Photodynamic Therapy Using IR-783 Liposomes for Advanced Tongue and Breast Cancers in Humans

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Article

Photodynamic Therapy Using IR-783 Liposomes for Advanced Tongue and Breast Cancers in Humans

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Abstract: Photodynamic therapy (PDT) is a minimally invasive treatment that elicits tumor apoptosis using laser light exclusively applied to the tumor site. IR-783, an HMC dye, impedes the proliferation of breast cancer cells, even without light. However, the mechanism by which IR-783 exerts its antiproliferative effects remains unclear. We aimed to determine the efficacy of PDT using IR-783 liposomes. A heptamethine cyanine (HMC) dye excited by long-wavelength infrared light and with high tissue permeability was used for PDT after liposomization to enhance tumor tissue accumulation. PDT was performed using IR-783 in two patients with tongue and breast cancers. IR-783 liposomes similarly inhibited cell proliferation in lung cancer cells even when not excited by light. Tumor size was markedly reduced in both cases, with no significant adverse events. Furthermore, the patient with tongue cancer exhibited improved respiratory, swallowing, and speech functions, which were attributed not only to the shrinkage of the tumor, but also to the improvement of airway narrowing. In conclusion, PDT using IR-783 liposomes effectively reduces tumor size in tongue and breast cancers.

Keywords: breast cancer; heptamethine cyanine dyes; Indocyanine Green; IR-783; liposome; lung cancer cells; photodynamic therapy; tongue cancer

1. Introduction

Despite research advances, cancer treatment remains challenging, and cancer incidence and mortality rates continue to increase worldwide [1]. The complexity of cancer treatment is compounded by several factors, including the presence of metastasis, drug resistance, and treatment discontinuation due to adverse drug events [2]. Photodynamic therapy (PDT) has been developed to address these complex factors that make cancer treatment difficult. PDT is a highly tumor tissue-specific therapeutic modality that targets cancer cells with internalized photosensitizers [3]. The lack of adverse events associated with the photosensitizers utilized makes PDT an exceptionally noninvasive modality, as it is unlikely to affect normal tissues [3]. Photosensitizers, such as sodium talaporfin or sodium porphyrin, excite the surrounding oxygen from its ground state to the singlet state when irradiated with a laser at the sensitizer's specific excitation wavelength [4]. This oxidant targets lipid membranes and induces ferroptosis in cancer cells as a result of the depletion of reduced glutathione, a substrate of glutathione peroxidase that reduces lipid peroxides in biological membranes to alcohol [5–7]. Damage-associated molecular patterns, such as high-mobility group box 1 and adenosine triphosphate, are released from cell membranes disrupted by ferroptosis and are then recognized by the Toll-like receptor family of dendritic cells. The cells subsequently elicit

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antigen-presenting responses through dendritic cell activation, leading to the activation of cytotoxic T cells [8,9]. It is therefore anticipated that the induction of ferroptosis by PDT will have an antitumor effect not only on light-irradiated tumor tissue, but also on cancers that have metastasized throughout the body and that this effect will be enhanced in combination with immune checkpoints [10].

Cytotoxic chemotherapeutic agents, including alkylating agents, platinum drugs, microtubule inhibitors, antimetabolite drugs, and topoisomerase inhibitors, function by impeding cell division and are thus not efficacious against slow-growing cancers such as cancer stem cells [11–13]. The efficacy of molecularly targeted drug therapy also depends on the expression of the target enzymes. The enzymes targeted by existing molecularly targeted drugs are not consistently expressed in cancer cells. Meanwhile, PDT has the potential to be an effective treatment for drug-resistant cancer cells because it can disrupt cancer cells with singlet oxygen [4,5], which in turn induces immunogenic cell death (H-I). In addition, photosensitizers that absorb near-infrared (NIR) light are expected to be effective against cancer cells that are resistant to reactive oxygen species owing to the combined effect of photothermal therapy (PTT) via thermal radiation and the generation of singlet oxygen via photoexcitation [14]. Accordingly, we focused on heptamethine cyanine (HMC) dyes that serve as photosensitizers when excited by NIR light for PDT because longer-wavelength light has the advantage of a relatively high tissue penetration [15].

We previously used indocyanine green (ICG) as the HMC for PDT [16], but IR-783 as the HMC has also been shown to have specific cytotoxic activity against breast cancer cells without being influenced by light [17,18]. Low-molecular-weight compounds, such as HMC, facilitate accumulation in solid cancerous tissue when encapsulated in liposomes or micelles [14,19]. This phenomenon, designated as the enhanced permeability and retention (EPR) effect, is characterized by the tendency of macromolecules to leak out of blood vessels and into the tumor stroma owing to the markedly higher vascular permeability of tumor tissue than that of normal tissue [20–22].

Despite evidence indicating the clinical efficacy and safety of PDT with IR-783, the precise mechanism by which IR-783 exerts its antiproliferative effects remains to be elucidated. Therefore, this study aimed to determine the efficacy of PDT using IR-783 liposomes in the clinical setting and to investigate the cytotoxicity of liposomized HMC in an environment devoid of laser irradiation.

2. Materials and Methods

2.1. Ethics

This study was approved by the Ethics Review Committee of the IGT Clinic on February 24, 2023 (approval number: 30) and was conducted in accordance with the Declaration of Helsinki and the Ethical Guidelines for Medical Research Involving Human Subjects established by the Japanese Ministry of Health, Labor, and Welfare. Informed consent was obtained from all patients prior to the clinical trial.

2.2. Study Design and Patients

This x study reported the PDT outcomes in two patients with breast and with tongue cancer. Patients with porphyria and with a history of photosensitivity to previous PDT with photosensitizers such as sodium talaporfin or 5-aminolevulinic acid were not eligible. The study endpoint was the patient ability to receive PDT without photosensitivity reactions or compromising their quality of life.

2.3. Preparation of Heptamethine Cyanine Liposomes

ICG and IR-783, as photosensitizers that reacted with the NIR light used in PDT, were used as HMC dyes. We purchased 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC) from Yu-ka-Sangyo Co., Ltd. (Tokyo, Japan) and IR-783 from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Diagnogreen (ICG) was purchased from Daiichi Sankyo Co., Ltd. (Tokyo, Japan). Liposomes were prepared as previously reported [16]. Briefly, DMPC was dissolved in a 5% glucose solution at a concentration of 8.85 mM using a Bransonic® CPX8800H-J Ultrasonic Cleaner (Branson Ultrasonic

 doi:10.20944/preprints202409.1164.v

Co., Ltd., Danbury, CT, USA) and then sonicated at 40 kHz for 60 min under 45 °C. Subsequently, the liposomes were purified using sterile filtration through a 0.20-μm pore size filter (Sartorius, Goettingen, Germany). The particle size of the liposomes was determined using ELSZ-2000 (Otsuka Electronics Co., Ltd., Osaka, Japan). HMC liposomes were prepared by mixing 5 mg IR-783 or ICG with 8.85 mM/10 ml liposomes using a stirrer until the reagent was completely dissolved, followed by sterile filtration through a 0.20-μm filter. HMC liposomalization was determined through gel filtration chromatography using a PD-10 Sephadex G-25 gel filtration column (Cytiva, MA, USA).

2.4. Cell Culture

The human lung adenocarcinoma cell line HCC827 was purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in Roswell Park Memorial Institute 1640 (RPMI 1640) medium (Merck & Co., Inc., Rahway, N.J., USA) supplemented with 2 mM L-glucose, 10% fetal bovine serum, and 100 U/mL penicillin-streptomycin at 37 °C in 5% CO_2.

2.5. Proliferation Measurement

HCC827 cells were seeded in 12-well plates at a density of 1×10^4 cells per well. After 24 h, ICG, ICG liposomes, IR-783, and IR-783 liposomes were treated with 10 μM of the respective HMC equivalents, and cell growth was monitored using an incubation monitoring system (Olympus CM30).

2.6. MTT Assay

To determine cell viability, 5 mg/mL tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was introduced to the culture medium of HCC827 cells and incubated for 3 h at 37 °C. Subsequently, the medium was removed, and the cells were lysed with 500 μL dimethyl sulfoxide. Absorbance was then measured at 570 nm.

2.7. IR-783 Liposome Therapeutic Intervention

IR-783 liposomes were diluted to 50 mL with 5% glucose (Hikari Seiyaku Co., Ltd., Tokyo, Japan) and infused intravenously at a rate of 2 mL/min through a vein in the middle of the patient's arm. A multilaser delivery system (Weber, Germany) was selected as the laser delivery device for PDT. PDT was performed at a laser power density of 25 mW/cm^2 using a fiber with a wavelength of 810 nm approximately 24 h after liposome administration.

2.8. PDT for Tongue Cancer

The outer circumference of the fiber for the MLDS infrared was wrapped with a wrapping film, and the patient was instructed to lightly bite the portion of the fiber that was in contact with the mantle of the syringe. Irradiation with an 810-nm laser for 20 min was subsequently performed. A nasal cannula (MC Medical, Inc., Tokyo, Japan) was attached to a valve with a medical oxygen flow regulator (Air Liquide Japan G.K., Tokyo, Japan), and oxygen was administered intranasally to the patient at approximately 2 L/min during the intervention.

2.9. PDT for Breast Cancer

Five MLDS infrared fibers were passed through the aperture in the masher, wrapped in a wrapping film (Fig. 4A). Irradiation with an 810-nm laser for 40 min at the limit of contact with the lesion was then performed.

2.10. Statistical Analysis

All results are expressed as the mean ± standard deviation. The statistically significant differences were analyzed using the one-way analysis of variance followed by Bonferroni's post hoc

 doi:10.20944/preprints202409.1164.v

test. All statistical analyses were performed using the add-in software Statcel4 (v4.0; OMS Publishing, Inc., Tokorozawa, Japan). Significance levels were set at $P < 0.05$ and $P < 0.01$.

3. Results

3.1. T Physicochemical Characteristics of HMC Liposomes

IR-783 showed higher NIR absorption than did ICG (Fig. 1A), consistent with previous reports [23]. Moreover, both liposomalized IR-783 and ICG demonstrated enhanced absorption in the NIR region after processing (Fig. 1A). The absorption wavelength of ICG liposomes undergoes a slight shift towards longer wavelengths because of the dissolution of ICG within the hydrophobic groups of the phospholipid bilayer [24]. Here, this phenomenon occurred not only with ICG, but also with IR-783 (Fig. 1A). Encapsulation of HMC in liposomes was investigated using gel filtration chromatography. The wavelengths of ICG, ICG liposomes, IR-783, and IR-783 liposomes, as determined by gel filtration chromatography, were identified based on the UV spectrum results. For clarity, only the liposomes are depicted on the right side of Figure 1B. In gel filtration chromatography, smaller particles were identified in subsequent fractions. ICG was below the limit of detection, whereas IR-783 was detected in an inconsistent manner across the 40–100 mL fractions (Fig. 1B). The same fractions in which liposomes were detected prior to inclusion contained both liposomized ICG and IR-783 (Fig. 1B). For the EPR effect to accumulate macromolecules in tumor tissues, a size of 5–100 nm is considered optimal [22] because this range is not subject to renal clearance. Recently, a particle size as small as 30 nm was identified as particularly effective as it can easily pass through the cancer stroma [25]. Accordingly, we modified liposomes to a diameter of approximately 20 nm (Table 1). Both ICG and IR-783 exhibited a change in charge upon liposomization, with a positive tilt (Table 1). It has been hypothesized that the incorporation of HMCs into liposomes modifies the surface potential. The results of our UV spectroscopy, gel filtration, and zeta potential analyses demonstrated that the IR-783 liposomes could effectively encapsulate IR-783.

Table 1. Physicochemical characteristics of HMC liposomes. The results are expressed as the mean ± standard deviation (n = 3).

Sample	Diameter (nm)	Zeta potential (mV)
ICG	N.D.	33.9 ± 3.46
IR-783	N.D.	18.8 ± 3.57
Lipo	25.8 ± 1.42	8.4 ± 0.77
ICG Lipo	20.1 ± 0.83	-9.0 ± 5.38
IR-783 Lipo	21.8 ± 1.65	-9.5 ± 0.41

ICG: indocyanine green; Lipo: liposomes.

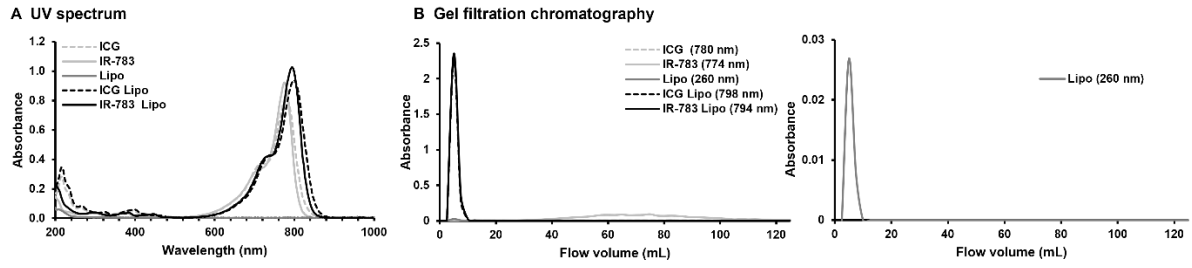

Figure 1. (A) UV spectrum results. The light-grey dotted line depicts ICG in isolation, whereas the straight line represents IR-783. The dark grey straight line represents liposomes alone, the black dotted line represents ICG liposomes, and the straight line represents IR-783 liposomes. The absorbance peaks of both ICG and IR-783 tends to shift to the right when liposomes are used. (B)

 doi:10.20944/preprints202409.1164.v

Results of gel filtration chromatography. The light-grey dotted line depicts ICG in isolation, whereas the straight line represents IR-783. The dark grey line represents liposomes alone, the black dotted line represents ICG liposomes, and the straight line represents IR-783 liposomes. The left side shows all liposome types, whereas the right side shows single liposomes with low absorbance values. ICG cannot be quantified at the limit of detection, and IR-783 is only sporadically detected in the latter half of the sample owing to its small size. Both liposomized ICG and IR-783 are observed at the same locations as the standalone liposomes rather than at the sites where they are detected independently. This finding confirms that ICG and IR-783 are liposomized. ICG: indocyanine green; Lipo: liposomes

3.2. Inhibitory Effect of IR-783 Liposome Treatment on Cell Proliferation

The inhibitory effects of HMC and HMC liposomes on the proliferation of HCC827 lung cancer-derived cells were observed in real-time using a culture monitoring system (Fig. 2A). The results demonstrated that only IR-783 liposomes significantly inhibited HCC827 cell proliferation at 36 h post culture. Similarly, ICG liposome-treated cells better inhibited cell proliferation than did control, although the difference was not significant. Furthermore, the inhibitory effect of IR-783 liposomes on cell growth was evaluated using the MTT assay (Fig. 2B). The results were comparable to those in the monitoring system, except in cells treated with liposomal IR-783 that exhibited significantly lower in viability than did the control group.

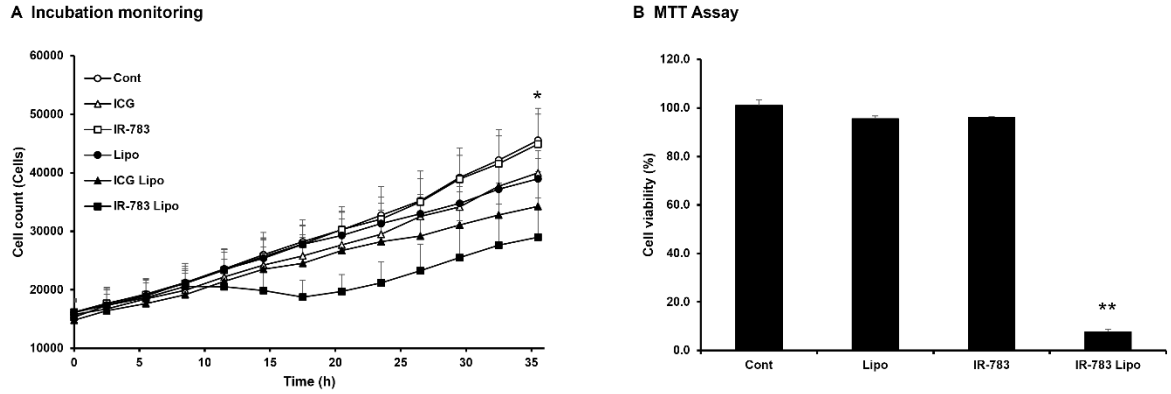

Figure 2. (A) Result of real-time measurement of HCC827 cell proliferation over 36 using an incubation monitoring system. HMC liposomes are treated with cells at equivalent concentrations to the respective HMC (10 μM), while controls and liposomes are treated at the same volume as the HMC (100 μL). The white circles represent the control group (5% glucose), while the black circles indicate the liposome group. The white triangles represent the ICG group, and the black triangles indicate the liposome group. The white squares represent the IR-783 group, and the black squares indicate the IR-783 liposome group. The vertical axis represents the number of cells, while the horizontal axis depicts time. Significant discrepancies in cellular proliferation after 36 h are determined exclusively between the control and IR-783 liposome groups. (B) Results of MTT assay for measuring the viability of HCC827 cells treated with IR-783 and IR-783 liposomes at 10 μM of IR-783 equivalent after 48 h. The controls and liposomes are treated with 100 μL, which is the same volume as that used for IR-783 and IR-783 liposomes. In comparison to the control cells, the cells treated with IR-783 liposomes show significantly lower viability. The results are analyzed via a one-way analysis of variance with Bonferroni's post-test and are expressed as the mean ± standard deviation (n = 5, * $P < 0.05$, ** $P < 0.01$). ICG: indocyanine green; Lipo: liposomes

3.3. PDT Using IR-783 Liposome for the Patient with Tongue Cancer

Despite being diagnosed with tongue cancer in 2022, the male patient in his 30s did not pursue professional cancer treatment and thus had disease progression without intervention. In December 2023, the lesion advanced, extending to the upper palate and causing respiratory distress (Fig. 3A, G). Consequently, the patient sought phototherapy in our hospital. In February 2024, 5 mg of IR-783 liposomes was administered intravenously at an IR-783 equivalent dose, and PDT was performed the

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following day at 60 J/cm² using two 810-nm probes (Fig. 3B). Owing to the improvement in lesions, pain, and normal biochemical test results after PDT, we decided to increase the IR-783 dosage. At 1 week after the initial dose, IR-783 liposomes (10 mg in 783 equivalents) were intravenously administered over 2 days. On days 2 and 3, we performed under conditions identical to those used for the initial dose. After three phototherapy sessions, the tumor in contact with his upper palate started to shed partially (Fig. 3C), and he reported an improvement in his breathing. Eighteen days after the initial intervention, the tumor in the upper portion of the tongue regressed, leaving only an ulcerative lesion on the right lateral aspect of the tongue (Fig. 3D). After 1 month, the ulcerated area had disappeared (Fig. 3F), and magnetic resonance imaging (MRI) T2 scans taken 3 months later showed no lesions. Additionally, an improvement in airway narrowing was observed on the initial scan (Fig. 3G–H).

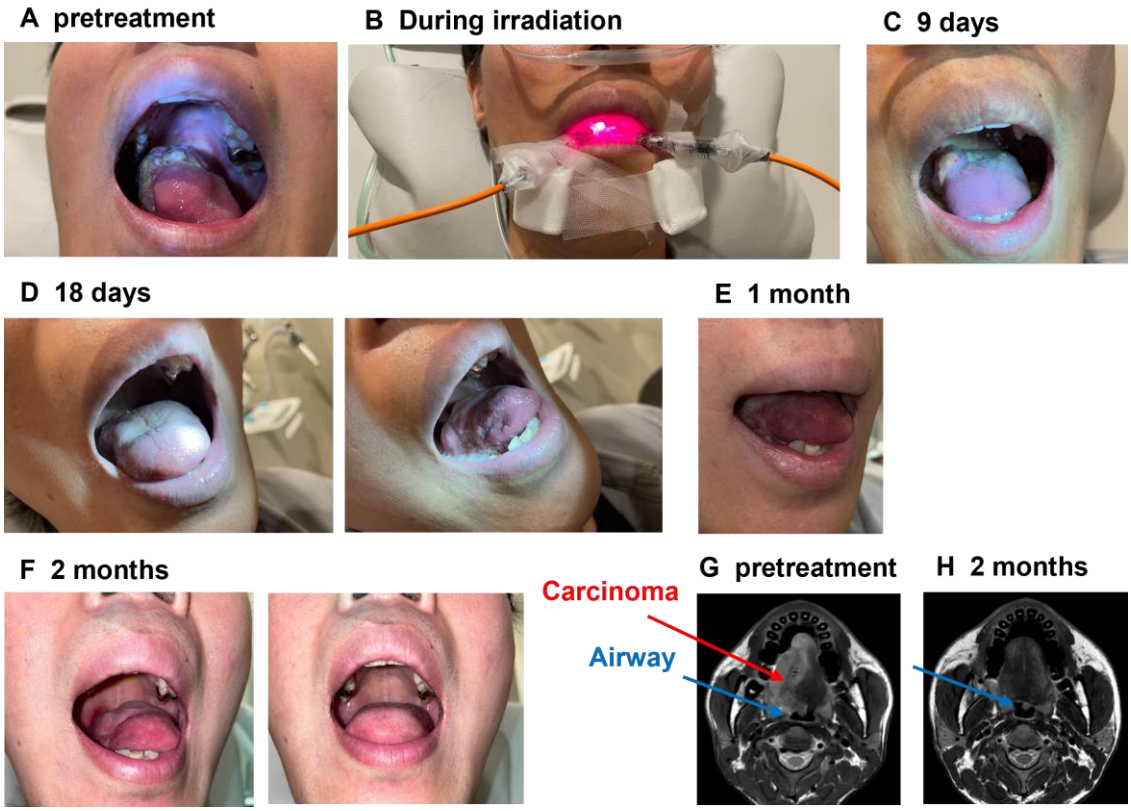

Figure 3. (A) Images showing the patient with tongue cancer prior to photodynamic therapy (PDT) using IR-783. (B) The patient undergoing infrared light irradiation. A large tumor is visible on the right middle part of the tongue. (C) Image obtained 9 days following the initial intervention showing a reduction in tumor size. (D) Image obtained 18 days following the initial intervention showing that the tumor in the upper portion of the tongue is no longer discernible, and only an ulcerative lesion in the right side of the tongue is visible. (E, F) Images obtained 1 and 2 months following the initial intervention, respectively. No lesions are identified, and there is no observable tendency for recurrence. (G) Magnetic resonance imaging (MRI) of the patient prior to photodynamic therapy using IR-783. (H) MRI of the patient 2 months following the initial intervention. The red line denotes the tumor tissue, while the blue line represents the airway. After 2 months, the tumor is no longer discernible on the MRI, and the stenotic airway has recovered.

3.4. PDT Using IR-783 Liposome for the Patient with Breast Cancer

A woman in her 60s was diagnosed with breast cancer in 2015 and was initially treated with a combination of taxane and pembrolizumab. In 2022, the patient developed massive breast discharge and pelvic metastasis and required radiation therapy for pain relief. In 2023, her physician determined that the pharmacological intervention was ineffective and thus withdrew treatment. In May 2024, she was admitted to our hospital because of the lack of alternative treatment options for

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her medical condition following the cessation of medication. When she visited our clinic, computed tomography (CT) confirmed the presence of a mass in her left breast (Fig. B, E). With her informed consent, we decided to perform PDT with IR-783 liposomes because the lesion was visible. On the first 2 days, 12.5 mg of IR-783 liposomes was intravenously administered at an equivalent dose of IR-783, and PDT at 300 mJ/cm^2 was subsequently performed using five 810-nm fibers in the next 2 days. During PDT, the lesion exhibited significant exudate secretion; however, the patient did not report any pain. Exudation immediately ceased after the initial PDT, and over the course of 1 week, the lesion surface slowly turned blackish and dry. As the lesion improved (Fig 4C), we administered IR-783 liposomes on day 8 for 2 more days under the same conditions, followed by two additional PDT sessions. When she returned to our clinic approximately 6 weeks later, CT imaging showed that the raised mass previously observed had been dislodged and had significantly decreased in size than that at the initial visit (Fig. 4D, F).

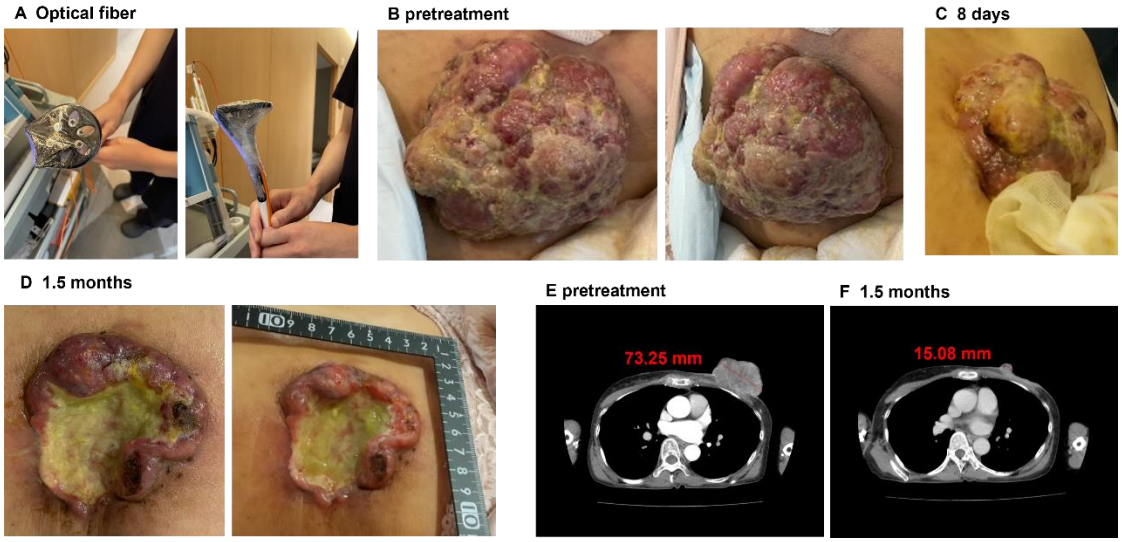

Figure 4. (A) Image of the irradiation equipment used in PDT. Five optical fibers for irradiation are grouped together in a washer and wrapped with wrapping film. (B) Pretreatment image of the tumor in the breast cancer patient. A large-sized tumor is seen in the left breast. (C) Image captured 7 days after the first treatment. The tumor is slightly smaller than that before PDT. (D) Image obtained approximately 6 weeks after the initial therapeutic intervention. The tumor size is markedly reduced. (E) Pretreatment CT image. (F) CT image obtained 1.5 months after the initial treatment. The tumor is markedly reduced.

4. Discussion

The findings of this study indicate that IR-783 liposomes can impede the proliferation of cancer cells without the need for photoexcitation, and they have antitumor effects against both tongue and breast cancers as photosensitizers for PDT. Although several anti-tumor effects of PDT and PTT using IR-783 have been documented in cell and animal studies [26–28], to the best of our knowledge, this is the first clinical report of PDT using IR-783 in humans.

IR-783 can inhibit the growth of cancer cells in the absence of laser irradiation [17,18]. This study demonstrated that liposomalized IR-783, but not IR-783 alone, had a notable inhibitory effect on the proliferation of lung cancer cells (Fig. 2). This discrepancy may be because previous studies required treatment of cells with IR-783 at concentrations of 100 μM or higher to achieve cell death comparable to ours [17,18], whereas the present study used a significantly lower concentration of 10 μM. The previous findings indicate that although the survival rate in breast cancer cells treated with IR-783 at concentrations below 10 μM was reduced, it was largely unaltered when compared to that in control cells [17,18]. The inhibitory effects of both IR-783 and ICG on cancer cell growth through liposomalization (Fig. 2) support that these HMCs have enhanced efficacy owing to increased cellular

 doi:10.20944/preprints202409.1164.v

uptake facilitated by liposomalization. In this clinical trial, PDT was performed with IR-783 liposomes as these liposomes demonstrated a superior anti-tumor effect compared to ICG liposomes.

A patient with tongue cancer presented with various symptoms, including dyspnea, dysphagia, dysgeusia, taste disorders, and speech disorders, all of which were attributed to the tumor. Pre-treatment MRI observations indicated that surgical intervention and radiotherapy were unlikely to be effective in improving cancer-related complications. However, lesion size was markedly reduced with PDT treatment (Fig 3), and this improved his breathing, taste, and speech functions. These findings indicate that PDT may represent a promising modality for the future treatment of tongue cancer. Further, the patient with breast cancer who previously demonstrated no improvement with drug therapy exhibited a notable reduction in tumor size following the PDT intervention (Fig. 4). These findings suggest that PDT using IR-783 liposomes may be an effective treatment tumors resistant to cytotoxic chemotherapeutic agents and immune checkpoint inhibitors. Although not reported in this report, the patient's lesions are improving and will continue to be monitored and, if necessary, treated in the same manner.

Despite the evidence indicating the clinical efficacy and safety of PDT with IR-783, several challenges remain unresolved. The precise mechanism by which IR-783 exerts its antiproliferative effects remains to be elucidated, and further investigation is required to gain a deeper understanding of this process. With the enhancement of endocytosis through IR-783 liposomalization in this study, further studies will be conducted to investigate the previously reported mitochondrial mitogenic effect of IR-783 [17,18]. With the limited number of patients reported in this study (only two), we will expand the scope of our research through collaboration and other means as more patients are accumulated. Ultimately, we intend to conduct further statistical analyses, including 5-year survival rates. Invasive procedures such as laparotomy and thoracotomy are required to treat visceral tumor tissues with PDT, given that this treatment is essentially aimed at tumor tissue confined to the superficial layers of the body. Such a procedure would be challenging to perform in a clinic of our size and would require the resources of a larger hospital. Sonodynamic therapy (SDT), which employs ultrasound waves that penetrate deep into the body to stimulate photosensitizers, has garnered considerable attention in recent years [29]. The inhibitory effect of SDT with IR-780, a derivative of IR-783, on cancer cells was demonstrated in both cell and animal experiments [29, 30]. Further studies are planned to investigate the potential of SDT using IR-783 as a treatment modality for pancreatic and liver cancers. Subsequent studies will be conducted at our clinic using SDT with IR-783, including in patients with pancreatic and liver cancers.

5. Conclusions

In conclusion, this study demonstrates that IR-783 liposomes inhibit cell growth and shrink tongue and breast cancer cells. The utilization of IR-783 as a photosensitizer for PDT is anticipated to represent a novel approach to cancer eradication, particularly for patients with refractory advanced cancer, without imposing undue burden.

Author Contributions: Conceptualization, Y. K. and S. K.; methodology, Y. K.; software, S. K., T. K., and F. Y. ; validation, Y. K., S. K., Y. H., T. K., A. T., F. Y., S. Y., and H. M.; formal analysis, Y. K. and S. K.; investigation, Y. K. and S. K.; resources, Y. K. and Y. K.; data curation, S. K.; writing—original draft preparation, S. K.; writing—review and editing, O. I. and K. H.; visualization, H. M.; supervision, O. I. and K. H.; project administration, Y. K.; funding acquisition, none. All the authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of the IGT CLINIC (protocol code 30 and date of approval: February 24, 2023).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data necessary to evaluate the conclusions of the study are presented in this paper. Additional data are available upon request from the corresponding author [KH].

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Conflicts of Interest: The authors declare no conflicts of interest.

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