1 ***From molecules to nanovectors: current state of the art and***
2 ***applications of photosensitizers in photodynamic therapy***

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12 **Graphical abstract**

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Abstract

Photodynamic therapy (PDT) is a concept based on a selective activation by light of drugs called photosensitizers (PS) leading to reactive oxygen species production responsible for cell destruction. Mechanisms of photodynamic reaction and cell photo-destruction following direct or indirect mechanisms will be presented as well as PS classification, from first generation molecules developed in the 1960s to third generation vectorized PS with improved affinity for tumor cells. Many clinical applications in dermatology, ophthalmology, urology, gastroenterology, gynecology, neurosurgery and pneumology reported encouraging results in human tumor management. However, this interesting technique needs improvements that are currently investigated in the field of PS excitation by the design of new PS intended for two-photon excitation or for X-ray excitation. The former excitation technique is allowing better light penetration and preservation of healthy tissues while the latter is combining PDT and radiotherapy so that external light sources are no longer needed to generate the photodynamic effect. Nanotechnology can also improve the PS to reach the tumor cells by grafting addressing molecule and by increasing its aqueous solubility and consequently its bioavailability by encapsulation in synthetic or biogenic nanovector systems, ensuring good drug protection and targeting. Co-internalization of PS with magnetic nanoparticles in multifunctional vectors or stealth nanoplateforms allows a theranostic anticancer approach. Finally, a new category of inorganic PS will be presented with promising results on cancer cell destruction.

Key-words: phototherapy dynamic, photosensitizer, cancer management, nanotechnology

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41 **1. Introduction**

42 Phototherapy in its ancestral form has been used empirically in Chinese, Indian, Egyptian and
43 Greek civilizations. Simple photosensitizer-free phototherapy was performed mainly using the
44 beneficial effects of sunlight, for example in the prevention of local skin disease (Daniell and
45 Hill, 1991). Light is the source of many therapies among which we find photodynamic
46 therapy (PDT), which requires oxygen (Rkein and Ozog, 2014). PDT is a concept based on a
47 selective activation by light of drugs called photosensitizers (PS) (Gomes et al., 2018). These
48 drugs, non-toxic in the absence of excitation light have the ability to absorb light energy,
49 leading to an energy transition and transfer to the surrounding environment. This transfer
50 leads to reactive oxygen species (ROS) production, such as singlet oxygen, responsible for
51 cell destruction (Donohoe et al., 2019) and to luminescence properties (Celli et al., 2010). It is
52 accepted that the main agent responsible for the PDT effect is singlet oxygen (Fig. 1), even if
53 other agents are involved (Ochsner, 1997). Due to their very short shelf life and low diffusion
54 in biological media, the reactivity of ROS will be limited to their intracellular localization.
55 Using a localized light irradiation, the selectivity of PDT action allows to lyse cells while
56 preserving healthy cells. Light sources used can be lamps, fluorescent tubes, lasers, and more
57 recently electroluminescent diodes, possibly fitted with optic diffusers (Brancaleon and
58 Moseley, 2002). The fluorescence properties of PS are also an advantage allowing
59 visualization of patient tissues during PDT coupled with surgery. Oxidative stress induced by
60 PDT causes cell death by necrosis or apoptosis and can produce a variety of effects on
61 immune cells (Agostinis et al., 2011). The type of PS, its concentration, its subcellular
62 localization as well as the dose of light applied influence the effects of the photodynamic
63 treatment (Ratkay et al., 2000).

High dose PDT (i.e. high levels of PS and / or light) acts directly on tumor cells and indirectly by destruction of the vascular endothelium following the release of tumor antigens and activation of anti-tumor immune response (Agostinis et al., 2011). In contrast, low dose PDT therapy may have immunomodulatory effects capable of treating different models of autoimmune diseases or inflammatory disorders, while keeping cell viability intact (Doix et al., 2019). The immunosuppressive effect of PDT was first noticed in 1986, with the suppression of a contact hypersensitivity reaction in mice previously exposed to a hapten, one of the two building blocks of an antigen (Elmets and Bowen, 1986).

2. Principles of photodynamic therapy

PDT is a technique whose biophysical principles were experimentally established 130 years ago by Oscar Raab (Daniell and Hill, 1991; Raab, 1900). Preliminary work from Tappeiner and Jesoniek showed that porphyrins were preferentially picked up by cancer cells, causing red fluorescence when exposed to light with appropriate wavelength. Porphyrins were also able to interact with light energy causing reactions leading to cell death (necrosis, apoptosis) (Tappeiner and Jesoniek, 1903). Thus, PDT consists in sensitizing cancerous tissues to the action of light by the prior injection of a photosensitizing molecule preferentially captured and retained in cancerous tumors. Dougherty *et al.* made their first application in digestive cancerology with hematoporphyrin derivative in 1978 (Dougherty et al., 1978). Following intravenous injection, the inactive PS will reach and be captured preferentially by cancerous tumors for various reasons partly linked to the histopathological texture of the cancerous tissue (vascular and lymphatic richness, large interstitial sector and abundance of macrophages), the hydro and liposolubility of the PS facilitating its penetration into neoplastic cells (Chen et al., 2017). Then, the exposure of the tumor to colored laser-like light will cause a photo activation defined by the change of state of the chromophoric molecules induced by

photons. By light absorption, the molecules acquire energy and pass from a S_0 ground state to different excited states (S_1 , S_2 ...), the first state called “singlet” (S_1) is occupied directly or after very rapid partial non-radiative deactivation of the higher states (Fig. 2). The excited molecule has an excess of energy that will be released spontaneously either in the form of heat or light. Two pathways of de-excitation by emission of light can be distinguished: i) a quick de-excitation (order of nanoseconds) from the lowest singlet excited state S_1 leading to a fluorescence (photo diagnosis) ii) a slow de-excitation (order of microseconds), from an intermediate state known as “triplet” T_1 responsible for the phenomena of photosensitivity. The PS can return to the fundamental state either by the emission of light or by transmitting the excess of energy to molecular oxygen in its triplet ground state (3O_2) which is thus excited affording singlet oxygen (1O_2). Then, the PS returns to its initial state and is ready for a new collection of light energy. From the triplet state, in addition to singlet oxygen, free radicals such as superoxide and hydroxyl are also formed (Chen et al., 2014). Although the entire mechanism is not fully known, it seems that singlet oxygen and, to a lesser extent, free radicals are the main cytotoxic agents. PDT-induced cytotoxicity is therefore mainly due to oxidation phenomena leading to the degradation of amino acids, nucleic bases, lipids composing cell membranes and mitochondria (Moan et al., 1998). PDT-induced cytotoxicity is influenced by oxygen concentration in the tumor tissue, anoxic areas may exist within a tumor explaining the unequal and partial efficacy of the treatment requiring additional exposure (Weston and Patterson, 2014).

2.1. Mechanisms of photodynamic reaction

Photo-oxidation is the basic process of the photodynamic effects which are actually made up of many chemical and biological reactions. In 1976, Weishaupt *et al.* associated the photo

inactivation of tumor cells to the singlet oxygen action as cytotoxic agent (Weishaupt et al., 1976). Photo-oxidation can be divided into two stages which are presented below.

2.1.1. Passage of the photosensitizer from the S_0 fundamental state to the T_1 excited triplet state following light absorption

The photoreactive molecule is initially in the S_0 fundamental state. Following the absorption of one photon, it will first find itself at a higher S_1 singlet type energy level. From this level, it can return to the ground state following different pathways. The de-excitation can be either non-radiative in the form of heat or radiative in the form of light which is called photoluminescence. On the one hand, when the decay is done preserving the multiplicity of spin (S_1 to S_0 transition) the emission of light is called fluorescence. This emission is fast in the order of nanoseconds. On the other hand, an intersystem conversion between the singlet state S_1 and the triplet state T_1 can be possible for which the multiplicity of spin is not preserved. Although this non-radiative transition is normally prohibited, the PS on triplet T_1 state can then either return to the S_0 ground state emitting light through a slow phosphorescence process (in the order of microseconds) or participate in the second stage of the photo-oxidation reaction. It must be remembered that from S_1 singlet state level, the cross-system conversion from S_1 to T_1 is not the only type of transition carried out as we can for example observe fluorescence phenomena (Fig. 2). For the photodynamic effect to be significant, it is necessary that the efficiency of the PS inter-system conversion from S_1 to T_1 is higher to the effectiveness of other S_1 deactivation processes. Thus, the quantum yield of the intersystem crossover from S_1 to T_1 , noted Φ and defined as the ratio of the number of reacting molecules according to the photo-oxidation process on the number of photons absorbed by the system during the same time, will have to be high enough to allow the T_1

triplet state to be populated effectively and others deactivation processes will then constitute secondary phenomena (Castano et al., 2005; Robertson et al., 2009).

2.1.2. Sensitized photo-oxidations from T₁ triplet photosensitizer

The PS triplet state T₁ is very reactive and presents two possible modes of interactions, either directly with the molecules of the tissue where it is found, or with the molecular oxygen in the triplet state (³O₂) (endogenous and exogenous) present in the media. Consequently, there are two types of reactions for this stage, type I and type II reactions (Foote, 1991).

2.1.2.1. Type I reaction

In this case, PS in the triplet state generates radicals that react directly with O-containing substrates basically by electron transfer. This process leads to the production of ROS (OH[•], H₂O₂ ...). One of the most common reaction uses molecular oxygen as a substrate affording superoxide anion radical (O₂^{•-}). The initiated cascade of reactions leads to the oxidative stress resulting in the destruction of cancer cells (Braun et al., 1986; Kwiatkowski et al., 2018; Luksiene, 2003; Nowak-Stepniowska et al., 2013).

2.1.2.2. Type II reaction

In this case, the PS in the triplet state T₁ will transfer its energy to an oxygen molecule that will pass from the fundamental state triplet ³O₂ to the excited singlet state ¹O₂ (Braun et al., 1986; Nowak-Stepniowska et al., 2013). The singlet oxygen formed ¹O₂ will then interact with different electron-rich organic molecules that will oxidize and cause damage to the organelles of the cell such as the plasma membrane, mitochondria, lysosomes and nucleus, giving rise to tumor necrosis (Dougherty et al., 1998; Kirakci et al., 2019).

In conclusion, PS and light are the initiating agents of a series of various chemical reactions resulting in the production of oxygenated free radicals leading to the tumor destruction. To fight against this photo-oxidation and the increase in oxidant compounds, the cell has several defenses to maintain the $O_2^{\cdot-}$ level and to prevent its conversion to OH° , such as superoxide dismutase enzyme (SOD) that will eliminate $O_2^{\cdot-}$ to form H_2O_2 and O_2 . Catalase, an enzyme localized in peroxisomes catalyzes disproportionation of H_2O_2 to O_2 and H_2O . Likewise, glutathione peroxidase, an enzyme present in mitochondria and in the cytosol of cells reduces H_2O_2 and organic peroxides formed (Ochsner, 1997). However, if SOD is stable when facing photosensitization, glutathione peroxidase and catalase are much less stable and will be greatly degraded allowing an increase of oxidizing agents in the cell and therefore its gradual destruction (Castano et al., 2005).

3. Mechanisms of cell photo-destruction

PDT is therefore based on several elements such as tumor area previously charged with PS, photons exposure, PS activation, production of reactive oxygen species and concentration in molecular oxygen. These ROS are responsible for cell destruction by the following mechanisms.

3.1. Role of singlet oxygen

Singlet oxygen 1O_2 is formed by the combined action of a PS, light and oxygen; It is a powerful oxidant which reacts with many cellular components such as membrane (phospholipid bilayer and cholesterol), amino acids (histidine, tryptophan, and methionine) and nucleic bases (guanine). Due to its very short lifetime (0.01 to 0.04 μs) and its high reactivity, 1O_2 acts directly on tumor area and does not have time to diffuse further off-sites (Vrouenraets et al., 2003).

Photo-cytotoxic activity will therefore depend on several factors:

- quantum yields of S_1 state formation
- conversion yields from S_1 to T_1
- efficiency of energy transfer from PS T_1 state to 3O_2
- PS localization and subcellular concentration

PS used in PDT have the ability to focus mostly in tumor cells (Dellinger, 1996; Dos Santos et al., 2019), this seems to come from tumor with larger permeable neo-blood vessels and an increase in the number of LDL (Low Density Lipoproteins) receptors. PS are usually small molecules, but they are bind to plasma LDL and therefore react like macromolecules which can then bind to larger receptors number in tumor cells (Cruz et al., 2013).

The destruction of tumor cells is done by photodestruction following direct mechanisms such as necrosis or apoptosis or indirect mechanisms such as vascular damage or immune response (Kessel, 2019; Kessel and Oleinick, 2018, 2010).

3.2. Direct mechanisms

The direct cellular photodestruction mechanisms combine two categories, necrosis and apoptosis. This direct cell damage depends on factors such as the dose, the location of the PS in the cell and the cell line.

3.2.1. Necrosis

Necrosis is a non-programmed death cell. In PDT, this cellular reaction is caused by ROS (type I reaction) and by singlet oxygen (type II reaction). Necrosis leads to an increase of permeability and a loss of integrity for cell membranes. The cellular content also diffuses into the surrounding tissues causing inflammatory reactions (Kessel and Oleinick, 2018).

3.2.2. Apoptosis

Apoptosis or programmed cell death, is a normal programmed cellular mechanism for which cells self-destruct in response to a signal. This phenomenon results in the death of individual cells, in certain places, at a specific time. The body uses apoptosis mechanism to get rid of unusable, unwanted, or potentially harmful cells. When using PDT, ROS and singlet oxygen can cause mitochondrial apoptosis or "death receptors" by activating caspases proteins (Kessel, 2019).

3.3. Indirect mechanisms

These mechanisms of indirect cellular damages include targeted reactions to tumor vascular tissue as well as inflammatory responses and immune systems.

3.3.1. Indirect damages to the tumor vascular tissue

As PS are mainly hydrophobic, they bind to plasma LDL and therefore accumulate at the level of LDL receptors in the proliferating endothelial tissue in the case of tumor tissue. With this accumulation in the neo-vessels and after PS activation, vascular damage occurs around the tumor causing cells death by oxygen and nutrients depletion (Huang et al., 2008; Krammer, 2001).

3.3.2. Indirect damages due to inflammation and the immune response

When activated PS makes damages to tumor cells, there is a release of inflammatory mediators. A local inflammatory reaction occurs with an influx of neutrophils, mast cells, monocytes and macrophages. Subsequently these macrophages can serve as antigen presenting cells recognized by lymphocytes which opens the way for immunologic therapy or even the possibility of on-site vaccination (Castano et al., 2006; Reginato et al., 2014).

4. Photosensitive materials

The choice of PS is very important to allow an effective photosensitization. Thus, the photosensitizing compound must meet the following general criteria (Braun et al., 1986; Kwiatkowski et al., 2018):

- the absorption domain of the PS must be located in the visible or UV and must imperatively be located in a spectral region where the compound to be oxidized does not absorb, to avoid unwanted side reactions.
- the PS must be inert towards oxygen singlet $^1\text{O}_2$ and compounds to be oxidized so it will not participate in photochemical reactions.
- the energy of the T_1 triplet state of the PS must be greater than that of singlet oxygen $^1\text{O}_2$ so that the transfer energy is efficient.
- the lifetime of the T_1 triplet state must be long enough to allow a good interaction between the PS and oxygen in the triplet state $^3\text{O}_2$.
- the quantum yield of the conversion from S_1 to T_1 (ϕ) must be the largest possible to allow good stand of T_1 triplet state thus allowing other deactivation processes to be of minor importance.

Whatever the type of PS, these conditions must be respected in order to allow an effective PDT. In addition to these requirements, the products used in PDT must have certain properties more specific to photochemotherapy such as few side effects and a specificity oriented towards type II reaction generating singlet oxygen and therefore ROS. Currently, PS can be classified into three generations (Kwiatkowski et al., 2018).

4.1. Classification of photosensitizers

4.1.1. First generation photosensitizers

In the 1960's, Schwartz developed a mix based on hematoporphyrin (Hp) and derivatives (Davis and Schwartz, 1967; Modell and Schwartz, 1964a, 1964b; Sternberg et al., 1998). This preparation, whose composition was often variable, was called hematoporphyrin derivatives. Lipson and Baldes have used hematoporphyrin derivatives in combination with other porphyrins, protoporphyrin (PpIX) and uroporphyrin and their irradiation with light to treat breast cancer successfully. This was really the beginning of photochemotherapy based on the use of porphyrins (Lipson and Baldes, 1960). At the end of the 20th century, various preparations of hematoporphyrin derivatives have been used in many clinical trials. In 1998, Sternberg *et al.* developed Photofrin II[®], an ultrafiltrate of hematoporphyrin derivatives (Sternberg et al., 1998). Subsequently, the Canadian QLT laboratories synthesized Photofrin[®], a mixture resulting from numerous purifications and considered as the gold standard of PDT. Photofrin[®] is the trade name of porfimer sodium which is a mixture of oligomers forming dimers and trimers connected by ester or ether bonds. The determination of the active fraction of this product was not easy, many works and studies were conducted for this purpose (Tsukagoshi, 1995).

It seems that only the dimers and trimers with ether bonds are exhibiting effective photocytotoxic activity, as ester bonds are instable under physiological conditions (Pandey et al., 1988). In addition, ether bonds are destroyed in acidic media like tumor pH, it is therefore probable that these ether-bridged dimers are cleaved into monomers when they enter the tumor cells (Pandey et al., 1990).

Although Photofrin[®] has effective photocytotoxicity, it also exposes to certain limits such as:

- a strong accumulation in the skin causing important side effects up to six weeks after treatment.

- an absorption and photoactivation wavelength near 630 nm, the wavelength range at which endogenous tissues chromophores in particular oxyhemoglobin will also absorb light thus reducing Photofrin[®] photoactivation. In addition, light scattering within the tissue will also limit Photofrin[®] tissular penetration (Miller, 1999).

These limits of Photofrin[®] led to the numerous works and studies to discover new PS products from the 1980's. One of the objectives of this research is to find compounds absorbing at wavelengths in the red or even in the near infrared to avoid interference mentioned above and to improve the penetration of light into the cancerous matrix therefore extending PDT to deeper tumor treatments.

4.1.2. Second generation photosensitizers

Second generation PS have been developed to overcome the disadvantages of hematoporphyrin derivatives. These synthetic molecules, with a defined chemical structure, have an optimized light absorption spectrum allowing biggest size tumors treatment, but have no selectivity towards tumor cells. Several compounds have obtained a marketing approval or are currently being evaluated. They are activated with a light at a wavelength between 600 and 800 nm, allowing an increase in the light penetration depth in the tissue. Besides, these are pure compounds, quickly eliminated by tissues, thus limiting the period of skin photosensitization (Nowak-Stepniowska et al., 2013). Currently, the second generation photosensitizers group includes 5-aminolevulinic acid (Effala[®], Ameluz[®], Gliolan[®], Alacare[®]) (Fukuhara et al., 2020), methyl-aminolevulinate (Metvix[®], Metvixia[®]), meso-tetrahydroxyphenylchlorin (Foscan[®]) (Posadzki and Car, 2018), benzoporphyrine derivatives (Visudyne[®]) (Di Nicola et al., 2020), rotoporphin (Purlytin[®]) (Hunt, 2002), padeliporfin (Tookad[®]) (Fukuhara et al., 2020), mono-L-aspartylchlorine e6 (Laserphyrin[®], Aptocine[®])

(Aizawa et al., 1987), phthalocyanines (Rak et al., 2019), lutetium texaphyrin (Lutex[®], Lutrin[®]) (Young et al., 1996), lutetium motexafin (Antrin[®]) (Chen et al., 2001), gadolinium motexafin (Xcytrin[®]) (Evens, 2004), methylene blue (Dos Santos et al., 2017) and rose bengal (Dini et al., 2010).

4.1.3. Third generation photosensitizers

Second generation PS are not very specific for tumor cells, which is a limiting factor for the implementation of an effective and selective PDT. Several studies were performed to develop vectorized PS using chemical species recognized specifically by one type of tumor cell. One of the axis of research on new PS consists in coupling a PS already synthesized with monoclonal antibodies to enhance PS affinity for the tumor cells and therefore to improve targeting while reducing side effects. For example, there are studies on new modified texaphyrins (Arambula et al., 2011; Preihs et al., 2013), new vectors by chemical coupling with small peptides (Johnson et al., 2014, 2013), estradiol-derived groups (El-Akra et al., 2006),...

All cellular pathways expand targeting and uptake of PS by receptor-target interaction. The main research lines focus on PS glycoconjugated, glycosylated or conjugated with estradiol to improve active targeting of tumor cells, for example the targeting of estrogen receptor alpha (Swamy et al., 2006). Another line of research is studying passive targeting of tumor cells by vectorization of PS using liposomes, nanoparticles (NPs) as well as emulsions or micelles, allowing PS to reach tumors at much higher concentrations (Chen et al., 2001; Dollo et al., 2020; Evens, 2004; Rak et al., 2019; Young et al., 1996).

4.1.4. Inorganic photosensitizers

Among new PS currently studied we can cite nano-sized metal clusters, aggregates of atoms of transition metals interconnected by metal-metal bonds like hafnium (Liu et al., 2016), niobium (Ivanov et al., 2020) or molybdenum (Jackson et al., 1996). In particular, hexanuclear molybdenum halide cluster complexes with the general formula $(\text{Mo}_6\text{L}^{\text{a}}_8\text{L}^{\text{i}}_6)^{2-}$, L^{a} =halogen; L^{i} =halogen or organic ligands (Fig. 3) have been widely studied due to their photo-physical and photo-chemical properties (Kirakci et al., 2018, 2012; Solovieva et al., 2016). They have interesting optical, magnetic, electrical and catalytic properties currently used in many fields such as electronics (as insulators or metallic conductors) but also have interesting potential for biotechnology and nanotechnology (optics, imaging, and theranostics). This combination of a drug and a diagnostic test is called theranostics (Lee et al., 2012). Indeed, their possibility of generating singlet oxygen after activation is a real advantage.

4.2. Current clinical applications

PDT has reached its climax in the 1980's when multiple clinical teams, primarily in the United States, Canada and Japan, have shown its effectiveness in destroying usually inoperable tumors (Dougherty, 1984). However, despite marketing authorizations (Photofrin®, 1997; Foscan®, 2002), clinical sites using these techniques are unusual. Among the reasons advanced we can cite the lack of knowledge of pathologies eligible for PDT. However, companies continue to invest in this area and we can mention the launching on the market of methyl-aminolevulinate (Metvixia®) and 5-aminolevulinic acid (Effala®) in dermatology (Pariser et al., 2003). In ophthalmology, PDT using benzoporphyrine derivatives (Visudyne®) experienced a strong enthusiasm in treatment of patients with neovascular form (still called wet or exudative and characterized by an abnormal growth of blood vessels) of age-related macular degeneration (Brown et al., 2005). However, since 2006, intravitreal

injections of bevacizumab (Avastin[®]), a humanized monoclonal antibody with anti-angiogenic activity, have dramatically reduced the use of PDT.

In urology, the French company Steba-Biotech has developed a new PS, padeliporfin (Tookad[®]) for the treatment of prostate cancer (Azzouzi et al., 2013). Computer-controlled planning combined with real-time dosimetry is an essential research route to facilitate application, reduce local toxicity and increase anticancer efficacy. However, the concept of partial treatment or focal therapy for prostate cancer is recent and still controversial in the urological community due to the frequency of tumor multifocality. If the functional results of the first clinical trials are encouraging, evidence of long-term cancer efficacy is pending.

In gastroenterology, PDT benefits from a renewed interest due to the development of diode lasers (which advantageously replace the first dye lasers pumped by argon laser) and to the demonstration of its effectiveness i) for the treatment of superficial esophageal cancers in patients inaccessible to another treatment, with a post-radiotherapy recurrence (Wu et al., 2019), ii) for severe dysplasia in Barrett's mucosa (Panjehpour and Overholt, 2006), and iii) for inoperable cholangiocarcinoma, with the aim of prolonging the survival of patients receiving palliative biliary drainage (Gao et al., 2010).

In gynecology, the value of PDT was shown in the treatment of high grade cervical dysplastic lesions after application of a 5-aminolevulinic acid solution (5-ALA) (Hillemanns et al., 2014; Matoba et al., 2018). The PDT applied to the endometrium seems promising, particularly in the treatment of functional menorrhagia in the premenopausal period. Use of a local PDT after 5-ALA intra-uterine instillation has shown that this could be an alternative to the surgical treatment currently offered.

In neurosurgery, several studies reported encouraging results in the clinic using interstitial photodynamic therapy (iPDT) to treat first-line non-operable glioblastomas (brain tumors) or their recurrences. Survivals greater than two years following iPDT have been reported while

the median survival in the event of recurrence is estimated to be three months (Beck et al., 2007).

In pneumology, the number of studies on the treatment of lung cancer is still limited and the place of PDT in therapeutic arsenal remains to be investigated (Moghissi and Dixon, 2003).

On the other hand, PDT appears to be a promising treatment for malignant pleural mesothelioma (MPM) or primary cancers pleura. Thus, PDT has been tested in phase I and II clinical trials for MPM patients in combination with extra-pleural pneumonectomy or pleurectomy/decortication followed by intravenous chemotherapy. The first work carried out by Professor Friedberg's team (University of Pennsylvania, Philadelphia, USA) has shown promising results with median overall survival 31 months (Du et al., 2010).

In dermatology, PDT is gradually becoming the standard treatment for actinic keratosis (precancerous skin lesions), basal cell carcinomas superficial and Bowen's disease (intra-epidermal cancer, *in situ*). It is efficient, relatively easy to perform (although progress is expected on the sources of light), well tolerated and inexpensive, generating no aesthetic consequences. In the literature, response rates up to 90% are reported for PDT of actinic keratosis treatment (Morton et al., 2013). The growing interest in this technique is confirmed by the recent marketing of products competing with methyl-aminolevulinate (Metvix®), such as 5-aminolevulinic acid (Ameluz®) developed by the Biofrontera company (Reinhold et al., 2016). The different drugs actually on the market for photodynamic therapy is presented (Table. 1).

5. Areas of improvement for PDT

5.1. Irradiation improvement

5.1.1. Biphotonic excitation

407 The use of a classic PDT excitement, i.e. based on the excitation of the PS by absorption of a
408 photon has the drawback of requiring the use of excitation wavelengths located most often in
409 the visible, which results in a low penetration of light into the tissues, i.e. less than 5 mm. The
410 spectral window operable in biological media is between 700 and 1100 nm due to the weaker
411 diffusion in this area and also the lower absorbance of biomolecules such as melanin,
412 hemoglobin and water. Two photons excitation PDT (2PE-PDT) can be interesting to excite a
413 PS with classic activation in the visible with two processes, i.e. two-photon absorption (TPA)
414 and up-conversion (Sivasubramanian et al., 2019). TPA is a nonlinear optics phenomenon
415 based on simultaneous absorption of two lower energy photons (and therefore of longer
416 wavelength) to lead to the excited state (He et al., 2008). This TPA approach has also shown
417 its interest in studies carried out *in vivo* (Collins et al., 2008). The advantages of this
418 technique compared to the classic PDT results in a better light penetration in a biological
419 environment and an important spatial selectivity, minimizing the risk of accidental destruction
420 of healthy tissue. It is however necessary to rationally design new PS with sufficiently large
421 TPA cross section, having good solubility in biological aqueous media, good photostability
422 and safety in the absence of light and ability to cross biological membranes. The molecular
423 two-photon cross-section $\sigma^{(2)}$ is usually quoted in of Goeppert-Mayer unit (GM) where 1 GM
424 is $10^{-50} \text{ cm}^4 \cdot \text{s} \cdot \text{photon}^{-1}$, resulting from the product of two areas (one for each photon, each in
425 cm^2) and a time (within which the two photons must arrive to be able to act together).
426 Recent studies have demonstrated the potential of supramolecular buildings based on
427 porphyrins, leading to huge $\sigma^{(2)}$ values, typically of several orders of magnitude greater than
428 those of the former PS. Arena-Ru (II) complexes have shown their potential interest in the
429 treatment of cancers and metastasis (Scolaro et al., 2005), where Ru (II) polypyridyl
430 complexes have attracted great attention as potential PS in PDT. Their stability and chemical
431 inertness in solution, possible engineering for the modularity of their photophysical properties

such as TPA, as well as the relatively long shelf life of their excited state triplet MLCT (Metal-to-Ligand Charge Transfer) (Girardot et al., 2007) give them clear advantages as potential PS in excited two-photon PDT. Furthermore, hybridized functionalized silica nanoparticles by molecular PS presenting strong TPA responses were also developed. These nano-objects have demonstrated strong potential *in vivo*, in two photon excitation PDT (Gary-Bobo et al., 2011).

5.1.2. X-ray excitation

Nanoparticles can take advantage of their physical and chemical particular properties to envisage an innovative therapeutic strategy which can combine PDT and radiotherapy via the use of nanoparticulate scintillators excitable by X-rays (Kamkaew et al., 2016). The concept consists in using scintillator loaded nanoparticles which, excited by X-rays, can in turn emit photons reabsorbed by the PS itself, grafted onto the NP matrix. It is therefore no longer necessary to use an external light source to generate the photodynamic effect. This concept was demonstrated by Chen and Zhang in 2006 (Chen and Zhang, 2006). Recent publications show that this kind of nanoscintillators allows to consider the use of nanoparticles for PDT, excitable by radiotherapy (Sun et al., 2020). Recently, a new generation of inorganic PS based on octahedral molybdenum cluster complexes have proven their ability to produce ROS after exposure to X-rays (Kirakci et al., 2018). This feature eliminates energy losses during energy transfer from the scintillating nanoparticles to the surrounding PS occurring in the original systems (Chen and Zhang, 2006), and considerably simplifies the PS architecture. Upon excitation with light, they form long-lived triplet states that relax via red-NIR luminescence. In another recent application, Kirakci et al. showed that for a new water-soluble molybdenum cluster complex with iodine inner ligands and carboxylated apical ligands bearing ethylene oxide groups, the X-ray-induced toxicity on cancer cells as well as the *in vivo* toxicity were

low or moderate, confirming the pertinence of octahedral molybdenum cluster complexes as singlet oxygen radiosensitizers for X-PDT (Kirakci et al., 2021). These findings could be a source of considerable ramifications in the treatment of cancers.

5.2. Nanotechnology

A major challenge in cancer therapy is to override body's defense mechanisms to bring the drug to its biological target (Sanhai et al., 2008). The question is to limit non-specific effects while ensuring a high concentration in the tumor. Some compounds are under investigation, obtained by conjugation of a PS to a tumor addressing molecule (sugars, peptides and ligands). However, one of major issue for PS remains their hydrophobicity which promotes aggregation in biological media and limits their bioavailability. One solution could be to encapsulate the PS in nanoparticles to improve its bioavailability (Shi et al., 2017). They have the advantage of increasing PS penetration in tissues and cells as well as the efficacy of PDT (Mallidi et al., 2016). It is also possible to combine a PS and a labeling agent within the same nanoparticles. Nanovector systems, which ensure good drug protection and targeting, are among the most relevant candidates to become the future generation of anti-cancer vectors (Zhao et al., 2020). Liposomes are an effective vectorization strategy for PDT (Derycke and de Witte, 2004). However, these synthetic vectors must be designed very carefully to overcome biocompatibility issues of the carrier and protection of the drug. Ratajczak *et al.* developed a strategy using cell microvesicles as a platform for transport. After their isolation, PS are loaded in these vesicles by co-incubation using different concentration gradient. In the case of hydrophobic compounds, the internalization could be reached by a simple passive diffusion process (Susa et al., 2019). The idea is to take advantage of a natural transportation system to design a biogenic nanovector, i.e. a cell-derived nanovector. These microvesicles, naturally released by most cells under physiological conditions or under the effect of different

stimuli, circulate freely in the organism where they act as natural carrier of cellular material. PS can use these intercellular taxis to travel from one cell to another (Ratajczak et al., 2006), thus internalized by cells, they can come out through these microvesicles, which will then be picked up by other cells. It is also possible to co-internalize PS and magnetic NPs, making of these microvesicles true multifunctional vectors capable of being detected by MRI (Magnetic Resonance Imaging) and manipulated or guided by magnetic forces and following release into tumor cells, to couple PDT and treatment by magnetic hyperthermia. This type of stealth nanoplatform is therefore part of a theranostic anticancer approach (Silva et al., 2015).

Preliminary studies for the formulation and evaluation of NPs of organic copolymer of lactic and glycolic acid (PLGA) containing several inorganic molybdenum clusters have been carried out (Brandhonneur et al., 2018; Dollo et al., 2020). PLGA nanoparticles containing $\text{Mo}_6\text{I}_8 (\text{OCOC}_2\text{F}_5)_6$ cluster (CMIF) in the form of cesium salt were prepared by a solvent displacement technique. Cell viability was evaluated *in vitro* on the A2780 ovarian cancer cell line after treatment by the free or encapsulated cluster in NPs for concentrations varying from 5 to 100 $\mu\text{g} / \text{ml}$ and for different incubation times (24, 48 and 72h). A strong photo-toxicity was obtained for CMIF loaded nanoparticles with a half maximal inhibitory concentration (IC_{50}) value near 1.8 μM (Brandhonneur et al., 2020). Preliminary work therefore presents the octahedral clusters of molybdenum as new PS for PDT of ovarian cancer and suggest that PLGA polymer nanoparticles constitute a delivery system suitable for targeting cells tumor. Additionally, Vorotnikov et al. used targeting antibodies C7b and trastuzumab for grafting of Mo_6 -containing silica nanoparticles for *in vivo* photodynamic treatment of cancer on xenografted mice (Vorotnikov et al., 2020).

Conclusion

Many second or third generation photosensitizers are cited in the literature, but very few are approved for clinical use. The most recent developments are oriented towards the design and study of PS targeted towards several cancers according to their biological characteristics. A second line of research, in full swing, consists in selectively destroying the neo-vessels supplying nutrients to the tumor in order to eradicate them by privation of energy or oxygen resources. One of the only real drawbacks of PDT is the low penetration of light in the tissues as well as the relatively low specificity of this method. However, ways of improvement are in development (X-ray, nanosystems ...). In conclusion, the combination of nanotechnology and new inorganic PS is a promising technique to optimize PDT treatment of tumors.

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844 **Table 1:** Summary of the different drugs on the market for photodynamic therapy. EMA: European Medicine Agency, FDA: U.S. Food and Drug
845 Administration, MHLW: Japanese Ministry of Health, Labour and Welfare. (*): drug withdrawn from the market

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Photosensitizers	Classification of photosensitizer	Proprietary Names and Owner	Pharmaceutical form	Marketing authorisation	Pathology
Porfirmer Sodium	First generation	Photobarr [®] (Pinnacle)	injectable solution	2004 (EMA)*	Lung and esophageal cancer
		Photofrin [®] (Axcan)	injectable solution	2003 (FDA)	
5-Aminolevulinic Acid	Second generation	Alacare [®] (Galderma)	medicated plaster	2011 (EMA)	Moderate actinic keratosis, basal cell carcinoma
		Ameluz [®] (Biofrontera)	gel	2011 (EMA) 1999 (FDA)	
		Effala [®] (Galderma)	medicated plaster	2011 (EMA)	
		Gliolan [®] (Medac)	powder for oral solution	2007 (EMA) 1999 (FDA)	Malignant glioma
Hexaminolevulinate Hydrochloride		Hexvix [®] (Ipsen)	powder for intravesical solution	2013 (EMA) 2010 (FDA)	Diagnosis of bladder cancer, Treatment of cervical dysplasia
Methyl-aminolevulinate		Metvix [®] (Galderma)	cream	2011 (EMA)	Moderate actinic keratosis, basal cell carcinoma
		Metvixia [®] (Galderma)	cream	2011 (EMA) 2004 (FDA)	
Meso-tetrahydroxyphenylchlorin (temoporfin)		Foscan [®] (Biolitec Pharma)	injectable solution	2001 (EMA)	Advanced head and neck squamous cell carcinoma
Verteporfin		Visudyne [®] (QLT)	powder for injectable solution	2000 (EMA) 2002 (FDA)	“Wet” form of age-related macular degeneration (AMD), choroidal neovascularisation caused by pathologic myopia
Padeliporfin		Tookad [®] (STEBA Biotech)	powder for injectable solution	2017 (EMA)	Prostate cancer
Mono-L-aspartylchlorine e6 (Talaporfin)		Laserphyrin [®] (Meiji Seika)	injectable solution	2004 (MHLW)	Early-stage lung cancer

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848 **Figure 1.** Mechanism of the photodynamic reaction and biological effects

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Figure 2. Modified Jablonski diagram showing the formation of singlet oxygen.

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861 **Figure 3.** General structure of hexanuclear molybdenum halide cluster unit ($Mo_6L^i_8L^a_6$) where L^i and L^a represent inner and apical ligands
 862 respectively and A^+ represents the counter cation, from (Dollo et al., 2020)

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