



# Photodynamic Therapy as a Treatment to Nasopharyngeal Carcinoma

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## Abstract

NPC is highly prevalent in Hong Kong with more than 800 new cases reported annually. The epidemiologic evidence implies that Epstein-Barr virus (EBV) infection, environmental factors and genetic factors play roles in the tumorigenesis of NPC. Conventional treatment of NPC is chemoradiotherapy, yet the treatment outcomes in patients with advanced stage of NPC are unsatisfactory. Therefore development of new treatment strategies is crucial for patients with Nasopharyngeal Carcinoma (NPC), particularly for patients with drug resistance properties or distance recurrence. Photodynamic therapy (PDT) is an FDA approved cancer regimen which employs a combination of light-activated photosensitizer visible light and molecular oxygen to selectively destroy the biological targets. 3<sup>rd</sup> generation of photosensitizers with light activation by advance light sources such as coherent light source (laser) and non-coherent light source (LED) could be one of novel strategies in for NPC patients. In-depth investigation for selected PSs mediated PDT on NPC model is still underway.

**Keywords:** Photodynamic therapy; Nasopharyngeal carcinoma; Epstein barr virus; Microna; Drug resistance

## Introduction

### Overview of nasopharyngeal carcinoma (NPC)

Nasopharyngeal carcinoma (NPC) is endemic in Asia and is one of the top ten cancers highly prevalent in Hong Kong. The overall incidence is 6.5/100,000 person-years in southeastern Asia. Yet the incidence rate increase sharply in Hong Kong to 12.2/100,000 person-years [1-4]. Nasopharyngeal Carcinoma (NPC) encompasses any squamous cell carcinoma arising in the epithelial lining of the nasopharynx and is characterized by poor or undifferentiated carcinoma [5,6].

### Classification of NPC

The histopathological classification of NPC was distinguished into three types by The World Health Organization according to the degree of differentiation. Type I is keratinizing squamous-cell carcinoma similar to carcinomas that arise from other sites of the head and neck. Type II is non-keratinizing epidermoid carcinomas. Type III represents the undifferentiated carcinomas [2,7]. Among these, NPC Type I is uncommon in endemic areas while type II and type III NPC are more common and are closely related to EBV infection [8].

NPC can be also staged clinically according to the 7<sup>th</sup> edition of the International Union against Cancer (UICC) and the American Joint Committee on Cancer (AJCC) staging-system manual. AJCC classifies NPC into IV stages according to the TNM system. TNM system contains 3 key pieces of information, includes T) describes whether the primary tumor has invaded into nearby tissues or organs, N) describes whether the primary tumor has spread to nearby lymph nodes, and M) describes whether the cancer has metasized. Different score are given to the TNM system in order to provide more details about each of these descriptions. The number 2 to 4 indicate the degree of spread, while X will be given to those cases which cannot be assessed. Stage I NPC is T1-N0-M0, indicates tumor is in the nasopharynx and may spread to nearby soft tissues. Stage II NPC is T2-N0-M0 or T1/T2-N1-M0, indicated the tumor has spread into nearby tissue. Stage III NPC is T3-N0 to N2-M0 or T1/T2-N2-M0, indicates the tumor has spread to lymph nodes. Stage IV NPC is T4-N0 to N2-M0 or Any T-N3-M0 or Any T-Any N-M1, indicates the tumor may have spread to distant sites [9].

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## NPC Tumorigenesis

It is widely accepted that Epstein-Barr virus (EBV) infection plays a major role in the tumorigenesis of NPC. EBV is a herpes virus that infects over 90% of adult population and is consistently detected in NPC patients [10,11]. Studies reported that EBV is one of the most potent transforming agents for human cells and is associated with a number of malignancies, including Burkett's lymphoma and nasopharyngeal carcinoma [12-14]. It is rare to observe viral replication in EBV-infected cells as EBV establishes a latent infection with a restricted set of latent gene being expressed, including two EBV-encoded nuclear RNAs (EBER1, EBER2), six EBV-encoded nuclear antigens (EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C, EBNA-LP), and three latent membrane proteins (LMP1, LMP2A, LMP2B). These latent gene expression is now identified at three patterns, which is known as latency I, II and III. Only the EBERs and ENBA1 are expressed in latency I; the EBERs, EBNA1, LMP1 and LMP2 are expressed in latency II; and all latent genes are expressed in latency III [12,15-19].

In NPC, EBV replicates and hides in cells followed by type II latency infection cycle, with expression of a limited number of viral proteins, including the latent membrane proteins (LMP1, LMP2A and LMP2B) and EBV-determined nuclear antigens (EBNA1 and EBNA2). Among these, LMP1 is the principal oncogene involves in the process of EBV-associated oncogenesis of NPC [17,20-26]. LMP1 is a 66kDa integral membrane protein consists of a 6 transmembrane domains and a carboxyl-terminus containing 3 signaling domains called C-terminal activating regions 1, 2 and 3 (CTAR 1, CTAR 2 and CTAR 3). The three CTAR domains provide docking sites for signaling adaptor proteins. Among these, CTAR 1 and CTAR 2 are two of the distinct functional domains responsible for the possess of most of the LMP1 signaling activity via directly activate a number of signaling pathways including nuclear factor kappa B (NF-kB), mitogen-activated protein kinases (MAPK) and Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway [8,23,27,28]. The LMP1 induced signal pathways is one of the elemental factors controlling the biological behaviors of NPC. Signaling pathways involved are mainly control cell functions such as the inhibition of apoptosis; induction of cell immortality; promotion of cell proliferation and influence the cell invasion and metastasis [7,16,29,30].

## EBV Encoded Micro RNAs

In additional to the well-established viral protein expression, EBV has been found to express various RNAs. These have been referred to as complementary strand transcripts (CSTs), BamHI A rightward transcripts (BARTs) or the BARF0 RNAs [31,32]. The possible role of these RNAs may affect viral transformation as high levels of CST and BARTs expression have been detected in NPC tumors [20,32,33]. The recent discovery of BART micro RNAs (miRNA) has shed new light on the function of these transcripts. miRNAs are a class of 19-24 nucleotide non-coding RNAs which modulate gene expression. miRNAs are produced by endogenous enzymatic (Dicer) digestion of RNA transcripts containing hairpin. Protein translation is inhibited by forming complementary duplexes of miRNA with their target mRNAs and cause degradation of these mRNAs [29,34]. The number of EBV miRNAs made up 23.2% of the total miRNAs in the biopsy samples in NPC patients, whereas only 0.1% EBV miRNAs is found in adjacent normal nasopharynx tissue

[35]. These potent gene regulators are through to control a wide range of biological functions, including differentiation, cell growth and cancer development [34,36,37]. Several studies reveal the function of EBV encoded miRNAs to modulate both viral and cellular gene expression in NPC cells, alternating the anti-apoptotic function, immune evasion, and viral protein expression patterns. MiR-BART5-5p was found to be down regulated the expression of a pro-apoptotic protein, PUMA in C666-1 cells. It can keep the cell survive and thus the period of latency increase [38]. miR-BART16 could down regulate TOM22, a mitochondrial receptor for the pro-apoptotic protein Bax. miR-BART2-5p could help the host cell to escape from NK cell recognition by repressing the expression of cellular stress-induced immune molecule (MICB). miR-BART 3 targets IPO7, a nuclear importer receptor which has been showed to be responsible for the nuclear translocation of transcription factor and is involved in cytokine and early gene expression in activated T cells. miR-BART6-5p could suppress viral onco-protein EBNA2, preventing the transition from latency I and II to III [39]. miR-BART2-5p can inhibit EBV lytic replication by targeting the viral DNA polymerase BALF5. miR-BART22 can down regulate the expression of LMP2A. It can inhibit telomerase reverse transcription and induce anti-proliferation by NF-kappa B suppression.

EBV miRNAs demonstrate differential expression in different latent stages. EBV BARTs produce two clusters of miRNA and that the BART Cluster 1 miRNAs target the LMP1 protein expression and BART Cluster 2 miRNAs target EBV DNA polymerase BALF5 for degradation, effectively inhibiting lytic replication and help EBV establish a type II latency in infected cells [29,36,38,40]. EBV constitutively express up to 44 mature miRNAs and their main target is its oncogene LMP1 [29]. Recent report indicate three BARTs cluster 1 miRNAs (ebv-miR-BART1-5p, 16 and 17-5p) targeting the LMP1 gene and down regulating the LMP1 protein expression [27,34,41]. One possible reason for the high level expression of miRNAs in EBV is to avoid over expression of LMP1 protein. The over expression of LMP1 protein could results in the inhibition of cell proliferation and increase in apoptosis ref. Therefore, introducing miRNAs could inhibit excessive MP1 expression in NPC cells and results in resistant to the apoptosis [27,34,41-45].

## Drug Resistance Mechanisms in NPC

Multidrug resistance is the major obstacle to chemotherapy in tumor patients. Development of MDR may be intrinsically prior to treatment or acquired during treatment [46]. Increase drug efflux from the cells via the adenosine triphosphate (ATP) binding cassette transporters (ABC), inactivation of drugs via detoxifying enzymes, and defective apoptotic pathways are three most common cause of MDR development [47,48]. Among these, the increase drug efflux via ABC membrane transporters is one of the leading mechanisms of MDR development in tumor cells [49,50]. ABC membrane transporters includes P-glycoprotein (P-gp/ABCB1), multidrug resistance associated protein 1 (MRP-1/ABCC1) and breast cancer resistance protein (BCRP/ABCG2). Among these, P-gp is the best studied mechanisms of MDR phenotype [51,52].

## Multidrug Resistance Mechanisms Developed in Nasopharyngeal Carcinoma

There are only few articles reported the correlation of multidrug resistance protein expression on EBV infected NPC cells. A few studies demonstrated that P-gp and MRP1 were found to be expressed in

NPC cells but in different levels. Study has shown that a small portion of NPC express MDR1. Study demonstrated a 12.6% NPC patients and 32.6% recurrent NPC patients expressed MDR1 [53]. Another group of researcher reported that only 3.3% of tested NPC patients expressed MDR1 [54]. Study also reported a significantly higher expression level of MDR1 in keratinizing squamous cell carcinoma type NPC than those with non-keratinizing and undifferentiated types NPC. MDR1 is expressed at the apical surface of normal nasopharyngeal epithelial cells, which protects normal tissue against exogenous toxins and hydrophobic xenobiotics.

Different from MDR1 expression, the expression of MRP1 varied among the NPC samples. Study reported that 40% of the tested NPC patient samples expressed MRP. The high expression rate of MRP is proved to be correlated with the clinical stage. The MRP1 expression could be one of the prognostic markers at the time of diagnosis before treatment. However, study fails to illustrate the correlation between MRP and chemo-sensitivity testing with selected anticancer drugs, such as CDDP, 5-FU, PEP, MMC and ADM. Furthermore, concerning the overall 5-year survival rate, there was no difference between NPC exhibiting MRP and NPC not exhibit MRP [55]. Similar result was obtained by another group of researcher [54].

### ABCB1/P-glycoprotein (P-gp)

Phenomenon of multidrug resistance was initially noticed in 1948 in leukemia patients by Farber [56]. In 1976, Ling's group reported that the MDR phenotype is related to decreased intracellular drug accumulation mediated by a 170kDa plasma membrane glycoprotein, which known as the P-glycoprotein [57]. The association of high MDR1 expression levels with intrinsically resistant cancers and the increased expression of the MDR1 gene with cancers acquired drug resistance during treatment confirm the important role of MDR1 in human cancers. Recent studies indicated that P-gp is the most typical ATP-dependent drug efflux pump contributes to multidrug resistance in cancer cells and is the best-characterized mechanism of MDR. P-gp is a 170kDa trans membrane protein consists of two hydrophobic trans membrane domains (TMDs), three membrane spanning domain (MSD) and two nucleotide binding domain (NBDs). It is encoded by MDR1 gene and is located on chromosome 7. [58,59]. The TMDs region that form a pore-like structure contains the drug binding sites for different drugs while the NBDs responsible for the ATP binding and hydrolysis that drives drug transport [60,61].

### Mechanism of Action of P-gp

Drug binding to the pore-like structure (high-affinity drug binding site) initiates the transport cycle via binding and hydrolysis of ATP coupled with P-gp. ATP is bound to nucleotide binding domains leads to conformational change of P-gp. Changes in P-gp conformation results as reduced affinity for drug binding and re-orientation of the site so that it is exposed to the extracellular medium [60,62,63]. Studies have also shown that the binding of ATP, rather than hydrolysis of ATP, provide the energy for drug translocation [59,64-66]. The hydrolysis of ATP and release of ADP from P-gp does affect its conformation, returns the drug binding site to high drug affinity status.

### Conventional Treatment of NPC

Treatment selected for NPC patients were based on the AJCC classification system. The conventional treatment for NPC is chemo-radiotherapy because of high radio- and chemo-sensitivity with a

5-years overall survival of 70-80% for stage I and II NPC. Unfortunately majority of NPC patients were diagnosed with locally advanced stages as it is difficult to detect early because of its complex anatomical location [7,67]. The treatment outcomes in patients with stage II NPC become less favour that with stage I NPC because of the distance recurrence. Local recurrence, distant recurrence and development of multi-drug resistance properties are the most common cause of treatment failure [8,9, 54]. Complications always resulted after cheom-radiotherapy, such as hearing impairment, endocrinological dysfunctions, temporal lobe necrosis, cranial neuropathy, haemorrhage, and bone necrosis [68,69]. Thus development of new treatment strategies is crucial for patients with NPC, particularly for those patients with multi drug resistance properties.

### Overview of Photodynamic Therapy (PDT)

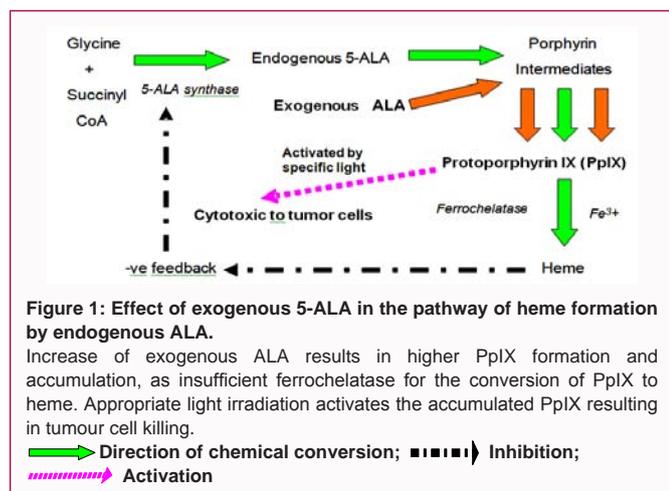
Photodynamic therapy (PDT) is an evolving cancer treatment regimen with approved for use in USA, EU, Canada, Russia and Japan [70-72]. PDT uses a combination of photosensitizing agents (PS), visible light and molecular oxygen to selectively destroy the biological target. None of these is individually toxic, but together they initiate photo-destruction to biological target. In general, tumour localizing photosensitizers will absorb photon to produce photo-toxin such as singlet oxygen ( $^1O_2$ ) and reactive oxygen species (ROS). Depends on the cellular organelles where PSs localized, ROS generated could oxidize many biological molecules, such as protein, lipids and nucleic acids and lead to *in vivo* and *in vitro* tumour cell disruption through apoptosis, necrosis and autophagy [73,74]. The antitumor effects of PDT derive from 3 mechanisms; including direct cytotoxicity effects on tumour cells, destruction of tumour associated vasculature, and induction of inflammatory reaction against tumour cells [70,73-84].

### Photosensitizers

The prerequisites of an ideal photosensitizer including: chemical purity, low dark toxicity, high quantum yield of singlet oxygen, selective accumulation in tumour cells, short time interval between drug administration and maximal accumulation within target cells, rapid clearance from the body, and being activated by longer wavelength with better tissue penetration [85].

### Hematoporphyrin Derivative (HpD) – Photofrin

The first FDA approved photosensitizer was Hematoporphyrin derivative (HpD). It was developed in the 1970s and early 1980s and is now known as the 1st generation photosensitizers for the treatment of tumor, such as early and late endobronchial lesions, Barrett's esophagus and esophageal obstructing lesions with high response rate and promising result obtained [86-89]. It is a mixture of monomers, dimmers and oligomers of hematoporphyrin synthesized by chemical manipulation and are the photosensitizer that brought PDT to a worldwide audience. The first report of the preparation of HpD was published by Dougherty in 1983[90]. Afterward, a number of HpD formulas were available in commercial and Photofrin from Axcan Pharma was the one commonly used for tumor treatment. Photofrin mediated PDT is performed as follows: i) Photofrin is administrated via intravenous injection with 24-48 hours incubation, ii) activated Photofrin with light at wavelength 630 nm. It has had promising results in controlling recurrence in breast cancer, brain tumors, and head and neck neoplasms [91-93]. However, drawbacks of Photofrin such as low singlet oxygen quantum yield at 630 nm and long clearance time limited the application of Photofrin in cancer treatment. The



drug is ineffective to generate singlet oxygen at 630 nm activation and require long time for treatment. A 24-48 hours drug incubation time favors concentration of Photofrin in rapid proliferating tissue such as tumor and to maximize the difference of Photofrin concentration in normal tissue and rapid proliferating tissue. Slow clearance time is another drawback of Photofrin mediated PDT. HpD remains in tissue for 4-6 weeks after injection and thus patients are advised to protect themselves from exposure to sunlight or bright light for a 4-8 weeks period in order to avoid skin photosensitization [94,95].

## 5-aminolevulinic Acid Hexyl Ester Derivatives and Protoporphyrin (PpIX)

A number of 2<sup>nd</sup> generation photosensitizers of different chemical families were synthesized in the late 1980s to offer potential advantages over the 1<sup>st</sup> generation photosensitizers, including higher chemical purity, better tumour selectivity and faster clearance [86,96].

5-aminolevulinic acid (5-ALA), the precursor substance of protoporphyrin, is one of the FDA approved 2<sup>nd</sup> generation photosensitizers and is popularly used for in vivo and in vitro studies over the past decade. It has been applied on cancers such as skin cancer, gastrointestinal adenocarcinoma, Bowen disease and basal cell carcinoma and promising result was obtained [97,98].

5-ALA is the precursor (also known as pro-drug) of an endogenous photosensitizer, protoporphyrin IX (PpIX), involved in the heme bio-synthesis pathway. 5-ALA accumulated in mitochondria region will be converted into PpIX through oxidation (Figure 1) and will further convert into iron (II) protoporphyrin (protoheme) by ferrochelatase in the presence of iron. By adding excess exogenous 5-ALA, more PpIX will be generated and accumulated in the cells as the rate of ALA transform into PpIX is greater than the rate of PpIX convert into protoheme. PpIX is temporarily accumulated in cells with high exogenous ALA, thus within tumour cells (Figure 1) [97]. Cells will become cytotoxic when PpIX is photo-activated with specific wavelength. The wavelength applied depends on the absorption wavelength of photosensitizer. Low wavelength will have a poor penetration rather than high wavelength. However, if the light applied has a too high wavelength may cause photo-damage to the surface cells. Thus the optimum wavelength applied is always between 620-650 nm [97,99]. There are several factors affecting the PpIX accumulation in tumour cells, such as the uptake of 5-ALA, concentration of iron and activity of the enzyme ferrochelatase [100].

The major side effect of 5-ALA refers to its poor penetration

ability via the biological membrane. The poor biological membrane penetration of 5-ALA was due to its hydrophilic properties [97]. Therefore, a more effective and powerful derivative of 5-ALA was developed and is known as the 5-ALA hexyl ester (H-ALA). H-ALA is one of the 5-ALA derivatives with an increase in lipophilic properties by adding a long lipophilic chain (hexyl group) to 5-ALA. The hexyl group added to 5-ALA results in better penetration of 5-ALA into the cytoplasm. Study shows that a 60-fold increase of ALA is needed to produce the same amount of PpIX accumulation inside cells compared to H-ALA [101-109].

## Foscan® and FosPeg® (Biolitec AG)-Meso-tetrahydroxyphenylchlorin (mTHPC) and Its Pegylated Liposomes Form

Meta-tetra (hydroxyphenyl) chlorine (mTHPC) is another 2<sup>nd</sup> generation photosensitizer with a hydrophobic nature that has excellent photocytotoxicity. It is a clinically approved photosensitizer in USA, Europe and UK and has been shown to be highly effective in treating diseases like basal cell carcinoma, prostate and pancreatic cancer [110-113]. The typical mTHPC mediated PDT is performed as follows: i) mTHPC is administered via intravenous injection, ii) a 24-96h drug incubation followed by light activation at 652 nm wavelength. The mTHPC, a chlorine-like photosensitizer, contains an active ingredient temoporfin and is derived from the reduction of porphyrins. Bonnet was the one who first synthesized temoporfin as a pure compound by a reduction reaction and reported its photophysical properties and photo-cytotoxicity in 1989 [114]. It has a hydrophobic nature which ensures rapid penetration across biological membranes and localization at critical intracellular membranous organelles [115]. The absorption peak of mTHPC shifts to the longer wavelength of 652 nm in the red spectrum, which favors deeper tissue penetration. It showed a 10 times higher extinction coefficient than that of Photofrin and results in a shorter incubation time [116]. Therefore, it is a more potent photosensitizer (approximated 100 times greater) than Photofrin or 5-ALA [95,117-119].

However, the major drawbacks of mTHPC are the bio-distribution, clearance and selectivity of tumour uptake. These problems are related to the photochemical properties of mTHPC. The hydrophobicity leads to poor solubility of mTHPC in physiologically acceptable media, which complicates its formulation and administration. It is soluble in inorganic solvent but is insoluble in all aqueous media. Another side effect of mTHPC is the bio-distribution. Hydrophobic mTHPC forms aggregates, which decrease in photo-activity and bind strongly to serum protein. mTHPC will also accumulate in subcutaneous fat tissue near intravenous administration and prolongs the clearance time to 4-6 weeks after injection. All these problems of mTHPC urge the development of a drug delivery system – liposomal formulation of mTHPC [120,121].

Current development of photosensitizers, also known as the 3<sup>rd</sup> generation of photosensitizers, aims to improve drug delivery approaches, such as biological modifications like antibody conjugate or liposome conjugate [77,84,122-124]. The aim of using liposomes for carrier and delivery systems is to improve its therapeutic effects by solubilizing the photosensitizer at a suitable concentration, increase drug uptake as well as tumour eradication [125-128]. The formation of mTHPC contained in pegylated liposomes is known as FosPeg® (Biolitec AG). The lipocompatible polymer Polyethylene glycol (PEG) has been chemically linked to the outer surface of the liposome. They function as i) stabilizer to stabilize the liposome, ii)

increases its hydrophilicity to minimise the binding of liposomes to opsonins (minimize the loss of liposome from circulation), iii) inhibit the release of hydrophobic PSs to the liposomal membrane system via binding with the serum protein, iv) avoid recognition by the host's immune system [120,129-131]. Modification of liposomes with long-circulating poly ethylene glycol (PEG) could improve the bioavailability of m-THPC and could improve the therapeutic index of encapsulated drugs [115,120,125-127,132,133].

## Light Sources and Light Dose for PDT

Development of new light source is one of the important factors to improve the efficiency of PDT. Light sources commonly used for PDT including laser, laser diodes, light-emitting diodes and filtered board-band light. Three main criteria must be fulfilled for an ideal light source, including i) light spectrum emitted must correspond to the absorption spectrum of the photosensitizer selected, ii) the wavelength must be long enough in order to achieve a deep tissue penetration, iii) sufficient photon energy should be provided to maximize the quantum yield of singlet oxygen [134-136]. The choice of light source depends on the photosensitizer and the depth of tissue need to be penetrated. In early days, conventional light sources such as halogen lamps and xenon lamps were employed for the *in vitro* and *in vivo* PDT studies. As board-band light sources, the specific wavelength output is achieved by adapting different filters. Usage of these non-coherent conventional light sources to activate photosensitizers mainly due to the following, i) the ease of use, ii) low cost iii) and large treatment area [124]. Incoherent and coherent light are commonly employed for PDT and usually show similar efficiencies [137].

Advanced light sources such as laser, laser diodes and light-emitting diodes (LED) have become the light source of choice as they could produce coherent light with monochromatic wavelength that allows easy calculation of light dosimetry. Light generated from these light sources could be delivered down an optical fibre to the desired site of treatment to achieve a greater selectivity [138,139]. Among these, LED based light sources become more important and raise peoples' concern in recent time because of the advantages such as relatively cheap, considerably small in size and relatively mobile, produce cool light which need not to have cooling units, and reliable [140]. Studies of light dosimetry focus on the calculation of power needed for a PDT treatment. The power applied for a PDT treatment is defined as the light dose, which expressed as the energy delivered per unit area ( $J/cm^2$ ). The term light dose is used to describe the relationship between light power density and irradiation time in order to reflect the amount of light that reaches the surface of the tumour in a period of time. There are some criteria for selecting an appropriate light dose, including the penetration depth to the tissue, low light intensity and short treatment time to avoid heating [141-143]. In parallel to the light dose, drug dose is also important to formula the overall photodynamic dose (light dose x drug dose). Factors affect the outcome of PDT including, photosensitizers (PSs) applied, intracellular concentration of PSs, localization of PSs, quantum energy obtained by the PSs and concentration of molecular oxygen presence.

## PDT Effect on NPC – Molecular Targets and Drug Resistance

The development of improved therapeutic strategies, such as PDT and immunotherapy, shed light on the development of NPC treatment

[108,144-148]. Our group demonstrated promising outcomes from a number of *in vitro* studies concerning the PDT effect using several PSs including hypericin, mTHPC, merocyanine 540, 5-ALA and hexyl-ALA on NPC/HK1, NPC/CNE1 and NPC/CNE2 cells. We found that such photo-cytotoxicities were mediated through the mitogen activated protein kinase (MAPK) signal pathways. In response to PDT, expression of MAPK signals - ERK and p38 and Epidermal Growth Factor receptor (EGFR) signal pathways were found to be inhibited and associated with the apoptotic tumor cell death. Our studies also revealed the PDT effect on EBV viral components. PDT triggered LMP1 related mRNA and miRNAs expression, which inhibit NPC cell proliferation and cell migration. The PDT efficacy was also proved to be independent to the drug resistance properties [108,119,132, 149-158]. Other study reported the association between suppression and anti-proliferative activities induced by PDT using different cell models. Lai and his colleagues showed that PDT has an immuno-enhancing effect in NPC patients by increasing natural killer cells and interleukin-2 [159]. Another group from Hong Kong has illustrated similar outcomes by using other PSs curcumin and Zn-BC-AMon NPC/CNE2 cells and NPC/HK1 cells respectively [156,158,160]. Preliminary clinical studies using hematoporphyrin and temoporfin for the treatment of the local and recurrence of NPC after curative radiotherapy found encouraging result for residual or recurrent NPC restricted locally to the nasopharynx [161,162]. Studies also revealed that PDT could modulate the inflammatory cytokine production and angiogenic factors production [163-165].

## Conclusion

PDT could be one of the best choices over the conventional cancer therapies for NPC patients, particularly for those with distance recurrence and multi drug resistance properties developed.

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