Cancer Metastasis Rev (2015) 34:563–573
DOI 10.1007/s10555-014-9544-y

NON-THEMATIC REVIEW

Retinoblastoma: might photodynamic therapy be an option?

Ricardo Teixo · Mafalda Laranjo · Ana Margarida Abrantes · Gonçalo Brites · Arménio Serra · Rui Proença · Maria Filomena Botelho

Published online: 13 January 2015

Abstract Retinoblastoma is a tumor that mainly affects children under 5 years, all over the world. The origin of these tumors is related with mutations in the RB1 gene, which may result from genetic alterations in cells of the germ line or in retinal somatic cells. In developing countries, the number of retinoblastoma-related deaths is higher due to less access to treatment, unlike what happens in developed countries where survival rates are higher. However, treatments such as chemotherapy and radiotherapy, although quite effective in treating this type of cancer, do not avoid high indices of mortality due to secondary malignances which are quite frequent in these patients. Additionally, treatments such as cryotherapy, thermotherapy, thermochemotherapy, or brachytherapy represent other options for retinoblastoma. When all these approaches fail, enucleation is the last option. Photodynamic therapy might be considered as an alternative, particularly because of its non-mutagenic character. Photodynamic therapy is a treatment modality based on the administration of photosensitizing molecules that only upon irradiation of the tumor with a light source of appropriate wavelength are activated, triggering its antitumor action. This activity may be not only due to direct damage to tumor cells but also due to damage caused to the blood vessels responsible for the vascular supply of the tumor. Over the past decades, several *in vitro* and *in vivo* studies were conducted to assess the effectiveness of photodynamic therapy in the treatment of retinoblastoma, and very promising results were achieved.

Keywords Photodynamic therapy · Retinoblastoma · Cancer therapy · Photosensitizers

R. Teixo · M. Laranjo · A. M. Abrantes · G. Brites · M. F. Botelho
Unit of Biophysics, Faculty of Medicine, University of Coimbra, Azinhaga de Santa Comba, Celas, 3000-548 Coimbra, Portugal

M. Laranjo · A. M. Abrantes · M. F. Botelho
CIMAGO, Faculty of Medicine, University of Coimbra, Azinhaga de Santa Comba, Celas, 3000-548 Coimbra, Portugal

M. Laranjo (✉) · A. M. Abrantes · R. Proença · M. F. Botelho
IBILI, Faculty of Medicine, University of Coimbra, Azinhaga de Santa Comba, Celas, 3000-548 Coimbra, Portugal
e-mail: mafaldalaranjo@fmed.uc.pt

A. Serra
Chemical Engineering Department, Faculty of Science and Technology, University of Coimbra, Pólo II, Pinhal de Marrocos, 3030-290 Coimbra, Portugal

R. Proença
Centre for Integrated Responsibility in Ophthalmology, Coimbra Hospital and University Centre (CRIO-CHUC), Praceta Mota Pinto, 3000-075 Coimbra, Portugal

1 Introduction

Retinoblastoma (RB) is the most common intraocular neoplasia in children [1]. This disease appears mostly before the age of five. The bilateral form is usually diagnosed earlier (14–16 months), while the unilateral form is diagnosed between 29 and 30 months of life. This tumor has an incidence of 1 per 20,000 births, with no significant differences between races, geographical location, and industrial development (developed *vs.* developing countries) [2]. The survival rate in the United States of America (USA) is almost 100 %. However, in other countries, particularly in developing countries, this rate has significantly lower values, such as those found in Latin American countries (80–89 %), Iran (83 %), China (81 %), India (48 %), and African countries (between 20 and 46 %) [3]. Table 1 summarizes RB mortality in some countries.

Retinoblastoma can be classified according to two criteria: bilateral or unilateral and hereditary or non-hereditary.

Table 1 Retinoblastoma-related mortality in representative countries (adapted from [4])

	Mortality (%)
Europe—all countries	5–11
Taiwan	36
Kenya	73
Mexico	11
Canada	1
Honduras	35–73
Brazil	5–22

Retinoblastoma is associated with a gene, the retinoblastoma gene (RB1), which is located at the long arm of chromosome 13 (13q14). It is a recessive tumor suppressor gene important for cell growth and development. For tumor development to occur, a loss, deletion, inactivation, or mutation of both alleles corresponding to this gene must happen [5]. The "two-hit" hypothesis, presented by Knudson and colleagues, states that this loss of function of the RB1 gene may occur through a mutation in germ cells and subsequent mutation in the somatic cells of the retina (hereditary retinoblastoma) or two mutations in somatic cells of the retina at different times (sporadic retinoblastoma) [6]. Most recently, it was demonstrated that the loss of function of the RB1 gene initiates a retinoma and causes genomic instability. However, this event is insufficient to cause retinoblastoma. David Comings hypothesized that the retinoblastoma causative gene was retina specific, and it is well known that loss of RB1 function in many human cancers could promote cancer development, probably by loss of cell cycle control and genomic instability [4, 7].

2 Clinical features, classification, and diagnosis

Because retinoblastoma development occurs essentially in very young children, their carriers are not able to transmit their symptoms, which means that the identification of a retinal problem arises only with the onset of leukocoria and/or strabismus. Leukocoria is characterized by the appearance of a tumor of white color, which produces a reflection of light on the pupil. Retinoblastoma can be curable if diagnosed 3–6 months after the first sign of leukocoria. The identification of this sign could be difficult in health centers because it is identified more easily in a dim-lighted place. A United Kingdom (UK) study demonstrated that one in each four children waited more than 4 months for primary care referral to an ophthalmologist, which could diminish the chances of survival [4]. Strabismus results from the loss of central vision in one or both eyes, causing the ocular misalignment.

Other signs such as heterochromia, hyphema, glaucoma, inflammation, and orbital cellulitis are less common as signs of retinoblastoma [1, 6, 8, 9].

Retinoblastoma presents three characteristic growth patterns: endophytic (growing intravitreal), exophytic (subretinal, invading the choroid), and diffuse (difficult to diagnose, since it is similar to an inflammation or bleeding). The most common route of cell invasion observed in retinoblastoma is through the retina, affecting the optic nerve. Thereafter, the tumor continues its invasion, affecting the optic chiasm, or even to the pia mater to the subarachnoid space. There may also exist extension of the tumor to extraocular zones which can lead to its spread until the lymphatic vessels, with subsequent development of metastases, decreasing the chances of survival [1].

In the 1960s, a classification system for retinoblastoma was created by Reese and Ellsworth (The Reese-Ellsworth Classification, 1963), based on the stage of intraocular tumor and predicting preservation of the eyeball, after treatment with external beam radiation. However, this type of therapeutic approach was falling out of use, so there was a need to develop a new classification system best suited to the new reality of treatments. In 2003, the International Classification of Retinoblastoma (ICRB) was completed [10].This system is based mainly on the way tumor spreads to the vitreous and subretinal space and also taking into account tumor size and location, as it is detailed in Table 2.

In order to get a proper diagnosis, clinicians must perform several ocular examinations, imaging studies and investigation for metastasis. The ocular examination should be performed under general anesthesia by indirect ophthalmoscopy and should include evaluation of the cornea, anterior chamber, and iris which is easily performed using a surgical or binocular handheld slit lamp microscope. This observation could lead to the identification of the type of retinoblastoma growth: endophytic retinoblastoma grows towards the vitreous and it is associated with vitreous and subretinal seeds; exophytic retinoblastoma grows outwards and produces secondary serous retinal detachment. Other factors that should be characterized are the number and tumor size, the laterality and distance from the optic disk and macula, and the presence of subretinal fluid and subretinal/vitreous seeds [8].

The diagnosis of retinoblastoma using imaging methods is achieved by ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI) [8, 12].

The US is non-invasive, easily available with low costs. In this technique, the retinoblastoma can be observed as an intraocular mass more hyperechoid than the vitreous, with calcifications indicated by hyperreflectivity. The monitoring of the tumor size during chemoreduction could be done with this type of imaging method, but it is more sensitive for small calcifications and useless for identification of extraocular spread. CT is able to demonstrate an intraocular mass with a higher density than the vitreous body which is calcified in 90 % of the cases. These structures are slightly heightened after injection with an iodine contrast agent. The presence of calcifications is a

Table 2 The International Classification System for Retinoblastoma and the corresponding management strategy at the University of California, San Francisco (retired from [11])

	Characteristics	Prognosis	Treatment	Follow-up
Group A, small	≤3 mm in height. ≥2 disk diameter (3 mm) from fovea, ≥1 disk diameter from optic nerve	Usually eradicated by treatment. Good visual and overall prognosis	Focal therapy only (argon/YAG laser, cryotherapy, hyperthermia, or brachytherapy)	Follow Q6 weeks because of local recurrences and new tumors
Group B, medium	>3 mm in height, clear subretinal fluid ≤3 mm from tumor margin	Too large to be treated with focal therapy alone. Visual prognosis excellent with treatment	1) Vincristine + low-dose carboplatin up to 6 cycles 2) Focal therapy with 2 to 6 cycles	Repeat examination under anesthesia after cycles 2, 4, and 6
Group C, confined, medium	Localized vitreous seeding (C1) or subretinal seeding (C2; ≤3 mm from tumor margin) or both (C3)	Visual prognosis variable depending on location of tumor	1) Three-agent chemotherapy (vincristine, high-dose carboplatin, etoposide, granulocyte colony-stimulating factor) up to 6 cycles 2) With or without sub-Tenon or carboplatin (currently on hold) 3) Focal therapy	Reassessment after every cycle
Group D, diffuse, large	Diffuse vitreous (D1) or subretinal seeding (D2; >3 mm from tumor margin) or both (D3). Subretinal fluid >3 mm from tumor margin	Visual prognosis guarded depending on tumor location. Morbidity from focal therapy is high	1) Three-agent chemotherapy (vincristine, high-dose carboplatin, etoposide, granulocyte colony-stimulating factor) up to 6 cycles 2) External beam radiation (EBR) 3) With or without sub-Tenon or carboplatin (radiation is now off the protocol)	Often still requires enucleation
Group E, enucleation, advanced	No visual potential or presence of ≥1 of the following: tumor in anterior segment, tumor in ciliary body, neovascular glaucoma, vitreous hemorrhage, phthisical eye, orbital cellulitis-like presentation, involvement of optic nerve, extraocular disease on neuroimaging	No visual potential. Morbidity with treatment is high	1) Enucleation 2) Prophylactic 3-agent chemotherapy	Rule out extraocular spread on pathologic examination

characteristic of retinoblastoma in children below 3 years of age. A variant of CT, named spiral CT, is considered a better method for the diagnosis of retinoblastoma since it is a volumetric acquisition that can be applied without general anesthesia and is associated with a lower radiation exposure. The MRI studies usually use gadolinium enhancement and fat suppression to evaluate extraocular or optic nerve invasion, subarachnoid seeding, and intracranial involvement. This method is also used for the diagnosis of trilateral retinoblastoma, which consists in bilateral retinoblastoma and pinealoblastoma [8]. The specific investigation for metastasis is only carried out when there are significant evidences suggesting extraocular extension pointing to possible metastasis. In the group of image methods, we can include analysis of cerebrospinal fluid, bone marrow biopsy, and bone imaging [8, 12].

3 Treatments

Currently, retinoblastoma, like other cancers, is the target of several therapeutic approaches, depending on the stage of the disease and the patient's response. The main therapies used to retinoblastoma are radiotherapy (external beam radiation and brachytherapy), chemotherapy, thermotherapy, cryotherapy, chemothermotherapy, and enucleation.

3.1 External beam radiation

External beam radiation was the first globe-salvaging treatment for retinoblastoma [13]. This is a highly effective treatment for retinoblastoma, and it is an advisable treatment for children who suffer from the bilateral form of the disease and when the chemotherapy failed, with recurrence of the disease. The doses normally used range from 42 to 46 Gy, and with this methodology, the preservation of eye reaches 58–88 %. However, patients with the hereditary form of the disease who received this treatment have an increased risk of developing secondary tumors, reaching an incidence of 35 % of secondary malignancies, mainly including osteosarcoma. This risk is even higher for children under 1 year of age [6, 14]. This treatment could also lead to undesired effects such as orbital hypoplasia, dry eye, and cataract [13].

3.2 Brachytherapy

Brachytherapy consists in placing a radioactive implant in the sclera adjacent to the base of the tumor. The radioactive agents used in this therapeutic approach are cobalt-60 (^{60}Co), iodine-125 (^{125}I), gold-198 (^{198}Au), strontium-90/yttrium-90 (^{90}Sr/^{90}Y), palladium-103 (^{103}Pd), ruthenium-106/rhodium-106 (^{106}Ru/^{106}Rh), iridium-192 (^{192}Ir), and ruthenium-109 (^{109}Ru) [15, 16]. This therapy aims to expose the tumor to ionizing radiation with a dose of 40 to 45 Gy for a period that can range from 2 to 4 days [6, 12].

This approach is recommended for patients who failed initial treatment including a first treatment with external beam radiation, chemofailure, tumor recurrence, or where the chemotherapy is not indicated [8]. However, it is not recommended in cases of large tumors or in tumors that reach the macula. In addition, side effects from this treatment revealed to be less common than those that arise with external beam radiation, specially the appearance of optic neuropathy, retinopathy, and cataract formation [6]. This therapy allows the decrease of radiation spread to the orbit and periorbital area, preventing problems associated with the external beam radiation [8].

3.3 Chemotherapy

Chemotherapy is used in the treatment of retinoblastoma with the aim of reducing tumor size in order to apply other therapies such as thermoablation or cryoablation with the purpose of completely eradicating the disease [17, 18]. Chemotherapy could be delivered for different routes of administration such as intravenous, intra-arterial, periocular, or intravitreal.

Intravenous chemotherapy is recommended for cases of large intraocular retinoblastoma or for cases of unilateral disease with small tumors, where other localized therapies had no effect. The main chemotherapeutic agents used for treatment of retinoblastoma are carboplatin, vincristine, and etoposide, in which standard regimen consists of six cycles with a combination of vincristine, etoposide, and carboplatin, with standard dose based on patient weight [8, 19]. This regimen is used worldwide and it has been demonstrated as effective for intraocular retinoblastoma control [19]. In a study with 78 patients, this standard regimen resulted in a complete remission in 72 % of the cases that were treated with a chemotherapy agent alone. A high response rate of 84 % in macular tumors was also observed [12]. The secondary effects are expected such as bacterial infections, and there is a high risk of development of new tumors in other organs, particularly with etoposide. Therefore, some less toxic chemotherapeutic agents have been developed in the last years, such as topotecan, an inhibitor of DNA topoisomerase-1, and 2-deoxy-D-glucose (2-DG), a glycolytic inhibitor [17, 18].

Intra-arterial chemotherapy (IAC), which consists of the ophthalmic artery infusion therapy, has arisen as a promising approach for management of eyes with retinoblastoma, namely in unilateral retinoblastoma [10, 20]. The technique consists of selective catheterization of the cervical segment of the internal carotid artery followed by propelling of a microballoon distal to the ophthalmic artery which is followed by an infusion of melphalan [20]. Intra-arterial chemotherapy has been described as a safe and effective treatment for retinoblastoma. Used as a primary procedure, IAC could achieve ocular survival at 2 years in 82 % of the cases and 58 % if used as a secondary treatment. This therapy could be extremely efficient in cases of group C and D (see Table 2) retinoblastomas and could also be helpful for retinal detachment from retinoblastoma. However, this approach can also be related to local ocular toxicity, namely vascular compromise of the ophthalmic artery, retinal artery, or choroidal vessels [19].

Periocular chemotherapy with injection of carboplatin is often used simultaneously with systemic chemotherapy for the control of retinoblastoma to boost the local dose of chemotherapy in the vitreous. This approach could lead to some complications, namely orbital and eyelid edema and ecchymosis, orbital fat atrophy, muscle fibrosis leading to strabismus, and optic atrophy [19, 20].

Intravitreal chemotherapy has been used against retinoblastoma, with melphalan being the most effective chemotherapeutic agent. In a study performed by Kivela and colleagues, this approach was shown to be effective using methotrexate as a chemotherapeutic agent, but numerous injections in the eyes of children over a 1-year period was necessary, which could be a disadvantage. Tumor control achieved with intravitreal chemotherapy is highly dependent on the dose used, and its role in the treatment of retinoblastoma is still under research, but it could be an interesting alternative as a second-line therapy for recurrent vitreous seeding [19, 20].

3.4 Thermotherapy

Thermotherapy consists in the application of a heat source directly into the tumor, in the form of infrared radiation. This radiation causes hyperthermia of tumor cells and leads to cell death by apoptosis [8]. In this treatment, temperatures between 45 and 60 °C are reached which do not cause blood coagulation in the vessels of the retina. This technique is used specially for retinoblastomas of small dimensions [6].

3.5 Cryotherapy

Cryotherapy or cryoablation aims the destruction of vascular endothelium that supports the tumor due to rapid cooling. Consequently, it allows a higher influx of chemotherapeutic agents into the vitreous cavity [8]. It can be used to target peripheral tumors or small recurrent tumors after treatment

with other therapeutic approaches. This therapy can cause undesirable side effects such as conjunctival edema and retinal detachment [6]. This method consists in triple freeze techniques that are delivered in one or two sessions [12].

3.6 Chemothermotherapy

Chemothermotherapy is a therapeutic approach which combines thermotherapy with chemotherapy, and it is used for cases of large tumors or for tumors that have already spread to subretinal areas. Both techniques are applied with a few hours apart, to separate them, reaching tumor control rates of around 89 %. The adverse effects that are due to chemothermotherapy are mainly focal atrophy of the iris, retinal detachment, and corneal edema. This therapy is especially advantageous for cases of tumors of small dimensions adjacent to the optic nerve and fovea [6]. A study where the therapy consisted of chemoreduction followed by thermotherapy or cryotherapy resulted in complete control of 394 tumors [12].

3.7 Enucleation

Enucleation consists in the removal of the affected eye. It is the most appropriate treatment for children with unilateral retinoblastoma in advanced stage or for eyes that have not responded to other treatments in bilateral cases [6, 8]. The removed eye is replaced by an orbital implant of silicone, plastic, or hydroxyapatite through the myoconjunctival technique, in which the surgeon places the implant posteriorly in the orbit and attaches the rectus eye muscles to the conjunctival fornices which results in normal movement of the implant. Timely enucleation reduces the risk of metastatic spread, morbidity, side effects of chemotherapy and focal laser treatment, and repeated examinations under general anesthesia [4].

Despite the efficacy demonstrated by previous treatments, the adverse effects remain a fear factor, particularly for parents of children affected with retinoblastoma, due to the high risk of vision loss or even loss of the eye itself. Additionally, there is the possibility of developing secondary tumors in patients treated with external beam radiation, especially in the first year of life [21]. Thus, development of a non-mutagenic therapy such as photodynamic therapy could be interesting, and the effect of photodynamic therapy in the treatment of retinoblastoma has already been investigated.

4 Photodynamic therapy

4.1 Historical perspective

Light has been used as a therapeutic agent since ancient times, particularly by the Egyptians, Indians, Chinese, and Greeks in the treatment of psoriasis and vitiligo [22]. In 1903, Finsen was awarded with the Nobel Prize due to effective treatment of disease using light, thus appearing phototherapy [23]. However, the first experimental evidence of photosensitization process dates back to the early twentieth century, in 1900, when Oscar Raab found that the combination of acridine with light was toxic to paramecium [24]. This discovery was made by accident, since acridine would only function as a fluorescent probe, but when subjected to a light source caused the death of protozoa. Raab and his teachers Jodlbauer, Jesionick, and Von Tappeiner found that acridine had functioned as photosensitizing agent, i.e., a compound that absorbs light initiating a photophysical or photochemical reaction [23, 25]. Von Tappeiner and colleagues concluded that this photosensitization would be dynamic and different from that observed on photographic plates with other chromophores. The term photodynamic reaction was then created for this type of reactions in the presence of molecular oxygen (O_2). Von Tappeiner appeared to predict the antitumor potential of photosensitization and verified the effect of light exposure of a tumor in the presence of eosin. However, a modern approach, now called photodynamic therapy (PDT), has emerged only decades after the investigations carried out by Von Tappeiner, when Lipson and his colleagues, as well as Schwartz's team, used a mixture of fluorescent porphyrins in a localized tumor, calling this mixture as hematoporphyrin derivative [22].

4.2 Principles

The PDT is now a recognized strategy to combat cancer, being used in the treatment of several solid tumors and other lesions [26]. The activation promoted by light will lead to the electronic excitation of the molecule, a photosensitizer (PS), from its ground state (^{1}PS) to a singlet excited state (^{1}PS*). After the activation, we can observe the electronic decay to the ground state with the formation of fluorescence, without photodynamic effect. To make this happen, the conversion of the ^{1}PS* to the spin triplet excited state (^{3}PS*) is required [27]. The interaction of this molecule with other triplet-state molecules (like molecular oxygen) can give rise to the generation of two types of reactive species that can be part of different photooxidative reactions, as shown in Fig. 1. The type I reaction may involve electron or proton transfer producing excited PS and substrate radicals. These intermediates, reacting with molecular oxygen, may form peroxides, hydroxyl radicals, and superoxide ions, triggering the activation of a free radical chain. On the other hand, a type II reaction may occur. This process is mediated by energy transfer to molecular oxygen generating a singlet oxygen molecule. The formation of singlet oxygen by this pathway seems to have a key role in photodynamic cytotoxicity, since there is a considerable interaction between this form of oxygen and several other biomolecules [29].

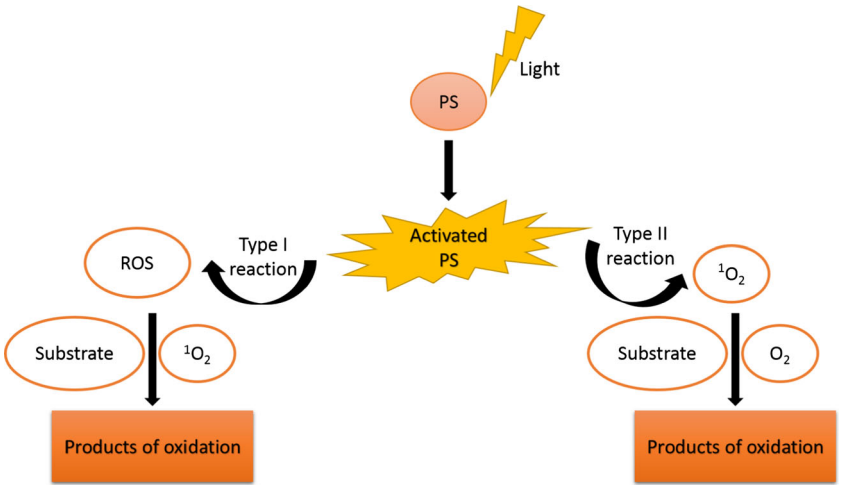

Fig. 1 Reactions of photodynamic therapy (PDT). PDT can induce two types of reactions: type I and type II reactions. The first one involves the interaction of radicals produced by the transfer of a hydrogen atom from the activated photosensitizer to a substrate. The second involves the direct transfer of energy from the sensitizer to oxygen to form singlet oxygen that can further oxidize other substrates (adapted from [28])

PDT specificity is due not only to the focalized light exposure used to treat but also to some preferential accumulation of the PS in tumor cells. Tumor cells have special characteristics that differentiate them from normal cells. For example, tumor cells have a lower pH than normal tissues, due to high levels of lactate resulting from the increased glycolytic activity, which will promote a greater accumulation of drugs that can protonate in an acidic medium. Moreover, there are macrophages in the tumor tissue that can swallow aggregates of the drug alone or linked to lipoproteins. On the surface of tumor cells, there is higher expression of receptors for low-density lipoproteins which correlated with the fact that most lipophilic PSs associated with these lipoproteins accumulated in tumors. In fact, there are many other characteristics of tumor tissues leading to an increase of PS internalization. This feature, together with the production of the therapeutic action only by tumor lighting, makes PDT a safe therapy. Furthermore, PDT significantly reduces the problems associated with the damage of normal cells and systemic effects that occur in other therapeutic approaches such as radiotherapy and chemotherapy [24].

4.3 Light sources

The effectiveness of PDT is dependent of the use of suitable light source. The effectiveness of therapy depends on the greater or lesser matching between the emission spectrum of the light source with the absorption spectrum of the PS [24]. Moreover, tissue light penetration is very important: light having a wavelength on the blue region penetrates less into the tissue than a light source corresponding to the range of red or infrared regions. The values of wavelength between 600 and 1200 nm is called optical window of tissue and is the preferable region to irradiate [25]. However, not all wavelengths within this range are able to trigger a photodynamic effect, since transfer of energy from PS to oxygen singlet state is necessary, which is only possible with the use of light sources with a wavelength up to 850 nm. Regarding these factors, it is also necessary to determine the spectrum of activity of the PS, which describes the relative effectiveness of a PS for different values of wavelength [24].

Nowadays, the light sources used in PDT belong to three major groups: broad-spectrum lamps, diodes, and lasers, and factors such as the total light dose to be applied, exposure time, and mode of delivery of the light also must be take into account [25, 30] The broad-spectrum lamps in the beginning of application of PDT were widely used due to their low cost and easy handling, but otherwise, its coupling to optical fibers for delivery of light to internal organs without loss of effectiveness is difficult [27]. Afterwards, light diodes have been developed, resulting in cheaper, easily transportable systems, but they only are able to send a single wavelength, significantly reducing its versatility [27]. Lasers have contributed significantly to improving the usage of PDT since they allow a higher intensity and the selection of precise wavelength as well as the precise application of light by the use of optical fibers. Two types of lasers can be used: continuous-wave lasers or pulse lasers, such as gold vapor laser [29]. As mentioned previously, the optical fiber technology provides an excellent aid in PDT, since it allows reaching deeper regions of the body that only with the illumination surface would not be possible to treat with this therapeutic approach.

4.4 Photosensitizers

Nowadays, there are several PSs approved for clinical use. A PS to be virtually ideal must possess certain characteristics, such as being a chemically pure compound with preferential accumulation in the tumor, fast clearance from the body, and a large absorption value in the region of 600 nm [26]. Moreover, an ideal PS should not show toxicity in the absence of light irradiation and must allow the formation of singlet oxygen in higher yields, i.e., gives preferentially a type II reaction, which

is described as being more effective in destroying tumor tissue [29].

Hematoporphyrin (Hp) was considered a powerful PS in 1912 by Meyer-Betz, and in the 1950s, it was proven that it possesses characteristics of preferential accumulation in tumor tissues by Figge and colleagues. By chemical synthesis, Hp would form a hematoporphyrin derivative (HpD), which had even better antitumor characteristics than Hp. The HpD is not a pure compound. It was posteriorly purified by chromatographic methods by Dougerty and colleagues, giving rise to Photofrin® which is still the most widely used PS in clinical practice [24]. Photofrin® consists of a set of derivatives of Hp and shows several peaks of absorption, including at 630 nm, but with a reduced intensity. Therefore, to compensate for the low light intensity of the absorption peak of Photofrin® for this wavelength, high doses are need and the light sources used with this wavelength allow a good penetrating power. Another disadvantage of this photosensitizer is the occurrence of patient photosensitivity for long periods of time, from 1 to 3 months after administration [27].

Another PS widely used is the 5-aminolevulinic acid (ALA). This compound is a precursor of protoporphyrin IX (PpIX) a natural porphyrin. ALA is the only biochemical precursor of PpIX, which turns out to be true PS. PpIX by itself has low toxicity since the body has endogenous mechanisms for their removal. The administration of ALA will induce an excessive accumulation of PpIX in the tumor tissue, leading to the desired toxicity. In addition to the antitumor effect, ALA can be used as a diagnostic tool due to its greater accumulation in the tumor tissue and by the fact that the tumor in these circumstances could be visualized when illuminated by blue light through endoscopic fluorescence. However, as ALA is hydrophilic and presents limitations of penetration through the cell membranes, leading to the development of different chemical formulations, including esters of ALA for improving this aspect. These compounds proved to be more effective in the accumulation of PpIX in the tumor cells, due to their greater hydrophobic character [24].

Another family of PSs currently used is the family of chlorins, of which meta-tetrahydroxyphenyl chlorin (m-THPC) is a representative. The m-THPC is a quite potent PS, since it produces a high amount of singlet oxygen when irradiated at the wavelength of 652 nm, and for this reason, a greater light penetration is obtained compared to the 630 nm used in the treatments mentioned above. Due to this fact, the light energies required for m-THPC activation are lower than those used for ALA and Photofrin®. The use of easily transportable PS is referred to cause some pain during treatments. This PS is used in the palliative treatment of tumors of the head and neck, as well as in the treatment of tumors of the oral cavity, esophagus, stomach, pancreas, and lung [23, 27].

Benzoporphyrin derivatives (BpD) are also used as photosensitizers in PDT. An example is verteporfin, a hydrophobic compound, which can be formulated with liposomes. Its activation occurs with light of wavelength 690 nm, allowing a significant penetration into the tissues. Their removal from the body occurs fast, only a few hours period, which is an advantageous characteristic for the patient treatment. This compound acts primarily by destruction of blood vessels surrounding the tumor. Verteporfin is currently used for the treatment of ophthalmic astrocytoma, choroidal melanoma, choroidal neovascular membranes, and several cutaneous neoplasias [23].

Apart from these PSs, there are many others in development and in study, such as tin ethyl etiopurpurin (SnET2), mono-L-aspartyl, chlorin e6 (Npe6), and lutetium texaphyrin (Lutex). All these PSs have absorption peaks at wavelengths relatively high (660, 664, 690, and 732 nm, respectively), and show lower skin photosensitivity [31].

4.5 Cytotoxicity

The half-life of singlet oxygen is very short, so its effect will be felt mainly at the place where it is produced by the photodynamic reaction. Due to this fact, the different PSs mentioned above have different modes of action according to PS subcelullar localization . For example, Photofrin® acts on lipid membranes, Npe6 at the level of lysosomes [25], verteporfin affecting mitochondria [29], and m-THPC the endoplasmic reticulum [32]. All these localizations can lead to cell death, since PDT may activate the three major pathways of cell death: apoptosis, necrosis, and autophagy. Apoptosis is the major pathway of cell death activated by PDT [25]. The mechanisms leading to apoptosis are known. Different PSs can activate NF-κB and stimulate the MAPK signaling pathway. In addition, apoptosis is activated preferentially by PSs that influence the mitochondria, since these are organelles which are involved in the regulation of this process of cell death. Apoptosis is triggered upon activation light, because there is an inhibition of ATP by ATP synthase, and may also affect the complexes I, III, and IV of the respiratory chain. This process causes a release of cytochrome c from the mitochondria, which will activate the signaling cascade of caspases leading to several processes of irreversible cleavage of proteins with important roles at the structural level, intracellular signaling and gene transcription [29].

Regarding the process of cell death by necrosis, the mechanisms involved are not yet fully understood. However, certain processes such as activation of protein RIP1, excessive production of reactive oxygen species, damage of lysosomes, and excessive intracellular calcium may be involved. If the PSs cause great harm in the inner mitochondrial membrane or cause a large excess of intracellular calcium, a change in the permeability of mitochondria may be promoted, favoring cell death by necrosis [25].

Autophagy (or macroautophagy) is a lysosomal pathway for degradation and recycling of proteins or intracellular organelles, which can be activated by various signs of stress, including oxidative stress, which may have a cytoprotective role or pro-cell death following chemotherapy. However, there are studies that relate autophagy as a mechanism to ensure cell viability after PDT. This can be counteracted by the use of PSs affecting the lysosomal pathway leading the cells to follow the apoptotic process [25].

In addition to cell death *per se*, with respect to antitumor activity, PDT plays another important role: the antivascular effect. The first study to demonstrate this effect was made in the 1960s, by Star and his colleagues. In this study, the effect of PDT in blood vessels surrounding the tumor implanted in an animal model (mouse) by using HpD as a photosensitizer was investigated. Vasoconstriction was observed followed by stagnation of blood flow, bleeding, and formation of platelet aggregates [25].

PDT may also influence the immunological system and has been demonstrated to reduce the severity of the symptoms of experimentally induced autoimmune diseases. It has been found that the use of the photosensitizers ALA, verteporfin, and HpD selectively sensitizes monocytes (CD14+), dendritic cells (CD83+), and Langerhans cells. Furthermore, they can also activate lymphocytes that have the receptor of interleukin-2 (IL-2). In other studies, it was found that treatment of tumors in mice with PDT led to an upregulation of the levels of interleukin-6 (IL-6) and decreased levels of messenger RNA (mRNA) of interleukin-10 (IL-10), with a large increase in the levels of this interleukin on the skin (which may explain the skin sensitivity, characteristic of PDT). These results demonstrate that IL-6 may play a key role in the modulation of immune response and the local inflammatory response associated with antitumor effect produced by PDT. Furthermore, this therapy proves to be less effective in treating tumors in mice with severe combined immunodeficiency (SCID mice) than in those with the immune system intact, which reveals the key role of immune response on the antitumor effect produced by PDT for a more effective treatment [29]. All these effects mentioned above are important for cell death in tumors but can also occur in cells from normal tissues, so that, once again, it is important to highlight the need for a treatment strategy with PDT in which the tumor site is as accurate as possible.

5 PDT in the treatment of retinoblastoma

As mentioned earlier, since its discovery, PDT revealed enormous potential as an antitumor therapy applied to various neoplasms. The retinoblastoma treatment with this therapy has also been the subject of study over the past three decades. Since it is not a mutagenic therapy, PDT would be ideal for retinoblastoma treatment, which is characterized by the appearance of secondary malignancies when it is treated with chemotherapy and radiation. *In vitro* studies were performed in order to evaluate the efficacy of different PSs in the death of retinoblastoma cell cultures. Being the longest known PS, the Hp was the first photosensitizer molecule being studied and proven to be effective in eliminating the different cell cultures when activated by white light, verifying that cell death was greater when the light exposure periods were longer. It was also discovered that inhibition of photodynamic effect was increased with the amount of serum present in the medium. This could be due to the presence of hemopexin, a protein responsible for transporting porphyrins in blood circulation to the liver to be degraded [33]. On the other hand, other researchers evaluated the effect of Photofrin II (resulting from the purification of HpD) in the death of retinoblastoma cell cultures. Results of these studies showed that low concentration of this PS and high doses of light energy can cause cell death in a dose-dependent manner while the amount of energy does not cause significant differences [34]. The success of PDT in the treatment of retinoblastoma highlighted the importance of hematoporphyrins in this process, namely the PpIX, which was proven in a study by Ruiz-Galindo and colleagues. These researchers observed an increase in expression of the proteins protoporphyrinogen oxidase (PPOX), uroporphyrinogen synthase (UROS), and aminolevulinic acid synthase (ALAS), enzymes involved in the synthesis of the heme group of PpIX after administration of ALA [35]. Verteporfin (derived from benzoporphyrins) was also evaluated as PS to PDT for treating retinoblastoma, having proved equally effective in inducing cell death in five different cell lines of retinoblastoma, among which one was resistant to etoposide. It was also found that reduced mass was needed for this PS to cause cell death (in the order of nanomolar), while increasing concentration decreased the amount of light energy required to induce cell death, and this happens in a dose-dependent manner [36, 37]. Walther and colleagues performed a study where they investigated the effect of PDT based on the photosensitizer tetrahydroporphyrin-tetratosylat in the retinoblastoma cell lines Y79 and WERI Rb-1. They observed that this photosensitizer could induce a decrease in cell proliferation in a dose- and time-dependent manner in both cell lines, with minimal cytotoxicity in the dark and in normal cells [38].

Furthermore, the investigation of the effects of PDT in the treatment of retinoblastoma was also conducted by *in vivo* studies involving various animal models. In these studies, different PSs and the way they affect the growth and the whole tumor microenvironment were also evaluated. Most studies have been conducted on the effectiveness of the PS family of porphyrins, highlighting the behavior of Photofrin II and dihematoporphyrin. In the latter case, there was a complete

remission in about 15 to 25 % of intraocular tumors induced in the animal model, but having adverse effects such as intraocular bleedings [39]. Regarding Photofrin II, it was observed that, when activated by a light source of wavelength corresponding to the red region, it was able to induce cell death in intraocular tumors in a dose-dependent manner for both the PS concentration and light dose. This therapy also affected blood vessels and surrounding normal tissues, causing adverse effects such as damage in the conjunctiva and cornea, being a limiting factor for this treatment. Studying the kinetics of tumor death, it was found that the mechanism induced by PDT was biphasic, having initially a process of direct tumor cell death followed by a secondary destruction of the tissue due to damage in the surrounding blood vessels [34, 40–42]. Verteporfin and m-THPC also have been tested with xenograft models derived from three different cell lines. There has been a high efficiency in all three models using m-THPC activated by laser, and in the case of verteporfin, it was effective in one of the models [43].

Research in this area has been developed until the clinical trials. Ohnishi and colleagues performed a study involving the treatment of retinoblastoma in five children using HpD and illumination with an argon laser. In this trial, destruction of tumor tissue as well as angio-necrosis was observed, and it is thought that this treatment would be sufficient to treat tumors of small dimensions. Conversely, to treat larger tumors, the combination of PDT and radiation was required, although the radiation doses were lower than those used when applying radiation treatment alone [44].

It should be noted that tumor cell death was not the only target of study over the past decades. The effect of the presence of oxygen in the efficacy of PDT, the antivascular effect, and the immune response triggered by PDT were also investigated by several groups. The importance of the presence of oxygen in the photodynamic effect has been demonstrated through the intracellular accumulation of lipid peroxide, resulting from oxidation of lipids caused by oxidative stress in retinoblastoma cell lines after PDT using HpD, under aerobic and anaerobic conditions. In this study, it was found that under anaerobic conditions, PDT revealed to be ineffective at inducing cell death [45].

In PDT, the tumor vasculature is one of its preferential targets, even being a central target in tumor destruction at *in vivo* conditions. This has been demonstrated by studies where the effect of PDT using Photofrin® in intraocular perfusion using the method of captured rubidium-86 chloride (^{86}RbCl) was evaluated. By this method, there is a decrease in uptake of ^{86}RbCl after application of PDT, demonstrating that the vasculature of an eye affected by a tumor was hampered due to the action of PS with subsequent irradiation [46]. In addition, another study by White and colleagues confirmed that the PDT was ineffective in treating an animal model of retinoblastoma with reduced vascularization, demonstrating again the essential role of the blood vessel damage involving the retinoblastoma for a successful PDT [47].

As already mentioned, the PDT can induce an antitumor immune response. In retinoblastoma, a group of researchers observed the activation of macrophages with an antitumor capacity for retinoblastoma cells coated with IgG. Researchers have used two irradiation sources, white light and red light [48, 49]. They proved that the white light was more effective in the macrophage activation, demonstrating the role of immune potentiation promoted by HpD, which can be crucial to the destruction of retinoblastoma [48, 49]. Another challenge in this area of research is the induction of a higher uptake of PSs by tumor cells. This can be achieved by adding different functional groups on PS molecules directed to the protein responsible for increased PS uptake by the tumor. For example, a study by Schmidt-Erfurth and his colleagues showed that the combination of PS chlorin e6 (Ne6) with LDL potentiated the uptake of PS by tumor cells, according to a mechanism of receptor-mediated uptake, as demonstrated by the saturation of receptors as well as by their competitive inhibition [50]. Other researchers have also found that the combination of a sugar (mannose and galactose) with porphyrins linked by diethylene glycol (TPP(p-Deg-O-α-GalOH)$_3$ or TPP(p-Deg-O-α-ManOH)$_3$) increases its photochemical activity, which is also a process mediated by an interaction between sugar and cell receptors [51]. Another interesting study evaluated the relevance of planar bilayers for modeling interactions between glycodendrimeric porphyrins and retinoblastoma cells. In this study, the authors used a concavalin A-grafted bilayer system as a model for retinoblastoma cell membranes and studied the porphyrin interaction with the common Y79 cell line and the adhesion of porphyrin-bearing liposomes to immobilized cells onto a proper sensor. They verified that the porphyrin-bearing liposomes were more phototoxic than the free porphyrin and the free liposomes. Moreover, they observed a strong tendency of these glycodendrimeric porphyrins to aggregate in solution or to interact with blood proteins. In this way, it was difficult to evaluate the real contribution of the molecular recognition, so they immobilized cells onto a Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D), with the cell developed without fetal calf serum before the injection of the porphyrins. With this experiment, they proved that the mannosylated porphyrins specifically interact with the mannose receptor borne by Y79 cells [52]. Gary-Bobo and colleagues performed a study where they evaluated the effect of multifunctionalized mesoporous silica nanoparticles (MSN) combining three different approaches: one-photon excitation photodynamic therapy (OPE-PDT), camptothecin delivery, and targeting using mannose or galactose in retinoblastoma cell line Y79. They also have designed a MSN encapsulating a photosensitizer specifically for two-photon excitation photodynamic therapy (TPE-PDT) and functionalized with

mannose on the surface. They observed high cell death with MSN functionalized with mannose even using a short period of irradiation (three scans of 1.25 s each) at a low fluence (10.6 J cm^{-2}). On the other hand, with the studies performing OPE-PDT combined with camptothecin delivery, they found a synergistic effect of these approaches in order to induce cell death [53].

6 Concluding remarks

Retinoblastoma is a tumor that mainly affects children all over the world. Despite the advances in the conventional therapies available, particularly radiotherapy and chemotherapy, which are often associated to other treatment modalities like thermotherapy, cryotherapy, and eventually, enucleation, and its high efficacy, they can cause mutagenic effects, leading to the development of secondary malignancies which are quite frequent in patients treated for retinoblastoma. This is of particular concern when patients are children under 5 years. Therefore, it is important to consider other treatment approaches that can overcome this limitation. PDT was considered an important strategy in oncologic therapy, and it was used with success in several types of cancer [51]. Understanding of its biology is advanced and several PS and light application systems are available. In the case of retinoblastoma, PDT might be considered particularly because of its non-mutagenic character. Furthermore, several *in vitro* and *in vivo* studies assessing the potential of photodynamic therapy in the treatment of retinoblastoma showed promising results that must be further explored.

Acknowledgments The authors thank The Foundation of Science and Technology for the financial support to Mafalda Laranjo (SFRH/BD/44957/2008).

References

1. Mahajan, A., Crum, A., Johnson, M. H., & Materin, M. A. (2011). Ocular neoplastic disease. *Seminars in Ultrasound, CT, and MR, 32*(1), 28–37. doi:10.1053/j.sult.2010.12.001.
2. DiCiommo, D., Gallie, B. L., & Bremner, R. (2000). Retinoblastoma: the disease, gene and protein provide critical leads to understand cancer. *Seminars in Cancer Biology, 10*(4), 255–269. doi:10.1006/scbi.2000.0326.
3. Houston, S. K., Murray, T. G., Wolfe, S. Q., & Fernandes, C. E. (2011). Current update on retinoblastoma. *International Ophthalmology Clinics, 51*(1), 77–91. doi:10.1097/IIO.0b013e3182010f29.
4. Dimaras, H., Kimani, K., Dimba, E. A. O., Gronsdahl, P., White, A., Chan, H. S. L., & Gallie, B. L. (2012). Retinoblastoma. *Lancet, 379*(9824), 1436–1446. doi:10.1016/S0140-6736(11)61137-9.
5. Shields, C. L., & Shields, J. A. (2004). Diagnosis and management of retinoblastoma. *Cancer Control: Journal of the Moffitt Cancer Center, 11*(5), 317–327.
6. Chintagumpala, M., Chevez-Barrios, P., Paysse, E. A., & Plon, S. E. (2007). Retinoblastoma: review of current management. *The Oncologist, 12*(10), 1237–1246. doi:10.1634/theoncologist.12-10-1237.
7. Comings, D. E. (1973). A general theory of carcinogenesis. *Proceedings of the National Academy of Sciences of the United States of America, 70*(12), 3324–3328.
8. Mehta, M., Sethi, S., Pushker, N., Kashyap, S., Sen, S., Bajaj, M. S., & Ghose, S. (2012). Retinoblastoma. *Singapore Medical Journal, 53*(2), 128–135. quiz 136.
9. Balmer, A., Zografos, L., & Munier, F. (2006). Diagnosis and current management of retinoblastoma. *Oncogene, 25*(38), 5341–5349. doi:10.1038/sj.onc.1209622.
10. Kiss, S., Leiderman, Y. I., & Mukai, S. (2008). Diagnosis, classification, and treatment of retinoblastoma. *International Ophthalmology Clinics, 48*(2), 135–147. doi:10.1097/IIO.0b013e3181693670.
11. Lin, P., & O'Brien, J. M. (2009). Frontiers in the management of retinoblastoma. *American Journal of Ophthalmology, 148*(2), 192–198. doi:10.1016/j.ajo.2009.04.004.
12. Ray, A., Gombos, D. S., & Vats, T. S. (2012). Retinoblastoma: an overview. *Indian Journal of Pediatrics, 79*(7), 916–921. doi:10.1007/s12098-012-0726-8.
13. Gombos, D. S., & Chevez-Barrios, A. P. (2007). Current treatment and management of retinoblastoma. *Current Oncology Reports, 9*(6), 453–458.
14. Khetan, V., Gupta, A., & Gopal, L. (2011). Retinoblastoma: recent trends. A mini review based on published literature. *Oman Journal of Ophthalmology, 4*(3), 108–115. doi:10.4103/0974-620X.91265.
15. Abouzeid, H., Moeckli, R., Gaillard, M.-C., Beck-Popovic, M., Pica, A., Zografos, L., … Munier, F. L. (2008). (106)Ruthenium brachytherapy for retinoblastoma. *International journal of radiation oncology, biology, physics*, *71*(3), 821–8. doi:10.1016/j.ijrobp.2007.11.004.
16. Ramasubramanian, A., & Shields Carol, L. (2012). Retinoblastoma. In: R. Aparna & Ls. Carol, Eds. Jaypee Brothers Medical Publishers (P) Ltd. doi:10.5005/jp/books/11629.
17. Shahsavari, M., & Mashayekhi, A. (2009). Pharmacotherapy for retinoblastoma. *Journal of Ophthalmic & Vision Research, 4*(3), 169–173.
18. Shields, C. L., & Shields, J. A. (2010). Intra-arterial chemotherapy for retinoblastoma: the beginning of a long journey. *Clinical & Experimental Ophthalmology, 38*(6), 638–643. doi:10.1111/j.1442-9071.2010.02297.x.
19. Shields, C. L., Fulco, E. M., Arias, J. D., Alarcon, C., Pellegrini, M., Rishi, P., … Shields, J. a. (2013). Retinoblastoma frontiers with intravenous, intra-arterial, periocular, and intravitreal chemotherapy. *Eye (London, England)*, *27*(2), 253–64. doi:10.1038/eye.2012.175.
20. Villegas, V. M., Hess, D. J., Wildner, A., Gold, A. S., & Murray, T. G. (2013). Retinoblastoma. *Current Opinion in Ophthalmology, 24*(6), 581–588. doi:10.1097/ICU.0000000000000002.
21. Aerts, I., Lumbroso-Le Rouic, L., Gauthier-Villars, M., Brisse, H., Doz, F., & Desjardins, L. (2006). Retinoblastoma. *Orphanet Journal of Rare Diseases, 1*, 31. doi:10.1186/1750-1172-1-31.
22. Pervaiz, S., & Olivo, M. (2006). Frontiers in research review: cutting-edge molecular approaches to therapeutics, (October 2005), 551–556.
23. Allison, R. R., & Sibata, C. H. (2010). Oncologic photodynamic therapy photosensitizers: a clinical review. *Photodiagnosis and Photodynamic Therapy, 7*(2), 61–75. doi:10.1016/j.pdpdt.2010.02.001.
24. Juzeniene, A., Peng, Q., & Moan, J. (2007). Milestones in the development of photodynamic therapy and fluorescence diagnosis. *Photochemical & Photobiological Sciences: Official Journal of the European Photochemistry Association and the European Society for Photobiology, 6*(12), 1234–1245. doi:10.1039/b705461k.

25. Agostinis, P., Berg, K., Cengel, K. A., Foster, T. H., Girotti, A. W., Gollnick, S. O., … Golab, J. (2011). Photodynamic therapy of cancer: an update. *CA: a cancer journal for clinicians*, *61*(4), 250–81. doi:10.3322/caac.20114.
26. Serra, A., Pineiro, M., Pereira, N., Rocha Gonsalves, A., Laranjo, M., Abrantes, M., & Botelho, F. (2008). A look at clinical applications and developments of photodynamic therapy. *Oncology Reviews, 2*(4), 235–249. doi:10.1007/s12156-008-0081-1.
27. Triesscheijn, M., Baas, P., Schellens, J. H. M., & Stewart, F. A. (2006). Photodynamic therapy in oncology. *The Oncologist, 11*(9), 1034–1044. doi:10.1634/theoncologist.11-9-1034.
28. Dolmans, D., Fukumura, D., & Jain, R. (2003). Photodynamic therapy for cancer. *Nature Reviews Cancer, 3*(May), 380–387. doi:10.1038/nrc1070.
29. Luksiene, Z. (2003). Photodynamic therapy: mechanism of action and ways to improve the efficiency of treatment. *Medicina (Kaunas, Lithuania), 39*(12), 1137–1150.
30. Calzavara-Pinton, P. G., Venturini, M., & Sala, R. (2006). Photodynamic therapy: update 2006. Part 1: photochemistry and photobiology. *Journal of the European Academy of Dermatology and Venereology : JEADV, 21*(3), 293–302. doi:10.1111/j.1468-3083.2006.01902.x.
31. Marchal, S., François, A., Dumas, D., Guillemin, F., & Bezdetnaya, L. (2007). Relationship between subcellular localisation of Foscan and caspase activation in photosensitised MCF-7 cells. *British Journal of Cancer, 96*(6), 944–951. doi:10.1038/sj.bjc.6603631.
32. Belzacq, A. S., Jacotot, E., Vieira, H. L., Mistro, D., Granville, D. J., Xie, Z., … Brenner, C. (2001). Apoptosis induction by the photosensitizer verteporfin: identification of mitochondrial adenine nucleotide translocator as a critical target. *Cancer research*, *61*(4), 1260–4.
33. Sery, T. W. (1979). Photodynamic killing of retinoblastoma cells with hematoporphyrin and light. *Cancer Research, 39*(1), 96–100.
34. Winther, J. B. (1990). The effect of photodynamic therapy on a retinoblastoma-like tumour. An experimental in vitro and in vivo study on the potential use of photodynamic therapy in the treatment of retinoblastoma. *Acta ophthalmologica. Supplement*, (197), 1–37.
35. Ruiz-Galindo, E., Arenas-Huertero, F., & Ramón-Gallegos, E. (2007). Expression of genes involved in heme biosynthesis in the human retinoblastoma cell lines WERI-Rb-1 and Y79: implications for photodynamic therapy. *Journal of Experimental & Clinical Cancer Research: CR, 26*(2), 195–200.
36. Stephan, H., Boeloeni, R., Eggert, A., Bornfeld, N., & Schueler, A. (2008). Photodynamic therapy in retinoblastoma: effects of verteporfin on retinoblastoma cell lines. *Investigative Ophthalmology & Visual Science, 49*(7), 3158–3163. doi:10.1167/iovs. 07-1016.
37. Jin, C., Wu, Z., Li, Y., Chen, H., & North, J. (1999). The killing effect of photodynamic therapy using benzoporphyrin derivative on retinoblastoma cell line in vitro. *Yan ke xue bao = Eye science / "Yan ke xue bao" bian ji bu, 15*(1), 1–6.
38. Walther, J., Schastak, S., Dukic-Stefanovic, S., Wiedemann, P., Neuhaus, J., & Claudepierre, T. (2014). Efficient photodynamic therapy on human retinoblastoma cell lines. *PloS One, 9*(1), e87453. doi:10.1371/journal.pone.0087453.
39. Winther, J. (1987). Porphyrin photodynamic therapy in an experimental retinoblastoma model. *Ophthalmic Paediatrics and Genetics, 8*(1), 49–52.
40. Winther, J., & Overgaard, J. (1987). Experimental studies of photodynamic therapy in a retinoblastoma-like tumour. *Acta Ophthalmologica. Supplement, 182*, 140–143.
41. Winther, J., & Overgaard, J. (1989). Photodynamic therapy of experimental intraocular retinoblastomas—dose-response relationships to light energy and photofrin II. *Acta Ophthalmologica, 67*(1), 44–50.
42. Winther, J., & Ehlers, N. (1988). Histopathological changes in an intraocular retinoblastoma-like tumour following photodynamic therapy. *Acta Ophthalmologica, 66*(1), 69–78.
43. Aerts, I., Leuraud, P., Blais, J., Pouliquen, A.-L., Maillard, P., Houdayer, C., … Poupon, M. F. (2010). In vivo efficacy of photodynamic therapy in three new xenograft models of human retinoblastoma. *Photodiagnosis and photodynamic therapy*, *7*(4), 275–83. doi:10.1016/j.pdpdt.2010.09.003.
44. Ohnishi, Y., Yamana, Y., & Minei, M. (1986). Photoradiation therapy using argon laser and a hematoporphyrin derivative for retinoblastoma—a preliminary report. *Japanese Journal of Ophthalmology, 30*(4), 409–419.
45. Ohnishi, Y., Murakami, M., & Wakeyama, H. (1990). Effects of hematoporphyrin derivative and light on Y79 retinoblastoma cells in vitro. *Investigative Ophthalmology & Visual Science, 31*(5), 792–797.
46. Horsman, M. R., & Winther, J. (1989). Vascular effects of photodynamic therapy in an intraocular retinoblastoma-like tumour. *Acta Oncologica (Stockholm, Sweden), 28*(5), 693–697.
47. White, L., Gomer, C. J., Doiron, D. R., & Szirth, B. C. (1988). Ineffective photodynamic therapy (PDT) in a poorly vascularized xenograft model. *British Journal of Cancer, 57*(5), 455–458.
48. Yamamoto, N., Hoober, J. K., & Yamamoto, S. (1992). Tumoricidal capacities of macrophages photodynamically activated with hematoporphyrin derivative. *Photochemistry and Photobiology, 56*(2), 245–250.
49. Yamamoto, N., Sery, T. W., Hoober, J. K., Willett, N. P., & Lindsay, D. D. (1994). Effectiveness of Photofrin II in activation of macrophages and in vitro killing of retinoblastoma cells. *Photochemistry and Photobiology, 60*(2), 160–164.
50. Schmidt-Erfurth, U., Diddens, H., Birngruber, R., & Hasan, T. (1997). Photodynamic targeting of human retinoblastoma cells using covalent low-density lipoprotein conjugates. *British Journal of Cancer, 75*(1), 54–61.
51. Laville, I., Pigaglio, S., Blais, J.-C., Doz, F., Loock, B., Maillard, P., … Blais, J. (2006). Photodynamic efficiency of diethylene glycol-linked glycoconjugated porphyrins in human retinoblastoma cells. *Journal of medicinal chemistry*, *49*(8), 2558–67. doi:10.1021/jm0580151
52. Makky, A., Daghildjian, K., Michel, J.-P., Maillard, P., & Rosilio, V. (2012). Assessment of the relevance of supported planar bilayers for modeling specific interactions between glycodendrimeric porphyrins and retinoblastoma cells. *Biochimica et Biophysica Acta, 1818*(11), 2831–2838. doi:10.1016/j.bbamem.2012.07.003.
53. Gary-Bobo, M., Mir, Y., Rouxel, C., Brevet, D., Hocine, O., Maynadier, M., … Durand, J.-O. (2012). Multifunctionalized mesoporous silica nanoparticles for the in vitro treatment of retinoblastoma: drug delivery, one and two-photon photodynamic therapy. *International journal of pharmaceutics*, *432*(1-2), 99–104. doi:10.1016/j.ijpharm.2012.04.056