Chapter

Laser Ablation and Immune Stimulating Interstitial Laser Thermotherapy

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Abstract

Based on nineteenth-century findings that showed that heat (fever) could be used to treat cancer, local hyperthermia has been developed as a tool to eradicate local tumors when surgical excision is deemed impossible. Nonetheless many cancer patients with advanced disease still lack effective treatment. During the last decades, data has emerged indicating that in situ destruction of tumors in some cases may induce tumor antigen release which can stimulate antigen-specific cellular immunity. Immune stimulating interstitial laser thermotherapy (imILT) is a method for local hyperthermia using laser light to increase tissue temperature with a specific protocol which can result in in situ vaccination. In vivo studies have shown that the method can induce an immune response that is effective against re-challenging, therefore indicating abscopal effect. Data was collected during clinical studies to assess the safety and feasibility of the method.

Keywords: local hyperthermia, laser ablation, LITT, ILT, imILT, immunooncology, laser, abscopal effect, laser ablation, local treatment

1. Introduction

The use of both light and heat in medicine has roots that reside long back in history. In ancient times, sunlight was used to treat different kinds of skin and mental diseases. These treatments mimic, amplify, and in some cases focus on natural occurring phenomena to achieve a therapeutic goal.

During the nineteenth century, it was observed that prolonged heating, as fever or locally externally induced hyperthermia, could cause cancerous formations to disappear [1–4]. Since then, many methods to treat cancer with heat were introduced, from whole body to local methods such as microwave ablation, radiofrequency ablation, and laser ablation. The main goals with innovative treatments that utilize heat are to give an alternative to patients that are not suitable for surgery and minimize the impact of the intervention on the patient. In addition, many of these methods have a lower economical impact on the treating institution budget, which enables clinics to offer treatment to a larger number of patients.

Other methods that do not make use of heat as treating source were also developed, such as cryogenic ablation that uses subfreezing temperatures to kill the tumor cells or photodynamic therapy (PDT) that uses a selective combination of light and photoactivatable drugs to induce radicals in the tumor.

Interest in focal ablation of tumors increased significantly in the last decades because of indications that local treatment may cause shrinkage of untreated, in some cases distant, tumors suggesting the involvement of the immune system in the process [5–7]. The so-called abscopal effect evoked by local treatments could be used to treat patients that lack effective treatments to date. Immune stimulating interstitial laser thermotherapy is an innovative hyperthermia treatment that uses a specifically tailored treatment protocol based on lower temperature heating for a prolonged period of time and designed to maximize the probability of triggering the immune system response to the treated tumor type. The medical device system uses laser as heat source; the same system is also used for interstitial laser ablation to burn tumorous and non-tumorous formation when imaging is challenging given its natural MR compatibility.

2. Laser-induced hyperthermia

Laser-based hyperthermia, known as laser thermotherapy or laser ablation, is a focal hyperthermia technique that uses laser light as heat source. Its minimally invasive version for treatment of tumors located deeper in the body is called interstitial laser thermotherapy (LITT or ILT). The main goal in oncological treatments is to achieve tumor destruction without damaging tissue and structures surrounding the neoplastic lesion to be treated. Different factors concur to the tissue destruction, among these direct cell death and coagulation.

During laser-induced thermotherapy, light causes damage in tissue due to absorption of light and through heat conduction into the tissue of the absorbed energy. Laser thermotherapy therefore produces a lesion that is larger than the volume where light is absorbed due to this heat conduction.

These two phenomena, direct light absorption and heat conduction, determine the modality and the parameters to be used to control the tumor heating and are dependent on the characteristics of the tissue to be treated.

2.1 Direct tissue absorption

The penetration depth, which is defined as the distance at which the light is attenuated to $1/e$ (37% of original intensity), can be used to describe the volume in which the main part of the laser energy is absorbed in tissue, i.e., where direct absorption is the dominant factor. Penetration depth can also be used to determine whether the possibility for carbonization during ablation is affected by, for example, the choice of the wavelength. Low penetration depth indicates a higher power density in tissue and thereby a higher risk of carbonization. Therefore it is an important factor to be taken into consideration both when designing the optical fibers to deliver the light and when deciding on suitable treatment parameters.

Penetration depth depends on the tissue type since the optical properties are dependent on tissue composition and structure. For a generic tissue composition, the effective attenuation coefficient and the penetration depth can be calculated as follows:

$$\mu_{eff} = \sqrt{3\mu_a\left(\mu_a + \mu_s(1-g)\right)} \tag{1}$$

$$\delta_{eff} = 1/\mu_{eff} \tag{2}$$

Values for μ_a, μ_s, and g, in different tissue types, are available in textbooks dealing with optical properties in tissue, e.g., [8].

The absorption, μ_a, of a specific tissue depends on the tissue composition. Each component has a specific absorption spectrum. Biological tissue has a relatively low absorption in the interval 600–1200 nm. This is due to the fact that the absorption spectrum of the main component of tissues, water, has a minimum in this region. Other tissue components, especially blood, must also be considered. In **Figure 1**, only the major absorbers for the specific wavelength in use are shown.

The scattering, μ_s, depends on the tissue structure, for example, on the cell size and shape, and how they are arranged in the tissue. Light scattering in tissue has two contributions, Rayleigh and Mie scattering; the latter is usually predominant due to the scattering of particle size. The scattering is inversely proportional to the wavelength: as the wavelength increases, the scattering coefficient diminishes. As the two wavelengths considered are spectrally close, the scattering coefficients are similar. The scattering spectrum for a generic soft tissue is shown in **Figure 2** and was calculated according to [9]:

$$\mu_s = \frac{a'}{(1-g)}\left(f_{Ray}\left(\frac{\lambda}{500\,[nm]}\right)^{-4} + (1-f_{Ray})\left(\frac{\lambda}{500\,[nm]}\right)^{-b_{Mie}}\right) \quad (3)$$

The equation takes into consideration different scattering contributions mainly due to the different sizes of the scattering centers.

All the parameters are tissue dependent. The values for a generic soft tissue in **Table 1** were used in **Figure 2**.

2.2 Heat conduction in biological tissue

The energy deposited in tissue causes an increase in temperature in the portion of tissue where laser light is absorbed. Naturally, the difference in heat evens out over time. The heat is removed from the volume where absorption of light occurs by active or passive cooling. Active cooling is achieved through blood perfusion, which varies during time according to response of the tissue to heat and is dependent on the perfusion rate and therefore on the tissue type. Passive cooling is due to heat

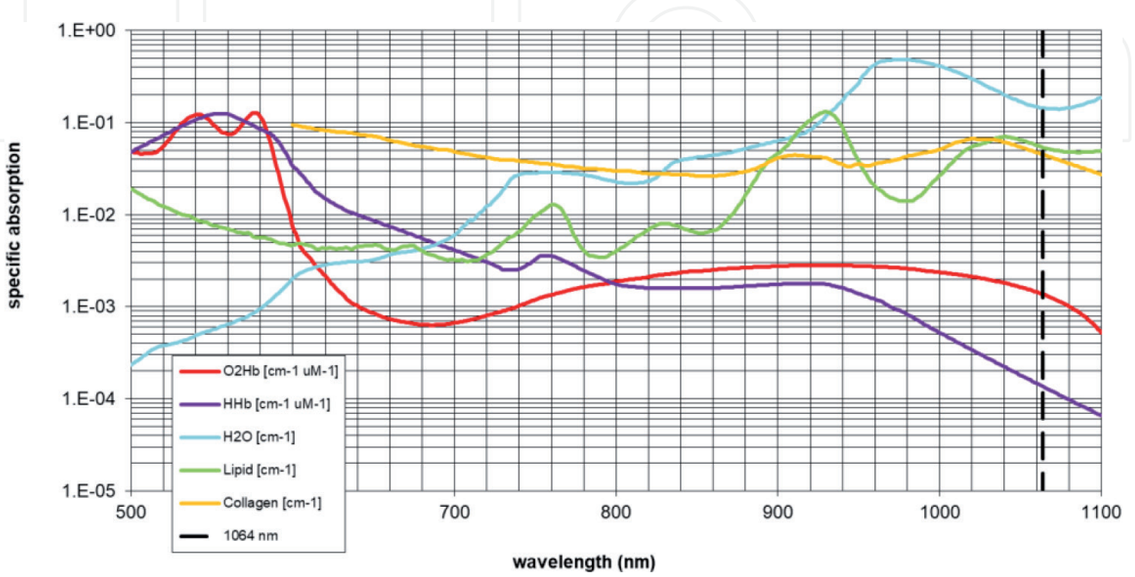

Figure 1.
Absorption spectra of tissue components in the window 500–1100 nm. Dotted line at 1064 nm.

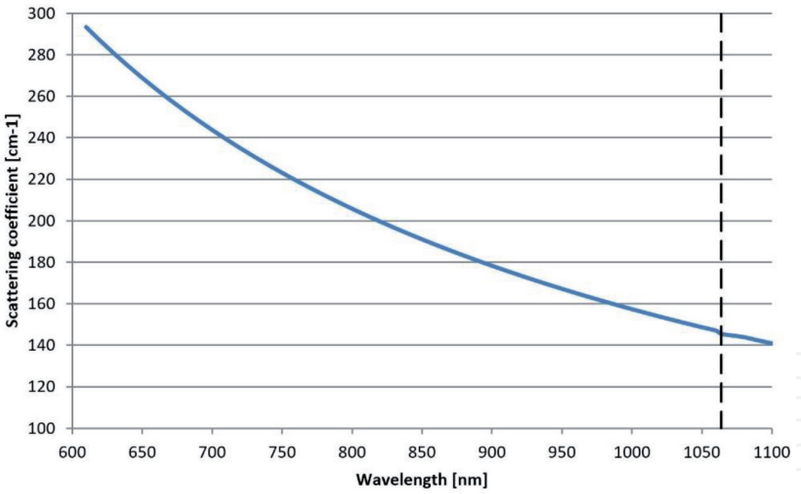

Figure 2.
Scattering coefficient for a generic soft tissue in the window 500–1100 nm, data from literature. Dotted line at 1064 nm.

g	0.95
a′ [cm^{-1}]	19.1
f_{Ray}	0.153
b_{Mie}	1.091

Table 1.
Scattering parameters for a generic tissue [9].

conduction and is described by the second law of thermodynamics which asserts that heat flows spontaneously from hot to cold bodies, in this case from the heated portion of tissue to the portion of tissue at body temperature.

If the delivered energy is high enough, the heat conduction concurs to the progression of the damage since heat conduction can cause tissue temperatures to rise well above the threshold for permanent damage. The threshold for permanent tissue damage is discussed in the following paragraphs.

Pennes' equation models heat distribution in the tissue:

$$\rho c \frac{\partial T}{\partial t} + \nabla(-k\nabla T) = \rho_b c_b \omega_b (T_b - T) + Q_{met} + Q_{ext} \quad (4)$$

The equation describes the heat flow in the tissue as the combination of (passive) heat conduction, (active) heat transport due to blood perfusion and dependent on the temperature difference, metabolic heat source which is the heat produced by the tissue itself, and the external heat source, in this case the laser energy [10–12].

2.3 Laser-induced tissue effects

Effects on biological tissues induced by lasers can vary in nature and can be classified in several groups among which are photochemical damage, when light triggers a chemical reaction in the tissue, and thermal effects, when heat is the cause of the outcome. Photochemical damage includes radical formation and tissue inflammation, while examples of thermal damage are protein denaturation and burning. The type of damage triggered depends mainly on the characteristics of the

light beam (wavelength, power, pulse properties, exposure time, spot size) and if the beam is collimated, i.e., laser source.

Thermal effects are caused when the temperature in the tissue is locally increased over the physiological temperature; the threshold is generally set to 40°C. Conditional to the specific tissue properties, beam characteristics and exposure times, the tissue can undergo hyperthermia (<60°C), coagulation, vaporization, carbonization, or pyrolysis. Hyperthermia can be reversible or irreversible depending on the combination of temperature reached and exposure time. Local ablation techniques, such as microwave, radiofrequency, or laser ablation, aim at achieving a temperature of at least 60°C in the whole treated volume, therefore inducing cell death by coagulation; vaporization and carbonization may occur.

3. Laser ablation and immune stimulating interstitial laser thermotherapy (imILT)

Classic laser ablation is used to treat solid tumor masses in a variety of organs and aims at heating the whole tumor volume at a temperature of at least 60°C in order to coagulate the tissue in the area to be treated. In this way, near to instant cell death is achieved. An optical fiber is placed in the center of the region of interest, and light is delivered for a period of time of 1–10 minutes depending on the volume to ablate and the device used. The treatment can be repeated directly after to achieve larger coagulation volume either inserting the fiber in a new position or utilizing the so-called pull-back technique, meaning performing a new ablation along the insertion track by pulling the fiber back.

Immune stimulating interstitial laser thermotherapy (imILT) is a local ablation method that works at non-coagulating temperatures at the tumor border. The technique consists in creating a temperature gradient in the tumor that results in a heating to 46°C at the tumor border or some millimeters outside it. The temperature is then kept for a prolonged period of approximately 30 minutes to achieve an immunogenic cell death (ICD) at the tumor border, visible only 48–72 hours after treatment, which activates an immune response [13, 14]. An example of ablation achieved performing an imILT treatment is shown in **Figure 3**. The biological process is not fully understood to date, but the hypothesis is that imILT creates inflammation in the tumor. Damage-associated molecular pattern (DAMP) signal is created, and antigens, which are not coagulated due to the low temperatures, are released [7, 15–17]. The antigens are picked up by antigen-presenting cells (APCs) that in turn trigger an immune response [18–21].

The method can in principle be used to treat all types of solid tumors, but some types will be more responsive than others depending on the tumor biology, which is true for immunotherapies in general. Some results from proof-of-concept preclinical and clinical studies are presented in this chapter.

3.1 Technical solutions for interstitial laser thermotherapy

The CE-marked and FDA-approved TRANBERG® Thermal Therapy System for imILT consists of three main parts: a laser generator, a laser applicator, and a thermometry system. The laser generator is a diode-based system that emits light at a wavelength of 1064 nm and with a maximum accessible power of 25 W continuous wave. The unit has a built-in temperature feedback system that is able to measure the temperature in the tissue by means of a minimally invasive temperature probe and to drive the laser emission in order to maintain a stable temperature, set by the user between 43 and 50°C, for a treatment time of up to 30 minutes. The laser

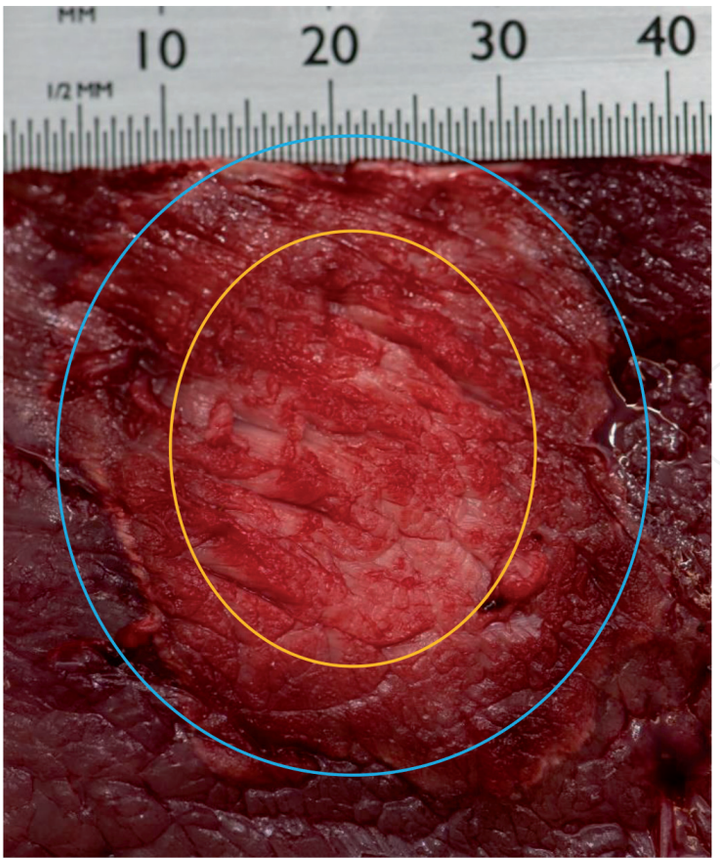

Figure 3.
Effect of imILT treatment on porcine healthy skeletal muscle tissue. Coagulation is achieved within the yellow circle, and immunogenic cell death (ICD) is achieved along the ablation border, between the yellow and the blue line.

applicator consists of a non-cooled optical fiber and an introducer to enable insertion of the fiber in the tissue. The non-cooled optical fiber is available in different tip designs tailored to the ablation volume and shape to be achieved and the tissue to be treated.

All the procedures are performed under image guidance, using MRI, ultrasound, computed tomography (CT), or a combination of the previous depending on the availability of these techniques at the clinic. While it is only possible to perform imILT treatments using ultrasound or CT guidance due to limitations in the temperature probe design, the design of the laser applicator allows laser ablation procedure to be performed with MRI guidance, for example, when performing a focused laser ablation (FLA) for the treatment of early prostate cancer or benign prostatic hyperplasia (BPH).

3.2 In vivo studies on abscopal effect of imILT

Extensive preclinical studies were performed to prove the immune stimulating effects of imILT. One specific study aimed at comparing the immunologic memory evoked by imILT if compared to resection [22].

Research was conducted on 280 rats divided in four groups: (1) rats with tumor implanted in the liver that were treated with imILT, (2) rats with tumors implanted in the liver that were treated with surgical resection, (3) rats without tumor that were treated with imILT ablating normal liver tissue (sham imILT), and (4) rats without tumors that were treated with resection of a part of a healthy liver (sham resection).

Rats in groups 1 and 2 were implanted with adenocarcinoma and treated after 6–8 days. A second challenging tumor of the same kind was implanted in another lobe 2, 5, or 10 weeks later, and the animals were followed for up to 48 days after rechallenge unless they showed signs of inactivity or distress earlier. Vital tumor at sacrifice was evaluated together with other immune system markers. Group 1, tumor treated with imILT, showed a distinct behavior if compared with the other three groups. In groups 2, 3, and 4, the challenging tumor, second implanted, displayed a growth so substantial that none of the rats survived for 48 days. On the contrary, rats in group 1 showed eradication of the challenging tumor at day 48. The extent of the tumor burden for the four groups is represented in **Figure 4**. These findings, combined with results from immunology markers from blood tests, indicate that imILT invokes a strong immune response and an immunologic memory against the treated cancer.

3.3 Clinical results

A number of pre-marketing clinical studies on imILT were performed at Lund University Hospital, Lund, Sweden, where the method was developed for the first time. These studies demonstrated the recruitment of immunocompetent cells in breast cancer patients which indicate a favorable antitumor activity [23–27].

More recently, initial findings from the clinical study program designed to evaluate the safety and the usability of the method performed using the TRANBERG®|Thermal Therapy System (Clinical Laserthermia Systems, AB, Sweden) were published [28]. A variety of solid tumors are included in the study program; the data was reported after 12 patients were treated, out of which 4 were female and 8 were male. Indications treated were breast cancer (n = 1), breast cancer metastasis (n = 1), colon cancer metastasis (n = 2), malignant melanoma metastasis (n = 2), pancreatic carcinoma (n = 1), and primary pancreatic carcinoma (n = 5); the latter two were treated in open surgery, while the other percutaneously. All the treatments were performed using CT or ultrasound guidance. All patients included in the study underwent numerous previous treatments due to comorbidity.

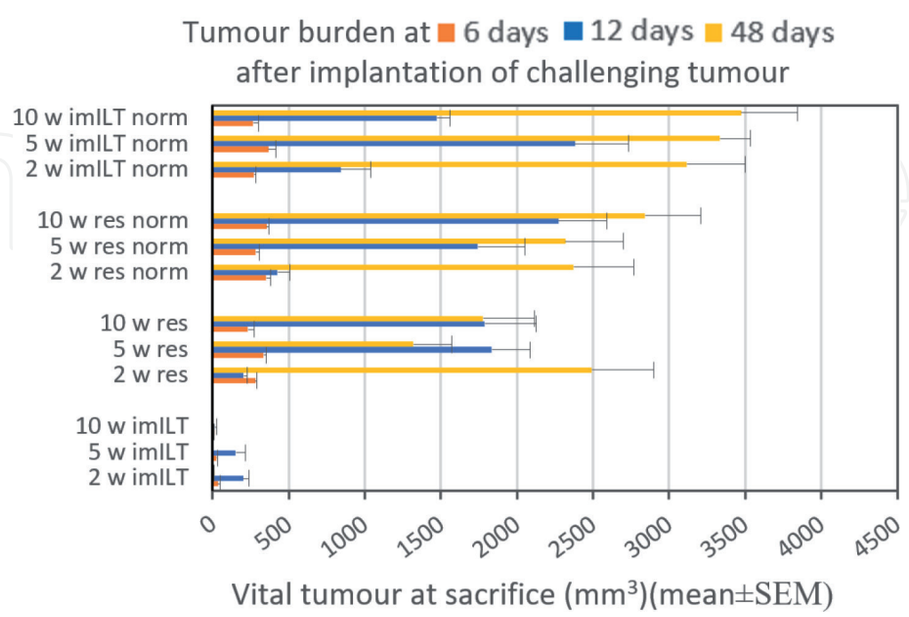

Figure 4.
Tumor burden after implantation of challenging tumor. Only rats having been treated with imILT of primary tumor survived for 48 days after implantation of challenging tumor. All other rats in the 48-day study group had to be euthanized within 10–30 days after the tumor challenge due to extensive tumor. Image: Mats Ekelund.

Immunotherapy was delivered on two malignant melanoma patients before imILT treatment but not during the study period.

One serious adverse event was reported out of nine patients within the sponsor initiated clinical study; the frequency of serious adverse events is in line with previous data on other local ablative techniques, including laser ablation [29, 30], indicating that the procedure can be safely performed.

Usability results vary among the different study clinics. Preliminary indications suggest that insertion and placement of the instrumentation within the volume to be treated are the main challenge, while sterile access, removal from the tissue, and handling of disposable are perceived as less complicated. Handling of the laser unit needs further investigation as the data is spread [28].

The safety studies were not designed to collect statistically significant efficacy results. Each study included different indications to gather safety data and input to future efficacy studies as extensive as possible leading to a low number of patients per indication, and therefore no indication-based data was published. Future ongoing publications will include indicative efficacy and quality-of-life results from these studies.

3.3.1 Case report

This case is a 53-year-old patient with pancreatic cancer diagnosed about 2 years before and treated with first-line chemotherapy, FOLFIRINOX 16 cycles, for tumor reduction. Disease progression was registered after 12 cycles. Due to intolerable toxicity, the treatment regimen was changed to second- and third-line chemotherapies, gemcitabine and protein-bound paclitaxel 16 cycles, after which partial response was achieved. At the time of the first imILT treatment 2 years after the diagnosis, the patient presented with pancreatic carcinoma and three liver metastases (stage IV). PET-CT showed a hypermetabolic focus around the biliary stent, but no clearly visible tumor in the pancreas, and three metastases in the liver (segments VI, V/VI, and V/ peri-gallbladder area).

The first treatment was performed on a 19 mm liver metastasis in segment VI that was metabolically active; see **Figure 5**. The intervention was performed percutaneously under CT guidance, and a first treatment was performed by placing the tip of the radial laser applicator in the metastasis—see **Figure 6**—and a temperature

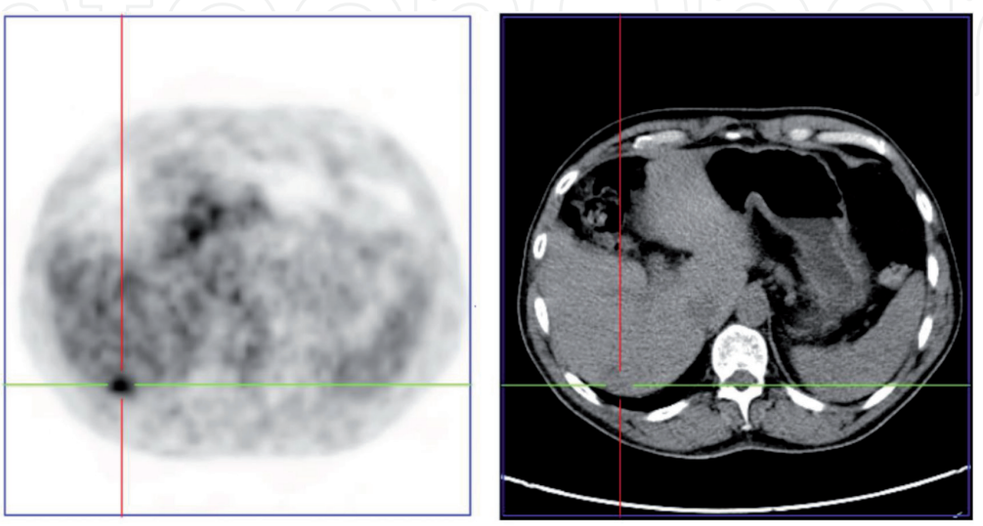

Figure 5.
PET-CT (left) and CT (right) scans showing the position of the treated metastasis during the first treatment session [31].

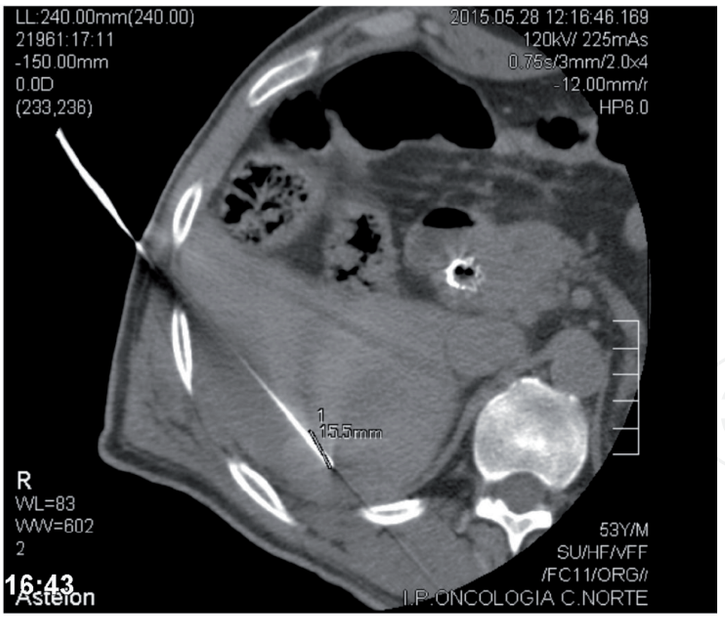

Figure 6.
Laser applicator positioning visualized using CT scan while placing the instrumentation for the first treatment [31].

needle at a distance of approximately 10 mm. The temperature needle was used to regulate the laser emission based on the measured temperature and achieve ICD in a region of the lesion that presented as metabolically active from the PET scan. A temperature of 44–45°C was kept during a period of 30 minutes according to the imILT protocol. A second overlapping ablation was performed after repositioning the laser applicator to necrotize the whole volume of the metastasis. Track ablation was performed to minimize risk for track seeding of tumor cells along the insertion track. A post-procedure CT scan was performed to ensure the ablation of the entire tumor, which was achieved as shown in **Figure 7** (black arrow). The patient suffered slight pain and rise in temperature (38°C) posttreatment, but no other

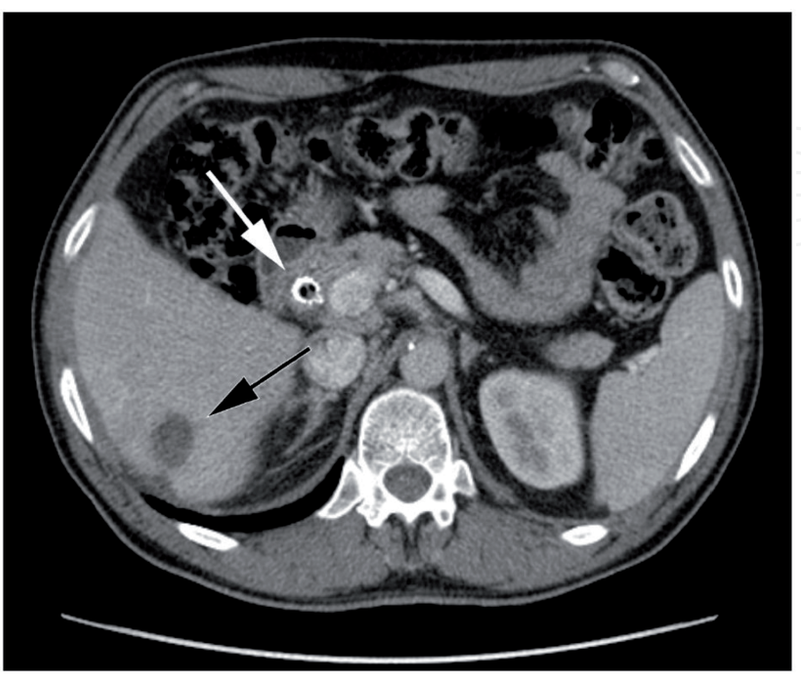

Figure 7.
Posttreatment CT that shows the ablation cavity (black arrow) and the biliary stent (white arrow). First treatment session [31].

discomfort was registered; the patient was discharged after 3 days. No complications were reported during the first 3 months following therapy [31].

Partial response in liver metastasis and total response in pancreas primary tumor were registered 21 months later. However, 3 months later disease progression was noticed, and the patient was treated with imILT for a second time 24 months after the initial treatment. The targeted metastasis was a 35 × 50 mm liver metastasis evaluated at ultrasound at the time of the treatment. The metastasis was treated performing one imILT treatment combined with an overlapping LITT treatment of about 5 minutes to necrotize the whole metastatic mass; the imILT treatment was achieved positioning the radial laser applicator off center within the tumor and the temperature probe at a distance of approximately 11 mm from the applicator. The temperature measured by the probe was kept at 43–45°C for 20 minutes.

Lastly, a third imILT treatment was performed after 40 months from the first treatment because of new disease progression. A new 20 mm liver metastasis was treated using a diffuser laser applicator combined with an introducer with built-in temperature sensors, which resulted in only one puncture. The laser applicator was inserted in the center of the metastasis, and the sensors were positioned 25 mm from the applicator tip to achieve a lesion of 25–30 mm in diameter. To date, 4 months after the last treatment, no complications connected to the laser treatment have been reported [32].

4. Conclusion

Local ablation of tumors is receiving increasing attention for the treatment of metastatic disease because of observed effects on distant tumorous masses suggesting the involvement of the immune system following local therapy.

One technique for local tumor eradication is laser ablation which kills the tumor mass by heating the tissue through direct light absorption and heat transfer resulting in tissue coagulation. imILT is an interstitial laser ablation method tailored to evoke an immune response against the treated tumor. The technique utilizes a laser applicator to deliver energy in the form of laser light to the tissue; the energy delivered to the tissue is precisely controlled based on the temperature measured by a sensor inserted in the tissue at the periphery of the tumor to obtain a lower temperature ablation that aims at maximizing the immune cell death (ICD) volume of the ablation.

Preclinical results indicate that imILT invokes an immune response against the treated tumor, if compared with resection in a rat tumor model. Clinical studies suggest that the procedure can be safely performed since the frequency of the adverse events is in line with previous data on other local ablation techniques. The case of a pancreatic cancer patient treated with imILT was presented.

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Conflict of interest

Cristina Pantaleone is the Technical Manager of Product Development at Clinical Laserthermia Systems, AB.

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Nomenclature

μ_a	absorption coefficient
μ_s	scattering coefficient
g	anisotropy factor
a'	scaling factor that equals the reduced scattering coefficient at 500 nm
f_{Ray}	fraction of Rayleigh scattering
b_{Mie}	scattering power (Mie scattering)
ρ	tissue density
ρ_b	blood density
k	tissue thermal conductivity
c	tissue heat capacity
c_b	blood heat capacity
ω_b	blood perfusion rate
T_b-T	difference between the heated tissue and the blood or the surrounding tissue
Q_{met}	metabolic heat
Q_{ext}	external heat sources
BPH	benign prostate hyperplasia
DAMP	damage associated molecular pattern
CT	computed tomography
ICD	immunogenic cell death
ILT	interstitial laser thermotherapy
imILT	immune stimulating interstitial laser thermotherapy
LITT	laser-induced thermotherapy
PDT	photodynamic therapy

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References

[1] Busch W. Uber den Einfluss, welchen heftigere Erysipl zuweilen auf organisierte neubildungen ausuben. Vol. 23. Rhein. Westph: Verhanl. Naturh. Preuss; 1866. pp. 28-30

[2] Coley WB. The treatment of malignant tumors by repeat inoculations of erysipelas: With a report of ten original class. The American Journal of the Medical Sciences. 1893;**105**:487-511

[3] Coley WB. The therapeutic value of the mixed toxins of the streptococcus of erysipelas and bacillus prodigiosus in the treatment of inoperable malignant tumors. American Journal of Surgery. 1896;**112**:251-280

[4] Westermark N. Uber die Behandlung des ulcerierenden Cervix-carcinoms mittels konstanter Wärme. Vol. 22. Zbl Gynäk. 1898. pp. 1335-1339

[5] Isbert C, Boerner A, Ritz JP, Schuppan D, Buh HJ, Germer CT. In-situ ablation of experimental liver metastases delays and reduces residual intrahepatic tumour growth and peritoneal tumour spread compared with hepatic resection. The British Journal of Surgery. 2002;**89**:1252-1259

[6] Yantorno C, Soanes WA, Gonder MJ, Shulman S. Studies in cryoimmunology. I. the production of antibodies to urogenital tissue in consequence of freezing treatment. Immunology. 1967;**12**:395-410

[7] den Brok MHMGM, Sutmuller RPM, van der Voort R, Bennink EJ, Figdor CG, Ruers TJM, et al. In situ tumor ablation creates an antigen source for the generation of antitumor immunity. Cancer Research. 2004;**64**:4024-4029

[8] Roggan A, Dörschel K, Minet O, Wolff D, GSL M. The optical properties of biological tissue in the near infrared wavelength range - review and measurements. SPIE Press. 1995;**PM25**:10-44

[9] Jacques SSL. Optical properties of biological tissues: A review. Physics in Medicine and Biology. 2013;**58**:R37-R61

[10] Nguyen TH, Park S, Hlaing KK, Kang HW. Temperature feedback-controlled photothermal treatment with diffusing applicator: Theoretical and experimental evaluations. The Journal of the Optical Society of America. 2016;**7**(5)

[11] Lakhssassi A, Kengne E, Semmaoui H. Modifed Pennes' equation modelling bio-heat transfer in living tissues: Analytical and numerical analysis. Natural Science. 2010;**2**(12):1375-1385

[12] Saccomandi P, Schena E, Silvestri S. Design of fiber optic applicators for laser interstitial thermotherapy: Theoretical evaluation of thermal outcomes. In Proceedings: IEEE Engineering in Medicine and Biology Society. 2013;**2013**:3733-3736. DOI: 10.1109/EMBC.2013.6610355

[13] Galluzzi L, Buqué A, Kepp O, Zitvogel L, Kroemer G. Immunological effects of conventional chemotherapy and targeted anticancer agents. Cancer Cell Review. 2015;**28**(6):690-714

[14] Cook AM, Lesterhuis WJ, Nowak AK, Lake RA. Chemotherapy and immunotherapy: Mapping the road ahead. Current Opinion in Immunology. 2016;**39**:23-29

[15] Chu KF, Dupuy DE. Thermal ablation of tumours: Biological mechanisms and advances in therapy. Nature Reviews. 2014;**14**(3):199-208

[16] Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature. 2011;**480**(7378):480-489

[17] Zerbini A, Pilli M, Penna A, Pelosi G, Schianchi C, Molinari A, et al. Radiofrequency thermal ablation of hepatocellular carcinoma liver nodules can activate and enhance tumor-specific T-cell responses. Cancer Research. 2006;**66**(2):1139-1146

[18] Gallucci S, Lolkema M, Matzinger P. Natural adjuvants: Endogenous activators of dendritic cells. Nature Medicine. 1999;**5**:1249-1255

[19] Sauter B, Albert ML, Francisco L, Larsson M, Somersan S, Bhardwaj N, et al. Consequences of cell death: Exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. Journal of Experimental Medicine. 2000;**191**(3):423-434

[20] Sturesson C, Ivarsson K, Andersson-Engels S, Tranberg KG. Changes in local hepatic blood perfusion during interstitial laser-induced thermotherapy of normal rat liver measured by interstitial laser doppler flowmetry. Lasers in Medical Science. 1999;**14**:143-149

[21] Sturesson C, Ivarsson K, Stenram U, Andersson-Engels S, Tranberg KG. Interstitial laser thermotherapy of a rat liver tumour: Effect of hepatic inflow occlusion. Lasers in Surgery and Medicine. 2011;**43**:29-35

[22] Ivarsson K, Myllymäki L, Jansner K, Stenram U, Tranberg KG. Resistance to tumour challenge after tumour laser thermotherapy is associated with a cellular immune response. British Journal of Cancer. 2005;**93**:435-440

[23] Tranberg KG, Möller PH, Hannesson P, Stenram U. Interstitial laser treatment of malignant tumours: Initial experience. European Journal of Surgical Oncology. 1996;**22**:47-54

[24] Tranberg KG, Ivarsson K, Willner J, Håkansson L, Stenram U. Induction of a distant anti-tumour effect by interstitial laser thermotherapy (imILT) in a patient with malignant melanoma. In: 2nd International Conference on Tumor Microenvironment: Progression, Therapy and Prevention; Baden, Austria. 2002. p. 49

[25] Haraldsdottir KH, Ivarsson K, Götberg S, Ingvar C, Stenram U, Tranberg KG. Interstitial laser thermotherapy (ILT) of breast cancer. European Journal of Surgical Oncology. 2008;**34**:739-745

[26] Haraldsdottir KH, Ivarsson K, Jansner K, Stenram U, Tranberg KG. Changes in immunocompetent cells after interstitial laser thermotherapy of breast cancer. Cancer Immunology, Immunotherapy. 2011;**60**:847-856

[27] Haraldsdottir KH, Ingvar C, Stenram U, Tranberg KG. Long-term follow-up after interstitial laser thermotherapy of breast cancer. Anticancer Research. 2015;**35**:6147-6152

[28] Axelsson J, Pantaleone C, Åstrom S. Initial findings of immunostimulating interstitial laser thermotherapy of solid tumours. Journal for Clinical Studies. 2017;**9**(4):28-31

[29] Vogl TJ, Straub R, Eichler K, Woitaschek D, Mack M. Malignant liver tumors treated with MR imaging-guided laser-induced thermotherapy: Experience with complications in 899 patients (2,520 lesions). Radiology. 2002;**225**:367-377

[30] Paiella S, Salvia R, Girelli R, Frigerio I, Giardino A, D'Onofrio M, et al. Role of local ablative techniques radiofrequency ablation and irreversible electroporation in the treatment of pancreatic cancer. Updates in Surgery. 2016:2-6

[31] Axelsson J, Pantaleone C, Dymling S, Bergenfeldt M, Sousa MJ, Machado M, et al. Successful Treatment of Pancreatic Cancer Liver Metastasis Using Immunostimulating Interstitial Laser Thermotherapy (imILT). Available from: https://clinicallaser.se/wp-content/uploads/2017/05/Poster_CLS_Porto_May_2017.pdf

[32] Gonçalves B, Sousa MJ, Machado M, Santos L. P-66 Abscopal effect of immunostimulating laser thermotherapy on liver metastasis in pancreatic cancer: A case report and study protocol for a randomised controlled trial. In: European Conference on Interventional Oncology; 8-11 April 2019; Amsterdam, The Netherlands. 2019