REVIEW ARTICLE

Drugs 1999 May; 57 (5): 725-734
0012-6667/99/0005-0725/$10.00/0

Photodynamic Therapy in the Treatment of Cancer

Current State of the Art

R. Alex Hsi, David I. Rosenthal and *Eli Glatstein*

Department of Radiation Oncology, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, USA

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Abstract

Photodynamic therapy (PDT) is a treatment modality using a photosensitising drug and light to kill cells. The clinical use of PDT requires the presence of a photosensitising agent, oxygen and light of a specific wavelength which matches the absorption characteristics of the photosensitiser. When the photosensitiser is activated by the appropriate wavelength of light, it interacts with molecular oxygen to form a toxic, short-lived species known as singlet oxygen, which is thought to mediate cellular death. The appeal of PDT in oncology is that the photosensitiser tends to be retained in tumour tissues for a longer period of time as compared with normal tissues resulting in a large therapeutic index. This potential for minimal normal tissue toxicity has prompted an interest in studying PDT as a cancer treatment. Furthermore, the use of PDT is not precluded by prior radiotherapy, chemotherapy or surgery. The development of PDT has been hampered by the limitations of the older photosensitisers, namely limited depth of tissue penetration, and extended skin phototoxicity which limits the number of applications during a course of treatment. However, newer photosensitisers are being developed which allow greater depth of tissue penetration and have minimal skin phototoxicity allowing for multiple fractionated treatments. With such advancements, PDT has great potential to become an integral part of cancer treatment in the future.

The origin of PDT can be traced back to the turn of the century when Raab described the lethal effect of light on paramecia treated with an acridine dye. In 1961, Lipson et al.[1] reported on the use of haematoporphyrin derivative (HPD) for fluorescent detection of tumour tissue. When cells containing photosensitiser are exposed to light of certain wavelengths, fluorescence of tumour occurs and may aid in diagnosis, whereas at other wavelengths, cytotoxicity results. Dougherty[2] first reported in 1975 the eradication of transplanted animal tumours with HPD and red light without excessive damage to surrounding uninvolved skin. The first human therapeutic use was in a patient with recurrent bladder cancer before cystectomy. Since that time, the therapeutic applications of PDT have been expanded to include skin, gastrointestinal, pulmonary, head and neck, gynaecological and various intraperitoneal malignancies.

1. Light Sources

Any type of light source can be used for PDT. However, the laser has unique properties that make it the most efficient source. The laser consists of an external power source (light, electricity or radio waves) acting upon a laser medium (helium, argon, neon, CO_2, Nd:YAG, ruby crystal) which is contained in an optical cavity to cause excitation of the atoms of the laser medium with the consequent emission of photons. These emitted photons constitute the laser beam and are unique because of 3 properties: monochromaticity, coherence and collimation. Monochromaticity refers to the fact that all waves of the laser beam have virtually the same wavelengths and energies. Coherence means that all waves are exactly in phase with each other both in space and in time. Collimation implies that the rays are virtually parallel and the laser beam does not diverge.[3]

The electromagnetic waves generated by lasers used in PDT do not produce ionising radiation (x-rays and γ-rays) but operate in the visible light and infrared regions of the electromagnetic spectrum. Light dose or fluence is expressed in joules per unit length or area (J/cm or J/cm^2) and fluence rate is expressed in (milli)watts per unit length or area (mW/cm or mW/cm^2).

Lasers and optical fibres allow light delivery to deep-seated tumours through endoscopic, interstitial or intracavitary techniques. Clinical PDT lasers include argon pumped dye lasers or quasipulsed metal vapour lasers, which can yield up to 5W of usable light.[4] More recently, solid-state lasers with even more power have been developed that may also offer greater reliability at lower costs than currently employed systems.

2. Photobiology

2.1 Mechanisms of Tumour Destruction

Although the exact mechanisms of action of PDT are not entirely defined, it is clear that PDT involves the interaction of oxygen, photosensitiser and light (fig. 1). The photosensitiser is excited and activated by the light and interacts with molecular oxygen to yield reactive singlet oxygen ($^1O_2$), the proposed mediator of photodynamic cytotoxicity (type 2 photo-oxidation). Singlet oxygen further reacts with biomolecules to form cytotoxic oxyproducts. Singlet oxygen has both a short lifetime (<0.04 μsec) and short radius of action (< 0.02μm).[5]

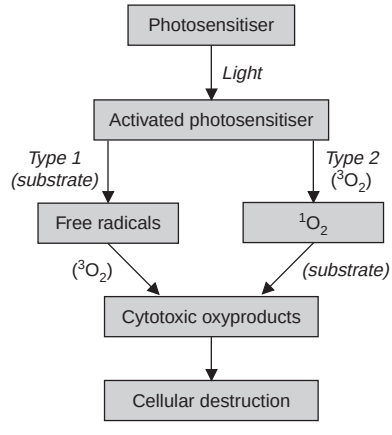

Fig. 1. Type 1 is a direct reaction of the excited sensitiser with a biomolecule by a mechanism involving electron transfer to yield free radicals. A type 2 reaction results from energy transfer from the excited sensitiser to molecular oxygen to produce singlet oxygen ($^1O_2$).

Alternatively, an excited sensitiser may react directly with a biomolecule (type 1 photo-oxidation) to form free radicals which further react with molecular oxygen. An activated photosensitiser also has the potential to fluoresce when returning to the ground state, thus providing an opportunity to localise occult tumours via fluorescence detection techniques.

The cellular and subcellular location where the photodynamic effect occurs has been the subject of much investigation. It appears that both direct cytotoxic activity and microvascular damage may contribute to the destruction of tumour tissue. The direct cytotoxic effect is the result of incorporation of the photosensitisers into cellular membranes such as the plasma membrane. This damage is manifested by swelling, bleb formation, shedding of vesicles containing cytosolic enzymes and inhibition of membrane enzymes such as Na^+, K^+-ATPase.[6-8] Other subcellular targets include lysosomes, Golgi apparatus and rough endoplasmic reticulum. It is felt that the association of photosensitisers to low density lipoproteins (LDL) is, at least in part, responsible for localisation within these subcellular sites.

In addition, mitochondria have also been shown to be targets of certain photosensitisers such as porfimer sodium (Photofrin®) and 5-aminolevulinic acid (ALA). In the case of ALA, the heme biosynthetic pathway of mitochondria is utilised to convert ALA to its active metabolite protoporphyrin IX.

Photosensitisers do not appear to accumulate within cellular nuclei and DNA damage does not seem to play a significant role in the action of PDT. This is supported by the observations that PDT is not mutagenic in *in vitro* systems[9,10] and the incorporation of broxuridine (bromodeoxyuridine) does not sensitise cells to PDT as it does with ionising radiation whose effect is primarily on DNA.[11,12]

The vascular effect of PDT also contributes to varying degrees to tumour control. The mechanisms of this effect vary with different photosensitisers although the end result of tumour hypoxia and anoxia is the same for all photosensitisers. Porfimer sodium PDT induces platelet activation and release of thromboxane, resulting in vessel constriction and thrombus formation.[13,14] Various phthalocyanine compounds have been shown to cause primarily vascular leakage,[15] and mono-L-aspartyl chlorin e6 results in platelet aggregation and blood flow stasis.[16]

Damage to the vascular endothelium as well as inhibition of production or release of nitric oxide by the endothelium may also play a role in the damage of tissue microvasculature after PDT.[17] Administration of agents inhibiting nitric oxide synthase or scavenging nitric oxide appears to enhance tumour cure presumably by enhancing the effect of PDT on vascular perfusion.[18]

More recently, it has been shown that an apoptotic response may also contribute to the antitumour effect of PDT. Apoptosis is a mechanism by which normal genetically programmed cell death occurs within an organism. Malignant cells tend to lose this mechanism and hence have no natural inhibition to their growth. PDT has been associated with an enhanced apoptotic response.[19] It has been shown that the release of cytochrome c and other mitochondrial factors into the cytoplasm can initiate an apoptotic response.[20,21] Thus, sensitisers which localise in the mitochondria such as porfimer sodium or are produced in the mitochondria such as protoporphyrin IX (via ALA) are likely to induce apoptosis.

A PDT-induced immune reaction which sensitises the host to tumour antigens may also play a role in long term tumour control. After the PDT reaction, destroyed tumour cells are phagocytosed by macrophages which then act as antigen presenting cells. Under the direction of inflammatory mediators, these cells process tumour-specific antigens and present them on their membrane surface, thus inducing T lymphocyte mediated cellular immunity.[22] Once immunity is acquired, the activity of these lymphocytes can extend beyond the primary tumour site to possible metastatic sites.

2.2 Photosensitiser Localisation

The mechanism of preferential photosensitiser localisation in tumours is not fully understood. However, it appears that factors such as LDL receptor uptake, hydrophobicity, tumour pH, leaky vasculature and poorly developed tumour lymphatics play a role in tumour-specific uptake and retention. In addition, the drug delivery vehicles used may significantly improve the tumour specificity of the photosensitisers.

Photosensitisers have clearly been shown to associate with LDL and are taken up by cells via LDL receptor-mediated endocytosis.[23] This pathway delivers the photosensitiser rapidly to the lysosomal compartment which, when exposed to light, releases lysosomal hydrolases into the cytoplasm.[24]

Many types of tumour cells express a high number of LDL receptors which may result in an increased uptake of photosensitiser relative to normal cells.[25] However, the importance of this mechanism has been debated as some compounds which have a high specificity for tumour cells do not bind to LDL while others which are not very specific for tumour cells have a high affinity for LDL.[26] The affinity of photosensitisers for tumour tissue also increases with their increasing degree of hydrophobicity.[27,28] Liposome-associated photosensitisers have shown improved efficiency of cellular uptake as compared with the same photosensitisers in an aqueous solution.[29] In addition, tumour tissues tend to exhibit a lower pH as compared with normal tissues,[30,31] and cell uptake has been found to increase with decreasing pH.[32] Leaky tumour vasculature may also lead to increased concentrations of photosensitiser in tumour tissue with decreased clearance caused by the poor tumour lymphatic drainage. Finally, the drug delivery vehicles can greatly influence the biodistribution of the photosensitiser.

In addition to lipid-associated vehicles already mentioned, monoclonal antibodies and microspheres directed at tumour cell surface antigens can be used to improve tumour specificity.[33,34] This technology, though still in its infancy, could be used both as an aid to photodiagnostic as well as phototherapeutic purposes.

2.3 The Oxygen Effect

The presence of oxygen is a critical factor in determining the effectiveness of the photodynamic effect. The rate of oxygen consumption, and therefore singlet oxygen production, can be affected by both light fluence rate and fractionation. At high fluence rates, mathematical modelling indicates that oxygen consumption can outpace the rate of oxygen diffusion from capillaries thus resulting in a decrease in the surrounding volume of oxygenated tissue.[35] Lowering the fluence rate and, presumably, oxygen consumption has been shown in preclinical studies to improve the efficiency of tumour response to PDT.[36,37] although the practicality of lengthening treatment times must also be taken into consideration.

Another way to maintain tumour oxygenation is to fractionate light delivery. Alternating intervals of light and dark allow for reoxygenation of hypoxic tissues and thus improved tumour cell killing.[37,38] The optimal fractionation schedule, however, remains to be determined. Factors such as sublethal damage repair during dark periods may affect tumour response when fractionated light schedules are used. The time course for repair of sublethal damage appears to be similar to that seen for ionising radiation, indicating that dark periods of 6 hours or more may decrease tumour response. Preclinical studies by Bellnier and Lin[39] showed an increased cell survival with increasing length of dark periods with maximum cell survival occurring at dark intervals of 4 to 9 hours. It has been suggested that intervals of less than 1 hour would be insufficient to allow for sublethal repair.[37] Other factors such as photobleaching (a chemical reaction which leads to destruction of the photosensitiser during light exposure) as well as vascular destruction after the first light exposure may also reduce the effectiveness of fractionation schemes.

2.4 Dosimetry

One of the difficulties with PDT has been the verification of light energy delivery. In contrast to radiation therapy, where uniform standards have been developed to describe the dose of photon energy delivered to tissues, no commonly accepted standard exists for reporting light dose with PDT. In the case of PDT for the oesophagus and lung, which are currently the only Food and Drug Administration approved uses of PDT in the US, dose is typically prescribed based on light energy output from the diffusing tip of a fibreoptic light source. The diffusing tip is usually cylindrical and output is reported as energy (in Joules) per centimetre of the diffuser length. However, this method does not take into account scattered/reflected light and therefore does not necessarily represent the actual light dose delivered to the tissue. This issue is further complicated when fractionated light treatment schemes are used resulting in progressive inflammation and necrosis causing subsequent changes in the optical properties of the tissue after each light treatment.

Direct measurements of light *in vivo* have been developed using photodetectors. Bays et al.[40] have developed an oesophageal light diffusing system which incorporates an optical detector fibre within the same housing as the diffuser which can more accurately measure the dose of light delivered to the tissue.

At the US National Cancer Institute, a system of flat photodiodes was developed for use in the peritoneal cavity where PDT was used for patients with intraperitoneal carcinomatosis from a variety of malignancies.[41] This system allowed for real time measurements of light fluence and fluence rate. Dose scheduling was based on these measurements rather than output from the light source.

Another system developed in the Netherlands[42] also allows for real time online measurements but employs isotropic optical detectors. This system represents the next generation in clinical photodetection systems and is currently being used both in the Netherlands and at the University of Pennsylvania in trials of PDT in the chest cavity for malignant mesothelioma. No well developed system exists for the measurement of light for interstitial PDT applications. Fenning et al.[43] have developed a model for light distribution based on preclinical data, but the measurement of interstitial light dose is complicated by the continuous changes in blood flow (and thus oxygen concentration) and photosensitiser concentration occurring within tissue. Much work remains to develop an adequate system of light measurement within solid organs treated with PDT.

2.5 Second Generation Photosensitisers

Although porfimer sodium is the most widely used sensitiser for PDT, its drawbacks include extended skin photosensitivity (about 4 to 6 weeks) as well as its limited depth of tissue penetration caused by its relatively low activation wavelength. These factors have led to the development of a second generation of synthetic photosensitisers which produce shorter periods of photosensitivity, longer activation wavelengths which translate into increased depth of tissue penetration, as well as higher quantum yields of singlet oxygen and higher tumour to normal tissue concentrations. These newer agents include chlorins, purpurins, phthalocyanines and texaphyrins.

Benzoporphyrin derivative (BPD) is a chlorin synthesised from protoporphyrin which is activated at 690nm. At this wavelength, tissue penetration is approximately 1cm (versus about 0.5cm with porfimer sodium and 630nm red light). It exists in both a monoacid and diacid form, but the monoacid form has greater photodynamic activity. Peak tissue levels are reached at 3 hours after intravenous infusion and diminishes to 50 to 60% by 48 hours after injection.[44] Skin photosensitivity lasts no more than 1 week. Trials are ongoing using BPD for skin cancers, endometrial ablation, psoriasis and age-related macular degeneration.[45,46]

Another chlorin known as meta-tetrahydroxyphenylchlorin (mTHPC) is a synthetic compound which is activated by 652nm red light as well as 514nm green light. *In vivo* studies[47] have shown that PDT of the murine RIF-1 tumour with porfimer

sodium required a light dose 4 to 13 times higher when compared with mTHPC for a similar antitumour effect. This more efficient cell killing presumably would lead to shorter treatment times for the same biologic effect. In addition, photosensitivity lasts for only 1 to 2 weeks. Studies are ongoing using mTHPC in human mesothelioma, as well as bronchial, prostate and nasopharyngeal cancer.[48]

The phthalocyanines are synthetic porphyrins which are activated at a wavelength of about 675nm (red). They can be chelated with a variety of metal ions such as aluminum and zinc which enhance their phototoxicity. The kinetics of these compounds are much more rapid than porfimer sodium with maximal tumour to tissue ratios reached after 1 to 3 hours. They are also more rapidly eliminated (about 24 hours) and skin photosensitivity is also much shorter than for porfimer sodium.[49]

Texaphyrins are tri-pyrrolic pentazza expanded porphyrins which are activated in the 730 to 770nm range. Texaphyrins can complex large metal cations such as lutetium and gadolinium. Interestingly, gadolinium texaphyrin has been developed as a radiation sensitiser as well as a diagnostic magnetic resonance imaging (MRI) contrast agent for localising tumours.[50]

Lutetium texaphyrin has been developed as a photosensitiser for use in PDT. A recent phase I study using this sensitiser for patients with unresectable or metastatic cancers of the skin and subcutaneous tissue was recently reported,[51] with promising results for patients with chest wall recurrence of breast cancer after mastectomy as well as melanoma and other skin cancers. Skin photosensitivity was mild and transient (less than 72 hours). A phase II study is now underway.

ALA is an early precursor in the heme biosynthetic pathway. After several enzymatic steps, ALA is converted to the endogenous photosensitiser protoporphyrin IX, which is activated by 630nm red light as well as green light. Advantages of ALA include its short duration of skin photosensitivity (1 to 2 days) as well as the ability to administer the drug in topical, oral and intravenous formulations. ALA has been used to treat a variety of diseases including skin cancers, oral mucosal lesions as well as dysplasia and superficial tumours of the oesophagus.[52,53]

3. Clinical Applications

PDT has been tested clinically in a variety of tumours including intraperitoneal, gastrointestinal, genitourinary, pulmonary, head and neck and skin cancers.

A phase I study has been completed at the National Cancer Institute for patients with refractory intraperitoneal malignancies.[54] 51 patients with either primary ovarian tumours, gastrointestinal tumours, sarcomas or pseudomyxomas were treated with surgical debulking followed by intraperitoneal PDT. Porfimer sodium and a combination of red and green light were used to treat the entire peritoneal surface. The major toxicity noted in the study was bowel perforation. Because of the transmural penetration by red light, a switch was made to the less penetrating green light with no further bowel perforations. The maximum tolerated dose of porfimer sodium and light were determined for various intraperitoneal organs and a phase II study is currently underway.

PDT for gastrointestinal malignancies has focused mainly on oesophageal cancer. Both palliative and curative therapy has been reported. Patients receive photosensitiser followed by delivery of light via optical fibres which are passed through a flexible endoscope. A phase II trial[55] randomising 218 patients to palliative oesophageal PDT using porfimer sodium versus Nd:YAG laser ablative therapy revealed equivalent improvement in dysphagia with fewer perforations in the PDT group (1 *vs* 7%, $p < 0.05$).

Curative therapy for patients with superficial early stage carcinomas as well as Barrett's oesophagus with high grade dysplasia have also been reported. Overholt and Panjehpour[56] reported a series of 55 patients with dysplasia and/or early carcinoma in Barrett's mucosa treated with porfimer sodium 2 mg/kg followed at 48 hours by 100 to 300 J/cm of red light. All patients were followed for a minimum of 6 months. The extent of Barrett's mucosa was

reduced by 75 to 80%, with replacement of the glandular mucosa by squamous epithelium and relocation of the squamocolumnar junction distally by approximately 6.4cm. In 43 patients with high grade dysplasia and/or adenocarcinoma, 40 (93%) had ablation of their high grade dysplasia/tumour and 11 of 12 patients with low grade dysplasia had no dysplasia on follow-up endoscopies. However, a major complication was oesophageal stricture (53%) requiring multiple dilation procedures in 4 patients.

Early and advanced stage lung cancer has also been treated with PDT. Bronchogenic tumours can be treated in a manner similar to oesophageal lesions by delivering light via optical fibres which are introduced via a flexible bronchoscope. Moghissi et al.[57] reported a prospective randomised trial of PDT versus Nd:YAG laser ablation for advanced malignant bronchial obstruction. 26 patients with Stage III inoperable non–small-cell lung cancer with >50% intraluminal bronchial obstruction received porfimer sodium (2 mg/kg IV) and 200 J/cm 630nm red light (n = 15) or Nd:YAG laser given by 3 to 5 second exposures at 40 to 50 watts power (n = 11). At 1 month after treatment, the luminal diameter was significantly greater in the PDT group ($p < 0.0006$).

In a series of 21 patients with operable early stage endobronchial squamous cell lung cancer, Cortese et al.[58] noted a 52% complete response rate, with 43% (9/21) of patients spared an operation (which was performed for recurrent disease or subsequent primary lung cancers).

Initial reports have also demonstrated the feasibility of combining surgical resection and PDT for malignant pleural mesothelioma.[59] Baas et al.[42] reported the results of 5 patients treated with surgical resection followed by intraoperative PDT using mTHPC and 652nm red light. With follow-up of 9 to 11 months, 4 of 5 patients were alive with no signs of recurrent tumour.

Low dose PDT (15 to 20 J/cm^2) has been used to treat carcinoma *in situ* or microscopic bladder disease.[60,61] A complete response rate of approximately 75% for papillary lesions has been reported. However, for lesions larger than 1.5cm, the complete response rate is significantly lower (33%).[62,63] Preliminary animal data on the use of PDT for prostate cancer have also been reported. Chang et al.[64] used mTHPC and 650nm red light delivered via the transurethral or interstitial route to treat canine prostatic tissue. Glandular atrophy was noted with no stromal disruption and no change in the ultimate size or shape of the gland. This treatment holds promise both as primary therapy as well as for local recurrence of prostate cancer after radiation therapy.

Cutaneous malignancies, such as basal and squamous cell carcinomas, Bowen's disease, as well as chest wall recurrences of breast cancer have been treated with PDT. Wilson et al.[65] reported an 88% complete and 12% partial response rate in 151 basal cell lesions using 630nm light doses of 133 to 180 J/cm^2 48 to 72 hours after injection of 1 mg/kg porfimer sodium. With 12 months' minimum follow-up, recurrences were seen in <10% of complete responders.

Kennedy and Pottier[66] reported a 90% complete response rate in 80 lesions using topical ALA activated by broad band (600 to 800nm) red light, although follow-up was only 3 months. ALA is particularly advantageous because in aqueous solution it passes readily through abnormal but not normal keratin, thus inducing photosensitisation primarily to abnormal tissue. With longer follow-up, however, tumour regrowth has been reported anywhere from 3 to 50%.[67-69]

PDT using topical ALA for Bowen's disease (squamous cell carcinoma *in situ*) has also been shown to be effective. Cairnduff et al.[68] reported a 97% complete response rate (35/36 lesions) with a 9% recurrence rate after 18 months.

A phase I trial of PDT for chest wall recurrences of breast cancer was carried out at the National Cancer Institute using 1.5 mg/kg porfimer sodium and 630nm red light at doses from 20 to 359 J/cm^2.[70] Responses were seen (20% complete, 45% partial), but the duration of response was short (average 2.5 months). Complications included chest wall pain, erythema, ulceration and necrosis of skin grossly

involved by tumour. Another phase I trial[51] using lutetium texaphyrin and 732nm light reported both a complete and partial response rate of 33% (66% overall response rate). Phase II studies are underway.

PDT has shown some promise in the treatment of head and neck tumours. The accessibility of these tumours via endoscopy as well as the tendency to develop mucosal 'field cancerisation' make them a good target tissue for PDT. Gluckman[71] treated a total of 41 patients with either carcinoma *in situ*, early or advanced squamous cell carcinoma of the head and neck. Using HPD or DHE (dihaematoporphyrin ether) and 50 to 100 J/cm^2 of 630nm light, 8 patients with oral carcinoma *in situ* were treated, with a complete and partial response rate of 87.5 and 12.5%, respectively. Carcinoma recurred in two patients after follow-up of 5 to 53 months. 25 early stage patients had a complete response rate of 56% and a partial response rate of 24%. The best results were obtained in the oral cavity and oropharyngeal patients, where 11 of 13 had complete response and 2 had a partial response. However, 4 patients had tumour recurrence within 1 year of treatment. Eight patients with advanced tumours were treated palliatively, but results were no more effective than standard therapeutic regimens.

4. Future Directions

PDT is a modality with significant potential as a cancer treatment. The recent development of new second generation photosensitisers with decreased toxicity, improved selectivity and longer activation wavelengths will improve the efficacy of PDT and broaden its applications. The development of techniques for interstitial delivery of light will also make it possible to treat nonsuperficial tumours. Further investigation into light dosimetry will be necessary in both interstitial and superficial delivery systems. In addition, the changes in sensitiser concentration, oxygen tension and blood flow which occur during PDT must be defined to optimise conditions for maximal tumour cell killing effect.

Finally, studies combining PDT with other therapeutic modalities such as surgery, radiation therapy, chemotherapy and immunotherapy are in their initial phases and will hopefully improve outcome and minimise toxicity of cancer treatment.

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Correspondence and reprints: Dr *R. Alex Hsi*, Department of Radiation Oncology, 2 Donner Bldg, Hospital of the University of Pennsylvania, 3400 Spruce St, Philadelphia, PA 19104, USA.
E-mail: Hsi@xrt.upenn.edu