic cell death based on Monte Carlo stochastic simulations to explore further fluctuations in apoptosis signaling to predict outcomes (34). Mathematical modeling can be used as a tool to investigate the sequence and probability of events following certain intrinsic or extrinsic stimuli. It is fair to propose by designing PS to localize mitochondria using mitochondrial markers; apoptotic cell death can be optimized (28). Another possible approach to promote apoptosis is developing photoactivated chemical factors that bind to cell death receptors triggering apoptotic cell death pathway. This is already been attempted with the aid of photoCORMs (Carbon Monoxide Releasing Molecules) (26).

**Necrosis**

Necrosis is considered as accidental and uncontrolled in the manner in which cell death proceeds. It is believed to be operating without the underlying signaling events, but the accumulating evidence suggests the existence of caspase-independent pathways. The occurrence of cellular necrosis proceeds through cytoplasmic and organelle swelling, followed by loss of membrane integrity followed by cellular content discharge, which characterizes necrotic cell death (1,62,68).

Apoptosis operates as the default cell death mechanism, while necrosis will only occur when the activation of caspases fails. Necroptosis is the programmed form of necrotic cell death that proceeds either through FasLigand (FasL), a member of the tumor Necrosis Factor (TNF) family of proteins and tumor necrosis factor-related Apoptosis-Inducing Ligand-Receptor-1 (TRAIL-R) or other members of tumor Necrosis Factor (TNF) family. Fas-Associated protein with Death Domain (FADD) plays an essential role as an adaptor protein in Fas and TRAIL-R-induced necrosis. Still, its part in TNF-induced necrosis remains controversial (62,65,69,70). The basic format of cell death caused by PDT switches to necrosis with PS localized in the plasma membrane and lysosomes. The biochemical pathway leading to necrosis after PDT has yet to be identified. Factors such as intracellular $Ca^{2+}$ and specific ROS have been recognized as necessary in promoting necrotic cell death following PDT (1). Studies conducted so far have not provided sound evidence on the factors at play during necrosis, so it cannot be adapted successfully into a mathematical model to develop simulations for further examination; the molecular biological approach remains the only option available.

**Autophagy**

Autophagy is a catabolic pathway that allows the degradation of eukaryotic cells and recycles cellular contents. In a basic sense, autophagy contributes to maintaining intracellular and cellular homeostasis. The role of autophagy in causing cell death has raised controversy since its discovery; autophagy is known to accompany cell death, while its pro-survival role is well established (71,99). Autophagy acts as a defense mechanism against ROS-induced damage following PDT by clearing the cells of all the damaged organelles, but its effect on the overall outcome of PDT is yet to be revealed (1,96). Studies on PDT-induced autophagy with different cancer cell lines and PS led to the following conclusions: PDT is capable of direct induction of autophagy independent of PS target. Apoptosis frequently occurs in cells that are already undergoing autophagy following PDT. In cells that can undergo apoptosis, autophagy performs a pro-survival function. In contrast, cells less likely to experience apoptotic cell death promote cell death via necrosis(62,65). In our view, autophagy needs to be studied in the light of increasing overall cell death; existing literature vaguely indicates the possibilities of autophagy with little evidence to support the

claims, the mechanism, and the factors leading to autophagy are yet to be understood.

**Vascular Damage**

Cytotoxicity and tumor regression have effectively resulted from microvascular stasis and consequential hypoxia. Blood flow stasis following PDT occurs due to combined damage to sensitive microvasculature locations and the consequent physiological responses. It is generally hypothesized that vessel stasis mechanisms begin with perturbation and damage to endothelial cells during light activation of photosensitizers in tissues. The physiological cascade of responses, including platelet aggregation, the release of vasoactive molecules, leukocyte adhesion, an increment of vascular permeability, and vessel constriction, combine to produce blood flow stasis, and the formation of thrombogenic sites within the vessel lumen is a result of endothelial cell damage (72). Studies have revealed that second-generation PS, MV6401 evokes a biphasic vascular response in experimental animals after PDT. The late formation of a thrombus and necrosis following vasoconstriction was the most rapid response observed (73). A delay in tumor growth usually accompanies the vascular effects; similar vascular effects were observed with Photofrin–PDT (1,73). It was noted that angiogenic factors such as vascular endothelial growth factor (VEGF) and cyclooxygenase (COX-2) were upregulated during PDT (74). The action by which blood vessels are formed from the existing ones is termed angiogenesis. Angiogenic factors promote it. The use of specific inhibitors for these angiogenic factors can influence the overall outcome of PDT positively. An investigation conducted utilizing benzoporphyrin derivative monoacid ring A (BPD–MA, Verteporfin) as the PS led to the unveiling of a correlation between the timing of vascular damage and cure, which implies the significant role played by blood flow stasis in tumor destruction following PDT (1,70,73).

Standish *et al*. used interstitial Doppler optical coherence tomography (IS-DOCT) to investigate the microvascular changes during PDT. The study results indicated a dependence of microvascular closure on irradiance rate and total irradiance during PDT. While faster vascular shutdown rates were associated with increasing PDT irradiance rate and total irradiance, a threshold effect at irradiance rates above 66 $mW/cm^2$ was recorded. No further increase in vascular shutdown rate was reported. Use of irradiance or total irradiance value that causes an abrupt vasculature shut down during PDT limits the supply of molecular oxygen to the region of interest, leading to ineffective treatment, as will a shallow irradiance rate. It is understood that microvascular closure takes place at different rates, and a correlation was found between PDT total irradiance and irradiance rates. These dependencies can be put into effective use in PDT treatment planning, feedback control for treatment optimization, and post-treatment assessment.

PDT is a complex and dynamic process that requires accurate, real-time assessments of treatment delivery and therapeutic response. IS-DOCT may be a suitable option for real-time monitoring. Still, the difficulty remains in deriving the optimal IS-DOCT/PDT monitoring metrics and predicting treatment response and outcome based on them (37). Only a few biochemical factors leading to blood flow stasis following PDT haven't been revealed experimentally, but available evidence has established a positive correlation between blood flow stasis and tumor cell killing. More *in vivo and in vitro* investigations need to be carried out to identify specific factors and their specific contributions to tumor microvascular closure so that they can be manipulated in favour of increased overall tumor cell death.

**Immune Response**

One of the first events after PDT at the tumor site is the generation of damage-associated molecular patterns (DAMPs) or so-called 'danger' signals, which contribute as warning signals for innate immune response. Studies on the release of DAMPs following PDT indicated that DAMP associated with PDT could differ in the same cancer cells between in vivo and in vitro settings (73). It was observed that DAMPs released after PDT correlated well with the sub-cellular localization of PS since it's the origin of ROS-induced stress (75). A good correlation between DAMPs and PDT can be established only following further research into the molecular and cellular mechanisms (73). DAMPs released following PDT will be detected by the innate immune cells such as monocytes or macrophages, neutrophils, and dendritic cells (DCs) recruited to the tumor site treated with PDT. Then these innate immune cells infiltrate in massive numbers to attack the damaged tumor cells (75). The innate immune cells' primary function is to neutralize the DAMPs by engulfing and eliminating the cellular debris and compromised tissue components, promoting local healing by restoring normal tissue function.

Investigations have been conducted to identify the factors mediating the crosstalk between the immune system's innate and adaptive sections following PDT. It has been revealed that the enhancement of adaptive anti-tumor immunity by PDT involves the activation of dendritic cells (DCs), which are stimulated by the recognition of DAMPs/Cell Death Associated Molecular Patterns (CDAMPs) discharged by dying tumor cells (75). 70 Kilodalton heat shock proteins(Hsp70) are among the best-characterized DAMPs released following PDT, form stable chaperone complexes with cytoplasmic tumor antigens by HSP-antigen complexes binding to the danger signal receptors, Toll-like receptors 2 and 4on the surface of dendritic cells (73, 75). DCs remain in an immature state in the absence of inflammation. Following tissue inflammation and release of DAMPs, the dendritic cells (DCs) mature and rush to the draining lymph nodes in massive numbers. The transition to the mature state of DC is correlated with the elevation in the numbers of

surface major histocompatibility class I and II molecules (MHC I and MHC II) and the costimulatory molecules CD80 and CD86. Costimulatory molecules are a heterogeneous group of cell surface molecules capable of amplifying or counteracting the initial activating signals given to T cells by the T cell receptor (TCR) following interaction with an antigen/major histocompatibility complex (MHC). These changes permit the DCs to express peptide-MHC complexes at the cell surface and $CD4^{+}$T helper cells and $CD8^{+}$cytotoxic T lymphocytes (CTLs) to trigger an adaptive immune response (75). It is not only antigen-specific T cells that can provide post-PDT adaptive immunity but also B cells that produce antigen-specific immunoglobulins, thus mounting the so called humoral immune response. So far, there is only one study conducted by Preise *et al.* displaying the activation of humoral immunity as an implication of PDT-induced systemic antitumor protection (77).

The first clinical case of systemic PDT-immune response was observed and published in 2007, recording PDT of multifocal angiosarcoma of the head and neck located on the right upper limb of a patient, causing spontaneous regression of the untreated distant tumors on the contralateral left upper limb, accompanied by increased immune cell infiltration (78). Kabingu *et al.* (2009) reported that PDT treatment of BCC lesions enhanced the reactivity of patients' lymphocytes against Hip1, a known BCC-associated TA (tumor Antigen) (79). Post PDT adaptive immune response and increased immune cell infiltration are required to be studied extensively in clinical studies before any valid conclusion can be made.

The immunology of tumor cells has been extensively studied with a broad scope of molecular biological approaches as it is crucial to all forms of anticancer therapies. The existing theoretical basis provides a sound background for any future research on post-PDT tumor immune response. Grace *et al.* developed a mathematical model to understand and explore tumor immune cell interactions (38). Our belief is such models integrating PDT will give more insights into how immune response can be regulated to optimize PDT outcome. Although important insights can be gained from mathematical modeling, the development of such models incorporating patient-specific data remains a vital goal yet to be realized for potential clinical benefit.

**Effects on Cells Surviving PDT**

There is strong evidence suggesting that PDT can cause considerable damage and inhibit the growth rate of tumor cells that survive the PDT procedure. Cancer cells are highly invasive and display a rapid growth rate. A study conducted using ALA\PDT has been able to provide evidence for a deceased cellular invasion in surviving cancer cells (1). In addition to that, reduced mitochondrial function and suppressed cellular invasiveness were the notable phenotypic changes in the surviving tumor cell lines. A correlation was found between the surviving cells' reduced invasive ability and the downregulation of the Epidermal Growth Factor Receptor (EGFR). Researchers confirm that there is a considerable probability that these effects pass on to the progeny (1). Surviving tumor tissue demonstrates a lower growth rate, which may require a cell cycle arrest in the surviving tumor cells. A study conducted with the non-small lung cancer cell line H1299, which used Photofrin /PDT, targeted the G0/G1 phase of the cell cycle, resulting in a notable reduction of Bcl-2 expression (76).

In addition to this, early proteasome malfunction induced G2/M phase arrest, which was time-limited (1). Only lung cancer cell lines A549 and H1299 delivered consistent results concerning the G0/G1 phase arrest hypothesis. An upregulation of p-21 and expression of p-53 and a temporary reduction in Bcl-2 were noted simultaneously with this arrest (80). Ahmad et al. reported similar results with human epidermoid carcinoma cells A431 treated with Pc4/PDT, in addition to cell cycle arrest through G0/G1 phase arrest. It was observed that the expression and activity of cyclin-dependent kinases CDK2, CDK6, and the inhibition of regulatory counterparts cyclin E and cyclin disassociated with G0/G1 phase arrest (1,74,81). Research data provides strong evidence for reduced expression and activity of CDKs in cells following PDT, explaining the observed decreased Rb phosphorylation (1,75).

The role of MAPKs in the survival or death of cells following PDT remains controversial. Research data from time to time reports either promotion or protection from apoptosis caused by MAPK involvement (82,83). NF-κB plays a role in modulating anti-apoptotic gene expression, which may negatively impact tumor destruction (1). NF-κB is also suspected to be involved with tumor recurrence since it may upregulate the expression of specific factors promoting proliferation and angiogenesis. The apparent dichotomy in NF-κB activity has not yet been completely understood (84). The proteasomes' act on substrates as tumor suppressors, signaling molecules, cell cycle regulators, transcription factors, and anti-apoptotic proteins. Thus Synthesis of NF-κB precursor or the degradation of NF-κB suppressor can be controlled by the proteasome (85,86). Arrest or retardation of tumor cell progression may inhibit the proteasome since it would interrupt the systematic degradation of cell cycle proteins and factors such as NF-κB (1,74). Chiaviello *et al.* extensively studied the proteasome activity of lung adenocarcinoma cells with sub-lethal Photofrin/PDT and recorded a reversible inhibition of proteasome activity shortly after photosensitization (87). The effects that are expected to have on the cells surviving PDT are mainly based on the understanding of the cell cycle and cytology. Still, there is a complex network of parameters at interplay at the cellular level. The available evidence is too few, inconclusive, and contradictory to establish the factors determining the effects on cells surviving PDT. Multiple *in vitro*

studies have been done to investigate the effects of cells surviving PDT. It has been found that under the influence of PDT, cellular migration and attachment were suppressed in many cell lines (89,90). It has also been proven that PDT decreases cellular invasion in lung adenocarcinomas, melanoma, breast carcinoma, and head and neck cancer cell lines (91,92). Malignant lesions are treated repeatedly with PDT to achieve a reasonable efficacy; a sound understanding of the effects on the surviving cells and the causative factors might open up possible avenues to induce post-treatment cell death, leading to enhanced overall efficacy.

## CLINICAL APPLICATIONS OF PDT

Even though the first use of PDT in treating skin cancer was attempted in the 1980s, it helped spread slowly as an alternative treatment technique for other cancer types. The PS used for PDT has come to their third generation, and the search is underway for better and more effective PS with minimum side effects. PDT has been attempted for different types of cancer, and studies have been carried out via clinical trials. PDT is ideally suited to treat skin cancer. The first large clinical trial using hematoporphyrin derivative (HpD) illuminated by red light resulted in more than 85% of complete recovery (CR) rates. Numerous studies have shown that PDT has been able to achieve response rates equivalent to those achieved by conventional anticancer treatment methods for superficial skin cancers (58). Patients with a few localized lesions are treated with a procedure that follows a topical application of ALA a few hours before the illumination. This procedure has reportedly generated excellent CR rates of 86% to 100% for basal cell carcinoma (BCC) (93). One significant drawback of ALA PDT is that the illumination is very painful during the first few minutes. Cold air and local anesthesia can be used to alleviate the pain (88).

Multiple skin-cancer lesions are treated with PDT using a systemic application of PSs such as porfimer sodium or mTHPC, with a recorded CR of 91% for BCC. Treatment periods are found to be shorter for BCC treated with mTHPC PDT (88). The only primary skin neoplastic condition that is not treatable with PDT is malignant melanoma. This type must be surgically uprooted for extensive histopathological examination, prognostic evaluation, and continued management. One treatment session is adequate for neoplastic lesions with a thickness of up to 3 mm. Thicker lesions are usually retreated after follow-up, or pretreated, e.g., with curettage, which means that a layer of the tumor is removed surgically, and PDT is performed on the tumor bed (15).

Clinical trials have proved HpD/PDT and porfimer sodium/PDT to be effective for superficial and recurrent bladder cancer. Response rates were initially high, about 70 to 100%, and the long-term response rates were about 30%-60%. PDT has not become an established treatment method for bladder carcinoma due to the high incidence of side effects such as urinary frequency, pain, and persistent reduction in bladder capacity. But these side effects were caused mainly by the excessive and non-uniform light doses delivered during early clinical studies (88). A standardized procedure with lower drug and light doses or less penetrating light of 514nm for illumination resulted in reasonable CR rates with side effects such as transmural bladder injury and treatment-related morbidity (88). More recent efforts of using ALA/PDT for recurrent superficial bladder cancer produced CR rates of 40%-52% at 18-24 months without a persistent reduction in bladder capacity. In this attempt, ALA /PDT was given as a single treatment and in combination with mitomycin C (94-96). PDT done using green light for illumination coupled with appropriate dosimetry has proven to be a promising in situ treatment option for superficial bladder carcinoma.

Conventional treatment for early-stage head and neck carcinoma is surgery or radiotherapy, while chemoradiation is the standardized treatment for advanced stages. These traditional treatment procedures have their limitations and drawbacks. The surgical treatment procedure for head and neck cancer requires a wide margin, leading to functional damage to adjacent tissue resulting in difficulties in swallowing and speech. Also, radiotherapy has a risk of xerostomia, trismus, and osteonecrosis. At the same time, chemoradiation is related to high morbidity.

In contrast, trials have proved PDT to be equally effective as conventional treatment methods for small superficial tumors, but PDT spared the healthy tissue beneath the tumor. PDT generated excellent long-term functional and cosmetic results in clinical trials, and it could also be utilized in the palliative treatment of recurrent head and neck carcinoma (97). CR rates have been recorded as 85% at year 1 and 77% at two years for early-stage primary tumors in the oral cavity and oropharynx. For lip carcinoma, it was recorded as 96% (97-99). Patients with head and neck cancers have a lifetime risk of 20%-30% of developing second or multiple cancers after the treatment for the primary. In such cases, PDT can be used following radiotherapy or surgery since there is no cumulative tissue toxicity after PDT (97-99). When conventional therapy fails, PDT can also be used as a salvage treatment for recurrent head and neck cancers (63-65). In treating large tumors PDT was used interstitially (97).

Numerous studies have established the therapeutic use of PDT in endobronchial cancer of different stages. Palliative treatment of obstructive endobronchial cancer using HpD or porfimer sodium PDT has been recorded to relieve symptoms in the vast majority of patients (100-103). Side effects included cough, expectoration of necrotic debris, and dyspnea, in addition to skin photosensitivity, which lasted a few days following the treatment. PDT was recorded to be employed as a curative

treatment for early-stage lung cancers, where survival rates were 56%-70% for five years, and for carcinoma-in-situ (CIS), the 5-year survival rate was about 90% (101,104,105). Sometimes, after endobronchial cancers are treated via resection and irradiation, field cancerization or recurrence of tumors may occur. Due to limited pulmonary reserve, these patients cannot undergo irradiation or resection once more, and such patients can be conveniently treated with PDT.

The standard treatment procedure for esophageal cancer is esophagectomy (88), a surgical technique of complete or partial removal of the esophagus via an incision made in the chest or abdomen. Esophagectomy is associated with high morbidity and mortality, leading to the development and application of less invasive techniques to treat esophageal cancer. Endoscopic mucosal resection, coagulation, and PDT were notable among these less invasive methods. Initial studies of PDT were conducted on obstructive esophageal tumors as palliative treatment, and subsequent studies confirmed the efficacy of PDT as a treatment for such tumors (106-108). PDT has an observed CR rate of 87% at six months for treating small superficial tumors in the esophagus. This was achieved by using porfimer sodium as PS, and even mTHPC gave comparable results (109,110). Despite the high efficacy, PDT caused severe side effects in most clinical trials since the esophagus is a thin-walled structure. These side effects ranged from transient skin photosensitivity to stenosis, fistulas, and perforations and were reported in 57% of the patients treated with PDT using red light. It was noticed that when PDT was carried out using less penetrable green light and m-THPC as the PS, fistulas, and perforations were not observed as side effects, and the procedure did not compromise the efficacy (108-110).

Barrett's esophagus is considered a serious complication of GERD (Gastroesophageal reflux disease). In this condition, the normal tissue lining of the esophagus changes to a form that resembles the tissue lining of the intestine. Patients with this condition have a higher risk of developing esophageal cancers, specifically esophageal adenocarcinoma. Researchers have attempted to treat Barrett's esophagus via PDT; the studied document shows a notable reduction in the risk of developing esophageal cancer when patients are treated with porfimer sodium-PDT. In clinical practice, endoscopic mucosal resection is widely used to treat severe conditions of Barrett's esophagus over highly invasive esophagectomy. A combination of endoscopic mucosal resection and PDT has proved to be as effective as esophagectomy in producing CR rates of 83 to 100% in 1 year (111,112). It is fair to conclude PDT as a potential candidate for an alternative anticancer treatment procedure as it demonstrates average CR rates exceeding 60% for superficial cancers on the skin, head, neck, and in hollow organs, given these rates are highly variable and dependent on parameters such as PS, illumination, tumor size, and tissue oxygenation.

In a systematic review and meta-analysis, Patel *et al.* reported that PDT had a 14% better chance of complete lesion clearance at three months post-treatment than cryotherapy for thin AKs (Actinic keratoses) on the face and scalp (114). A clinical study conducted by Chhatre *et al.* on the survival outcomes of stage III and stage IV Non-Small Cell Lung Cancer (NSCLC) patients observed a lower risk of mortality in the PDT group and radiation with chemotherapy group compared to the radiation alone group (50% and 53% lower, respectively). Among NSCLC (Non-Small Cell Lung Cancer) patients with stage III or stage IV disease not eligible for surgery, the addition of PDT to chemotherapy and radiation therapy offered survival benefits over radiation therapy alone (115). The results of this clinical study provide evidence for the potential use of PDT in later-stage lung cancer to improve survival rates. Due to the large sample size of 147 participants, it is a relatively effective outcome in terms of reliability. Li Bo Li *et al.,* in a retrospective study comparing PDT and chemotherapy on advanced esophageal cancer, concluded; PDT combined with chemotherapy for advanced esophageal cancer is superior to PDT alone and chemotherapy alone (116). It is not about which therapeutic technique triumphs over cancer. It is about ensuring the defeat of cancer. The future of anticancer therapy appears to be complex combination treatments, and PDT has already approved itself as a viable and potent candidate for the blend.

## LIMITATIONS, ADVANTAGES, AND DISADVANTAGES OF PDT

PDT has many advantages, potentially promoting its use as an anticancer treatment. In comparison with other treatment modalities used for anticancer therapy, PDT is characterized by the following:

- Selective action on the sensitized tumor
- Minimally invasive technique
- Possibility of being repeated
- No accumulation of toxicity
- The meager mutagenic potential
- Healing is fast with sound cosmetic effects
- Organ functionality is retained
- Short treatment time
- Compared to conventional anticancer treatments, fewer adverse effects
- Cost-effectiveness

Light is delivered selectively to the tumor, which initiates the photodynamic action, so the overall activity of PDT is selective and localized. Due to fewer adverse effects and the absence of accumulated toxicity, the procedure can be repeated in the case of recurrent tumors. The treatment duration of PDT is shorter compared to conventional treatment methods, and it is also cost-effective. PDT causes minimum damage to the

healthy tissue in the vicinity, so the organ functionality remains undisturbed, and the cosmetic effect is also high (113,117).

One of the significant limitations of PDT is that light used to photoactivate the PS cannot penetrate a tissue thicker than 1 cm, which limits the use of PDT in treating tumors on or just below the skin or on the lining of internal organs or cavities (113). PDT is less effective in treating large tumors due to the inability of light penetration deep into large tumors (113,117). PDT is a localized treatment and generally cannot be employed to treat metastasized cancers (113). In clinical practice, PDT is faced with many challenges, including accurate identification of cancer within a respective tissue, precise prediction of the behavior of that cancer, and definitive treatment of the identified target volume. Furthermore, the hurdles included assessing the tissue after treatment to determine whether the planned treatment volume received the intended treatment and providing appropriate follow-up for the untreated part of the tumor, which may lead to the recurrence.

PDT results in residual photosensitivity in patients, which may last for several days after treatment. The therapeutic efficacy of the PDT procedure depends heavily on the accuracy of light delivery to the target site. Tissue oxygenation acts as a limiting factor for the therapeutic efficacy of PDT. Among the adverse effects of PDT, pain is the most prominent one, apart from photosensitivity (117).

## SIDE EFFECTS OF PDT

### Early-Onset Side Effects

Pain has been an issue of general concern since it is the most common and limiting side effect of conventional PDT. During the clinical trials, it is recorded that about 58% of the patients who have undergone PDT procedures complain about severe pain (93). A painful burning sensation starts almost immediately during the illumination process, which rapidly becomes intense and reaches a peak during the first few minutes. The pain usually decreases with time and subsides towards the end of the treatment procedure (2). Pain can be severe in some instances, stopping light exposure prematurely, leading to inadequate therapeutic results. Thus patients experiencing severe pain are hardly satisfied with the effectiveness and the convenience of PDT, eventually negatively influencing them to discontinue further treatments (93).

PDT-induced pain results from an interplay between intrinsic and extrinsic factors, yet the exact mechanism of PDT-induced pain is unknown. Reactive Oxygen Species (ROS) have been identified as the primary mediators of pain during PDT. The intensity of the pain will depend primarily on the depth of skin at which singlet oxygen is produced, which is, in turn, dependent on the wavelength of light used for illumination and on the PS. Studies have not reported any correlation between PDT-related pain and age or gender. Some studies vaguely reported a higher intensity of pain in fair-skinned patients, although in general, skin phototype seems not to have any effect on the pain experience (93). Regarding PS, many studies investigated the intensity of pain while using ALA or MAL, but the results are hard to interpret since these drugs are used differently in clinical practice (93).

Other factors that are notable in influencing pain during PDT are lesion type, location, and size of the treatment area. Studies have identified actinic keratosis (AK) as the most painful lesion to treat using PDT. In contrast, the head and neck as the location have the most significant impact on pain perception due to the high nerve density. Researchers also noticed that the lesions on the limbs caused a greater degree of pain during treatment than the lesions in the trunk (117). As a result of many studies conducted, researchers were able to derive a positive correlation between the intensity of the PDT-induced pain and the size of the treatment area (117).

Among Local Skin Reactions (LSRs) developed on the local skin area exposed to light during PDT, erythema and edema are the main phototoxic effects. Erythema is the appearance of redness in the skin, and edema is characterized by swelling of tissue or inflammation. These are the typical inflammatory responses to phototoxicity. A clinical study carried out to investigate the adverse effects using a large sample group of patients undergoing topical PDT over five years recorded 89% of erythema and edema occurrence, 80% of scaling and itching, 9% of crusting, 6% of pustules, 1.2% of erosions and 0.4% of infections (93). Research involving ALA-PDT demonstrated that acute inflammatory response causes immediate stinging, followed by prolonged erythema. The study also revealed the role of histamine as a mediator in bringing about an acute inflammatory response to PDT. It was observed that post-treatment dermal histamine level peaks about 30 minutes after the illumination, remains stable till about 4 hours, then gradually returns to baseline level within about 24 hours following treatment (93).

Clinical research carried out to study the effect of oral H1 antihistamine therapy on reducing LSR didn't find any reduction in the inflammatory response or the therapeutic efficacy of ALA-PDT. So the role of histamine as the key mediator of the post-PDT inflammatory response is still under dispute (93). PDT is also capable of causing local and systemic immunosuppression and reducing delayed-type hypersensitivity (DTH) responses to recall antigens. It was observed that ALA-PDT and MAL-PDT are both immunosuppressive locally, even after one treatment procedure (93). Urticaria, also commonly called hives, is an upsurge of swollen, pale red bumps or plaques (wheals) on the skin. Literature has described urticarial reactions in response to ALA and MAL PDT, where 0.9% of

patients suffered from prevailing severe itching and wheal within the first minute of light exposure (93). Clinicians have recorded the early-onset side effects eagerly. A more systematic and integrated approach can be proposed for studying side effects, as they are mainly outcomes of the innate immune response. In light of such investigations, more insight can be gained on ways and means of minimizing and managing side effects.

**Late-Onset Side Effects**

PDT has only a few late-onset side effects, which appear to be rare in most cases. These can appear, ranging from a few weeks to months. In rare cases, PDT can induce hyperpigmentation and a sense of fear. Hyperpigmentation is usually a transient condition, displaying a slow resolution in the months following the treatment. The reason for hyperpigmentation is not recorded in the literature, though it is assumed to be a result of phototoxic damage caused to the melanocytes (93). Bullous pemphigoid is a rare skin condition marked by the formation of large, fluid-filled blisters. They are known to develop on areas of skin that often flex — such as the lower abdomen, upper thighs, or armpits. Literature available describes the appearance of BP at sites treated with PDT for Bowen's disease. Yet, the causative mechanism for this condition remains unknown (93).

PDT has the potential to induce or stimulate skin carcinogenesis in patients treated with the procedure. Several literature sources recorded the post-PDT onset of basal cell carcinoma (BCC), invasive squamous cell carcinoma (SCC), and keratoacanthoma (93). Various pathogenic mechanisms, including immunosuppression, mutagenesis, and isotopic response, may lead to carcinogenic risk. The mutagenic effect of PDT remains in dispute. Simultaneously, some researchers claimed that there is no direct effect of PDT on mutagenic DNA; others proposed that ROS generated during photosensitization can cause DNA mutations and oncogene activation (93). The occurrence of skin cancer at the sites exposed to PDT is explained by a concept known as an immunocompromised district (ICD). The idea suggests that a damaged skin area with an immune response imbalance is prone to distinct secondary disease. The role played by PDT as a promoter of skin malignancies is not fully understood, and further studies are required in that regard. Since late on-set side effects less frequently reported are given poor attention in the literature. Immunosuppression and mutagenic effects need to be further explored via in vivo and in vitro models to fully comprehend the causative factors and mechanisms before integrating PDT into mainstream anticancer therapies.

**Alleviation of Side Effects**

Managing pain is a major challenge in PDT. Different techniques are employed to manage pain during PDT treatment procedures, such as cold air analgesia, topical anesthesia, infiltration anesthesia, and nerve block hypnosis. But none of them has proved to be completely effective. Daylight photodynamic therapy (DL PDT), wherein the exposure to average daylight causes photoactivation of PS without using a directed beam, can be considered a painless alternative to conventional PDT (93,119). In a randomized clinical study conducted to investigate the effect of cold water and pauses in illumination to reduce pain during PDT, one area was cooled during the first half of the illumination. The other area was cooled during the second half of illumination. A three-minute pause was carried out between the two halves of illumination. An immediate fall in pain intensity has been recorded when illumination is stopped (120). A light delivery platform that supports programmable paused illumination equipped with a mechanism to cool the PDT site would be advantageous to promote the application of PDT.

Urticarial reactions are explained by the release of histamine from the mast cells of the dermis. These reactions can be controlled by administering an antihistamine before the treatment (93). Thanos *et al.* showed that the immune suppressive effects of PDT could be reduced by the administration of oral or topical nicotinamide (Vitamin B3) (93). More investigations are needed to verify the effect of antihistamine administration in suppressing urticarial reactions before it becomes a norm in clinical practice.

## FUTURE PROSPECTS OF PDT

PDT has excellent potential to be developed into a mainstream anticancer treatment procedure. In recent years, researchers have attempted to utilize modern imaging techniques coupled with molecular biology to monitor and guide PDT procedures. Simultaneously, focus has been given to improving the targeted and selective delivery of PS to the tumor cells. A combination of ground-breaking research and developments in cancer biomarkers, nanotechnology, and targeted molecular medicine has opened a new realm of possibility for anticancer PDT, which is more personalized and predictive than ever before. Combining PDT with conventional anticancer treatments has opened up new opportunities for improved therapeutic efficacy.

**Detection of Tumor Biomarkers**

Developing a technique to detect the overexpression of several tumor marker genes simultaneously, being aware that a single cell generally expresses more than one altered gene must have a high predictive value in identifying cancer cells amidst the typical cellular background. Fluorescent probes have been designed to detect the levels of expression of different biomarkers in tumor cells and tissues. The expression of biomarkers such as messenger RNAs (mRNAs) or the presence of a specific mutation in an oncogene in tumor cells can be detected via molecular beacons (MBs) capable of emitting fluorescent signals only after binding to their specific target mRNAs. These

biomarkers may work as indicators for a well-defined clinical outcome (118,121). A biomarker is defined as an objectively measured characteristic that describes a normal or abnormal biological state in an organism by analyzing biomolecules such as DNA, RNA, protein, peptide, and biomolecular chemical modifications (122).

A cancer biomarker provides a measurement of the risk of developing cancer in a specific tissue or the risk of cancer progression or possible response to anticancer therapy (122). Identification of these biomarkers using molecular beacon (MB) and fluorescence imaging will enable monitoring tumor growth, progression, and location, thus efficiently guiding PDT treatment. With the advent of molecular biology, cancer biomarkers have been studied at length, even to the extent of developing a new generation of PS that can selectively bind to tumor cells. However, there is a possibility of the variability of tumor marker expression depending on the type and stage of cancer, so the findings of a specific study cannot be generalized without broadly investigating the variability factors.

**Targeted and Effective Delivery of PS**

Many PS drugs in use are hydrophobic with poor solubility in water (124). As a result, they easily aggregate under physiological conditions, significantly reducing the quantum yields of ROS production (124). The development of effective delivery systems that include customized PS drugs and a mechanism to transfer them into target tissues/cells and addressing critical biological barriers for conventional PS delivery are crucial. In recent days**,** PS drugs conjugated with nanomaterials have gained attention in the field of PDT due to their ability to circumvent the critical limitations of conventional PS drugs as follows (124). Through hydrophilic properties, nanomaterials can significantly improve the solubility of PS drugs in water by increasing their cellular uptake. Once formed into nanoparticles with nanomaterials, PS drugs can achieve passive targeting of a tumor by the enhanced permeability and retention effect (EPR) (124), which is often attributed to the leaky tumor vasculature and poor lymphatic drainage of tumor tissues. Furthermore, the cell-specificity of PS drugs can be noticeably improved by surface modification of the nanoparticles to bind active targeting moieties such as antibodies, peptides, and aptamers (124).

Incorporating PSs in nanostructured drug delivery units, such as polymeric nanoparticles (PNPs), solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), gold nanoparticles (AuNPs), hydrogels, liposomes, liquid crystals, dendrimers, and cyclodextrins, is considered as a way of surpassing the limitations of conventional PS. In addition, nanotechnology-based drug delivery systems may improve the transcytosis of a PS through epithelial and endothelial barriers and permit the simultaneous co-delivery of multiple drugs (125).

The novel smart drug delivery and phototoxicity on/off nano-system proposed by Yanchun *et a*l. are based on graphene oxide (NGO) as the carrier and modified to implement subcellular targeting and attacking (126). In designing the nano-drug (PPa-NGO-mAb), NGO is modified with the integrin αvβ3 monoclonal antibody (mAb) for tumor targeting. Pyropheophorbide-a (PPa) conjugated with polyethylene-glycol is used to coat the surface of the NGO to induce phototoxicity (126). The polyethylene-glycol phospholipid is loaded to improve water solubility. The results verify that the phototoxicity of PPa on NGO can be switched on and off in organic and aqueous environments, respectively. This smart system also offers a potential alternative to drug delivery systems in anticancer therapy (126). Recent advances in light-activated drug release by various techniques such as photocage, photo-induced isomerization, optical upconversion, and photothermal releases by which different wavelength ranges can be successfully implemented in the effective delivery of PS to the tumor tissues. Light-activated drug release also contributes to controlling undesired photobleaching during the PDT procedure. Joanna *et al.* evaluated the influence of electroporation on the Photofrin uptake and distribution in breast adenocarcinoma cells (MCF-7) and healthy Chinese hamster ovary cells (CHO) lacking voltage-dependent channels in vitro (67). The uptake of Photofrin was measured using flow cytometry and fluorescence microscopy methods. Observations indicated that electropermeabilization of cells in the presence of Photofrin increased the uptake of the photosensitizer (128). Targeted delivery will significantly reduce the PS dose that needs to be administered, thus reducing post-treatment photosensitivity. The metabolism of nanoparticles intended to be used in PS drugs has been studied carefully before any clinical translations. We are optimistic about nano-particle-assisted drug delivery approaches to eventually breakthrough effective and efficient PS delivery during PDT.

**Combination Therapy**

Combining PDT with conventional anticancer therapies such as chemotherapy, radiotherapy, and novel approaches like immunostimulant and antioxidant agents have been explored in recent years. The studies aimed to find a combined outcome to be additive, synergetic, or antagonistic (131). Though the efficacy of combined therapy is empirical, systematic methods were also employed to analyze its effectiveness. Graphic isobologram and finding combination index are two such methods used (132). Varriale *et al.* and Crescenzi *et al.* mentioned the specific applications of combination index and isobolographic analysis in developing PDT as a combined modality (133).

PDT has a good potential of triggering an anti-tumor immune response by specified mechanisms described early in this review. Combining this anti-tumor immune response with immunostimulants to generate a combined effect has been attempted.

These studies have been carried out across various cancer models, including lung cancer, colon cancer, squamous cell carcinoma, melanoma, and breast cancer, and have displayed promising in vivo results with increased survival and reduced tumor volumes (134). To generate combined effects, immunostimulants were administered during PDT intratumorally, intravenously, or topically depending on the type and location of the tumor (135). Although studies did provide substantial evidence that the therapeutic effect of the combination therapy was independent of the type of PS used, many studies used Photofrin as the PS (136,137). Brodin *et al.* summarized the results of recent studies on the applications of PDT as a combined modality with immunostimulants, ionizing radiation, and chemotherapy (134).

PDT combined with ionizing radiation (IR) has shown synergetic effects, but early reports were limited to purely additive results (149,150). Investigations related to Bowmen's Disease (BD) highlighted the synergetic impact of PDT with IR (151). The IR and ALA- PDT combination therapy proved to have improved the therapeutic efficacy of the IR treatment for BD while reducing the irradiation dose with no recorded side effects on the skin (152). It was noted in many studies that the irradiation dose and the time elapsed between the administration of PS and the irradiation played a key role in PDT –IR interaction (153). The possible mechanisms for the high toxicity of the combined therapy may be due to the loss of a critical number of tumor cells and altered biochemical microenvironment, leading to late tissue changes and additive toxicity (154). It is noteworthy that the combination of PDT with IR also utilizes the potential of sure PSs to function as radiosensitizers (149). The results yielded by research to some degree are ambiguous, and it is reasonable to conclude that the interaction between PDT and IR are dependent on numerous parameters such as type of pathology, dose and dose rate of both ionizing radiation and light, and the sequence and time of the treatments (118). Luksiene et al. (149) and Allman et al. (159) reported additive effects, while Sazgarnia et al. (162) found the outcomes to be exceeding that of an additive effect in contrast to Sharma et al. (160), reporting effects less than additive, in their respective *in vitro* tumor models tested with IR – PDT combined therapy.

Another promising emerging approach that uses nanoparticles to enable interaction of PDT with radiotherapy (RT) in treating cancers located in deep tissues was proposed by Chen and Zhang in 2006 (156). This proposed technique uses luminescent nanoparticles to deliver the PS to the target tissue. When irradiation with an appropriate dose of X rays, the nanoparticles scintillate, activating the PS. Thus, the method eliminates the need for an external light source to activate the PS. As the high-energy radiation beams can penetrate deep tissues, this approach might be a feasible way to treat deep tumors (157). The enhanced performance of the combination was established by the investigations executed using Lanthanide doped nanoparticles. Yet, some concerns contribute to the disturbingly reduced efficacy. Out of which most notable, Lanthanide doped nanoparticles exhibit a strong emission between 450 nm and 600 nm while most of the PSs used are porphyrins or their derivatives, which have a maximum absorption at about 400 nm. As a result, lanthanide-based nanoparticles are unable to activate the PS efficiently through scintillation (155). Utilization of the afterglow luminescence combined with scintillation luminescence in photoactivation has yielded positive results in improved PDT- RT combined outcome (157). The metabolic activity-based PET (Positron Emission Tomography) probe, 2-deoxy-2($^{18}$F) Fluoro-D-glucose ($^{18}$FDG) has been attempted to be employed as a substitute for a light source for photoactivation, has proved to be a promising new approach to treat deep tumors (158).

In combining PDT with chemotherapy, many possible options are available in achieving a combined or synergetic outcome (134). Evidence suggests that PDT affects cell membrane permeability, causing better delivery of cytotoxic drugs, leading to a mixed result. Some chemotherapy drugs act as cytotoxic agents and a PS, enabling illumination following the chemotherapy drug administration, giving room to synergetic products (162). Additive and synergetic effects of PDT-chemotherapy combination reduce the required chemotherapy dose, minimizing the possibility of severe side effects. Recent research carried out in the PDT-Chemotherapy combination suggests vastly improved therapeutic efficacy. Table 1 summarizes all the recent studies carried out in that regard (134).

**Table 1:** Studies conducted on PDT and chemotherapy combination.

| Implemented therapy Combination | PS used | Outcome | Reference |
|---|---|---|---|
| Cisplatin (6.25 mg/mL in vitro and 2mg/kg in vivo) along with the photosensitizer prior to PDT was evaluated | In vitro: cells were incubated with 5-aminolevulinic acid(5-ALA) at 25 or 50 mg/mL for 24 h In vivo: 375 mg/kg 5-ALAwas administered 6 h before PDT | (1) Combined 5-ALA PDT and cisplatin increased cytotoxicity (2) greater efficiency against tumor recurrence | Ahn et al. (163) |
| treatment with 24 h incubation with low-dose cisplatin followed by PDT, evaluated through cell viability and cell death mechanisms | After incubation with cisplatin, cells were incubated with 5-aminolevulinic acid (5-ALA) for 4 hr prior to PDT | (1 with cisplatin doses >1mg/L synergistically enhanced cytotoxicity(2) Increased apoptosis rate, related to upregulation ofp53 and changes in p21, Bcl-2, and Bax expression | Wei et al. (164) |
| doxorubicin of varying concentration (4-16 mmol/L) on a multidrug-resistant cell line was evaluated in vitro | Cells were incubated with pheophorbide a (Pa) photosensitizer 2 h before PDT | (1) Synergistic effect on cytotoxicity from combined doxorubicin þ PDT mediated by intracellular ROS generation (2) A synergistic effect is observed only in the multidrug-resistant line | Cheung et al. (165) |
| Gefitinib, which can inhibit ABCG2 protein-mediated efflux of porphyrin out from neoplastic cells, was assessed at different concentrations in vitro in combination with PDT | Following gefitinib incubation, cells were incubated with 1 mmol/L 5-aminolevulinic acid (5- ALA) for 6 h prior to PDT | (1) outcome was a dose-dependent reduction of the surviving glioma fraction(2) Effect due to decreased ABCG2 expression and subsequent increase in intracellular porphyrin levels | Sun et al. (166) |
| The apoptosis-inducing protein apoptin experimented in combination with PDT via PVP3 plasmid administration | Cells were incubated with 5-aminolevulinic acid (5-ALA) at 1 mmol/L for 6h before PDT, and the mice were administered 5-ALAat 100 mg/kg 3 h prior to PDT | Notably, stronger antitumor effects in vitro and in vivo compared to monotherapies | Fang et al. (167) |
| 5-FU, gemcitabine, oxaliplatin and cis-diammine dichloroplatinum chemotherapy in combination with PDT in vitro and gemcitabine and oxaliplatin in combined with PDT in vivo | Cells were incubated for 24 h with 20 mg/mL talaporfin sodium (TPS) photosensitizer. The mice were injected with 5 mg/kg TPS at 2 h before PDT | (1) Significant increase in tumor necrotic area and apoptosis-positive cells (2) Synergistic cytotoxicity increase from oxaliplatin and gemcitabine þ PDT | Nonaka et al. (168) |

| **Implemented therapy Combination** | **PS used** | **Outcome** | **Reference** |
|---|---|---|---|
| PDT was combined with 5 mg/kg Adriamycin to investigate increased antitumor effects through potentially enhanced apoptosis and inhibited tumor angiogenesis | The photosensitizer benzoporphyrin derivative monoacid ring (BPD-MA) was intravenously injected 24 h before PDT at 1 mg/kg | (1) Adriamycin PDT resulted in significantly reduced tumor volumes<br>(2) Also, a considerable increase in survival compared to separate Adriamycin or PDT | Tong et al. (169) |
| Combining doxorubicin or vincristine with PDT in the treatment of sensitive or resistant murine leukemia cells was experimented | Cells were incubated for 4 h prior to PDT with 1 mmol/ L 5-aminolevulinic acid (5-ALA) | (1)Chemotherapy-resistant LBR-D160 and LBR- V160 cell lines were sensitive to 5-ALA PDT<br>(2) No increase in treatment efficacy | Diez et al. (170) |
| The polytherapy combination of Navelbine or cisplatin chemotherapy followed by PDT, and by adoptive immunotherapy with splenic lymphocytes from PDT-treated mice was investigated | mTHPC (Foscan) was administered post-chemotherapy and 24 h prior to PDT at 0.3 mg/kg | (1) Chemotherapy, PDT and adoptive immunotherapy was successful against this aggressive metastatic tumor<br>(2) PDT or chemotherapy alone showed no survival advantage over control | Canti et al. (171) |

Other than studies conducted by Diez et al. and Canti et al., all further investigations reported positive outcomes in terms of increased cytotoxicity and tumor control. Cheung et al. and Nonaka et al. observed synergetic effects between PDT and chemotherapy in their respective *in vivo* and *in vitro* studies. Chemotherapy is widely used as an anticancer therapy; the combined use of PDT can be promoted as a way forward to familiarize PDT. Since the synergetic effects are highly dependent on the PS used, the outcomes of these investigations cannot be generalized. An acceptable range of *in vivo* and *in vitro* studies to unveil the variables that determine synergetic and increased cytotoxic effects, followed by clinical investigations, will be a feasible way forward for this combined modality.

The use of antioxidant agents or radical scavengers ought to nullify or counteract the effects caused by PDT, but several studies propose otherwise (132). Buettner e*t al.* reported having metal traces (in their case, iron). Ascorbate combined with Photofrin/PDT caused a heightened production of radicals and decreased cell survival of various cell lines (132). A cooperative therapeutic outcome was observed when ascorbate was associated with other photosensitizers in other systems under different conditions (132). Many studies have proposed various interpretations and explanations. Finally, it was concluded that the enhanced toxicity of the photodynamic action results from the augmented formation of highly diffusible hydrogen peroxide and other toxic radicals on the addition of ascorbate to cells expressing high myeloperoxidase levels followed by photosensitization (172). Melnikova *et al.* recorded the efficacy of m-tetrahydroxyphenylchlorinmTHPC/PDT could be synergistically improved in the presence of alpha-tocopherol, but only at the elevated concentrations of vitamin in a study with HT29 adenocarcinoma cells and MRC-25 normal fibroblasts (173). While the final therapeutic outcome of incorporating antioxidants with PDT may depend on many variables, including antioxidant concentration, the presence or absence of catalytic trace metals, the order and the time interval between the administration of the drug and the light exposure, the light fluence, the oxygen accessibility and more (132). Since variables are too many use of antioxidants for enhanced PDT is not a promising area for future explorations.

## CONCLUSIONS

Over the past several decades, many researchers have committed to making PDT a viable alternative treatment procedure for cancer. Still, PDT has not become a mainstream anticancer therapeutic procedure, owing to its poor efficacy and inability to treat deeper lesions; despite the past, we can be hopeful for the near future. New approaches are being looked into to increase the therapeutic efficacy of PDT and the reach of PDT to deep tumors. Most of the studies conducted on the novel approaches have yielded promising results consistently. In our view, combining PDT with conventional anticancer therapies, enhanced light delivery, and dosimetric systems, 3rd generation PS coupled with nanotechnology based targeted drug delivery, and effective and systematic management of side effects are the key areas where a breakthrough can be expected. The use of mathematical modeling as a tool, where possible, will contribute immensely to quickening the pace of broader investigations conducted to validate previous findings. It is crucial that adequate *in vivo* and *in vitro* testing should be performed prior to any clinical interpretation.

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