ABSTRACT

With the growing number of cancers cases over the years the need for effective treatments are becoming more demanding. Though many of the advancement in the development of cancer therapies are reliable, the side effects that accompany these like the lack of specificity have been a cause of concern and hence alternative therapies have now become the focus of research. Photodynamic therapy is one such therapy which is based on the combined effect of mainly three components including light, oxygen and a photosensitizes drug. The ability of the drug to be taken up by cancer cells and only become reactive on exposure to light of specific frequency, gives the therapy the advantage of being specific thus avoiding normal cells from damage. The use of photodynamic therapy for breast cancer is currently in clinical trials. This review focuses on the general aspect of photodynamic therapy and the current scenario of photodynamic therapy in the treatment of breast cancer.

Key Words: Breast Cancer, Photodynamic Therapy (PDT), Photosensitizer, Apoptosis.

INTRODUCTION

Cancer accounts for 8.2 million deaths in 2012 making it one of the leading causes of death in our present generation as estimated by the World Health Organisation [1]. Cancers are caused by abnormalities in the genetic material of the transformed cells. Carcinogens such as chemicals, infectious agents, radiation and tobacco smoke may be some of the causative...
factors attributed to these genetic abnormalities. Faulty DNA replication or through inheritance are other factors that lead to the development of cancer. The cancer-promoting genetic abnormalities may randomly occur through errors in DNA replication, or are inherited, and thus present in all cells from birth. The complex interactions between carcinogens and the host's genome affect the hereditability of cancer. Lung, liver, stomach, colorectal and breast cancers cause the most cancer deaths each year. Approximately 30% of cancer deaths are due to the five chief behavioural and dietary risks which include high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use, and alcohol use. More than 60% of world's total new annual cases occur in Africa, Asia and Central and South America. These regions account for 70% of the world’s cancer deaths. It is expected that annual cancer cases will rise from 14 million in 2012 to 22 within the next two decades.

The genetic link to cancer is currently undergoing a lot of research and is improving our understanding of cancer biology, helping to identify “at-risk” individuals, assisting the ability to characterize malignancies, establishing treatment tailored at molecular levels of diseases and leading to the development of new therapeutic modalities. Alterations in three types of genes are responsible for tumorigenesis: oncogenes, tumour-suppressor genes and stability genes. Though in certain diseases such as cystic fibrosis or muscular dystrophy, the mutations in one gene can cause the disease there is no evidence of single gene defect that can cause cancer. Mammalian cells have multiple safeguard mechanism to protect them against the potentially lethal effects of cancer gene mutations, and only when several genes are defective does an invasive cancer develop. Thus it is more convenient to assume that mutated cancer genes as contributing to, rather than causing, cancer.

Cancer develops from one single cell. It transforms from a normal cell into a tumorous cells by a multistage process which typically involves succession from a pre-cancerous lesion to malignant tumours. These changes are the result of the interaction between a person's genetic factors and three categories of external agents, including

- physical carcinogens (ultraviolet and ionizing radiation)
- chemical carcinogens (asbestos, components of tobacco smoke, aflatoxin and arsenic)
- Biological carcinogens (infections from certain viruses, bacteria or parasites)

Ageing is another primary factor for the development of cancer. The number of cancer cases rises exponentially with the age probably due to the build up of risks for specific cancers over the years and due to immunosenescence experienced by older people.
In a normal cell, if there is DNA damage it will induce cell cycle arrest to provide time for repair and enhance cell survival and if repair cannot be made it will undergo apoptosis but in cancerous cells they move through the checkpoints without being repaired and results in unrestrained proliferation of mutated cells. The outcome of a cell in response to DNA damage is situation specific with most tissues showing a preference for cell cycle arrest, whereas thymocytes and splenocytes are susceptible to apoptosis.

**Breast cancer** is the second leading cause of cancer death in women, exceeded only by lung cancer. The chance that breast cancer will be responsible for a woman's death is about 1 in 36 (about 3%). Death rates from breast cancer have been declining since about 1989, with larger decreases in cases of women younger than 50. These decreases are believed to be the result of earlier detection through screening and increased awareness, as well as improved treatment. A recent study of breast cancer risk in India revealed that 1 in 28 women develop breast cancer during her lifetime. This is higher in urban areas being 1 in 22 in a lifetime compared to rural areas where this risk is relatively much lower being 1 in 60 women developing breast cancer in their lifetime. In India the average age of the high risk group in India is 43-46 years unlike in the west where women aged 53-57 years are more prone to breast cancer.

The proportion of individuals carrying a mutation who will manifest the disease is referred to as penetrance. A generalised view is that the common genetic variants that are related with cancer susceptibility have a lower penetrance than rare genetic variants. Penetrance is usually identified by the individual carrier's age and sex in the case of adult onset diseases. The penetrance for breast cancer in female BRCA1/BRCA2 mutation carriers is often assigned by 50 and 70 years of age. Though a number of methods to estimate penetrance exist, mostly all are biased to an extent, and determining an individual mutation carrier's risk of cancer involves some level of imprecision.

All cancers carry somatic mutations in their genomes. A subset known as driver mutations, are an advantage on cancer cells which bestow clonal selectiveness and are normally involved in oncogenesis, while the rest are known as passenger mutations. Somatic driver substitutions and small insertions/deletions (indels) were identified in cancer genes previously implicated in breast cancer development, such as AKT1, BRCA1, CDH1, GATA3, PIK3CA, PTEN, RB1 and TP53. A few driver mutations are commonly mutated, but many infrequently mutated genes jointly play a role and contribute significantly in myriad different combinations. Multiple somatic mutational processes have been operative.
Ultimately, characterization of the genomes of breast cancer and other types of cancers will provide an accurate and scientifically relevant classification generating input onto a vastly unknown area to better understand clinical heterogeneity of the disease and to ultimately develop new strategies to find new modes of prevention and treatment.

Many commonly used management options for breast cancer exist including: chemotherapy, radiation therapy, surgery, immunotherapy, monoclonal antibody therapy amongst other methods. Which therapies are used depends upon factors like the position of the tumour, grade of the tumour the stage of the disease as well as the general state of a person’s well being.

Complete removal of the cancer without damage to the rest of the body is the goal of treatments. Sometimes this can be accomplished by surgery, but positive effect of such procedures is often outweighed by the ability of cancer to invade adjacent tissues or spread to distant sites by microscopic metastasis\(^\text{10}\). The effectiveness of chemotherapy is often limited by toxicity to other tissues in the body. Radiation can also cause damage to normal tissue. So research is being carried out to find new ways as to how to improve the treatments of cancer with lesser side effects and more accuracy.

Breast cancer recurrences after mastectomy pose a threat to the patient as surgical options is limited\(^\text{11}\). If disease is localized, then surgical removal can be performed but if the lesions which in most case are widespread throughout the chest wall or involve heavily irradiated tissue then this pose a therapeutic challenge. Aggressive chemotherapy is an option which patients normally acquire but there is little to no local response most avenues for local control have been exhausted. Multiple studies show that photodynamic therapy (PDT) provides good tumour kill for primary cutaneous malignancies\(^\text{12}\) and suggest its effectiveness in ablating dermal lymphatic recurrences of breast cancer\(^\text{11}\). The main advantages of PDT over other methods of treatment is that unlike for instance oncotherapies there is a higher significant degree of selectivity of drug accumulation in the tumour tissue followed by the absence of systemic toxicity of the drug alone, the capability to irradiate only tumour, the prospect to simultaneously treat multiple lesions and the ability to retreat a tumour in order to improve the response.
PHOTODYNAMIC THERAPY

The first known effort of using photosensitizing drugs to cure various diseases goes back to ancient Egypt, India, and Greece, where it is believed a psoralen-containing plant extracts and light were applied to treat psoriasis and vitiligo. In 1904 the term “photodynamic” was coined by Von Tappeiner to describe oxygen-dependent chemical reactions induced by photosensitization.

Photodynamic therapy is a medical treatment used for the treatment of neoplastic and non-malignant lesions. Photodynamic therapy (PDT) is a form of photo chemotherapy and needs the presence of light, a photosensitizer, and molecular oxygen for treatments. It was in the early 1900s that combination of photosensitizer and light as therapeutic agents came into the spotlight but it was only much later the food and drug administration (FDA) of USA was the first in the world to approve PDT using a pure form of Photofrin®. Suitable dye sensitizers for PDT are mainly porphyrinoid compounds, including chlorins, bacteriochlorins, phthalocyanines, and related structures which have extended conjugation and absorb light in the visible region and thus as a result they can be used to make coloured compounds or dyes.

PDT has an advantage over other conventional methods by the fact that its treatment is localised and it does not affect other cells or tissues but the light needed to activate most photosensitizers cannot penetrate through more than one third of an inch (1 cm) of tissue using standard laser technology and low powered LED technology. The treatment to PDT for tumour is limited to certain areas of the body like on or under the skin or on the lining of some internal organs. Using PDT as treatment of large tumours and metastasis has been unsuccessful for the same reason. A great deal of research and clinical study is now underway to determine optimal combinations of photosensitizers, light sources, and treatment parameters for a wide variety of different cancers.

Moreover, large numbers of current researches show possible practical usefulness of photosensitization in the broad field of different sciences such as dermatological diseases, atherosclerosis, infectious diseases, rheumatoid arthritis, age-related macular degeneration, restenosis, AIDS, haematological diseases all of which can be successfully treat by PDT.

Three requisite non-toxic components for photosensitization are as follows;

a. Photosensitizer
b. Light
c. Oxygen

**Photosensitizers**

The physico-chemical properties of the photosensitizer are important considerations that need to be addressed to achieve good results in photosensitization. Some important characteristics of photosensitizers which are desirable for efficacy include chemical purity, capability to localize specifically in neoplastic tissue, short time interval between the administration of the drug and its maxima accumulation in hyperproliferating tissue, rapid clearance from normal tissues, activation at wavelength with optimal tissue penetration, high quantum yields for the generation of singlet oxygen, and lack of dark toxicity[13]. The basic precondition to attain a desirable response to photosensitization is for a sufficient amount drug to be localised in the target tissue. Before being retain in hyperproliferating cells photosensitizers are first taken up by normal cells as well[19]. Increased blood vessel permeability as well as poor lymphatic drainage in neoplastic tissues is factors which are assumed to contribute to the retention of the drug in neoplastic lesions; however the exact mechanism of retention is still a topic of research.

Localization of the photosensitizers is an important step in Photodynamic therapy and is also important to facilitate drug development. Since the introduction of second generation sensitizers tend to be pure compounds not mixtures, loci of localization can often be identified. Mitochondria, lysosomes, plasma membrane, and nuclei of tumour cells and tumour vasculator have been evaluated as potential PDT targets[20]. A distinguishing aspect of PDT is vascular shutdown[19], and therefore identification an optimal subcellular target remain relevant since both vasculature and tumour are composed of individual cells. Up till now, clinical efficacy has been described for only a small group of agents. Though current research generalise a theory relating to localization and efficacy, it is still unclear whether a single target will prove advantageous in all instances[20].

In one specific form of treatment, porphyrin, which is a breakdown component of red blood cells, were administered to a patient to try to identify surface cancer cells. Porphyrin which has a strong absorption of orange-red light was successful in identifying cancer cells probably because the cancer cells would take up the porphyrin, thereby producing strong absorption to the red-orange light. The application of light to that colour to the person's skin, porphyrin which has been taken up can be identified and therefore the location of cancer cells could
possibly be identified. This procedure had the disadvantage that the porphyrin was injected into a patient and therefore, the entire patient's body was subjected to the porphyrin.  

In more recent times, many advances have been made so chemicals have been developed to localise porphyrin. For example ALA (amino laevulenic acid) is such chemical which is typically used. A basic requirement for such procedures is that the chemical applied to the area to be treated is applied only to that area, normally 8-20 hours before treatment. Light of a specific wavelength range, such as 580 to 680 nm illuminates the treated area. Blood cells are not damaged by such wavelength as blood cells reflect light in this wavelength band.

A methyl ester of ALA is a modified version of basic ALA which is being used nowadays. This chemical takes up more quickly than ALA, and typically in 3-5 hours, thereby reducing the time period between the application of the chemical and the treatment.

Other chemicals are currently being developed which can be used in conjunction with photodynamic therapy in order to treat a patient.

**Table 1.** A list of commercially approved photosensitizers for photodynamic therapy.

<table>
<thead>
<tr>
<th>Commercial name</th>
<th>Chemical definition</th>
<th>Absorption maximum</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photofrin</td>
<td>Mixture of di hematoporphyrin esters and ethers</td>
<td>630 nm</td>
<td>Systemic</td>
</tr>
<tr>
<td>Foscan</td>
<td>Meta-tetrahydroxyphenylchlorin (m-THPC)</td>
<td>652 nm</td>
<td>Systemic</td>
</tr>
<tr>
<td>Visudyne</td>
<td>Benzoporphyrin derivative</td>
<td>690 nm</td>
<td>Systemic</td>
</tr>
<tr>
<td>Levulan</td>
<td>5-Aminolaevulinic acid (ALA) converted into protoporphyrin IX (PPIX)</td>
<td>635 nm</td>
<td>Oral/topical</td>
</tr>
<tr>
<td>Metvix</td>
<td>Methyl 5-aminolevulinate</td>
<td>-</td>
<td>topical</td>
</tr>
</tbody>
</table>

Photosensitizers for PDT of cancer have been classed into three generations:

a. First generation: Haematoporphyrin derivative (HpD) and its analogues.

b. Second generation: Structurally distinct compounds with long-wavelength absorption.

c. Third generation: Second generation photosensitizers bound to carriers for selective accumulation in the tumour.
Though the introduction of first generation photosensitizers was a breakthrough in PDT it had some defects. PDT sensitizers such as Photofrin exhibited prolonged patient photosensitivity and possessed the inability to absorb long wavelength \[24\]. These were a few of the main limitations of first generation photosensitizers thus paving the way for second generation photosensitizers. The synthesis of improved (second generation) photosensitizers lead to the modification of tetryrrolic (porphyrin) compounds such as benzoporphyrin (Visudyne), chlorine (Temoporfin) and Porphycene (ATMPn), which have the capability to absorb longer wavelength \[24\]. The use of metallated derivatives have been studied and scientist have synthesized metallated derivatives such as Al, AlPcS\(_4\) and Si, SiNC(Nc napthalocyanine) \[25\]. However no correlation has been observed between metallation and increased photodynamic activity \[26\]. New research has shed light to the idea that targeted strategies increases the affinity of the photosensitizer for tumour tissues \[27\]. Even targeting subcellular component like mitochondria or plasma membrane have been studied \[28\]. These targeting approaches have led to the development of third generation photosensitizer and some of the best available results \[26\].

**Light Source**

Since PDT rely on localised light delivery, it can be useful only to tumours that can be reached by light either directly or through an optical fibre. The effectiveness of PDT is limited by the ability of light to penetrate into the tissues as a result of the treatment gets confines to the treatment of superficial cancer only and cannot kill cancer present deep in the tissues \[29\].

Light source and light delivery are two important features in PDT. The choice of light source for PDT can be determined by the location of the tumour, by the light dose delivered and by the choice of photosensitiser. The most preferred light source has an emission spectrum (i.e. the plot of power output at any single wavelength) matching the absorption spectrum (i.e. the plot of the absorption coefficient at any single wavelength) of the photosensitizer.

Conventional light sources which are used in photodynamic therapy are not particularly flexible and are commonly used to deal with only a single radiation bandwidth. The conventional systems does not possess any system to provide a feedback in relevance to the treatment process or progress made by a patient, thus making it difficult to provide records of progress of treatment. \[21\]
Light sources available for PDT belong to three broad groups: broadband lamps, diode lamps and lasers. Broadband metal halogen e.g. slides projector lamps or fluorescent lamps have superior power density that keeps light exposure times within reasonable limits and are relatively inexpensive. Light emitting diodes are cheap, easy to use, have a narrow bandwidth of 20-50 nm.

Lasers and lamps have both been employed to perform PDT and the superiority of one source over the other has not been demonstrated, therefore the use of lasers or lamps depends on the specific application. Although PDT has been traditionally performed using lasers, the availability of broadband sources (lamps) is challenging the use of lasers where light can be directly delivered to the tumour (skin, oral cavity, etc.) without the need to couple the source to an optical fibre. 30

**Table 2. Types of lamps available for clinical PDT**

<table>
<thead>
<tr>
<th>Types of lamps available for clinical PDT</th>
<th>Wavelength(s)</th>
<th>Bandwidth</th>
<th>Irradiance</th>
<th>Light delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tungsten filament</td>
<td>400–1100 nm</td>
<td>10–100 nm (depending on filters used)</td>
<td>Up to 250 mW/cm² or typically up to 1.8 mW/cm²/nm</td>
<td>Direct or via liquid light guide</td>
</tr>
<tr>
<td>Xenon arc</td>
<td>300–1200 nm</td>
<td>10–100 nm (depending on filters used)</td>
<td>Up to 300 mW/cm² or typically up to 3 mW/cm²/nm</td>
<td>Normally liquid light guide</td>
</tr>
<tr>
<td>Metal halide</td>
<td>Depending on the metal, lines between 250–730 nm (can be phosphor coated)</td>
<td>10–100 nm (depending on filters used)</td>
<td>Up to 250 mW/cm² or typically 1.2 mW/cm²/nm</td>
<td>Direct or liquid light guide</td>
</tr>
<tr>
<td>Sodium (phosphor coated Fluorescent)</td>
<td>590–670 nm</td>
<td>10–80 nm (depending on filters)</td>
<td>Up to 100 mW/cm²</td>
<td>Direct illumination</td>
</tr>
</tbody>
</table>
Table 3. Types of lasers available for clinical PDT

<table>
<thead>
<tr>
<th>Types of lasers available for clinical PDT</th>
<th>Wavelength(s)</th>
<th>Bandwidth</th>
<th>Irradiance</th>
<th>Pulse duration</th>
<th>Light delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argon laser</td>
<td>488 and 514.5 nm</td>
<td>Monochrom</td>
<td>0.5–1 W/cm²</td>
<td>CW</td>
<td>Direct or optical fibre</td>
</tr>
<tr>
<td>Dye laser pumped by argon laser</td>
<td>500–750 nm (depending on the dye)</td>
<td>5–10 nm</td>
<td>10–200 mW/cm²</td>
<td>CW</td>
<td>Direct or optical fibre</td>
</tr>
<tr>
<td>Metal vapour laser</td>
<td>UV or visible (depending on metal)</td>
<td>Monochrom</td>
<td>Up to 10 W/cm²</td>
<td>10–50 ns quasi-CW</td>
<td>Direct or optical fibre</td>
</tr>
<tr>
<td>Dye laser pumped by metal vapour laser</td>
<td>500–750 nm (depending on the dye)</td>
<td>5–10 nm</td>
<td>10–500 mW/cm²</td>
<td>10–50 ns quasi-CW</td>
<td>Direct or optical fibre</td>
</tr>
<tr>
<td>Solid state</td>
<td>For a Nd:Yag 1064, 532, 355, 266 nm</td>
<td>Monochrom</td>
<td>Up to 10 W/cm²</td>
<td>10 ps–30 ns quasi-cw</td>
<td>Direct or optical fibre</td>
</tr>
<tr>
<td>Dye laser pumped by solid state laser</td>
<td>400–750 nm (depending on dye)</td>
<td>5–10 nm</td>
<td>10–500 mW/cm²</td>
<td>10 ps–30 ns quasi-cw</td>
<td>Direct or optical fibre</td>
</tr>
<tr>
<td>Solid state optical parametric oscillator</td>
<td>250–2000 nm</td>
<td>Monochrom</td>
<td>Up to 1 W/cm²</td>
<td>10 ps–30 ns</td>
<td>Direct or optical fibre</td>
</tr>
<tr>
<td>Semiconductor diode lasers</td>
<td>600–950 nm</td>
<td>Monochrom</td>
<td>Up to 700 mW/cm² CW</td>
<td>CW</td>
<td>Optical fibre</td>
</tr>
</tbody>
</table>

**Oxygen**

Various studies have backed the concept that the efficacy of photosensitization is directly related to the yield of O₂ in the tumour environment and the yield of O₂ depends on the concentration of oxygen in the tissue \(^{[13]}\). Hypoxic cells are very resistant to photosensitization and the photodynamic reaction mechanism itself may consume oxygen at a rate which will inhibit further photosensitization effects \(^{[13]}\).

Singlet oxygen is understood to play the major role in this effect, and application of this effect to cancer therapy is of increasing importance. Direct spectroscopic evidence of singlet oxygen in PDT is hard to come across, probably due to the rapid reaction of singlet oxygen
with biomolecules. Nevertheless, it is a generalised assumption that O$_2$ is the major contributor.\textsuperscript{23}

Although there are many methods for the generation of O$_2$, the photosensitized generation is a straightforward and convenient method for the production of 1O2, requiring only 3 components i.e oxygen, light of an appropriate wavelength, and a photosensitizer capable of absorbing and using that energy to excite oxygen to its singlet state.\textsuperscript{23} The excitation of the photosensitizer is achieved by transition of a photon from the ground state to a singlet excited state. On relaxation the singlet excited state yields the photosensitizers lowest singlet state (singlet ground state). This is followed by intersystem crossing that generates the sensitizer triplet state. The lifetime of the triplet state is longer than that of the singlet ground allowing this excited state to react in one of two ways, defined as Types I and II mechanisms.\textsuperscript{23}

**Type I pathway** follows the pathway of transfer of electron or hydrogen atom which leads to the production of radical forms of the photosensitizers or the substrate. Peroxides, superoxide ions, and hydroxyl radicals are formed by the reaction of these intermediates with oxygen thus initiating the free radical chain reactions.

**Type II pathway** is facilitated by the energy transfer process with accompanying the return of the sensitizer to its ground state. The insitu generation of singlet oxygen via type II pathway appears to play the central role in photodynamic cytotoxicity because of the highly efficient interaction of the O$_2$ species with different biomolecules.\textsuperscript{23}

**MECHANISM OF ACTION**

The mechanism of action of the drug involves uptake of photosensitizers by the body followed by localization on the drug (Fig.1). Then on irradiation of the drug induces cell death by either damage the cells or by cell cycle arrest, the details of which are explained below.

When light of specific wavelength is delivered to the photosensitizer drug, the molecules gets excited from ground state to singlet state. On relaxation the molecules move back to ground state producing fluorescence. Some molecules enter the triplet state from where two paths are possible. Type 1 where the triplet state lead to the production of hydroxyl radicals which leads to cell death or the Type II where the triplet state on lowering to ground state converts
triplet oxygen to singlet oxygen which induces oxidative stress which ultimately causes cell death.

**Figure 1- Mechanism of Photosensitizer in Photodynamic therapy**

**Uptake and Localization of PDT**

The cellular sites of photosensitizer localization and photodamage are very important factors as target identification contribute significantly in the drug development process. Photosensitizers that are clinically accepted contain several porphyrin components with different lipophilicity and different intracellular localization. Second–generation sensitizers are more pure compounds and as a result, loci of localization can often be easily identified. Mitochondria, lysosomes, plasma membrane, endoplasmic reticulum have been evaluated as potential PDT targets in the tumor cells. It is a universally accepted fact that the photosensitizer’s lipophilicity and aggregation degree are the main factors that determine the accumulating efficiency and localization specificity in the tumor cells.

As explained by Živilė Lukšienė in 2003 the mechanism of uptake of PDT is as follows. The first step is the intravenous administration of hydrophobic sensitizers. These sensitizers are normally bound to lipoproteins which aid in the transfer of the photosensitizers by the blood system to the tumor specific site. The tumor selectivity is more as the lipophilic characteristic of the drug is increased. Živilė Lukšienė goes on to explain the reason the sensitizers which are lipophilic have a tendency to accumulate in tumors is that neoplastic cells express a
particularly large number of lipoprotein membrane receptors. Following receptor-mediated endocytosis, the sensitizer molecules preferentially accumulate in the lipophilic compartments of tumor cells, including plasma, mitochondrial, endoplasmic reticulum, nuclear and lysosomal membranes.\textsuperscript{23}

The pH value of most tumors is low due to their poor oxygen supply and high glycolytic activity, as a result lower tumor pH is associated with an enhanced uptake of photosensitizers. After intravenous injection of hydrophilic photosensitizers, the drugs are mostly carried by albumin and other serum proteins. Through microscopic measurements it has shown that a preferred accumulation of these sensitizers within the interstitial space and the vascular stroma of the tumor tissue. The hydrophilic behavior of the sensitizers makes it difficult to diffuse across the plasma membrane and move into the cytoplasm. In general, it is by direct interaction that hydrophobic drugs attack tumor cells. On the other hand hydrophilic sensitizers kill hyperproliferating cells indirectly by damaging blood vessels and interrupting the supply of oxygen and other essential nutrients.\textsuperscript{23}

To understand the mechanism of PDT on cancer it is important to understand the cell cycle of cancer cells. Cancer, a group of diseases is characterized by uncontrollable growth of cells. It is different from normal cells by the fact that, unlike normal cells, these cancerous cells do not follow the normal path of cell cycle. In a normal cell, if there is DNA damage it will induce cell cycle arrest, to provide time for repair and enhance cell survival and if repair cannot be made it will undergo apoptosis but, in cancerous cells they move through the checkpoints without being repaired and results in unrestrained proliferation of mutated cells. The ultimate destiny of a cell in response to DNA damage is specific to individual circumstances with most tissues showing a preference for cell cycle arrest, whereas thymocytes and splenocytes are susceptible to apoptosis.\textsuperscript{29}

**PDT Induced Apoptosis**

The migration of ground state oxygen from its formation sites is kept to a minimal so as a result the damage caused to the tissues are kept around the vicinity where the photosensitizers have been introduced and as they do not accumulate in the cell nuclei, photosensitization has generally a low potential of causing DNA damage, mutations and carcinogenesis.\textsuperscript{23}
Mitochondria mediated and Cell death mediated apoptosis

Photodynamic therapy induces apoptosis via two major pathways: mitochondria-mediated or intrinsic pathway, and death receptor-mediated or extrinsic pathway. The mitochondria mediated is when the photosensitizers localize in the organells of mitochondria while the intrinsic pathway is when other structures are direct targets for photosensitizers.

In mitochondria mediated the mitochondrial transmembrane potential is disrupted and this results in the release of cytochrome c and cytosol, which in turn facilitates the formation of a complex called apoptosome and activation of hydrolytic enzymes — caspases. Activation of these hydrolytic enzymes leads to the cleavage of multiple cellular proteins, DNA fragmentation and eventually cell death. Death receptor-mediated apoptosis occurs normally when the cell membrane are targets for the photosensitizers. Almeida explains this pathway is activated by multimerization of cell membrane receptors belonging to the tumor necrosis factor (TNF) receptor superfamily. Fas receptor is considered to play a major role in PDT induced apoptosis. “Multimerization of Fas receptors allow formation of a “death inducing signalling complex” consisting of Fas, FADD adaptor protein and procaspase-8. In these conditions, procaspase-8 activates itself proteolytically and activates downstream effector caspases.”

Signalling mediated apoptosis

PDT damage of plasma membrane can be observed within few minutes after light exposure. This happens as most hydrophobic photosensitizers accumulate in the plasma membrane and as a result the plasma membranes are targets for phototoxic events. This type of damage is manifested as swelling, blebbing, shedding of vesicles containing plasma membrane marker enzymes, cytosolic and lysosomal enzymes, reduction of active transport, depolarization of plasma membrane, inhibition of activities of plasma membrane enzymes such as Na+ K+ -- adenosine triphosphatase (ATPase), a rise in Ca²⁺, up- and down- regulation of surface antigens, etc. Plasma mitochondria and nuclear membrane are severely damaged by oxidation of unsaturated fatty acid residues and cholesterol.

Almeida explains that there are three different signaling pathways that may be involved in photodynamic action originating at the plasma membrane, including activation of phospholipase C (PLC), phospholipase A2 (PLA2) and ceramides. PLC cleaves phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). Calcium ions from intracellular stores are released with the
involvement of IP₃, DAG activates protein kinase C (PKC), which facilitates in the induction of cell death or survival. ³⁰

**Cell cycle arrest mediated apoptosis**

Events of the cell cycle are triggered by an independent cell cycle control system, which guarantees that the events are timed accurately occur in the right sequence and occur only once per cell. The control system is responsive to various intracellular and extracellular signals, in order that the cell-cycle progression can be arrested when the cell either fails to complete an essential cell-cycle process or comes across undesirable environmental factors. ³²

The central components of the cell-cycle control system are cyclin-dependent protein kinases (Cdks), whose activity relies on connection with cyclins which are regulatory subunits. Differences in the activities of various cyclin-cdk complexes lead to the initiation of various cell cycle events. So, activation of S phase cyclin cdk complexes initiates S phase, while activation of M phase cyclin-cdk triggers mitosis. Two enzyme complexes, SCF and APC are also crucial components of the cell cycle control system, they induce the proteolysis of specific cell-cycle regulators by ubiquitylating them and thereby trigger several critical events in the cycle ³³.

Cell damage of PDT can be caused due to growth arrest. Various studies have been carried out to study the cell cycle arrest caused by PDT. The cascades of events involved are as follows. After photosensitizing the cells, electrons are excited from ground state to an excited singlet state and then enter into the triplet excited state through an intersystem crossing. On retrieving back to the ground state, highly reactive singlet oxygen is produced. Singlet oxygen causes oxidative stress to the cells forcing the cells to undergo cell death or causing difference in regulation of the proteins. Among the proteins, p53 protein induces the release of p21 protein and as a result proteins like cyclin dependent kinase as a result stopping the progression of the cell cycle to the G0/G1 phase. Various studies have shown that chemotherapeutic drugs like cisplatin, taxol, vinblastin and colchacin follow similar paths where due to the induction of photosensitizer, p53 protein induces the release of p21 protein and as a result proteins like cyclin B are repressed causing the cell cycle to stop at the G2/M phase.³³

**CONCLUSION**

Photodynamic therapy is a very promising field in the development of cancer treatment. PDT
is an approved method for treatment for four types of cancers including skin, lung, early or late cancers of head and neck and oesophageal. Its quick procedure and cost efficient method are some of the added advantage the treatment posses besides the fact it does not possess the unpleasant side effects the current treatments have. Besides re treatment is another advantage to PDT when compared to radiation which is limited to the number of doses. For the treatment of breast cancer the studies are still in clinical trials and give promising results. Rosa et al in 2004 studied the effect of photorin based PDT on breast cancer with chest wall progression. In the study 14 patients with a total of 500 truncal metastases were treated with PDT with total light dose of 150 to 200 J/cm2 at 48 hours. The result showed that all patients exhibited tumor necrosis. They concluded that PDT for chest wall recurrence of breast carcinoma provides a platform for good long-term local tumor control. With a high response rate, few complication and high level of tolerance patient can be treated on an outpatient basis making it less traumatic and an excellent alternative for cancer therapy.

REFERENCES


