Photodynamic therapy for cancers

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Photodynamic therapy (PDT) is a new treatment modality for cancers. After intravenous injection, photosensitizer is selectively retained by the tumor cells. There is more sensitizer in the tumor than in the normal tissue. When exposed to light, the sensitizer produces an activated oxygen species, that oxidizes critical elements of neoplastic cells. In this article we review a large body of evidence suggesting that photodynamic therapy represents a convenient and effective approach to the treatment of malignant tumor. Besides that, PDT is applied to benign diseases. The studies on PDT have shown that cellular immune modulatory effects can be achieved in the absence of cell killing, thus expanding the potential applications of PDT to completely new fields.

DURING the past decade, photodynamic therapy (PDT) has been increasingly recognized as a therapeutic modality used in the treatment of malignant tumors. The first systematic clinical studies of PDT were initiated by Dougherty and colleagues[1] in 1976, while Hayata *et al.*[2] were the first to apply fiberoptic endoscopic laser irradiation to treat early stage bronchogenic carcinoma with PDT 1980. Increasing attention has been paid to the potential of PDT in the treatment of malignant tumor. The past few years have witnessed lot of interest in PDT and over 10,000 papers on the subject have now been published. PDT with Photofrin (Quadra Logic Technologies, Inc., Vancouver, Canada) is now on the verge of becoming an established cancer treatment modality.

In 1993, Canada received the Board of Health approval for use of Photofrin-mediated PDT for treating recurrent superficial bladder cancer. The Netherlands has permitted licenses for treating lung and esophageal cancers with Photofrin PDT. In Japan, PDT with Photofrin and excimer dye laser obtained government approval in October 1994 and finally obtained national insurance reimbursement status in April 1996 for early stage lung, esophageal, gastric and cervical cancer for the first time in the world. Further regulatory submissions for a variety of applications have been made in the United States, France, Germany and Italy. The most promising treatment sites may be those where there is limited thickness of tumor, such as in superficial skin lesions or early-stage carcinomas involving the aerodigestive tract, bronchus, or genitourinary tract. Other potential uses include those in which PDT could be combined with surgery or chemotherapy, treatment techniques that are common in pleural mesothelioma or peritoneal carcinomatosis and novel applications of PDT, such as adjuvant PDT for advanced bronchogenic carcinoma and bone marrow purging. We review in this article the most recent clinical developments in the field of oncology.

Mechanism of photodynamic therapy

Porphyrins have an intense absorption in the blue region around 400 nm (Soret band) and 4 additional absorption bands between 500 nm and 650 nm. The penetration depth of light into tissue is defined as the depth at which the light is reduced by a factor of e^{-1}. However, effect of PDT may occur at two to three times more than these depths. As the penetration of light in tissue increases at higher wavelengths[3], the weakest absorption band at about 630 nm is normally used for illumination of porfimer sodium sensitized tissue. Although 630 nm has been applied most commonly, Star *et al.*[4] have recently shown that the light of 625 nm wavelength has a higher biological activity.

The tissue damage induced by PDT is believed to occur primarily as a result of singlet oxygen ($^1O_2$) formation out of the available tissue oxygen ($^3O_2$) (ref. 5). Several studies have documented the requirement of oxygen for photosensitizing action. Gomer and Razum[6] reported complete resistance of hypoxic tumor tissue to PDT, which has been confirmed by several other studies *in vivo* and *in vitro*[7–9] and singlet oxygen scavengers were found to suppress the photocytotoxic effect[10].

The singlet oxygen is generated via the so-called type II mechanism of photosensitized oxidation. On absorption of photon, the porphyrin molecule is brought to an excited singlet state that has an extremely short half-life. From this singlet state the photosensitizers can decay back to the ground state and emit light in the form of fluorescence.

For the photodynamic effect, however, the photosensitizers should undergo intersystem crossover to the excited triplet state of the molecule. This triplet species is more stable with a longer lifetime than the singlet species. Therefore, the excited triplet state has a high probability of interacting with ground state oxygen. The transfer of

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energy from the triplet photosensitizer generates the highly reactive singlet oxygen (Figure 1).

The electrophiric nature of singlet oxygen makes it very efficient at producing oxidized forms of biomolecules. After transfer of electronic energy to oxygen, the sensitizer returns to its ground state. The excitation efficiency of a photosensitizer is defined as the triplet state quantum yields, i.e. the probability of triplet state formation per photon absorbed.

Photosensitizers

Choosing the sensitizers for clinical trials is a highly selective process. Great care must be taken in selecting fluorescent materials that are safe and have an affinity for malignant tissues. Photofrin synthesized from hematoporphyrin is now the most widely used photosensitizer. Photofrin is composed of active components produced from hematoporphyrin derivative (HpD), *bis*-1-8-(hydroxylethyl)deutro-porphyrin-3-etyl-ether (DHE). The many studies conducted on fluorescent materials have shown Photofrin to be a highly appropriate sensitizer in terms of safety, stability, low toxicity and tumor selectivity. Research on new photosensitizers that efficiently absorb longer wavelength of light and show more effective cytocidal activity is now underway. Among the second-generation photosensitizers being developed, tin etiopurpurine, SnET2 (ref. 11), mono-aspartyl chlorine e6, Npe6 (ref. 12), phthalocyanine (ref. 13), meta-tetra (hydroxyphenyl) chlorine, m-THPC (ref. 14) and ALA (ref. 15) are highly expected for clinical use. Mono-aspartyl chlorine e6 (NPe6) is a chlorine photosensitizer with properties of very short-term photosensitivity and a high extinction coefficient at 664 nm. Maximal tumor response was achieved at 4–6 h after sensitizer administration. NPe6 has been examined in a preliminary clinical study of patients with superficial malignancies[16]. Another expectable photosensitizer is 5-amino-levulinic acid (ALA). Administration of exogenous ALA enhances the biosynthesis of endogenous protoporphyrinIX (PpIX) for production of heme in certain types of cells and tissues[17]. The subsequent conversion of PpIX to heme is a relatively slow step, resulting in transient accumulation of protoporphyrin to sufficient levels that it can act as a photosensitizer. Preclinical studies have been carried out to investigate ALA administration by topical application, intradermal injection, subcutaneous injection, intraperitoneal injection and orally. Systemic routes produce generalized photosensitivity, but are required for tumors that are too thick to be reached by local administration. Loh *et al.*[18] found comparable kinetic of PpIX in animals after intravenous and oral ALA administration. PpIX predominantly accumulated in mucosa of colon, stomach and bladder, with little in the submucosa and smooth muscle layers. Subsequent light treatment resulted in mucosal ablation only. Three patients were administered oral ALA, and biopsy samples demonstrated preferential PpIX accumulation after 4–6 h. Following topical application only in normal skin after 4 h, a 12 h interval was required in order for tumor cells situated in lower dermis to become maximally fluorescent[19].

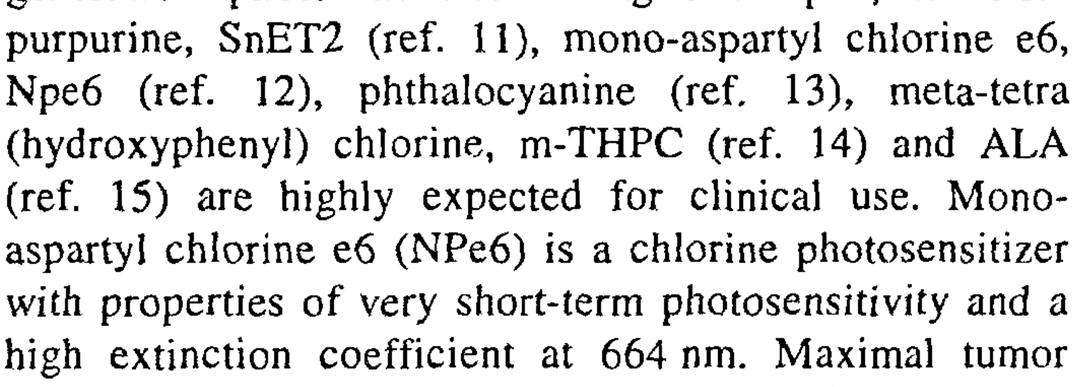

Figure 1. Scheme of the type II photochemical reaction.

Laser systems

PDT can be performed with any light source with an appropriate spectrum. Through the emission of a monochromatic form of intense collimated light energy, lasers offer an especially effective way to deliver light. The argon dye laser is commonly used to provide the red beam required for photoradiation of tumor tissue sensitized by photoactive drugs or dyes[20]. In this process, a dye, usually Rhodamine B or Kiton red, is pumped into an appropriate optical cavity to produce red laser light tunable to a specific wavelength. This laser has drawback, however, primarily its limited ability to penetrate tissue. We developed a new diagnostic and therapeutic endoscopic laser system in 1982. This system utilizes an excimer dye laser capable of emitting a high-energy pulsed laser beam[21]. This laser appears to have many advantages over other PDT delivery systems. As demonstrated in the murine m-KSA sarcoma model, the excimer dye laser was shown to have greater tissue penetration than the argon dye laser[22]. High energy photons capable of exciting Photofrin in tumor tissue to significant levels are provided by pulsed dye-laser systems in as little as 10 ns. Another pulsed beam laser, the gold vapor laser, was developed in the hope of achieving greater tissue penetration, but maintaining the laser equipment to ensure optimal performance is difficult.

The characteristic high-energy beam of the excimer laser is generated from a gas mixture of 0.9% Xe, 0.1% HCl and 99% He at 2 atm pressure. At 308 nm, the optimal performance of the excimer laser is 30 mJ/pulse at one-half peak power for 10.9 ns. Generation of the 630 nm high energy beam used in PDT requires coupling the excimer laser to a system containing 2 molar Rhodamine B dye in ethanol[23].

Recently, a new high-power red laser diode and system (Panasonic, Osaka, Japan) was developed for PDT. This

system has a wavelength of 664 nm and a power output of 500 mW/cm^2 (CW) in tissue. Diode laser has considerable potential for PDT involving current sensitizers, especially Npe6. Furthermore, this system is compact (49 × 20 × 40 cm), light weight (20 kg), easy to operate and only needs 100 VAC·3 A power supply with no maintenance.

New applications for malignant disease

PDT for lung cancer

The lung cancer mortality rate still remains high, despite increased screening and early detection. This disease is thought to be multicentric, patients have a high risk of developing another primary lung cancer even after complete resection of the original lesion. This means that surgical treatment of initial early stage lung cancer has become as conservative as possible to preserve pulmonary function. Surgical resection can be totally successful at removing the original lesion, but patients frequently have coexisting pulmonary or cardiovascular disease, making them a highly surgical risk[24]. Conservative treatment of initial early stage lung cancer in order to preserve lung tissue is essential for the quality of life of the patient.

The bronchoscopic PDT procedure

Approximately 48 h following intravenous injection of 2.0 mg/kg body weight of Photofrin, bronchoscopic PDT is performed under topical anesthesia. Patients should avoid direct sunlight for at least 2 weeks after Photofrin injection. The laser beam is transmitted through a quartz fiber (400 μm) inserted through the instrumentation channel of a fiberoptic bronchoscope. Held 1–2 cm perpendicularly from the target, the fiber tip produces a 4–8 mm circular area of illumination. When using the argon dye laser, power output at the fiber tip is adjusted to 80–400 mW. Energy is adjusted to a 4 mJ/pulse at 30–60 Hz frequency when using excimer dye laser. Illumination time generally ranges from 10 to 40 min at energy densities of 100–400 Joules per cm^2 for surface irradiation in the treatment of early-stage lung cancer. With the fiber tip inserted in the tissue, interstitial radiation was performed in advanced cases of cancer with endobronchial obstruction[25]. We employ two types of fiber-optic tips for laser light delivery. One of them is the microlens-tipped fiber, which provides a uniform field of light for front-surface illumination. And another is the cylindrical diffuser-tipped fiber, which permits delivery of light around the wall of the bronchus when a microlens-tipped fiber cannot be aimed directly at a lesion. Occasionally, these fibers are inserted directly into the tumor tissue for effective, tumor-specific interstitial light delivery, especially in cases of advanced obstructing tumors. After PDT, necrosis of the tumor occurs, and the necrotic debris and associated secretions must be removed through toilet bronchoscopy for a few days after initial treatment. In lung cancer cases, complications that occurred following PDT included skin photosensitivity (90%), obstructive pneumonia (5%) and massive bleeding (1%). Complications after PDT can be avoided or minimized if patients avoid direct sunlight for a minimum of two weeks and receive regular toilet bronchoscopy[26]. Light dosage should also be restricted in cases of large tumors reaching beyond organ walls.

Early stage lung cancer

Despite the increasing incidence of lung cancer internationally, overall therapeutic results in these patients have not improved significantly during the past decade. More than 150,000 men and women developed lung cancer in the United States, and it is estimated that 90% or more of these patients will die of their disease within five years of diagnosis[27]. In Japan, there are approximately 48,000 lung cancer deaths annually and the 3-year survival rate is estimated[28] to be less than 20%. Since there is no significant damage to normal tissue, PDT is one of excellent modality to treat early bronchial cancer. Since 1980, we have used our PDT techniques to treat 139 lesions of 112 patients who were endoscopically suggested of early-stage lung cancer. Tumor response to PDT including advanced case was evaluated endoscopically, histologically and cytologically. Complete remission was obtained in 36.6% and partial remission in 62.2%. In advanced lesions, opening of bronchi obstructed by the tumor was achieved in 75.4%. This group continued many cases of 5-year survival, including one patient who was the first cancer case in the world to survive for 5 years after undergone treatment consisting of PDT alone[29].

Most patients have advanced disease at the time of diagnosis, and the results of therapy for this population are disappointing. However, an increasing number of early-stage lung cancer cases are being detected as a result of improved survey and diagnostic techniques. In cases of early detection, it is generally possible to perform curative resection. However, there is high surgical risk for many patients due to concurrent cardiovascular or chronic obstructive pulmonary disease. Conservative treatment of initial early-stage lung cancer in order to preserve lung tissue is essential for the quality of life of the patient. We used PDT to treat 133 lesions in 110 cases of endoscopically detected early-stage lung cancer (stage '0') between 1978 and 1998. The age of the patients ranged from 36 to 82 years, with a mean age of 65. All but one was male. Except for one adenocarcinoma, all lesions were squamous cell carcinomas. Though the treatment of

choice for early stage lung cancers is usually surgical resection, PDT was performed. Many patients who underwent PDT have poor pulmonary function or refuse surgery. In the treatment of these patients, we used an argon- or excimer-dye laser employing Rhodamine-B dye to generate 630 nm light.

Three grades of tumor response were noted—(i) complete remission (CR): no visible presence of a tumor through biopsy and/or brushing cytology for at least 4 weeks; (ii) partial remission (PR): over 50% reduction in tumor volume but cancer still detectable on biopsy or brushing for at least 4 weeks after therapy; and (iii) no change (NC): tumor size remained the same and cancer was still recognizable on biopsy or brushing. Tumor response to PDT was evaluated endoscopically, roentgenographically and histologically one month after treatment. Endoscopic and histologic examinations were conducted on the treated areas in surgically resected or autopsied cases.

The results of PDT in endoscopically detected cases early-stage lung cancer are shown in Table 1. Ninety-two cases (114 lesions) out of 110 cases (133 lesions), or 85.7%, achieved complete remission but in 18 other cases (19 lesions), the entire extent of the lesion could not be seen endoscopically. Therefore, radiotherapy and/or peroral chemotherapy or surgical resection was administered to these patients to prevent recurrence and ensure curative effect. Twelve cases (9.0%) experienced recurrence and were treated by surgery and radiotherapy. Follow-up showed 87 patients (105 lesions) to be disease-free from one to 198 months, but 5 deaths occurred due to lung cancer.

A typical CR case is presented in Figure 2. In this 78-year-old man, squamous cell carcinoma of the lung was initially diagnosed based on positive sputum cytology. All roentegenographic examinations were negative. The tumor was thickened type, located in the right upper lobe bronchus and 0.5 × 0.5 cm in size (Figure 2 *a*). Since the patient's pulmonary function was very poor, he was subsequently treated by PDT. Figure 2 *b* shows the same site 1 week after PDT. The lesion was covered with

Table 1. Results of PDT in early stage central lung cancer

Cases	Results		Disease free	Recurrence	Death
110 (133*)	CR: 92 (114*) (83.6%) (85.7%)		87 (105*) (2–208 m)	12 (12*)	23 (17 other cause)
	PR: 18 (19*) (16.4%) (14.3%)				
	Combined therapy				
	Surgery	13			
	Radiation	5			
	Chemotherapy	1			

*Lesions

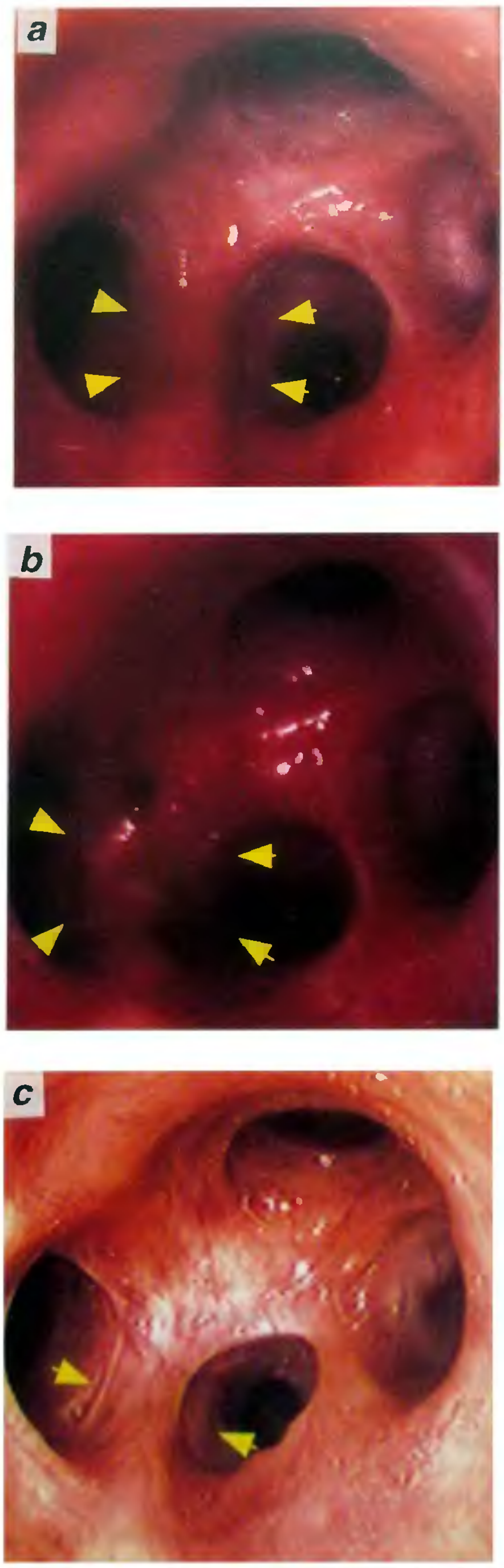

Figure 2. Early central bronchial cancer case treated by PDT. *a*, 78-year-old man squamous cell carcinoma of Rt B^2a; *b*, 1 week after PDT covered by necrosis tissue; *c*, 3 years after PDT, no evidence of recurrence.

necrotic tissue. He is now apparently disease-free 39 months after PDT (Figure 2 *c*).

The results of our study have shown the following conditions to be essential for successful PDT in early-stage lung cancer. (i) The entire lesion must be visible endoscopically. (ii) The tumor must be situated where sufficient laser beam photoradiation can be delivered. (iii) The lesion should be superficial and 1 cm or less in longest dimension. (iv) The histologic type should be squamous cell carcinoma. (v) There should be no lymph node involvement. Identification of cases with tumors limited to the bronchial wall and without involvement of lymph nodes is the most difficult aspect of this treatment method. We examined resected specimens to investigate the effect of the presence or absence of lymph node involvement in early-stage central-type lung cancer. No involvement of lymph nodes was found in 13 lesions of carcinoma *in situ* resected at our hospital[30]. We histologically examined the basis of resected specimens of lung cancer cases including an early-stage case treated by PDT that did not show complete remission. Complete remission was not obtained when lesions were (i) at an anatomical site difficult to photoirradiate, (ii) located submucosally and photoradiation could not be performed at a 90° angle to the surface of the lesion, (iii) located beyond the cartilage, and (iv) extensive. To overcome these difficulties, increased laser power and PDT using cylindrical quartz fibers with 360° diffusion should be used. PDT can be an effective alternative to surgical resection as the primary treatment of patients with early-stage central type lung cancer. The effectiveness of utilizing PDT with Photofrin should be a consideration when deciding on cancer treatment.

Due in part to advances in technologies for the detection of lung cancer and therapeutic achievements in its management, there has been a greater frequency in reports of multiple primary lung cancers[31,32]. From 1980 to 1998, 75 out of 2811 lung cancer patients (2.7%) at Tokyo Medical University Hospital were diagnosed with multiple primary lung cancer. Martini's criteria were used to diagnose either synchronous or metachronous carcinoma of the lung[33]. Of the 75 multiple primary lung cancer patients, 46 were treated with PDT. Table 2 shows the results of PDT for multiple primary lung cancer. Complete resection was achieved in 69 out of 81 lesions.

Our series of patients with synchronous multiple lung cancers underwent PDT for treatment of all biopsy-proven early-stage cancerous foci (i.e. all lesions that were diagnosed by biopsy when discovered to be grossly superficial during bronchoscopy). In cases where lesions were more advanced but surgically curable (i.e. less than stage III_B), resection was performed. A considerable amount of pulmonary function was preserved in patients who were able to receive a sleeve lobectomy after they underwent PDT to decrease the extent of resection. Transbronchial aspiration cytology of lymph nodes nos 3, 7, 11 and 12 was performed simultaneously. A CT scan for staging was then conducted. PDT was done on all superficial ($TisN_0M_0$) lesions for complete tumor elimination. It was used palliatively on others to allow more definitive resection.

In cases of metachronous primary bronchogenic carcinoma, there was a 5 to 204 month interval between the diagnosis of the first and second cancers. PDT was utilized to reduce the extension of resection prior to surgery for the first cancers in two cases. Figure 3 shows a case of metachronous cancer in a 67-year-old male. Initial diagnosis showing carcinoma of the lung was based on positive sputum cytological findings during a mass-screening program for individuals at risk for having lung cancer. All X-rays were negative. Due to poor pulmonary function, PDT was used to treat the first tumor, which was nodular and located at the left B^{1+2}_{a+b}. During a routine bronchoscopical examination five years after initial treatment, a second granular tumor was discovered at the orifice of the left B^6. Endoscopic PDT was used to treat this tumor. A third tumor was detected in the right B^2_{ab} five months after endoscopic treatment of the second, it was also treated with PDT and disappeared completely. A fourth tumor was discovered 39 months later in the orifice of left B^{1+2}_{a+b} orifice and was also treated by PDT. The fifth lesion was detected 42 months later in the left B^{1+2}_{a+b} and it was treated by PDT again. The sixth tumor was detected 20 months later in the left B^6_{a-b+c}. It was treated by PDT again.

From our therapeutic results, we have determined the indications for PDT in multiple primary lung cancers to be the following: (i) All bronchoscopically-accessible superficial ($TisN_0M_0$) lesions which the distal margin of the lesion can be photoirradiated. (ii) Stage I lesions in patients who refuse surgery or have poor pulmonary functions. (iii) Stage I lesions as palliative treatment in curative resection cases. (iv) Advanced lesions requiring palliation[32]. Moreover, 28% and 11% five-year disease-

Table 2. Results of PDT in multiple primary lung cancer

Cases (lesion)	46	(111)
2 lesions case	35	(70)
3 lesions case	6	(18)
4 lesions case	3	(12)
5 lesions case	1	(5)
6 lesions case	1	(6)
Therapeutic procedure	Lesion	
PDT alone	69	
Surgery alone	28	
Radiation alone	2	
PDT → surgery	8	
PDT → radiation	4	
Result		
Lesions treated by PDT	81	
CR	69 (86.4%)	
PR	12 (13.6%)	

free survival rate can be expected in patients with synchronous and metachronous multiple primary lung cancers, respectively, if conventional treatment methods are employed. The survival data in our institute suggest that PDT may be a viable option in treating multiple primary lung cancer. Coupled with advances in therapeutic techniques, the survival rate will ultimately increase. The result is a greater possibility of detecting a second or third primary cancers. Effective control of the disease and therapeutic flexibility for the surgeon are the benefits of PDT. Either as a single therapeutic method or as an adjunct to major surgery and/or radiation therapy, PDT gives the surgeon effective control and therapeutic flexibility in treating cancer.

Esophageal cancer

Early stage esophageal cancer is treatable by surgery. But advanced lesion involving varying degrees of esophageal obstruction carries a mortality of 10–20% after surgery. Many different palliative procedures have been introduced to relieve dysphasia. PDT appears promising for treating early or superficial esophageal tumors and as a palliative treatment for malignant dysphasia[34]. PDT is also being evaluated for the condition known as Barrette's esophagus, in which columnar epithelium replaces normal malpighian epithelium[35]. The incidence of carcinoma is 10% in these patients. Currently, two patients with Barrette's esophagus with early adenocarcinoma have been treated with PDT[36]. Variation in response was noted because of insufficient light delivery to esophageal folds.

Gastrointestinal cancers

Radical surgical treatment has been the first choice for early gastric cancer, but high resolution endsonography allows *in situ* diagnosis with a high sensitivity and specificity and in consequence the option of local endoscopic treatment. In Japan, early gastric cancer is still treated by conventional surgery, and patients have received PDT who refused surgery. In our institute, 19 cases (20 lesions) with stage I–III gastric cancer were treated by HpD or Photofrin PDT. Argon-dye-laser or excimer-dye-laser beam was delivered through a fiber passed down the instrument channel of a gastrofiberscope. A complete response was obtained in 11 of the 19 patients (60%). Incomplete responses were thought to be due to inadequate light dosage, either because of the tumor's location or because of extensive or invasive growth into the muscular layer[37].

Also it is possible to destroy experimental colon cancers without producing any damage to normal tissue exposed to similar light dosage[38]. Barr *et al.*[39] reported the results of ten patients with inoperable colorectal disease treated with PDT as an alternative to Nd : YAG laser therapy. The advantage of PDT over thermal ablation appeared to be preservation of the submucosal collagen layer. As a result, the colon retained mechanical strength, which removed the risk of perforation and healing by rapid regeneration occurred. Nd : YAG laser should be applied for tumor debulking and PDT for small or residual disease. Recently, a pilot study using second-generation sensitizers for PDT in gastrointestinal and colorectal cancers was reported. Mlkvy *et al.*[40] compared three photosensitizers; ALA, Photofrin and m-THPC, and Photofrin and m-THPC works better, but cause cutaneous photosensitivity lasting more than 5 weeks. Better results with ALA are possible when using higher drug doses or modified light dosimetry[40].

Bladder cancer

Sensitizers are selectively retained in human bladder tumors, compared with normal mucosa[41]. In case of carcinoma *in situ* (CIS), Ta and Tl bladder cancer, tumor destruction of superficial transitional cell cancers, which do not involve muscular layer, can be achieved with good response with PDT. Papillary bladder cancer is conventionally treated by transurethral resection (TUR), but high recurrent rate still remains. PDT is also suggested for these cases. Irradiation of the whole bladder is necessary, because bladder cancer sometime exists multifocally. The superficial tumors of bladder are often difficult to detect cystoscopically, so there is a risk of missing tumor with focal irradiation only[42]. Nseyo *et al.*[43] described the effectiveness of PDT in the management of patients with recurrent superficial bladder cancer in 58 patients to assess the long-term role of PDT in the management of resistant superficial transitional cell carcinoma (TCC) including Ta, Tl, and refractory CIS of the urinary bladder. All 58 patients had failed at least one course of standard intravesical therapy or had contraindication for intravesical chemotherapy or immunotherapy. Patients undergoing prophylactic PDT after complete resection were confirmed to be tumor-free by cystoscopy and bladder cytology before PDT. These 58 patients

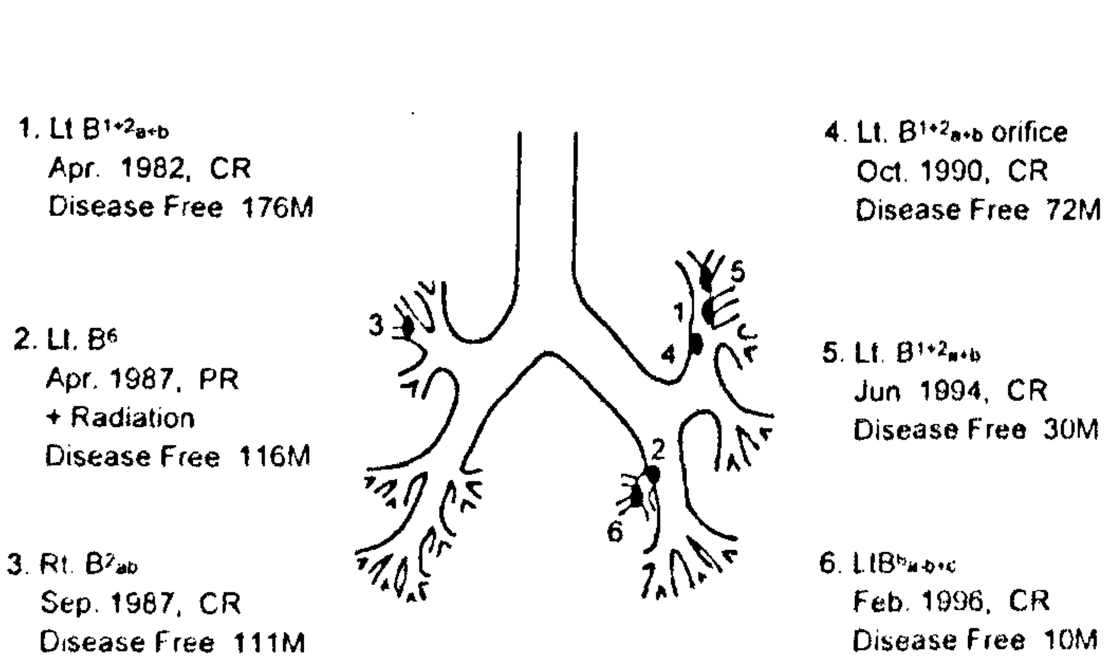

Figure 3. Six lesions of early stage multiple lung cancer case.

underwent a single PDT treatment with 2.0 or 1.5 mg/kg of Photofrin and 10–60 J/cm^2 light (630 nm). At three months, complete response rates were 84% and 75% for residual resistant papillary TCC and refractory CIS respectively; and 90% of patients treated prophylactically had not had recurrences. At a median follow-up of 50 months (range 9–110), 59% (34/58) of the responders are alive, with 31/34 still disease-free.

Gynecological cancer

Cervix vaginal and vulva neoplasm are good indication for PDT in this field. In Japan, the incidence of carcinoma *in situ* (CIS) and dysplasia of the uterine cervix has been increasing among young women in recent years. Most of these patients want to preserve their fertility. Also, to accommodate high-risk patients with complications, elderly patients, and those who refuse surgery, PDT as a method to preserve fertility could be a considerable modality. Muroya *et al.*[44] reported their studies using the PDT with new irradiation method. They treated 56 patients (39 CIS and 17 dysplasia patients). There were 54 CR (96.4%), only one NC, and one PR with very limited remnants but most of the lesions had disappeared. The NC was highly suspected to be invasive carcinoma and the PR was CIS. In the CIS case, some remnant was evident at the end of the cervical canal, and PDT was administered again. Five patients became pregnant after the treatment. Four had normal deliveries and one had a cesarean section. With almost no side effects except skin photosensitivity, PDT is considered to be the best therapy for treating CIS and dysplasia while preserving fertility.

Cutaneous and subcutaneous cancer

Conventional treatments for cutaneous and subcutaneous cancers include surgery, radiation and chemotherapy. Satisfactory cure rates can be achieved with current modalities, but alternative modalities are necessary for extensive or multiple lesions such as superficial basal cell carcinomas, squamous cell carcinoma and Bowen's disease[45]. The results of widespread surgical excision and irradiation can be cosmetically unacceptable for a patient. PDT is a noninvasive treatment for skin cancer. But following intravenous or oral administration of the photosensitizer, generalized photosensitivity is the major side effect. Topical application of the photosensitizer under occlusive foil is a novel method[46]. Topical PDT (TPDT) is most investigated with ALA as photosensitizer. ALA is a precursor of endogenous porphyrins in the biosynthetic pathway for heme. This new modality is increasingly and successfully used to treat precancerous and cancerous epithelial skin tumors. Another approach of ALA–TPDT are nontumoral applications, especially psoriasis. ALA–TPDT is well tolerated by patients and makes excellent cosmetic results. It is an alternative treatment for various superficial skin tumors.

Brain tumor

PDT for the treatment of a variety of brain tumors, particularly gliomas, has been extensively investigated in laboratory studies and has been studied in clinical trials. The main advantage of PDT lies in its ability to select out tumor cells that are infiltrating brain parenchyma and that are responsible for local tumor recurrence, the major therapeutic dilemma in the treatment of gliomas. PDT has been shown to be safe clinically, but adequate trials have yet to be undertaken to prove its efficacy and much work remains to be done to optimize treatment. Popovic *et al.*[47] discussed about the laboratory studies and clinical trials involving PDT in the treatment of cerebral tumors, particularly the commonest brain tumors, gliomas. Muller *et al.*[48] reported the use of PDT in the treatment of 20 patients with newly diagnosed malignant supratentorial gliomas. Eleven patients had glioblastoma multiforme (GBM) and 9 had malignant astrocytoma (MA). Intravenous porphyrin photosensitizer was administered 12–36 h prior to surgery and photoillumination. At operation all patients had the tumor subtotally resected followed by intraoperative cavitary photoillumination. Interstitial photoillumination using fibers with 2 cm diffusing tips supplemented the cavitary illumination in 3 patients. The total light energy delivered ranged from 570 to 4050. The energy density ranged from 15 to 110 J/cm^2. All but two had postoperative radiation therapy. No untoward effects of radiation in conjunction with PDT were identified. There was 1 postoperative death and 1 patient had a persistent increase in postoperative neurological deficit. The median survival of these 20 patients with newly diagnosed malignant gliomas was 44 weeks with a 1- and 2-year survival of 40 and 15%, respectively. The median survival of these patients with newly diagnosed GBM was 37 weeks with a 1- and 2-year survival of 35 and 0%, respectively and the median survival for MA was 48 weeks with a 1- and 2-year survival of 44 and 33%, respectively. Six patients with a Karnofsky score of > 70 who received a light dose of > 1260 J (mean energy density = 62 ± 20 SEm J/cm^2) had a median survival of 92 weeks with a 1- and 2-year survival of 83 and 33% respectively. Patients with malignant astroscytic tumors (GBM and MA) have a very poor prognosis. Nevertheless, PDT is safe in newly diagnosed patients with supratentorial malignant gliomas who undergo postoperative radiation and appears to prolong survival in selected patients when an adequate light dose is used. Further improvement in survival may be expected with higher light doses.

Intracavitary PDT

Laser treatment of malignancies in the peritoneal and pleural cavity via intraoperative PDT is currently being examined.

Intraperitoneal malignancy

Disseminated malignant disease within the peritoneal cavity is a difficult clinical problem that can cause chronic pain, can lead to intestinal or genitourinary obstruction, and also to visceral perforations. While radiotherapy and chemotherapy may have therapeutic benefit in some patients, the rate of treatment-related toxic effects can be high, and peritoneal carcinomatosis usually is resistant to most therapeutic efforts. Interest in PDT for disseminated intraperitoneal tumors was stimulated by the work of Tochner *et al.*[49] who demonstrated treatment of a murine ascitis tumor with HpD given intraperitoneally 2 h before laser treatment (10 mW, 514 nm) for 16 cases. Uptake studies 2 h after HpD injection showed 5–12 fold greater concentration of HpD in tumor cells than in normal tissue. A total of four PDT sessions, given at 2-day intervals, resulted in 100% complete response and a cure rate of 85%.

A pilot study was done to determine the feasibility of adjuvant PDT in recurrent retroperitoneal sarcoma in Roswell Park Memorial Institute[50]. Ten patients, who had recurrence after conventional methods of treatment, had repeated resections of the tumor and intraoperative PDT to the tumor bed. In 8 of 10 patients, a complete resection was possible, and 2 patients are alive without recurrence at 28 and 24 months. There were no complications from the therapy.

Sindelar *et al.*[51] reported the results of 23 patients (13 with ovarian cancer, 8 with sarcoma, and 2 with pseudomyxoma peritoneii) who underwent intra-abdominal photodynamic therapy. Following resection, 630 nm light was delivered to peritoneal surfaces at escalating doses ranging from 0.2 to 3 J/cm^2. Five of 8 patients cleared positive peritoneal cytologies after treatment. Six patients remained clinically free of disease for up to 18 months, and five patients had treatment-related complications.

Wierrani *et al.*[52] treated 3 cases of recurrent ovarian cancer using mesotetrahydroxyphenylchlorin (m-THPC). Two of the 3 patients were treated solely with PDT via laparoscopy. All the three patients remained free of relapse more than two years after PDT. This study also indicated some advantages of the new photosensitizer (m-THPC) such as lower photosensitivity and deeper penetration.

DeLaney *et al.*[53] reported the results of 54 patients who had intraperitoneal dissemination and treated as part of a phase I study. Initially, 630 nm light at 2.8–3.0 J/cm^2 was used, but small bowel edema occurred with perforation in three cases. Light dose escalation up to 3.75 J/cm^2 was achieved by using green (514 nm) light and small bowel complications occurred.

The increasing use of laparoscopes for abdominal procedures offers the possibility of additional light treatments via the peritoneoscope after initial debulking surgery but prior to formation of adhesions. It is reasonable to continue studies of intraperitoneal PDT to determine maximum tolerable doses and deliver booster doses to limited anatomic areas at risk of residual disease. Phase II and III studies will be required to demonstrate conclusively whether intraperitoneal PDT has therapeutic benefit in disseminated intra-abdominal neoplasm.

Intrapleural malignancy

The number of men dying from mesothelioma in Western Europe each year will almost double over the next 20 years[54], from 5000 in 1998 to about 9000 around 2018. But the treatment of patients with pleural malignancies, specially mesothelioma, remains disappointing. The need of innovative treatment is clear because there is no universally accepted standard treatment for malignant mesothelioma, and the efficacy of current therapies do not yield enough median survival rate. Radical resection can seldom be performed. Macroscopically, the resection may appear complete but microscopically tumor cells are often evident at surgical margin. For those cases that are considered to be surgical candidates, adjuvant treatment such as radiation and/or chemotherapy have generally been given. Despite some positive results, the overall survival was not significantly improved, whereas adverse reactions increased. In the largest study by Pass *et al.*[55], 42 patients were treated with PDT using HpD in a phase I study. Thirty-one of the 42 patients (74%) died and no increased survival (mean 12.4%) was observed. Forty-eight hours after 2 mg/kg of Photofrin injection, PDT was performed with two argon dye lasers. The actual laser administration time was 68 min to achieve a total light dose of 25 J/cm^2.

Ris *et al.*[56] performed a pilot study in eight patients with 0.3 mg/kg of m-THPC and 10 J/cm^2 of laser irradiation. In 7 patients, good local control of their thoracic disease was obtained but distant metastasis developed after 4–18 months. In one patient who died of pulmonary embolism 8 days after resection, post-mortem examination showed extensive necrosis in the remaining tumor but no damage to normal structures such as the heart and the esophagus. Baas *et al.*[57] also treated 5 patients with a pleural malignancy using PDT, performed with light of 652 nm from a high power diode laser, and m-THPC as the photosensitizer. The light delivery to the surface of the thoracic cavity was monitored by *in situ* isotropic light detectors. The position of the light delivery fiber was adjusted to achieve optimal light distribution, taking account of reflected and scattered light in the thoracic

cavity. With these systems, light delivery to large surfaces for adjuvant PDT is feasible in a relatively short period of time. *In situ* dosimetry ensures optimal light distribution and allows total doses to be monitored at different positions within the cavity[57]. This combination of light delivery and dosimetry is well suited for adjuvant treatment with PDT in malignant pleural tumors. There is little doubt that further experimentation with this technique will help us to use it more effectively.

Bone marrow purging

High-dose chemotherapy and autologous bone marrow transplantation are an effective combination for treating leukemia and selected solid tumors. Clinical trials have demonstrated a potential role for this regimen in the management of acute leukemia and non-Hodgkin's lymphoma. Autologous bone marrow transplantation offers several advantages, notably avoiding the risk of graft rejection, viral infections and lymphoproliferative disorders from graft manipulation. However, relapse rates tend to be higher in autologous marrow graft than allogenic bone marrow transplantation firstly because autologous transplant lacks the immunologic 'graft-versus-host' advantage of allogenic transplants and secondly, autologous grafts have the possibility of tumor cell contamination. Methods to reduce tumor cell contamination in autografts include exposure to chemical agents or monoclonal antibodies[58].

Bone marrow grafts consist of free-flowing single cells in suspension, which are amenable to uniform exposures to photosensitizer and light. A significant advantage of this technique is that the drug can be removed before reinfusion of the treated cells into the patient, thus avoiding systematic photosensitization[59]. Merocyanine 540 (MC540)-mediated PDT inactivates experimental leukemia, lymphoma, and neuroblastoma cells by a singlet oxygen-mediated mechanism. MC540 is currently undergoing phase I clinical testing for the extracorporeal purging of autologous bone marrow and peripheral blood stem cells[60]. Encouraged by these favorable preclinical studies, clinical trials evaluating PDT in bone marrow transplantation have been developed. A phase I trial investigating the toxicity of PDT with MC540 purging of leukemia and lymphoma cells is under way.

Summary and conclusion

In this article we have reviewed a large body of evidence suggesting that photodynamic therapy represents a convenient and effective approach to the treatment of a number of malignant diseases, and attempted to describe the new trials and applications for various other diseases currently ongoing. Besides the malignant tumor, PDT is applied to benign diseases. The studies on PDT have shown that cellular immune modulatory effects can be achieved in the absence of cell killing, thus expanding the potential applications of PDT to completely new fields. At this time, it appears that future non-oncological applications for PDT lie in two general areas: treatment modalities that successfully ablate disease tissue involving either hyperplasia or neovascularization, and in the modulation of cellular behavior without causing tissue ablation or cell death. In the past two decades, several thousand cancer patients have received PDT, although the majority have not been part of prospective clinical trials, but it is extremely difficult to compare the results, because different photosensitizers, light sources, and treatment parameters were used. Furthermore, either the paucity of long-term follow-up data or histologic confirmation of complete response limits comparison of these trials with standard cancer treatment modalities.

Clinical PDT trials in the coming years will undoubtedly expand the range of indications for this novel form of therapy, not only for oncologic conditions but also for non-oncologic conditions.

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