

Review

Photodynamic Therapy of Breast Cancer in Animal Models and Their Potential Use in Clinical Trials—Role of the Photosensitizers: A Review

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Abstract

In this article, we reviewed the use of photodynamic therapy (PDT) for breast cancer (BC) in animal models. These *in vivo* models imitate the cancer disease progression, aid diagnosis, as well as create opportunities to assess treatment during the approval process for the new drug. BC ranks first among women's cancers. Nowadays, there are many diagnostic methods and therapy options for BC but the majority of them have severe side effects. This article discusses the advantages and some disadvantages of the use of small and large animals used for BC models. A literature review showed that the majority of studies have used large animal models, and recently there has been more interest in developing BC in small animal models. BC cell lines such as MCF-7, BT-474, MDA-MB-231, and 4T1 are commercially available for two-dimensional and three-dimensional *in vitro* cell cultures and subcutaneous models. The purpose of this article is to discuss the performance of PDT in animal models and its further clinical implications. PDT is known to be a non-invasive therapy, which uses monochromatic light and energy to excite photosensitizers (PSs) for the generation of reactive oxygen species as the required factors. Herein, we discuss the use of five photosensitizers in BC models such as chlorin e6 (Ce6), methylene blue, indocyanine green, 5-aminolevulinic acid, and meta-tetra(hydroxyphenyl)chlorin. The database PubMed and Scopus were searched for keywords: 'photodynamic therapy', 'breast cancer', 'animal model', 'clinical studies', and 'photosensitizer(s)'. The PDT search results in animal experiments and its effect on a living organism indicate the possibility of its application in clinical trials on women with local and disseminated BC. The availability and accessibility of small and large BC animal models enable the progress and trial of cancer drugs for innovative technologies and new diagnostics and treatments.

Keywords: photodynamic therapy; photosensitizer; breast cancer; animal model; *in vivo*; clinical studies

1. Introduction

1.1 Breast Cancer and Therapeutic Methods

Cancer is the main problem of 21st-century medicine. Animal research is the basis for the development of new forms of cancer treatment. The appropriate animal model is selected based on the type of cancer, the purpose of the study, the cost, and the time required to develop the model. Moreover, the similarity to human diseases in terms of the appearance of metastases, stages of the disease, and processes in the immune system should be taken into account [1]. Among the large number of animal models used in breast cancer (BC) research, rats and mice are the most common, due to their genetic and physiological homology with humans (about 98% genetic similarity), and therefore, widely used in research on nanoparticles (NPs). The results obtained in these murine models are highly reproducible [2–4].

BC tumors are determined by the timing of screening, correct diagnosis, and medical intervention [5]. BC is the most common cancer and has the highest mortality rate in

the Eastern and Western world. Breast cancer ranks first among diagnosed tumors. In the USA, the incidence of BC increased from 0.102% in 1980 to 0.142% in 1999, and a ~0.131% decrease from 2011 to 2017 [6,7]. An extensive group of women where BC ranks first is Chinese women, accounting for approximately 2% of newly diagnosed BCs and are the cause of nearly every 10 deaths [8]. In India, the incidence of BC has surpassed cervical cancer, causing the most deaths to date, and is the leading cause of cancer mortality [9]. In Europe, one in three cancers diagnosed in women is BC [8]. In total, BC accounts for more cancer deaths among American women than even lung cancer [6]. For instance, the incidence of BC in the USA was 12.9% in 2017 [6,7]. Breast cancer is the principal cause of female cancer in Europe and higher socioeconomic status has been linked to higher occurrence [10].

Yedjou *et al.* [11] analyzed the effect of racial differences in the American population on BC and inferred that younger Black women have more invasive BC, and is characterized by a higher mortality rate than in White



women, which might be related to barriers to early health care access, detection or screening, and different way of treatments. Furthermore, different gene pools have a strong influence.

The World Health Organization (WHO) histologically distinguishes 19 subtypes of breast cancer [12], among them ductal carcinoma (70–75% cases) and lobular carcinoma (10–14% cases) are the most common [12].

The *BRCA1* gene was cloned in 1994 by Miki and his team [13]. This gene is located on the long arm of chromosome 17 (17q21). It is a large gene comprising 80 kb of DNA [14] and encoding a protein of 1,863 amino acids. *BRCA1* is a suppressor gene that controls the cell cycle through mechanisms such as apoptosis and DNA repair. Its role is to maintain the stability of the genome [15]. *BRCA1* is a transcription activator, an element of the DNA double-strand break repair system, and participates in chromatin remodeling in the SWI/SNF complex [16,17]. In large animal models, correlations between genes and phenotypes may be easier to explain. In addition, human medical equipment such as magnetic resonance (MR), computed tomography (CT), endoscopes, bronchoscopes, and many surgical instruments can be successfully used.

There is no doubt that the years 2020 and 2021 passed under the pandemic COVID-19. Since the beginning of the pandemic, the number of cancer diagnoses, including BC, has decreased, which does not imply that the incidence has also decreased. Furthermore, diagnostic breast imaging, surgical consultation, genetic research, and treatments including radiation therapy, chemotherapy, and surgery have also reduced during the pandemic. Yin *et al.* [18] noted a decline in the average weekly imaging of 94.6%–61.7%, surgery of 20.5%, surgical consultation of 11.5%, and genetics consultations dropped to 39.9% in the period from February to April 2020. The reason may be due to the limited access to medical services but moreover, Vanni *et al.* [19] proved that fear of COVID-19 infection delayed further procedures upon BC appearance. All medical data show an increase in the percentage of newly detected, untreated, and metastatic BC over the next few years [20].

The most common treatment for breast cancer is the surgical removal of the tumor. During the procedure, a margin of non-cancerous tissue (the so-called healthy area) is also removed to minimize the risk of recurrence and metastasis. Although the biopsy procedure is well tolerated by patients, it may cause bleeding or damage to organs in the vicinity of the punctured organ. Another method of treatment is radiotherapy, and one commonly used radiotherapy is brachytherapy. Brachytherapy is a procedure that includes placing radioactive sources near the tumor or in the area where the tumor was surgically removed. Despite its high efficiency, it requires a large dose of radiation. A relatively new and emerging therapeutic method is photodynamic therapy.

1.2 Fundamental Photodynamic Therapy Mechanism

Photodynamic Therapy (PDT) appears to be a promising alternative in the localized treatment of breast cancer [21,22]. PDT is a minimally invasive way of treating gastrointestinal cancers and precancerous lesions. Since it is known that photosensitizers (PSs) accumulate selectively in BC, PDT may be able to selectively destroy neoplastic tissue foci, while maintaining surrounding healthy tissues surrounding [23]. PDT can be used in the case of multifocal lesions and repeated without the risk of cumulative toxic effects. Moreover, the therapy is well tolerated by patients and delivers an excellent cosmetic effect in the case of skin lesions [24]. The antitumor activity of PDT includes its effects on the immune and inflammatory response of the body, the direct cytotoxic mechanism, and the indirect mechanism of the occlusion of blood and lymph vessels [25].

PDT is achieved by a photodynamic reaction that is induced by the excitation of photosensitizer exposed to light [26–29]. PDT uses light with low and medium energy values [29,30] and commercially available PSs. The minimum amount of light can be delivered to a tissue depth of 1 cm [31]. PDT action can take place in two ways. In a type I reaction, the excited PS reacts with biomolecules such as lipids, proteins, and amino acids to form superoxide radicals by electron transfer. In a type II reaction, the excited PS gives singlet oxygen ($^1\text{O}_2$) by direct energy transfer to molecular oxygen. Type I and II reactions can occur simultaneously, but type II is predominant during PDT. The reactive oxygen species (ROS) affect all intracellular components, including proteins and DNA, and can destroy the neoplastic cell by necrosis and apoptosis [32,33]. The cell's response to PDT depends on PS concentration, energy dose, wavelength of laser light exposure, and internal factors including cell metabolism, cell cycle phase, amount of cellular adenosine triphosphate, and genetic makeup of the cell [34]. Fig. 1 shows the mechanism of singlet oxygen and ROS generation by photodynamic therapy and their functions.

Delivery of PS to the tumor occurs either actively by targeting molecules for the uptake and delivery in specific tumors, or passively, by enhancing the permeability of cell membranes [35]. Reactivity with ground-state molecular oxygen ($^3\text{O}_2$) results in the formation of singlet oxygen ($^1\text{O}_2$). Mechanisms that produce singlet oxygen are important to photodynamic therapeutics, anti-cancer agents, and other skin treatments.

2. Materials and Methods

Review articles, research articles, and short essays from the PubMed and Scopus databases were analyzed from their first appearance in the literature until 2022. The selection of articles includes animal models and clinical trials on the use of photodynamic therapy (PDT) in breast cancer (BC). The databases were searched for the keywords

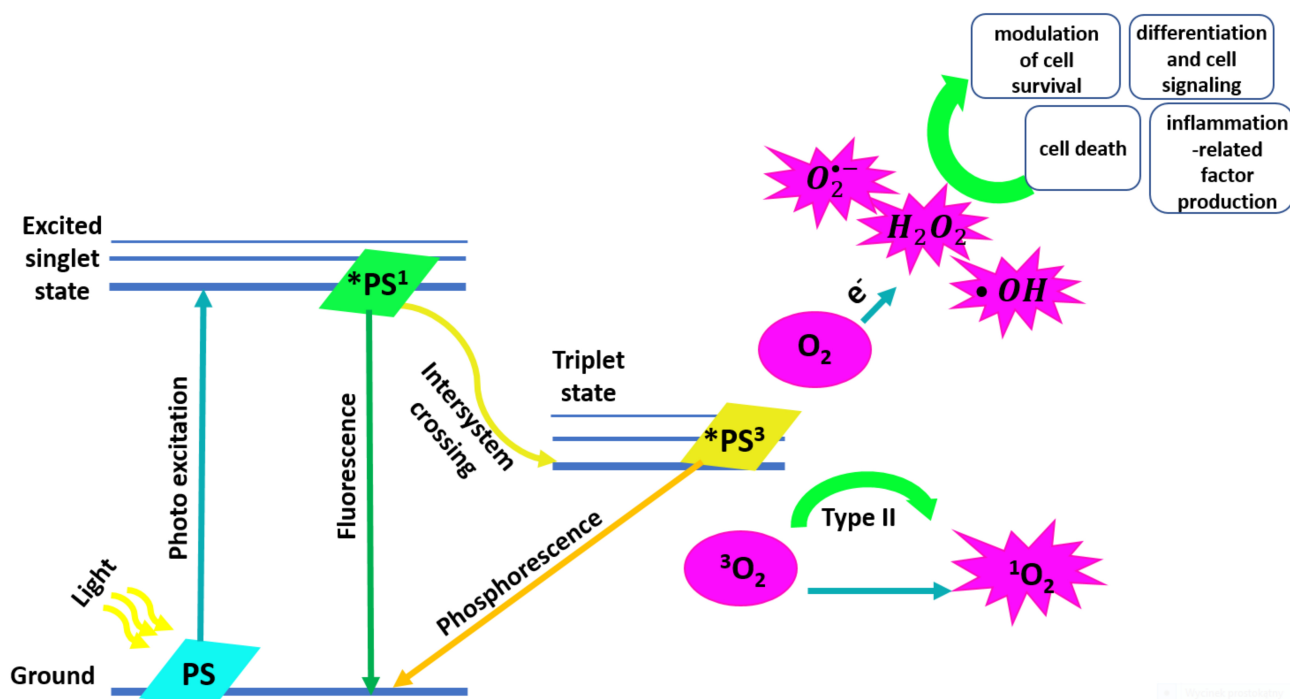


Fig. 1. Mechanism of reactive oxygen species (ROS) generation by photodynamic therapy and their influence on the cell death and differentiation, cell signaling and inflammation.

“photodynamic therapy”, “breast cancer”, “animal model”, “clinical trials” and “photosensitizer(s)”. This systematic review is based on The Cochrane Collaborative Recommendations and Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA). The articles were selected based on their relevance to the topic. The basic criterion was free-access articles and articles written in English or Polish (Fig. 2). The article aims to review the use of PDT in the treatment of BC on an animal model and the results of clinical trials available in the literature, which is a consistent continuation of the manuscript presented by us at the level of *in vitro* studies.

3. The Position of the Photosensitizer in the Treatment of Breast Cancer—An Animal Model

In this review, we consider existing models employed for the pre-clinical use of nanomedicines for BC treatment—taking in both *in vitro* two-dimensional (2D) [36] and three-dimensional (3D) [37], and animal models. The advantage of nanomedicine is the increase in the concentration of the drug in the tumor tissue, while limiting the amount of the drug in non-cancerous cells [38]. Table 1 (Ref. [39–68]) presents PSs excited by light of the appropriate wavelength that does not damage healthy tissue. Generated $^1\text{O}_2$ reacts with cellular components and causes cell damage and ultimately death of cancer cells.

Technological progress in using light in treatment has contributed to the development of innovative techniques

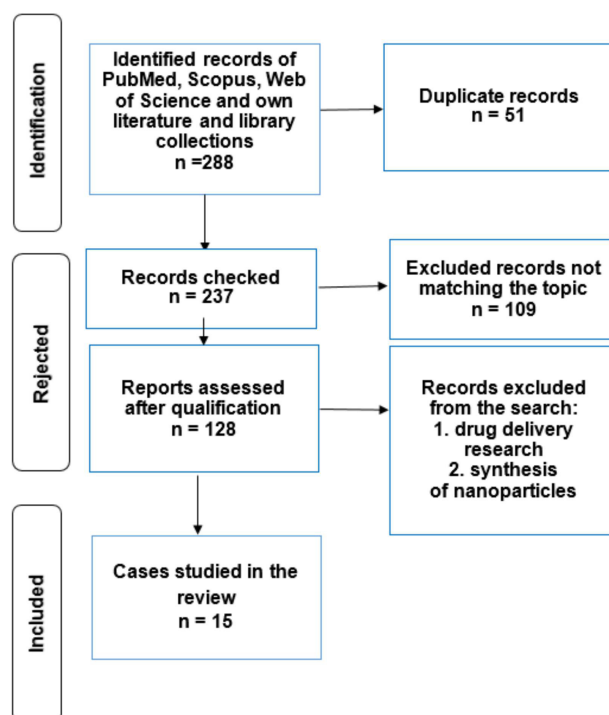


Fig. 2. Preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement.

in medical sciences using spectroscopic methods [69,70]. Chemiluminescence is an analytical method that tracks drugs in the body. It allows the diagnosis and understand-

Table 1. The use of PSs in animal model photodynamic therapy (PDT).

References	Photosensitizer(s)	Wavelength [nm]	Fluence [mW/cm ²]
[39,40]	Chlorine e6	660	100
[40]	Chlorine e6	635	100
[41]	Chlorine e6 + NPs	600	-
[42]	Chlorine e6 + NPs	638	300
[43]	Photodithazine®	-	100
[44]	Chlorine e6	652	-
[45]	Methylene Blue + NPs	660	-
		808	-
[46]	Curcumin + NPs	450	-
[47]	5-aminolevulinic acid	633	105
[48]	5-aminolevulinic acid + NPs	630	-
[49]	Hexaminolevulinate	450–490	-
		510–540	-
[50]	5-aminolevulinic acid	633	250
[51]	Indocyanine green	808	140
[52]	Indocyanine green + NPs	NIR	-
[53]	Phthalocyanine	690	-
[54]	Ryboflavin	975	-
[55]	5-aminolevulinic acid	400–700	100
[56,57]	Benzoporphyrin	690	150
[58]	Benzoporphyrin	690	150
[59]	Verteporphyrin	690	100
[60]	Chlorine e6 + NPs	655	2
[61]	2-(1-Hexyloxyethyl)-2-devinyl pyropheophorbide-	658	90
[62]	Benzoporphyrin	690	-
[63]	Photofrin	630	-
[64]	Pulitin	660	150
[65]	Porforin sodium	630	-
[66]	Photofrin	630 nm	-
[67]	Mono-L-aspartyl chlorin e6	664	-
[68]	Mmeta-tetra(hydroxyphenyl)chlorin	652	-

NP, nanoparticle; NIR, Near-Infrared Light.

ing of cellular processes in real-time. Chemiluminescence is the emission of light methods resulting from a chemical reaction. For instance, a luminol derivative coupled with boron-dipyrrromethene (BODIPY) can be used to obtain a glow in the form of green light.

Luminescence consists of the emission of electromagnetic radiation with an intensity greater than the intensity of thermal radiation at a given temperature and with a finite duration of lighting that does not disappear immediately after the excitation is interrupted [71]. Luminescence includes photoluminescence—caused by electromagnetic radiation, e.g., by a laser or employing a halogen, xenon, deuterium, or other lamps; electroluminescence—caused by an electric field, e.g., in the flow of electrons in a p-n junction; chemiluminescence—glow occurs as a result of excitation by chemical reactions [72]; bioluminescence—caused by biological processes; radioluminescence—caused by ionizing radiation; X-ray luminescence—glow occurs as a result of X-ray excitation; triboluminescence—occurs

as a result of excitation by friction and electrostatic forces; sonoluminescence—caused by ultrasonic radiation; cathodoluminescence—excitation by electron stream; electrochemiluminescence—caused by chemical processes and electric field and thermoluminescence—caused by increasing the temperature.

Breast cancer (BC) animal models are classified by implantation site into three classes: generation of primary tumors [73], generation of experimental metastasis [74], and classification by host immunological state [75]. BC also is classified by the origin of implanted cells in syngeneic mouse models [76], cell-derived xenografts [77], and patient-derived xenografts [78]. Moreover, spontaneous murine breast cancer models are also known as genetically engineered mouse models [79] and carcinogen-induced models [80].

4. A Review of Photodynamic Therapy Treatment of Breast Cancer in Animal Models

The popularity of PDT testing is related to the design of animal models of disease. Many efforts have been made to study PDT in animal models. The use of animals for PDT purposes is important in medical research. The remarkable anatomical and physiological similarities between humans and animals, especially mammals, have prompted researchers to investigate a wide range of mechanisms and evaluate new PDT treatments in animal models. However, not all results obtained in animals can be directly translated to humans. There exist fundamental differences between the BC model in large and small animals in the parameters affecting the absorption, distribution, metabolism, and excretion of the drug. The main difference between large and small animals is the mass, of approximately 50 kg on average, which affects the accuracy of the results and pharmacokinetic differences.

4.1 Xenograft Models (MCF-7, BT474, MDA-MB-231)

Breast tumor xenografts are performed in immunodeficient mice.

Most BC cell lines have been recovered from metastatic lesions or isolated from pleural effusion. The reason for this phenomenon is the difficulty of obtaining lines from solid tumors. Less-invasive BC cell lines are underrepresented [81]. Cell-derived xenografts enable the analysis of nanomedicines. Widely used cell lines in pre-clinical studies are MCF-7 (luminal A) and MDA-MB-231 (triple negative breast cancer (TNBC)). To minimize the risk of graft-versus-host disease (GvH), immunocompromised animals are used to implant human BC cells. Unfortunately, genetic mutations, differences in telomere dynamics and regulation, and differences in ligand-receptor interactions result in imperfect animal models [82–84]. Mouse BC are significantly less hormone-dependent, due to the lower number of cellular estrogen/progesterone receptors (ER/PR) receptors [85]. The types and grades used to classify female BC are not the same as murine BC owing to the difference in the detailed morphological structure of both tumors [86,87]. The respiration rate is one of the most significant differences between humans and mice, in which smaller animals consume more oxygen per cell. Notably, this factor leads to the induction of hypoxia-sensitive genes and changes the kinetics of its growth in large and hypoxic tumors [88].

Mark Greens proved that monoclonal antibodies inhibit ErbB-2 receptor expression in breast tumors *in vivo* [89]. New technologies are an opportunity to improve models of BC carcinogenesis [90]. Animal models should be characterized by high reproducibility and represent the most suitable model of the human tumor [91,92]. Considering the above shortcomings of existing animal models and the fact that their tumor genetics do not perfectly match, it is es-

sential to monitor tumor growth. Therefore an experimental system providing controlled conditions and allowing reproducible experimental set-up, as well as the quantification of processes is required. Molecular differences between tumors are more visible in 3D models than in 2D models, in which prototypes of cell cultures such as cell co-cultures, advanced 3D cell cultures, and patient-derived cells can be analyzed.

The general maintenance of cells to design animal models of BC is described here [91–93]. Briefly, BC cells line MCF-7, BT474, or MDA-MB-453 were packed in a sterile tissue transport medium at the time of surgical resection. The tumors were dissected under aseptic conditions using scalpels. The samples were formed into small pieces up to 2 mm², and washed with saline with PBS, followed by incubation in a glass flask with the medium. The cells were washed with PBS and then counted manually in the hemocytometer chamber. The cell suspension was placed in a plastic T-flask at a density of $0.5\text{--}1 \times 10^6/\text{mL}$ and incubated in the medium with antibiotics. After overnight incubation, the supernatant was removed. The adherent cells were used to generate tumor cell lines for the respective samples and cultured in the tumor medium. The 3D cell cultures are diverse culture systems, and the majority of them share key features that are crucial for the development of nanomedicine [94].

Cells were centrifuged and cultured in medium with antibiotics (density $1\text{--}2 \times 10^6/\text{mL}$) until a density of $5\text{--}6 \times 10^6/\text{mL}$ was reached. Combinations of cells in the medium were possible owing to the constant circulation of the pump in the capillaries. The condition of the medium was monitored daily (replaced at a concentration of 1000 U lactate/mL). The remaining cells not used to inoculate the bioreactor can be inoculated into a new tissue culture bag of a density $1 \times 10^6/\text{mL}$. The 3D cell cultures in the field of nanomedicine give a lot of important *in vitro* information on apoptosis, cell migration or other mechanisms. This allows a more accurate understanding of some mechanisms of pharmacotherapy [95], specifically of its effect on cell morphology (e.g., shape, size, and cell density) or systemic integrity. In addition, 3D cultures provide a broader picture of the distribution of nanodrugs in the tumor and individual cells, considering the physicochemical characteristics of NPs such as shape, deformability, Z potential and their influence on 3D culture [96,97].

4.2 3D Model Mice

Nowadays, research using animals arouses extreme emotions. However, it is necessary to thoroughly understand the safety, stability, and direction of biochemical processes of anti-cancer drugs in animal models, which possess similar prototypes to the human body regarding pharmacodynamics/pharmacokinetics and distribution of the substance. The use of new technology can also advance drug discovery and the development of individualized treatment strategies.

Duanmu *et al.* [98] analyzed chlorin e6 (Ce6) (Fig. 3) treatment of chemoresistant BC xenografts in nude mice. Tumors were irradiated with a 635 nm fiber-coupled diode laser (100 mW/cm^2). The tumor growth was inhibited with no apparent damage to healthy cells as observed in histological examinations [98].

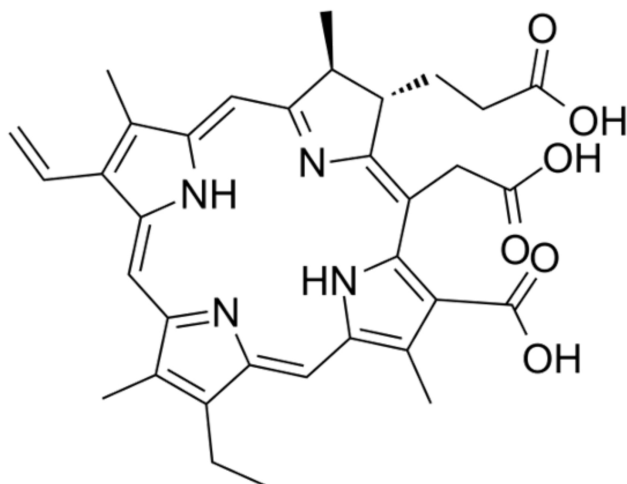


Fig. 3. Chlorin e6 (ce6).

Li *et al.* [99] have used DOX and Ce6 as the model chemotherapeutic drug and PS, respectively, which were encapsulated. The *in vitro* and *in vivo* study used a connection of chemotherapy Doxorubicin (DOX), photothermal therapy PTT (NIR), and photodynamic therapy (PDT) (Ce6) with a positive anti-cancer effect and no-side effect [98,99]. Yu *et al.* [100] created laminarin-based nanoplateforms (HLDM) to deliver PS (Pp IX) in BC, and performed *in vivo* and *in vitro* research. A wavelength laser was used as the light source. DNA destruction, nuclear lysis, and subsequent death of MCF-7 BC cells were observed *in vitro*. Micelles showed greater phototoxicity under light conditions than in its light. *In vivo* study showed that Pp IX-loaded HLDM micelles could effectually deliver PS into tumor cells and produce ROS-mediated, subsequently reducing tumor volume and overcoming instability of Pp IX [100].

Mesoporous silica NPs (MSNs) have facilitated the potential for drug delivery system (DDS)⁺ due to several characteristics. NPs that are similar to red blood cells (RBC) were created for transporting DOX to BC by using Ce6 in PDT. To evaluate the PDT effect on DOX *in vivo*, tumors were treated by the 655 nm laser at 2 W/cm^2 in a 4T1 mouse BC metastatic model. The use of light in PDT resulted in a $10 \times$ increase in DOX release with tumor and metastatic inhibition effect [101]. NPs have higher solubility, bioavailability, selectivity, and lower cellular toxicity and drug resistance, positively impacting pharmacokinetics and systemic biodistribution [102].

The collagen in the tumor also reduces the effectiveness of the treatment. Tang *et al.* [101] observed that after initial treatment with losartan, *in vivo* collagen (type I) level was reduced by 53% compared to placebo. Further, the combination of losartan with the Ce6-PMO nanoplateforms showed therapeutic efficacy and an increase in the suppression rate of tumor volume to up to 82% [103]. To enhance photothermal therapy, polydopamine (PDA) was synthesized with TiO₂ nanoparticles. Then, synergistic phototherapy nanoprobe were constructed by coupling Ce6 with Mn^{2+} for simultaneous PDT/PTT [104]. Chemical conjugation of chitosan (CS), octadecanoic acid (OA) and gadopentetic acid (GA) with Ce6 led to the creation of complex Gd-CS-OA/Ce6. The complex showed strong tumor ablation *in vivo* in 4T1 tumors in mice [105]. Kim *et al.* [104] synthesized a conjugate composed of the antibody Trastuzumab and Ce6 for the treatment of human epidermal growth factor receptor 2 (HER2). Trastuzumab-chlorin e6 conjugate obtained a high $^1\text{O}_2$ concentration under laser irradiation. In addition, *in vivo* studies in mice with HER-2 have shown an increased penetration of Trastuzumab-chlorin e6 conjugate into the tumor than the antibody itself [104]. Walt *et al.* [45] considered cytokeratin 18 as an early and stable marker indicating a response to PDT treatment. In the initial stage of apoptosis, caspase breaks down cytokeratin 18. Thus, M30 antibodies against the neoepitope of cytokeratin were used, and a decrease in S-phase fraction (SPF) 2 hours after PDT (40 J/cm^2 of 652 nm laser light) and recovery of SPF within 96 hours were observed.

Accordingly, the indicator M30/SPF may be useful in monitoring tumors after PDT [37]. Kim *et al.* [106] produced cancer-targeting peptide p 18-4/Ce6-conjugated polyhedral oligomeric silsesquioxane (PPC) NPs for improving the targeting ability of Ce6 to BC cells, thereby enhancing PDT efficacy. *In vitro* and *in vivo* studies confirmed their accumulation in the tumor and inhibition of BC growth [106]. Rollakanti *et al.* [51] proved that Vitamin D boosted the performance of PDT in a small animal model of BC. Mice were injected subcutaneously into MDA-MB-231 BC cells. Tumors were preconditioned with calcitriol, $1 \mu\text{g/kg}$ i.p (low and safe dose) once for 3 days. PS used 5-aminolevulinic acid (ALA) and the tumor received 250 J/cm^2 of 633 nm light. Analysis results indicated a 4.0 ± 0.7 -fold increase in the number of apoptotic cells following ALA-PDT, compared to a 36.8 ± 7.4 -fold increase in the number of apoptotic cells after Vit.D/ALA-PDT treatment [50].

Many studies have shown that nanographene oxide conjugated with different PS are effective PDT/PTT agents against different tumors. Dos Santos *et al.* [46] used methylene blue (MB) to create nanoplateforms to study their effect in combination with PDT/PTT on murine BC metastasis and primary BC (4T1-Luc). This experiment used an LED with a wavelength of 660 nm (fluence 90.8 J/cm^2) and a laser with a wavelength of 808 nm (fluence 8.3 kJ/cm^2).

The experimental results after observing animals for 30 days revealed that the combined therapy had a beneficial effect. Explicitly, the therapy resulted in the complete ablation of the tumor, while separate therapy did not exhibit such an effect [45].

4.3 Syngeneic Models

Mouse tumor allografts (syngeneic mouse models) are particularly important due to the efficient immune system, undisturbed by the foreign gene pool derived from the tumor. They are particularly relevant for studies of immunotherapies.

Phuong *et al.* [42] increased the effect of Ce6 by combining it with albumin nanoparticles (NPs) created from bovine serum albumin (BSA). BC 4T1 cells from the murine model were irradiated using 660 nm laser light. The complex displayed a remarkably enhanced tumor suppression effect when irradiated by 660 nm light compared with free Ce6 (tumor volume 90 ± 39 versus 487 ± 69 mm³ respectively) [34]. Gao *et al.* [54] used prodrug to combat adaptive immune resistance for PDT in an animal model of BC (4T1) and colon cancer (CT26). Scientists rationally designed a tumor-microenvironment-sheddable prodrug vesicle by integrating a PEGylated PS and a reduction-sensitive prodrug of IDO-1 inhibitor. In opposition to PDT, the prodrug-vesicle-mediated combination immunotherapy provoked augmented antitumor immunity to eradicate the tumor [53].

The expression of tissue factor (TF) was detected in endothelial cells of pathological capillary blood vessels associated with solid tumors. A study has proved that fVII is a natural ligand for TF. Liang *et al.* [43] increased the antioxidant effect of NPs composed of gambogic acid-grafted hyaluronic acid (HA-GA) and Ce6. The treatment was carried out in mice with BC composed of 4T1 cells when the tumor was 155 mm³ in size. Mice included in the experiment were divided into six research groups by applying different concentrations of photosensitizers. The light source was a laser with a length of 638 nm and a power of 0.3 W/cm². HA-GA@Ce6 + laser group inhibited the tumor after 3 intravenous injections, a remarkable inhibition of tumor expansion that was not observed in the absence of laser irradiation. In the groups subjected to free GA, only a slight increase in ASPAT was observed, and nephrotoxic and hepatotoxic effects were absent with the HA-GA@Ce6 application [35]. Ashkbar *et al.* [47] loaded curcumin into Fe₃O₄-SiO₂ NPses and used it in BC mice Balb/c (4T1cells) model. CW diode lasers at 450 nm for PDT and 808 nm for PTT were used. The tumor volume of the nanocomposite (NC) + PDT + PTT group showed a 27% decrease compared to its initial amount in the absence of side effects [48]. Akens *et al.* [107] assessed the effectiveness of 5-ALA and benzoporphyrin-derivative monoacid ring A (BPD-MA) for the possible treatment of bone metastases. Vertebral metastases in a rat model were

generated by intracardiac injection of human BC cells. Afterward, animals were sacrificed at various times, and tissues were removed from lumbar vertebrae, kidneys, livers, and ovaries. Compared to BPD-MA, 5-ALA displayed a notably difference in the intensity of tumor vertebra/spinal cord uptake, suggesting its potential healing potential [107].

5-ALA has been also proposed as a sonosensitizer for sonodynamic therapy (SDT) with promising results on BC [108]. Female severe combined immunodeficiency (SCID) mice bearing MDA-MB-231 tumor xenografts were used for animal model experiments by Fahey and Girotti [48] to examine the use of iNOS/NO in resistance to PDT. Yu *et al.* [100] created nanocycles with Hematin-Laminarin-Dithiodipropionic Hematin-Laminarin-Dithiodipropionic Acid-MGK, named (HLDM) and Protoporphyrin IX (Pp IX). *In vivo* experiments revealed that the Protoporphyrin IX (Pp IX) -loaded HLDM micelles could induce a remarkable anti-tumor effect, simultaneously making laminarin a potential drug delivery system (DDS) [100]. Čunderlíková *et al.* [50] explored the applicability of hexyl-ester of ALA hexaminolevulinate (HAL) for PDT purging of BM grafts from BC cells in the murine 4T1 BC model. HAL has greater lipophilic properties, which makes it easier to pass through cell membranes than ALA. Mice with *ex vivo* bone marrow cleansing by HAL-PDT had been shown to possess a lower number of lung metastases and a longer survival time (the filter combination consists of a 450–490 nm band-pass excitation filter, a 510 nm beam splitter, and a 510–540 nm band-pass emission filter) [51]. Gao *et al.* [103] analyzed whether integrin $\alpha v \beta 6$ -targeted PDT of tumors using a phthalocyanine dye-labeled probe (DSAB-HK) was able to affect the immune system.

Mice with BC 4T1 were used and divided into 4 groups: control, DSAB-HK PDT, anti-PD-1, and DSAB-HK PDT + anti-PD-1 (70 J/cm², 690-nm laser), and mice in the anti-PD-1 groups were irritated by anti-PD-1 antibody. Mice were implanted with 4T1-fLuc cells to induce lung metastases and divided into groups as above. In both cases, researchers confirmed the effect of PDT by suppressing an immune checkpoint [104]. Xu *et al.* [109] developed conjugates by synthesizing zinc phthalocyanine (ZnPc) with GnRH analogs. Conjugates demonstrated significant antitumor efficacies in a BC *in vivo* [101]. In an experiment performed by Khaydukov *et al.* [55], SK-BR-3 cells were inoculated subcutaneously into ten immunodeficient Balb/c Nu/Nu mice. In order to extend the Riboflavin (Rf) photosensitization depth in cancer tissue to 6 mm in depth, scientists designed core/shell upconversion nanoparticles (UCNPs, NaYF₄:Yb³⁺:Tm³⁺/NaYF₄) capable to change 2% of the deeply-penetrating excitation at 975 nm to ultraviolet-blue power. This innovative combination inhibited the growth of BC *in vivo* [54].

Ahn *et al.* [110] researched the effects of Cisplatin (Cis) on PDT in BC using a mouse model. To activate

PS (Cis at the concentration of 3 mg/kg), a diode laser 660 nm/80 J/cm² was used. Mice were arranged into four groups: control, Cis, PDT, and Cis/PDT. On day 3rd, a higher rate of tumor growth was observed during the combination than a single treatment, which may have been caused by the greater inflammatory response following the PDT reaction; therefore it decreased on day 7th. The largest decrease in BC was observed in the combined group (77% and 52% on days 3rd and 7th, respectively). The results of the study suggest a positive effect of Cis/PDT combination therapy in the treatment of BC [110]. Huang *et al.* [111] conducted *in vitro* and *in vivo* experiments and showed a positive effect of purine-18 in mice with TNBC. The *in vivo* study conducted using a subcutaneous 4T1 BC animal model revealed a visible reduction in tumor size without any harmful effect on the body of mice after the administration of Pur-18 and PDT [111].

Ren *et al.* [112] designed polyethylene glycol (PEG) modified hematoporphyrin (HPP)-based NPs system to load DOX (HPPD) and achieved a synergistic effect of chemotherapy and PDT. HPPD/PDT in mice with BC led to significant tumor ablation with reduced cardiotoxicity. A group of scientists headed by Subramaniyan [113] considered that a local tumor would be suppressed by locally released patitaxel (PTX) + PDT and a distant tumor by systemic action. The rats (Fischer 344) with local large tumors (16 mm) and smaller distant tumors (4–6 mm) were injected with PTX prodrug (dose: 1 μ mole kg⁻¹, i.v.), and tumors were treated with illumination using a 690 nm laser (75 or 140 mW/cm² for 30 min, cylindrical light diffuser, drug-light interval 9 h). In the two-tumor model, the large tumor was eliminated, also curing the untreated tumor through adaptive immune activation. For about a year, no recurrence was observed [113].

Hypericin (HY) has the advantage of being active in the dark and has a high tendency to accumulate in primary and metastatic tumors. This feature was proved by Blank *et al.* [114] on mice with breast adenocarcinoma (DA3), squamous cell carcinoma (SQ2) and lung metastases. DA3 survival increased from 15.6% to 34.5%, SQ2 from 17.7% to 46.1% in SQ2 [114]. The concentration of MDA-MB-231-mCherry cells was set at 2×10^7 /mL in 50 μ L PBS mixed with 50 μ L Matrigel (#354262; Corning, NY, USA) for orthotopic tumor implantation in femal nude mice (aged 5–6 weeks; weighing 18–22 g). After 15 days, Weng *et al.* [56] used 5-ALA as a PS that was injected intraperitoneally and irradiated the tumor by white light (400–700 nm, 100 mW/cm²) for 20 minutes for a single time, at that time tumor was surgically removed in some animals. Subsequently, the number of circulating tumor cells (CTC) was systematically monitored by *in vivo* flow cytometry (IVFC). In this experiment, scientists observed that CTC levels decreased after PDT treatment, delaying tumor recurrence, thus a better treatment than surgery. Lung and liver metastasis were not observed in animals after PDT,

proving the antimetastatic potential of PDT [55]. Burch *et al.* [57] administered intravenously BPD-MA and used 150 mW of 690 nm light illumination in mice models. PDT demonstrated an ablative effect on vertebral metastases with light energies (25–150 J) [56]. In the research on the anti-metastatic potential of PDT groups, scientists analyzed metastatic lesions within vertebrae and long bones which were treated with differing regimens of 690 nm laser light (2 metastases to femurs and lumbar vertebrae). As an experiment, BC rats were injected with BPD-MA and then irradiated with 690 nm light (25–150 J). The best ablation results were obtained at 150 J [50].

PDT uses the existence of NPs to clarify and enhance its effectiveness. Brezániová *et al.* [115] synthesized thermosensitive NPs. These NPs were bound to the PS temoporfin to improve *in vivo* anti-cancer effect in Nu/Nu mice BC MDA-MB-231 tumors [115]. Cathepsin is a subsection produced by several types of cancer, including BC, which represents their potential as biomarkers for treatment. Ben-Nun *et al.* [116] created a nanoprobe with PS (qABP) targeting cathepsin. Scientists evaluated the advantages of the probe on cell apoptosis after irradiation (710–760 nm) in mice with 4T1 BC [116]. Liu *et al.* [117] used liposomal nanoplateforms with platinum nanoparticles (nano-PT) verteporphyrin (VP) to enhance PDT by increasing oxygen delivery. The designed model was analyzed in mouse tumor models *in vitro* and *in vivo*. It was concluded that this therapy inhibited primary tumor growth and lung metastasis, with no side effects [117]. Spontaneous regression or spontaneous remission of cancer is one of the determinants of cancer treatment. Understanding the physiology and mechanisms of cancer regression can facilitate the selection and most effective form of treatment. Available literature reveals that selected types of cancer have an increased tendency to spontaneous regression and these cancer types include lung cancer, blood cancer, and one of the most common skin cancers, melanoma [118].

Table 2 (Ref. [98,101,109,112,113]) summarizes the characteristics of the analyzed animal models along with the percentage effectiveness of the applied PDT therapy.

Dib *et al.* [119] used the nanotechnology bridged silsesquioxane nanoparticles (BSNs) that have been functionalized with PEG and mannose (PORBSNs-mannose) to target BC in zebrafish embryos bearing human tumors *in vivo*. After injection of BC cells into the body tumor area of zebrafish, larvae were irradiated by laser light at 800 nm, power 3 W in two-photon PDT. Tumor death was observed indirectly by increasing the caspase 3 concentration [119]. PDT was used with laser ablation to intensify the therapeutic effect in groups of mice with BC. The new method showed superior results to single treatment techniques that use only one of these approaches [120,121]. Sine *et al.* [62] administered the anticancer drug 2-(1-hexyloxyethyl)-2-devinyl pyropheophorbide (HPPH) (Ex/Em 410/670 nm) together with calcein and liposomes in mice model and ob-

Table 2. Efficacy of PDT therapy in animal models.

Type of animal model	Efficiency of PDT	References
Chlorin e6 photodynamic therapy in mice	12-fold increase	[98]
Periodic mesoporous organosilicon nanoplatfrom loaded with chlorine 6 in mice	administration of losartan and Ce6-PMO increased the effectiveness of PDT compared to treatment with Ce6-PMO alone	[101]
Nude mice with a breast tumor	increase in efficiency by 27%	[109]
Rat breast cancer model	increase by 47%	[112]
BALB/c mice aged 9–10 weeks	treatment with Hypericin cured approximately 35% and 46% of animals that carried adenocarcinoma	[113]

served its effect. Animals irradiation was performed for 5 minutes with a diode laser at 658 nm. A reduction in tumor volume and a decrease in luciferase concentration were observed [120].

5. Photodynamic Therapy Clinical Breast Cancer Treatment Examples

Xu *et al.* [109] researched PDT in oncoterapies of gonadotropin-releasing hormone (GnRH) receptors. The combination of zinc phthalocyanine (ZnPc) with GnRH analogs showed greater specificity for BC cells *in vivo* [109]. Morrison *et al.* [122] utilized continuous low-irradiance photodynamic therapy (CLIPT) in a Phase I clinical trial with 630 nm laser energy and intravenously administered porfimer sodium as the PS. Cuenca *et al.* [67] used a diode laser (light with a wavelength of 630 nm) in combination with Photofrin (porfimer sodium) as a PS to reduce metastases in the chest in patients with BC. Tumor necrosis which is photogenerated by $^1\text{O}_2$ [123,124] was observed in all patients after the follow-up period [67]. Khan *et al.* [125] reduced the doses of photofrin II and increased the doses of light in PDT chest treatment. The activity of a new PS, mono-L-aspartyl chlorin e6 (Npe6) was assessed in the Phase I study. Tumor reduction was observed in all 14 patients including patients with BC by using Npe6/PDT (25–100 J/cm², 664 nm) [68]. Li *et al.* [126] observed an objective response rate of 62.5% and the clinically beneficial response rate of 75% in 8 patients with late-stage BC. In 11 patients before surgery, Frei *et al.* [127] used PDT to identify the sentinel node in patients with BC. Higher fluorescence intensity (FI) was observed in diseased tissues after the application of ALA, an effective identification method as they displayed a 5 times higher FI compared to the nodes without changes [127].

6. Potential Side Effects of Photodynamic Therapy—Photosensitivity and Effects on Cells Adjacent to the Cancer

According to the literature, vitamin D deficiency is a sign of photosensitivity. One of the methods to reduce the discomfort associated with photosensitivity is vitamin D supplementation. Physicians most often recommend oral supplementation in doses of 2000 to 5000 IU a day [128].

The main goal of PDT is to destroy malignant (disease/cancer) cells. PDT influence is also observed in the cells surrounding the tumor. There are a few validated studies that highlight the fact that PDT also affects normal cells. However, the undeniable fact is that the evaluation of the normal cells after photoreaction can help in better understanding the principle and mechanism of PDT. There is an example in the literature where human epidermal keratinocytes were used to analyze the effects of PDT treatment. Studies have confirmed that PDT promotes autophagocytosis and induces the apoptotic process of keratinocytes [129].

In the literature, a study on animal models suggests that liposomal aluminum phthalocyanine sensitized many cells to light, a process that was associated with the effect of PDT, i.e., extensive, irreversible apoptosis, the occurrence of which is crucial for the effectiveness of the treatment [130].

Animals have many features similar to humans, particularly in their physiology and behavior. The similarity in the brain structure is reflected in external features. Therefore, there are many scientific studies in which the authors used animal models in analogy to humans. We believe that it is important to develop research on syngeneic models, which, due to their simplicity and lower cost of acquisition, are a promising alternative to the popular xenograft models.

7. Conclusions

PDT is used in many cancers as a radical, or palliative treatment, offering minimal side effects, and good cancer specificity compared to alternative and conventional oncological treatments, such as surgery, chemotherapy, or radiation therapy. Research is anticipated to enhance PDT as a treatment for BC and to rationally select conventional, and modern combination therapies, such as cancer vaccines, immunotherapy, oncolytic viral therapy, and immunotherapy.

This article highlights both the advantages and some disadvantages of the use of small and large animals for BC models.

The photosensitizers localize in mitochondria, near nuclear areas, and endoplasmic reticulum, making them good candidates for BC PDT. Due to the action of PDT primarily through apoptosis, PDT is characterized by low side

effects. However, it is worth considering the conjugation of photosensitizers with other molecules, which is a further challenge in treatment.

Another advantage of PDT is its potential property to induce immunity against recurrence and damage of metastases distant from the treated sites, which provides additional benefits over existing treatments.

PDT can also be used as a complimentary adjuvant therapy but further research is required to investigate if PDT can be used successfully in BC.

On the other hand, the main disadvantage of using PDT in BC is the absence of large randomized controlled trials. Therefore, treatment schedules still need to be improved and standardized to achieve better therapeutic efficacy. The proper selection of the photosensitizer, a suitable wavelength of light, the irradiation duration, and intervals between PS and PDT, would improve the effectiveness and safety of BC PDT. Based on the data collected in our review, we would like to highlight the promising potential of nanomedicine in the treatment of BC. Nanoparticles demonstrated promising *in vitro* tumor targetability, therefore it is necessary to apply them in studies on animal models to fully understand the pharmacokinetic/pharmacodynamic of PDT.

Author Contributions

MCC, AKK, DA and GC designed the research study. MCC, MKO and KD analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

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Conflict of Interest

The authors declare no conflict of interest.

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