Photodynamic therapy for melanoma:a multifaceted anti-cancer treatment against malignant melanoma

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ABSTRACT— The treatment of patients with malignant melanoma remains complex and unsatisfactory. Conventional treatment strategies have been almost ineffective. Dacarbazine (DTIC) is identified as one of the most effective chemotherapy drugs against melanoma with a response rate of around 15–20%. Numerous patients with melanoma whom their primarily response to surgery subsequently relapse. Obviously, there is an essential need for better identification, where the improvement of potential treatments is warranted. One such treatment is photodynamic therapy (PDT), in which an agent (photosensitizer, PS) produces reactive oxygen species (ROS) for post-irradiation of cancer cells (containing PS). This study reviews the currently discovered potential of PDT and their combinations in treating metastatic melanoma with discussion of the impact of different PDT strategies on cancer treatment . Based on different studies, we realize that PDT with different strategies has shown to have powerful anti-cancer effects on reducing cell viability, metastasis ability, and the induction of apoptosis in different types of melanomas (in vitro and/or in vivo studies). More Important, promising outcomes have been reported in the reduction of recurrence rate after PDT-treatment. Also, PDT is a manageable therapeutic approach that may offer an additional powerful option for adjuvant therapy of patients with melanoma. Combinations of PDT with doxorubicin, temozolomide, and DTIC have revealed better response rates, suggesting that PDT is as effective as the other combination treatments. PDT, both as a single treatment strategy and in combination with conventional treatment strategies, can be a promising treatment option for patients with metastatic melanoma. In this paper we summarize the importance of PDT and its multifaceted activation response in melanoma. More importantly, potential PDT strategies as an adjuvant therapy are reviewed to provide a break through to the limitation of current anti-melanoma therapies.

KEYWORDS: Malignant melanoma, Photodynamic therapy, Adjuvant therapy, Metastasis; Apoptosis.

I. INTRODUCTION

Melanoma is one of the most highly metastatic and resistant types of cancers. This type of cancer occurs as melanocyte malignancy in uveal [1], mucosal [2], and cutaneous [3]. The most common form of melanoma is the cutaneous type [4]. Various risk factors support melanoma incidence and progress, such as genetic backgrounds, fair skin, hair color, lighter eyes, sunburn susceptibility, arsenic, and exposure to ultraviolet (UV) radiation [5, 6]. According to World Health Organization (WHO), there were 287,723 new melanoma cases and 60,712 melanoma related-deaths in 2018. Also, the rate of mortality and incidence was lower in females than in males. Unfortunately, wordwide [7, 8] melanoma is responsible for most skin cancer-related deaths.

Notwithstanding that considering the immense melanoma treatment-research, the survival rate of patients remains unsatisfactory where metastatic melanoma has reduced the overall survival of patients to 6–10 months [9].It is clear that the detection of melanoma in early-stages may be treated by surgerical procedures. However, there are no reliable treatment options for melanoma with metastatic capacity

or metastatic melanoma. Moreover, relapse rates of cured melanoma with surgery (alone) remain high [10]. So, the essential challenge is in the treatment of patients with metastatic melanomas. The systematic treatment for metastatic melanoma started with chemotherapy. The first chemo-drug, dacarbazine (DTIC), was approved in the USA in 1976 to treat metastatic melanoma. Even today, all randomized trials use DTIC as a reference chemo-drug (a comparator). Meanwhile, the response-rate of controlling melanoma with this chemo-drug was less than 7.5% [11].

The other chemo-drug, temozolomide (TMZ) is an (orally administered) alkylating drug, which can traverse the blood-brain barrier and has the powerful potential in controlling the process of metastasis in melanoma, especially cerebral metastases [12]. Also, this chemo-drug shows the same effect as compared to DTIC when used in metastatic melanoma[12].

On the other hand, taxanes as a single chemo-drug or in combination with DTIC offered as a useful chemotherapy option for metastatic melanoma [13]. Nevertheless, it should be mentioned that DTIC is the only approved chemo-drug by the United States Food and Drug Administration (FDA) for melanoma up to this point [14]. Also, chemo-drugs usually induce long-term and serious side-effects in patients, especially with high doses, where the prolonged administration of chemo-drug leads to the increased rates of chemo-resistance form in melanomas [15, 16].

In addition to chemotherapy, radiation therapy as a primary and adjuvant treatment option is typically used in melanoma patients [17]. Though melanoma is an approximately radio-resistant cancer, radiation therapy plays an effective role in controlling melanoma [18]. Literature of adjuvant-radiation therapy for melanoma with (highest risk) has revealed improvements in decreasing the risk of local recurrence but has failed to demonstrate a meaningful increase in overall survival rate [19-22].

As a whole , a conventional chemotherapy and radiation therapy have not been a successful one .Treatment options for advanced melanoma have been developed to include targeted therapy or immunotherapy (alone or in combination with other treatments) [23]. During the recent decade, there has been a positive evolution in immunotherapy as an adjuvant-therapy or treatment option for metastatic melanoma. A primary strategy of immunotherapy, interleukin-2, has revealed a powerful potential in controlling cutaneous melanoma [24]. Hence, the FDA validation was approved in 1998 due to its excellent response rates. However, this treatment approach has induced extensive toxicities in patients with melanoma, although studies [25] show some good outcomes.

T cell has an axial role in the immune-mediated tumor suppression. The immune checkpoints (such as CTLA-4 and PD-1) is a principal mechanism that can terminate the T cell-specific response. So, the development of immune checkpoint inhibitors has improved the immunotherapy of advanced melanoma [26]. Immune checkpoint blockers were the primary strategy of treatment revealed to improve overall survival in patients with metastatic melanoma. Nowadays, the antibodies ,anti-PD-1 (nivolumab and pembrolizumab), the anti-CTLA-4 (ipilimumab), are all used as the standards of care in clinical practice [27] for treatment.

Despite the approval of anti-PD-L1 and anti-CTLA-4 treatments, these agents alone improve only a fraction of patients. Also, the evidence show that some patients whom have been receiving the immune checkpoint blockers ,have shown to experience serious side effects. Side effects are induced principally due to immune checkpoints blockage, that supports normal physiological barriers to autoimmunity, resulting in various local or systemic autoimmune responses [14].

Notwithstanding that with these treatments, there is still no set procedure and requirements that is recognized for effective cancer treatment. Present article investigates the

effectiveness of photodynamic therapy (PDT) as a powerful treatment approach in combination with other therapeutic approaches or alone for melanoma.

II. PHOTODYNAMIC THERAPY (PDT)- A POWERFUL TREATMENT APPROACH FOR VARIOUS HUMAN CANCERS

PDT is a negligibly invasive therapeutic approach that is based on the interaction of a harmless light-sensitive agent, recognized as photo-sensitive agent (photosensitizer, PS), with the visible light-irradiation at a wavelength specific to stimulate the PS in order to induce cellular photo-damage. The primary registered utilization of visible-light for medical purposes goes back to the ancient Egypt three thousand years ago,where the use of the use of sun-light for treatment of the vitiligo (skin-disease) after the administration of plant extracts (containing psoralens as light-sensitive agents) can be seen in the so-called Ebers Papyrus [28]. In this way, the joint administration of a plant extract (containing light-sensitive agents) to the skin of a patient along with exposure to sun-light, was used for treatments. However, the foremost research-based report of the usage of light-sensitive agents was published over a hundred years ago, when it was proven that irradiation of light after administration of acridine orange could destroy protozoan cells [29]. In the 1970s, Thomas Dougherty and et al. examined anti-cancer effects of PDT. It was then that they were able to reveal partial or total suppression of the growth of the numerous malignant tumors, say recurrent colon, metastatic breast, basal cell cancers, and metastatic melanomas [30].

The limitations of current melanoma treatments may be reduced by an exact understanding of effective therapeutic approaches such as PDT, where a clinical study for treatment of the cancer is necessary. Hence, we have reviewed the advantages of PDT as a promising therapeutic approach for metastatic melanoma.

A. *Mechanisms of PDT – anti cancer effects*

As mentioned above, PDT has been described as a hopeful clinical option for treating different malignant tumors. It depends on the administration of PS, which is excited by using visible light of a certain wavelength after a specified incubation time [31]. The long incubation time of PSs provides optimally localized of the PS in different intracellular organelles. Conversely, the short incubation time of PSs mainly targets tumor vasculature [32].

The appropriate wavelength considered for PDT usually matches with the absorption peak of the applied PS, theoretically occurring among 650 and 850 nm (phototherapeutic window). Phototherapeutic window corresponds to maximum tissue penetration of visible-light (minimum overlapping with endogenous PSs) as well as enough energy to produce excited states of PS. In the presence of molecular oxygen, the photo-excited PS results in generation of the reactive oxygen species (ROS), that are extremely cytotoxic agents to cancer cells where the PS administers (Fig. 1) [33].

The main ROS produced during PDT is singlet oxygen ($^1O_2$), generated by energy transfer from the triplet state of used PS (type II reactions). Other ROS, such as the hydroxyl radical ($OH^{\bullet}$) and superoxide ion ($O_2^{\bullet-}$), generated by electron transfer reactions (type I reactions), can also be produced. These agents have the same very short half-life, reacting with substrates only limited to a micron of their production site [34].

Anti-cancer outcomes of PDT are based on the combination of three mechanisms: 1) direct cytotoxic effects on the tumor cells, 2) the demolition of the tumor blood vessels and, 3) the trigger of anti-tumor immunity. These three mechanisms provide the long-term cancer management and count as the main benefit over common treatments, including chemotherapy and/or surgery, which are usually immunosuppressive or immunologically silent [31, 35].

Major PSs (clinically and pre-clinical) accepted for PDT are phthalocyanines, bacteriochlorins, chlorins, and porphyrins. The 5-aminolevulinic acid (ALA), porfimer sodium, verteporfin, temoporfin, and talaporfin are commonly used in clinical application but display incomplete absorptions in the PDT phototherapeutic window and decreased capacity of PDT in the elimination of deep tumors. Moreover, long-term (weeks or even months) skin photo-sensitivity is mostly reported with several of these PSs, especially porphyrins. This is due to their slow body clearance processes resulting in the requisite of times without sun-light for patients, thus reducing their quality of life [36, 37].

So, the clinically-accepted PSs are not fully perfect for an ideal PDT. This has initiated the improvement of PSs with better photochemical and spectral properties. In fact, several of the limitations of accepted PSs were relatively overcome in the past several years. For example, padeliporfin is a negatively charged palladium bacteriochlorophyll PS, prepared from freebase extraction using benthic bacteria. It shows high absorption at the phototherapeutic window (~763 nm) and body clearance rate for about a few minutes. The rapid clearance rate of PS reduces the photo-sensitivity of skin after PDT but needs clinical procedures with infusion simultaneous and irradiation administration [38, 39]. Another PS, redaporfin, is an amphiphilic and synthetic bacteriochlorin with a higher ROS quantum yield, and absorption peak at ~749 nm, which provides the PDT-treatment of deep tumors [40].

A case report recently demonstrated a patient with malignant head and neck squamous cell cancer, which was uninhibited after chemotherapy, radiotherapy, and surgery but observed significant benefit from PDT with redaporfin. In a preclinical study, using three periods of redaporfin-PDT, have shown a considerable tumor eradication. Also, two years after the use of redaporfin and consecutive administration of nivolumab (an immune checkpoint inhibitor), no signs of the cancer tumor was evident [41].

In addition to PSs, the light source is one of the other main factors of PDT, and its selection has a significant impact on the efficiency of PDT. In fact, the right selection of light source as the heart of this treatment depends mainly on PS absorption peak and location of the tumor (type of cancer). While conventional LEDs and lamps are still used in PDT, they have been replaced with lasers because of the unique properties such as high intensity, excellent coherency, monochromatic beam, and good penetration range [35]. Among all kinds of lasers, diode lasers have been under much the attention because of their lightweight, small size, low cost, easy installation, simple operation, and facile mobility. Therefore, diode lasers have been developed as the most common light sources used in PDT [42, 43].

The third main factor in the PDT is the capacity of ROS generation during treatment and consequently the degree of oxidation induction. As mentioned, ROS is able to damage biomolecules and eventually induce cancer cell death (apoptosis and/or necrosis) by triggering a shut-down of intra-tumoral vasculature and an anti-tumor immune response. In this way, by stimulation of PS with an appropriate wavelength for the light source (especially around phototherapeutic window) along with the application of molecular oxygen in the target site, one can produce ROS (e.g., $^1O_2$, $OH^{\bullet}$, and $O_2^{\bullet-}$). These agents' increasing presence triggers a cascade of biochemical and molecular events, causing target cell death [44, 45].

So, PDT performance depends on three factors, including PS, light source, and molecular oxygen rate, for sufficient ROS generation (Fig. 2). PDT optimization is complex since all factors are correlated. Nevertheless, sufficient generation of ROS remains the utmost important factor since after that PDT success on it. However, other factors (e.g., PS and light source) need to be considered in treatment strategy if the improvement is to be made toward effective PDT. The main superiority of PDT over traditional anti-cancer therapies is (1) PDT has negligible systemic toxicity and patient tolerating repeated dosing, (2) PDT

capacity to demolish cancer cells selectively (owing to this selectivity capacity, side effects of PDT to normal cells is negligible). Lastly, (3) PDT can be utilized separately or in combination with other anti-cancer treatments (as an adjuvant anti-cancer therapeutic approach) such as surgery, chemotherapy, immunotherapy, and radiotherapy[46, 47]. These advantages have result to PDT receiving increased attention from cancer researchers, especially dermatology research.

III. PDT AND ITS MULTIFACETED ACTIVITY IN MELANOMA- AS A POWERFUL AND ADJUVANT THERAPEUTIC APPROACH

A. *PDT as an effective inhibitor of melanoma cell proliferation*

It is well recognized that one of the main features of cancer cells such as melanoma cells is their capacity to defeat control points of the cell cycle and cell proliferation. In melanocytes, cell proliferation is affected by the interaction of various growth factors such as epidermal growth (EGF), hepatocyte growth (HGF), stem cell (SCF), and fibroblast growth factor (FGF), that leads to a maintained extracellular receptor kinase (ERK) activity [48]. In other words, the RAS/Raf/MEK/ERK signaling pathway is one of the important regulating signaling pathways in cell proliferation, with ERK being up-regulated in human malignant melanomas (> 90%) [49].

Also, PTEN and BRAF mutations are co-occurrence in approximately 20% of human melanomas [50]. The BRAF mutations leads to amplification of ERK signaling pathway causing uncontrolled proliferation and viability for melanoma cells [51].

Mohammad Amin Doustvandi et al. [52] defined ZnPc-PDT as an effective treatment in which the viability of SK-MEL-3 melanoma cells are decreased at different doses of ZnPc-PDT. This result show similarity to those of Zhaohai Pan et al. [53], who used TBPoS-2OH as PS to perform novel PDT treatment. The study showed that TBPoS-2OH-PDT could prevent the proliferation capacity of two different melanoma cells (A375 and B16 cell lines) by increasing the intracellular ROS levels. In another study [54], authors used curcumin as a natural PS to perform PDT on the A375 and C32 melanoma cells; they found considerably inhibiting cell proliferation after curcumin-PDT than after curcumin treatment (alone). Although curcumin treatment (separately) somewhat inhibits cell proliferation of two melanoma cells, but harmless curcumin concentrations are photo-cytotoxic in curcumin-PDT.

In an overview, many studies confirmed the effective roles of different PDT protocols in inhibiting cell proliferation for melanomas. Metastatic melanoma shows higher resistance to traditional treatment, which may be due to the rapid proliferation of these cells, contributing to acquiring the aggressive behavior. So, this cytotoxic impact of PDT on melanoma cells proliferation has to be considered for the successful treatment strategy of this cancer.

B. *PDT as an effective inhibitor of cell migration of metastatic melanoma cells*

Almost all melanoma types have radial growth with negligible metastasis capacity in early phases, followed by vertical growth with high metastasis capacity (especially dermal metastasis). For all variants of melanoma at the early-stage, the risk of recurrence and death of patients is closely related to Breslow depth (depth of invasion) at the time of detection of melanoma and starting the curing process.

Compared with non-melanomas skin cancers such as cutaneous squamous cell carcinomas and basal cell carcinomas, melanomas show a great metastasis capacity (e.g., local, zonal, and distant invasion). The metastasis process stages in melanomas have been well described and characterize as a series of steps, including local invasion, intravasation, survival in the circulation, arrest at different organ site, extravasation, micro-metastasis formation, and metastatic colonization of target organs. Also, tumor blood vessels in melanoma contribute to inflammation and neo-angiogenesis, leading to

the acquisition of cell migration, metastasis, and invasion. It has also been established that tumor treat performance relies strongly on vessel destruction around and in the treated tumor [55].

With these descriptions, PDT can impact tumor vessels by photo-damage of the endothelium. In other words, this treatment can extensively impact the tumor vasculature through induction of endothelial cell death, vascular leakage, and coagulation, resulting in the blockage of the tumor blood vessels [56]. Many studies in previous years exploring the mechanisms of PDT efficiency have shown that the vascular disruption triggered by PDT is mainly responsible for advantages of the treatment, depending on the incubation time of PS before light source irradiation (short incubation time of PSs mainly targets tumor vasculature) [57-59].

Altogether, it seems that using different incubation times of PS in PDT on the inhibition of cell migration ability and invasion is perceptible. In this regard, Tammela et al. investigated the effects of PDT in eradicating melanoma in mice models by using verteporfin as PS after irradiation with a light source at 635 nm. The study demonstrated extensive damage of tumor-connected lymphatic vessels, which inhibited metastasis and melanoma recurrence [60].

Recently, Jie Zhou et al. examined the efficiency of PDT using Au@MTM-HA as a novel PS platform on metastatic melanoma (an in vitro and in vivo study). After specified incubation times, the melanoma cells and tumors were exposed to laser light of 532 nm (0.8 W/cm^2, for 10 min). Au@MTM-HA-PDT weakens the cell migration and invasion ability of B16-F10 melanoma cells via control cells. Most importantly, in vivo findings showed effective inhibition of lung metastasis of melanoma cells in the animal model [61].

Also, Sheleg et al. investigated the performance of PDT by chlorin e6 as PS on metastases of melanoma (especially dermal metastases). During the administration (intravenous) of chlorin e6 (5 mg/kg) to 14 patients, tumor sites were exposed to the light source (80—120 J/ cm^2). With twice application PDT, all derma metastases from melanoma tumors regressed with a non-recurrence rate. This therapeutic approach did not display any hepatic or renal injury [62]. Clinically, PDT has revealed promise potential in the treatment of metastatic melanomas. Nevertheless, further clinical studies are need before PDT approval for the common treatment strategy of melanoma.

C. *PDT as an effective inducer of apoptosis cell death in melanoma cells*

PDT can result in oxidative stress that eventually activates different types of cell death. Our understanding of different types of cell death continues to develop with new signaling pathways of cell deaths being continuously revealed. Autophagy, necrosis and apoptosis are the best known types of cell death although terms like, lysosome-dependent cell death and immunogenic cell death, necroptosis, pyroptosis, ferroptosis and etc. have also being discovered[63].

Apoptosis, is an important type of cell death after anti-cancer treatment, is described as biological event including chromatin condensation, DNA fragmentation, membrane bubbling, cell shrinkage, and triggering of different caspase cascades [64]. This type of cell death shows an essential role in keeping homeostasis and eliminating unwanted cells [65]. Not unexpectedly, impaired normal regulation of apoptosis triggers the pathogenesis of numerous human diseases [66, 67]. The most notable of this irregularity is observed in cancer. Unfortunately, cancer is one of the leading causes of death worldwide. One of the features of different cancers such as melanoma is their ability to escape apoptosis cell death[68].

Therefore, numerous treatment strategies for melanoma purpose to overcome apoptosis escape by targeting different apoptosis molecular pathways such as caspase-dependent and caspase-independent apoptosis pathways. While most treatment strategies do so directly

or indirectly on the induction of apoptosis, emerging insights into PDT based on directly triggering different apoptosis pathways are considered the most promising treatment strategy used to cure melanoma.

In this regard, targeted cell death after PDT may result from apoptosis and/or necrosis (necrosis as the unwanted cell death), depending on the PS type, cancer cell type, light source dosage, and oxygen level [49, 69-71]. Several studies have demonstrated that PDT with different PSs (e.g., zinc phthalocyanine and hypericin) utilize the caspase-dependent apoptosis pathway for induction of apoptosis (as classic apoptosis pathway) [72-74]. However, this treatment strategy has also been shown to induce caspase-independent apoptosis pathways [52, 75].

So, suppose some cancer cells, such as melanomas, blocked the main apoptosis pathways (caspase-dependent) owing to different genes mutation involved in these pathways. In this case, the apoptosis cell death can still occurre in the cells by the caspase-independent apoptosis pathway. After PDT, the overall mechanisms of induction apoptosis may be a combination of different apoptosis pathways [76].

Jingjing Cai et al. studied photo-toxic effects of ALA on human A375 melanoma cell line. In this study, the survival rate, apoptosis rate, and apoptosis molecular pathways were considered for assessing ALA-photo-toxicity after irradiation with a light source at 643 nm (0.58 J/cm^2). The study established that ALA-PDT could prevent the survival ability and strongly trigger apoptosis cell death of malignant melanoma A375 cells. Their findings also indicated the induction of apoptosis associated with the caspase-dependent pathways (death-receptor and mitochondrial pathways of apoptosis)[77].

In another study, Joanna Nackiewicz et al. studied the photo-toxicity of zinc octacarboxyphthalocyanine toward Me45 melanoma cells after irradiated with a light source 685nm. This in vitro study revealed that zinc octacarboxyphthalocyanine-PDT is an effective treatment strategy for malignant melanoma. In this way, zinc octacarboxyphthalocyanine (30 µM) with the combination of light source (dosage 2.5 J/cm^2), was sufficient to damage melanoma cells through apoptosis, with negligible cytotoxic effects on normal fibroblasts (healthy NHDF) [78].

Also, Mohammad Amin Doustvandi et al. studied the effects of different dosages of light sources toward SK-MEL-3 melanoma cells. After 24 hour incubation period with different concentrations of zinc phthalocyanine, the SK-MEL-3 cells were exposed to a light source of 675 nm with the doses of 8, 16, and 24 J/cm^2, the apoptosis effectively was induced in the treated melanoma cells. Zinc phthalocyanine-PDT with the doses of 16, and 24 J/cm^2 caused induction of the caspase-dependent apoptosis pathway. More importantly, with a low dosage of a light source (8 J/cm2), this treatment strategy caused induction of the caspase-independent apoptosis pathway [52] observably.

So, we can realize the powerful potential of PDT on the induction of different intracellular apoptosis pathways. These results and many studies in this area may be a promising start point for the effective treatment of melanomas, especially resistant melanomas.

D. *PDT as a hopeful adjuvant therapeutic approach for melanoma*

There is a high risk of metastasis and recurrence in the surgical resection of metastatic melanoma tumors. After removing locoregional metastases in different patients with melanoma by using surgery, recurrence rates for 1-year were from 8% to 76%, depending on the tumor bulk [79-81]. For decades, several adjuvant therapy approaches were available to decrease mortality and recurrence in resected metastatic melanomas. Recent progress in PDT as an effective adjuvant therapy approach has improved the standard of care for managing patients with the cancer. PDT that opens up novel possibilities and may be commonly used as an effective treatment strategy for adjuvant therapy in advanced chemotherapy and post-

resected metastatic melanomas in the future [82].

In 2017, FA Nsole Biteghe et al. studied the synergism outcomes of combination therapy between DTIC and Hypericin-PDT on human metastatic melanoma cells (UCTMel-1 and A375 cell lines). This combination therapy design obviously decreased metastatic melanoma cell viability percentage. Furthermore, this treatment strategy weakens the renewal ability of metastatic melanoma cells – a feature that melanoma cells used to trigger clonogenic growth and tumorigenicity. This study indicates that PDT as a powerful adjunctive therapy combined with DTIC may be useful as a therapeutic modality for cancer patients [83].

Also, in 2019, an in vitro study [74] indicated that ZnPc–PDT increases the sensitivity of human melanoma SK-MEL-3 cells to doxorubicin about several hundred times through a pre-treatment strategy, especially at the low dose of light source. Based on this study, it appears that the promising result of PDT in melanoma management as adjuvant therapy will be dealt with overcoming melanoma's protective capacity against chemotherapy drugs.

Furthermore, investigation in animal models have already revealed that the combination of PDT with temozolomide resulted in a better anti-cancer response [84]. So, based on these studies and other studies in this field, PDT as a hopeful adjuvant therapy option can be administered as an adjunctive approach (in combination with chemo-drugs or even alone) in post-resection treatment regimes. As our understanding of the best strategies to combine PDT with other therapeutic approaches is developing, further clinical PDT improvement should be expected.

E. *Melanin as a specific endogenous PS in melanoma*

The one feature that sets melanoma separately from other malignant cancers is the presence of the melanosome organelle and melanin pigment (its related product). It is consequently not unexpectable to believe that the complexity of PDT of this cancer may in several condition be associated to this melanosome and its product [85]. It realizes logically then that for optimum design protocols of PDT should be considered the melanosome as one of the potential challenges in the PDT against melanoma [86].

This organelle is one of the membrane-bound organelles in melanocyte cells, which accommodate the biological pathway that results in melanin production, a polymeric pigment [87, 88]. So, the PDT-treatment of malignant melanomas immediately proposes this endogenous molecule (melanin) interaction with the light source that absorbs light (acts as an endogenous PS). According to this, the most appropriate wavelength of light source for PDT of melanoma is 650–850 nm, known as the phototherapeutic window (as mentioned above). Melanin is an endogenous pigment in pigmented melanomas that shows significant absorption across the spectrum's visible region, but this absorption falls to minimum levels in the phototherapeutic window [89]. Table 1 lists examples of PSs that can confidently be used for in vivo and/or in vitro PDT treatment in various melanoma cell lines without interference from melanin.

IV. CONCLUSION AND FUTURE PERSPECTIVE

Over the last years, PDT has been identified as a powerful therapeutic approach to show extensively anti-tumor effects and has opened novel therapeutics options against many types of cancers, including malignant melanomas (whether alone or even in combination with other anti-cancer treatment).

The concept of "adjuvant therapy" in treating malignant melanomas for PDT with existing anti-tumor impacts could extend the possible PDT applications for clinical applicability. In fact, different strategies of PDT can potentiate the cytotoxic effects of chemo-drugs through combination therapy. So, localized PDT-treatment combined with the approved formulation of PS can effectively manage the toxicity caused by the traditional anti-cancer

treatments (e.g., chemotherapy) with enhanced sensitivity of melanoma cells to lower doses therapeutic approaches.

Interestingly, identification roles of key PDT modulators from incubation times of PSs, novel PSs and dosage of light sources can significantly reduce cell viability and tumor angiogenesis capacity in different types of malignant melanoma. Furthermore, the preferential killing of melanoma cells through the induction of apoptosis cell death with negligible necrosis and prevention of metastasis ability during PDT-treatment may result in decreased off-target toxicity, improved patient outcomes, lowering the risk of melanoma recurrence, and prevention of tumor metastasis.

Furthermore research to reveal the role of different strategies of PDT in the management of malignant melanoma can help to develop and design powerful treatment options targeting melanoma cells in pathway-specific manner, tumor-specific, and cell-specific, that will have resilient therapeutic strategy in melanoma treatment.

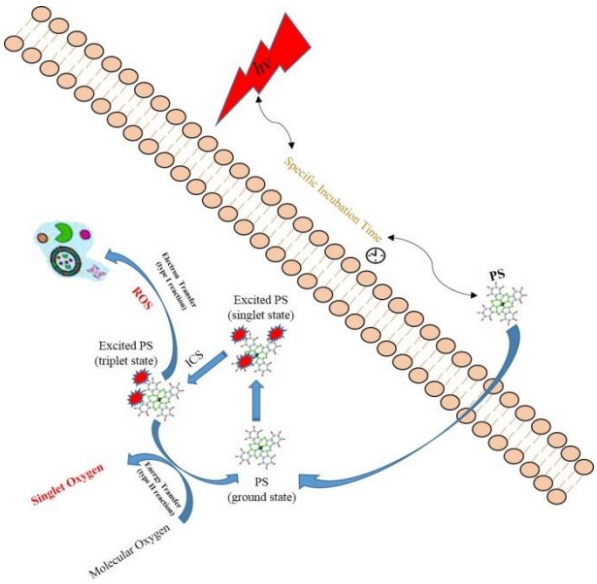

Fig. **1** Mechanism of PDT. Both reactions (Type I and type II) are triggered when the PS absorbs a photon and is excited to the singlet state ($^1PS^*$, relatively short-lived). The $^1PS^*$ may undergo intersystem crossing (ISC) to form a triplet state ($^3PS^*$, relatively long-lived). The $^3PS^*$ can transfer an electron to biomolecules, resulting in ROS production (type I reaction). On the other hand, in a type II reaction, energy of the $^3PS^*$ may be directly transferred to molecular oxygen, to form singlet oxygen.

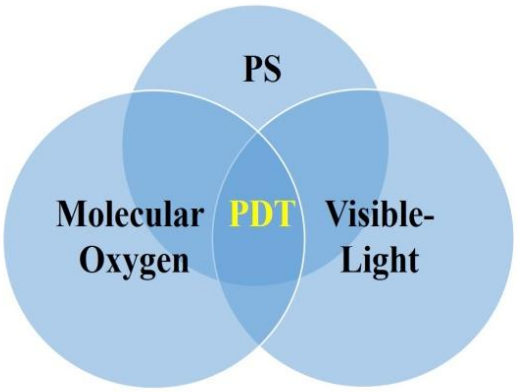

Fig. **2** Interaction between three main parameters of PDT.

Table **1** Examples of PSs approved for clinical use or under clinic trials [90].

PSs	Drug Name	λ max (nm)	Marketed by
N-aspartyl chlorin e6 (NPe6)	Laserphyrin	~664	Meiji Seika Pharma Co., Ltd. (Japan)
Meta-tetra(hydroxyphenyl)chlorin(m-THPC)	Foscan	~652	Biolitec Pharma Ltd. (Dublin,Ireland)
5,10,15,20-Tetrakis(2,6-difluoro-3-Nmethylsulfamoylphenyl)-bacteriochlorin	Redaporfin	~750	Luzitin SA (Portugal)
2-(1-Hexyloxyethyl)-2-devinylpyropheophorbide (HPPH)	Photochlor	~665	Roswell Park Cancer Insitute (NY, USA)
Palladium bacteriopheophorbide (WST-11)	Tookad-Soluble, Stakel	~753	Steba Biotech (Luxembourg)
Palladium bacteriopheophorbide (WST-09)	Tookad	~763	Steba Biotech (Luxembourg)
Verteporfin	Visudyne	~689	QLT Inc. (Canada)
Disulfonated tetraphenyl chlorin (TPCS2a)	Fimaporfin	~652	PCI Biotech (Norway)
silicon phthalocyanine PC4	PC4	~675	Not licensed and produced by NCI (USA).
Aluminum phthalocyaninetetrasulfonate (AlPcS4)	Photosens	~676	General Physics Institute (Russia)

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