International Journal of PharmaO2
Journal Home Page: http://www.ijpo.in/
(IJPO: A Peer-reviewed Bi-monthly online journal)

Review

A Review on the Therapeutic Potential of Nature derived Cholrin Photosensitizer and its Synthetic Counterparts for Photodynamic Therapy in the control of Neoplastic Diseases.

Mohamed Ali-Seyed*[1] Siddiqua Ayesha[2]

[1]Department of Clinical Biochemistry, Faculty of Medicine, University of Tabuk, Tabuk, Saudi Arabia, Tabuk 71491, Saudi Arabia.
[2]School of Public Health, SRM University, SRM Nagar, Kattangulathur, Chennai, India.

ARTICLE INFO

Article history:
Received: 05/08/2020;
Accepted: 21/08/2020;
Available online: 02/09/2020.

Key Words:
Photosensitizer
Chlorin e6
Photolon
Anticancer
PDT
Cell death
Apoptosis

Please cite this article as: Seyed M.A., (2020). A review on the therapeutic potential of nature derived cholrin photosensitizer and its synthetic counterparts for photodynamic therapy in the control of neoplastic diseases. *International Journal of PharmaO2*, 2(5), 0315-0327.

ABSTRACT

In the past three decades, photodynamic therapy (PDT) has accepted as an alternative modality for the management of wide variety of diseases including cancer, skin and bacterial, viral and fungal infections. It is minimally invasive treatment, which involves the interaction of a non-toxic photosensitizer (PS), light of an appropriate wavelength and tissue oxygen to remove unwanted cells by generating free radicals mediated by light receptors, which is more prevalent when the plant-produced metabolites are heterocyclic/polyphenols in nature. Until date, more than hundred photosensitizers or photosensitive drugs were identified various natural sources including plants. Many bioactive principles have shown in recent years to be potential photosensitizers, i.e. their toxic activities against various microorganisms, insects or cells are dependent on or are augmented by light of certain wavelengths. PDT considered often as selective and target specific, which led to the novel concept of therapeutic prospects in the control of infectious and other diseases including cancer. Photosensitization mechanisms commonly involve singlet oxygen and radicals, which causes photo damage to membranes or macromolecules. Although many existing PSs developed in the last 30 years, only a handful of them employed in human clinical applications. The main classes of natural photosensitizers reviewed in this chapter are chlorins and their synthetic counterparts because of their therapeutic efficacy by employing various search engines such as PubMed, Scifinder, and Web of Science. The continued progress in the development of novel photochemical is essential to advance targeted delivery of PS and efficacy of PDT, which consequently expands the range of clinical applications. The constant development of new photosensitizers is required to improve site-specific delivery for therapeutic efficacy of PDT, which consequently expands the range of clinical applications. If successful, these efforts will provide PDT therapy for infectious, cancer and other diseases with minimal risk to healthy tissue.

*Corresponding author: Dr. Mohamed Ali-Seyed Department of Clinical Biochemistry, Faculty of Medicine, University of Tabuk, Tabuk, Saudi Arabia, Tabuk 71491, Saudi Arabia.e-mail: sdmdali.ali@gmail.com

Introduction

Natural products have proven templates for the development of new drug entities (Beutler, 2010). It is widely accepted that plant derived natural compounds are harmless because of their less toxic nature in comparison with pure chemicals. However, it is possible that some constituents nullify the toxic effects of other components of a plant and the whole plant extract becomes less toxic and more useful (Wagner and Ulrich, 2009). In the last few decades, medicinal chemists have isolated many clinically important bioactive principles/phytocompounds from various traditional medicinal plants (Geysan *et al.* 2003; Seyed *et al.* 2017). The progress of medicinal drug development from herbal plants sources face numerous problems such as absorption, therapeutic efficacy and poor compliance. Despite having above problems, still crude herbs/plants mostly formulated in various preparations. The drug discovery from plants has traditionally been time-consuming, faster and better methods for plant collection, bioassay screening, compound isolation and compound development need incorporation of advanced techniques (Jantan *et al.* 2015; Seyed and Vijayaraghavan, 2020). It is well established that the drug leads of plant origin involves simple stepwise preparation and therefore less expensive than the synthetic drugs (Katiyar *et al.* 2012; Seyed, 2019). In this regard, by employing, various approaches like combinatorial and synthetic chemistry and modeling to obtain compounds for drug discovery including isolation from plants (Geysan *et al.* 2003; Mouhssen, 2013).

PDT is an emerging alternate mode of regulatory approved site-specific cancer treatment and it involves the introduction of a non-toxic photosensitizer (PS) that accumulates selectively more in cancer cells (Dougherty, 1993; Ali *et al.* 2001, 2002; Robertson *et al.* 2009) with minimal risk to healthy tissue than surrounding cells. In PDT, combining PS that absorbs specified wavelength of light, which in turn to produce free radical molecules to eliminate neoplastic cells and tissues. PDT can be used to treat a variety of diseases including skin diseases, bacterial, viral and fungal infections (Hamblin *et al*. 2004), various malignancies (Gitika *et al*. 2012), cardiovascular, dermatological, ophthalmic, and immunological disorders (Ali-Seyed *et al*. 2011; Geltzer *et al.* 2013). There are three well established PDT mediated biological mechanisms involved in the destruction of tumorous tissue: cellular, vascular and immunological (Buytaert, 2007). Several cellular organelles like mitochondria and plasma membrane are known sites of photosensitizers location (Luo and Kessel, 1997; Piette, 2003; Buytaert *et al.* 2007). However, a given photosensitizer might not usually bind to a specific intracellular structure and/or localization, which would lead to the diversity of cell death pathways involved in PDT (Yoo and Ha 2002; Ali-Seyed *et al*. 2011). Compared with conventional surgery, the approach is non-invasive, enables accurate targeting, repeated administration without total-dose limitations associated with radiotherapy, and results in little or no scarring after healing (Nowak *et al*. 2011). For successful clinical outcome, photosensitizer should meet certain requirements.

Although many studies in the last few decades have focused on PDT for various applications based on its successful outcomes, however, less interest or attention has been paid to plant derived extracts or molecules of natural origin studied for their phototoxic activity to date. Herbal research recently gained momentum to explore plants as sources of new phytotherapeutic agents (Marrelli *et al*. 2014; Seyed *et al*. 2017; Vijayaraghavan and Seyed, 2020) because many photoactive biocompounds are natural products (Newman and Cragg, 2016). For years hematoporphyrin derivative namely Photofrin, food and drug administration (FDA) approved sensitizer extensively employed for clinical treatment of various types of cancer including bladder, breast (Juarranz *et al*. 2008; O'Connor *et al*. 2009) and to kill various microbial organisms (Hamblin and Hasan, 2004). However, it has some restrictions like (i) its cutaneous tissue retention time for 4-10 weeks after uptake that

leads to long-term skin photosensitivity, (ii) patients need to stay at for a considerable length of time to avoid light (iii) its insufficient low wavelength activation compromises tissue penetration and finally (iv) its badly defined molecular formula. Hence, the above limitations and drawbacks have encouraged the quest for new novel superior characteristics sensitizers (O'Connor *et al*. 2009; Pervaiz and Olivo, 2006).

In recent years, many established studies have enumerated various merits of ideal photosensitizers (Ali and Olivo, 2002). An ideal PS need to be hydrophilic in nature for easy absorption, non-toxic till exposed to light and activated by an appropriate wavelength by tunable laser light source (Brancaleon and Moseley, 2002). More importantly, a good PS should generate a good photodynamic outcome based on its cellulular localization and selectivity (Boyle and Dolphin, 1996). The following are some of the characteristic features such as (a) it would be a chemically pure drug with specific uptake by the target tissue (b) minimal dark toxicity (i.e., activated only upon irradiation), (c) high photo activity (high quantum yield of ROS), (d) rapid clearance to avoid phototoxic side effects, and finally (e) strong absorption at relatively long wavelengths (~630–800 nm) (Detty *et al*., 2004; Juarranz *et al*. 2008; O'Connor *et al*. 2009). Based on the above characteristics, most of currently available synthetic or natural photosensitizers have been identified and employed for various applications (O'Connor *et al*. 2009; Palumbo, 2007).

From the available phytochemical literature, the photosensitizing metabolites isolated from 35 families of plants belong to 15 different phytochemical classes (Kelsey et al. 1995). These secondary metabolites are products of four biosynthetic pathways like fatty acid, polyketide, shikimate and terpenoid (Thirumurugan et al. 2018). Many studies have established that these light-activated extracts of plants belonging to a variety of taxonomically disparate 44 families that have been found to contain photosensitizers or exhibit phototoxic activity which include Acanthaceae, Campanulaceae, Gesnariaceae, Loganiaceae, Malpigiaceae, Papaveraceae, Phytolaccaceae, Piperaceae and Sapotaceae (Kelsey et al. 1995).

A spectrum of plant derived extracts have been screened for chemotherapeutic properties but their potential as source of photosensitizers in PDT has been very rarely investigated (Marrelli *et al*. 2014; Jong *et al*. 2013). Looking for potential novel photosensitizers is a significant prerequisite step in PDT studies because, to date, there are only a few clinically approved PDT drugs, including Photofrin®, Foscan® and Levulan® which are used mainly for skin, gynecological, gastrointestinal, and head and neck (H&N) cancers (Dhaneswar *et al*. 2014). Over the past two decades, a large number of natural and synthetic dyes have been developed and tested *in vitro* and *in vivo* as photosensitizers in PDT experiments (Berlanda *et al*. 2010; Buytaert *et al*. 2007; Palumbo, 2007).

Looking for possible novel photosensitizers (PS) is a crucial first step in PDT investigations because, to date, there are only insignificant number of approved PDT drugs are available including Levulan®, Foscan® and Photofrin®, which are used mostly for H&N, gastrointestinal, gynecological and skin cancers (Baskaran *et al*. 2018). In this line, chlorophylls, porphyrins, furocoumarins, cholorins (Ce6) and few other emerging PS are of interest as they have shown superior therapeutic efficacy. The continuous development of novel new photosensitizers is required to improve site-specific delivery for therapeutic efficacy of PDT, which consequently expands the range of clinical applications.

It is conceivable that no current PS which would meet all clinical demands. Most of the PS have many disadvantages like limited cell specificity or selectivity, skin sensitivity to prolonged irradiation and unpredictable efficacy (Allison *et al*. 2004). Other requirements, which include the following: (i) PS should be water-soluble for intravenous injection, (ii) they should exhibit stronger absorption of light mostly in the red or near-

infrared region, which is required for deep penetration into tissues, (iii) have high yield of singlet oxygen generation (SOG) and produce low dark cytotoxicity, and finally can be rapidly eliminated from the body. However, chlorins (Ce6), hypocrellins, hypericin and curcumin properties seem to be advantageous as compared to other commonly used photosensitizers. The following cellular organelles are prime target locations for photosensitizers, which include mitochondria, lysosomes, ER, plasma membrane and Golgi, etc., (Kessel and Luo, 1998; Piette, 2003; Buytaert *et al*. 2007). However, a given photosensitizer might not usually bind to a specific intracellular structure and/or localization, which would lead to the diversity of cell death pathways involved in PDT (Yoo and Ha 2002; Galanou *et al*. 2008; Ali-Seyed *et al*. 2011).

Many PSs such as chlorophylls, furocoumarins, hypocrellins including hypericin, chlorins and curcumin have gained attention in recent years because of their efficacy (Baskaran *et al*. 2018). Based on the available literature and efficacy, this review will focus mainly on chlorin type of natural photosensitizers and their counterparts because of their efficacious pharmacokinetic and photodynamic activities. Most compounds or molecules that absorb light, and hence acquire energy in the process, subsequently lose the acquired energy through radiationless decay by internal conversion mechanism. However, PS are molecules in which internal conversion is not efficient. Rather PS molecules transfer electron from one to another. In other words, PS transfer energy of excitation to other molecules, often to molecular oxygen. In most cases, energy transfer is very efficiently populate in their excited triplet states because this state allows prolonged time for energy and/or electron transfer to occur. So, most highly effective photosensitizers used in the clinics exhibit high quantum yields of excited triplet state (Zhao *et al*. 2013).

Generally, photosensitizers in their absorption maxima at the red region, specifically at 668 nm, which is within the optical window of biological tissues (between 600 and 800 nm). Low range of light fail to penetrate the tissue into deeper regions and produce no results. Similarly, very long wavelengths (800 nm and above) also not useful as they have insufficient energy to excite tissue oxygen to become singlet then to to generate substantial yield of ROS (Yoon *et al*. 2013). Avoiding side effects, minimal or no destruction to nearby/surrounding healthy tissue but PS specific localization to neoplastic lesions is an essential consideration for clinical PDT. But photosensitizers are rarely elective given that ROS do not discriminate between cancerousand non-cancerous tissue. Though the selectivity may not be achieved by any natural PS extracts, however, the selectivity could be maximized by using focused lasers as light source or precise delivery tools to target the tumor region (Yoon *et al*. 2013).

Although many clinically approved PS but few under clical trials (Anand *et al*. 2012) are currently in use to treat various types of cancer. Chlorins are promising agents for PDT compare to other PS, due to their absorption and emission in the red spectrum range, where the light penetrates deep enough into the tissues, high phototoxicity resulting in usage of low drug and light doses (Spikes, 1990). There are several chlorin-type photosensitizers in clinical use today are Temoporfin (Foscan, mTHPC, 5,10,15,20-Tetra(m-hydroxyphenyl) chlorin), Talaporfin (LS11, MACE, N-aspartyl chlorin e6, NPe6), Photolon®, Radachlorin (a mixture of three chlorins), and Photodithazine (glucosamine salt of chlorine e6) (Gijsens and De Witte, 1998; Brasseur *et al*. 1999). Chorines including bacteriochlorines absorption band width fall in the red and near-infrared region permit deeper penetration of light of appropriate wavelength into tissue, thus making these photosensitizers interesting candidates for PDT of various cancer (Bonnet and Martinez, 2001).

Chlorins

Photosensitivity and poor absorption of tissue-penetrating red light, have led to the development of new PS with many novel

characteristic features, especially longer absorption wavelength for deeper penetration into tissue and faster clearance from normal tissue (Sibata *et al*. 2000). The second-generation photosensitizers have shorter periods of photosensitization, longer activation wavelengths (and, therefore, are activated deeper within tissues), higher yields of singlet oxygen, and tumor selectivity (Zhang *et al*. 2018). For example, chorines including bacteriochlorines fall in the bandwidth of red and near-infrared permitting deeper tissue penetration for light, therefore qualifying these PS ideal candidates for PDT of neoplastic tissues (Ferreira *et al*. 2008).

The choice of chlorin-type photosensitizers for the selective destruction of cancer cells has been known for past few decades because of their selective potential phototoxic nature compound with a their strong absorption falls in the range of red region of the visible spectrum leading to destruction of diseased tissue in deeper tissue layers. Although chlorines exhibit photophysical properties equivalent to those of the porphyrin type of PS macrocycles. However with intensified and redshift of Q bands, making chlorine-PS more better candidates for photodynamic diagnosis (PDD) and PDT (Allison *et al*. 2006).

Chlorin e6

Chlorin e6 (Ce6) is a natural molecule and a member of the chlorin family.It is usually made from live Spirulina chlorophyll (Chlorella ellipsoidea) and other green plants. other green plants (Yoon *et al*. 2013). Ce6 is lipophilic in nature and exhibits asymmetric structure with three ionizable carboxylic groups in it but pH dependent. (Mojzisova *et al*. 2007; Shim *et al*. 2011). Chlorin e6, (17S,18S)-18-(2-carboxyethyl)-20-(carboxymethyl)-12-ethenyl-7-ethyl-3,8,13,17-tetramethyl-17,18,22,23-tetrahydroporphyrin-2-carboxylic acid, its molecular structure is C34H36N4O6 with a molecular weight of 596.67. Chlorin e6 represents an interesting class of tetrapyrrole compounds as far as their plant origination and photophysical properties are concerned (Battersby, 2000). It is well established that tetrapyrrole backbones present in several important biomolecules like chlorophyll, bacteriochlorophyll and haem and they have been termed the 'pigments of life' (Battersby, 2000). Generally, tetrapyrrole PSs (with the exception of bacteriochlorins) tend to produce predominantly. Type II free radical species (singlet oxygen) and Type I (hydroxy radicals), which are often produced by PSs with other structures. Although many tetrapyrrole PS compounds have beeen used for PDT applications, only few of them have exhibited their superior actions in the clinic as well as in clinical trails (Copley *et al*. 2008; Seyed *et al*. 2011).

Chlorin e6 exhibits advantageous photophysical properties for PDT candidate because of its high absorption in the red spectral region (Li *et al*. 2013), and its low cost to make compared to other porphyrin-based PDT drugs and also having long lifetimes in their photoexcited triplet states (Sun *et al*. 2018). It is evident that longer wave length laser light always penetrate deeper than the lower one (633 nm) commonly used for Photofrin. by a high sensitizing efficacy and rapid elimination from the body (Kostenich *et al*. 1994). They are known to combine high values of inter conversion coefficient (and, consequently yields high quantum of singlet oxygen when compare to porphyrins (610-620 nm).

Photolon

Photolon® (1, 3, 5, 8 - tetramethyl-4-ethyl-2-vinyl-chlorin-6-carbonic-acetic-7-propion acid sodium-vapor salt), a chlorin e6 hydrophile photosensitizing compound linked with polyvinylpyrrolidone (PVP) in the ratio of 1 : 1 (Chin *et al*. 2006). Although, its chemical structure corresponds to a partially reduced porphyrin moiety, but its molecular structure shares with chlorine e6, which separates pheophorbide exocyclic dimethyl amine β-ketoester by hydrolysis. The creation of Photolon® by joining Ce6 and PVP, exhibit better stability and solubility in water and, therefore, also better bioavailability than Ce6 alone (Coply *et al*. 2008). It has a partially

reduced porphyrin moiety and its molecular structure is comparable to chlorin e (Waidelich *et al*. 2010; Jian Hua *et al*. 2013), which can be isolated after hydrolysis of the 5-membered exocyclic beta-ketoester moiety of pheophorbide a. Unlike first generation porphyrins, second generation chlorins (Chlorin e6 and derivative of Photolon®) demonstrated a higher ability to accumulate in neoplastic tissue and faster removal time from the body and strongly absorb in the red (between 640 and 700 nm), and thus provide a possibility of treatment of relatively massive and deep-lying tumors. The intravenous administration of Photolon improves high uptake rate in target tissues which produce not only high tumorotropic but also low phototoxicity and removed completely from the body during several days.

Previous studies have proven that Photolon has better therapeutic outcome with increased wavelength to match with its absorption peak because the photosensitizer can reach deeper localized lesions without increase of phototoxicity (Detty *et al*. 2004; Isakau *et al*. 2008). Ali-Seyed *et al*. (2011) and others (Sobaniec *et al*. 2013) that proved that Photolon® preferentially localizes in intracellular organelles in the following sequence: nucleus, mitochondria, lysosomes, and Golgi apparatus. A study by Ali-Seyed et al. (2011) demonstrated that Photolon-PDT specifically inuced apoptosis in CT-26 cells, this apoptotic cell death implies physiologically correlates with low drug toxicity Anticancerous properties of Photolon® were also proved in studies of other authors (Juzeniene, 2009; Trukhachova *et al*. 2011).

Both Ce6 and Photolon® have a shorter tumor accumulation time, more rapid clearance, and higher singlet oxygen generation efficiency, compared against first generation PS (Sharma *et al*. 2004; Horibe *et al*. 2011) and are activated by near-infrared wavelengths (eg, 664 nm), enabling the molecule to reach deep tissue layers (Spikes, 1990), compare to 630-nm laser light used for Photofrin or porphyrins (Huang *et al*. 2011). Both of them are attractive PDT drug candidates because of (1) their high absorption in the red spectral region, and (2) their low cost to make compared to other porphyrin-based PDT drugs. Chlorin e6 and Photolon® exhibit advantageous photophysical properties for PDT like higher molar absorption in the near infra red spectrum and prolonged photoexcited triplet states. Previous studies have reported that local application of Photolon® in a form of an ointment/patch/oral rinse and subsequent irradiation with the laser light (665 nm) was effective in case of xenograft tumors and at the clinical trials (Coply *et al*. 2008; Cabrera *et al*. 2012). To support this claim, chlorin e6 based photosensitizers have recently received more attention due to their high photodynamic activity and therapeutic efficacy (Trukhachova *et al*. 2011) against various cancers, including melanoma, bladder cancer, and nasopharyngeal (Chin *et al*. 2007; Thong *et al*. 2008; Ali-Seyed *et al*. 2011).

Foscan/M-tetrahydroxophenyl chlorine (mTHPC)

Foscan® (Biolitec Pharma Ltd., Dublin, Ireland/Germany) is a plant based chlorine derivative is a photosensitizing agent, which contains temoporfin. This drug shows increased singlet oxygen formation compared to porphyrins as well as high selectivity due to its high hydrophobicity leading to more incorporation in cells (Dobson *et al*. 2018). However, it is synthetically pure and can produce a rapid and significant photodynamic reaction (PDR), but its treatment time is very short in terms of seconds. Since the drug is so active that after infusion patients must stay in a dark room for 24 hours because light exposure including normal room light is sufficient to activate this drug and produce significant severe burn (dark toxicity). Although Foscan-PDT is very effective and this PS found a special place for the treatment of primary and recurrent head and neck cancers (Meier *et al*. 2017). The biggest disadvantage with Foscan-PDT is so painful even under anesthesia for most patients who undergo Foscan illumination.

Mono-L-aspartyl chlorine e6 (NPe6)

Marketed under different names such as MACE, LS11, NPe6, this derivative called Fotolon (RUE Belmedpreparaty, Minsk, Republic of Belarus); a plant-based chlorine (Spikes and Bommer, 1993) is a very effective agent to generate the photodynamic reaction (PDR). It has other generic branded names such as MACE, LS11, and NPe6. It is important to note that unlike Foscan, NPe6 do not cause dark toxicity and allows treatment time several hours after infusion (Aizawa *et al*. 1987). Moreover, NPe6 allows same day infusion and therapy, which is very convenient for patients and practitioners.

Radachlorin

Radachlorin® (Rada-Farma) and Photoditazine® (Veta-Grand) are hydrosoluble chlorines produced in Russia (Ferreira *et al*. 2008). Photoditazine® has only chlorine e6 in its composition, however Radachlorin® presents in its composition three types of chlorines e6 (90 – 95%), p6 (5 –7%), and other not published (1-5%). In Russia, Radachlorin® and Photoditazine® have beenused in clinical studies of PDT in malignant tumors of the skin, oral cavity, larynx, stomach, bronchus, esophagus, vulva and others (Mirzaei *et al*. 2015; Ghoodarzi *et al*. 2016). Radachlorin-PDT does not produce either local or systemic complications, and the treatment had good results without skin photosensitization. Radachlorin® also have few disadvantages like photo instability similar to other PSs such as porphyrins and phthalocyanines (Kochneva *et al*. 2010). This PS suffers degradation by light that can be visualized by decreasing of their initial absorption and fluorescence intensity (Kochneva *et al*. 2010) while in simple solutions and complex environments.

Mechanism of Action

PDT may be a promising treatment for patients with tumors. Despite recent advancements, the mechanism of its action of many PS is poorly understood and is different from the cytotoxic effects induced by known antitumor drugs because the mechanism of the terminal effect of PDT is still subject to many research efforts. However, it is known that multiple factors play a role in governing the outcome of PDT as well as the effective mechanism of action of irradiated PS including chlorins (Dougherty, 1998; Gupta *et al.* 2010). C6-PDT targets not only neoplastic cells, microvasculature and inflammatory and immune systems of hosts. It appears clear that the combination of all these components is required in order to achieve long term control of the tumor. Many well demonstrated results have suggested that the better outcome of PDT treatment mainly depends on the type, concentration and intracellular localization of the photosensitizer (Oleinick *et al*. 2002; Seyed *et al*. 2011). In addition, light wavelength, light fluence and fluence rate are important to ensure sufficient oxygen availability and supply (Dougherty, 1998; Sibata *et al*. 2000; Zhu and Finlay, 2008). Besides the above, it is necessary to ensure sufficient light reaches target and how the PS interacts with cells subcellular localization in the target tissue or tumor and retain a certain time interval between its application and irradiation of the tested cells (Postiglione *et al*. 2011). Also, it depends to a significant degree on the conditions in which the photodynamic reaction takes place as they are crucial to determine the outcome of PDT to a certain extend (Buytaert *et al*. 2007, Misiewicz *et al*. 2009).

For many PSs, mainly chlorins found mitochondria as an important sub-cellular target used in PDT because these PS able to induce mitochondia mediated apoptosis and cellular damage after illumination (Morgan and Osero, 2001). In most cases, it is accepted that the accumulation of a PS including Ce6 in mitochondria but less with endoplasmic reticulum (ER) (Merlin *et al*. 2003; Li *et al*. 2014) leads to the activation of the apoptotic pathway in the cell. Kessel and Poretz (2000) reported that chlorin e6 (Ce6) was located to plasma membrane and/or mitochondria and later by Seyed *et al*. (2011) confirmed their findings and also reported some additional sub-cellular targets including nucleus, lysosomes and Golgi apparatus. However, this study failed

to demonstrate both Ce6 and Photolon in ER. The molecular mechanism of Ce6-based PDT was not clear previously because most studies concentrate on tumor cells, but its detailed mechanism is mostly started clear now. The generation of highly reactive singlet oxygen and the formation of reactive oxygen species (ROS). The resulting oxidative stress leads to impairment of mitochondria and endoplasmic reticulum function and, consequently, to the execution of all kinds of cell-death programs, from classical apoptosis to programmed necrosis or autophagy (Buytaert *et al.* 2007; Kessel and Poretz 2000; Kessel *et al*. 1995; Seyed *et al*. 2011). Besides the above, chlorin-PDT induce hypoxia by altering tumor vasculature, which lead too microvascular shutdown (Broekgaarden *et al*. 2015; Dang *et al*. 2017); and induce inflammatory and immune responses (van Straten *et al*. 2017).

Programmed cell death or apoptosis is very often initiated by or related to loss of function by the mitochondria, which is reflected by a decrease in mitochondrial membrane potential (Elmore, 2007). Many studies observed significant impairment of mitochondrial potential in about 50% of the cells 3 h after PDT. It is known that the disruption of mitochondrial potential by free radical accumulation leads to cytochrome c (Cyt.C) release into the cytoplasm, which in turn activates caspase cascades, which include effector caspase-3 at the bottom. Moon *et al*. (2009) have shown an efficient antitumor activity of Ce6-induced PDT (Ce6-PDT) both in vitro and in vivo using a rat tumor model. In this study, three-week-old male Sprague-Dawley (SD) rats were inoculated s.c. on the right flank with RK3E-ras cells. The animals administered (i.v) with Ce6 for 24 h and beyond, PDT was performed using a laser diode at a light dose of 100 J/cm2. Ce6-PDT induces apoptosis through the activation of caspase-3 and its downstream target such as PARP cleavage and the reduction of anti-apoptotic bcl-2. The in vivo experiments, confirmed the above and demonstrated Ce6-PDT led to a significant reduction of tumor size. These findings suggest that Ce6-PDT can effectively arrest tumor growth by inhibiting cell proliferation and inducing apoptosis.

Conclusion

In summary, currently, PDT has emerged as an alternative therapeutic options for a variety of malignant tumors. Photosensitizer chlorin e6 and its synthetic counterparts have proven to be useful in designing PDT as most promising agents for clinical use against various types of cancer. Besides, various established investigations showed that PDT under conditions, which are optimal and effective on cancer treatment by inducing apoptotic cell death. However, one cannot rule out the possibility of potential complications of chlorin e6-based PDT in vivo, which targets multiple cell populations including normal cells if an inappropriate wavelength of light sources are used. Though a discussion of every prospective of chlorin PSs on their anti-cancer tested in the last few years is beyond the scope of any single review, yet the present review discussed mostly the anti-cancer prospects not the other important potentials like anti-microbial efficacy of this photosensitizer chlorin e6 and its synthetic counterparts.

References

1. Aizawa K, Okunaka T, Ohtani,T, Kawabe H, Yasunaka Y, O'Hata S, Ohtomo N, Nishimiya K, Konaka C, Kato H, Hayata Y, Saito T (1987). Localization of Mono-L-Aspartyl Chlorin e6 (npe6) in mouse tissues. Photochem Photobiol. 46: 789-793.

2. Ali SM, Olivo M (2002). Bio-distribution and subcellular localization of Hypericin and its role in PDT induced apoptosis in cancer cells. *Int J Oncol*. 21(3):531-540.

3. Ali SM, Olivo M, Yuen GY, Chee SK (2001). Photodynamic-induced apoptosis of human nasopharyngeal carcinoma cells using Hypocrellins. Int J Oncol 19 (3): 633-643.

4. Allison RR, Bagnato VS, Cuenca R, Downie GH, Sibata CH (2006). The future of photodynamic therapy in oncology. Fut Oncol 2 (1): 53-71

5. Allison RR, Sibata C, Mang TS (2004). Photodynamic therapy for chest wall

recurrence from breast cancer. Photodiagnosis Photodyn Ther. 1(2):157-171.
6. Anand S, Rollakanti KR, Brankov N (2017). Fluorouracil Enhances Photodynamic Therapy of Squamous Cell Carcinoma via a p53-Independent Mechanism that Increases Protoporphyrin IX levels and Tumor Cell Death. Mol Cancer Ther. 16(6):1092-1101.
7. Baskaran R, Lee J, Yang SG (2018). Clinical development of photodynamic agents and therapeutic applications. Biomater Res. 22: 25.
8. Battersby AR (2000). Tetrapyrroles: the pigments of life. Nat Prod Rep. 17: 507-526.
9. Berlanda J, Kiesslich T, Engelhardt V, Krammer B, Plaetzer K (2010). Comparative in vitro study on the characteristics of different photosensitizers employed in PDT. J Photochem Photobiol B. 100(3):173-180.
10. Beutler JA (2009). Natural Products as a Foundation for Drug Discovery. Curr Protoc Pharmacol. Author manuscript; available in PMC 2010 Sep 1. Published in final edited form as: Curr Protoc Pharmacol, 1; 46: 9.11.1-9.11.21.
11. Bonnett, R., Djelal, B.D. and Nguyen, A. (2001), Physical and chemical studies related to the development of m-THPC (FOSCAN®) for the photodynamic therapy (PDT) of tumours. J. Porphyrins Phthalocyanines, 5: 652-661.
12. Boyle RW, Dolphin D (1996). Structure and biodistribution relationships of photodynamic sensitizers. Photochem Photobiol. 64: 469-485.
13. Brancaleon L, Moseley H (2002). Laser and non-laser light sources for photodynamic therapy. Lasers Med Sci. 17:173–186.
14. Brasseur N, Ouellet R, Madeleine C, van Lier JE (1999). Water-soluble aluminium phthalocyanine–polymer conjugates for PDT: photodynamic activities and pharmacokinetics in tumour-bearing mice. Br J Cancer 80: 1533-1541.
15. Broekgaarden M, Weijer R, van Gulik TM, Hamblin MR, Heger M (2015).Tumor cell survival pathways activated by photodynamic therapy: a molecular basis for pharmacological inhibition strategies. Cancer Metastasis Rev. 34: 643–690.
16. Buytaert E, Dewaele M, Agostinis P (2007). Molecular effectors of multiple cell death pathways initiated by photodynamic therapy. Biochim Biophys Acta. 1776(1):86-107.
17. Cabrera H, Castro J, Grassi HC, Andrades EDJ, López-Rivera, SA (2012). The Effect of Photodynamic Therapy on Contiguous Untreated Tumor. Dermatol Surg. 38 (7): 1097-1099.
18. Chin, WW. Heng PW, Olivo M (2007). Chlorin e6 – polyvinylpyrrolidone mediated photosensitization is effective against human non-small cell lung carcinoma compared to small cell lung carcinoma xenografts. BMC Pharmacol 7: 15.
19. Chin, WWL, Heng PWS, Bhuvaneswari R, Lau WKO, Olivo M (2006). The potential application of chlorin e6-polyvinylpyrrolidone formulation in photodynamic therapy. Photochem Photobiol Sci. 5:1031-1037.
20. Copley L, Pauline W, Karel W, Parker M, Virna L (2008). Photolon™, a chlorin e6 derivative, triggers ROS production and light-dependent cell death via necrosis. The Int J Biochem Cell Biol. 40. 227-235.
21. Dang J, He H, Chen D, Yin L (2017). Manipulating tumor hypoxia toward enhanced photodynamic therapy (PDT).Biomater. Sci. 5: 1500-1511.
22. Detty MR, Gibson SL, Wagner SJ (2004). Current clinical and preclinical photosensitizers for use in photodynamic therapy. J Med Chem. 47(16):3897-3915.
23. Dhaneshwar S, Patil K, Bulbule M, Kinjawadekar V, Joshi D, Joshi V (2014). Photodynamic therapy for cancer. Int J Pharm Sci Rev Res 27(2): 125-141.
24. Dobson J, de Queiroz G, Fernandes, Golding JP (2018). Photodynamic therapy and diagnosis: Principles and comparative aspects. The Veter J. 233: 8-18.
25. Dougherty TJ (1993). Photodynamic therapy. Photochem Photobiol 58: 895-900.
26. Dougherty TJ, Gomer CJ, Henderson BW, Jori G, Kessel D, Korbelik M, Moan J and

Peng Q (1998). Photodynamic therapy. J Natl Cancer Inst 90: 889-905.
27. Elmore S (2007). Apoptosis: a review of programmed cell death. Toxicol Pathol. 35(4):495-516.
28. Ferreira J, Menezes P, Kurachi C, Sibata C, Allison R, Bagnato V (2008). Photostability of different chlorine photosensitizers. Laser Phys Lett 5: 156-161.
29. Ferreira S, Juliana Menezes PFC, Kurachi C, Sibata C, Allison RR, Bagnato V (2008). Photostability of different chlorine photosensitizers. Laser Physics Letters. 5: 156-161.
30. Galanou, MC, Theodossiou TA, Tsiourvas D, Sideratou Z, Paleos CM (2008). Interactive transport, subcellular relocation and enhanced phototoxicity of hypericin encapsulated in guanidinylated liposomes via molecular recognition. Photochem. Photobiol. 84: 1073-1083.
31. Geltzer A, Turalba A, Vedula SS (2013). Surgical implantation of steroids with antiangiogenic characteristics for treating neovascular age-related macular degeneration. Cochrane Database Syst Rev. 1(1):CD005022.
32. Geysen H, Schoenen F, Wagner D, Wagner R (2003). Combinatorial compound libraries for drug discovery: An ongoing challenge. Nat Rev Drug Discov. 2. 222-230.
33. Ghoodarzi R, Changizi V, Montazerabadi AR, Eyvazzadaeh N (2016). Assessing of integration of ionizing radiation with Radachlorin-PDT on MCF-7 breast cancer cell treatment. Lasers Med Sci 31: 213–219.
34. Gijsens A, De Witte P (1998). Photocytotoxic action of EGF-PVA-Sn(IV)chlorin e6 and EGF-dextran-Sn(IV)chlorin e6 internalizable conjugates on A431 cells. Int J Oncol 13 (6): 1171-1178.
35. Gitika BK, Sharma SK, Huang YY, Dai T, Hamblin MR (2011). Photodynamic Therapy for Infections: Clinical Applications. Lasers Surg Med. Author manuscript; available in PMC 2012 Sep 23. Published in final edited form as: Lasers Surg Med. 43(7): 755–767.
36. Gupta S, Dwarakanath BS, Muralidhar K, Sengul TK, Jain V (2010). Non-monotonic changes in clonogenic cell survival induced by disulphonated aluminum phthalocyanine photodynamic treatment in a human glioma cell line. J Transl Med. 8: 43.
37. Hamblin MR, Hasan T (2004). Photodynamic therapy: a new antimicrobial approach to infectious disease? Photochem Photobiol Sci. Author manuscript; available in PMC 2011 Apr 5. Published in final edited form as: Photochem Photobiol Sci. 3(5): 436-450.
38. Hamblin MR, Hasan T (2004). Photodynamic therapy: a new antimicrobial approach to infectious disease? Photochem Photobiol Sci. 3(5):436-450.
39. Horibe S, Nagai J, Yumoto R, Tawa R, Takano M (2011). Accumulation and photodynamic activity of chlorin e6 in cisplatin-resistant human lung cancer cells. J Pharmaceut Sci. 100(7): 3010-3017.
40. Huang DL, Wang XL, Wang HW, Huang Z (2011). Successful treatment of refractory facial acne using repeat short-cycle ALA-PDT: Case study. Photodiagnosis Photodyn Ther. 8(4):343-346.
41. Isakau HA, Parkhats MV, Knyukshto VN, et al. Toward understanding the high PDT efficacy of chlorin e6-polyvinylpyrrolidone formulations: photophysical and molecular aspects of photosensitizer-polymer interaction in vitro. J Photochem Photobiol. B. Biol. 92(3):165-174.
42. Jantan I, Syed Nasir AB, Mohamed Ali SM, Wai LK, Mesaik MA (2015). The evolving role of natural products from the tropical rainforests as a replenishable source of new drug leads. Drug Discovery and Development-From Molecules to Medicine. pp 3-38.
43. Jian Hua L, Chen Z, Huang Z, Zhan Q, Ren FB, Liu J, Yue W and Wang Z (2013). In vitro study of low intensity ultrasound combined with different doses of PDT: Effects on C6 glioma cells. Oncol Lett 5: 702-706.
44. Jong W, Tan P, Kamarulzaman F, Mejin M, Lim D, Ang I (2013). Photodynamic activity of plant extracts from Sarawak, Borneo.Chem Biodivers. 10(8): 1475-1486.

45. Juarranz A, Jaén P, Sanz-Rodríguez F, Cuevas J, González S (2008). Photodynamic therapy of cancer. Basic principles and applications. Clin Transl Oncol. 10(3):148-154.
46. Juzeniene A, Thu Tam TT, Iani V, Moan J (2009). 5-Methyltetrahydrofolate can be photodegraded by endogenous photosensitizers. Free Radical Biol Med. 47(8):1199-1204.
47. Katiyar C, Gupta A, Kanjilal S, Katiyar S (2012). Drug discovery from plant sources: An integrated approach. *Ayu*. 33(1):10-19.
48. Kelsey R. Downum and Jinghai Wen (1995). The Occurrence of Photosensitizers among higher plants. Light-Activated Pest Control. Chapter 11. pp 135-143.
49. Kessel D, Luo Y (1998). Mitochondrial photodamage and PDT-induced apoptosis. J Photochem Photobiol. B. Biol. 42(2): 89-95.
50. Kessel D, Poretz R. (2000). Sites of Photodamage Induced by Photodynamic Therapy with a Chlorin e6 Triacetoxymethyl Ester (CAME). Photochemistry and photobiology. 71: 94-96.
51. Kessel D, Woodburn K, Gomer GJ, Jagerovic N, Smith KM (1995).Photosensitization with derivatives of chlorin p6. J Photochem Photobiol B 28: 13-18.
52. Kochneva EV, Filonenko EV, Vakulovskaya EG, Scherbakova EG, Seliverstov OV, Markichev NA, Reshetnickov AV (2010). Photosensitizer Radachlorin®: Skin cancer PDT phase II clinical trials. Photodiagnosis Photodyn Ther. 7(4):258-267.
53. Kostenich GA, Zhuravkin IN, Zhavrid EA (1994). Experimental grounds for using chlorin e6 in the photodynamic therapy of malignant tumors. J Photochem Phtobiol B. Biol. 22(3):211-217.
54. Li Y, Yu Y, Kang L, Lu Y (2014). Effects of chlorin e6-mediated photodynamic therapy on human colon cancer SW480 cells. Int J Clin Exp Med. 7(12):4867-4876.
55. Li Z, Wang C, Cheng L, Gong H, Yin S, Gong Q, Li Y, Liu Z (2013). PEG-functionalized iron oxide nanoclusters loaded with chlorin e6 for targeted, NIR light induced, photodynamic therapy. Biomaterials. 34(36):9160-9170.
56. Luo Y and Kessel D (1997). Initiation of apoptosis versus necrosis by photodynamic therapy with chloroaluminum phthalocyanine (Review). Photochem Photobiol 66: 479-483.
57. Marrelli M, Menichini G, Provenzano E, Conforti F (2014). Applications of Natural Compounds in the Photodynamic Therapy of Skin Cancer. Current Medicinal Chemistry. 21: 1371.
58. Marrelli M, Menichini G, Provenzano E, Conforti F (2014). Applications of natural compounds in the photodynamic therapy of skin cancer. Curr Med Chem 21(12): 1371-1390.
59. Meier D, Botter SM, Campanile C, Robl B, Gräfe S, Pellegrini G, Born W, Fuchs B (2017), Foscan and foslip based photodynamic therapy in osteosarcoma in vitro and in intratibial mouse models. Int J Cancer 140: 1680-1692.
60. Merlin JL, Gautier H, Barberi-Heyob M, Teiten MH, Guillemin F (2003). The multidrug resistance modulator SDZ-PSC 833 potentiates the photodynamic activity of chlorin e6 independently of P-glycoprotein in multidrug resistant human breast adenocarcinoma cells. Int J Oncol. 22(4):733-739.
61. Mirzaei H, Djavid G, Esmaeeli, Hadizadeh M, Jahanshiri-Moghadam M, Hajian P. (2015). The efficacy of Radachlorin-mediated photodynamic therapy in human hepatocellular carcinoma cells. J Photochem Photobiol. 142: 86-91.
62. Misiewicz K, Skupińska K, Graczyk A, Kasprzycka-Guttman T (2009). Influence of protoporphyrin IX amino acid substituents on affinity to human breast adenocarcinoma MCF-7 cells, Biotechnic Histochem. 84:1, 17-23.
63. Mojzisova H, Bonneau S, Christine VB, Brault D (2007). Cellular uptake and subcellular distribution of chlorin e6 as functions of pH and interactions with membranes and lipoproteins. Biochimica et Biophysica Acta Biomembranes. 1768 (11): 2748-2756.
64. Moon YH, Kwon SM, Kim HJ, Jung KY, Park JH, Kim SA, Kim YC, Ahn SG, Yoon JH (2009). Oncology Reports. 22(5). 1085-1091.

65. Morgan J, Oseroff AR (2001). Mitochondria-based photodynamic anti-cancer therapy. Adv Drug Deliv Rev. 49:71-86.
66. Mouhssen L (2013). The Success of Natural Products in Drug Discovery. Pharmacol Pharm. 4: 17-31. 10.4236/pp.2013.43A003.
67. Newman DJ, Cragg GM (2016). Natural Products as Sources of New Drugs from 1981 to 2014. J Nat Prod. 79 (3): 629-661.
68. Nowak AS, Wiktorska KM, Maciej M, Anna P (2011). Cytotoxicity of PP (Arg)(2)- and Hp(Arg)(2)-mediated photodynamic therapy and early stage of apoptosis induction in prostate carcinoma in vitro. Acta biochimica Polonica. 58: 497-505.
69. O'Connor AE, Gallagher WM, Byrne AT (2009). Porphyrin and nonporphyrin photosensitizers in oncology: preclinical and clinical advances in photodynamic therapy. Photochem Photobiol. 85(5):1053-1074.
70. Oleinick NL, Morris RL and Belichenko I (2002). The role of apoptosis in response to photodynamic therapy: what, where, why, and how. Photochem Photobiol Sci 1: 1-2, 2002.
71. Palumbo G (2007). Photodynamic therapy and cancer: a brief sightseeing tour. Expert Opin Drug Deliv. 4(2):131-148.
72. Pervaiz, S, Olivo, M (2006). Art and Science of photodynamic therapy. Clin Exp Pharmacol Physiol. 33: 551-556.
73. Piette J, Volanti C, Vantieghem A, Matroule JY, Habraken Y, Agostinis P (2003). Cell death and growth arrest in response to photodynamic therapy with membrane-bound photosensitizers. Biochem Pharmacol. 66(8):1651-1659.
74. Postiglione, I.; Chiaviello, A.; Palumbo, G (2011). Enhancing Photodynamyc Therapy Efficacy by Combination Therapy: Dated, Current and Oncoming Strategies. Cancers 3: 2597-2629.
75. Robertson CA, Evans DH, Abrahamse H (2009). Photodynamic therapy (PDT): a short review on cellular mechanisms and cancer research applications for PDT. J Photochem Photobiol B. 96(1):1-8.
76. Seyed MA (2019). A comprehensive review on Phyllanthus derived natural products as potential chemotherapeutic and immunomodulators for a wide range of human diseases. Biocatalysis and Agricultural Biotechnology 17: 529-537.
77. Seyed MA, Bhuvaneswari R, Khee Chee Soo, Malini Olivo (2011). Photolon™ - Photosensitization induces apoptosis via ROS-mediated cross-talk between mitochondria and lysosomes. Int J Oncol 39: 821-831.
78. Seyed MA, Bhuvaneswari R, Soo KC, Olivo M (2011). Photolon™ - Photosensitization induces apoptosis via ROS-mediated cross talk between mitochondria and lysosomes. International Journal of Oncology 39: 821-831.
79. Seyed MA, Vijayaraghavan K (2020). Dengue virus infections and anti-dengue virus activities of Andrographis paniculata. Asian Pacific Journal of Tropical Medicine 13 (2): 49.
80. Seyed MA, Vijayaraghavan K, Rajkumar J, Bukhari SN, Al-Sayed B (2017). Chromolaena odorata: A neglected weed with a wide spectrum of pharmacological activities (Review). Molecular Medicine Reports 15 (3), 1007-1016.
81. Sharma S, Bakal J, Oliver-Fernandez A, Blair J (2004). Photodynamic therapy with verteporfin for subfoveal choroidal neovascularization in age-related macular degeneration: results of an effectiveness study. Arch Ophthalmol. 122(6):853-856.
82. Shim G, Lee S, Kim YB, Kim CW, Oh YK (2011). Enhanced tumor localization and retention of chlorin e6 in cationic nanolipoplexes potentiate the tumor ablation effects of photodynamic therapy. Nanotechnol. 22 (36): 365101.
83. Sibata CH, Colussi VC, Oleinick NL, Kinsella TJ (2000). Photodynamic therapy: a new concept in medical treatment. Braz J Med Biol Res. 33(8): 869-880.
84. Sobaniec S, Bernaczyk P, Pietruski J, Cholewa M, Skurska A, Dolińska E, Duraj E, Tokajuk G, Paniczko A, Olszewska E, Pietruska M (2013). Clinical assessment of the efficacy of photodynamic therapy in the treatment of oral lichen planus. Lasers Med Sci. 28(1):311-316.

85. Spikes JD (1990). Chlorins as photosensitizers in biology and medicine. J Photochem Photobiol B. 6(3): 259-274.
86. Spikes JD, Bommer JC (1993). Photosensitizing properties of mono-L-aspartyl chlorin e6 (NPe6): a candidate sensitizer for the photodynamic therapy of tumors. J Photochem Photobiol B. Biol. 17(2):135-143.
87. Sun L, Li Q , Hou M, Gao Y, Yang R, Zhang, Xu Z, Kang Y, Xue P (2018). Light-activatable Chlorin e6 (Ce6)-imbedded erythrocyte membrane vesicles camouflaged Prussian blue nanoparticles for synergistic photothermal and photodynamic therapies of cancer. Biomat Sci. 6(11):2881-2895.
88. Thirumurugan D, Cholarajan A, Raja SSS, Vijayakumar R (2018). An introductory chapter: secondary metabolites. Second metab-sources Appl, pp1-21,
89. Thong PS, Olivo M, Kho KW, Bhuvaneswari R, Chin WW, Ong KW, Soo KC (2008). Immune response against angiosarcoma following lower fluence rate clinical photodynamic therapy. Journal of Environmental Pathology, Toxicology and Oncology: Official Organ Int Soc Environ Toxicol Cancer. 27(1):35-42.
90. Trukhachova T.V., Shliakhtsin SV, Cerkovsky DA, Istomin YP (2011). A novel finished formulation of the photosensitizer Photolon® for topical application. Evaluation of the efficacy in patients with basal-cell carcinoma of the skin. *Photodiagn Photodyn Ther.*, 8, 200-201.
91. Van Straten D, Mashayekhi V, de Bruijn HS, Oliveira S, Robinson DJ (2017). Oncologic Photodynamic Therapy: Basic Principles, Current Clinical Status and Future Directions. Cancers (Basel), 9(2), 19.
92. Wagner H, Ulrich-MG (2009). Synergy research: approaching a new generation of phytopharmaceuticals. *Phytomed*, 16(2-3), 97-110.
93. Waidelich R (2010). Laser-induced lithotripsy and photodynamic therapy in urology: A short introduction to current laser applications. *Med Laser Appl.*, 25 (1), 14-19.
94. Yoo JO, Ha KS (2012). New insights into the mechanisms for photodynamic therapy-induced cancer cell death. *Int Rev Cell Mol Biol.*, 295,139-174.
95. Yoon I, Li JZ, Shim YK (2013). Advance in Photosensitizers and Light Delivery for Photodynamic Therapy. *Clin Endosc*, 46(1), 7-23.
96. Zhang J, Jiang C, Figueir JP, Longo, Azevedo RB, Zhang HB, Muehlmann LA (2018). An updated overview on the development of new photosensitizers for anticancer photodynamic therapy. *Acta Pharmaceutica Sinica B*, 8 (2), 137-146.
97. Zhao J, Wu W, Suna J, Guoa S (2013).Triplet photosensitizers: from molecular design to applications. *Chem Soc Rev*, 42:5323-5351.
98. Zhu TC, Finlay JC (2008). The role of photodynamic therapy (PDT) physics. *Med Phys*, 35, 3127-3136.