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COMBINED EFFECTS OF SELECTIVE PHOTOTHERMAL THERAPY AND IMMUNOADJUVANT AGAINST STAGE IV BREAST CANCER

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Metastasis to distant sites is a severe treatment challenge and a major cause of death for breast cancer patients. Laser immunotherapy (LIT) is a novel technique, combining a selective photothermal therapy with local application of glycated chitosan, a potent immunoadjuvant. The pre-clinical studies of LIT have shown its unique characteristics in generating specific antitumor immunity. The clinical application of LIT in the treatment of melanoma patients has achieved preliminary success. Recently, LIT has been used to treat late-stage breast cancer patients. Here we report for the first time the clinical results of this combination therapy in breast cancer patients. The LIT treatment procedures are presented and the medical history of two stage IV breast cancer patients is reviewed. Most of the breast cancer lesions and the metastasis of lung and brain disappeared after repeated treatments of LIT. One patient achieved complete

response; the other achieved partial response at the time of this report. Although there is still a long way for LIT to become a standard modality for breast cancer treatment, the results of this study indicated its promising future.

Keywords: Laser immunotherapy; near-infrared laser; glycated chitosan; indocyanine green; metastasis cancer; stage IV breast cancer patients; clinical trials.

1. Introduction

Breast cancer is a leading cause of death among women worldwide according to the latest report from World Health Organization. Each year it accounts for about 519,000 deaths, which is projected to continue rising.[1] Furthermore, metastasis is prevalent in breast cancer patients, which is the major cause of treatment failures. Many approaches such as chemotherapy, radiation therapy, hormonal therapy, and targeted therapy have been applied to manage the metastasis of breast cancer.[2] However, challenges remain in the treatment of metastatic breast cancer and, more importantly, in the control of metastasis and prevention of cancer recurrence.

Cancer immunotherapy has become more and more promising in the treatment of metastatic breast cancer. Particularly, cancer vaccines have held great promise as the tool to treat breast cancer.[3] Combining a selective photothermal therapy with an active immunological stimulation, laser immunotherapy (LIT) was developed to induce systemic immune responses through local interventions.[4] The strategy of LIT is to directly destroy tumors at the treatment site and to induce systemic antitumor immune responses in the host, based on the concept of *in situ* autologous tumor vaccination using the whole tumor cell as the source of tumor antigens.

LIT contains three major components[4]: (1) a near-infrared laser (for non-invasive tumor irradiation), (2) indocyanine green (ICG, a light-absorbing dye), and (3) glycated chitosan (GC, a potent immunostimulant). It synergizes two major interactions: (1) a local, selective laser photothermal interaction using the combination of laser and ICG, and (2) a targeted immunological stimulation using the combination of local administration of GC and tumor antigens released by the photothermal interaction.

Pre-clinical studies showed that the combination of the selective photothermal and immunological interactions, both applied locally, could destroy the treated primary tumors as well as eradicate untreated metastases at distant sites.[5,6] LIT has already been applied to treat late-stage melanoma patients in a proof-of-concept study, and the preliminary data indicated that the efficacy of LIT was significantly better than currently available modalities.[7,8] Here we report, for the first time, the clinical procedures of LIT in the treatment of breast cancer patients. Specifically, we present the treatment outcomes of two patients who were diagnosed with late-stage breast cancer and have failed other available modalities. Clinical effects and adverse reactions of LIT in those patients are also presented.

2. Methods

2.1. *Eligibility of patients*

Patients were eligible if they were more than 18 years of age and had histologically confirmed metastatic breast cancer according to the criteria of modified American Joint Commission on Cancer (AJCC) staging system.[9] The treatment protocol was developed according to the Declaration of Helsinki and Good Clinical Practice guidelines. All participants were required to comprehend and sign an informed consent form approved by the Institutional Review Board before treatment. Patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of no more than two.

2.2. *Laser immunotherapy*

Laser immunotherapy (LIT) was carried out with the following four steps: (1) asepsis followed by local administration of anesthetic (lidocaine 2% with adrenaline); (2) local injection of 0.25% ICG, which was obtained from Akorn Inc. (Buffalo Grove, IL); (3) laser irradiation with an 805 nm laser (provided by ImmunoPhotonics Inc., Columbia, MO) for 10 minutes, with a power density of $1.0\,W/cm^2$; and (4) local administration of GC, which was developed by our research group.[4] The injection volumes of ICG and GC were determined according to the

sizes of the tumors, usually between 1/4 to 1/2 of tumor volume. LIT was applied with one month interval between each laser treatment, based on the evaluation of the tumor progression.

CT scan and ultrasound tomography were used for the evaluation of the primary lesions and metastasis. Follow-up examinations included adverse events (AEs) review and analysis of hematology, blood chemistry, and baseline coagulation. Toxicity was assessed according to National Cancer Institute Common Toxicity Criteria.[10] Baseline laboratory studies were performed at the beginning and at the end of each LIT treatment.

3. Results

We started our preliminary clinical studies in September 2009. So far, we have treated 11 stage IV breast cancer patients. Although the follow-up treatment and evaluation are ongoing, we have observed significant tumor responses (both primary and metastasis) in the majority of the patients. Only two patients experienced tumor progression and the rest of the patients had either complete responses or partial responses. More importantly, no patients died since they started LIT treatment, significantly increasing the survival time.

Here, we present two cases of patient studies to demonstrate the effectiveness of LIT.

3.1. *Patient* 1

This patient was a 43-year-old female, diagnosed with two tumors in the right breast and multiple pulmonary metastases on May 2008 (AJCC stage IV). Pathological anatomy showed the following: mucoid carcinoma of breast with fibroadenoma fragments, adenosis, and microcalcifications. Estrogen receptors: ++/+++ (60%), Progesterone receptors: +/+++ (15%), and HER2 neu negative.

This patient did not receive prior surgery or radiation therapy. However, she received chemotherapy, with AC (doxorubicin/cyclophosphamide) for four cycles from June 2008 to August 2008, and with Paclitaxel for three cycles from September 2008 to December 2008. Due to the progression of pulmonary metastasis, she received chemotherapy with capacitabine and ixabepilone for three cycles until May 2009, and then hormonal therapy with tamoxifen. The chemotherapy with different agents showed no or little effect on the tumor progression. She was also resistant to hormonal therapy.

Clinical observations before LIT showed that the sizes of the two tumors in the right breast of the patient were $6\,\text{cm} \times 4.5\,\text{cm}$ and $2\,\text{cm} \times 2\,\text{cm}$. Pulmonary metastases were observed prior to LIT. This patient was considered to have no traditional modality available and the prognosis was extremely poor.

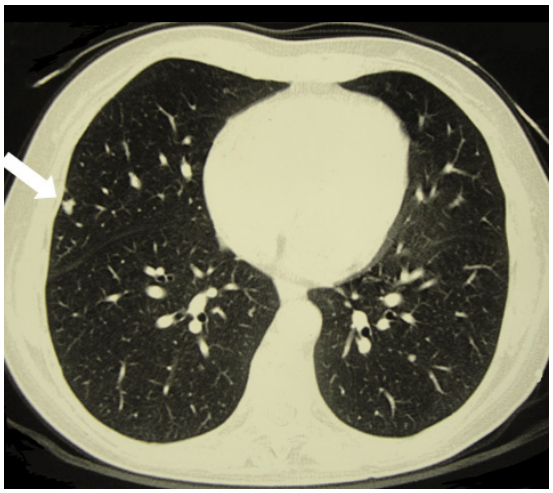
She started to receive LIT in September 2009. The occurrence of blistering, edema and redness was observed after LIT, which could disappear spontaneously. The blistering and redness could be attributed to the topical laser application. Cellulitis of the treatment area occurred after the first two LIT treatments, and was resolved by using dicloxacillin. No other adverse effect was observed. CT scan shortly after LIT showed that there was a remarkable increase in the volume of right mammary gland with heterogeneous appearance, which was supposed to be induced by the thermal effect of LIT.

This patient received four LIT treatments. Two and a half months after the first LIT treatment, CT scan showed that the lungs were normal without any metastasis. The development of one of the pulmonary metastases is shown in Fig. 1. As shown in Fig. 1, within a short time span of 2.5 months, a lung metastasis of a size close to 0.5 cm completely disappeared. A thorough evaluation was taken five months after the first LIT. No metastasis was observed in any organs. Bone scan at the same time indicated that there was no bone metastasis. The latest tomography examination showed the same result. The follow-up duration was ten months at the time this paper was written. The clinical outcome of this patient was determined as a complete response (CR).

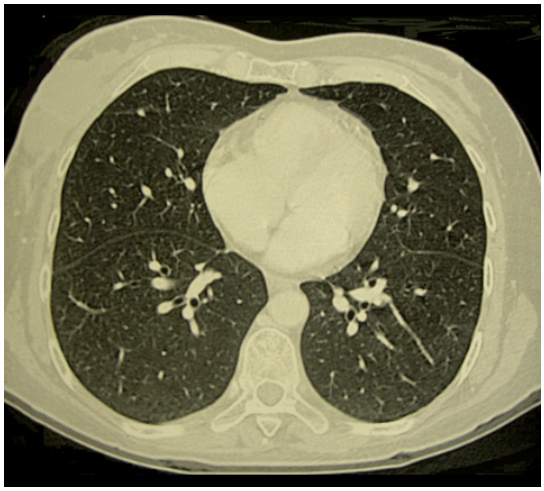
3.2. *Patient* 2

This patient was an 85-year-old female with stage IV breast cancer. She was diagnosed with triple-negative breast cancer, which is a subtype of breast cancer that is clinically negative for expression of estrogen, progesterone receptors (ER/PR), and HER2 protein. The tumors occurred in both breasts, and had metastasized to the lungs and the brain.

Due to the age of the patient, the stage of cancer and the poor prognosis at the initial diagnosis, she did not receive any surgery, chemotherapy or radiation therapy. Clinical observations before LIT showed that the tumor sizes were $3.8\,\text{cm} \times 3.1\,\text{cm}$



(a)



(b)

Fig. 1. Computed tomography of the pulmonary metastasis of Patient 1 before LIT (a), arrow, and 2.5 months after LIT (b). The pulmonary metastasis disappeared after treatment.

in the right breast and 3.6 cm × 3.1 cm in the left breast. CT scan indicated a brain metastasis of 1-cm diameter and a 5.0-cm metastasis in the left lung, as well as 28 nodules with sizes between 0.2 cm and 2.9 cm distributed in both lungs.

This patient started to receive LIT in September 2009. The change of the tumor size was summarized in Table 1. The breast tumors decreased in size by about 1 cm after the first treatment of LIT. After the second LIT treatment, the tumor size increased to the level of the original size. However, the breast tumors remained stable for several months, and no new lesion was observed. Meanwhile, no severe adverse effect occurred during the treatment. After two more LIT treatments, the tumor in the left breast completely disappeared and the tumor in the right breast was divided into two separate nodules, and the size of each one was 1 cm × 1 cm. Then, the two separate small residual tumors were treated individually by LIT. In total, this patient received five LIT treatments. A thorough examination was performed one month after the last LIT treatment. The contrast CT scans showed no tumor in the brain of the patient; only one nodule with a diameter of 1 cm × 1 cm in the right breast remained. The 5.0 cm tumor in the left lung had shown shrinkage to 4.3 cm at the 3rd month, 3.6 cm at month 6, and 3.2 cm at month 8. Additional treatment is planned at the time of this report, if the results of continued observation warranted it. Since the patient is currently being evaluated, the clinical response of this patient was determined as a partial response (PR), although favorable responses are expected based on the progression of the tumors (both primary and metastases) during the LIT treatment.

4. Discussion

For late-stage breast cancer patients, the tumor cells have already spread to distant organs. Conventional methods, such as surgery, chemotherapy, and radiation therapy, are not very effective in treating such patients.[12] Moreover, the prognosis of metastatic breast cancer patients is often very poor.[12] The promising results of these two patients indicated that the application of LIT is not restricted by the previous treatment history. Because its immunological effect depends on the patients' immune system, the only requirement

Table 1. Change of the tumor size of Patient 2 during the treatment of laser immunotherapy.

Time after the first cycle of treatment (month)		0	2	4	6	7	8
Tumor size (cm)	Right breast	3.8 × 3.1	3 × 2.5	4.2 × 3.8	3.7 × 3.0	1.0 × 1.0 1.0 × 1.0	1.0 × 1.0 —
	Left breast	3.6 × 3.1	2.5 × 1.5	3.7 × 2.9	3.7 × 2.0	1.0 × 1.0	—

is that their immune systems are not drastically compromised.

Our results also indicate that LIT treatment is safe in breast cancer patients. Edema and redness were the common adverse effect. Cellulitis of the treatment area occurred, which could be cured by using dicloxacillin. No severe adverse effect was observed. Because of the low toxicity, the treatment of LIT can be repeated many times as long as viable tumor tissue can be found and targeted for the treatment. Repeated treatments could help overcome the immunosuppression, even when the cancer cells mutate during the course of LIT. This feature sets LIT apart from other cancer immunotherapies.

Based on our pre-clinical studies, it is assumed that LIT can generate systemic immune response. LIT provides a unique approach to generate specific antitumor immune response by combining selective photothermal tumor disruption and immunological stimulation.[13] It is believed that LIT-induced immune responses are induced by the released antigens from interrupted tumor cells by the selective photothermal interaction.[14,15] Glycated chitosan works as an immunoadjuvant to further amplify its specific antitumor immunity.[16] The novelty of LIT lies in the process of inducing immune responses through a combination of laser photothermal therapy and active immunological stimulation *in situ*.

ICG was applied in the study to further enhance the absorption of laser light to generate selective photothermal effect. Actually, the laser energy itself with high enough power can significantly increase the temperature in the target tissues.

In conclusion, LIT is a promising modality for the treatment of late-stage breast cancer. The results showed that LIT was well tolerated, and capable of reducing the sizes of treated primary breast tumors and untreated metastases in the lungs and brain.

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