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Targeted Photodynamic Therapy for Improved Lung Cancer Treatment

Anine Crous and Heidi Abrahamse

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Abstract

Cancer develops from the outgrowth of a clonal population of cells with a genetic pathology to evade cell death and exponential proliferation. It has become a global burden with increasing mortality rates. Lung cancer is a major contributor to cancer fatalities. Conventional therapies have shown advances in treating lung cancer, but the successful eradication of cancer lies in targeting both cancer and cancer stem cells. Cancer stem cells (CSCs) are a ration of cells found within the tumour bulk, capable of cancer initiation, therapy resistance, metastasis and cancer relapse. Photodynamic therapy (PDT) has proven effective in treating lung cancer. PDT exerts selective cell death mechanisms toward cancerous cells. With the use of a photosensitizer (PS) which becomes excited upon irradiation with laser light at a specific wavelength, the PS forms reactive oxygen species (ROS) in turn killing neoplastic cells. Leading therapeutic sequel can be obtained by transcending PDT though combination therapies such as immunotherapy and nanotechnology which will enable PDT to target lung CSCs preventing lung cancer recurrence.

Keywords: lung cancer, lung cancer stem cells, PDT, targeted PDT

1. Introduction

Cancer is a global burden affecting millions of people. The yearly death toll for cancer surpasses AIDS, tuberculosis and malaria combined [1]. Cancer is characterised by mutational development of cells that lead to uncontrolled cell proliferation and tumour formation [2]. Tumours are classified according to tissue type and origin [3]. Lung cancer is one of the most frequently diagnosed diseases, having the highest fatality rate amongst all cancers [1]. Carcinoma of the lung arises due to risk factors; such as smoking, corrosive chemical inhalation and air pollution; leading to accumulated mutations of normal lung tissue. These mutations cause

genetic modifications that can alter cell cycle regulation, leading to increased cell proliferation, tissue invasion, tumour formation and metastasis [4]. Lung cancer treatment options are: chemotherapy, radiation, surgery, targeted and immunotherapy [5].

It has been hypothesised that a small group of cells residing within a tumour are responsible for tumour initiation and development. These cells; called cancer stem cells (CSCs); arise from normal stem cells (SCs) that have acquired several mutations, due to their extended life span as compared to differentiated cells [6]. Dysregulation of pathways controlling SCs are seen in CSCs, which lead to exponential cell proliferation, evasion of apoptosis, infinite replication capacity, angiogenesis, metastasis and immune response evasion [7]. CSCs have been identified and characterised using various biochemical assays and techniques. Lung CSCs with tumorigenic potential have been identified [8]. They can be characterised and isolated using CSC identification methods [9, 10]. Due to the identification of CSCs in lung cancer it has become apparent to re-evaluate and develop target specific therapies for lung cancer. Evidence suggests that conventional therapies fail in complete cancer eradication due to lung CSCs and their abilities of drug efflux, treatment resistance and metastasis.

Photodynamic therapy (PDT) is a low cost, minimally invasive therapeutic model that has previously been used for lung cancer treatment. PDT uses a non-toxic photochemical dye/ photosensitizer (PS) that is administered orally or intravenously and absorbed by the cancer. The dye localises in the cellular organelles, whereby upon activation by light at a specific wavelength causes cell death [11, 12]. Even though PDT has shown many successes treating lung cancer [12], there are still some complications that need to be addressed such as photo-sensitivity and low tumour selectivity [13]. New advancements addressing the complications seen in PDT have been made by developing a PS that is cell specific which can target CSCs in particular by using immunoconjugates and carrier molecules in the form of antibodies (Abs) and nanoparticles (NPs), respectively.

2. Cancer

2.1. Cancer

Malignancy or cancer is a term used for diseased cells. These cells characteristically evade cell death through rapid proliferation and can metastasize by travelling through the blood and lymphatic systems invading distant tissues [14]. Collectively, cancer has more yearly fatalities than diseases such as AIDS, tuberculosis and malaria. According to the International Agency for Research on Cancer the most frequently diagnosed cancers were lung (1.8 million, 13.0% of the total), breast (1.7 million, 11.9%), and colorectal (1.4 million, 9.7%). The most prevalent cancer-related fatalities included lung (1.6 million, 19.4% of the total), liver (0.8 million, 9.1%), and stomach (0.7 million, 8.8%) malignancies. Population growth and ageing affects the cancer related outcome. By 2030, it could be expected that there would be 27 million cases of cancer, 17 million cancer deaths annually and 75 million persons living with cancer within 5 years of diagnosis [1]. Cancer arises from progressive transformation of normal cells that encounter

genomic damages leading to mutations in their DNA sequence. Corruption of the DNA can be endogenous caused by errors in replication of DNA, the intrinsic chemical instability of certain DNA bases or from attack by free radicals generated during metabolism. Exogenous DNA damage can be caused by ionising radiation, UV radiation and chemical carcinogens. Although cells have the ability to repair unwanted changes in the genome, errors may occur leading to permanent mutations. Errors such as inactivation of regulatory genes maintaining genomic integrity facilitate additional mutations [15].

Tumorous cells can overpower their normal functioning neighbouring cells eventually forming a tumour as it overcomes normal regulation of cell growth leading to clonal evolution [2]. Neoplastic cells are self-sustainable, making them able to relocate to any space of the body and multiply. This is due to activation of certain enzymes, specifically telomerase, which is normally active only in SCs. Telomeres control cell death by shrinking during every mitoses until the cells eventually die. Therefore cancer cells are able to evade cell death through up regulation of telomerase as it avoids telomere shrinkage, preventing it from shortening leading to elongated telomeres. In addition, telomerase can prevent cell senescence and apoptosis [16].

Cancer classification is based on their tissue type and origin. Carcinomas encompass more than 80% of all cancer cases. These are cells that are epithelial in origin, and usually include breast, colon, prostate and lung. Carcinomas are subdivided into adenocarcinoma and squamous carcinoma [3].

2.2. Lung cancer

Lung carcinomas are neoplastic cells showing unrestrained development of mutated lung cells that are formed in the lung tissue lining the air passages. The mutated cells divide rapidly leading to tumour formation. As tumour formation progress, the numerous abnormal cells start undermining the lungs primary function preventing the lungs from providing the bloodstream with oxygen. Lung cancer can be categorised into two broad groups namely: Small cell lung cancer (SCLC), which is characterised by its neuroendocrine appearance. It encompasses 15% of lung cancer cases. Non-small cell lung cancer (NSCLC), accounts for the remaining 85% of cases. It is classified into subtypes including: adenocarcinoma (38.5%), squamous cell carcinoma (20%), and large cell carcinoma (2.9%). 52% of Patients have a 5 year expectancy when diagnosed with localised disease. Over 52% of patients with distant metastasis at diagnosis have a 5-year survival rate of 3.6% [17].

Regulatory circuits maintaining normal cell proliferation and homeostasis have defects in lung carcinoma. A multistep transformation is followed from a normal lung cell to malignant lung cancer phenotype, altered by a series of genetic and epigenetic modifications, leading to aggressive cancerous expansion. Subsequent to the primary cancer development, constant addition of genetic and epigenetic abnormalities follow during cancer proliferation, leading to tissue invasion, metastasis, and resistance to conventional therapies. Cancer prevention, early detection and treatment rely on the identification and characterisation of these molecular changes. Information on tumour characteristics and genetics will significantly advance prognosis and ideal treatment selection [18].

Contributing carcinogenic risk factors for lung cancer include: smoking, passive smoking and radon; occupational exposures such as asbestos; inhalation of corrosive chemicals like cadmium, silica and vinyl chloride; and long-term and accumulated exposure to air pollution. Lung cancer can also be congenital, where family history of lung cancer increases the risk of development [4].

Therapeutic modalities for NSCLC include: surgery, radiofrequency ablation (RFA), radiation therapy, chemotherapy, targeted therapies and immunotherapy. Therapeutic options depend on the cancer stage, patient's health and lung function and cancer characteristics. Treatments used for SCLC include: chemotherapy, radiation, surgery and palliative care. Surgery is less likely to be a primary treatment for SCLC as by the time diagnoses are made it would have metastasised [5].

3. Cancer stem cells

3.1. The CSC hypothesis

It is hypothesised that tumour development and progression is maintained by a small subset of cancer cells having SC characteristics. These CSCs are capable of self-renewal and differentiation, playing a significant role in malignant proliferation, invasion, metastasis, and tumour recurrence. Cancer cells have accumulated several mutations during their cell cycle, acquiring significant characteristics called the hallmarks of cancer. These specific traits include evasion of growth signalling pathways impeding proliferation, anti-apoptotic functions, infinite replication capacity, angiogenesis and metastasis with distant organ invasion, as well as immune response evasion. In order for a cell to acquire these mutations, its cell cycle needs to be longer than that of somatic cells. Cells that are maintained throughout an organism's lifespan are adult SCs, making them susceptible to neoplastic conversion [19]. SCs divide either symmetrically producing two daughter SCs, or asymmetrically producing one progenitor and one SC, having the ability to differentiate into multiple cell types while self-renewing and overcome senescence [6].

Dysregulation of the pathways maintaining SC function can lead to uncontrolled cell division and differentiation leading to CSC formation and tumour progenitors [7]. A major pathway involved in cell cycle proliferation and arrest is Wnt-β-catenin, which promotes SC renewal by signalling transcription genes. [20]. SC self-renewal is regulated by Notch signalling [21]. The Sonic Hedgehog (Shh) pathway promotes SC proliferation, activating various SCs [7]. Studies have found that these signalling pathways are not always activated in normal SCs but rather in CSCs where the genetic programs governing self-renewal are stimulated in SCs when the need for rejuvenation and repair arises where as in CSCs it is differentially active [22].

3.2. CSC identification and characterisation

Improved identification and isolation of CSCs will lead to enhanced studies on CSCs and targeted therapies. To date, various methods have been implicated in this regard, having different levels of success in common malignancies [23].

First identification of CSCs where made by Bonnet and Dick in 1997, who identified an arrangement of stem like cells that simulated the normal hierarchy of haematopoietic SCs. They identified rare carcinogenic cells in human acute myeloid leukaemia (AML) that was able to repopulate the entire original disease over serial transplantations. The subpopulation characterised by CD34 +ve and CD38 −ve had the capacity to self-renew and differentiate [24]. This study formed the basis for CSC research in both hematologic malignances and solid tumours. Breast CSCs from a solid tumour was first identified by Al-Hajj et al., using CD44 and CD24 markers [25]. Since then, CSCs have been identified in a variety of solid tumours, including lung cancer [26, 27]. Common characteristics from these different tumour types are shared between the isolated CSCs. Characteristics include drug resistance, propagation of tumours, and asymmetric division. CSCs can be isolated and characterised by means of the following methodologies: isolation using CSC-specific cell surface markers by flow cytometry [28]; detection of side-population (SP) phenotypes by Hoechst 33,342 exclusion [29]; assessment of aldehyde dehydrogenase (ALDH) activity [30]; characterisation by tumourigenicity evaluation [31] and stem-ness gene expression and transcriptional factors [32].

3.3. Lung CSCs

Lung cancer's ability to recur, regardless of putative treatment, proposes that a small population of the disease contain the capacity for self-renewal and regeneration. This sub/side population (SP) of CSCs portray tumorigenic potential. With therapeutic targeting, treatments may have the potential to eliminate tumour recurrence [8].

The lung being highly compartmentalised, have led to various epithelial cell types being labelled as presumed lung precursors due to their stem/progenitor cell-like responses to injury. The behaviours and characteristics of these cells also include repopulation of injured tissue. Cell type AEC2 have been characterised as a limited, epithelial progenitor for the alveolus, as they are said to be the progenitor of AEC1, which is involved in gas exchange in the alveolus. Studies have indicated that the bronchio-alveolar stem cell (BASC) a less differentiated cell located in the bronchio-alveolar duct junction act as an injury-responsive, limited progenitor for the distal airway-alveolar epithelium. BASCs have been implicated in lung cancer tumour genesis due to their overexpression of oncogenic K-ras and rapid proliferation by K-ras signalling. Clara cell and AEC2 markers are found in tumour formation of BASCs that have been expressing long term activation of K-ras, both Clara cell secretory protein (CCSP) and surfactant protein C (SP-C) have been identified respectively. Studies have indicated that cancer cells portraying a distinctive combination of the clara cell and AEC2 markers present in BASCs can be isolated from lung tumours. Supporting evidence shows that BASCs constituting of these double positive tumorigenic cells may be responsible for adenocarcinoma development. Along with BASCs and AEC2 being exceedingly receptive to proliferative stimuli, they show resistance to cell damage and injury by expanding within the epithelium following lung tissue damage and repair. These characteristics are critical for both normal tissue and CSCs. As injury resistance of these cells in lung cancer, could serve as a stem cell-like reservoir for generating additional tumours [19].

Lung CSCs can phenotypically be identified and characterised using CSC identification methods. One such method includes the SP phenotyping where efflux of Hoechst 33,342 dye

is measured due to the differential ability of the cancer cells imparted by the ATP-binding cassette family of transporter proteins present on the cellular membrane [9]. Increased ALDH activity is connected to cellular drug resistance, through detoxification of cytotoxic agents and oxidation of retinol to retinoic acid. It is also involved in early SC development and can be used as a reliable CSC marker [8].

Lung CSCs can also be tested for up regulation of SC genes. In a study conducted by Zakaria et al. they investigated CSCs isolated from lung cancer cell lines expressing SC transcription factors Sox2, Oct 3/4, Nanog, c-Myc, and Klf4. Gene expression in the lung CSCs were compared to the expression levels in normal SCs (PHBEC). Sox2, Oct4, c-Myc, and Klf4 were all detected and up-regulated in the CSCs. Currently, specific cell surface markers derived from the surface markers known to be present on normal haematopoietic or embryonic SCs are used to identify and isolate CSCs. For lung CSCs, CD133, CD166, EpCAM, CD90, and CD44 have been used as markers [10].

4. Photodynamic therapy

4.1. Fundamentals of PDT

PDT is a low cost, clinically approved, minimally invasive therapeutic procedure that can exert selective cytotoxic activity toward malignant cells. The procedure involves administration of a photosensitizing agent followed by irradiation at a wavelength corresponding to an absorbance band of the PS. In the presence of oxygen, a series of events lead to direct tumour cell death, damage to the microvasculature and induction of a local inflammatory reaction [11].

Molecular oxygen (O_2) is the terminal electron acceptor of the mitochondrial electron transport chain performing aerobic respiration. In the mitochondrion oxygen serves as an electron acceptor [33]. During PDT a photochemical reaction uses the free O_2, generating a highly reactive product termed singlet oxygen ($^1O_2$) and reactive oxygen species (ROS) which can rapidly cause significant toxicity leading to cell death via apoptosis or necrosis. Ground state/molecular oxygen has two unpaired electrons residing separately in the outermost antibonding orbitals. Depending on the electron configuration there are three possible states for O_2, the ground state of oxygen is called a triplet state $^3O_2$. Singlet oxygen is produced when undergoing photo-oxygenation, by inverting the spin of one of the outermost electrons (**Figure 1**). This type of oxygen is highly reactive and is the predominant cytotoxic agent produced during PDT [34].

A PS or photosensitizing agent is a chemical compound that can be excited by monochromatic light having a specific wavelength matched to an absorption peak of the administered compound. The excited PS subsequently transfers energy to a chosen reactant. This is commonly molecular oxygen [35, 36]. PSs commonly used in cancer are based on the tetra-pyrrole backbone simulating protoporphyrin found in haemoglobin. Naturally occurring tetra-pyrrol structures are found in haem (porphyrins), chlorophyll and bacteriochlorophyll. Synthetically synthesised tetra-pyrroles include phthalocyanines. As pyrrole-ring double bonds are successively reduced starting in porphyrins and going to chlorins and bacteriochlorins, the Q-band
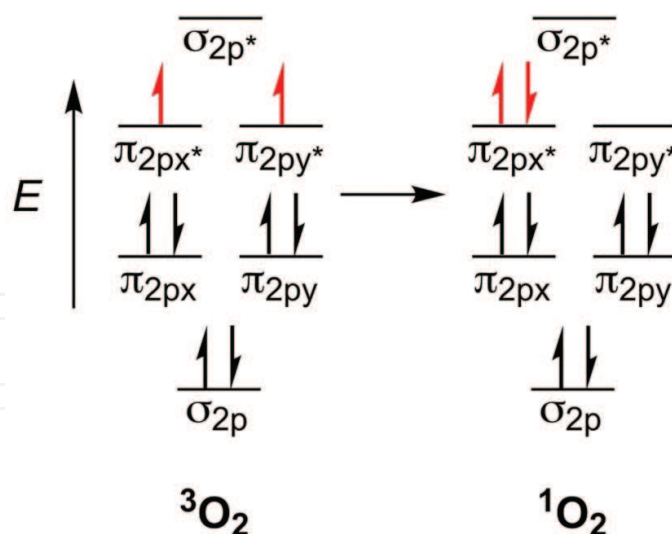
Figure 1. Molecular orbital diagrams showing the electron distribution in triplet and singlet oxygen [34].
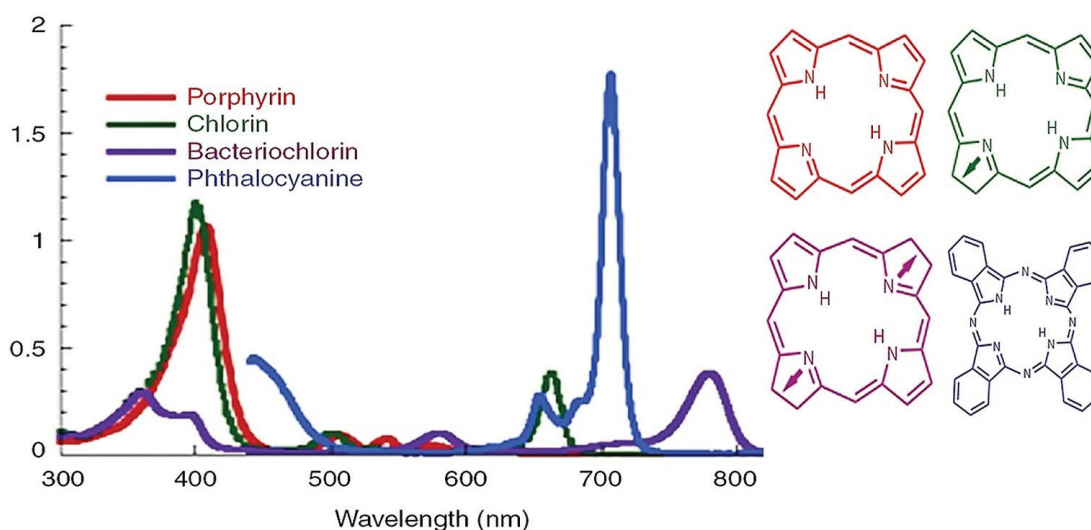

Figure 2. Tetrapyrrole absorption spectra showing porphyrins, chlorins, bacteriochlorins, and phthalocyanines [37].

moves to longer wavelengths and increases in size as seen in **Figure 2** [37]. Indicating that the structure of the PS has an influence on absorbance bands.

Efficient PSs should have a strong absorbance peak ranging from 600 to 800 nm in the deep-red to near-infrared (NIR) spectral region, which will allow for tissue penetration, as penetration tend to increase with wavelength. However, wavelengths longer than NIR are avoided, due to having a lower frequency and delivering too little energy for sufficient oxygen excitation. Ideally a PS should have suitable photo-physical characteristics. It should have a high-quantum yield of triplet formation ($\Phi T \geq 0.5$), a high singlet oxygen quantum yield ($\Phi \Delta \geq 0.5$), a relatively long triplet state lifetime (τT, μs range), and a high triplet-state energy (≥ 94 kJ mol^{-1}). Low dark toxicity and negligible cytotoxicity in the absence of light. Preferential accumulation in diseased/target tissue over healthy tissue. Rapid clearance from the body

post-procedure, to decrease side effects. High chemical stability: single, well-characterised compounds, with a known and constant composition. Soluble in biological media as effective PSs tend to be relatively hydrophobic compounds that rapidly diffuse into tumour cells and localise in intracellular membrane structures such as mitochondria and endoplasmic reticulum (ER). It should produce a marked inflammatory response via apoptosis, causing an immuno-genic effect against cancerous cells [36, 38].

Light source and light delivery are fundamental aspects in PDT. The light source depends on tumour location and the PS used. To date visible light ranging from 400 to 900 nm has been used in PDT. It has been noted that longer wavelengths in the visible red spectrum ranging between 600 and 810 nm are preferred due to optimum tissue penetration as well as PS structure mediating the use of red-shifted light. Historically PDT depended on low intensity lasers for a light source due to their valuable characteristics of monochromaticity, coherence, directionality and low power output (<100 mW), which removes the variable of heat that might have an influence on PDT. Lasers emit narrow beams of intense electromagnetic radia-tion that is monochromatic giving access to the wavelength region for excitation of PSs [37]. Coherence and directionality is correlated to the laser beams' divergence property. This is a qualitative measure of the laser irradiation to remain concentrated over a distance. Another important factor in choosing the light source is the fact that tissues have various optical properties depending on their bio-components. Tissue can both absorb and scatter visible light, this tend to decrease as the wavelength used increases [39].

4.2. Mechanisms of PDT

Three fundamental components act simultaneously in PDT (**Figure 3**): molecular oxygen, a light source and a PS. None of these is individually toxic.

During PDT, when a PS is absorbed it is still in its ground singlet state. A PS reaches its first excited singlet state through wavelength specific light activation. This first excited singlet state is unstable and can either deteriorate through energy loss by emitting fluorescence or, it can reach its excited triplet state accomplished through intersystem crossing of molecular oxygen, which is long lived and more stable [40] (**Figure 4**). In solution, intersystem crossing is increased by the probability of the presence of paramagnetic species such as molecular oxygen. When the PS reaches its excited triplet state it can follow two pathways. These pathways are named Type I and Type II reactions.

Phostosensitizer   Visible red Light   O2

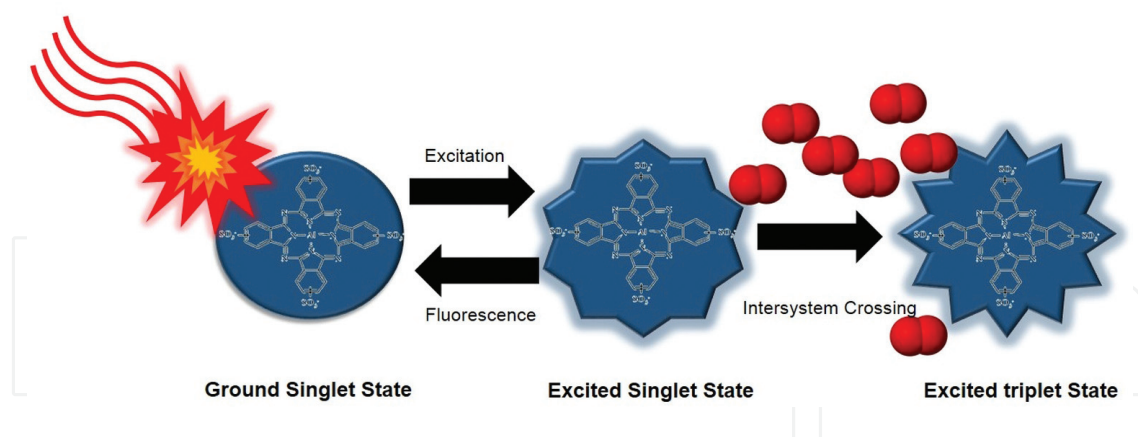**Figure 3.** Fundamental components of PDT.

Figure 4. Activation of a PS in its ground singlet state to its excited triplet state via light activation and intersystem crossing with molecular oxygen.

Type I reactions generate ROS, whereby adjacent biomolecules (i.e. lipids, proteins, and nucleic acids) and the PS in its excited triplet state undergoes an acid-base reaction transferring hydrogen ions. Depending on the target molecule, i.e. lipids, proteins, or nucleic acids, free radicals and radical ions are generated that then react with oxygen forming ROS [41] (**Figure 5**).

Type II reactions are based on a phenomenon called triplet–triplet annihilation. This involves the production of highly reactive singlet oxygen which is also extremely cytotoxic. Singlet oxygen is generated through the PS in its excited triplet state reacting with ground state molecular oxygen [41] (**Figure 6**).

Type I and II reactions happen simultaneously. The oxygen species generated between the two reactions depend on the components, i.e., the PS and amount of oxygen available to react with as well as PS localization in the biomolecules. Type II is considered the primary mechanism of cell death due to singlet oxygen generation. ROS and singlet oxygen have a high reactivity and short half-life, affecting only the biomolecules the PS had localised in or are close to the region where these species are generated, usually within a 20 nm radius. PS localization promotes selective sensitization and is therefore a primary factor in drug release studies to target tissues [41].
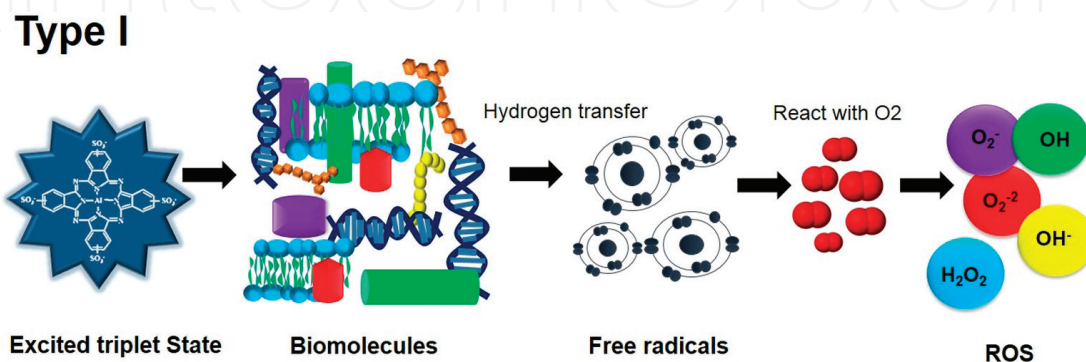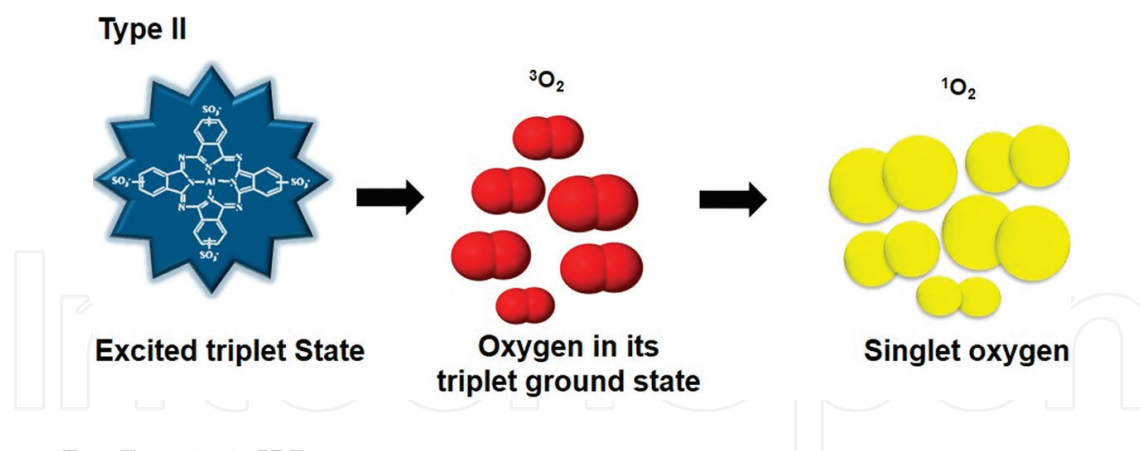

Figure 5. Type I reaction in PDT.

Figure 6. Type II reaction in PDT.

4.3. PDT and lung cancer

Currently PDT, alone or as an adjunct therapy is increasingly being used to treat thoracic malignancies. Effects exerted include both apoptosis and necrosis, damage to tumour vasculature as well as inducing an inflammatory reaction. It does not lose efficacy with repeated treatments and allow for combination treatment [42].

Porfimer sodium is the PS most commonly used to treat lung and other thoracic malignancies. It has been approved by the US Food and Drug Administration (FDA) for cases of NSCLC where standard therapies are not appropriate and to palliate symptoms from airway obstruction [43]. It is also reported to be a safe and effective neoadjuvant treatment, where it has been reported to significantly decrease tumour size, convert tumour operability and improve complete surgical resection [12]. Over the past decade, prospective clinical studies evaluated a variety of PSs, in treating early and advanced stage NSCLC. Early-stage disease had a complete response range from 72% to an impressive 94 and 100%. Advanced disease, local control and partial response ranged from 78 to 100%, respectively [12]. Key indications for the use of PDT to treat lung cancer include: Intraoperative PDT by transthoracic or thoracoscopic irradiation after tumour resection and complete removal of the macroscopic disease, where the margins in the surgical bed are illuminated before wound closure to treat undetected viable cancer cells, which could lead to a reduction in local recurrence [44]. Interstitial PDT, where intra-tumor light delivery is required to activate the PS, using image guidance and treatment planning, when the tumour is deep-seated and larger than 1 cm [45]. Definitive PDT treatment where indication includes early stage, superficial, and centrally located endobronchial NSCLC tumours, where the treatment option used is admittance of the PS and activation through bronchoscopic irradiation [12].

PDT has shown to be a safe and minimally invasive therapy designed as an anti-cancer drug, but still have room for improvement. Current PSs lack sufficient tumour selectivity which may result in uptake of the PS in non-cancerous tissue that can lead to adverse effects [46]. Another major trial in medicine today is drug delivery, this includes PDT. When administering a PS it is taken up by the blood and lymphatic systems, which can lead to photosensitivity [13].

5. Targeted PDT

5.1. Immunotherapy and PDT

Photoimmunotherapy (PIT) actively and specifically targets antigens via monoclonal antibodies (MAb) or antibody fragments (AbFs) used for drug delivery. PIT uses a PS conjugated to an Ab against a tumour, tumour associated (e.g. CSC) or tumour vasculature antigen [47]. Some of the advantages seen in PIT, compared to conventional cancer treatment such as chemotherapy/chemo-immunotherapy, is that during laser activation of the Ab-PS conjugate there is cell specific cytotoxicity of cancerous cells sparing the surrounding healthy tissue. Additionally PIT is neither immunosuppressive nor does it have an affinity for rapidly dividing cells, making it less likely to develop treatment induced resistance due to neoplastic cells up regulating alternative or circumventive pathways commonly seen in chemotherapy [48].

PIT has shown to be effective in various studies conducted on cancer using a variety of MAbs. This includes a study conducted by Savellano et al., where they conjugated a clinically approved benzoporphyrin derivative (verteporfin) to the anti-EGFR MAb cetuximab. Results showed that conjugated verteporfin had an affinity for EGFR-overexpressing A431 epidermal carcinoma and ovarian cancer cells, killing them via PDT mediated mechanisms, whereas free verteporfin exhibited no specificity [49]. In another PIT study they explored the effectiveness of PIT on metastatic lung carcinoma *in vitro* and *in vivo* using a mouse model. IRDye700DX is a silica-phthalocyanine dye that is extremely hydrophilic. It has an excitation wavelength of 690 nm which is NIR, allowing for enhanced tumour penetration of light. IR700 conjugated to MAbs showed *in vitro* results of target cell specificity with little to no toxic effects on non-target adjacent tissue. Targeted cells demonstrated cell membrane rupture within minutes of exposure to NIR-light activating the Ab-PS conjugate [50, 51]. *In vivo* experiments using trastuzumab-IR700 was used to treat early-stage lung metastases in a murine model. Results indicated specific binding, rapid induction of necrotic cell death, target specific cell death and prevented metastasis by target-specificity [52].

5.2. Nanomedicine and PDT

NPs are biomolecules synthesised for drug delivery and is used in nanomedicine today. Incorporating nanostructured drug delivery systems of PSs conjugated to NPs may have advantages that include improvement of transcytosis across epithelial and endothelial barriers, optimise delivery of low water soluble PSs and co-delivery of PSs into cells [41]. Other advantages of using PS-NP conjugates are defence against enzymatic degradation, controlled PS release into cancer cells, its small size allow for cellular penetration, NPs are biocompatible and photos table [53]. NPs can be classified according to their composition, morphology or structure [54]. Covalently binding the PS to the NP can enhance delivery of the PS to cancerous cells, as well as increase singlet oxygen production [37].

NPs are susceptible to engulfing by macrophages after intravenous administration. This can be overcome by polyethylene glycol (PEG) coating, enhancing bloodstream circulation time and

allowing for tumour accumulation. Studies have showed encouraging results for the use of PS-NP conjugates, whereby different compositions of NPs have been proposed [55]. One such study indicated that the use of dendrimer phthalocyanine (DPc)-encapsulated non active polymeric micelles have been successfully used both *in vivo* and *in vitro* treating human and subcutaneous mouse lung adenocarcinoma A549. Both experimental systems had a significant increase in PS-NP conjugate PDT efficiency as compared to PDT alone, where mitochondrial localization was observed [56]. Another method of enhancing PDT is by improving on the conjugation methods for PSs and NPs. Instead of using PS-NP encapsulation, PS-NP conjugation can be achieved through covalent binding [57]. One NP in particular that can be applied by covalent bonding of PSs to its surface is gold-NPs (Au-NPs). Au-NPs has enhanced surface-plasmon resonance (SPR) effects due to the non-linear optical fields found in metal NPs being very close [58]. Au-NPs have good biocompatibility, versatile surfaces, and unique optical properties [59], whereby their optical field can be enhanced by the SPR by changing the shape of the NP specifically to a ring [60]. Studies have conjugated Au-NPs to Abs, for specific cell surface receptor targeting, in anti-cancer treatments whereby the use of NIR-light produced photo-thermal heat destruction. Results showed a significant increase in apoptosis induction as compared to unconjugated NPs [61]. A drawback in using Ab-NP conjugates alone for cancer treatment was that to induce photo-thermal heat destruction a high power density laser had to be used. This led to unselective damage of normal tissue in the laser path surrounding the target of interest [62]. However, cancer cell death induction using TPDT requires low power lasers that are efficient in activating the PS avoiding destruction of normal tissue. Coating the NP with polyethylene glycol (PEG) have also enhanced conventional methods of Ab conjugation to NPs that led to poor orientation of the functional group of the Ab. NP PEGylation allows for covalent attachment of an Ab to the outer end of the PEG chain, thus maintaining availability of Ab binding sites to cell surface receptors. Studies using this method of Ab conjugation showed efficient internalisation into cancerous cells [63, 64]. TPDT involves
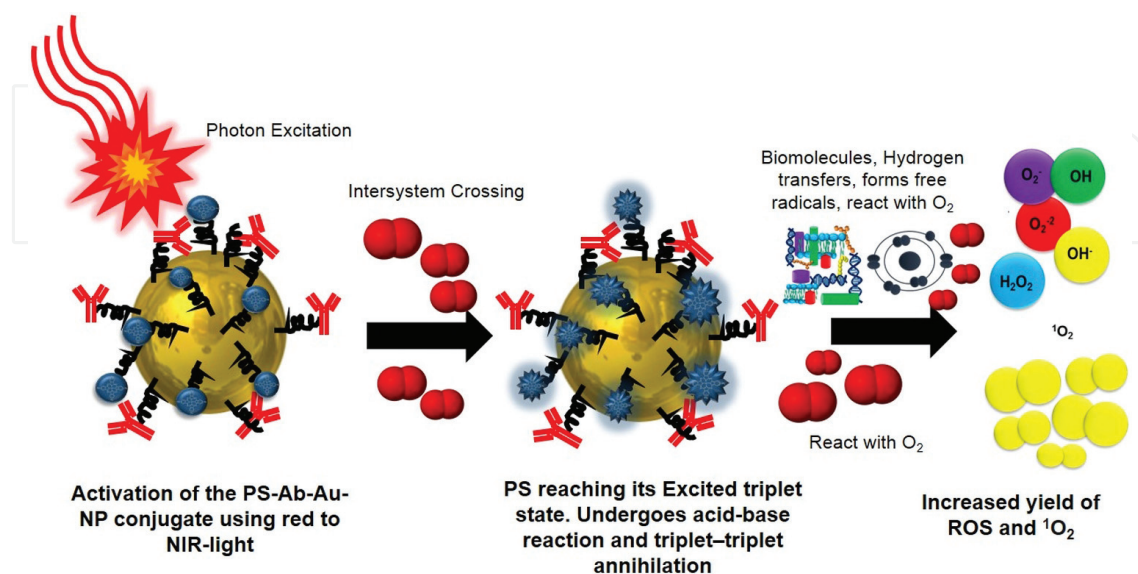

Figure 7. TPDT using a PS-Ab-Au-NP (PEG) conjugate.

the use of either/both an Ab or NP conjugated to a PS. The mechanisms still follow Type I and Type II reactions (**Figure 7**).

6. Conclusion

Despite intensive research and development of therapies, lung cancer still remains a primary contributor to cancer related deaths, with survival rates of patients diagnosed being dismal. Prompt diagnosis and effective treatment will radically improve patient outcome. Due to late diagnoses of lung cancer, conventional therapies show to be ineffective [18]. Lung cancer initiation and progression mechanisms have been identified that will be able to drive future research on molecular and biological targets. Conventional therapies are limited by drug resistance. Characterising and evaluating the mechanisms as well as lung CSCs leading to the acquisition of drug evasion, can aid in the development of therapies that will combat therapeutic resistance [65].

According to the CSC hypothesis, these cells are involved in tumorigenesis. This is because of their stem-cell like abilities that include indefinite self-renewal, slow replication, intrinsic resistance to chemotherapy and radiotherapy, and an ability to give rise to differentiated progeny. Studies have been able to identify CSCs in various cancers, including lung. Lung CSCs have been phenotypically identified using bio-markers typically expressed by normal SCs. Some of the markers include CD133, CD166, CD44 and ALDH1. Molecular pathways regulating SC proliferation, differentiation, and apoptosis are found to be active in CSCs as well, all giving rise to CSCs unique capability of drug evasion and metastasis or cancer relapse [66]. Due to conventional therapeutic strategies only targeting rapidly dividing cells destroying the bulk of the tumour, complete eradication of rare CSCs also need to be addressed. Therapies that aim to identify CSCs and overcome drug resistance due to CSCs having increased levels of efflux pumps need to be developed [27].

A potential therapy that can be advanced to treat CSCs is PDT. PDT involves the use of a nontoxic PS that localises in cellular organelles and when activated using light of a specific wavelength, reacts with oxygen to form free radicals leading to cell death. PSs have an affinity for malignant cells, inducing apoptosis via caspase reactions, mitochondrial damage and cytochrome c release. Unlike chemo and radiation inducing cell death via DNA damage. Another advantage of PDT is that cells that become resistant to chemo and radiation does not cause cross-resistance to PDT and there is no toxic accumulation [67]. Several modes of clinical PDT application has been defined, pertaining to localization and tumour density, as these factors play a role in PDT efficacy. One mode of concern is interstitial PDT, which is used on tumours larger than 1 cm in size [45]. This mode of PDT which is indicated for multicellular tumours has been explored previously *in vitro.* The efficacy of PDT concerning a monolayer as compared to multicellular spheroids indicated that spheroids are more resistant to PDT, however this can be overcome using a dose dependent manner of inducing cell death [68]. Although PDT has successfully been used to treat lung cancer a major pitfall still include low tumour selectivity, especially in a scenario where the lung cancer's genomics are predisposed to malignant metastatic tumours [42].

PIT uses a PS conjugated to and Ab allowing for cell specific cytotoxicity and does not develop treatment induced resistance. Interest has grown in the biomedical field for the use of NPs as a drug delivery vehicle specifically Au-NPs, due to their biocompatibility, high surface area and functionalized facile surface supporting self-assembly of thiolates [69]. Au-NPs can be synthesised to a structure supporting absorption in the far red to NIR wavelength. The combination of using an Ab-NP conjugate allows for all the significant contributions and advantages to be applied in one treatment, TPDT, having improved cell specific targeting as well as allowing significant accumulation of PS in the tumour site by using Abs to direct the PS to CSC specific markers for example, and NPs enhancing PS uptake that can increase singlet oxygen yield and effective cancer/ CSC death. Results indicate that TPDT might prove to be a promising treatment modality for lung cancer and targeting lung CSCs. As TPDT can be used as a primary or adjuvant therapy for lung cancer depending on the morphological state and tumour localization. Targeted PDT can lead to complete cancer eradication and prevent cancer relapse by destroying the bulk of the tumour as well as targeting the underlying CSCs. Improving the overall survival rate of patients diagnosed with lung cancer as well as increase quality of life through minimal side effects when receiving treatment.

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Conflict of interest

The material in this paper, submitted to *InTechOpen* is original work from the author and is not being considered elsewhere for publication. The authors indicate no potential conflict of interest.

Author details

Anine Crous and Heidi Abrahamse*

*Address all correspondence to: habrahamse@uj.ac.za

University of Johannesburg, Gauteng, South Africa

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