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**Breast cancer as photodynamic therapy target: Enhanced therapeutic efficiency by overview of tumor complexity**

Lamberti MJ *et al.*Photodynamic therapy on breast cancer

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**Abstract**

Photodynamic therapy, a minimally invasive and clinically approved, procedure permits to eliminate selected malignant cells from organism with a specific light activation of a photosensitizer agent. While the majority of approved photodynamic therapy protocols treat superficial lesions of skin and luminal organs, interstitial and intra-operative approaches have been investigated for the ablation of a broad range of superficial or bulky solid tumors such as breast cancer. This review article will discuss recent progress in research focused mainly on the efficacy’s assessing of different photosensitizers used in photodynamic therapy as well as the combinatory strategies of various therapeutic modalities to improve the index of treatments of parenchymal and/or stromal tissues of breast cancer solid tumors. When treating cancer, cytotoxic agents are intended to exert their effect on rapidly proliferating cancer cells. However, often cancer therapeutics lack specificity which can lead to toxicity and undesirable side effects. Many approaches have been designed to target tumors. Selective therapies can be established by focusing on distinctive intracellular (receptors, apoptotic pathways, multidrug resistance system, nitric oxide mediated stress) and environmental (glucose and pH) differences between tumor and healthy tissue. A rational design of effective combination regimens for the treatment of patients suffering breast cancer involves a better understanding of the mechanisms and molecular interactions of cytotoxic agents that underlying drug resistance and sensitivityhe.

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**Key words:** Photodynamic therapy**;** Breast cancer**;** Tumor microenvironment**;** Treatment combination**;** Synergism

**Core tip:** Breast cancer is the most common women's cancer in the world. The effective therapies that would not only reduce the high mortality rate associated with the disease, but also improve the quality of life patients with breast cancer are still searching for. In recent years possibilities of photodynamic therapy (PDT) using in breast cancer treatment are analyzed. PDT, a new and promising antitumor strategy has a potential range of applications alone or in combination with other approved or experimental therapeutic approaches that is definitely not exhausted yet.

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**PHOTODYNAMIC THERAPY ON SOLID TUMORS: FOCUS ON BREAST CANCER**

Photodynamic therapy (PDT) is one of the clinical approved and minimally invasive alternative method of cancers treatment, such as bladder, esophagus, respiratory tract and gynaecological cancers. PDT eliminates tumor cells by the combination of non-toxic per sephotosensitizers (PS) and light use[1]. Light activation of a PS results in energy transfer cascades that ultimately yield cytotoxic reactive oxygen species (ROS) which can then render cell death[2]. Antitumor effects of PDT derive from three interrelated mechanisms: direct cytotoxic effects on tumor cells, indirect damage to the tumor vasculature and induction of a inflammatory responsethat can activate systemic immunity[3].

Much progress has been seen in both basic research and clinical application in recent years. While the majority of approved PDT protocols treat superficial lesions of skin and luminal organs, such as actinic keratosis and Barrett’s esophagus, interstitial and intra-operative approaches have been investigated for the ablation of a broad range of superficial or bulky solid tumors located in the head and neck, brain, breast, lung, gastrointestinal, and genitourinary regions[2].

The photosensitizer is considered to be a critical element. In general, for solid tumor PDT an ideal PS should meet at least some of the following criteria: a commercially available pure chemical, low dark toxicity but strong photocytotoxicity, good selectivity towards tumor cells, longer wavelength allowing deeper light penetration, rapid removal from the body, and multiple administration routes (oral, intravenous, intratumoral or inhalational). Although some PSs satisfy all of or some of these criteria, there are currently only a few PDT photosensitizers that have received official approval around the world. Photofrin (630 nm, Axcan Pharma, Inc.), Levulan (prodrug of protoporphyrin IX; 630 nm, DUSA Pharmaceuticals, Inc.), Metvix (prodrug of protoporphyrin IX; 630 nm, PhotoCure ASA.), Foscan (652 nm, Biolitec AG), Laserphyrin (664 nm, Meiji Seika Kaisha, Ltd.), Visudyne (693 nm, Novartis Pharmaceuticals). Several 2nd generation PSs (*e.g*., HPPH - 665 nm, SnET2 - 665 nm, Lu-Tex - 732 nm) have been investigated in many preclinical and clinical trials for various solid tumors, and in particular SnEt2[4,5] and LuTex[5]are US clinically applied for breast cancer[3]. These photosensitizers show the selectivity towards tumor cells and are ideal for cellular and vascular-targeting PDT and interference with cytoprotective molecular responses is of growing interest.

Breast cancer is the most common women's cancer in the world. The effective therapies that would not only reduce the high mortality rate associated with the disease, but also improve the quality of life patients with breast cancer are still searching for.

In recent years possibilities of PDT using in breast cancer treatment are analyzed, and its full-potential range of applications alone or in combination with other approved or experimental therapeutic approaches nedded to be defined.

This review article will discuss recent progress in researches focused mainly on the efficacy’s assessing of different photosensitizers used in photodynamic therapy as well as the combinatory strategies of various therapeutic modalities with non-overlapping toxicities in order to improve the therapeutic index of treatments of parenchymal and/or stromal tissues of breast cancer solid tumors. Any interactions between PDT and PDT-sensitizing agents will be confined to the illuminated area. Therefore, the potentiated toxicity of the combinations is not systemic.

**CONVENTIONAL BREAST CANCER THERAPIES COMBINED WITH PHOTODYNAMIC THERAPY**

Most women with breast cancer have some type of surgery as main option in order to remove a breast tumor, which can be breast-conserving surgery or mastectomy (removal of breast). The breast can be reconstructed at the same time as surgery or later on. Radiotherapy or systemic therapy is commonly given as adjuvant treatment after surgery[6]. In addition, synergistic treatments offer favorable outcomes, increasing the efficacy, decreasing the dosage avoiding toxicity and minimizing the development of drug resistance[7].

Radiation therapy, or radiotherapy, a treatment which applies high-energy rays (ionizing rays) to destroy cancer cells. Radiation to the breast is often given adjuvantlyafter surgery to help lower the ofrecidive. Radiation therapy can be given internally or externally. The later is the most common type of radiation therapy for women with breast cancer.The main short-term side effects of external beam radiation therapy are swelling and heaviness in the breast, sunburn-like skin changes in the treated area, and fatigue[6].

In recent years, researchers have become increasingly interested in combining antitumor therapies in order to improve the patient outcome and to avoid, at least in part, side unwanted effects. In this context, there are reports indicating that some PSs can act as radiosensitizers[8,9]. Regarding breast cancer, several *in vitro* studies have shown a synergism between PDT and ionizing radiation in killing cells. The combined application of non toxic doses of indocyanine green (ICG)[10], rhodamine 123 (Rh-123) and its platinum complex Pt(Rh-123)2[11], zinc phthalocyanine (ZnPC) and meso-tetrahydroxyphenylchlorine (m-THPC)[12] with light proved to be very effective and resulted in a nearly complete reduction of survival. These reports suggest that a combined treatment of tumors with both PS-mediated PDT and ionizing radiation could be superior to the use of the single modalities of PDT and ionizing radiation alone. PDT and ionizing radiation interaction could enhance the therapeutic effect, thus reducing the ionizing radiation dose and thereby lowering the potential side effects.

Systemic therapy, better known as chemotherapy, is a treatment with cancer-killing drugs that may be given intravenously (injected into a vein) or by mouth. The drugs travel through the bloodstream to reach cancer cells in most parts of the body.There are several situations in which chemotherapy may be recommended in breast cancer patients: after surgery (adjuvant chemotherapy), before surgery (neoadjuvant chemotherapy) or for advanced breast cancer. In most cases (especially adjuvant and neoadjuvant treatment), chemotherapy is most effective when combinations of more than one drug are used. Many combinations are being used, and it's not clear that any single combination is the best. The most common chemo-drugs used for early breast cancer include the taxanes (such as docetaxel and paclitaxel) and the anthracyclines (epirubicin and doxorubicin). These may be used in combination with certain other drugs, like cyclophosphamide and fluorouracil (5-FU).Another chemo-drugs are useful in treating women with breast cancer, such as platinum agents (cisplatin, carboplatin). Chemotheray related-side effects depend on the type of drugs, the amount taken, and the length of treatment: .nausea and vomiting, mouth sores, easy bruising, hair loss, loss or increased appetite, , increased chance of infections, low blood cell countsor bleeding and fatigue[6].

The effect of combining PDT with traditional chemotherapy on breast cancer has been studied in recent years. Low doses of cisplatin*in vitro*, which probably will not cause severe side effects, shown to be more effective when combined appropriately with ICG based-PDT[13]. Additionaly, another*in vitro* study showed that the combination of mTHPC-mediated PDT and 5-ﬂuoro-2-deoxyuridine (5FdUr), a chemotherapeutic drug, resulted in a lower cell survival than single-mode treatment[14].Benzoporphyrin derivative monoacid ringAbased-PDT plus doxorubicin exerted enhanced *in vivo* antitumor effect on breast cancer, which was associated with the cooperative regulation of extrinsic apoptotic pathways and the inhibition of tumor angiogenesis[15]. How chemotherapeutic drugs and PSs interact and how these two can be combined in order to result in increased cell killing with reduced side effects must be examined in more detail.

We have recently reported that β-Lapachone, a pre-clinical chemotherapeutic drug, synergistically interacted methyl aminolevulinic acid-PDT on breast cancer *in vitro*. However, we demonstrated that the application scheme of both therapies have relevance in the outcome. Synergism was observed when chemotherapy was applied 24 h after PDT, due to the photodynamic induction of NQO1, the principal determinant of β-Lapachone cytotoxicity. The combination PDT followed by β-Lapachone treatment is a potentially promising modality for the treatment of cancer[16].

When treating cancer, cytotoxic agents are intended to exert their effect on rapidly proliferating cancer cells. However, often cancer therapeutics lack specificity which can lead to toxicity and undesirable side effects. Many approaches have been designed to target tumors. Selective therapies can be established by focusing on distinctive intracellular and environmental differences between tumor and healthy tissue. Additionaly, combining drugs represent a strategy to treat breast cancer. The molecular interactions with cytotoxic agents, combined with increasing knowledge of the molecular mechanisms underlying drug resistance and sensitivity, allow a rational design of effective combination regimens for the treatment of patients[17]. In this sense, the combinations between PDT and tumor-targeted strategies will be reviewed.

**PHOTODYNAMIC THERAPY AND BREAST CANCER RECEPTOR-TARGETED AGENTS**

Breast cancer cells are known to over-express estradiol receptor (ER), human epidermal growth factor receptor 2 (HER2), gonadotropin-releasing hormon receptors (GnRHR) and tisular factor (TF, factor VII receptor); they are strongly associated with increased disease recurrence and a poor prognosis[18]. Thus, therapies have been developed in order to remove [their](http://www.cancer.gov/Common/PopUps/popDefinition.aspx?id=45713&version=Patient&language=English) ligands or blocks their action so stops cancer cells from growing[6]. Intrestingly, photodynamic researchers started to employ these particularities as vehicles to selectively deliver photosensitizing agents. These receptors represent a potential site for directing photosensitizers, and increasing cellular uptake compared to normal cells *via* a receptor mediated process. So, over-expression of receptor in breast cancer was harnessed synergistically with the tumor-migrating effect of several PSs to selectively deliver target molecule-PS conjugates into breast tumor cells, and preferentially kill the tumor cells upon exposure to red light[19–32]. Table 1 summarizes the main results of PDT plus targeted-ligands on breast cancer. Developments in these specific types of receptor targeting approaches highlight the potential advantages of these conjugates in the discovery of more effective cancer photochemotherapy agents.

**PHOTODYNAMIC THERAPY COMBINED WITH ANTI-APOPTOTIC STRATEGIES**

PDT leads to the generation of cytotoxic oxygen species that appear to stimulate several different signaling pathways, some of which lead to cell death, whereas others mediate cell survival. In this context, we observed that PDT mediated by methyl-5-aminolevulinic acid as the photosensitizer resulted in over-expression of survivin, a member of the inhibitor of apoptosis (IAP) family that correlates inversely with patient prognosis. The role of survivin in resistance to anti-cancer therapies has become an area of intensive investigation. In this study, we demonstrated a specific role for survivin in modulating PDT-mediated apoptotic response. Silencing survivin expression increased apoptotic indexes and cytotoxicity exhibited by PDT on metastatic breast human cancer cells. In contrast, the overexpression of survivin increased cell viability and reduced cell death[33]. Regarding another antiapoptotic protein, Bcl-2 expression was suppressed by genistein and the PDT with hypericin might be a benefit from mutual therapeutic combination favoring apoptosis. Presented experiment considers combination of genistein and PDT with a view to achieve higher therapeutic outcome in human breast adenocarcinoma cell lines previously identified as PDT resistant[34].

**PHOTODYNAMIC THERAPY AND MULTIDRUG RESISTANCE INHIBITORS**

One of the principal requirements of successful PDT is enough intracellular accumulationof the photosensitizer drug. Mechanisms of anticancer drug elimination (or multidrug resistance, MDR) by tumour cells are mostly linked to the elevated expression and activity of drug efflux transporters that constitute a dominant impediment to curative cancer chemotherapy. Hence, novel strategies that overcome MDR modalities are considered a major goal of cancer research. The ATP-binding cassette protein ABCG2 (breast cancer resistance protein) effluxes some of the PSsused in PDT, thus, it has been associated with photodynamic resistance. It was reported *in vitro* and *in vivo* experiments, that the tyrosine kinase inhibitor (Imatinibmesylate) that blocked ABCG2 function increased intracellular PS levels and may enhance efficacy and selectivity of clinical PDT on breast cancer[35]. The tyrosine kinase inhibitor enhanced the efficacy ofphotodynamic therapy by inhibiting ABCG2[35].

Additionally, ABCG2 is a putative cancer stem cell (CSC) marker. CSCs, also known as tumor-initiating cells, are a small group of cancer cells involved in drug resistance, metastasis, relapse of cancers and tumor drug resistance[36]. Hence, it is of importance to develop PSs that are not subtrates of ABCG2 or to design strategies to avoid ABCG2-mediated antitumor therapies resistance[37].

Recently it was identified a mechanism that target and kill cancer cells with MDR fenotipe. The MDR mediates extracellular vesicles (EVs) rich in ABCG2 in attached breast cancer cells that highly concentrate chemotherapeutics, thereby sequestering them away from their intracellular targets. The authors showed that the EVs membrane damage that accumulated photosensitive cytotoxic drugs such as imidazoacridinones (IAs) and topotecanresulted in tumor cell lysis. Furthermore, IAs accumulated in lysosomes efficiently killed MDR cells byorganellerupture upon photosensitization. Therefore, a synergistic cytotoxic effect resulting in MDR reversal is elicited by combining targeted lysis of IAs-loaded EVs and lysosomes. In contrast, a selectivephotocytotoxic effect exerted by topotecan is achievedbyaccumulation into EVs of MDR cells. Thus, MDR modalities can be converted to a pharmacological lethal Trojan horse to selectively eradicate MDR cancer cells by ABCG2-dependent drug sequestration within EVs[38].

**PHOTODYNAMIC THERAPY AND NITRIC OXIDE SCAVENGERS**

As result of photodynamic intervention, oxygen reactive species are generated which can destroy tumor cells. Nitric oxide (NO) produced by photosensitized cells could be pro-carcinogenic by inhibiting apoptosis. It was shown that NO from chemical donor (SPNO) or activated macrophages maked breast tumor cells more resistant to photokilling sensitized by 5-aminolevulinic acid (ALA)-generated protoporphyrin IX (PpIX) by providing substantial protection against apoptosis[39,40]. Additionally, it was demostrated that PDT-treated breast cancer cells adquired the ability to upregulate inducible-nitric oxide synthase (iNOS) expression[41]. In this sense, apoptotic cell killing was strongly enhanced by an iNOS inhibitor or knockdown and a NO scavenger[42]. These finding strongly indicated that stress-elicited NO in PDT-treated breast tumors could compromise therapeutic efficacy and suggest NOS-based pharmacologic interventions for preventing this.

**PHOTODYNAMIC THERAPY AND BREAST TUMOR MICROENVIRONMENT INTERVENTION**

The tumor microenvironment (TME) of solid neoplasias is very different from those of normal tissues. TME, a well-defined ecosystem, comprises parenchymal (tumor) and stromal (non-tumor) populations that coexisted andestablished interspecific interactions. The cooperation between the mentioned population contribute to malignancy [43]. The implementation of interstitial and estimation of PDT dosimetryrelies on the complexity of solid tumor. Moreover, should be studied if stromal cellsaffected by photodynamic regimes extinguish the tumor ecosystem by extinguish the network that they establish with tumor cells. In this sense, we have recently reviewed the term “Ecological Photodynamic Therapy” to emphasize the need to modulate the regimens of application of PDT to take advantage of the effect it can have on interspecific relationships and thus achieve complete tumor eradication[43].

Endothelial cells (ECs) are the stromal population whose principal function is to supply TME with oxygen, hormones, nutrients, circulating cells and other fluids. The growth of blood vessels by pre-existing ones is called “angiogenesis”[44]. It has been observed a mutualistic interaction between ECs and tumor cells within TME: ECs provide nutrients and tumors cells develop a paracrine stimulation to finally sustain the angiogenic endothelial process[43]. Therefore, researchers have developed strategies to target the vasculature sorrounding breast tumor cells. It was synthetized a novel and effective ligand-targeted PDT for breast cancer by conjugating factor VII (fVII), a natural ligand with high affinity and specificity for tisular factor (TF), with the PSs veterporfin[28] or chlorin e6[29] (Figure 1). The reason for targeting TF is based on its over-expression in breast cancer and its selective expression in pathological neovascular ECs in cancer but not on normal tissues. fVII-targeted PDT improves the selectivity and efficacy of free PDT for the treatment of breast cancer and induces apoptosis and necrosis as the underlining mechanisms of action. Moreover, fVII-targeted PDT was effective and safe for treatment of chemoresistant breast tumors in vivo, presumably by simultaneously targeting both the tumour neovasculature and chemoresistant cancer cells[28,29]. Strategies to favor the vascular effect of PDT by targeting tumor vasculature are constantly evaluated. Recently it was developed a PSs conjugated to a peptidase-resistent-peptide targeting neuropilins (NRPs) over-expressed specially in tumor angiogenic vessels. Peptides-conjugated PSs allowed a selective accumulation into vascular cells and no degradation was observed in plasma *in vivo* after intravenous injection[45] (Figure 1). This finding provided useful information for the future design of stable targeted-molecules to improve the outcome of PDT-treated patients.

Treatment with PDT alone is often non-curative due to tumor-induced immune cell dysfunction and immune suppression. This phenomenon has motivated a new approach by combining immunostimulants with PDT to enhance anti-tumor immunity. Thus, it was combinated the application of PDT mediated by verteporfin with an immunomodulation approach using CpG oligodeoxynucleotide for the treatment of metastatic breast cancer in in vivo. CpG primed immature dendritic cells (DC) *via* toll like receptor 9 to phagocytose PDT killed tumor cells leading to DC maturation and activation. Peritumoral injection of CpG after PDT in mice gave improved local tumor control and a survival advantage compared to either treatment alone (Figure 1). In conclusion, CpG may be a valuable dendritic cell targeted immunoadjuvant to combine with PDT[46].

Regarding TME, not only cellular or biotic factors modulate the photodynamic response, also the abiotic components have strong influence on PDT outcome. In this sense, the effect of chronic hypoglycaemia on sensitivity to aminolaevulinic acid-induced PDT in vitro was studied in human breast cancer cells. It was shown that photodynamic therapy sensitivity was reduced in glucose deprived cells[47] (Figure 1). Additionally, tumors, due to a abnormal vasculature, are characterized by a more acidic environment compared to their surrounding normal tissues. The low pH can enhance the lipophilicity of several PSs, such as hematoporphyrin IX (HpIX)[48]. It has been shown that increasing the lipophilicity of a drug leads to increased tumoruptake[48] (Figure 1). As a result, within the breast TME, it’s possible to find a concentration gradient of the drug between the tumor tissue and the normal surrounding tissue. By injecting glucose it is possible to further reduce the extracellular pH value of tumors selectively[49], and to make tumor cells more sensible to PDT treatment[47]. This will in turn increase the pH gradient between tumor and normal tissue and finally result in an increased concentration gradient between these tissues for drugs that becomes more lipophilic at low pH values. If the low tumor pH turns out to be a general explanation for the selective localization of such drugs, the clinical outcome of PDT can be improved by combining the PDT therapy with glucose injections. It is therefore neccesary to characterize the biotic and abiotic components and its interactions in order to achieve the disruption of ecological networks which finally can lead to the destruction of the ecosystem.

**CONCLUSION**

Despite major advances, in terms of knowledge and treatment of breast cancer, this illness is still consider a huge problem in terms of morbility and mortality. It is expected that the involvement of the pharmaceutical industry and research institutes will continue to launch numerous clinical trials to evaluate applications of PDT in conjunction with or as a replacement for traditional methods for treating solid tumors.

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**Table 1 Receptor-targeted photodynamic therapy on breast cancer cells**

|  |  |  |  |
| --- | --- | --- | --- |
| **Targeted-receptor** | **Photosensitizer** | **Result** | **Ref.** |
| Estradiol receptor | Tetraphenylporphyrin | High-affinity conjugate-protein binding | James *et al*[19] |
| Estradiol receptor | Tetraphenylporphyrin | High-affinity conjugate-cell binding | Swamy *et al*[20] |
| Estradiol receptor | Pyropheophorbide a | Conjugateselective-celldeath | Fernandez Gacio *et al*[21] |
| Estradiol receptor | Pheophorbide a | Conjugateselective-celldeath | El-Akra *et al*[22] |
| Estradiol receptor | Pyropheophorbide | High conjugate-internalization | Sadler *et al*[23] |
| Estradiol receptor | Chlorin e6-dimethyl ester | Conjugateselective-celldeath | Swamy *et al*[24] |
| Human epidermal growth factor receptor  | Verteporfin and pyropheophorbide-a | Conjugate selective but less phototoxic  | Savellano *et al*[25] |
| Human epidermal growth factor receptor  | Verteporfin and pyropheophorbide-a | Conjugateselective-celldeath | Bhatti *et al*[26] |
| Human epidermal growth factor receptor | Zinc phthalocyanine (plus nanoparticules) | Conjugateselective-celldeath | Stuchinskaya *et al*[27] |
| Human epidermal growth factor receptor  | Sn-(IV)chlorin e6 monoethylenediamine | Conjugateselective-celldeath | Gijsens *et al*[32] |
| Tisular factor (factor VII receptor) | Verteporfin | Conjugateselective-celldeath | Hu *et al*[28] |
| Tisular factor (factor VII receptor) | Chlorine e6 | Conjugateselective-celldeath | Duanmu *et al*[29] |
| Gonadotropin-releasing hormone receptor | Zinc phthalocyanine | Conjugateselective-celldeath | Xu *et al*[30] |
| Gonadotropin-releasing hormone receptor | Protoporphyrin IX | Conjugateselective-celldeath | Rahimipour *et al*[31] |



**Figure 1 Photodynamic therapy combined with tumor microenvironment intervention on breast cancer.** Photodynamic therapy is one of the alternative method of breast cancers treatment. PDT involves: (1) administration of a PS which can be internalizated into tumor cells or into sorrounding vasculature, regarding PS characteristics; (2) local irradiation at a wavelength corresponding to the absorbance peak of the PS; and then (3) light activation of the PS promotes cell death, mainly by apoptosis.Targeting tumor vasculature has several benefits in order to improve PDT outcome. Thus, researchers have developed strategies to target the vasculature sorrounding breast tumor cells by conjugating the PS with endothelial specific-ligands.Immunoactivationof dendritic cells using CpG increased phagocytose of PDT killed tumor cells leading to DC maturation and activation, thereby promoting antitumor immune response. Regarding abiotic environmental factor, it was shown that photodynamic therapy sensitivity was reduced in glucose deprived cells, and that lower extracellular pH led to increased PS uptake, reinforcing photodynamic response.

PS: Photosensitizer; DC: Dendritic cells; ECs: Endothelial cells; PDT: Photodynamic therapy.