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9 **Fiorentini G. et al.:** Modulated Electrohyperthermia in Integrative Cancer Treatment for Relapsed Malignant Glioblastoma and Astrocytoma: Retrospective Multicenter Controlled Study

21 **Szasz O:** Bioelectromagnetic Paradigm of Cancer Treatment-Modulated Electro-Hyperthermia (mEHT)

34 **Prasad B. et al.:** Quantitative Estimation of the Equivalent Radiation Dose Escalation using Radiofrequency Hyperthermia in Mouse Xenograft Models of Human Lung Cancer

45 **Minnaar A.C. et al.:** The effect of modulated electro-hyperthermia on local disease control in HIV-positive and -negative cervical cancer women in South Africa: Early results from a phase III randomised controlled trial

69 **Chung H.J et al..:** Iron-dextran as a thermosensitizer in radiofrequency hyperthermia for cancer treatment

79 **Havas M.:** Integrative Therapeutic Options for Treating Stage Four Breast Cancer

85 **Minnaar A.C. and Kotzen J.:** Modulated electro-hyperthermia as an immune-modulator with checkpoint inhibitors and radiotherapy

87 **Danics L. et al:** Repeated treatment with modulated electro-hyperthermia inhibits tumor growth in a triple negative mouse breast cancer model

90 **Forika G.:** Elevated apoptosis and tumor stem cell destruction in a radioresistant pancreatic adenocarcinoma cell line when radiotherapy is combined with modulated electro-hyperthermia

99 **Karaszi A. et al:** Modulated Electro-Hyperthermia As Additional Treatment Option In Oncology - Feasibility Study On Quality-Of-Life, Imaging And Biomarkers To Support Treatment Planning

102 **Minnaar C.A. et al.:** Effects of modulated electro-hyperthermia on disease control and 3 year disease-free survival in locally advanced cervical cancer patients: Preliminary results

114 **Schvarcz Cs. et al.:** Short term treatment with modulated electro-hyperthermia in triple negative breast cancer bearing mice

117 **Vancsik T. et al.:** Modulated electro-hyperthermia promotes doxorubicin cytotoxicity in a C26 colorectal carcinoma cell line model

120 **Van Gool W.S. et al** Can we monitor immunogenic cell death (ICD) induced with modulated electrohypertheria and oncolytivc virus injections?

126 **Van Gool W.S. et al:** Modulated electrohyperthermia as part of immunogenic cell death treatment in pediatric neuro-oncology

128 **Wust P. et al.:** Physical potentials of radiofrequency hyperthermia with amplitude modulation

139 **Szasz A.:** Immune-effects with local hyperthermia

149 **Szasz O.:** Molecular basis of modulated electro-hyperthermia combination with radio- and chemo-therapies

Editorial

Dear Readers, Dear Fellow Researchers, Dear Colleagues, Dear Friends,

I am pleased to introduce the recent 26th volume of our Oncothermia Journal. As usually we are continuing to distribute the results reached with oncothermia worldwide, reprinting open access articles and showing presentations of expert conferences. We are glad to know, that you may use it as a reference in your practice and also you may discuss it openly, sending a "Letter to Editor" or write directly to the authors.
The clinical part of the present volume has many new results again. The study on glioblastoma and astrocytoma, or the cervical cancer stage-four study and breast cancer article give a useful presentation of the results with the oncothermia method. We are excited with the more widespread applications of immune-oncology, which has increasingly emphasized role in the present oncothermia applications, including the viral boosting of the immune system too. Clarifying the molecular basis of oncothermia together with the new results of electric field effects are also placed in the spectrum of this volume.
We had taken special attention to the ESHO'19 (Warsaw, Poland) showing some presentations. Especially the presentation of the renowned expert, Prof. Peter Wust's talk was in the center of attention, and we are happy to show his work in this volume. His approach explaining the effect of modulation is a remarkable addition to our present knowledge. His remark, correcting the old slogeen "Biology is with us, but physics is against us" shows a new way of understanding: "Physics is our friend, but we have to notice it."
The present volume offers again the high level of scientific and medical articles to the readers, representing the best values of the hyperthermia community and it well shows the great development possibilities as well as the bright future of the method.
I hope this volume serves your benefits and you will read it with enjoyment.

Sincerely yours,
Prof. Dr. Andras Szasz

Liebe Leserinnen und Leser, liebe Kolleginnen und Kollegen aus Forschung und Praxis,

Ich freue mich, Ihnen den 26. Band unseres Oncothermia-Journals vorstellen zu können. Wie üblich verbreiten wir weiterhin weltweit die mit Oncothermie erzielten Ergebnisse, drucken Open-Access-Artikel nach und zeigen Präsentationen von Expertenkonferenzen. Wir freuen uns zu wissen, dass Sie es als Referenz in Ihrer Praxis verwenden können. Sie können es außerdem auch offen diskutieren, einen „Brief an den Herausgeber" senden oder direkt an die Autoren schreiben.
Der klinische Teil des vorliegenden Bandes beeinhaltet wieder viele neue Ergebnisse. Die Studie zu Glioblastomen und Astrozytomen , die Studie zu Gebärmutterhalskrebs im vierten Stadium und der Artikel zu Brustkrebs ergeben eine nützliche Darstellung der Ergebnisse mit der Onkothermie-Methode.Wir sind begeistert von den sich immer weiter verbreiteten Anwendungen der Immunonkologie, die bei den gegenwärtigen Onkothermie-Anwendungen eine immer wichtigere Rolle spielen - einschließlich der viralen Stärkung des Immunsystems. In das Spektrum dieses Bandes wird auch die Aufklärung der molekularen Grundlagen der Onkothermie zusammen mit den neuen Ergebnissen der elektrischen Feldeffekte aufgenommen.
Wir haben der ESHO'19 (Warschau, Polen) besondere Aufmerksamkeit geschenkt und einige Präsentationen gezeigt. Besonders die Präsentation des renommierten Experten, Prof. Peter Wusts Vortrag, stand im Mittelpunkt. Wir freuen uns, seine Arbeit in diesem Band zu zeigen. Sein Ansatz, den Effekt der Modulation zu erklären, ist eine bemerkenswerte Ergänzung unseres gegenwärtigen Wissens. Seine Bemerkungbezüglich des alten Slogens "Biologie ist bei uns, aber Physik ist gegen uns", zeigt eine neue Art des Verstehens: "Physik ist unser Freund, aber wir müssen es bemerken."
Der vorliegende Band bietet den Lesern wieder ein hohes Maß an wissenschaftlichen und medizinischen Artikeln, die die besten Werte der Hyperthermie-Community repräsentieren, und zeigt die großen Entwicklungsmöglichkeiten sowie die glänzende Zukunft der Methode auf.
Ich hoffe, dieser Band gefällt Ihnen und Sie werden ihn mit Vergnügen lesen.

Mit freundlichen Grüßen,
Prof. Dr. Andras Szasz

Imprint

Editor-in-Chief

Prof. Dr. András Szász
Head of the Department of Biotechnics, St. Istvan University, Godollo, Hungary
Chief Scientific Officer (CSO), Oncotherm GmbH, Belgische Allee 9, 53842 Troisdorf, Germany
Tel: +49 2241 31992-0; + 36 23 555 510; E-mail: Szasz@oncotherm.de

Managing Editors

Dr. Janina Leckler
Oncotherm GmbH, Belgische Allee 9, 53842 Troisdorf, Germany
Tel: +49 2241 31992-0; E-mail: Leckler@oncotherm.de

and

Ms. Zsófia Hahn
Oncotherm Kft., Gyár u. 2. 2040, Budaörs, Hungary
Tel: + 36 23 555 510, E-mail: hahn.zsofia@oncotherm.org

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Rules for submission

As the editorial team we are committed to a firm and coherent editorial line and the highest possible printing standards. But it is mainly you, the author, who makes sure that the MEHT Journal is an interesting and diversified magazine. We want to thank every one of you who supports us in exchanging professional views and experiences. To help you and to make it easier for both of us, we prepared the following rules and guidelines for abstract submission.

Als redaktionelles Team vertreten wir eine stringente Linie und versuchen, unserer Publikation den höchst möglichen Standard zu verleihen. Es sind aber hauptsächlich Sie als Autor, der dafür Sorge trägt, dass das MEHT Journal zu einem interessanten und abwechslungsreichen Magazin wird. Wir möchten allen danken, die uns im Austausch professioneller Betrachtungen und Erfahrungen unterstützen. Um beiden Seiten die Arbeit zu erleichten, haben wir die folgenden Richtlinien für die Texterstellung entworfen.

1. Aims and Scope
The MEHT Journal is an official journal of the Oncotherm Group, devoted to supporting those, who would like to publish their results for general use. Additionally, it provides a collection of different publications and results. The MEHT Journal has an open-minded character, but it should particularly contain complete study-papers, case-reports, reviews, hypotheses, opinions, and all the informative materials which could be helpful for the international Oncotherm community. Advertisement connected to the topic is also welcome.

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Further information about the Journal, including links to the online sample copies and content pages can be found on the website of the journal: www.MEHT-Journal.com.

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Das MEHT Journal ist das offizielle Magazin der Oncotherm Gruppe und soll diejenigen unterstützen, die ihre Ergebnisse der Allgemeinheit zur Verfügung stellen möchten. Das MEHT Journal ist neuen Inhalten gegenüber offen, sollte aber vor allem Studienarbeiten, Fallstudien, Hypothesen, Meinungen und alle weiteren informativen Materialien, die für die internationale Oncotherm-Gemeinschaft hilfreich sein könnten, enthalten. Werbung mit Bezug zum Thema ist ebenfalls willkommen.

- Klinische Studien, regionale, lokale oder multilokale Oncothermie oder Electro Cancer Therapy (ECT) Behandlungen, Fallstudien, praktische Erfahrungen in komplexen Behandlungen, klinische Versuche, physiologische Effekte, Oncothermie in Kombination mit anderen Modalitäten und Behandlungsoptimierungen.
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Contents

Contents

Modulated Electrohyperthermia in Integrative Cancer Treatment for Relapsed Malignant Glioblastoma and Astrocytoma: Retrospective Multicenter Controlled Study 9

Bioelectromagnetic Paradigm of Cancer Treatment-Modulated Electro-Hyperthermia (mEHT) 21

Quantitative Estimation of the Equivalent Radiation Dose Escalation using Radiofrequency Hyperthermia in Mouse Xenograft Models of Human Lung Cancer 34

The effect of modulated electro-hyperthermia on local disease control in HIV-positive and -negative cervical cancer women in South Africa: Early results from a phase III randomised controlled trial 45

Iron-dextran as a thermosensitizer in radiofrequency hyperthermia for cancer treatment .. 69

Integrative Therapeutic Options for Treating Stage Four Breast Cancer 79

Modulated electro-hyperthermia as an immune-modulator with checkpoint inhibitors and radiotherapy 85

Repeated treatment with modulated electro-hyperthermia inhibits tumor growth in a triple negative mouse breast cancer model 87

Elevated apoptosis and tumor stem cell destruction in a radioresistant pancreatic adenocarcinoma cell line when radiotherapy is combined with modulated electro-hyperthermia 90

Modulated Electro-Hyperthermia As Additional Treatment Option In Oncology - Feasibility Study On Quality-Of-Life, Imaging And Biomarkers To Support Treatment Planning 99

Effects of modulated electro-hyperthermia on disease control and 3 year disease-free survival in locally advanced cervical cancer patients: Preliminary results 102

Short term treatment with modulated electro-hyperthermia in triple negative breast cancer bearing mice 114

Modulated electro-hyperthermia promotes doxorubicin cytotoxicity in a C26 colorectal carcinoma cell line model 117

Can we monitor immunogenic cell death (ICD) induced with modulated electrohypertheria and oncolytivc virus injections? 120

Modulated electrohyperthermia as part of immunogenic cell death treatment in pediatric neuro-oncology 126

Physical potentials of radiofrequency hyperthermia with amplitude modulation 128

Immune-effects with local hyperthermia 139

Molecular basis of modulated electro-hyperthermia combination with radio- and chemo-therapies **149**

Advertisement **159**

Modulated Electrohyperthermia in Integrative Cancer Treatment for Relapsed Malignant Glioblastoma and Astrocytoma: Retrospective Multicenter Controlled Study

Giammaria Fiorentini, MD[1], Donatella Sarti, PhD[1], Carlo Milandri, MD[2], Patrizia Dentico, MD[2], Andrea Mambrini, MD[3], Caterina Fiorentini, MD[4], Gianmaria Mattioli, MD[1], Virginia Casadei, MD[1]and Stefano Guadagni, MD[5]

[1]Azienda Ospedaliera "Ospedali Riuniti Marche Nord", Pesaro, Italy
[2]Nuovo Ospedale San Giuseppe, ASL Toscana Centro, Empoli, Florence, Italy
[3]Carrara General Hospital, Carrara, Italy
[4]University of Siena, Siena, Italy
[5]University of L'Aquila, L'Aquila, Abruzzo, Italy

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Modulated Electrohyperthermia in Integrative Cancer Treatment for Relapsed Malignant Glioblastoma and Astrocytoma: Retrospective Multicenter Controlled Study

Giammaria Fiorentini, MD[1], Donatella Sarti, PhD[1], Carlo Milandri, MD[2], Patrizia Dentico, MD[2], Andrea Mambrini, MD[3], Caterina Fiorentini, MD[4], Gianmaria Mattioli, MD[1], Virginia Casadei, MD[1] and Stefano Guadagni, MD[5]

Abstract

Background: There are interesting studies on glioma therapy with modulated electrohyperthermia (mEHT), which combines heat therapy with an electric field. Clinical researchers not only found the mEHT method feasible for palliation but also reported evidence of therapeutic response. **Purpose:** To study the efficacy and safety of mEHT for the treatment of relapsed malignant glioma and astrocytoma versus best supportive care (BSC). **Methods:** We collected data retrospectively on 149 patients affected by malignant glioma and astrocytoma. Inclusion criteria were informed consent signed; >18 years old; histological diagnosis of malignant glioma or astrocytoma; relapsed after surgery, adjuvant temozolomide-based chemotherapy, and radiotherapy; and indication for treatment with mEHT in palliative setting. mEHT was performed with capacitive coupling technique keeping the skin surface at 26°C and the tumor temperature at 40°C to 42.5°C for > 90% of treatment duration (20-60 minutes). The applied power was 40 to 150 W using a step-up heating protocol. Results from patients treated with mEHT were compared with those treated with BSC. **Results:** A total of 149 consecutive patients were enrolled in the study, 111 (74%) had glioblastoma multiforme (GBM), and 38 (26%) had astrocytoma (AST). mEHT was performed for 28 (25%) of GBM and 24 (63%) of AST patients. Tumor response at the 3-month follow-up was observed in 29% and 48% of GBM and AST patients after mEHT, and in 4% and 10% of GBM and AST patients after BSC, respectively. The survival rate at first and second year in the mEHT group was 77.3% and 40.9% for AST, and 61% and 29% for GBM, respectively. The 5-year overall survival of AST was 83% after mEHT versus 25% after BSC and 3.5% after mEHT versus 1.2% after BSC for GBM. The median overall survival of mEHT was 14 months (range 2-108 months) for GBM and 16.5 months (range 3-156 months) for the AST group. We observed 4 long-term survivors in the AST and 2 in the GBM group. Two of the long survivors in AST and 1 in GBM group were treated by mEHT. **Conclusions:** mEHT in integrative therapy may have a promising role in the treatment and palliation of relapsed GBM and AST.

Keywords

relapsed malignant glioma, modulated electrohyperthermia, survival, tumor response, astrocytoma

Submitted May 22, 2018; revised October 9, 2018; accepted October 12, 2018

Introduction

Hyperthermia has been applied since the 1970s when heat was shown to kill tumor cells because they are more sensitive to heat than healthy cells. In the past decade, hyperthermia has been applied to several types of cancers with encouraging results in both tumor response and safety.[1] The most frequently used methods of hyperthermia for cancer treatment are magnetic nanoparticles (mNPs), external radiofrequency, hyperthermic perfusion, modulated electrohyperthermia (mEHT), frequency enhancers

[1]Azienda Ospedaliera "Ospedali Riuniti Marche Nord", Pesaro, Italy
[2]Nuovo Ospedale San Giuseppe, ASL Toscana Centro, Empoli, Florence, Italy
[3]Carrara General Hospital, Carrara, Italy
[4]University of Siena, Siena, Italy
[5]University of L'Aquila, L'Aquila, Abruzzo, Italy

Corresponding Author:
Giammaria Fiorentini, Onco-Hematology Department, Azienda Ospedaliera "Ospedali Riuniti Marche Nord", Via Lombroso 1, Pesaro, 61122, Italy.
Email: g.fiorentini@alice.it

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associated with a magnetic field, and catheter-mediated hyperthermia.[2-5]

The combination of traditional hyperthermia (41°C to 43°C) to radiotherapy (RT) or systemic chemotherapy improves and prolongs the clinical benefits of the single methods.[6,7] This synergistic effect is due to apoptosis induction, angiogenesis inhibition, chemo- and radiosensitivity activation, high drug concentration induction inside the lesion, and an increase in the tumor sensitivity to immunotherapy.[8,9] Gliomas form the majority of brain tumors (80%), and glioblastoma multiforme (GBM) represents 65% of these tumors.[10] The prognosis is rather poor due to the infiltration of the surrounding brain tissues and the resistance to chemotherapy and ET.[11] Astrocytoma tumors (AST) have a better prognosis than GBM[12]; however, the incidence is lower than GMB.

Surgery, adjuvant temozolomide (TMZ) and RT is the first treatment option for gliomas, and RT combined with chemotherapy and TMZ is the standard therapy when surgery is not indicated.[13,14] Adjuvant therapy with RT in combination with TMZ is effective in prolonging survival[13]; however, the disease recurrence rate is very high (37%).[14]

There is no standard treatment option for recurrent gliomas. Choosing the therapy after progression is very challenging and options include combinations of surgery, re-irradiation (re-RT), chemotherapy, anti-angiogenic agents, and combination therapies of hyperthermia with chemotherapy or RT.[15-18] In this case, surgery can be performed only for young and fit patients with small-size relapses.

There are interesting studies on glioma therapy with mEHT, which combines the heat therapy with an electric field.[21-24] and the literature shows that radiofrequency combined with conventional hyperthermia is an effective treatment for brain tumors.[19,20] Brain tumor therapy with an electric field is approved by the US Food and Drug Administration (FDA).[23] Clinical researchers have not only found the mEHT method feasible for palliation but have also reported evidence of therapeutic response.[16,25,26,27] Literature reports good tolerability with rare adverse events and improvement of survival and quality of life.[24-26] We have previously reported beneficial effects from mEHT treatment in our previous study on palliation, with evidence of tumor response and a slight increase of overall survival.[16,25] In this article, we describe the efficacy and safety of mEHT on relapsed malignant GBM and AST in a larger number of patients, and compare the results with results obtained from patients treated with conventional palliation as best supportive care (BSC).

Materials and Methods

Patient Selection

This was a retrospective observational multicenter cohort-controlled study, including 4 Italian hospitals: Pesaro, Carrara, Empoli, and L'Aquila, 3 of which had mEHT devices (Pesaro, Carrara, and Empoli). Inclusion criteria were diagnosis of GBM or AST relapsed after surgery, adjuvant RT and TMZ therapy and no second line therapy performed; >18 years old; informed consent signed; Eastern Cooperative Oncology Group (ECOG) performance status 0 to 3; and normal values of standard hematological parameters.

Table 1. Description of AST Patients Group.

Parameters (AST)	n	%
Males	20	52.6
Females	18	47.4
mEHT treated	24	63.2
MGMT methylated	11	28.9
MGMT nonmethylated	11	28.9
MGMT ND	16	42.2
IDH1 mutated	13	34.2
IDH1 wild type	11	28.9
IDH1 ND	14	36.9
Events	15	39.5
Age, years (range)	22-80	—
Survival, months (range)	3-156	—

Abbreviations: AST, astrocytoma; mEHT, modulated electrohyperthermia; MGMT, O6-methylguanine-DNA methyltransferase; IDH1, isocitrate dehydrogenase 1; ND, non detected.

From April 2003 to January 2018, 187 patients with GBM and AST were sent to our centers, 31 of these were lost to follow-up and were not evaluable for tumor response and survival. Seven were not refractory to treatment and were still in remission, and mEHT was used to delay tumor progression. Finally, 149 patients were included in the study: 111 (74%) had GBM and 38 (26%) had AST. The mEHT was administered as palliative care to 28 (25%) GBM patients and 22 (58%) AST patients (Tables 1 and 2). The remaining patients (83 GBM and 14 AST) received BSC, including dexamethasone, 18% glycerol infusion, mannitol, holistic therapy, and psychosocial support. Nitrosoureas or cisplatin-based chemotherapies were allowed in the BSC protocol. The BSC group included 28 (30%) patients (GBM) that received chemotherapy: 12 rechallenge of TMZ, 8 nitrosurea, and 8 cisplatin-based chemotherapies. Chemotherapy was not used for mEHT group. The patient's intention to treat was the selection basis for the mEHT group.

Device Description and mEHT Protocol

The study was performed with modulated electro-hyperthermia (mEHT) (EHY-2000 Plus device; CE0123, Oncotherm, Troisdorf, Germany). mEHT was used to obtain predefined heating with an electric field,[27,28] increasing the field power in an energy-controlled manner.[16,17,24,25] The

Table 2. Description of GBM Patients Group.

Parameters (GBM)	n	%
Males	68	61.3
Females	43	38.7
mEHT treated	28	25.2
MGMT methylated	25	22.5
MGMT nonmethylated	27	24.3
MGMT ND	59	53.2
IDH1 mutated	13	11.7
IDH1 wild type	19	17.1
IDH1 ND	79	71.2
Events	86	77.5
Age, years, range	27-86	—
Survival, months, range	2-108	—

Abbreviations: GBM, glioblastoma multiforme; mEHT, modulated electrohyperthermia; MGMT, O6-methylguanine-DNA methyltransferase; IDH1, isocitrate dehydrogenase 1; ND, non detected.

therapeutic temperature used in the mEHT treatment was estimated to range from 40°C to 42.5°C as previously reported.[16,17,24-27] The mEHT technique operates with amplitude-modulated short-wave radiofrequency at a carrier frequency of 13.56 MHz. The technique combines impedance coupling with the technique of capacitive coupling. The device has 2 electric capacitive plates (electrodes): one under the patient in the treatment bed, and one movable electrode, which is positioned over the patient's target anatomical area to be treated. The electrodes have a water bolus to make the best transfer of energy, to distribute the electric current inside the tissue following the actual form of the patient's body, and to keep the skin temperature safely cooled by cooling the surface with the bolus. The preset skin temperature was 26°C and it did not exceed 39°C at the point of skin contact during the treatment. The electrode was 20 cm in diameter and round in shape.

Preprocedural medication administered to all patients before each mEHT to avoid brain edema was 250 mL of glycerol 18% and dexamethasone 12 mg. The selected area was treated 3 times per week for 8 weeks. At 3 months, the tumor response was assessed on computed tomography (CT) or magnetic resonance imaging (MRI) scans and if the response was positive (complete response [CR], partial response [PR], or stable disease [SD]) then the 8-week course of treatment was repeated. The treatment time and the applied power were increased in each session according to the patient's tolerance to heat. The first treatment was always performed applying 40 W for 20 minutes (48 kJ energy). Time was gradually raised from 20 to 60 minutes and the power was increased according to a step-up power protocol from 40 up to 150 W (540 kJ) over 2 weeks.[25]

This protocol achieved an estimated average temperature inside the tumor volume of >40°C for more than 90% of the mEHT sessions, which was indicated by the device and controlled by the phantom measurement. The median number of mEHT treatments/patient were 22 (range 11-62). Summary of the technical mEHT parameters of the treatments is shown in Table 3.

Table 3. Treatment Parameters.

Practical Parameters	Value
Step-up power, W (from-to)	40-150
Average energy dose, kJ	540
Therapeutic temperature, °C	40-42.5
Treatment time/session, minutes	60
Treatment frequency (weekly)	3
Treatment cycle, weeks	8
Follow-up time, months	16

Outcome Measures

MRI or CT scans were performed every 3 months to study the efficacy of the treatments. Tumor response was assessed with the response evaluation criteria in solid tumors (RECIST, v.1.1). The ECOG performance status scale was used to evaluate the functional recovery. Overall survival (OS) was computed from the date of initial diagnosis to last follow-up or death of the patient. Adverse events were recorded indicating intensity and duration, using the Common Terminology Criteria for Adverse Events v3.0 (CTCAE). Data on O6-methylguanine-DNA-methyltransferase (MGMT) methylation[28] and isocitrate dehydrogenase 1 (IDH1) mutational status[29] were collected when available (Table 3). The median total follow-up time was 16 months (range 1-156 months).

Statistical Analysis

We used descriptive and Kaplan-Meier nonparametric statistical estimates. The statistical significance was measured by Student's t test, z test for proportions, and log-rank test for Kaplan-Meier curves; $P \leq .05$ indicated statistically significant difference between samples.

Results

Sample Characteristics

Median age was 60 years for GBM (range 33-86 years) and 50 years (25-71 years) for AST group. Both subgroups (GBM and AST) followed the normal distribution and the gender ratio had no significant differences, so the statistical evaluation had an optimal basis. Data for MGMT methylation and IDH1 mutational status were not available for many patients. A summary of the demographic data is shown in Tables 1 and 2.

Table 4. Tumor Response and Survival of AST Group.

Results (AST)	mEHT (n)	mEHT (%)	BSC (n)	BSC (%)
CR	2	8	0	0
PR	8	33	1	7
SD	6	25	5	36
PD	4	17	9	64
ND	2	8	1	7
OS, months median (range)	16 (3-156)	—	16.5 (3-120)	—

Abbreviations: AST, astrocytoma; mEHT, modulated electrohyperthermia; BSC, best supportive care; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ND, non detected; OS, overall survival.

Table 5. Tumor Response and Survival of GBM Group.

Results (GBM)	mEHT (n)	mEHT (%)	BSC (n)	BSC (%)
CR	1	4	2	2
PR	6	21	2	2
SD	8	29	12	14
PD	13	46	62	75
ND	0	0	5	6
OS, months, median (range)	14 (2-108)	—	9 (2-84)	—

Abbreviations: GBM, glioblastoma multiforme; mEHT, modulated electrohyperthermia; BSC, best supportive care; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ND, non detected; OS, overall survival.

Tumor Response

At the 3-month follow-up period, the AST patients of mEHT group showed 2 (9%) CR, 8 (36%) PR, and 6 (27%) SD. SD was also considered a positive response due to the relapsed nature of the disease in this study. For this reason, the overall positive response of AST (CR + PR + SD) was 72% after mEHT (Table 4, Figure 3A) and is significantly higher than the 37% observed after BSC (PR = 6% and SD = 31%) ($P < .005$). Also, the objective response (CR + PR) (45% vs 6%, $P > .005$) was significantly higher than that of the BSC group. Progressive disease (PD) was observed in 4 (18%) patients in mEHT and in 9 (56%) of BSC. At the 3-month follow-up period, the GBM showed 1 (4%) CR, 6 (21%) PR, and SD 8 (29%), with an overall positive response of 54% in the mEHT group, and 13 PD (46%) (Table 5, Figure 3B). The GBM treated with BSC had 2 (2%) CR, 2 (2%) PR, and 12 (14%) SD; whereas PD was observed in 62 (75%). Overall positive response of GBM treated with BCS was 19% significantly lower ($P < .05$) than that of mEHT group.

Two characteristic cases of AST and 1 case of GBM treated with mEHT are shown in Figures 1 and 2, respectively. Figure 1A and B shows relapsed AST (grade III) cases in a 24-year-old man and Figure 1C-F shows relapsed AST (grade III) in a 32-year-old man. The tumors in both cases were well controlled locally. Figure 2 shows the MRI of a patient (male, 54 years old) with relapsed GBM (grade IV).

Survival

The response rates of AST and GBM are shown in Figure 3A and B, respectively. The Kaplan-Meier estimates of the treatment survivals are shown for AST and GBM in Figures 4 and 5, respectively. The median OS of AST was 16.5 months (range 3-120 months) and 16 months (range 3-156 months) in the BSC and mEHT groups, respectively ($P = .0065$) (Figure 6). Figure 6 shows the advantage of mEHT over BSC in terms of survival for AST. Survival rate of AST at the first and second year in the mEHT group was 77.3% and 40.9%, respectively. The 5-year OS of AST was 83% after mEHT versus 25% after BSC.

The median OS for GBM was 14 months (range 2-108 months) after mEHT and 9 months (range 2-84 months) after BSC (Figure 7) ($P = .047$).

We observed 4 long-term survivors in the AST and 2 in the GBM group. Two of the long survivors in the AST and 1 in the GBM group were treated by mEHT, with an OS of 156, 62, and 108 months, respectively. The long survivor of BSC group had a survival of 84 months.

Most patients reported a better quality of life (evaluated by subjective responses as reported during follow-up visits) after mEHT. Six patients had an objective clinical response and therapeutic benefit by mEHT measured by decreased ECOG values from 3 to 1 in 2 (9%) AST and 2 (7%) GBM patients and from 3 to 0 in 2 (9%) AST patients.

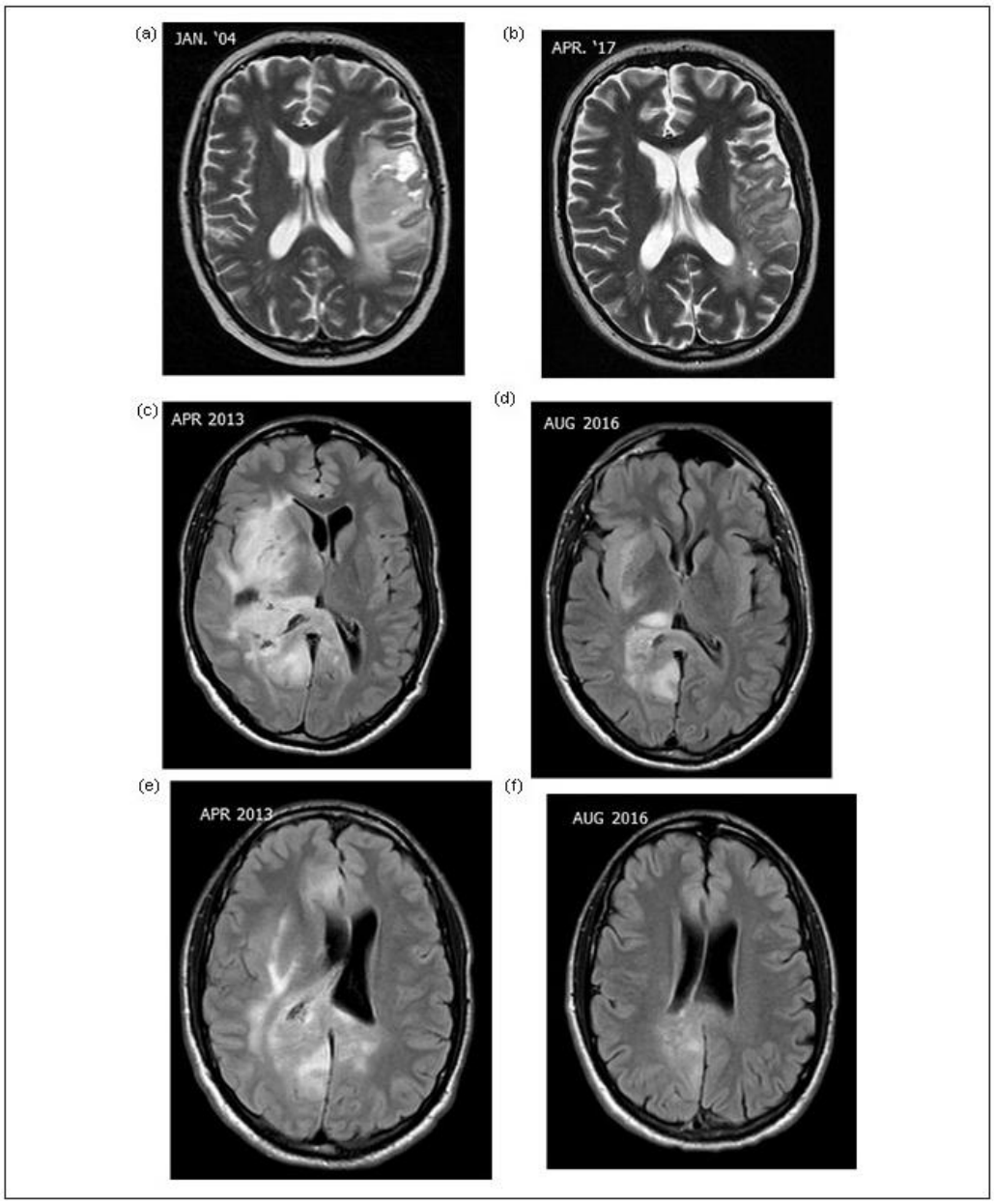

Figure 1. Magnetic resonance imaging of astrocytomas: case 1 (male 24 years), relapsed astrocytoma grade III (a, b) and case 2 (male, 32 years), relapsed astrocytoma grade III (c, d, e, f).

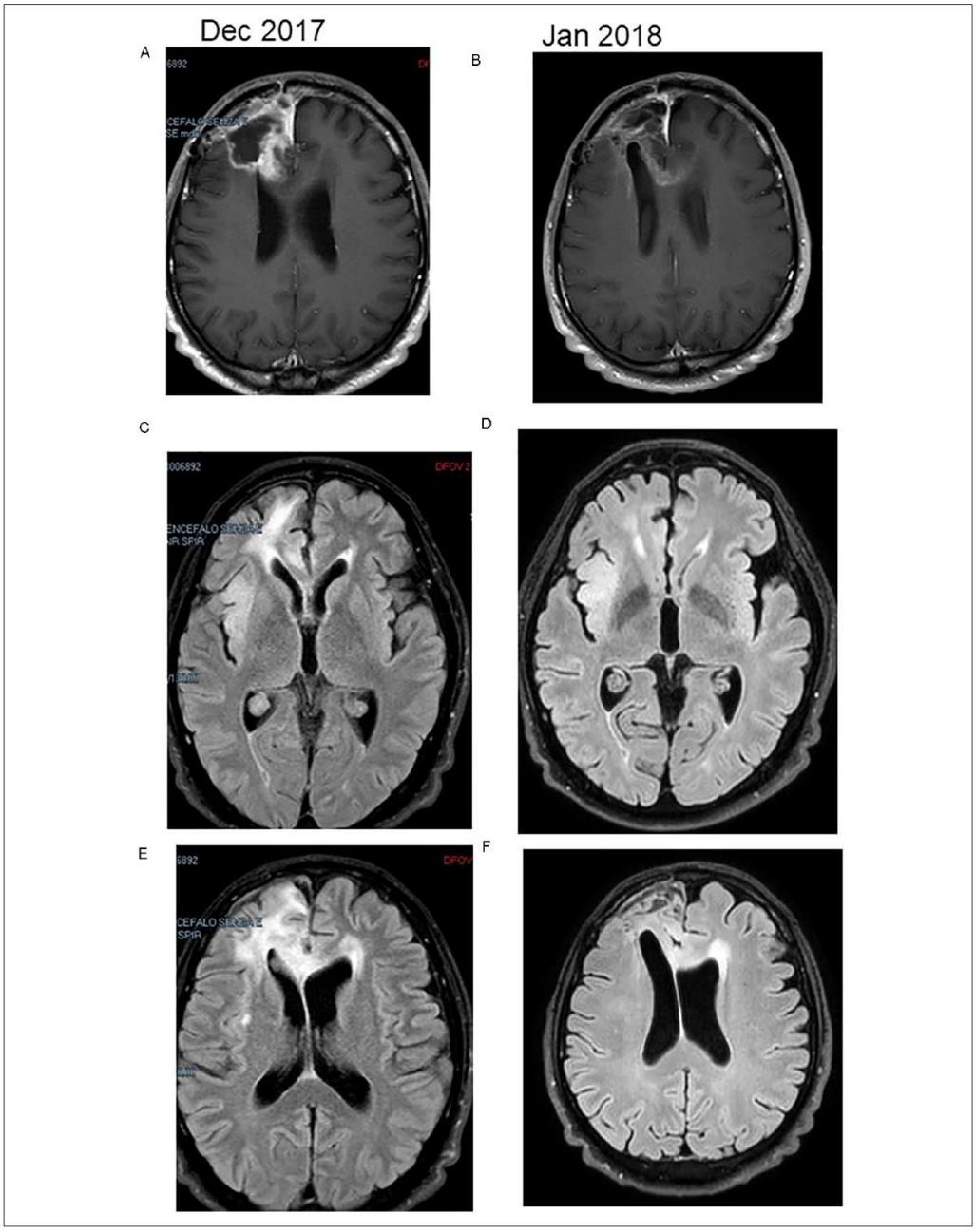

Figure 2. Magnetic resonance imaging of glioblastoma. Relapsed glioblastoma that received 20 hyperthermia sessions with partial regression of tumor and edema (B, D, F).

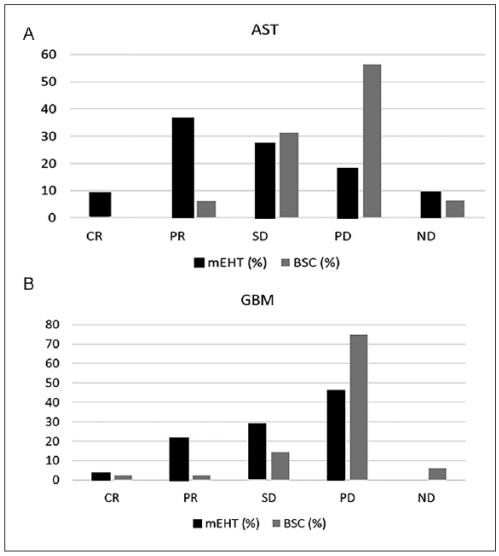

Figure 3. Response rates of (A) astrocytoma (AST) and (B) glioblastoma multiforme (GBM). mEHT, modulated electrohyperthermia; BSC, best supportive care; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ND, non detected

Safety

Safety of mEHT was also studied. Previously, it was hypothesized in some experts' opinions that mEHT could not safely heat tumor cells while keeping normal temperature in non cancerous tissues.

mEHT toxicity was mostly mild (grade 1). We observed in the entire mEHT group (28 GBM and 22 AST) 1 (2%) headache, 1 (2%) scalp burn, and 5 (10%) seizures. All patients who reported seizures had experienced this symptom from the beginning of the disease. Seizure occurred during mEHT treatment in 1 case. The others had a seizure more than 1 hour after the treatment. Three patients had absence seizures and 2 had tonic-clonic spasm for a few minutes (range 1-3). Seizures were resolved with medication, including diazepam 10 mg in 100 mL of saline and levetiracetam in tablets without any further episodes. The small total number of adverse events (5%) in this study supports the strong safety profile of mEHT.

Cardiac evaluation was performed for all patients with electrocardiography and echocardiography before and after the cycle of mEHT. No significant variations were observed.

Discussion

Relapsed glioma has a poor prognosis, and there are currently no standard treatments available. The main limitation of systemic drugs is blockage from reaching their target by the blood-brain barrier.[12] The absence of a standard treatment exposes the patients to physician choices, which range from surgery (not always indicated) to second-/third-line chemotherapies, re-irradiation, biodegradable carmustine wafers, gene therapy, and mEHT.[12,15-17,19,21,24-26]

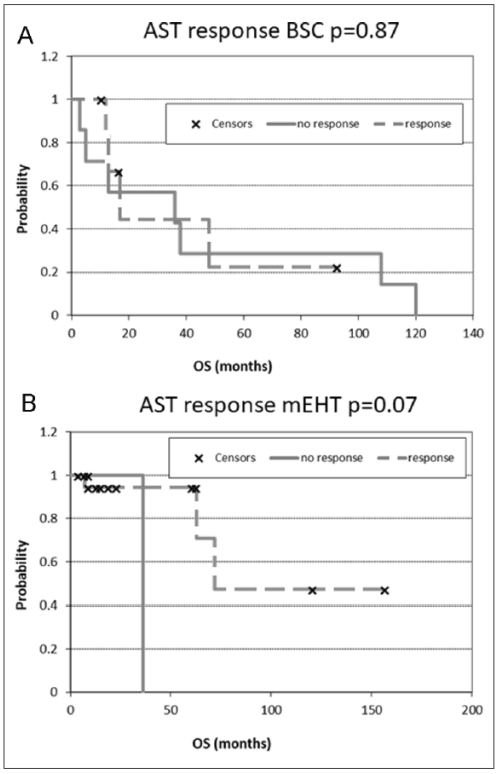

Figure 4. Kaplan-Meier plots for astrocytoma (AST). (A) after best supportive care (BSC) treatment and (B) after modulated electrohyperthermia (mEHT) treatment. OS, overall survival.

The method of mEHT is effective in relapsed gliomas because the electric field–induced heating damages the tumor cells[30,31] and increases apoptosis.[32,33] The basis of the hyperthermia effect is that tumor cells are more sensitive than healthy cells to heat with the synergy of an electric field. Conventional hyperthermia works by applying a uniform, homogeneous overall heating up to 42°C to 43°C to targeted areas, and it can be used in association with chemotherapy or RT.[5-7] The mEHT method heats selected malignant cells up to 42°C to 43°C, but the whole average temperature is lower than that obtained with the conventional method. This mild hyperthermia increases blood flow,[18-20,34,35] which is necessary to support complementary chemotherapy and RT.

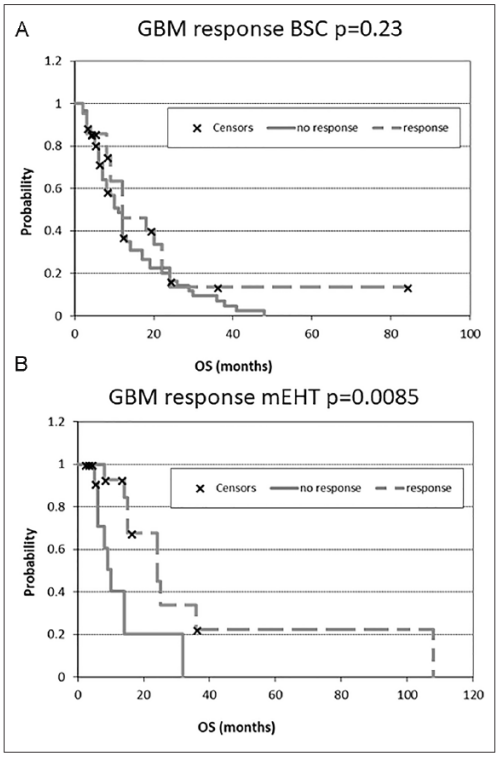

Figure 5. Kaplan-Meier plots for glioblastoma multiforme (GBM). (A) after best supportive care (BSC) treatment and (B) after modulated electrohyperthermia (mEHT) treatment. OS, overall survival.

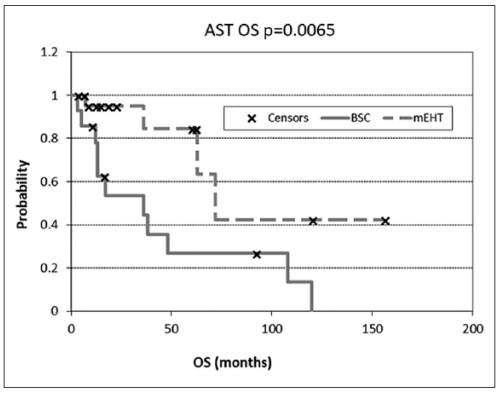

Figure 6. Overall survival (OS) of astrocytoma (AST) in best supportive care (BSC) and modulated electrohyperthermia (mEHT) groups.

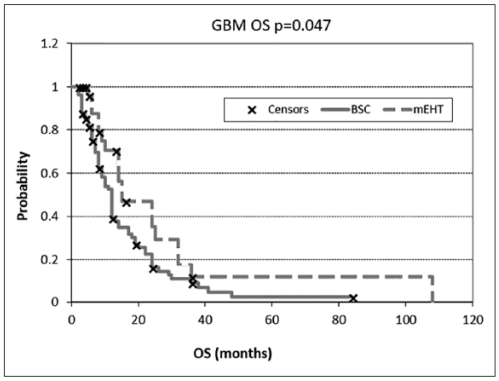

Figure 7. Overall survival (OS) of glioblastoma multiforme (GBM) in best supportive care (BSC) and modulated electrohyperthermia (mEHT) groups.

The main advantage of mEHT is the application of high temperature on selected cells, while keeping surrounding cells at moderate temperature. For this reason, mEHT is ideal for intracranial heating, where high temperatures of the brain could be life-threatening. The selective heating of mEHT is safer than the overall homogeneous heating of conventional hyperthermia and increases remission rate, OS, and progression-free survival.[16,17,24,25] This study shows that mEHT is safe and well tolerated, rarely resulting in mild pain, burns, or discomfort. The rare adverse events were observed for only 7 patients (14%) out of the whole mEHT group (28 GBM and 22 AST) and were temporary in duration. No signs of damage to healthy tissues during mEHT therapy were observed.

The overall positive response (CR + PR + SD) of AST after mEHT was 72% and is significantly higher than the 37% observed after BSC ($P < .005$), as is the objective response (CR + PR) (45% vs 6% $P > .005$). The overall PR of GBM after mEHT is 54% and is significantly higher than that of 18% obtained with BSC ($P = .001$) as is the objective response (25% vs 4%, $P = .016$). This may suggest a better effect of mEHT than BSC. Efficacy of mEHT in terms of tumor response and survival are comparable to those obtained in other studies and in our previously reported results of mEHT.[16,17,25] Some authors report a response rate 66% for GBM after mEHT; however, they observed only SD or PR and no CR.[16,17,24,26]

The median duration of relapse-free survival of the mEHT (AST + GBM) group was longer (16 months, range 6-120 months) compared with our previous study, which was 10 months.[16] This may be due to the larger number of patients enrolled. Tumor response was associated with an improvement in performance status in 6 (12%) patients of the mEHT group (AST + GBM).

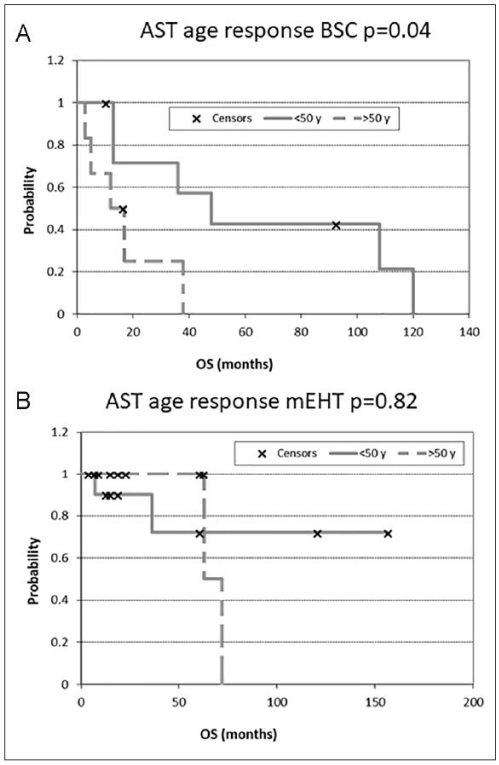

Figure 8. Overall survival (OS) according to astrocytoma (AST) patients' age: (A) after best supportive care (BSC) treatment and (B) after modulated electrohyperthermia (mEHT) treatment.

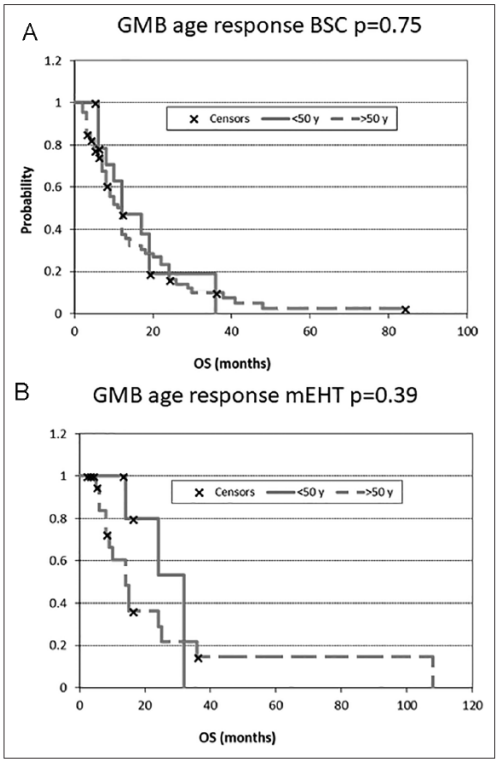

Figure 9. Overall survival (OS) according to glioblastoma multiforme (GBM) patients' age: (A) after best supportive care (BSC) treatment and (B) after modulated electrohyperthermia (mEHT) treatment.

The median OS for GBM is 14 months (range 2-108 months) after mEHT and 9 months (range 2-84 months) after BSC, whereas the OS of AST is 16 months (range 3-156 months) after mEHT and 16.5 months (range 3-120 months) after BSC. These results on the median OS are similar to that of 19.5 months reported in our previous study[25] and are higher than the 2.1 to 7.9 months reported by Silva et al[36] in their study on magnetic hyperthermia used as a palliative therapy in recurrent gliomas. OS was comparable to results reported by Sneed et al[22] (31% at the 2-year follow-up); however, that study used invasive processes, while mEHT treatment is a non invasive therapy. The difference in the 5-year OS of 83% after mEHT versus 25% after BSC in the AST group seems a relevant and important parameter.

The relationship of age to the results is interesting (Radiation Therapy Oncology Group [RTOG] classification[37]). In the case of BSC treatment for AST patients, age is a crucial parameter. BSC treatments are significantly better in survival for younger patients than for older ($P = .04$); while the mEHT treatment in AST patients is not affected by age as concerns survival ($P = .82$) (Figure 8). In the case of GBM patients, neither BSC nor mEHT treatments are influenced by the age of patients ($P = .75$ and $P = .39$, respectively) (Figure 9).

The main limitations of this study are the absence of randomization and the retrospective collection of data. The general sample of consecutive patients without arbitrary exclusions may be considered as control sample; however, there was no randomization as this was a retrospective observational study. Further multicenter randomized and prospective studies with a greater number of patients are needed to confirm these results.

Conclusions

Recurrent gliomas (GBM and AST) may benefit from mEHT, a safe and effective integrative therapy. Both GBM and AST patients showed higher response rates after mEHT than those who received best supportive care. Together with local control, the OS was also significantly improved by mEHT compared with the conventional BSC palliation in recurrent glioma patients. Only a few mild adverse events were reported. Quality of life is also improved, as assessed by subjective reports of the patients and a decrease in ECOG performance status scores. This study shows that mEHT is safe and well tolerated for the management of recurrent gliomas, rarely resulting in mild pain, burns, or discomfort. Further studies are required to confirm these results.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Oliver Szasz
Department of Biotechnics, St. Istvan University, Budaörs, Hungary.

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Oliver Szasz

Department of Biotechnics, St. Istvan University, Budaörs, Hungary
Email: biotech@gek.szie.hu

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Abstract

One of the most frequently applied bioelectromagnetic effects is the deep heating of the living species with EMF energy. Despite its long history, hyperthermia is a rarely applied oncotherapy. The reason is its controversial results and complicated control. One of the solutions is concentrating the electromagnetic energy nanoscopically on the parts of the malignant cells instead of heating up the complete tumor-mass. This approach is a kind of non-uniform energy absorption, providing energy liberation only in the selected regions. The energy-absorption of the malignant cells targets the membranes and creates a situation far from thermal equilibrium. The selection of the malignant cells is based on their decided differences from their healthy counterparts. The distinguishing parameters are the electromagnetic properties of the components of the malignant tissue which are the physiologic differences between the malignant cells and their healthy counterparts. The targets realize nano-range heating, using natural nanoclusters on the cell-membrane without artificially implementing them. This energy absorption generates consequent reactions, like programmed cell-death (apoptosis) continued by immunogenic cell-death involving extended immune reactions. The applied radiofrequency current is amplitude modulated by time-fractal modulation pattern. The accurately matched impedance realizes the self-selective mechanisms which are promoted by stochastic resonances. This complex method is a new kind of hyperthermia, named mEHT. Our objective is to analyze the problems of the selective, non-equilibrium energy absorption, and present a solution by the electromagnetic mechanisms for an effective and controllable hyperthermia in oncology.

Keywords

Electromagnetic Effects, RF-Current, Warburg-Effect, Szent-Gyorgyi-Effect,

Fractal-Physiology, Oncology, Hyperthermia, Nanoscale Heating, Modulated Electro-Hyperthermia, Nanothermia, mEHT, Apoptosis, Immunogenic Cell-Death

1. Introduction-Hyperthermia Challenges

Modern hyperthermia treatments in oncology use electromagnetic effects overheating the living object completely (whole body) or partly (regionally or locally). Oncological hyperthermia is intended to be an ideal combination therapy; it provides synergies with most of the conventional treatment modalities, boosts their efficacy and helps to desensitize the previously non-effective treatments.

The history of hyperthermia in oncology has been hectic and controversial [1]. In consequence, the general professional skepticism blocked its application for a long time. Hyperthermia is a constrain energy-delivery, forcing the homeostatic equilibrium to change. From this point the control of the processes became complicated. The adequate dose and protocol of the method and the reproducibility of the results are a complex task; its thermal status is far from equilibrium.

There are multiple physiologic feedbacks trying to re-establish the lost homeostasis. The main correction factors in the thermal control are the blood-flow and the surface regulation processes, like the sweating, orientation control of the hair in the skin, etc. The local heating immediately activates the physiological controls, first of all, the blood-flow, creating intensive heat-exchange conditions to construct a local out-of-equilibrium situation.

The locally or systemically increased blood-flow tries to compensate the growing temperature and cools down the target volume. The blood-flow drastically modifies the specific absorption rate (SAR), irrespective of how accurately it was focused on the tumor-mass. In consequence, the SAR and the temperature mapping of the targeted volume could be significantly different. Therefore, competition starts between the cell-killing potential of the heat and the cancer-supporting potential of the gained blood-supply by higher temperature of the targeted volume.

The focus of the RF-energy is not an easy task, but not impossible. However, the focus of the energy does not mean the focus of the temperature. The temperature is naturally spread by the convective and conductive heat-flow, derived from the temperature gradient and controlled by the physiologic constrains. Temperature-spread is a natural process, its termination is impossible. The time limit is only the heated volume. This is how the ablation techniques work. Also, the isotherms of the heating can be defined only with the help of the time-factor.

Following the temperature distribution is a safety issue, it is important to block the local burn of the healthy tissue, while allowing the temperature for necrosis in the tumor. The temperature control shows the temperature spreading

well, as well as the formation of the unwanted and uncontrolled hot-spots [2], [3].

Together with the problems of the temperature measurement in depth of the body to control the target, the technical solutions of the energy delivery are also challenging, due to the fact that the deep-heating needs complicated bioelectromagnetic considerations. The energy has to be delivered in depth without overheating the healthy layers between and has to be focused in depth on the heterogeneous and non-regular form of the tumor. The penetration depth of the biological material has frequency dispersion, quickly shortened by the growing frequency, and so contradicting with the demand of high frequency for precise beaming. The bolus-transmitters have to be constructed and non-absorbing but well transferring of the applied electromagnetic effects and the temperature of the body surface has to be limited under the blistering threshold.

To provide enough energy in depth, it needs power of >20 W/kg [4], which is far over the surface tolerance. Usually intensive cooling of the surface is applied to avoid the blisters on the skin. This method has also various physiological feedbacks. When the cooling is too large, the feedback isolates the skin from the deeper cooling, reducing the blood-flow in the subcutane layer under the cooled area. The extra impedance requests higher voltage to provide the same power in the depth as before, increasing the skin jump of the electric field vector perpendicular to the surface. The increased potential will increase the risk of burn.

Due to the challenges, many prospective clinical trials of conventional hyperthermia are questioned [5]. A recognized specialist of hyperthermia formulated a long time ago [6]: "The mistakes made by the hyperthermia community may serve as lessons, not to be repeated by investigators in other novel fields of cancer treatment."

The modulated electro-hyperthermia (mEHT, trademark: oncothermia) offers a new paradigm with nanoscopic heating having adequate answer to the present complex challenges.

2. Method-Selection Parameters in Nanoscopic Range

During conventional hyperthermia applications the macroscopic heating concentrates on the equal (homogeneous) temperature of the entire targeted volume or at least it constructs isothermal (isodose) patterns. The above described physiological feedbacks and the very inhomogeneous malignant target make this aim impossible. mEHT as new paradigm of hyperthermia technology declares the non-equal heating, it does not try to reach any macroscopic "isotherm" actionsas usual goal in ionizing radiation. The isotherms are consequences of the "bad reflex" of the equilibrium effects. Due to its heterogeneity, heating in biosystems are anyway far from equilibrium, when the physiological feedback as well as the normal thermodynamical rules are contracting of the macroscopic equilibrium. The method, mEHTdoes not heat the complete tissue in the targeted volume equally [7]. It concentrates the liberation of the absorbed energy

on the cellular membranes and on the extracellular electrolytes of malignant cells [8]. The microscopically inhomogeneous heating is far from the thermal equilibrium [9]. MEHT applies cellular approach selectively, heating up the malignant cells individually liberating the incident energy in nanoscopic range at the cell-membrane [10]. The energy-absorbers are the membrane rafts, which are clusters of transmembrane proteins, populating the membrane of malignant cells more densely than of the cells of the healthy counterparts. This method does not need any artificially implemented nanomaterials into the target, it uses natural nanoscopic clusters of membrane rafts [11]. Experimental verification of this method shows the significant inducement of apoptosis by this method on lower average temperatures than the isothermal heating does, [12].

The heating energy is not liberated in a sudden single step but is regulated in multiple small energy-absorption processes. This makes it possible to control the energy-liberation and to avoid the overheating the healthy parts. The accurate selection of malignant cells is a key step in the proper mEHT. There are robust electromagnetic differences between the malignant and healthy cells *in vivo*. The biological processes and structures of the healthy cells are distinguishably different from the malignant ones. These differences make it possible to accurately select the cancer-cells by their electromagnetic behaviors and actively destroy them without damages on their healthy neighborhood.

The main physiological differences between malignant cells and their healthy counterparts are:

1) Differences in the metabolic rate of the malignant and healthy cells (Warburg effect, [13]), which create higher conductivity of tumor than its neighborhood. The increase of the current density in the tumor could be visualized by the measurements of real processes by radiofrequency current density image (RF-CDI), [14] [15] [16] [17]. The Electric Impedance Tomography (EIT) measures and images the tumor based on the impedance differences [18]. This effect could be applied in prophylactics like mammography [19]. Further increase of the conduction selectivity is the positive feedback by growing temperature, [20]. The measured gain of selectivity is active [21], which means 14% increase by heating of 36˚C → 43˚C.

2) Differences in the dielectric constant of the extracellular electrolyte and membrane-bound water of the malignant and healthy cells (Szent-Gyorgyi effect, [22]); which allows the selection of malignant cells by their autonomy caused higher dielectric permittivity. The cell-cell adhesion is decreased by the increasing permittivity, [23], which harmonizes with the autonomic malignant cells (disorder in extracellular matrix [24]) and their decreased membrane potential, [25]. The order-disorder phase-transition indicates two different states of the cells: their autonomy status (called alpha-state) and their connected collective status (beta-state), [26].

3) The above two are combined with delta/beta dispersion (Schwan effect) [27]); to select the transmembrane protein clusters (rafts) of malignant cells. There are

various forms of frequency dependent energy-absorption mechanisms exist, [28], creating variation of the dielectric properties, [29]. The β-dispersion [30] (known as interfacial polarization effect) is between 0.1 - 100 MHz, it is characteristically determined by the membrane capacities of the cell and the intracellular organelles, bound-water to membrane etc. Responses in this interval are well-connected to the cell-membrane changes. The bound water to the membrane has the upper frequency part of the β-dispersion, denoted by δ, [31], and so this part is well-selective for the various cell-membrane states. Selective treatments have to be chosen in range of β-dispersion, [32]. Especially, the difference between the malignant and healthy tissue is that all the electrolyte and membrane properties differ, [33] [34]. The proper selection uses the dipole relaxation of beta-dispersion connected to the membrane bounded water, [35].

4) Structural differences (pathological pattern recognizing) between the malignant and healthy tissues (fractal physiology effect, [36]). The morphology is an important factor of the cellular organization, [37], and varies by kind of tissues. Cellular structures have coordination constrains preferring special coordination arrangements [38], and form self-organized collectivity [39] [40]. The tendency of proliferation is low in the population having small number of cells [41]. A critical cell density is necessary when starting significant cell division, similarly to the observed self-synchronization of chemical oscillators [42]. Dominantly, a favorable topological position (cyclic symmetry of the coordination number) chooses the actual division [43]; justified experimentally too, [44]. Healthy cells work collectively, their energy-consumption, as well as their life-cycles and the availability of resources are controlled in collective way by the various forms of the self-organizing, [45]. The organization process is governed by special "social" signals [46] commonly regulating and controlling the system. The self-organizing in topology is connected to a fractal-structure, which also appears in the dynamic self-similar stochastic behavior of the system, [47].

The special selection effects described above are accurately applied in mEHT, briefly summarized in **Figure 1**. However, the dose of energy is crucial for all the selection steps. Applying too much energy realizes the classical hyperthermia, it heats up all components of the target, the treatment loses its selection ability. The popular wisdom is valid: the difference between the medicine and poison is only the applied dose.

3. Results-Technical Realization of Nano-Heating

MEHT uses the above selection factors targeting the malignant cells with high efficacy, heating it to high temperature very locally (nanoscopic range) and stimulating the immune reactions against the malignant metastases. While the conventional hyperthermia focuses on and targets a macro region, the nano-heating of mEHT process, is similar to the ionizing radiation concept, which also acts nanoscopically on the DNA of the cells. Radiotherapy destroys the DNA strands, nanotherapy of mEHT destroys the cell-membrane of the malignant cell

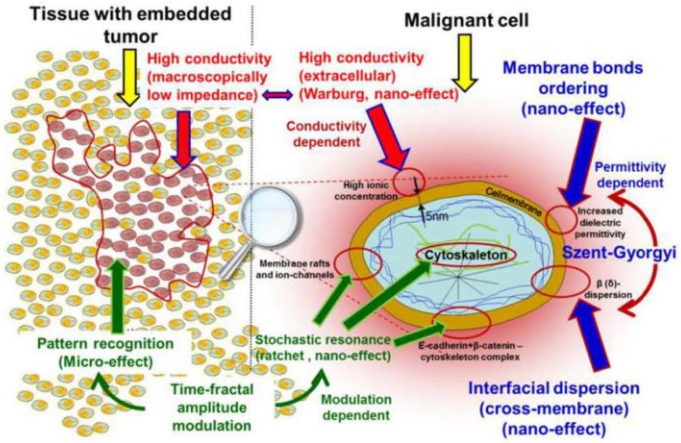

Figure 1. Main selection factors of modulated electrothermia (nanothermia, mEHT) method (to be clear, only those details are shown, which have role in the processes) {Adapted from [48]}.

or at least induces apoptotic cell death from there, due to the energy-absorption on the clusters of transmembrane proteins, as nanounits.

The electric effects are shown on a schematic impedance figure (Figure 2). The various actions are not independent, effects are overlapping and synergetic.

Heating of the ECM more intensively than the cytoplasm provides a spherical thermal gradient and consequently creates heat-flow through the membrane of all the selected individual cells. This centrally symmetric effect avoids the thermal limit of the external field application [49].

This nanoheating is far from thermal equilibrium. According to the Onsager's reciprocity relations [50], the induced heat-flow is coupled to charge current too, as well as the kinetics of the processes is also coupled, [51]. This current is ~150 pA/μm^2, [52]. This ionic current creates a zero-mode electric current, which in turn induces a zero-mode electric field in the cell membrane. Therefore, even small fields with zero-th mode components could elicit biological effects.

The temperature gradient through the cellular membrane pumps the non-equilibrium thermal processes. The gradient is quite large (~0.01˚C /nm [≈ 107˚C/m]), [46], creating a considerable heat-flow (~1.5 pW/μm^2). One of the actions is the change of the intracellular pressure (1320 kPa; [46]); by the electro-osmotic conditions. Due to the rigid cell membrane of the cancerous cells [53], the pressure could be fatal for the cell which has maximal tolerable lateral tensile stress of σmax ≈ (2 – 10)105 Pa [54]. The high pressure (when it is not enough to explode the cell) increases the membrane permeability, allowing the internal HSP chaperones to be expressed on the outer membrane to ignite immune reactions. This process is promoted by the temperature effect on the membrane permeability.

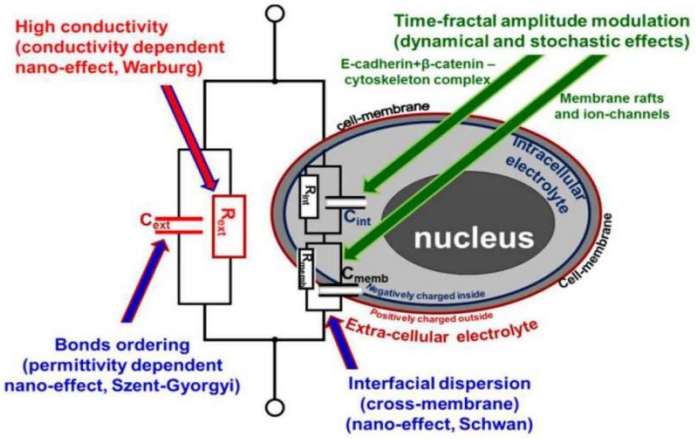

Figure 2. Action of the various selection factors in nanothermia shown in the impedance schematics of a malignant cell {Adapted from [48]}.

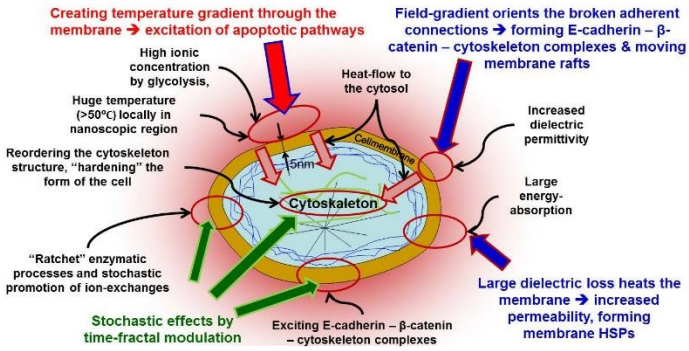

Figure 3. Summary of mEHT selection effects on malignant cell {Adapted from [48]}.

The RF-current anyway has special effects characteristically acting on the membrane of the cells. Its ohmic component directly affects the membrane, while the displacement current (imaginary component) deflects it, causing various mechanical effects on the outer membrane. The effect of the ohmic component is proportional with the square of the RF-current (Joule heat) while the capacitive component simply depends on the current itself. The summary of the effects is shown on **Figure 3**.

The synergy of electric field with the thermal effects selectively acts on the malignant cells and could be well followed from laboratory to the patient's bed, [55]. The certain differences between the mEHT and other hyperthermia with the same temperature are clearly shown by *in vitro* [56] and *in vivo* [57] measurements. The immune-simulative effect of the method is also proven [58]. The method has wide and successful clinical applications in various cancer types, like gliomas [59], colorectal cancers, [60] [61]; lung cancers [62] [63], in carcinoma

of uterine cervix [64], malignant ascites [65], sarcomas [66] [67], pancreas carcinoma [68] [69]; prostate cancer [70] [71].

4. Conclusion

Bioelectric explanation of heterogeneric heating of mEHT method is discussed. The method uses nanoheating technology to select and effectively heat the rafts of transmembrane proteins on the membrane of the malignant cells. The bioelectromagnetic selection focuses the electromagnetic absorption on the cell-membranes. The nano-range energy-liberation could be precisely controlled without considerable wasted energy and without having disadvantages because of the heating of the tumor-environment on average. MEHT results and its general benefits open a new kind of local heating to destroy the primary and metastatic tumor lesions.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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Quantitative Estimation of the Equivalent Radiation Dose Escalation using Radiofrequency Hyperthermia in Mouse Xenograft Models of Human Lung Cancer

Bibin Prasad [1,6], Subin Kim[2], WoongCho[2], Jung Kyung Kim [3], YoungA. Kim[4], Suzy Kim[2] & HongGyunWu[5]

[1] Department of Mechanical Engineering, Graduate School, Kookmin University, Seoul, 02707, Republic of Korea.
[2]Department of Radiation Oncology, SMG-Seoul National University Boramae Medical Center, Seoul, 07061, Republic of Korea.
[3]School of Mechanical Engineering and Department of Integrative Biomedical Science and Engineering, Graduate School, Kookmin University, Seoul, 02707, Republic of Korea.
[4] Department of Pathology, SMG-Seoul National University Boramae Medical Center, Seoul, 07061, Republic of Korea.
[5] Department of Radiation Oncology and Cancer Research Institute, Seoul National University College of Medicine, Seoul, 03080, Republic of Korea.
[6]Present address: Department of Radiation Oncology, SMG-Seoul National University Boramae Medical Center, Seoul, 07061, Republic of Korea.

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Bibin Prasad[1,6], Subin Kim[2], Woong Cho[2], Jung Kyung Kim[3], Young A. Kim[4], Suzy Kim[2] & Hong Gyun Wu[5]

Hyperthermia is a potent radiosensitizer, and its effect varies according to the different types of cancer cells. In the present study, the radiosensitizing effect of hyperthermia on lung cancer cell lines A549 and NCI-H1299 was determined based on the equivalent radiation dose escalation. *In vitro* cell experiments were conducted using lung cancer cell lines A549 and NCI-H1299 to determine thermal radiosensitivity. *In vivo* experiments were conducted using mouse heterotopic xenograft models to determine the treatment response and increase in the temperature of tumors using a 13.56 MHz radiofrequency (RF) hyperthermia device. Using the α and β values of the linear–quadratic equations of cell survival curves, numerical simulations were performed to calculate the equivalent radiation dose escalations. The dielectric properties of tumors were measured, and their effect on the calculated equivalent radiation dose was analyzed. Hyperthermia increased the equivalent radiation dose of lung cancer xenografts and a higher escalation was found in NCI-H1299 cells compared with that observed in A549 cells. An underestimation of the calculated equivalent radiation dose was observed when the dielectric property of the tumor was varied. This study may contribute to the effective planning of thermoradiotherapy in clinics.

Mild hyperthermia ranging from 41 °C to 45 °C, combined with radiation therapy (RT), has shown excellent clinical results[1,2]. Hyperthermia is normally used once or twice per week during RT. The combined effects of radiation and hyperthermia may be quantified in terms of equivalent dose, where the radiation dose distribution with hyperthermia is converted to an equivalent radiation dose[3–5]. Biological modeling is often applied to calculate the equivalent radiation dose distributions. The linear–quadratic (LQ) model, defining the number of lethal lesions as the sum of the lethal lesions produced from a single radiation track and those produced from two radiation tracks, is widely used for this purpose[6–8].

Radiosensitization caused by hyperthermia may be modeled as a temperature-dependent variation of the radiosensitivity parameters α and β in the LQ model and the equivalent radiation dose model combining the effects of hyperthermia. α and β vary according to the different types of cancer cells and radiation[9–11]. Thermoradiotherapy planning using the LQ model incorporates the DNA repair inhibition mechanism because it is the dominant form of radiosensitization using hyperthermia[5,12]. Other mechanisms, such as direct cell killing and reoxygenation,

[1]Department of Mechanical Engineering, Graduate School, Kookmin University, Seoul, 02707, Republic of Korea. [2]Department of Radiation Oncology, SMG-Seoul National University Boramae Medical Center, Seoul, 07061, Republic of Korea. [3]School of Mechanical Engineering and Department of Integrative Biomedical Science and Engineering, Graduate School, Kookmin University, Seoul, 02707, Republic of Korea. [4]Department of Pathology, SMG-Seoul National University Boramae Medical Center, Seoul, 07061, Republic of Korea. [5]Department of Radiation Oncology and Cancer Research Institute, Seoul National University College of Medicine, Seoul, 03080, Republic of Korea. [6]Present address: Department of Radiation Oncology, SMG-Seoul National University Boramae Medical Center, Seoul, 07061, Republic of Korea. Correspondence and requests for materials should be addressed to S.K. (email: suzy101@snu.ac.kr)

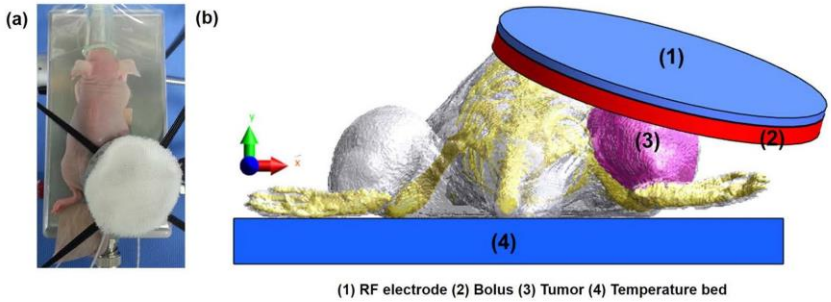

Figure 1. Mouse xenograft model used in this study: (**a**) Experimental mouse model for the measurement of temperature; (**b**) Computational mouse used for numerical simulation.

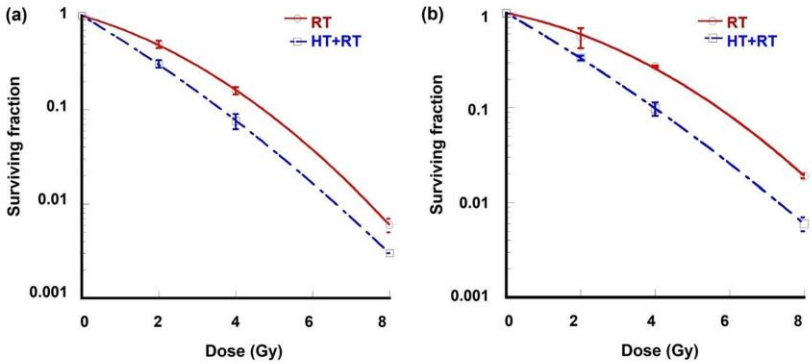

Figure 2. (**a**,**b**) Clonogenic cell survival curves of (**a**) A549 and (**b**) NCI-H1299 cells. Surviving fraction of cells treated with hyperthermia (42 °C for 30 min) prior to radiation were compared with those of radiation alone. *Points*: mean for three independent experiments, *bars*: standard errors.

should also be considered in more advanced and sophisticated thermoradiotherapy planning to achieve improved treatment outcomes[12].

Linear and exponential mathematical models determining the thermal sensitivity of cancer cell lines have been reported previously[3], and an escalation of the equivalent radiation dose was determined for patients with cervical and prostate cancer receiving simultaneous therapy with radiation and hyperthermia[3,5]. Equivalent radiation dose escalations of >10 and 7–11 Gy were reported previously in prostate[3] and cervical cancer[5]. These studies showed a useful method to compare the effectiveness of hyperthermia in RT and design/guide the dose escalation using hyperthermia in clinical studies.

The objective of the present study was to determine the equivalent radiation dose escalation in lung cancer cell lines A549 and NCI-H1299. *In vitro* experiments were performed to determine the thermal radiosensitivity parameters and propose a modified linear model of temperature dependency of α in the treatment of lung cancer. *In vivo* experiments and numerical simulations were performed using a mouse xenograft model (Fig. 1(a,b)) to evaluate the effect of varying dielectric properties of tumors and confirm the equivalent radiation dose escalation.

Results

Figure 2(a,b) Depicts the logarithmic fractional cell survival of A549 and NCI-H1299 cells treated with RT alone and combined with hyperthermia therapy (HT). The addition of HT to RT increased the fraction of cell death and enhanced the radiosensitivity of NCI-H1299 cells and A549 cells. The addition of HT treatment at 42 °C to RT (2–8 Gy) enhanced the linear parameter (α) to 0.53 from 0.23 Gy^{-1} for A549 and to 0.51 from 0.24 Gy^{-1} for NCI-H1299, respectively, which elucidates the radiosensitizing effect of HT on lung cancer cell lines. The average values of α and β of the cells treated with RT alone or with combined HT and RT are detailed in Table 1.

The temperature dependency of α in the lung cancer cell lines A549 and NCI-H1299 is illustrated in Fig. 3(a) based on the linear model calculation (equation 6) and input conditions in Table 2. It was observed that, in response to an increase in temperature from 37 °C to 42 °C, α tended to increase linearly from 0.2 to 0.5 Gy^{-1} for both A549 cells and NCI-H1299 cells. The increased α enhanced the radiation dose fraction from 2 to 3.08 Gy

Table 2. Description of GBM Patients Group.

Parameters (GBM)	n	%
Males	68	61.3
Females	43	38.7
mEHT treated	28	25.2
MGMT methylated	25	22.5
MGMT nonmethylated	27	24.3
MGMT ND	59	53.2
IDH1 mutated	13	11.7
IDH1 wild type	19	17.1
IDH1 ND	79	71.2
Events	86	77.5
Age, years, range	27-86	—
Survival, months, range	2-108	—

Abbreviations: GBM, glioblastoma multiforme; mEHT, modulated electrohyperthermia; MGMT, O6-methylguanine-DNA methyltransferase; IDH1, isocitrate dehydrogenase 1; ND, non detected.

therapeutic temperature used in the mEHT treatment was estimated to range from 40°C to 42.5°C as previously reported.[16,17,24-27] The mEHT technique operates with amplitude-modulated short-wave radiofrequency at a carrier frequency of 13.56 MHz. The technique combines impedance coupling with the technique of capacitive coupling. The device has 2 electric capacitive plates (electrodes): one under the patient in the treatment bed, and one movable electrode, which is positioned over the patient's target anatomical area to be treated. The electrodes have a water bolus to make the best transfer of energy, to distribute the electric current inside the tissue following the actual form of the patient's body, and to keep the skin temperature safely cooled by cooling the surface with the bolus. The preset skin temperature was 26°C and it did not exceed 39°C at the point of skin contact during the treatment. The electrode was 20 cm in diameter and round in shape.

Preprocedural medication administered to all patients before each mEHT to avoid brain edema was 250 mL of glycerol 18% and dexamethasone 12 mg. The selected area was treated 3 times per week for 8 weeks. At 3 months, the tumor response was assessed on computed tomography (CT) or magnetic resonance imaging (MRI) scans and if the response was positive (complete response [CR], partial response [PR], or stable disease [SD]) then the 8-week course of treatment was repeated. The treatment time and the applied power were increased in each session according to the patient's tolerance to heat. The first treatment was always performed applying 40 W for 20 minutes (48 kJ energy). Time was gradually raised from 20 to 60 minutes and the power was increased according to a step-up power protocol from 40 up to 150 W (540 kJ) over 2 weeks.[25]

This protocol achieved an estimated average temperature inside the tumor volume of >40°C for more than 90% of the mEHT sessions, which was indicated by the device and controlled by the phantom measurement. The median number of mEHT treatments/patient were 22 (range 11-62). Summary of the technical mEHT parameters of the treatments is shown in Table 3.

Table 3. Treatment Parameters.

Practical Parameters	Value
Step-up power, W (from-to)	40-150
Average energy dose, kJ	540
Therapeutic temperature, °C	40-42.5
Treatment time/session, minutes	60
Treatment frequency (weekly)	3
Treatment cycle, weeks	8
Follow-up time, months	16

Outcome Measures

MRI or CT scans were performed every 3 months to study the efficacy of the treatments. Tumor response was assessed with the response evaluation criteria in solid tumors (RECIST, v.1.1). The ECOG performance status scale was used to evaluate the functional recovery. Overall survival (OS) was computed from the date of initial diagnosis to last follow-up or death of the patient. Adverse events were recorded indicating intensity and duration, using the Common Terminology Criteria for Adverse Events v3.0 (CTCAE). Data on O6-methylguanine-DNA-methyltransferase (MGMT) methylation[28] and isocitrate dehydrogenase 1 (IDH1) mutational status[29] were collected when available (Table 3). The median total follow-up time was 16 months (range 1-156 months).

Statistical Analysis

We used descriptive and Kaplan-Meier nonparametric statistical estimates. The statistical significance was measured by Student's t test, z test for proportions, and log-rank test for Kaplan-Meier curves; $P \leq .05$ indicated statistically significant difference between samples.

Results

Sample Characteristics

Median age was 60 years for GBM (range 33-86 years) and 50 years (25-71 years) for AST group. Both subgroups (GBM and AST) followed the normal distribution and the gender ratio had no significant differences, so the statistical evaluation had an optimal basis. Data for MGMT methylation and IDH1 mutational status were not available for many patients. A summary of the demographic data is shown in Tables 1 and 2.

Table 4. Tumor Response and Survival of AST Group.

Results (AST)	mEHT (n)	mEHT (%)	BSC (n)	BSC (%)
CR	2	8	0	0
PR	8	33	1	7
SD	6	25	5	36
PD	4	17	9	64
ND	2	8	1	7
OS, months median (range)	16 (3-156)	—	16.5 (3-120)	—

Abbreviations: AST, astrocytoma; mEHT, modulated electrohyperthermia; BSC, best supportive care; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ND, non detected; OS, overall survival.

Table 5. Tumor Response and Survival of GBM Group.

Results (GBM)	mEHT (n)	mEHT (%)	BSC (n)	BSC (%)
CR	1	4	2	2
PR	6	21	2	2
SD	8	29	12	14
PD	13	46	62	75
ND	0	0	5	6
OS, months, median (range)	14 (2-108)	—	9 (2-84)	—

Abbreviations: GBM, glioblastoma multiforme; mEHT, modulated electrohyperthermia; BSC, best supportive care; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ND, non detected; OS, overall survival.

Tumor Response

At the 3-month follow-up period, the AST patients of mEHT group showed 2 (9%) CR, 8 (36%) PR, and 6 (27%) SD. SD was also considered a positive response due to the relapsed nature of the disease in this study. For this reason, the overall positive response of AST (CR + PR + SD) was 72% after mEHT (Table 4, Figure 3A) and is significantly higher than the 37% observed after BSC (PR = 6% and SD = 31%) ($P < .005$). Also, the objective response (CR + PR) (45% vs 6%, $P > .005$) was significantly higher than that of the BSC group. Progressive disease (PD) was observed in 4 (18%) patients in mEHT and in 9 (56%) of BSC. At the 3-month follow-up period, the GBM showed 1 (4%) CR, 6 (21%) PR, and SD 8 (29%), with an overall positive response of 54% in the mEHT group, and 13 PD (46%) (Table 5, Figure 3B). The GBM treated with BSC had 2 (2%) CR, 2 (2%) PR, and 12 (14%) SD; whereas PD was observed in 62 (75%). Overall positive response of GBM treated with BCS was 19% significantly lower ($P < .05$) than that of mEHT group.

Two characteristic cases of AST and 1 case of GBM treated with mEHT are shown in Figures 1 and 2, respectively. Figure 1A and B shows relapsed AST (grade III) cases in a 24-year-old man and Figure 1C-F shows relapsed AST (grade III) in a 32-year-old man. The tumors in both cases were well controlled locally. Figure 2 shows the MRI of a patient (male, 54 years old) with relapsed GBM (grade IV).

Survival

The response rates of AST and GBM are shown in Figure 3A and B, respectively. The Kaplan-Meier estimates of the treatment survivals are shown for AST and GBM in Figures 4 and 5, respectively. The median OS of AST was 16.5 months (range 3-120 months) and 16 months (range 3-156 months) in the BSC and mEHT groups, respectively ($P = .0065$) (Figure 6). Figure 6 shows the advantage of mEHT over BSC in terms of survival for AST. Survival rate of AST at the first and second year in the mEHT group was 77.3% and 40.9%, respectively. The 5-year OS of AST was 83% after mEHT versus 25% after BSC.

The median OS for GBM was 14 months (range 2-108 months) after mEHT and 9 months (range 2-84 months) after BSC (Figure 7) ($P = .047$).

We observed 4 long-term survivors in the AST and 2 in the GBM group. Two of the long survivors in the AST and 1 in the GBM group were treated by mEHT, with an OS of 156, 62, and 108 months, respectively. The long survivor of BSC group had a survival of 84 months.

Most patients reported a better quality of life (evaluated by subjective responses as reported during follow-up visits) after mEHT. Six patients had an objective clinical response and therapeutic benefit by mEHT measured by decreased ECOG values from 3 to 1 in 2 (9%) AST and 2 (7%) GBM patients and from 3 to 0 in 2 (9%) AST patients.

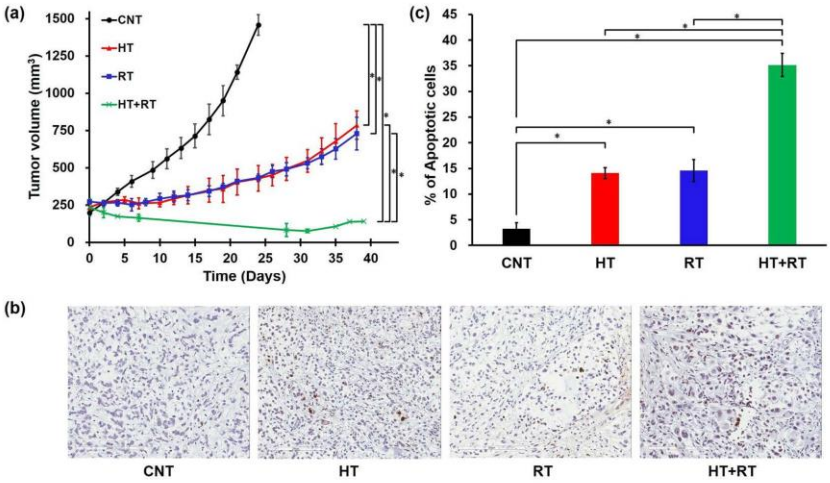

Figure 5. (**a**) Tumor growth curves of control (CNT), hyperthermia alone (HT), irradiation alone (RT), and hyperthermia followed by irradiation (HT + RT) groups (n = 3, *p < 0.05). Tumors of HT alone group were heated at 42 °C for 30 min at 2-day interval. Radiation dose was 5 Gy per fraction daily 2 fractions at 2-day interval. Tumors of HT + RT group were heated for 30 minutes at 42 °C prior to irradiation. Points: mean volumes of three tumors, bars: standard errors. (**b**) Apoptotic activity of each treatment group analyzed by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay. TUNEL-positive nuclei were counted in 3~4 fields randomly selected per tumor sections (original magnification, ×200; scale bar, 200 μm). (**c**) Comparison of apoptotic rate of each treatment group (error bars, standard errors; *p < 0.05). Image analysis was performed using QuPath software.

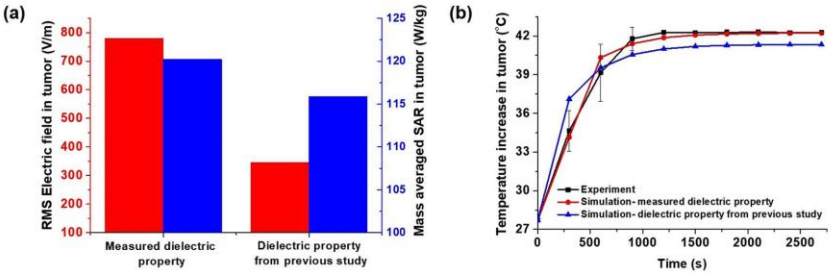

Figure 6. Comparison of electric field, energy absorption and temperature distribution: (**a**) Root mean square (RMS) electric field and the mass averaged specific absorption rate and (**b**) Temperature distribution obtained from the experiment and simulation of the mouse tumor model.

radiation dose escalation calculated for the NCI-H1299 xenograft was greater than that calculated for A549 tumors, indicating that radiation dose escalation during the addition of hyperthermia to the regimen may vary according to the different types of cancer.

The effect of combination treatment was verified with mouse xenograft model of A549 cells. HT before RT significantly enhanced the radiation-induced tumor growth delay. In a previous study with mouse fibrosarcoma, HT enhanced radiation-induced tumor growth delay. The radiosensitizing effect was more prominent when HT was applied before RT rather than after RT, and three times of repeated HT was more effective than single heating in suppressing tumor growth[13]. In our study, HT at 42 °C for 30 minutes was applied before each 5 Gy of RT and the combination treatment was more effective in suppressing tumor growth than HT or RT alone. HT was also found to enhance the apoptotic cell death of human lung cancer xenograft. Additive effect of HT and RT on the induction of apoptosis was shown by TUNEL assay (Fig. 5(c)). As for A549 tumors, similar effect may be obtained for NCI-H1299 xenografts. HT was reported to induce apoptosis of human hepatocellular carcinoma cells by

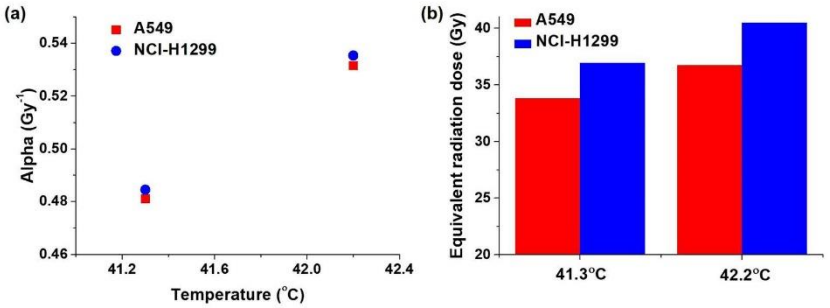

Figure 7. Comparison of the radiation dose escalation with temperature obtained from the dielectric properties measured in the present study and those obtained from a previous study: (**a**) effect on the radiosensitivity parameter α; (**b**) effect on the equivalent radiation dose.

up-regulating tumor suppressor SEPT4[14]. Increased cell death by combined HT + RT could be explained in part by increased apoptosis by HT.

In combined HT and RT treatment in clinics, addition of HT will change the homogeneity of tumor temperature from normothermic to hyperthermic range. This higher temperature distribution at mild hyperthermia range enhances the blood perfusion and thereby increases the oxygenation in tumor vessels which improves the efficacy of RT treatment[15]. Even though there is nonhomogeneous temperature distribution, previous studies reported that the T10, T50, and T90 (temperature achieved in 10, 50 and 90% of target tumor volume) were in the range 40–42 °C[3,5]. Therefore, most part of tumor could be in the hyperthermic range that has radiosensitizing effect, and these effects can be properly addressed with thermoradiotherapy planning in clinics.

In vivo measurement of temperature, along with electromagnetic and thermal simulations using mouse xenograft models, was performed to investigate the effect of dielectric property variation in temperature prediction and its corresponding equivalent radiation dose escalation. Studies reported that the dielectric properties of tumors vary according to the type and stage of cancer[16,17], and this variation may predict inaccurate SAR and distribution of the temperature[18]. For the combination of RT with HT, the equivalent radiation dose may vary according to the variations in the dielectric properties of tumors. The dielectric properties of tumors were measured for present study, and the values reported previously at 13.56 MHz were used[19] for comparison. Numerical simulations were performed to determine the SAR and temperature distribution using a mouse tumor model, comparing the dielectric properties of tumors measured in the present study with those obtained from a previous study. This comparison showed that the distribution was different[18]. When the calculated equivalent radiation dose was compared with the temperature achieved from two dielectric properties of the tumor and experimental measurement, the radiation dose escalation was underestimated from the predicted value.

Variations in the dielectric properties of tumors may either underestimate or overestimate the equivalent radiation dose escalation. Studies reported the dielectric property variation in liver and colorectal cancer on human tumors[16,17]. Patients with different type of liver tumor showed different dielectric property values with one patient having very large variation in permittivity and conductivity values[16]. A tumor property measurement on colorectal cancer considering different stages of cancer showed significant variations in the permittivity and conductivity values[17]. Hence, these variations should be considered carefully in the planning of clinical treatment. To improve the clinical treatment planning standards, noninvasive patient-specific dielectric property measurement techniques can be used, such as incorporation of magnetic resonance electrical properties tomography (MREPT)[20] and dictionary-based electric properties tomography (dbEPT)[21].

In the validation study, the α and β values for prostate cancer obtained from a previous study were used[3]. Three temperature points (minimum, mean, and maximum) obtained in the tumor tissues and their corresponding equivalent radiation doses were considered in this study. The results were in agreement with the MATLAB code produced for the present study. The linear model for temperature dependency in the validation study was determined using the equation provided in a previous study, and the equivalent radiation dose escalation was calculated by multiplying the equivalent fractional dose by the number of fractions[3]. For the lung cancer cell lines used in the present study, the linear equation was modified based on the cell experiment, and the calculations of the equivalent radiation dose were performed using the Lea–Catcheside protraction factor[4].

An accurate identification of radiosensitivity parameters and energy-source-dependent tissue properties is necessary for thermoradiotherapy treatment planning. In the present study, the radiofrequency (RF) energy source was used; thus, the tumor-specific dielectric properties were important. If thermoradiotherapy is performed using other energy sources such as laser or ultrasound, the absorption coefficient and the acoustic properties of the tumor tissues should be considered carefully for the accurate estimation of radiation dose escalation.

The present study uses mathematical models based on previous studies[3,4] for the calculation of the equivalent radiation dose. Further modifications may be required for determining the radiation dose escalation with high precision for lung cancer. However, the trend in the radiation dose escalation may be similar to that reported in the present and previous studies[3,5]. A modified linear model is proposed to determine the thermal sensitivity of lung cancer cell lines A549 and NCI-H1299. However, further studies are warranted to derive a general

formulation applicable to the treatment planning for lung cancer using the combination of RT with HT. In addition, studies considering the exponential increment of α, thermal dose threshold temperatures, time dependency, and the effect of perfusion or ventilation on tumor temperature are warranted to develop lung-cancer-specific treatment planning strategies in clinical practice.

Conclusion

The combined effects of RT and HT were quantified in terms of the equivalent radiation dose. The thermal enhancement of the radiosensitivity parameters of lung cancer cell lines A549 and NCI-H1299 was determined. Moreover, the estimated equivalent radiation doses were escalated using hyperthermia in both cell lines. The effect of enhanced equivalent radiation dose was confirmed with mouse xenograft models. The effect of the variation in the dielectric properties of tumors was also determined. The results showed that inaccurate estimations of the dielectric properties of tumors may lead to underestimation of the equivalent radiation doses. Therefore, the variation of the dielectric properties of tumors should be considered carefully in the planning of RF-induced thermoradiotherapy.

Materials and Methods

***In vitro* cell culture and treatment.** The human lung carcinoma cell lines A549 and NCI-H1299 were purchased from the Korean Cell Line Bank (Seoul, Korea). The cells were cultured in RPMI 1640 (HyClone, South Logan, UT, USA) supplemented with 10% inactivated fetal bovine serum (HyClone) at 37 °C in an atmosphere of 5% CO_2.

The LAB-EHY100 (OncoTherm, Budapest, Hungary) device was used for the hyperthermia treatment. The cells were placed in the heating chamber (LAB-EHY *in vitro* applicator) with culture medium at 42 °C for 30 min. Irradiation of cells was performed using a ^{137}Cs gamma-irradiator (MK 1-68, JL Shepherd, San Fernando, CA, USA) at a rate of 2.75 Gy/min.

The A549 and NCI-H1299 cells were plated into 60-mm dishes and exposed to 0, 2, 4, and 8 Gy radiation. For the combination treatment, cells were treated using the LAB-EHY100 at 42 °C for 30 min prior to irradiation. After 14 days of incubation, the colonies were stained with crystal violet, and those with more than 50 cells were counted. The plating efficiency of the control group and the surviving fraction of each treatment group were calculated. All experiments were performed in triplicate. The cell survival curve was fitted to the LQ model for the calculation of radiobiological parameters.

***In vivo* mouse experiment.** BALB/c nude mice were obtained from Koatech (Gyeonggi-do, Korea). The A549 and NCI-H1299 cells (5×10^6/100 μL) were subcutaneously injected into the right hind leg of the nude mice. After 1–2 months, the tumors were detected in the mice. The tumor volumes were calculated using the formula $V = (\pi \times L \times W \times H)/6$, with L, W, and H representing the length, width and height of tumor in mm, respectively.

Hyperthermia treatment (HT) in the mouse xenograft model was performed using the 13.56 MHz radiofrequency (RF) capacitive heating device LAB-EHY 100 as shown in Fig. 1(a) to determine the increase in temperature (41–42 °C) in the tumors[13,18]. Fiberoptic sensors were inserted into the tumor to determine the increase in temperature during RF heating. The tumor was heated to 42 °C and maintained at this temperature for 30 min.

The tumor growth delay was compared among HT or irradiation (RT) alone group and irradiation following hyperthermia (HT + RT) group using A549 xenografts. Mice of HT alone group received 2 times of HT at 2-day interval as described above. For irradiation, mice were immobilized in jigs with prone position and the tumors on the right hind legs were irradiated with a linear accelerator (Varian 21EX®, Varian Medical Systems, Palo Alto, CA, USA). 5 Gy per daily dose of radiation was delivered twice at 2-day interval. For mice in the HT + RT group, hyperthermia was followed by 5 Gy of radiation within 4 hours and the combination treatment was repeated at 2-day interval. Each group consisted of 3 mice.

Apoptotic activity was analyzed on the basis of TUNEL assay. Tumors from each group of three tumor-bearing mice, apart from the mice used for tumor growth study, were excised 3~5 days after treatment. For histologic detection, tumor samples were fixed in 10% neutral formalin and embedded in paraffin. The histological sections were subjected to TUNEL staining using an *in situ* ApopTag® kit (Millipore, Temecula, CA, USA) for apoptosis detection. The percentage of TUNEL-positive cells were calculated at 3~4 random area per sample. Image of tissue section was analyzed using QuPath, an open-source software for quantitative pathology[22].

The experimental protocol used in this study was approved by the Institutional Animal Care and Use Committee (17-0110-S1A1, Seoul National University Hospital) and performed in compliance with the committee guidelines and regulations. A detailed description of the experimental procedures performed in this study has been provided in our previous research[18].

Dielectric property measurement of tumor. The dielectric properties of tumors were measured using an impedance analyzer (E4991A; Agilent Technologies, Santa Clara, CA, USA), and the details of the measurement and analysis of the dielectric properties have been described in our previous research[18].

Electromagnetic and thermal simulations. Electromagnetic and thermal simulations were performed using the multiphysics simulation platform Sim4Life (Zurich Med Tech, Zurich, Switzerland)[23–25]. A three-dimensional mouse model, reconstructed from computed tomography images, was used for the simulations as shown in Fig. 1(b)[18,26]. Dielectric and thermal properties of tumor and other body sites are listed in Table 3[18,27]. The governing equations used for the electromagnetic simulations based on quasi-static approximation are provided below[23,28–30]:

Material	Density (kg/m³)	Electric Conductivity (S/m)	Relative Permittivity	Thermal Conductivity (W/m·K)	Specific Heat (J/kg·K)	Perfusion (mL/min/kg)	Heat Generation (W/kg)
Skin	1109	0.238	285.24	0.3721	3390.5	106.38	1.64
Rectum	1088	0.512	217.11	0.542	3657.5	786.23	11.85
Bone	1908	0.045	30.57	0.32	1312.8	10	0.15
Mouse Tumor (previous study)	1070	0.683	266.99	0.4949	3421.2	18.36	0.9
Mouse Tumor (measured)	1070	0.7847	278.85	0.4949	3421.2	18.36	0.9
Distilled Water	1000	0.00005	76.7	0.563	4181.3	—	—

Table 3. Dielectric (13.56 MHz) and thermal properties of tissues used in the simulations.

$$\nabla(-\varepsilon\nabla\phi) = 0, \quad E = -\nabla\phi \tag{1}$$

$$SAR = \frac{\sigma}{2\rho}|E|^2 \tag{2}$$

$$Q_r = \rho SAR = \frac{\sigma}{2}|E|^2 \tag{3}$$

In the equation above, ε is the permittivity, φ is the electric potential, E is the electric field strength, SAR is the specific absorption rate, σ is the electrical conductivity, ρ is the mass density, and Q_r is the heat source.

For the thermal simulation, Pennes' bio-heat transfer model was used[31]. The energy obtained from the electromagnetic simulation is provided as a user-defined heat source.

$$\rho c\frac{\partial T}{\partial t} = \nabla\cdot(k\nabla T) - \rho_b c_b \omega_b (T - T_b) + Q_r + Q_m \tag{4}$$

In the equation above, c is the specific heat, T is the temperature, t is the time, k is the thermal conductivity, Q_m is the metabolic heat-generation rate, ω_b is the perfusion rate, and ρ_b, c_b, and T_b correspond to the density, specific heat, and temperature of blood, respectively. The thermal properties, perfusion, and generation of metabolic heat used for the simulation are listed in Table 3[3,27]. A convective boundary with a surface heat transfer coefficient of 5 W/m²K and an ambient temperature of 25 °C were used as the boundary conditions. An initial temperature of 27.7 °C was applied to all body sites, and the duration of the simulation was 45 min. Grid independent studies were conducted and an optimum mesh of 1.98 M cells are used for the simulation.

Calculation of the equivalent radiation dose. A MATLAB code was used for the calculation of the equivalent radiation dose based on the linear–quadratic (LQ) model[32]. The LQ model may be expressed as[3]

$$SF(n, d, \alpha, \beta, T) = e^{-n(\alpha(T)d+\beta d^2)} \tag{5}$$

In the equation above, SF is the cell surviving fraction, d is the fractionated dose, n is the number of fractions, α and β are the radiosensitivity parameters, and T is the temperature.

Hyperthermia may alter α and β. In general, changes are more pronounced in α than in β because α is thought to represent the subset of DNA repair mechanisms. Hence, only an enhancement in α was considered in the present study. Temperature dependencies of α may be calculated using a linear model[3]. Considering the baseline temperature in this experiment and the thermal sensitivity of the lung cancer cells obtained from the experiments, the linear model was modified to provide an accurate estimation.

$$\alpha(T) = \alpha_{37} + \frac{2.17\alpha_{37} - \alpha_{37}}{42 - 37}(T - 37) \tag{6}$$

The equivalent fraction dose with hyperthermia (d_{HT}), independent of the number of fractions, may be expressed as[3]

$$d_{HT} = \frac{-\alpha_{37} + \sqrt{{\alpha_{37}}^2 + 4\beta_{37}d(\alpha(T) + d\beta_{37})}}{2\beta_{37}} \tag{7}$$

The equivalent radiation dose in hyperthermia (EQD_{RT}) for external beam radiotherapy considering the Lea–Catcheside protraction factor may be calculated using the following equation[4]:

$$EQD_{RT} = \frac{\alpha(T)D + G\beta_{37}D^2}{\alpha_{37} + \beta_{37}d} \tag{8}$$

In the equation above, D is the total radiation dose, and G is the Lea–Catcheside protraction factor. The input parameters used for the calculation of the equivalent radiation dose escalation are listed in Table 2.

Statistical analysis. *In vitro* or *in vivo* experimental data were expressed as mean ± standard error of mean. The statistical differences were assessed with Student's t-test. A threshold of $p < 0.05$ was defined as statistically significant.

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Author Contributions

S.K., J.K.K. and H.G.W. conceived and designed the study and oversight the experimental data analysis. S.B.K. performed the cell and mice experiments. B.P. performed the mice experiments and thermal simulations. B.P., S.K., W.C. and J.K.K. participated in data curation. B.P. wrote the manuscript. Y.A.K. reviewed the data and the manuscript. W.C., J.K.K. and S.K. revised and edited the manuscript.

Additional Information

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The effect of modulated electro-hyperthermia on local disease control in HIV-positive and -negative cervical cancer women in South Africa: Early results from a phase III randomised controlled trial

Carrie Anne Minnaar[1], Jeffrey Allan Kotzen[2], Olusegun Akinwale AyeniID[3], Thanushree Naidoo[2], Mariza Tunmer[2,4], Vinay Sharma[4], Mboyo-Di-Tamba Vangu[3,5], Ans Baeyens[1,6]

[1] Department of Radiation Sciences, Radiobiology, University of the Witwatersrand, Johannesburg, South Africa,
[2] Department of Radiation Oncology, Wits Donald Gordon Medical Centre, Johannesburg, South Africa,
[3] Department of Nuclear Medicine, Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg, South Africa,
[4] Department of Radiation Sciences, Radiation Oncology, University of the Witwatersrand, Johannesburg, South Africa,
[5] Department of Radiation Sciences, Nuclear Medicine, University of the Witwatersrand, Johannesburg, South Africa,
[6] Department of Human Structure and Repair, Radiobiology, Ghent University, Ghent, Belgium

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RESEARCH ARTICLE

The effect of modulated electro-hyperthermia on local disease control in HIV-positive and -negative cervical cancer women in South Africa: Early results from a phase III randomised controlled trial

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1 Department of Radiation Sciences, Radiobiology, University of the Witwatersrand, Johannesburg, South Africa, **2** Department of Radiation Oncology, Wits Donald Gordon Medical Centre, Johannesburg, South Africa, **3** Department of Nuclear Medicine, Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg, South Africa, **4** Department of Radiation Sciences, Radiation Oncology, University of the Witwatersrand, Johannesburg, South Africa, **5** Department of Radiation Sciences, Nuclear Medicine, University of the Witwatersrand, Johannesburg, South Africa, **6** Department of Human Structure and Repair, Radiobiology, Ghent University, Ghent, Belgium

* Ans.Baeyens@Ugent.be, Ans.Baeyens@wits.ac.za

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Abstract

Background

The global burden of cervical cancer remains high with the highest morbidity and mortality rates reported in developing countries. Hyperthermia as a chemo- and radiosensitiser has shown to improve treatment outcomes. This is an analysis of the local control results at six months post-treatment of patients enrolled in an ongoing study investigating the effects of the addition of modulated electro-hyperthermia (mEHT) to chemoradiotherapy for the treatment of HIV-positive and -negative cervical cancer patients in a low-resource setting.

Methods

This ongoing Phase III randomised controlled trial, conducted at a state hospital in Johannesburg, South Africa, was registered with the appropriate ethics committee. After signing an informed consent, participants with FIGO stages IIB to IIIB squamous cell carcinoma of the cervix were randomised to receive chemoradiotherapy with/without mEHT using a secure online random-sampling tool (stratum: HIV status) accounting for age and stage. Reporting physicians were blind to treatment allocation. HIV-positive participants on antiretroviral treatment, or with a CD4 count >200cell/μL were included. mEHT was administered 2/weekly immediately before external beam radiation. The primary end point is local disease control (LDC) and secondary endpoints are toxicity; quality of life analysis; and two year survival. We report on six month LDC, including nodes visualised in the radiation field on ^{18}F-FDG PET/CT (censored for six month survival), and six month local disease free survival

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(LDFS) (based on intention to treat). Trial status: Recruitment closed (ClinicalTrials.gov: NCT03332069).

Results

271 participants were recruited between January 2014 and November 2017, of which 210 were randomised for trial and 202 were available for analysis at six months post-treatment (mEHT: n = 101; Control: n = 101). Six month LDFS was higher in the mEHT Group (n = 39 [38.6%]), than in the Control Group (n = 20[19.8%]); $p = 0.003$). LDC was also higher in the mEHT Group (n = 40[45.5%]) than the Control Group (n = 20[24.1%]); ($p = 0.003$).

Conclusion

Our results show that mEHT is effective as a chemo-radiosensitiser for cervical cancer, even in high risk a patients and resource-constrained settings.

Introduction

The global burden of cervical cancer remains high, with 570 000 new cases and 311 000 deaths estimated to have occurred in 2018. It is the fourth most common cancer, and the cancer with the fourth highest mortality rate, amongst women. The mortality rate from cervical cancer is around 3 cases per 100,000 in developed countries and around 18 per 100,000 in developing countries.[1]

Current standard of care treatment for locally advanced cervical cancer (LACC), which includes International Federation of Gynaecology and Obstetrics (FIGO) stages IB2 to IVA, is a combination of chemotherapy (most commonly platinum-based), and radiotherapy (RT).[2] The treatment of cervical cancer in developing countries is complicated by limited resources, late stage of disease at presentation,[3] and the high prevalence of Human Immunodeficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS). Out of 54 African countries, 28 do not have access to radiotherapy treatments. Of those with access to RT, only 20 have access to brachytherapy (BT).[4] Where RT is available, the planning and delivery techniques are often sub-optimal, with many countries still relying on low energy machines, such as Cobalt60. There are usually not enough devices or staff to handle the high patient load, resulting in long waiting times for treatment.[5] The lack of radiology support restricts the availability of sophisticated planning techniques which require computed tomography (CT) or magnetic resonance imaging (MRI). Low-resource settings therefore rely on less labour-intensive 2D planning techniques that do not require sophisticated imaging to manage the burden of the high patient volumes. In our facility patients do have access to BT, however imaging techniques are not routinely available for the planning of oncology treatments. Treatment outcomes in low-to-middle-income countries (LMICs) are significantly worse than in less resource-constrained settings with an estimated 87% of cervical cancer-related deaths occurring in LMICs in 2018.[6] There is a scarcity of information on the outcomes and on the confounding prognostic factors unique to LMICs.[7] The protocols and outcome measures used are often sub-optimal in resource-constrained settings and therefore not comparable to international literature. For example in Uganda, 316 cervical cancer patients were evaluated and authors reported that HIV-positive patients (42%) had more than a two-fold higher risk of death than HIV-negative patients. More than two-thirds of the patients that died in the first

year, died in the first 6 months post-treatment and only 32% of the HIV-negative and 35% of the HIV-positive patients were alive at one year post-treatment. While this study did not report on local control, it does highlight the high mortality rates seen in low-resource settings.[8] HIV infection is associated with a higher risk of treatment interruptions and residual disease,[9] a higher risk of not completing the prescribed chemotherapy protocol,[10] and worse survival rates for cervical cancer patients.[11] The high burden of cervical cancer on already strained public health systems in Sub-Saharan Africa necessitates the investigation of feasible chemo-radiosensitisers that are effective, even under such difficult conditions.

Hyperthermia (HT) is well known for its radiosensitising effects and the benefits of its addition to RT for the management of LACC have been described in two meta-analyses.[12,13] Hyperthermia (HT) has been investigated as a chemo- or radiosensitiser in numerous studies on cervical cancer.[14–17] There is evidence that HT may be particularly effective in the treatment of Human Papilloma Virus (HPV) positive cancers as it promotes the degradation of oncogenic proteins.[18] HT as a radiosensitiser has shown to improve survival and local disease control (LDC) in LACC patients,[19] however at least one study has reported worse toxicity, with no improvement in local disease control.[20] As a chemo-sensitiser HT has demonstrated improved outcomes and acceptable toxicity combined with platinum-based cytotoxic agents in the management of residual or recurrent cervical cancer.[14] Phase I/II studies have shown that chemoradiotherapy plus HT for the management of LACC is tolerated well, with no significant increase in treatment related toxicity resulting from the addition of HT.[21,22] Two Phase III trials were published after recruitment for this study began. One was closed early due to slow recruitment.[23] Neither of the trials reported an increase in toxicity, however both trials reported no significant benefit to the addition of HT to chemoradiotherapy. One study used radiative heating,[23] and one study used a capacitive heating device with a power output of 800-1500W.[24] however two phase III trials published after recruitment for this study began, reported no benefit to the addition of HT to chemoradiotherapy.[23,24]

Radiative hyperthermia utilizes external antennas to induce an electromagnetic field using 70 to 120MHz which is coupled into the patient using a water bolus. Complex MRI guided planning systems and thermometry systems have been developed to support these treatments, minimize unwanted hot spot formation, and focus the energy on the tumour. Capacitive heating devices utilize two electrodes to produce an electro-magnetic field by capacitive coupling with the human body, resulting in heating. Both techniques are widely used clinically.[25] Challenges with radiative heating include the higher costs, complexity and sophisticated setting required to administer the treatments. Capacitive heating devices in comparison are more affordable[25] and carry less risk of hot spot formation. Capacitive heating devices frequently make use of intra-tumoral thermometers or locally applied temperature sensors to confirm temperatures, although the invasiveness of the procedures carries certain risks. Challenges associated with capacitive heating involve the effective heating of deep tumours through thick layers of adipose tissue (>2cm).

Hyperthermia faces challenges with acceptance and consistency in results. Szasz *et al* proposed a HT technique in which 13.56MHz capacitive-coupled modulated radiofrequency is applied to selectively target tumour cells based on the differences in the conductivity and permittivity of malignant tissue compared to healthy tissue.[26,27] This selective technique, known as modulated electro-hyperthermia (mEHT), combines the effects of heating and of the electric field to sensitise tumours to treatments.[28] The tumour, having different biophysical properties to healthy tissue, stores a charge thereby heating up selectively. Once the maximum energy has been absorbed, the excess energy passes through the tumour. This can also be described as extracellular heating where the extracellular matrix is heated with minimal impact on the intracellular environment. The device therefore uses the energy (measured in

Kilojoules) that is put into the system, rather than the temperature, as the measured dose. The energy output from the first electrode is compared to the energy received by the second electrode. Using algorithms the absorbed energy can be determined[29–31] and the tumour temperature is calculated. This technique therefore does not require intratumoural or MRI thermo-monitoring systems due to the real-time calculation of temperature by the device based on the energy dose. Confirmation of the heating capacity of the mEHT has been demonstrated.[32,33] The techniques is also able to safely heat tumors, regardless of tumour depth or adipose layer thickness.[30] Hyperthermia interferes with protein synthesis,[26,34] and inhibits DNA and RNA synthesis and repair[34,35] by inducing protein aggregation in the nucleus and changes in the DNA repair foci which inhibit the DNA double-strand break repair processes. This is synergistic for RT and for certain cytotoxic agents which cause DNA double-strand breaks.[36] Hypoxia is a central causative factor for radio-resistance.[37] Heating the tumour also results in changes in blood perfusion and re-oxygenation. [38] Enhanced perfusion results in improved oxygenation in the target tissue, which enhances the effects of the Reactive Oxygen Species induced by IR.[39] Increased temperatures result in an increased immune response at the heated site.[27,40] HT has direct actions on the immune system through direct heat-mediated cell killing, and indirect actions by modulating the innate and adaptive immune system.[41] The extra-cellular heating method used by mEHT minimises the stress on the intracellular environment, reducing the risk of thermo-tolerance. Heating up the extracellular matrix with low frequency mEHT has only a minor impact on the intracellular environment and the primary action and heating occurs in the extracellular matrix and at the cellular membrane.[42] According to research conducted by Andocs *et al*, cell stress induced by mEHT results in the generation of DAMP signals and the application of mEHT could potentially induce local immunogenic cell death.[43] mEHT has also been shown to induce apoptosis [44] more readily than HT.[45] Andocs *et al* hypothesise that the energy absorption at the cell membrane results in a non-homogenous energy distribution causing damage which activates the cell death-related signalling pathways during mEHT.[45]

mEHT has shown benefits and safety as a chemo-sensitiser for the treatment of recurrent or residual cervical cancer[17] and the increase in temperature achieved in cervical tumours using mEHT is similar to the increased temperature reported using other HT methods.[46] Locations in which the technique has shown safety include the brain,[47] head and neck,[48] lung,[49] and pancreas,[50] The different dose monitoring system and temperature calculations means that MRI monitoring is not required. This technique is simple to use in comparison to other technologies, making it suitable for investigating as a chemo-radiosensitiser in a low-resource setting.

In South Africa, cervical cancer is the leading cause of cancer death in women with a lifetime risk of 1 in 44 of developing cervical cancer.[51] According to UNAIDS, in 2017 there were 36.9 million people infected with the HIV world-wide with an estimated 7.1 million of those people living in South Africa.[52] The risk of cervical cancer is significantly increased in HIV-positive patients, likely a result of the of HIV-associated immune suppression[53] and the subsequent increased risk of chronic HPV infection.[54] This, combined with constrained resources, makes South Africa a particularly challenging setting in which to test chemo-radiosensitisers.

We report on an analysis of the local control results at six months post-treatment of patients enrolled in an ongoing study investigating the effects of the addition of modulated electro-hyperthermia (mEHT) to chemoradiotherapy for the treatment of LACC patients in a low-resource setting, with specific interest in the effects on the HIV-positive subgroup. Six month local disease-free-survival (LDFS) is also reported in order to account for the participants who did not survive six months post-treatment. To our knowledge, this is the first phase III

randomised controlled trial (RCT) on the trimodality treatment of cervical cancer using this form of HT, the first trial to apply HT twice a week for LACC, the first HT trial in Africa, and the first phase III HT study to include HIV-positive patients in a low-resource setting.

Materials and methods

This a single centre, phase III, randomised controlled (stratum: HIV status; accounting for stage and age) parallel group trial being conducted at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), a public hospital in Gauteng, South Africa, by the Radiation Sciences department of the University of the Witwatersrand. Approval from the Human Research Ethics Committee was obtained (M1704133) on 5th June 2012. According to the requirements of the local ethics board, the trial was registered on the South African National Clinical Trials Register before recruitment was started (ID: 3012). Registration of the trial ClinicalTrials.gov (NCT03332069) was finalised after recruitment began. The authors confirm that all ongoing and related trials for the intervention are registered on ClinicalTrials.gov.

Eligibility

Patients were recruited by the multi-disciplinary team of gynaecology and oncology specialists at the CMJAH (January 2014 to December 2017) after undergoing clinical staging according to FIGO staging criteria,[55] by chest x-ray, ultrasound and physical assessment. Written informed consent was obtained and participants were referred for additional haematological investigations (liver function; urea and creatinine levels; full blood count; pregnancy test with serum βHCG; HIV test; CD4 count if indicated), and a Positron Emission Tomography/Computed Tomography (PET/CT) scan with ^{18}F-Fluorodeoxyglucose (FDG), as part of the screening process.

Eligibility criteria. Primary, treatment-naïve, histologically confirmed invasive squamous or adeno-squamous cell carcinoma of the cervix; FIGO stages IIB (with invasion of the distal half of the parametrium), to IIIB (clinically staged); Eligible for CRT with radical intent; >18years old; Eastern Cooperative Oncology Group (ECOG) score <2; Creatinine clearance >60mL/min; Haemoglobin, platelet count and absolute neutrophil count within normal ranges; HIV positive patients on antiretroviral therapy or with a CD4 count >200cell/μL; Ability to understand and the willingness to sign a written informed consent document; Life expectancy >12 months; Patients who underwent emergency RT in the form of brachytherapy for haemostasis prior to enrolment were screened and enrolled provided they met all other eligibility criteria; Body Mass Index (BMI) within normal ranges; Life expectancy of greater than 12 months; Participants with para-aortic and other nodes outside of the radiation field visualised on the ^{18}F-FDG PET/CT scans were included.

Exclusion criteria. Bilateral hydronephrosis; Second primary malignancy; Extra-pelvic visceral disease visualised on the ^{18}F-FDG PET/CT; Vesicovaginal fistula or vesicorectal fistula that required a change in treatment protocols; Abnormal liver function tests; Currently pregnant or breast feeding; Prior hysterectomy; Prior malignancy treated in the preceding two years; Cardiovascular disease (excluding controlled hypertension); Acute or life-threatening infections or medical conditions or life threatening AIDS-defining illnesses other than cervical cancer; Evidence of resistance to antiretroviral therapy in HIV-positive patients; A medical or psychiatric illness that preventing the participant from being able to sign an informed consent or affecting the participant's ability to comply with the protocol stipulations; Carcinoma of the cervical stump; Contra-indications to mEHT (pacemakers, large metallic implants in the treatment area, inability to feel temperature in the treatment area, inability to express or vocalise pain); Any other contraindications to any of the prescribed treatments. Visualisation of para-

aortic lymph nodes on planning CT was also an exclusion criterion, however patients were not 3D planned.

Randomisation and masking

Once participants passed all screening evaluations, participant data was collected and captured by the research co-ordinator using REDCap (Research Electronic Data Capture), an online, secure web based application hosted by the University of the Witwatersrand. The REDCap stratified on-line software generated random-sampling tool was used to randomise participants to receive either CRT alone (Control Group) or combined with mEHT (mEHT Group); stratum: HIV status. Randomisation accounted for age (<30 years; 30–50 years; >50 years), and FIGO stage (IIB; IIIA; IIIB). Participants were informed in a private consultation by the research co-ordinator of their treatment group at treatment verification. The research co-coordinator was not involved in the treatment or clinical evaluations. Double blinding of the participants was not possible due to logistical challenges such as creating a "placebo treatment" on the mEHT device in which the participant would feel heat on the surface but not actually be treated. Clinicians examining the participants during follow-ups were blind to treatment allocation. Physicians reporting on the ^{18}F-FDG PET/CT images were blind to treatment allocation and did not interact with the participants, eliminating the risk of biased reporting.

Treatment

Chemotherapy (CHT). As per the institutional protocol, two doses of cisplatin (80mg/m^2) administered 21 days apart were planned during external beam radiation (EBRT), subject to the patient's ability to receive cisplatin (creatinine clearance >60mL/min; acceptable full blood count results; acceptable ECOG status). All participants who were treated with cisplatin received prehydration with normal saline and electrolytes as well as prophylactic antiemetics intravenously, as per the institutional protocol.

Radiotherapy. A haemoglobin (Hb) level above 10g/dL was required for EBRT and participants were transfused as needed. All participants were prescribed RT in the form of EBRT (50Gy in 25 fractions of 2Gy), and High Dose Rate (HDR) brachytherapy (BT) (3 fractions of 8Gy). The EMBRACE II study aims for an EQD2 dose of >85Gy. However this is not easily achievable without image guided brachytherapy.[56] The European Society for Medical Oncology Guidelines on Cervical Cancer management lists the optimal treatment as 80–90Gy at point A.[2] We planned an intended total equivalent RT dose of 86Gy. Standard planning for EBRT in our facility is 2D planning, and not the more labour-intensive planning techniques that are standard of care internationally. This is due to the limited staff capacity and resources available to treat the high volume of gynaecological oncology patients seen each year and due to the lack of access to imaging techniques, such as MRIs, for planning purposes. Participants for this study were planned using virtual simulation and standard fields were placed but volumes were not contoured as is standard of care for cervical cancer. EBRT was planned using CT guidance and volumes were determined using bony landmarks to encompass the entire pelvis including 2cm margins from the pelvic side wall laterally. The superior border was the midline of L5 and the inferior border extended to 2cm below the tumour. Posterior border encompasses the sacrum to the third sacral vertebra and the anterior border extended to the inferior border of the pubic symphysis. A four field box techniques was used for planning and the dose prescribed was normalized to 95%. The dose to the nodes was the same as the dose to the centre as it was a homogenous dose distribution to the entire field. 15MV or 18MV photon energy linear accelerators (Siemens Pty, Ltd) were used for the four field technique. ^{18}F-FDG PET/CT images were additions to the standard protocols with the sole

purpose of monitoring disease response and the costs of the imaging studies were covered by a research grant. The ^{18}F-FDG PET/CT images were not used to plan treatments. Para-aortic nodes seen on PET/CT images were therefore not targeted with EBRT. While extended fields to include para-aortic nodes significantly decreases the risk of distant metastases and of para-aortic failure, the effects on overall survival are not yet confirmed[57] and extended fields are not standard in a resource-constrained setting such as ours.

HDR BT was given as per the institutional protocol. Patients received 36Gy equivalent (EQD2 –equivalent dose in 2Gy fractions for an alpha-beta ratio of 10), to point A by intracavity BT using standard tandem-ring or tandem-ovoid vaginal applicators (source used: Iridium 192). 2-dimentional calculations were done based on orthogonal x-rays. Chemotherapy was not permitted on the days that BT was given. A 2D technique was used for the brachytherapy, due to lack of availability of imaging techniques. If the tumour was confined to the upper vagina, a ring-tandem applicator was used. Where the tumour extended inferiorly below the upper half of the vagina, a Joss-Flynn ovoid-tandem applicator was used to cover the whole extent of the vagina. The lower part of the vagina was kept within rectal tolerance when treating with a Joss-Flynn ovoid-tandem applicator. Standard IRCU38 bladder and rectal dose points were calculated for BT. More sophisticated BT techniques require sophisticated imaging techniques which are not available in low-resource settings. The first BT was planned to be administered after 10–13 fractions of EBRT with a week between each subsequent BT dose with the aim of completing all radiation treatments within 52 days.

Hyperthermia. Hyperthermia was delivered via a capacitive heating device (Model: EHY2000+; Manufacturer: Oncotherm GmbH, Troisdorf, Germany) which uses 13.56MHz modulated radiofrequency energy. The power was applied using two electrodes, one fixed in the bed and one in an adjustable arm placed over the treatment area. [58] A 30cm diameter applicator with electrode was used to cover as much of the pelvic field as possible. Participants randomised into the mEHT Group received two mEHT treatments (per week at 42.5°C for a minimum of 55 minutes, plus the prescribed CRT. A step-up heating protocol was used starting at a power output of 60W and increasing to the planned power over a period of five minutes. The planned power for the first treatment was 90W, for the second treatment the planned power was 110W, and the third and subsequent treatments were planned for 130W. An average energy dose of 360KJs over ten treatments was planned. mEHT treatments were administered at least 48 hours apart to reduce the risk of developing thermo-tolerance and EBRT was administered within 30 minutes of completing mEHT. mEHT was not administered on the same day as BT or cisplatin.

Follow-up. Participants were examined, and adverse events were documented, weekly on treatment and at six weeks, three months and six months post-treatment. Regular full blood count, renal, and liver function tests were performed. Toxicity was graded according the Common Toxicity Criteria for Adverse Events (CTCAE) version 4 and toxicities worse than Grade 2 were reported to the investigators. The six month follow-up period extended from February 2014 to August 2018 with the final participant completing treatment in January 2018.

Outcome measures

The primary outcome is LDC at six months post-treatment. Secondary outcomes reported in this paper include disease pattern analysis, early toxicity, and quality of life. As the participants were treated with EBRT to the whole pelvis and HT to the pelvis, we considered the entire region that was within the treatment field, assessing local disease control and not only tumour response. LDC was therefore considered a failure if the presence of disease in the radiation field was noted on any one of the evaluation methods post-treatment (Persistent Disease). The

same method of evaluation is described in the protocols of the multicentre (including our centre) study by the AIDS Malignancy Consortium (AMC) evaluating 2D planned EBRT, HDR BT and cisplatin in patients receiving ART (ClinicaTrials.Gov ID:NCT01590017).[59] The results on LDC from this trial are not yet available. Six month LDFS is also reported, taking into account death as a possible risk factor.

All participants were planned to have an ^{18}F-FDG PET/CT scan at six months post-treatment to evaluate local disease response and disease patterns presentation using the PERCIST 1.0 criteria (Complete Metabolic Response [CMR] SUV<2.5; Partial Metabolic Response [PMR] >30% reduction in SUV; Stable Metabolic Disease [SMD] <30% reduction in SUV; Progressive Metabolic Disease [PMD] increase in SUV).[60]. 13 participants were unable to have follow-up ^{18}F-FDG PET/CT scans due to poor ECOG status or renal dysfunction and were evaluated by Fine Needle Aspiration (FNA) of palpable nodes (n = 1), by CT scan (n = 3), and by clinical examination/PAP smear (n = 9). Evidence of disease on any of the evaluation methods was regarded as treatment failure. Six month LDFS was analysed according to the intention to treat principle and was considered a failure if there was a disease-related death or failure of LDC. We report on the tumour response determined clinically by examination, and on the level of agreement between tumour response determined by clinical exam and as visualised on ^{18}F-FDG PET/CT images. The early toxicity and quality of life are reported separately. Further papers on late toxicity and two year survival are planned.

Statistics

The sample size was calculated based on the estimated required sample sizes for a two-sample comparison of survivors' functions at two years (statistical power of 90%). We estimated an expected reduction in mortality at two years of 50%, based on survival of 20% in the control group and 40% in the experimental group. The statistical significance is defined as a two-sided alpha<0.05 for a log-rank test, with a constant Hazard ratio of 0.5693. Initially a 15% screening failure rate was predicted, however the screening failure rate was higher than expected (30%) and recruitment continued until the pre-determined required number of 200 eligible participants were enrolled.

Pearson's Chi squared test and Fisher's exact tests were used to determine the difference in frequencies between groups. T-test was used to compare the difference in means between groups. Logistic regression was used to test if prognostic factors (treatment arm, number of cisplatin doses; age; overall treatment time; RT dose; CD4 count; Body Mass Index [BMI]; number of mEHT treatments; energy dose administered), significantly predicted outcomes. The three stratification factors were used in the multivariate logistic regression model. Two sided p values are reported and p values <0.05 were considered significant. STATA 13.0 Statistics software program (Stata Corporation, College Station, Texas, USA), was used to analyse the data.

Results

Of 271 screened participants screened between January 2014 and November 2017, 210 were randomised for trial (Fig 1). The most common reasons for omission of the second cycle of cisplatin were renal dysfunction (mEHT: n = 19; Control: n = 11) and delayed in the administration of the first cycle (mEHT: n = 10; Control: n = 7), followed by non-compliance, anaemia, vomiting and dehydration, sepsis, poor ECOG, immunosuppression and other, including supply and technical problems. Reasons for all screening failures can be found in S1 Fig. By the stratification to tumour stage, participant age, and HIV status, the numbers of participants in each group were balanced. Four randomised participants did not arrive for treatment and no

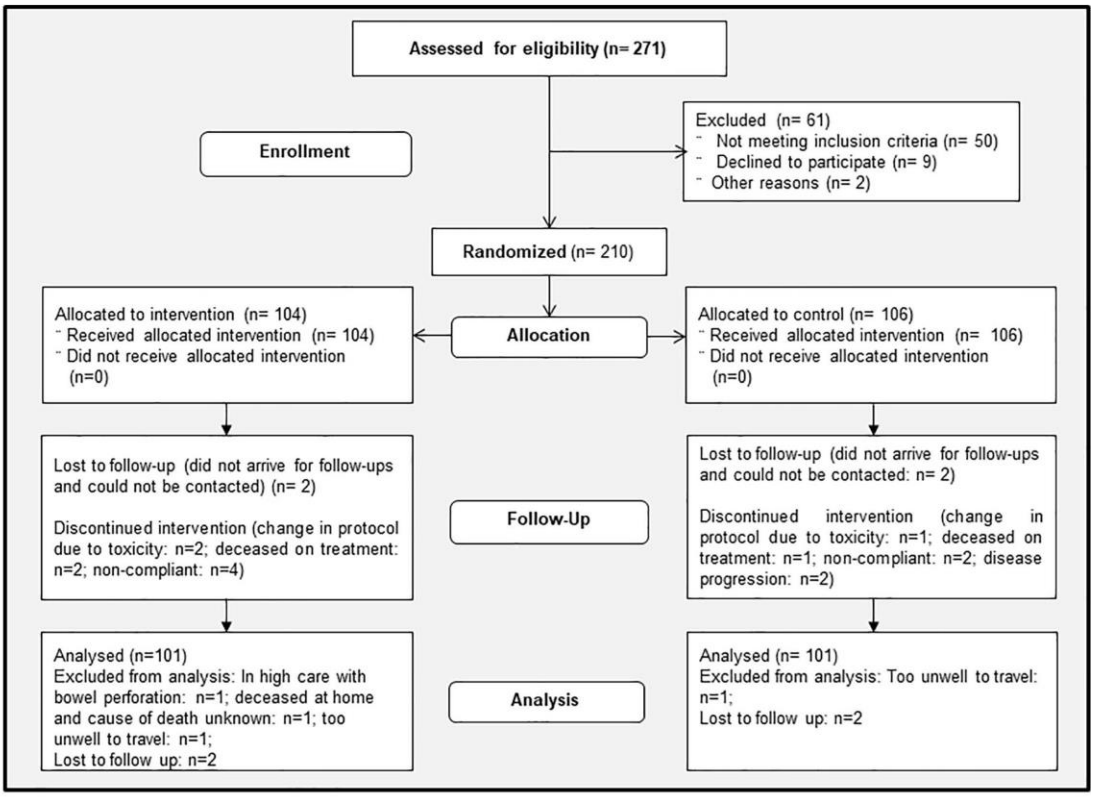

Fig 1. Trial profile: Consort flow diagram. Abbreviations: mEHT: Modulated Electro-Hyperthermia; LDFS: Local Disease Free Survival.

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further data is available on these participants. They were classified as "lost to follow up". Patient, tumour and treatment characteristics were balanced in both treatment groups with no significant difference in the means or frequencies (Table 1). There were 104 and 102 participants treated in the mEHT and Control Groups respectively. Four participants could not be evaluated for LDC at six months post-treatment (in high care with bowel perforation: n = 1; deceased at home and cause of death unknown: n = 1; too unwell to travel: n = 2). ^{18}FDG-PET/CT scans were conducted at a median of 186 days after completion of all RT treatments (range 154–610 days).

Treatment characteristics

Treatment characteristics are summarised in Table 2. Participants with a decrease in creatinine clearance to <60mL/min, and in whom measures to improve creatinine clearance failed, or whose CD4 count decreased to <150cells/uL, were not prescribed the planned cisplatin. Mean days to complete all radiation was 41.79 days (range 0–97). Six participants in the mEHT Group and four participants in the Control Group received less than 50Gy EBRT. Three participants died during on treatment (mEHT: n = 1; Control: n = 2), two did not arrive for

Table 1. Participant and treatment characteristics.

		OVERALL	mEHT	Control	P Value
	n	***206***	***104* [50.5%]**	***102* [49.5%]**	
HIV Status	Positive	*105* [51%]	*51* [49%]	*54* [53%]	*p = 575*
	Negative	*101* [49%]	*53* [51%]	*48* [47%]	
FIGO Staging	IIB	*75* [36%]	*40* [38%]	*35* [34%]	*p = 0.825*
	IIIA	*2* [1%]	*1* [1%]	*1* [1%]	
	IIIB	*129* [63%]	*63* [61%]	*66* [65%]	
ECOG	0	*10* [5%]	*3* [3%]	*7* [7%]	*p = 0.184*
	1	*196* [95%]	*101* [97%]	*95* [93%]	
Race	African	*191* [93%]	*96* [92%]	*95* [93%]	*p = 0.035*
	Caucasian	*5* [2%]	*4* [4%]	*1* [1%]	
	Other	*10* [5%]	*4* [4%]	*6* [6%]	
Age [years]	Mean	48.6	48.0	49.2	*p = 0.388*
	SD	10.49	10.77	10.22	
	Range	26–72	26–68	28–72	
BMI	Mean	27.45	27.81	27.08	*p = 0.431*
	SD	6.68	6.95	6.41	
	Range	15–49	15–49	15–41.7	
Hb [g/dL]	Mean	10.95	10.96	10.94	*P = 0.924*
	SD	2.26	2.13	2.39	
	Range	5.2–16.2	5.7–16.2	5.2–15.2	
CD4 count [cells/μL] in HIV positive	Mean	550	576.5	524.9	*P = 0.315*
	SD	261.76	251.57	270.97	
	Range	95–1524	148–1204	95–1524	

Abbreviations: mEHT: Modulated electro-hyperthermia; HIV: Human Immunodeficiency Virus; FIGO: International Federation of Gynaecology and Obstetrics; ECOG: Eastern Cooperative Oncology Group; SD: Standard Deviation; BMI: Body Mass Index; Hb: Haemoglobin.

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treatment, and two were moved to palliative care due to progression on treatment. Three participants who missed three or less fractions of EBRT cited transport difficulties as the reasons. There were no significant differences in early toxicities between the two groups. The mean dose of cisplatin received was 1.3. The main reasons for not receiving any cisplatin, a delayed first dose of cisplatin, or the cancellation of the second dose of cisplatin, are renal dysfunction (27 participants in the mEHT Group and 22 participants in the Control Group) and anaemia (n = 7 in both treatment groups). Other reasons for not receiving the required dose of cisplatin include leucopoenia; dehydration; sepsis; poor ECOG; and a low CD4 count. Three participants in the mEHT Group had a reduction in power during the mEHT treatments by more than 20% due to reported pain at the treatment site.

Local disease control and local disease free survival

In total, 202 participants were eligible for six month LDFS analysis (mEHT: n = 101; Control: n = 101), of which 171 participants (mEHT: n = 88 [87.1%]; Control: n = 83 [82.2%]), were alive at six months post-treatment. Complete metabolic response (CMR) of all local disease in the radiation field (LDC) in the participants who survived at six months was significantly associated with mEHT administration with a LDC seen in 40[45.5%] of the mEHT Group participants compared to 20[24.1%] of the Control Group (Pearson's chi2: $p = 0.003$). In a multivariate logistic regression model adjusted for HIV status, FIGO stage and age (Table 3),

Table 2. Treatment characteristics.

		OVERALL	mEHT	Control	P Value (t-test)
	n	*206*	*104* [50.5%]	*102* [49.5%]	
Total radiation dose	Mean	84.3Gy	84.7Gy	84Gy	*p = 0.624*
	SD	9.55	7.65	11.18	
	Range	2-86Gy	20-86Gy	2-86Gy	
Total external beam radiation	Mean	48.2Gy	49.4Gy	48.0Gy	*p = 0.724*
	SD	8.47	7.74	9.19	
	Range	2-50Gy	20-86Gy	2-50Gy	
Days to complete all RT	Mean	41.79	41.46	42.13	*P = 0.560*
	SD	8.16	5.88	9.98	
	Range	0–97 days	12–65 days	0–97 days	
No of Cisplatin doses	Mean	1.31	1.38	1.25	*p = 0.179*
	SD	0.69	0.67	0.58	
	0	*27* [13%]	*11* [11%]	*16* [16%]	
	1	*88* [43%]	*43* [41%]	*45* [44%]	
	2	*91* [44%]	*50* [48%]	*41* [40%]	
	Mean	2.88	2.89	2.87	*p = 0.778*
	SD	0.55	0.52	0.58	
No of brachytherapy fractions	0	*2* [1%]	*1* [1%]	*1* [1%]	
	1	*2* [1%]	*0* [0%]	*2* [3%]	
	2	*4* [2%]	*3* [4%]	*1* [1%]	
	3	*198* [95%]	*100* [95%]	*98* [95%]	
No of mEHT doses	Mean		9.09		
	SD		2.07		
	Range		1–10		
Average administered (KJ)	Mean		369.32KJ		
	SD		71.06		
	Range		237- 429KJ		

Abbreviations: mEHT: Modulated electro-hyperthermia; SD: Standard Deviation; RT: Radiotherapy; KJ: Kilojoules.

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participants in the mEHT Group were significantly more likely to achieve a LDC than participants in the Control Group (OR: 0.39, 95% CI: 0.20–0.77; p = 0.006). Age was also a predictor of LDC (OR: 1.04, 95% CI: 1.00–1.08; p = 0.040).

The intention to treat analysis showed a significant association between six month LDFS and the administration of mEHT (six month LDFS in mEHT Group: n = 39 [38.6%]; six month LDFS in Control Group: n = 20 [19.8%]; Pearson's Chi2: *p = 0.003*). A multivariate logistic regression model for six month LDFS (Table 4) also showed that participants in the

Table 3. Result of Multivariate logistic regression model to predict LDC (adjusted for HIV status, age, and stage).

Variable	Odds Ratio [OR]	P>|z|	95% Confidence interval [CI] range
Arm	0.39	0.006	0.20–0.77
HIV status	0.91	0.781	0.45–1.83
Age (years)	1.04	0.040	1.00–1.08
Stage (II or III)	1.09	0.604	0.78–1.54

Abbreviations: HIV: Human Immunodeficiency Virus

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mEHT Group were significantly more likely to achieve a six month LDFS than participants in the Control Group (OR: 0.36, 95% CI: 0.19–0.69; p = 0.002) and age was also a predictor of six month LDFS (OR: 1.04, 95% CI: 1.00–1.08; p = 0.031).

When analysed by HIV status, six month LDFS occurred in n = 27 [26.5%] of the HIV positive participants (n = 102), and n = 32 [32%] of the HIV negative participants (n = 100). The association between HIV status and six month LDFS was not significant (Pearson's Chi2: $p = 0.338$). When analysed by HIV status and treatment group, six month LDFS is higher in the HIV-positive and -negative participants treated with mEHT, with the highest six month LDFS seen in the HIV negative mEHT Group. The association between mEHT and six month LDFS was significant in the HIV-negative participants (Pearson's Chi2 $p = 0.006$), but was not significant in the HIV-positive participants (Pearson's Chi2 $p = 0.174$).

Tumour response

CMR of the tumour in the 158 participants (mEHT group: n = 85 [HIV-positive: n = 40; HIV-negative: n = 45]; Control Group: n = 73 [HIV-positive: n = 35; HIV-negative: n = 38), who were eligible for ^{18}F-FDG PET/CT scans at six months post-treatment was higher in the mEHT Group (n = 49 [57.6%]) than in the Control Group (n = 26 [35.6%]), with a significant association between the metabolic responses and the administration of mEHT (Fischer's exact: $p = 0.005$). These results are represented graphically in Fig 2. Complete metabolic response was higher in HIV positive participants (n = 40 [53.3%]), than the HIV-negative participants (n = 35 [42.2%], but this association was not significant (Fisher's exact: $p = 0.340$).

In an analysis by HIV status and by treatment group, the CMR was higher in the mEHT Group than in the Control Group in both HIV-positive and HIV-negative participants. The association between the CMR rates and mEHT was larger in the HIV-negative participants (mEHT: n = 25 [56.5%]; Control: n = 10 [26.3%]; Fisher's exact: p = 0.006), than in the HIV-positive participants (mEHT: n = 24 [60.0%], Control: n = 16 [45.7%]; Fisher's exact: p = 0.196), as seen in Fig 3.

Prognostic variables

The regression analyses show that age, number of cisplatin doses, treatment time, RT dose, and CD4 count, were not significant predictors of six month LDFS in our study (S1 Table).

In order to assess the efficacy of mEHT further, we considered the effect of BMI[31] (S2 Fig), energy dose (S3 Fig), and number of mEHT treatments on tumour response. Multivariate regression analyses showed that these variables were not a predictor of six month LDFS (S2 Table).

Disease pattern analysis

In the mEHT Group (n = 95), there were 15[37.5%] participants with extra-pelvic visceral disease visualised on the post-treatment ^{18}F-FDG PET/CT scans (HIV positive: 9[23%] out of 40;

Table 4. Result of logistic regression analysis to predict six month LDFS (adjusted for HIV status, age, and stage).

Variable	Odds Ratio [OR]	P>|z|	95% Confidence interval [CI] range
Arm	0.36	0.002	0.19–0.69
HIV status	0.91	0.788	0.45–1.83
Age (years)	1.04	0.031	1.00–1.08
Stage (II or III)	1.04	0.830	0.75–1.44

https://doi.org/10.1371/journal.pone.0217894.t004

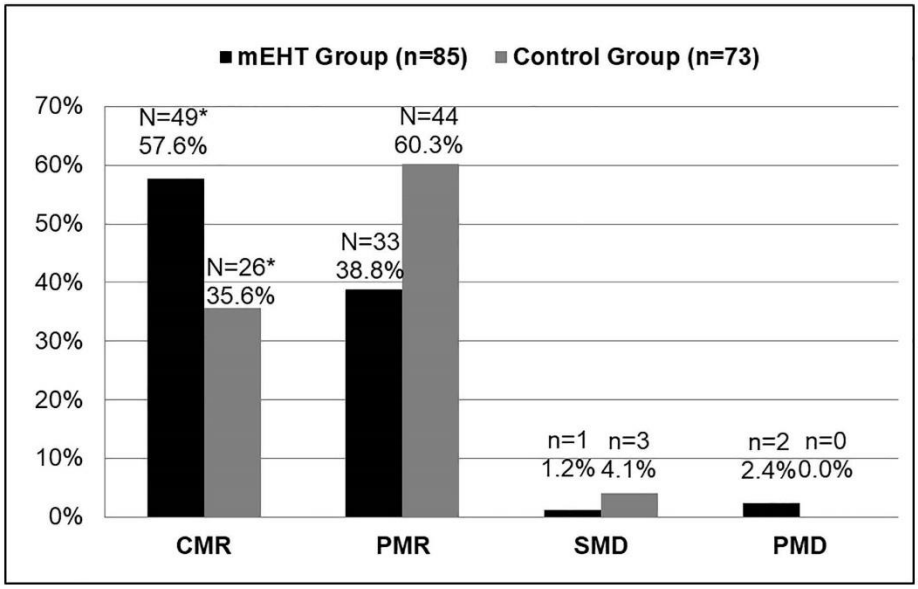

Fig 2. Tumour Response as Seen on ^{18}F-FDG PET/CT (PERCIST 1.0) by Treatment Group. mEHT: Fischer's exact table of association between all four metabolic responses and mEHT: $p = 0.005^*$. Abbreviations: mEHT: Modulated electro-hyperthermia; CMR: Complete Metabolic Response; PMR: Partial Metabolic Response; SMD: Stable Metabolic Disease; PMD: Progressed Metabolic Disease.

https://doi.org/10.1371/journal.pone.0217894.g002

HIV negative: n = 6[13%] out of 45). The Control Group (n = 73) had 16[45.7%] participants with visualised extra-pelvic visceral disease (HIV positive: 7[20%] out of 35; HIV negative: n = 9[24%] out of 38).The most common site for distant metastasis at six months was lung (n = 20), followed by liver (n = 12), bone (n = 9); soft tissue (n = 1), and brain (n = 1). Ten participants had multiple sites of distant visceral metastases.

54 Participants (64%) from the mEHT Group and 54 participants (74%) from the Control Group had nodes outside of the radiation field visualised on the pre-treatment ^{18}F-FDG PET/CT scans. The location of the nodes visualised on the pre-treatment scans are listed in Table 5. Para aortic nodes were visualised on the pre-treatment scans in 26 of the mEHT Group participants (25%) and 29 of the Control Group participants (28%). We compared the pre-treatment ^{18}F-FDG PET/CT scans of participants with extra-pelvic nodes to the post-treatment scans of the same participants in order to determine the response, if any, of the disease outside the radiation field. The percentage of participants who showed a systemic response to treatment, with resolution of all metabolically active disease (including extra-pelvic nodes which were not in the RT or mEHT fields, pelvic nodes and the primary tumour), was significantly higher in the mEHT Group: 24.1% (n = 13; HIV-positive: n = 6; HIV-negative: n = 7), versus the Control Group: 5.6% (n = 3; HIV-positive: n = 1; HIV-negative: n = 2) (Chi2, Fisher's exact: $p = 0.007$). One participant in each group did not receive cisplatin. In a multivariate analysis of age, cisplatin doses, total radiation dose and the number of days between the final radiation treatment and the follow-up ^{18}F-FDG PET/CT, none of the variables were indicators of an abscopal effect.

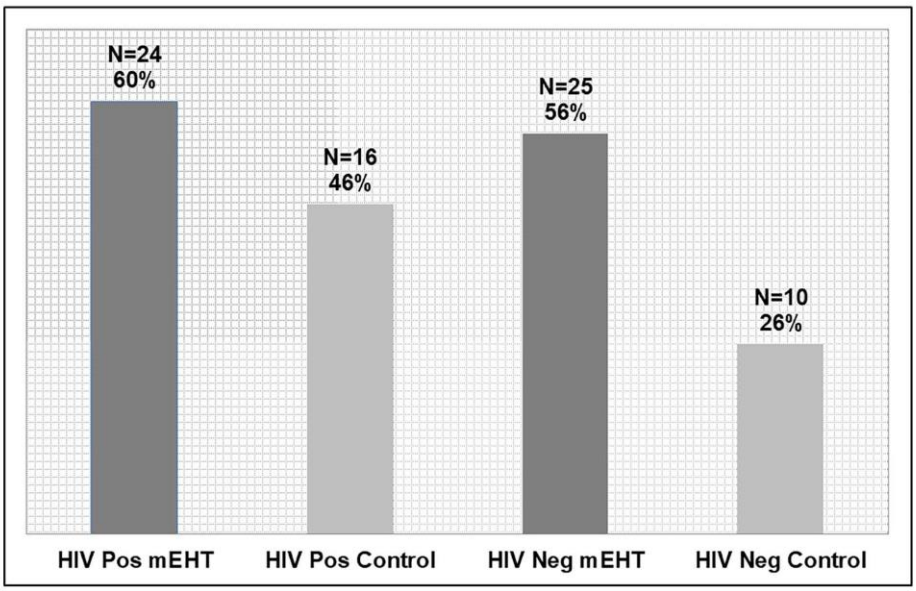

Fig 3. Tumour Response on ^{18}F-FDG PET/CT (PERCIST 1.0) by Treatment Group and HIV Status. Total participants in each subgroup: HIV-Positive mEHT: n = 40; HIV-Positive Control: n = 35; HIV-Negative mEHT: n = 45; HIV-Negative Control: n = 38. Abbreviations: mEHT: Modulated electro-hyperthermia.

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Toxicity

In our study, mEHT did not have any effect on the frequency of CRT-related early toxicities. The administration of mEHT was not associated with an increased risk in RT delays (OR: 1.614; p = 0.381; 95% CI: 0.553–4.712). Participant tolerance to mEHT treatments was excellent with 95 out of 104 (91.4%) participants receiving eight or more (≥80%) of the planned ten mEHT treatments. The reasons for not receiving more than eight mEHT treatments are adipose burns (n = 2), 1cm blister (n = 1), progressed on treatment (n = 1), moist desquamation resulting in RT and mEHT delay (n = 2), bleeding resulting in RT and mEHT delay (n = 1), did not arrive for RT or mEHT (n = 1), deceased on treatment (n = 1). Two participants in the control group also died during treatment. Eleven (10.6%) mEHT participants reported adverse events: Grade 1–2 adipose tissue burns (n = 10, 9.5%); grade 1 surface burns (n = 2, 2.0%). One participant in the Control Group also reported a subcutaneous burn. A multivariate

Table 5. Number of patients with nodes visualized in each region on pre-treatment scans.

	mEHT (n = 54)		Control (n = 54)	
Head and Neck	30	[56%]	26	[48%]
Thorax	29	[54%]	30	[56%]
Abdomen	32	[59%]	39	[72%]
Pelvis	43	[80%]	46	[85%]

Abbreviations: mEHT: Modulated Electro-Hyperthermia

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analysis showed increased BMI (OR: 1.78; p = 0.375; 95% CI: 0.499–6.335), were not predictive of adverse events associated with mEHT treatments.

Quality of life

At three months post treatment, fatigue and pain were significantly reduced in the mEHT Group and there was a significant improvement in social functioning (p = 0.049), and emotional functioning (p = 0.017) seen in the mEHT Group. There were no significant differences in quality of life between the groups while on treatment.

Discussion

Our results show that the addition of mEHT to chemoradiotherapy protocols for the management of LACC patients, results in a significantly higher six month LDC and six month LDFS, in a resource-constrained setting and in immunocompromised patients. Hyperthermia is a known radiosensitiser[61] and the heating effect of mEHT during the treatments is therefore likely to be the largest contributor to the improved outcomes seen in patients treated with mEHT in our study. Another possible contributing factor is the immunomodulating effect of mEHT described in the literature which may also contribute to the improved outcomes seen even in patients who are immunocompromised.

Due to resource constraints, the standard of care cisplatin schedule (40mg/m2 weekly), and standard of care of planning, using at least 3D-conformal radiotherapy (3DCRT) and extended fields (para-aortic), where indicated, was not used. It is not clear how much 3DCRT planning and extended radiation fields would improve the results in the treatment groups. Literature indicates that while extended fields on EBRT to include para-aortic lymph nodes significantly reduces the risk of distant metastases and the rate of para-aortic failure, the effects on overall survival are less significant.[57] Resource-constraints also affected factors such as time to complete treatment (listed as technical problems) and timing of the ^{18}F-FDG PET/CT scans in some cases (PET/CT down time, source supply problems). The control arm in our group had a lower response than what is generally achieved for LACC patients. Possible reasons include the evaluation of CMR using ^{18}F-FDG PET/CT studies compared to clinical evaluations of tumour response, a sample taken from a high risk population many of whom may be immunocompromised, and a resource-constrained setting where patient care and available treatments are substandard in comparison to less resource-constrained settings. Under-staging of cervical cancer patients is a well-documented challenge in low-resource settings where imaging techniques are not available.[62] There is a high risk that patients in our setting are being under-staged using only clinical staging methods, compared to the patients staged with imaging studies used in less resource-constrained settings and it is possible that many of our patients are in fact in Stage IV, based on the positive lymph nodes seen on pre-treatment ^{18}F-FDG PET/CT scans. The high rate of screening failure was unexpected and we did not expect that the so many of the patients were being under-staged and that there would be so many patients with visceral metastases and bilateral hydronephrosis on PET/CT images. This is an unfortunate challenge in low-resource setting where imaging studies to adequately stage patients are not routinely available.

The reviewed studies on HT for the treatment of cervical cancer, primary or recurrent, use local control, and not local disease control, as an outcome measure and the majority of the studies rely only on the clinical evaluation of local control with only a few using imaging studies.[14–17,20,22–24,63–67] The results from the two Phase III studies on the addition of HT to CRT reported a CR rate of 76% versus 86%(p = 0.54)[23] and 77.6% versus 88%(p = 0.192)[24] in the CRT versus the CRT plus HT groups respectively. In the Harima study[24] CR was achieved when no tumour was detected on physical examination or MRI, evaluated at one

month post treatment, although the number of patients evaluated using MRI was not reported. A multivariable logistic regression model did show that patients receiving CRT combined with HT in the Harmia study were more likely to have a CR ($p = 0.047$). Whole pelvis radiotherapy was applied however local disease control was not reported. The difference between this study and our study is the type of capacitive heating used and the frequency of HT treatments per week (one per week in the Harmia study and twice per week in our study). In the Harima study the tumour temperature was monitored rectally and vaginally, not intratumourally, compared to the real-time temperature calculations used by the mEHT device. Although 92% of participants in the Harmia study only received four to six HT treatments, the reasons for not completing HT (for example patient concerns or HT adverse events) are not given[24]. The compliance to mEHT treatments in our study was very good indicating good tolerance of the treatment, with more than 90% receiving eight or more mEHT treatments in our study. In the Phase III study by Flameling et al, the results posted did not include methods of evaluation, distribution of stages of disease, or patient characteristics. The study was closed prematurely due to slow recruitment, and was therefore underpowered, making drawing definitive conclusions impossible. In the Flameling study, HT was administered once per week and the primary tumour response was measured three months post-treatment. [23]

Challenges with clinical staging include the inadequate evaluation of the proximal tumour extension or side wall invasion, and inaccurate lymph node evaluation (especially deep pelvic lymph nodes).[68] With a sensitivity and specificity of lymph node evaluation of 100% and 99.6% respectively for nodes larger than 5mm, and the ability to detect metabolic changes before anatomical changes occur,[69,70] ^{18}F-FDG PET/CT is more effective at detecting lymph node involvement,[68] distant metastases,[71] and tumour activity.[72] LDC in our study included lymph nodes within the radiation field as visualised on ^{18}F-FDG PET/CT scans and we report on the loco-regional metabolic response, not the clinical response of the tumour.

The major challenges with the management of advanced cervical tumors are loco-regional control and distant metastases. Image guided adaptive brachytherapy (IGABT) has improved local and pelvic control rates for stage III/IVA disease to between 73% and 86%. Pötter et al report that the improved local and pelvic control compared to historical databases is associated with an overall survival benefit of around 10%.[56] These results are significantly higher than those seen in our study. Due to the differences in RT techniques, resources, staffing, patient characteristics, and confounding factors such as malnutrition and immune suppression, seen in our setting compared to less resource-constrained settings, a direct comparison of outcomes between the settings cannot be accurately made.

In our study, BMI and average energy dose were not predictors of six month LDFS, indicating that the mEHT treatments were able to target tumours regardless of the thickness of the adipose layer and that the required temperature and effect is still achieved in patients in whom the applied power needed to be lowered in order to achieve the prescribed treatment duration. In a phase I/II study investigating the effects of HT combined with CRT for the management of 68 LACC participants, adverse events directly attributed to HT included superficial burns (n = 12[18%]) and subcutaneous fatty necrosis (n = 5[7%]).[22] The number of superficial bones in our study was only two, and each was less than 1cm in diameter.

The significance of the results and benefits of HT for the treatment of LACC in the reviewed literature varies considerably. One possible reason is the lack of a fixed reference point for temperature and dose calculations, and the different heating methods.[73] Various heating techniques and protocols (treatment duration, frequency, and timing) are used which may contribute further to the variation in results. The two phase III trials on CRT plus HT which reported no benefit to the addition of HT, used a protocol of one HT treatment per week plus weekly cisplatin (40mg/m2),[23,24] compared to our protocols of two mEHT treatments per

week plus two doses of cisplatin (80g/m2) three weekly. Franckena *et al* reported that the number of HT treatments emerged as a predictor of outcomes with patients receiving more than three HT treatments showing a higher CR.[74] Our study did not show an association between the number of mEHT treatments and the CR, however the number of participants with three or less mEHT treatments was only three and the majority (91.3%) of participants could tolerate eight or more mEHT treatments. The need for mEHT treatment discontinuation was very low. Although not all participants could receive cisplatin in our study, the number of cisplatin doses was also not a predictor of outcomes.

Some studies have reported an increase in rates of distant metastases in participants treated with radiation plus HT.[20] We have not observed the same trend. In our study we observed the resolution of extra-pelvic nodal disease along with the pelvic disease in some of the participants. This rate was significantly higher in the mEHT Group. This may be due to the abscopal effect being potentiated by the addition of mEHT. A further analysis of these participants is planned.

According to the literature, treatment outcomes for HIV-positive patients are expected to be worse than in HIV-negative patients.[9–11] However, in our study the HIV-positive participants had a higher rate of complete metabolic tumour response to treatment than the HIV-negative participants, although the difference in CMR between the HIV-positive and -negative participants was not significant (53.3% versus 42.2%; Fisher's exact: $p = 0.340$). In a subgroup analysis of treatment groups in HIV-positive versus HIV-negative participants, the difference between the Control and mEHT Groups was larger in the HIV-negative participants. HIV-positive participants in each treatment group had a higher CMR rate at six months. This may suggest either less of an effect of mEHT in the HIV-positive participants, or a higher radiosensitivity of the tumours in HIV-positive participants. There are indications in the literature that HIV-positive patients have a higher chromosomal radiosensitivity[75] and this may contribute to the increased tumour response rates seen in these patients.

The difference between mEHT and other HT techniques is the dosing concept which relies on the energy dose applied to the patient during the treatment and the temperature is calculated based on the energy dose applied. Intratumoural or MRI thermo-monitoring systems are therefore not required, making the technique far simpler and more affordable than other techniques on the market, enabling the integration of the mEHT into the work flow in the resource-constrained setting. Without the need for increased staff to manage the treatments or the need for access to MRIs, theatres, or specialists, the use of the heating technique was viable. Although the device was loaned to the research team for research purposes, should the department be in a position to purchase the device, the cost to purchase the device is currently less than half the price of a BT device and does not require access to imaging techniques that the more advanced BT systems require. While mEHT is very clearly not a replacement for adequate EBRT and BT planning and treatments, it may be viable technique to improve outcomes in a setting where sophisticated RT techniques are not likely to be available for some time to come. A comparison between our results with mEHT and results from the use of mEHT in a less resource-constrained setting would be merited. Although the response rates varied between our study and the literature, the difference in CR between the two groups in our study is 22% and this improvement in outcomes, even in such high risk patients, is a strong indicator of the potential benefit to the use of mEHT.

Conclusion

This phase III RCT provides the first evidence for the use of mEHT to improve treatment outcomes when added to CRT for the treatment of LACC. The results offer evidence of efficacy of

a heating technique that is feasible and can be implemented in a low resource setting in a population sample in whom treatment and management of LACC is particularly challenging. The results indicate that mEHT can be integrated into the multimodal treatment regimens for LACC patients treated with radiotherapy with/without cisplatin, improving outcomes and potentially assisting in alleviating the burden of LACC on healthcare systems. There is also a potential benefit to the addition of mEHT to CRT for the treatment of LACC in less resource-constrained settings. A longer follow up period is required to assess the survival benefits and further papers are planned to report these results. The design of further phase III studies on mEHT as a chemo- or radiosensitiser for the treatment of other malignancies is warranted. The potential abscopal effect observed suggests that further investigations into the use of mEHT combined with immunotherapies are warranted.

Supporting information

S1 Fig. Reasons for screening failure before randomisation. *Abbreviations: VRF: Vesicorectal fistula; PET/CT: Positron Emission Tomography / Computed Tomography; ARV: Antiretroviral* *Bleeding: bleeding that could not be controlled by haemostatic brachytherapy and required admission and a change in protocols before completing the screening process; **Technical: ^{18}F-FDG supply difficulties.
(PDF)

S2 Fig. Patient BMI versus tumour response on ^{18}F-FDG PET/CT. Abbreviations: BMI: Body Mass Index; CMR: Complete Metabolic Response; PMR: Partial Metabolic Response; SMD: Stable Metabolic Disease; PMD: Progressed Metabolic Disease.
(PDF)

S3 Fig. Average energy dose versus tumour response on ^{18}F-FDG PET/CT. Abbreviations: KJ: Kilojoules; CMR: Complete Metabolic Response; PMR: Partial Metabolic Response; SMD: Stable Metabolic Disease; PMD: Progressed Metabolic Disease.
(PDF)

S1 Table. Logistic regression model for prognostic variables and six month local disease free survival. Abbreviations: RT: Radiotherapy; HIV: Human Immunodeficiency Virus.
(DOCX)

S2 Table. Logistic regression model of meht-related prognostic variables and six month local disease free survival. Abbreviations: mEHT: Modulated electro-hyperthermia; KJ: Kilojoules; BMI: Body Mass Index.
(DOCX)

S1 Appendix. CONSORT checklist.
(PDF)

S2 Appendix. LACC trial protocol.
(PDF)

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Author Contributions

Conceptualization: Carrie Anne Minnaar, Jeffrey Allan Kotzen, Ans Baeyens.

Data curation: Carrie Anne Minnaar, Jeffrey Allan Kotzen, Ans Baeyens.

Formal analysis: Carrie Anne Minnaar, Ans Baeyens.

Funding acquisition: Jeffrey Allan Kotzen, Ans Baeyens.

Investigation: Carrie Anne Minnaar, Jeffrey Allan Kotzen, Olusegun Akinwale Ayeni, Thanushree Naidoo, Mariza Tunmer, Ans Baeyens.

Methodology: Carrie Anne Minnaar, Jeffrey Allan Kotzen, Olusegun Akinwale Ayeni, Thanushree Naidoo, Mariza Tunmer, Ans Baeyens.

Project administration: Carrie Anne Minnaar, Jeffrey Allan Kotzen, Mboyo-Di-Tamba Vangu, Ans Baeyens.

Resources: Carrie Anne Minnaar, Ans Baeyens.

Supervision: Jeffrey Allan Kotzen, Mboyo-Di-Tamba Vangu, Ans Baeyens.

Validation: Mboyo-Di-Tamba Vangu, Ans Baeyens.

Visualization: Carrie Anne Minnaar, Jeffrey Allan Kotzen, Ans Baeyens.

Writing – original draft: Carrie Anne Minnaar.

Writing – review & editing: Carrie Anne Minnaar, Jeffrey Allan Kotzen, Olusegun Akinwale Ayeni, Thanushree Naidoo, Mariza Tunmer, Vinay Sharma, Mboyo-Di-Tamba Vangu, Ans Baeyens.

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Iron-dextran as a thermosensitizer in radiofrequency hyperthermia for cancer treatment

Hea-Jong Chung[1], Hyeon-Jin Kim[2], Seong-Tshool Hong[1]

[1] Department of Biomedical Sciences and Institute for Medical Science, Chon- buk National University Medical School, Jeonju, Chonbuk, South Korea

[2]JINIS BDRD Institute, JINIS Biopharmaceuticals Co., 913 Gwahak-Ro, Bongdong, Wanju, Chonbuk, South Korea

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Iron-dextran as a thermosensitizer in radiofrequency hyperthermia for cancer treatment

Hea-Jong Chung[1], Hyeon-Jin Kim[2] and Seong-Tshool Hong[1*]

Abstract

Radiofrequency hyperthermia is a recently rediscovered oncotherapy rising in popularity. However, lack of a proper thermosensitizer limits current radiofrequency hyperthermia to be only slightly effective, mostly being used as a subsidiary to a standard oncotherapy. Here, we report that iron-dextran delivers iron ion to cancer cells for cancer-selective accumulation of the iron ion, which functions as a thermosensitizer for radiofrequency hyperthermia. Intravenous injection of iron-dextran to tumor-xenografted mice resulted in selective accumulation of iron ion in the targeted cancer cells. The accumulated iron ion in cancer cells dramatically reacted to radiofrequency wave to result in tumor-selective dielectric temperature increment without harming the surrounding normal tissue. The oncotherapeutic effect of was evaluated using tumor-xenografted mice. The overall anticancer efficacy of radiofrequency hyperthermia after injection of iron-dextran as a thermosensitizer in breast cancer-bearing mice was much better than the efficacy of paclitaxel, a standard chemotherapy drug for cancer. Moreover, hyperthermia using iron-dextran as a thermosensitizer completely eradicated cancer in the tumor xenografted mice. This work suggests that iron-dextran is an ideal thermosensitizer for radiofrequency hyperthermia. We believe that the application of iron-dextran as a thermosensitizer would be a major progress in hyperthermia cancer treatments.

Keywords: Cancer, Hyperthermia, Iron-dextran, Radiofrequency, Thermosensitizer

Introduction

Treatment of cancer by temperature elevation has been developed from ancient time to modern medicine. The thermal cancer treatment in modern medicine is achieved by ablation and hyperthermia. Ablation uses high heat, typically >46 °C, to burn cancer tissue, in which laser lights are used. While, hyperthermia uses moderate temperature ranging from 39 to 46 °C. The purpose of hyperthermia is to increase temperature of interbody cancer to induce death of cancer cells either by apoptosis, necrosis or both.

One of the key characteristics of cancer cells is uncontrolled cell division. The uncontrolled cell division of cancer cells leads to the formation of an unorganized piling of cell mass which makes it impossible to properly develop neovascularization as in the case of normal tissues of the body [1]. Thus, cancer tissues are limited of blood circulation, leading to a constant hypoxic condition where glycolysis is facilitated to acidify the environment [2]. In addition to excessive glycolysis, poor blood circulation also makes cancer tissues vulnerable to high temperature. Unlike normal tissues, the excessive heat in the tumor tissues is difficult to be diffused into other parts of body via blood flow [3]. Based on such characteristics of cancer tissues, hyperthermic oncotherapies, in which cancer tissue is heated to high temperatures, were developed (Fig. 2a, b). Although the concept of hyperthermic oncotherapies was developed several decades ago, the therapeutic efficacy and benefit of hyperthermic oncotherapies have not noticed until recently [4]. Hyperthermic oncotherapies have reasonable therapeutic efficacy with almost no side effect so as to be getting

*Correspondence: seonghong@chonbuk.ac.kr
[1] Department of Biomedical Sciences and Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Chonbuk, South Korea
Full list of author information is available at the end of the article

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popularity currently. Hyperthermic oncotherapies especially in combination with radiotherapy showed excellent oncotherapeutic efficacy.

Elevation of the temperature in cancer can be achieved by various methods such as radiofrequency (RF), vibration of metal nanoparticles in alternative magnetic field, metal nanoparticle in an electromagnetic wave, pyrogenic chemicals, etc. Among these hyperthermal methods, magnetic or metal nanoparticles has got an attention in hyperthermal community and thus most thoroughly investigated so that some of these magnetic or metal nanoparticles even investigated in clinical trials [5]. Although magnetic or metal nanoparticles showed some efficacy, the insoluble nature of the magnetic or metal nanoparticles raised some concern [6].

Metal ions are known to interact well with electromagnetic waves [7]. Especially, interaction of metal ions which has a very strong dipole moment such as iron ion generates strong dielectric heat when radiated by RF wave [8]. Because of this characteristic, iron ion could be an excellent thermosensitizer in RF thermotherapy if iron ion can be delivered specifically to cancer cells. Considering the fact that X-ray and red light requires sensitizers for an effective oncotherapy, it would be reasonable to speculate that the efficacy of RF thermotherapy could be significantly improved when paired with a sensitizing reagent (Additional file 1: Table S1). To function as a tumor-targeting thermosensitizer, iron ion has to be accumulated more selectively in cancer cells than in normal cells. Iron-dextran, a medication for anemia, is a complex material in which iron ion form a complex with dextran through intermolecular bonding [9]. Because dextran has very strong tumor-targeting characteristics [10], we speculated whether the injection of iron-dextran would result in accumulation of iron ion in cancer cells. If iron-dextran is able to selectively deliver iron ion to cancer cells, iron-dextran would be an ideal thermosensitizer for RF thermotherapy. In this work, we showed that iron-dextran specifically delivered iron ion to cancer cells and thereby selectively increased the temperature of cancer tissue upon RF hyperthermic treatment. Our results confirmed successful eradication of cancer in a RF thermotherapy of tumor-xenografted animals when iron-dextran was used as a thermosensitizer, making iron-dextran a novel thermosensitizer candidate for RF thermotherapy to cure cancer.

Materials and methods

Cell lines and cell culture

The cell lines and cell culture protocol of this study using were followed as previously described [11]. The detailed protocol for culture is described Additional file 1.

Animal study condition

The protocol was approved by the Ethics Committee of Chonbuk National University Laboratory Animal Center (Permit Number: CBU 2012-0040) in accordance with the 'Guide for the Care and Use of Laboratory Animals', published by the National Research Council and endorsed by the ARRIVE Guidelines. The animal care protocol was followed as previously described [11–14].

Study of generating heat by iron-dextran in vitro

To evaluate the thermal performance of dextran and iron-dextran (Sigma-Aldrich, St. Louis, Missouri, USA), 0.1 mL of 5 mg/mL dextran or iron-dextran solutions were added onto 96-well plates in triplicate. After that, it was exposed for 10 min by the electromagnetic wave-dependent hyperthermia (LAB-EHY 100; Oncotherm, Budaörs, Hungary) at 50 W energy dose. The evaluation of heat capacity of solutions used by the thermal imaging camera (FLIR-E60; FLIR, Wilsonville, Oregon, USA) and the temperature of solutions measured by DirA Program (FLIR Tools; FLIR, Wilsonville, Oregon, USA). The iron-dextran in vitro condition protocol was followed as previously described [11]. The detailed protocol for iron-dextran in vitro condition is described in the Additional file 1. All data are presented as the mean ± SD and were compared using paired Student's *t-tests*. P value < 0.05 was considered as the statistical significance level.

Study of in vivo heat generation after iron-dextran injection

Two hundred μL of NCI-H460-luc2 (5×10^6) cells in saline solution was injected into the subcutaneous region of female nude mice with a BALB/c genetic background to generate cancer-bearing mice. Cancer growth was monitored daily. When the tumors reached a size of ~100 mm^3, the 24 cancer-bearing mice were grouped into 4 groups with 6 mice per group. Two cancer-bearing mice groups were *i.v.* injected with iron-dextran solution (10 mg/kg/day), and the other two groups were *i.v.* injected with dextran solution (10 mg/kg/day). Two mouse groups of iron-dextran and dextran were used for local hyperthermia experiment and the other two groups were used for whole-body hyperthermia. The iron-dextran in vivo condition protocol was followed as previously described [11]. The detailed protocol for iron-dextran in vitro condition and temperature measurement method on cancer and normal subcutaneous areas are described Additional file 1.

Organ distribution of iron ion concentration after iron-dextran injection

The organs (liver, heart, lung, kidney, muscle, brain, stomach, and tumor) distribution of iron ion were measured in tumor-bearing mice. A small piece (~1 g) of each organ including tumor was isolated from the cancer-bearing mice after injections of dextran or iron-dextran. The iron ion concentrations of each organ were measured by an Inductively Coupled Plasma Mass Spectrometry (Varian 800-MS; Varian Medical Systems, Palo Alto, CA, USA). The preprocessing protocol for measurement on iron ion concentration was followed as previously described [11].

The anti-tumor efficacy of iron-dextran in vivo

The anti-tumor efficacy of iron-dextran in vivo protocol was followed as previously described [11]. An IVIS imaging system (PerkinElmer, Waltham, MA, USA) was used to generate a bioluminescent image. The detailed preprocessing protocol for measuring in vivo imaging is described Additional file 1.

Histology of tumor tissue

The tumor tissue specimens from the cancer-bearing mice of the experimental groups were surgically isolated. After tumor tissues were fixed at 10% neutral-buffered formalin (Sigma Aldrich, St. Louis, MO, USA), followed by serial sectsion (5 μm) of tumor tissue specimens were cut and stained with hematoxyline and eosin (H&E) as previously described [13, 14]. The stained tissue images were scanned by using a slide scanner, Aperio Scanscope FL (Leica Biosystems, Wetzlar, Germany), and the scanned image was processed by ImageScope Software (Aperio Technologies, Vista, CA, USA).

Results

Iron-dextran generated dielectric heat in RF wave

Iron-dextran is a complex of iron ion and dextran. Interestingly, iron ion is capable of generating heat by RF waves and dextran is capable of targeting tumor tissues. It would be logical to speculate whether iron-dextran can be selectively delivered to cancer cells where it could generate dielectric heat upon irradiation with RF waves. Therefore, we performed a heat induction experiment on iron-dextran solution in a RF wave along with dextran solution as a control. As expected, the iron-dextran solution elevated the temperature of the solution greater than the dextran solution after irradiation of 13.56 MHz RF wave (Fig. 1a). This result demonstrated that the iron ion of iron-dextran reacted with RF to generate dielectric heat.

In addition to generation of dielectric heat, dextran is known to have cancer-targeting characteristics [15]. In order to test whether iron-dextran can selectively deliver iron to cancer cells, cancer and normal cells were exposed to iron-dextran, and heat generation was compared upon treating with RF thermotherapy (Fig. 1a). The exposure of cancer cells to a medium containing iron-dextran boosted temperature elevation in a dose-dependent manner in contrast to normal primary cells (Fig. 1b, Additional file 1: Fig. S1). Overall, these experiments indicated that cancer cells selectively accumulated iron ion after iron-dextran treatment to react with RF wave and induce dielectric heat more strongly. This result suggests that iron-dextran could be a possible candidate for a safe and effective thermosensitizer for RF thermotherapy.

The selectively accumulated iron ion in cancer cells reacted with a 13.56 MHz RF In order to evaluate iron-dextran's potential as a thermosensitizer for RF thermotherapy, we tested whether repeated injections of iron-dextran can selectively accumulate iron ion in cancer cells. Continuous injections of iron-dextran into cancer-bearing mice resulted in a gradually increasing concentration of iron ion in cancer cells in a dose dependent manner (Additional file 1: Fig. S2). We also measured the distribution of iron ion in cancer-bearing mice before and after *i.v.* injection of iron-dextran and dextran, respectively. As expected, iron ion successfully accumulated in tumor tissues after *i.v.* injection of iron-dextran compared to dextran injection (Additional file 1: Table S2). These results clearly demonstrate that iron-dextran is capable of selectively accumulating in tumor tissues when injected into tumor-bearing mice. After the confirmation of iron-dextran's selective accumulation, we conducted experiments using a tumor-xenograft model to confirm whether selective iron ion accumulation in the cancer cells will boost temperature elevation during RF thermotherapy. Various frequencies of RF have currently being adopted in modern RF thermotherapies, and iron-dextran should affect cancers similarly in each of RF frequencies [16]. In this work, we chose a RF hyperthermal machine using 13.56 MHz RF which frequency is most widely used currently [17]. Local 13.56 MHz RF hyperthermia after *i.v.* injection of iron-dextran increased the temperature of the cancer tissue of cancer-bearing mice by 9.96 °C, compared to 4.2 °C in the cancer tissue of the dextran injection group (the control group) (Fig. 2c, Additional file 1: Fig. S3).

Since in vitro experiment showed a positive correlation between the iron ion concentration and temperature elevation under RF hyperthermia (Fig. 1b), the in vivo effect of iron ion accumulation in cancer cells under RF hyperthermia was further investigated. Figure 3 shows that cancer

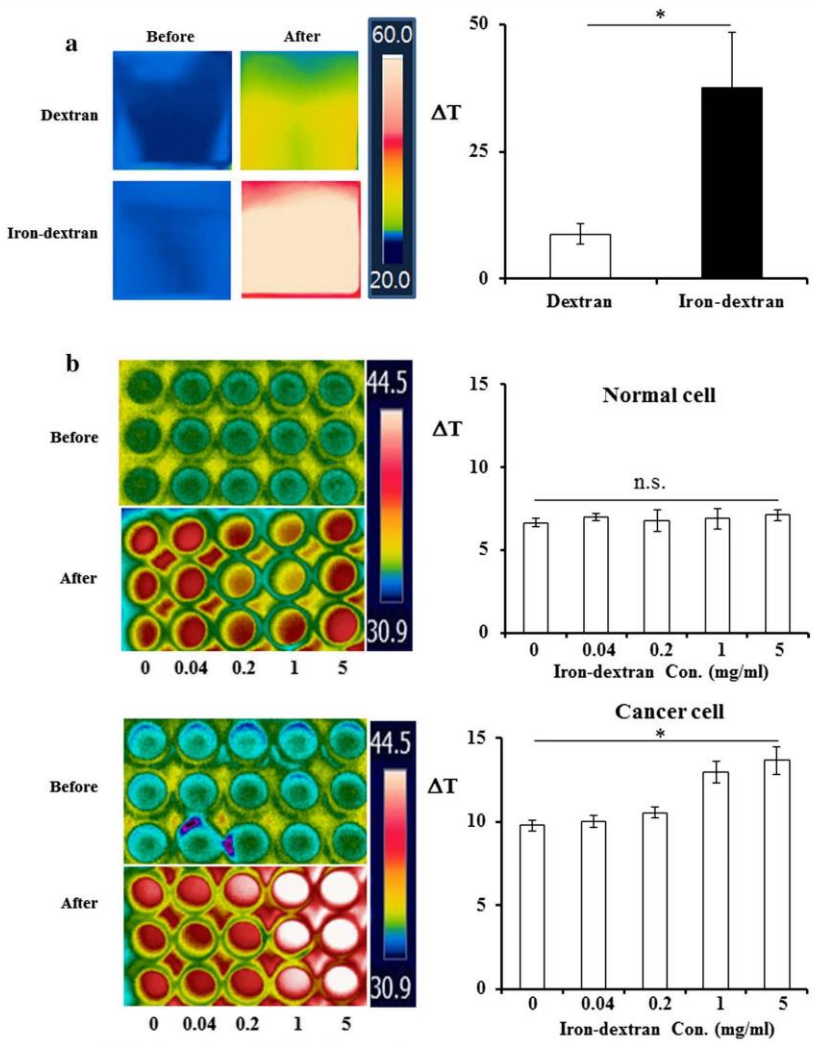

Fig. 1 Iron-dextran induced dielectric heat in the 13.56 MHz RF wave to boost temperature elevation. **a** The iron-dextran generated heating upon RF hyperthermia and the temperature was elevated significantly compared to iron-free dextran solution. **b** Exposure of human lung cancer NCI-H460 cells to iron-dextran boosted temperature elevation in a dose-dependent manner while this effect was not observed in normal cells. The values represent the mean ± SD (n = 6). A paired Student's t-test was used for the statistical analysis; $^{*}p < 0.05$

tissues reacted greater in 13.56 MHz RF hyperthermia as iron ion concentration was increased by repeated injections of iron-dextran into the mice. The local hyperthermia on the mice after the 8th injection resulted in a temperature increase of 15.3 °C in the cancer tissue, while the temperature only increased by 5.2 °C in the cancer tissue of the dextran-treated group (Fig. 3a). Similar to local hyperthermia, a whole-body hyperthermia on the cancer-bearing mice after 8th injection of iron-dextran elevated temperature by 3.1 °C in the cancer tissue, while only 1.2 °C

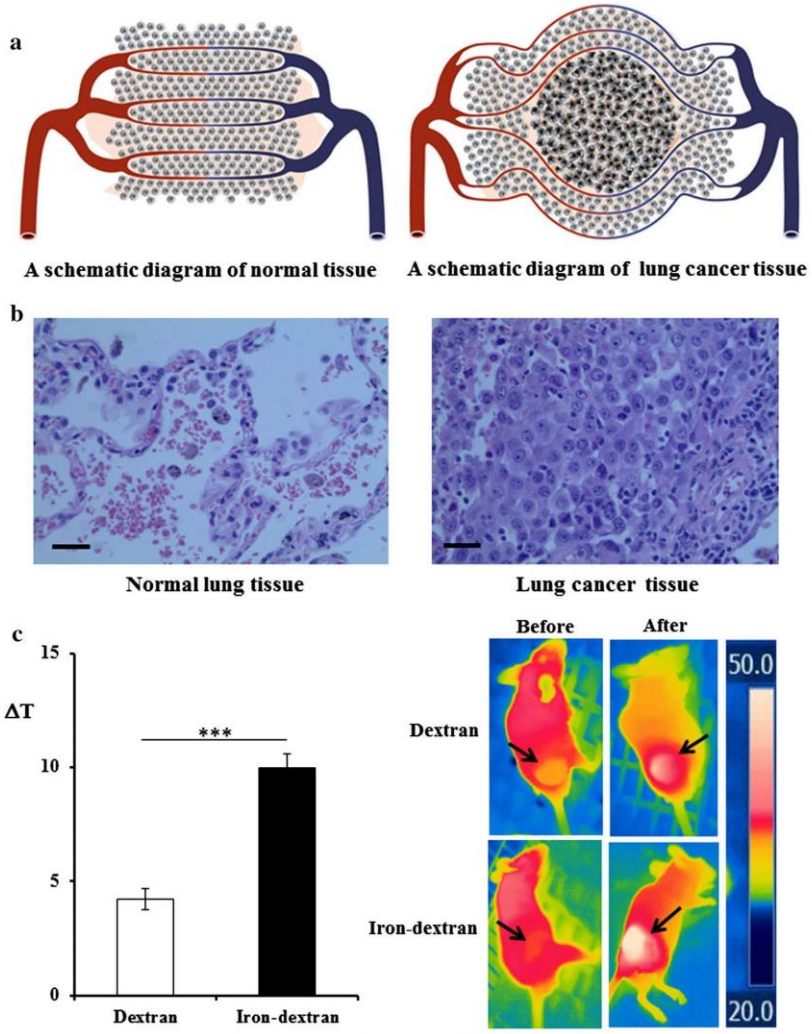

Fig. 2 Iron-dextran boosted temperature elevation in cancer-bearing mice upon 13.56 MHz RF hyperthermia. **a** Schematic drawing of vasculature in cancer and normal tissues. The excessive heat in the cancer tissues is difficult to be diffused into other parts of body via blood flow, resulting cancer tissues more vulnerable to hyperthermic oncotherapy. **b** H&E staining of cancer tissue showed the abnormal chaotic vasculature with active angiogenesis and slowed blood flow. Scale bar, 100 μm. **c** The temperature elevation in tumor tissue was dramatically boosted by local hyperthermia using 13.56 MHz RF wave with iron-dextran but not with dextran. Data represent the mean ± SD (n = 6). Asterisk indicates statistical significance as analyzed by a paired Student's t-test; ***$p < 0.001$ with the division

increased in the cancer tissue of the dextran-treated group (Fig. 3b, Additional file 1: Table S3). The cancer-selective accumulation of iron ion and subsequent tumor-selective heating by RF hyperthermia became more evident when the temperature change of normal subcutaneous tissue was compared to that of tumor tissue (Additional file 1: Fig. S4, Fig. S5). Temperature change over repeated injection of iron-dextran in local hyperthermia has increased up

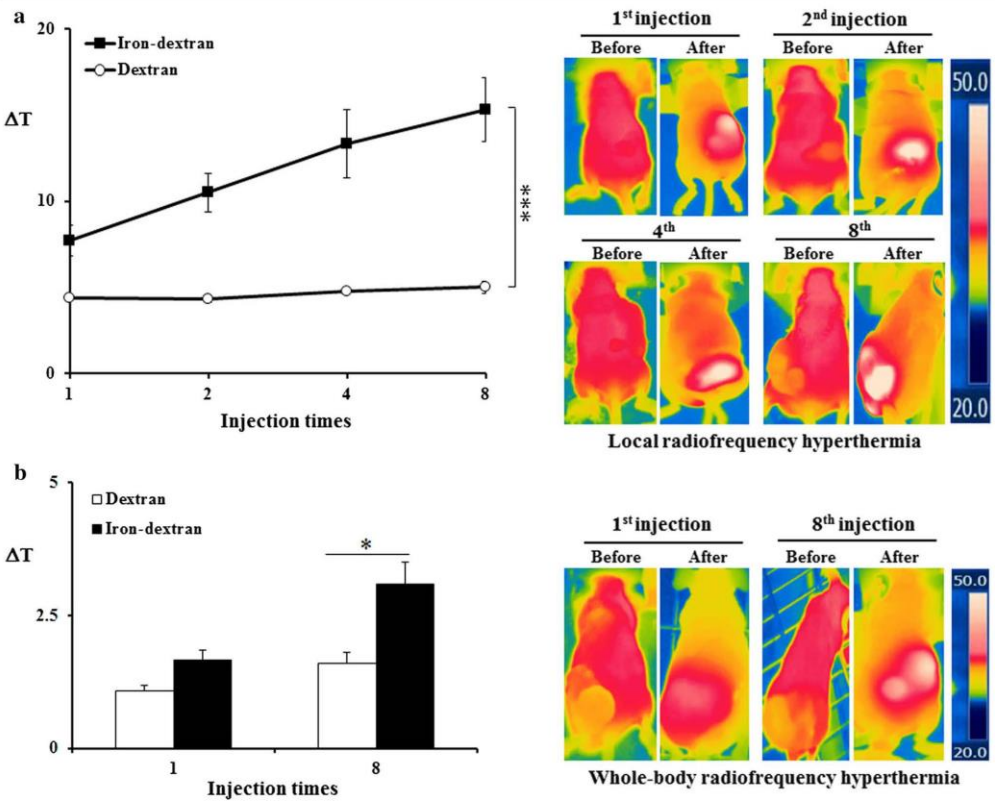

Fig. 3 The tumor tissues became more reactive to 13.56 MHz RF hyperthermia as *i.v.* injection of iron-dextran. **a** The repeated *i.v.* injection of iron-dextran to cancer-bearing mice boosted temperature elevation greater in a local 13.56 MHz RF hyperthermia as iron ion concentration was increased by repeated injections. **b** The repeated *i.v.* injection of iron-dextran boosted temperature elevation in a whole-body 13.56 MHz RF hyperthermia as iron ion accumulated. The values represent the mean ± SD (n = 6). A paired Student's t-test was used for the statistical analysis; $^{*}p < 0.05$, $^{***}p < 0.001$

to 15.3 °C in the cancer tissue but only to 3.4 °C in normal subcutaneous tissues (Additional file 1: Fig. S5). In accordance to the local hyperthermia, whole body hyperthermia after repeated injection of iron-dextran also resulted in tumor-selective temperature elevation (Additional file 1: Fig. S6, Fig. S7). These results clearly indicate that the selective accumulation of iron ion in the cancer cells leads to selective dielectric heating in cancer tissues in RF hyperthermia.

Application of iron-dextran as a thermosensitizer in RF hyperthermia

Since iron-dextran is able to induce tumor-selective temperature elevation under RF hyperthermia through selective accumulation of iron ion in cancer cells, we conducted animal experiments on the oncotherapeutic efficacy of iron-dextran as a thermosensitizer under RF hyperthermia. The cancer-bearing mice were treated every 3 days with local hyperthermia with 13.56 MHz RF after *i.v.* injection of 10 mg/kg/day iron-dextran as a thermosensitizer. The local RF hyperthermia after iron-dextran injection dramatically reduced the size of cancer tissue as thermotherapy continued and completely eradicated cancer after 5 weeks of treatment (Fig. 4a, b). The overall anticancer efficacy of RF hyperthermia with iron-dextran thermosensitizer was much greater than the oncotherapeutic efficacy of paclitaxel, a standard pharmaceutical drug for chemotherapy (Fig. 4a, b). In

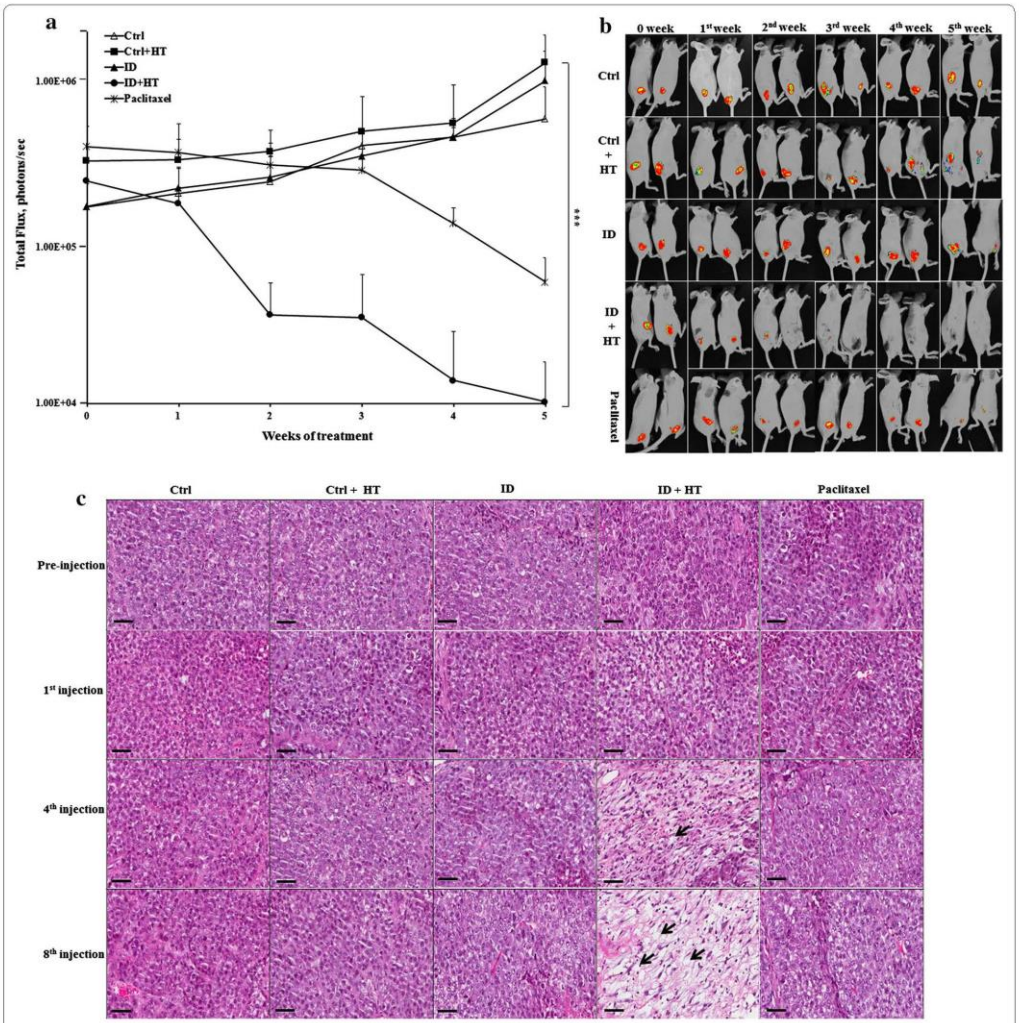

Fig. 4 The oncotherapeutic efficacy of iron-dextran as a thermosensitizer in a local 13.56 MHz RF hyperthermia. **a** The in vivo evaluation results of oncotherapeutic efficacy of iron-dextran as a thermosensitizer in the local 13.56 MHz RF hyperthermia in a tumor-xenografted mouse model. **b** The representative in vivo bioimage of the tumor-xenografted mice to evaluate the oncotherapeutic efficacy of iron-dextran as a thermosensitizer in 13.56 MHz RF hyperthermia. **c** The representative histological images of the cancer tissues of the tumor-xenografted mice after each treatment. The abbreviation in this figure, Ctrl; control mice with dextran injection, Ctrl + HT; control mice with dextran injection followed by local treatment of 13.56 MHz RF hyperthermia, ID; mice with iron-dextran injection only, ID + HT; mice with iron-dextran injection as a thermosensitizer followed by local treatment of 13.56 MHz RF hyperthermia, Paclitaxel; mice in which equivalent dose of paclitaxel was injected as a chemotherapeutic agent without RF hyperthermia treatment. Necrosis of tumor tissue is indicated by an arrow. The values represent the mean ± standard deviation ($n = 6$). A paired Student's t-test was used for the statistical analysis; *** $p < 0.001$. Scale bar, 50 μm

accordance with the dramatic efficacy in tumor abolishment, H&E staining revealed a typical display of massive cell death by necrosis on the tumor tissues after the therapy (Fig. 4c). Common necrosis features such as swollen cytoplasm, early loss of membrane integrity, and disintegration (swelling) of organelles were easily identifiable in cancer cells of the iron-dextran-treated group after multiple treatments (Fig. 4c). In contrast, such signs of necrosis were not observed in any other experimental groups (Fig. 4c). These results indicate that the RF thermotherapy using iron-dextran as a thermosensitizer caused massive necrosis of cancer cells, suggesting that iron-dextran is an ideal thermosensitizer for boosting the oncotherapeutic efficacy of RF hyperthermia.

Discussion

RF thermotherapy is a hyperthermal therapy that is mostly used as a subsidiary treatment to standard cancer therapies in modern medicine. Even though RF thermotherapy is used as a subsidiary treatment, RF thermotherapy is getting popularity in present clinics because of its safety and a modest efficacy. However, ironically, the main obstacle of RF thermotherapy in cancer treatments is the limitation on temperature elevation. The RF thermotherapy cannot selectively elevate the temperature in cancer tissues, limiting elevation of the temperature of cancer tissues to the safe range of 40–42 °C in which surrounding normal tissues are not damaged. Obviously, such a range of 40–42 °C temperature is not enough to kill cancer cells, instead only causing a few cancer cells to undergo apoptosis. The range of elevated temperature might explain a limit on oncotherapeutic efficacy of current RF thermotherapy.

RF thermotherapy kills cancer cells through apoptosis. It should be noted that this is a different kind of cell death than necrosis which was observed when *i.v.* injection of iron-dextran was used as a thermosensitizer in RF thermotherapy (Fig. 4c). Necrosis is the premature cell death, which is caused by infection, toxins, trauma, or high heat. Unlike apoptosis, which is triggered by gentle external factor, necrosis is triggered by harsh external factors such as high heat. RF thermotherapy alone elevates the temperature of cancer cells up to 40–42 °C at which only a few cells die through apoptosis. Considering the range of elevated temperature of current RF thermotherapy, it is unrealistic to expect that the current RF thermotherapy is a reasonable choice for an efficient cancer treatment. This work shows that the application of iron-dextran as a thermosensitizer in RF thermotherapy could selectively elevate temperature in cancer tissues up to 47 °C with minimal damage to normal tissues, which cannot be achieved by current RF thermotherapy. It should be noted that, however, IR thermography measurement used in this study has a limit in representing the defined temperatures in heterogeneous curved tumor tissue although it is one of most widely used method for thermotherapy. Consequently, it should be considered as the mean numbers representing 25 spots randomly chosen by program in tumor and normal tissues for comparative analysis.

Dextran is a complex branched polysaccharide made of various lengths of glucose residues, which is manufactured from bacterial fermentation [18]. Because of its higher molecular weight, biocompatibility, biodegradability and hydrophilic nature, dextran has been used in various pharmaceutical purposes such as plasma volume expanders, anticoagulants, drug carriers, a targeting moiety of nanoparticles for cancer, etc. [10, 19]. Especially, the large molecular weight as well as other physical characteristics of dextran made it ideal for tumor-targeting through enhanced and the enhanced permeability and retention effect. Also, glucose itself tends to be drawn by cancerous tissue. Because of these two reasons, dextran is used as one of the favored targeting moieties of current nanoparticles for cancer [20, 21]. In an agreement with those previously known feature of dextran, our experimental data clearly confirmed the cancer-targeting nature of dextran. Because of the excellent cancer-targeting ability, injection of iron-dextran make iron ion targeted into cancer, which resulted in selective accumulation of iron ion in the cancer tissue (Additional file 1: Table S2, Fig. S2).

In this work, we show that *i.v.* injection of iron-dextran into cancer-bearing mice selectively delivers iron ion to cancer cells, resulting cancer-specific accumulation of iron ion. The accumulated iron ions dramatically boost the cancer-specific dielectric heating in RF thermotherapy, selectively elevating the temperature of cancer cells strong enough to cause necrosis without damaging normal tissues so as to eradicate cancer. Iron-dextran is one of the safest drugs currently used for treating anemia [22]. These characteristics make iron-dextran an ideal choice for RF thermotherapy as a thermosensitizer. Considering current oncotherapeutic efficacy of RF hyperthermia even without a thermosensitizer as well as the oncotherapeutic efficacy showed in this work, the development of iron-dextran as a thermosensitizer in RF hyperthermia should be a major progress in cancer treatment.

Additional file

Additional file 1: Table S1. Electromagnetic waves widely used in modern medicine. **Table S2**. Distribution of ferric ion in each organ after single i.v. injection of iron-dextraninto tumor-bearing mice. **Table S3.** Temperature elevation of each organ after eight i.v. injection of iron-dextran into tumor-bearing mice. **Figure S1**. The effect of iron-dextran exposure to cancer and normal primary cells. **Figure S2**. The effect of repeated i.v. injectionof iron-dextran into tumor-bearing mice. **Figure S3**. Application of iron-dextran as a thermosensitizer in a local 13.56 MHz radiofrequency hyperthermia boosted cancer-selective temperature elevation in tumor-bearing animals. **Figure S4**. The positive co-relationship between the cancer-selective temperature elevation and ferric ion accumulation in a local 13.56 MHz radiofrequency hyperthermia. **Figure S5**. The selective temperature elevations of cancer tissue by a local 13.56 MHz radiofrequency hyperthermia were positively correlated with iron ion accumulations. **Figure S6**. The positive correlation between the cancer-selective temperature elevation and ferric ion accumulation in a whole-body 13.56 MHz radiofrequency hyperthermia. **Figure S7**. The selective temperature elevation of cancer tissue by a whole body 13.56 MHz radiofrequency hyperthermia were positively correlated with iron ion accumulations by repeated injections of iron-dextran.

Authors' contributions
HJC and STH analyzed the data and wrote the paper. STH provided technical assistance to HJC and helped with the data collection. HJK edited the manuscript. STH supervised the work. STH and HJC revised and edited the manuscript and supervised the work. All authors read and approved the final manuscript.

Author details
[1] Department of Biomedical Sciences and Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Chonbuk, South Korea. [2] JINIS BDRD Institute, JINIS Biopharmaceuticals Co., 913 Gwahak-Ro, Bongdong, Wanju, Chonbuk, South Korea.

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Integrative Therapeutic Options for Treating Stage Four Breast Cancer

Magda Havas

Environmental and Resource Studies, Trent School of the Environment, Trent University, Canada

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Integrative Therapeutic Options for Treating Stage Four Breast Cancer

*Magda Havas**

Environmental and Resource Studies, Trent School of the Environment, Trent University, Canada

Abstract

Traditional Western medicine treats breast cancer – the most commonly occurring cancer in women with some combination of surgery, chemotherapy and/or radiation therapy. Few doctors' council their patients to use complementary modalities and some forbid their use. However, complementary therapies in the form of agents or activities that minimize exposure to toxins, detoxify the body, strengthen the immune system, increase cellular energy, promote circulation, reduce inflammation, alleviate pain and create a hostile internal environment for the growth of cancerous cells without damage to normal healthy cells have been shown to enhance survival and reduce morbidity of cancer patients. Some of these complementary modalities are presented here as options for both oncologists and patients to consider.

Introduction

Breast cancer is the most commonly occurring cancer in women and the second most common cancer overall. In 2018, more than 2 million new cases of breast cancer were diagnosed globally [1]. Traditional Western medicine treats breast cancer with some combination of surgery, chemotherapy and/or radiation therapy. All three of these modalities have side effects and a woman diagnosed with breast cancer must decide which treatments she will use (if any) to prolong her life. The information available to patients is diverse and often contradictory, so this decision is far from simple. Women are advised to decide on their treatment shortly after being diagnosed, when they are emotionally most vulnerable and when they have the least amount of information available to them.

What few medical doctors share with their patients is, that complementary therapies can strengthen the body, promote the destruction of cancerous cells, minimize some of the side effects of chemoradiotherapy, and accelerate recovery following surgery. In this article some of those complementary modalities are presented for the benefit of doctors who treat cancer patients and for patients who want more options.

The complementary modalities that the author presents in this paper fall into five categories based on their function with many of the modalities having multiple functions. Cancer patients may use any of these options with or without conventional Western therapies. For a more comprehensive list of complementary cancer therapies, read Bollinger's book, Cancer: Step outside the box [2].

Remove the source of the cancer & detoxify the body

Source of cancer: While few of us know what caused our cancer, it is important to remove or avoid all possible carcinogens from our external environment. These include chemical pollutants found in air, water, food, cosmetics and household cleaners; mould and other microbes; electromagnetic pollution from wireless technology and electrical/electronic equipment; ionizing radiation; and stress associated with people, finances, etc. Constant stress prevents healing so minimizing stress, both physical and emotional, is critical for recovery.

Dental hygiene: Good dental hygiene is also important for recovery. Metal implants, silver/mercury amalgam fillings and root canals have been linked to illness and these need to be removed/replaced by a qualified holistic or natural dentist who can do it safely with proper follow-up. Mercury is a neurotoxin and leaches into the mouth. These fillings need to be removed safely with appropriate ventilation to protect both dentist and patient. Many chronic diseases are a result of root canal surgery and root canals have been associated with breast cancer [3,4]. Dr. Thomas Rau at the Paracelsus Clinic in Switzerland found that 98.5% of the 150 women he treated for breast cancer had one or more teeth with root canals on the same meridian (Chinese meridian system) as the original breast tumor (Figure 1) [5].

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***Correspondence:**
Magda Havas, Trent School of the Environment, Trent University, Peterborough, ON, K9L 1K3, Canada, E-mail: mhavas@trentu.ca

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Few dentists realize how dangerous root canals are. A root canal is a dead tooth that provides a breeding ground for microorganisms that can survive in an anaerobic environment. The microbes that inhabit the tooth release toxins leading to chronic ill health and an impaired immune system. With the constant release of toxins it is difficult to recover from cancer. Several books are now available on the dangers of root canals including root canal cover-up [3] and the toxic tooth [4].

Detoxification: Once toxins are present in the body it is important to remove them under the supervision of a qualified health care practitioner. Some combination of saunas, colonics, coffee enemas, probiotics, chelating agents, oxygen/ozone therapy, various supplements, and nutrient dense food high in fiber can be used to detoxify the body. The ideal order of detoxing is to start with the colon, followed by cleansing of parasite, kidney, liver/gall bladder, and blood [2].

Support the immune system

A strong immune system helps to eradicate cancerous cells in the body so strengthening the immune system is important. This can be done with high dose Intravenous (IV) vitamin C, ozone and/or oxygen therapy and exposure to the sun or UVB light bulbs for vitamin D production.

Vitamin C: Vitamin C acts as an antioxidant, kills cancer cells, and strengthens collagen which in turn interferes with metastasis [2]. Vitamin C IV (around 50 g per session twice weekly) minimizes fatigue and insomnia and enhances cognitive functioning, which are common side effects of chemotherapy [6]; enhances the cytotoxicity to cancer cells of chemotherapeutic drugs [7]; allows for the use of lower chemotherapeutic concentrations [8]; and reduces risk of mortality from breast cancer [8]. Oral liposomal vitamin C is also beneficial as this form passes through the stomach, is absorbed by the intestines, and released into the liver where it enters the blood stream. The common dosage recommended for oral vitamin C is 1,000 mg/day although dosages as low as 500 mg in combination with 400 mg of vitamin E have been shown to be beneficial [9]. In a meta-analysis that included 10 studies, post-diagnosis dietary vitamin C intake was statistically significantly associated with a reduced risk of total mortality and breast cancer-specific mortality [8].

Ozone: Ozone therapy is used globally but has received very little government support in the US. Doctors, especially those associated with the Society of Progressive Medical Education (SOPMED) hold medical conferences and training sessions on how to apply ozone for various ailments [10]. Because ozone is an oxidizing agent that leads to production of free radicals known to be biologically harmful, one would expect injection of ozone into the body to damage cells and organelles. However, when a small amount of ozone is slowly introduced to the body, it stimulates the production of antioxidants, and these beneficial chemicals repair free radical damage throughout the body.

Ozone can be used as an adjunct to cancer therapy and has numerous beneficial health effects [11], including the following: enhances reduction of free-radicals and strengthens antioxidant activity; increases metabolism of oxygen in the case of tissue hypoxia; facilitates transfer of oxygen trapped in the oxyhemoglobin in red blood cells; activates microcirculation; modulates autoimmune activity with production of cytokines (interferon, tumor necrosis factor, interleukins, etc.); kills microbes, especially those that are anaerobic; reduces pain; reduces inflammation; stimulates tissue regeneration and promotes healing after surgery.

For cancer, the best way to administer ozone is to introduce it into the blood stream by direct IV, major or minor autohemotherapy and rectal or vaginal insufflation. IV injections must be done in a doctor's clinic, although there are now ozone generators for in home use for rectal or vaginal insufflation. Pure oxygen from an oxygen tank needs to be used for ozonation since air has nitrogen compounds that can become toxic during air ionization and these should not be introduced into the body. It is also possible to inject ozone directly into a solid tumor.

Vitamin D & UVB radiation: We live in a society that has been taught to avoid the sun for fear of getting skin cancer. While excessive exposure to the sun may contribute to skin cancer for some, there is mounting evidence that we require UVB exposure and vitamin D to remain cancer-free. Vitamin D is believed to inhibit tumor development and growth and reduce mortality for at least 18 vitamin D-sensitive cancers [12]. For cancer of the breast, colon, rectum, esophagus, other biliary, and vulva the relative risk of mortality is higher than for incidence, possibly suggesting that the maintenance of adequate vitamin D levels is more critical for limiting tumor progression than for preventing tumor onset [13]. Indeed, the health benefits of sunlight appear to outweigh the risk of skin cancer [14]. In northern latitudes a deficiency or insufficiency of vitamin D is common especially during the winter and the lack of vitamin D production from minimal sunlight exposure may be associated with the higher risk of fatal breast cancer [15].

Clearly, vitamin D is another important part of complementary therapy. While the easiest is to eat foods rich in vitamin D (fish, eggs, milk, mushrooms, etc.) or to take vitamin D supplements or cod liver oil, a better method is to be exposed directly to sunlight for 20 min to 30 min daily around noon. In seasons or countries were sunbathing is not possible, exposure to UVB light bulbs at a wavelength of 311 nm promotes the body's ability to produce vitamin D and is especially important for people who are unable to absorb vitamin D from their food and supplements [16]. Full body exposure to a UVB light for a few minutes a day is sufficient to keep vitamin D at optimal levels. Ideally a UVB-narrowband lamp works best for vitamin D production [17] and these are readily available for in home use in countries that do not require a prescription for their use.

A review of the literature on the role of vitamin D in cancer prevention concluded that the majority of studies found a protective relationship between sufficient vitamin D status and lower risk of cancer. The evidence suggests that efforts to improve vitamin D status could reduce cancer incidence and mortality at low cost, with few or no adverse effects [18].

Increase cellular energy

Many of us have sluggish circulation and under-active mitochondrial activity within our cells and when cells don't have enough energy, they can't perform their work. Both Pulsed Electromagnetic Field Therapy (PEMF) and Light Therapy (LT) can help with circulation and cellular energy production and both have been successfully used as an adjunct to cancer therapy.

Pulsed Electromagnetic Field Therapy (PEMF): Pulsed electromagnetic field therapy has been used in countries around the world for decades and has been recently approved in Canada to improve circulation, reduce inflammation and alleviate pain.

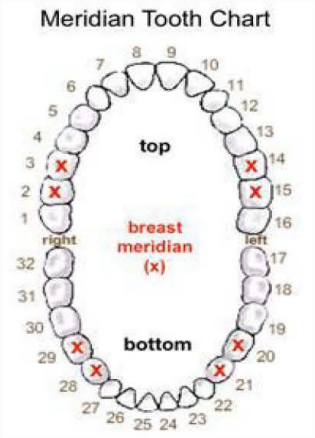

Figure 1: Meridian tooth chart showing teeth (×) that are on the breast meridians.

Many different products (mats, pads, probes, rings, cylinders) are now available for whole body exposure as well as localized exposure and these can be used daily or weekly to minimize the side effects of chemoradiotherapy and to speed recovery following surgery. Some of the technology has a pulse-based biofeedback option (ONDAMED, http://www.ondamed.net/en/) that enables the doctor/operator to select the specific frequencies that a person needs, while others have a built in systems that determines ideal frequencies based on body impedance (SEQEX, http://www.seqex.it/ion-resonance/?lang=en). Many PEMF devices have a bank of frequencies, waveforms, with adjustable intensities and exposure times.

Frequency, waveform, pulse rate, intensity, duration of exposure, dose, time of exposure and the local geomagnetic field are all important factors to consider when using PEMF therapy. Favorable and unfavorable electromagnetic frequencies have been identified for various types of cancers [19] and tumor-specific frequencies have been tested for different tumor types with promising results and no adverse side-effects [20]. In one study the greatest death of malignant breast cancer cells (MCF7) occurred at a PEMF frequency of 20 Hz, at 3 mT power density for 60 min of daily exposure over a three day period with no damage to non-malignant breast tissue [21]. PEMF therapy provides a promising therapy for different types of cancer alone or in combination with conventional and non-conventional (light, heat, diet, etc.) therapies. When used with conventional therapies (surgery, chemoradiotherapy) it minimizes myeloid toxicity and other side effects and enhances quality of life parameters [22,23]. Markov [24] discusses the history of PEMF therapy.

Light Therapy (LT): Light therapy is known by various names including heliotherapy, phototherapy, photo medicine, Photo Bio Modulation (PBM) and low Level Light/Laser Therapy (LLLT). Previous uses were restricted to rickets, tuberculosis, and treatment of various skin conditions, seasonal depression, and neonatal jaundice. During the past twenty years LLLT, especially in the red/infrared part of the visible light spectrum, is becoming a new treatment modality in supportive care for breast cancer. Red/IR wavelengths penetrate deeply into the body. These wavelengths stimulate cellular ATP energy production and increase nitric oxide concentrations, which in turn dilates blood vessels and improves circulation. LT is a non-invasive treatment option used to stimulate wound healing and to reduce inflammation and pain following surgery (mastectomy, lumpectomy, and lymphedema) although the optimal parameters for clinical applications have yet to be determined [25]. LT has been used to minimize side effects associated with radiation and chemotherapy such as oral mucositis, radiodermatitis, hemotherapy-induced peripheral neuropathy, and osteonecrosis of the jaw [26]. Karu [27] reviews the mechanisms of red and near infrared light therapy in mammalian cells and focuses on the role of cytochrome c oxidase, mitochondria and ATP production and signaling.

Both PEMF and LT are available for in home use without the need to visit a clinic for these treatments and the price of lasers and light emitting diodes is becoming more affordable. However, since red light stimulates cellular energy production, use of this directly on solid tumors should be done under the supervision of a doctor who can recommend the dose, duration, light intensity and can follow-up with scanning technology to ensure the light is not promoting cancer growth.

Starve cancer cells by removing their energy source

Glucose: Cancer cells feed on glucose. By reducing to a bare minimum sugars and starches in the diet, cancer cells can be starved of their energy source. Several "low carbohydrate" diets will work including the ketogenic diet, Atkins diet, and Mercola's fat for fuel diet [28]. There is also the Budwig diet [29], Gerson therapy [30] and Majalca's diet and cancer protocol that allows for more carbohydrates but also increases alkalinity in the body [31]. Therefore, a diet low in carbohydrates or one that makes the body more alkaline can facilitate recovery from cancer. Cancer wards that provide fruit juice and cookies to their patients undergoing chemotherapy are doing them a disservice as are doctors who don't provide dietary advice to their patients.

Create a hostile internal environment for cancer cells

Cancer cells prefer an acidic, anoxic environment at body temperature. Consequently, eating a diet or talking supplements that increase tissue alkalinity, increasing the oxygen content of cells, and increasing body temperature (above 42°C) create a hostile internal environment for cancer cells and can augment the cancer killing effects of conventional therapies without damage to healthy cells.

Oxygen: All cells in the body require oxygen and if the oxygen supply is limited, cancerous growths can result, a concept attributed to Warburg, a two-time Nobel Prize winner [32]. When cells don't receive enough oxygen they can morph into cancerous cells that rely on fermentation for their energy source. Consequently, increasing the oxygen content of the body creates unfavorable conditions for cancer development and growth. Oxygen content of tissues and cells can be enhanced using hyperbaric oxygen, ozone therapy and/or mild aerobic exercise. Ozone can be introduced to the body via ozone IV (at a doctor's clinic) or via rectal or vaginal ozone insufflation that can be done at home. The ten-pass ozone systems appear to be the most effective at oxygenating the blood as it provides more ozone than a one-pass system. Low level ozone oxygenates the blood and tissues, increases antioxidant activity in the body, and kills microbes that may contribute to tumor growth.

Hyperthermia: Temperatures above 42°C cause minimal damage to normal cells but are toxic to cancerous cells. Localized or full body hyperthermia provides a hostile environment for cancer growth.

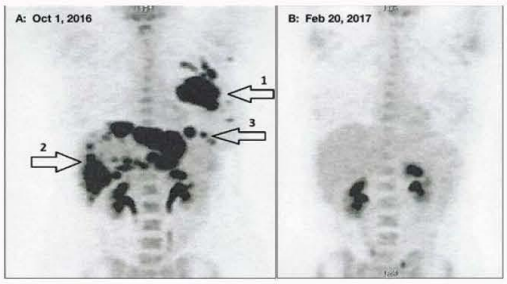

Plate 1: Whole body PET-CT scan of a 29-year-old female with stage 4, triple negative breast cancer. A: Shows a 77 mm × 55 mm primary tomor in the left breast (arrow 1); multiple widespread liver masses (arrow 2); and an upper left nodular abdominal lesion (arrow 3) prior to treatment (Oct 1, 2016); B: Is a follow-up scan 6 months later, Feb 20, 2017, showing complete remission. Source: Iyikesici et al. [38].

Heating can be done using saunas, hot tubs, heating pads or medical devices, like OncoTherm from Hungary that uses a combination of heat and Rife-like radio frequencies to destroy cancerous cells [33]. The American Cancer Society states that hyperthermia is currently being tested for various types of cancer including bladder, breast, cervical endometrial, head and neck, esophagus, kidney, liver, lung, leukemias, melanoma, neuroblastoma, ovarian, pancreas, prostate, sarcoma, and thyroid [34].

In one study [35], the control group-consisting of 105 post-surgery patients were treated with radiotherapy and/or chemotherapy. The hyperthermia group consisted of 35 patients who also received whole-body hyperthermia. Both groups were followed for 70 months. In the control group (105 patients), 12 (11%) died and 61 (58%) developed metastasis within a mean period of 36 months. In the hyperthermia group (35 patients) no patient died and only 3 (9%) developed metastasis within 52 months, clearly documenting the beneficial effects of hyperthermia in combination with traditional therapies.

Data from both preclinical and clinical trials have demonstrated improved antitumor immune responses with the addition of mild hyperthermia. The molecular mechanisms responsible for this improved immune response include the generation of heat shock proteins, the activation of antigens and changes in circulation of lymphocytes [36]. By better understanding the mechanisms involved doctors and scientists can perfect the use of hyperthermia as an adjunct treatment for cancer patients.

Example of integrated therapy to treat stage 4, triple-negative breast cancer

Stage 4 breast cancer-cancer that has spread to other parts of the body – is often lethal with only 22% of women surviving beyond 5 years [37]. Triple negative breast cancer is particularly aggressive, prone to metastasize, and more difficult to treat successfully than other types of cancer because it doesn't have receptor-sites for hormonal therapy.

Here is a case study of an overweight 29-year-old women with Stage 4, triple-negative breast cancer that had metastasized to lymph nodes, liver and abdomen (Plate 1A) [38]. She received integrated therapy consisting of: (1) insulin-potentiated chemotherapy that involved mild hypoglycemia achieved through a 12-hour fast and pharmacological doses of insulin prior to each administration of chemotherapy; (2) hyperthermia with body temperature raised to 45°C (12 sessions for 60 min each, using OncoTherm EHY-3010 HT device); (3) hyperbaric oxygen (12 sessions for 60 min each at 1.5 atmospheres using Quamvis 320 hyperbaric oxygen chamber); and (4) a ketogenic diet. She was told to avoid bread, pasta, rice, potatoes, sugar honey, and fruit; and was encouraged to eat eggs, leafy greens, above ground vegetables, high fat dairy, natural fats, meat, nuts and seeds. It was obvious she adhered to this diet as indicated by urinary ketones and a lower body mass index. This patient recovered after a six-month of treatment (Plate 1B). Two-months later, a mastectomy of the breast (which was not necessary) indicated no live cancer cells. Furthermore, this patient did not experience the adverse effects that are commonly associated with the current standard of care and this improved quality of life should also be considered when treating patients and designing research that compares integrated treatments to traditional therapy.

Conclusion

The purpose of this paper is to introduce the concept that complementary modalities are available to fight breast cancer (and other types of cancers) in addition to conventional treatments consisting of surgery and chemoradiotherapy. These techniques have been shown to be effective in promoting recovery and reducing the side effects of more conventional techniques and thus improving the quality of life for those suffering from cancer. The use of integrative medicine (conventional plus complementary treatments) shows considerable promise for cancer recovery and cancer patients and their families should be made aware of these treatments as should the nurses and doctors working in oncology. In this paper, activities that reduce exposure to cancer-causing elements in the environment, that detoxify the body, that support the immune system, that increase cellular energy, that starve cancer cells, and that create a hostile internal environment for cancerous growth without harming other cells in the body are presented. These activities/treatments include: removal of environmental toxins, removal of dental toxins, saunas to detox and to raise body temperatures, high dose vitamin C IVs, ozone therapy, exposure to natural sunlight or UVB lamps to enhance vitamin D production, hyperthermia, pulsed electromagnetic fields and light therapy to enhance circulation, increase cellular energy, reduce inflammation and pain. Many more complementary modalities are available to fight cancer than those presented in this paper and are provided elsewhere. Emotional support and stress reduction strategies are especially important and these are not discussed here.

Conflict of Interest

The author is not a medical doctor and is not offering medical advice. She is a research scientist and a breast cancer (stage 4) survivor and has used the therapies mentioned in this paper. The information presented here is meant to supplement information provided at a cancer clinic and to provide options for cancer patients to consider. No treatment gives guarantees but the chance of surviving cancer and reducing the side effects of traditional therapies is likely to be enhanced with one or more of the complementary modalities mentioned in this paper.

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Modulated electro-hyperthermia as an immune-modulator with checkpoint inhibitors and radiotherapy

Carrie Anne Minnaar[1], Jeffrey Allan Kotzen[2]

[1] University of the Witwatersrand, Radiobiology, Johannesburg, South Africa.

[2] Wits University Donald Gordon Medical Centre, Radiation Oncology, Johannesburg, South Africa

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Modulated electro-hyperthermia as an immune-modulator with checkpoint inhibitors and radiotherapy

C.A. Minnaar[1]; J.A. Kotzen[2];

[1] *University of the Witwatersrand, Radiobiology, Johannesburg, South Africa.*

[2] *Wits University Donald Gordon Medical Centre, Radiation Oncology, Johannesburg, South Africa*

INTRODUCTION:

Abscopal Effect: Ionizing radiation (IR) is known to occasionally induce a systemic response to local disease, which appears to be mediated by immune responses triggered by IR.

Modulated Electro-Hyperthermia Modulated electro-hyperthermia (mEHT) is a targeted and specialized HT technique which selectively affects the cell membrane of malignant cells, inducing several immunological processes which can be harnessed for the management of malignancies.

Relevant Research There is an ongoing Phase III randomized controlled trial in South Africa investigating the effects of the addition of mEHT to standard chemoradiotherapy protocols for locally advanced cervical cancer (including HIV-positive patients). 54 participants in each treatment group had disease outside of the radiation field before treatment. At six months post-treatment a complete metabolic response of all disease inside and outside of the radiation field was seen in the 13 mEHT group participants (24.1%), compared to 3 control group participants (5.6%), Chi2, Fisher's exact: p=0.013). The effect was seen equally in the HIV-positive and -negative participants.

The authors propose that this is due to the immune modulating effects of mEHT potentiating the abscopal effect of IR. These significant results prompted a literature search for ongoing or completed studies on HT or mEHT, immune checkpoint inhibitors (ICI), and IR.

OBJECTIVE:

To review available literature and studies, including ongoing studies on HT or mEHT, immune checkpoint inhibitors (ICI), and IR, in order to ascertain whether there is a gap in the knowledge which warrants further development of trials/studies on the combination of mEHT and ICI with IR.

METHOD:

We reviewed trials in clinicaltrials.gov and PubMed using the search words "Hyperthermia", "immune checkpoint inhibitors", and "cancer". Ongoing and completed studies were included on ICI-naïve patients from ClinicalTrials.gov. Studies using whole body irradiation or proton therapy were excluded.

RESULTS:

ClinicalTrils.gov: 17 trials met all the criteria except for the inclusion of HT. ICIs used included durvalumab + tremelimumab, nivolumab, ipillimumab, pembrolizumab and atezolizumab. Two studies included HT but not IR. No results included mEHT. No results were returned on HT or mEHT combined with ICI and IR in PubMed, although several reviews detailed the immunological effects of HT and mEHT and the suggested a benefit to its combination with IR and ICIs.

Phase II Studies involving Capacitive RF Hyperthermia

2017	recruiting/ ongoing	pembrolizumab	Mesothelioma	NCT03393858
2017	recruiting/ ongoing	pembrolizumab	Abdominal metastases	NCT03757858

RESULTS Continued:

Observational Studies on ICI and IR

2017	recruiting/ ongoing	Any tumours	metastatic tumours	NCT03042156

Phase I Studies on ICI and IR

2018	not yet recruiting	Ipilimumab Nivolumab	NSLC metastatic	NCT03509584
2018	recruiting/ ongoing	Durvalumab Tremelimumab	Metastatic gyn cancers	NCT03277482

Phase II Studies on ICI and IR

2016	recruiting/ ongoing	Nivolumab	Metastatic renal cell carcinoma	NCT03469713
2016	recruiting/ ongoing	Durvalumab	Metastatic colon and lung	NCT02888743
2016	completed	Ipilimumab	Metastatic melanoma	NCT02406183
2016	recruiting/ ongoing	Pembrolizumab	Metastatic melanoma	NCT02562625
2016	recruiting/ ongoing	Pembrolizumab	Upper GIT metastatic	NCT02830594
2017	recruiting/ ongoing	Tremelimumab Durvalumab	Metastatic	NCT03212469
2018	recruiting/ ongoing	Pembrolizumab	Metastatic unknown primary	NCT03396471
2018	recruiting/ ongoing	Durvalumab Tremelimumab	Metastatic SCC, Head and neck	NCT03283605
2018	not yet recruiting	Tremelimumab Durvalumab	Metastatic bladder	NCT03601455
2018	recruiting/ ongoing	Nivolumab Ipilimumab	Metastatic melanoma	NCT03354962

Phase III/Randomised Studies on ICI and IR

2018	not yet recruiting	Nivolumab\atezolizumab pembrolizumab	NSLC metastatic	NCT03774732

CONCLUSION:

mEHT has a clear and strong immune modulating effect, however it has not yet been investigated combined with ICI and IR.

The results of the South African trial on LACC, the literature on the immune modulating effects of mEHT, and the renewed interest in the abscopal effect induced by ICIs plus IR, strongly motivates the design of a trial on the combination therapy of mEHT, ICI and IR.

The authors declare that they have no conflict of interest.

Repeated treatment with modulated electro-hyperthermia inhibits tumor growth in a triple negative mouse breast cancer model

Lea Danics[1], Csaba Schvarcz[1], Zita Zolcsák[1], Zoltán Benyó[1], Tamás Kaucsár[1], Péter Hamar[1]

[1] Institute of Clinical Experimental Research, Semmelweis University, Budapest

Presented at the 33rd ESHO,Warsaw

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https://oncotherm.com/sites/oncotherm/files/2019-07/Repeated_treatment_with_modulated_electrohyperthermia.pdf

Introduction: The effective therapy of triple-negative breast cancer (TNBC) has not yet been achieved. Modulated electro-hyperthermia (mEHT) is a novel adjuvant antitumor therapy, based on selective tumor cell killing by a 13.56 MHz radiofrequency induced electric field.
Aims: Our aim was to investigate the effects of repeated mEHT treatment in a TNBC bearing mouse model.
Method: 4T07 cells were inoculated orthotopically in female BALB/c mice. Tumor growth was monitored in vivo by digital caliper and ultrasound (Phillips Sonos 5500). The mEHT (n=8) or sham (n=9) treatments started 7 days after inoculation and were repeated 5 times, on every other day. Mice were treated 30 minutes long with 1.0±0.5W power to achieve 40°C skin temperature above the tumor. Mice were euthanized 1 day after the fifth treatment and the tumors were dissected, weighed and processed for histology and molecular biology techniques. The ratio of the damaged area compared to the whole tumor area (Tissue Destruction Ratio, TDR) was evaluated on H&E and cleaved caspase-3 stained sections, while HSP70, a common damage-associated molecular signal, Ki67, a proliferation marker and p21, a tumor suppressor protein expression were analyzed on immunohistochemical staining with the HistoQuant module of the CaseViewer Software (3DHistech).
Results: There was a significant decrease in tumor growth (sham: 5.7x, mEHT: 2.4x relative to pre-treatment (day 6) size, $p<0.0001$) and weight (sham: 288.3±58.1 mg vs mEHT: 85.3±21.3 mg, $p<0.05$) in the mEHT treated group, compared to the sham group. The HSP70 stained area in the non-destructed tumor tissue was 5.2 fold higher in the mEHT treated group, compared to the sham group ($p<0.05$). Moreover, the Ki67 positive nucleus / mm^2 count was significantly lower (sham: 2823.4±211.9 pcs/mm^2 vs mEHT: 1736.7±315.3 pcs/mm^2, $p<0.05$) and the p21 positive nucleus / mm^2 count showed increasing tendency (sham: 127.0±25.3 pcs/mm^2 vs mEHT: 242.2±78.2 pcs/mm^2, p = 0,073) in the mEHT treated group, compared to the sham group.
Conclusion: Our findings suggest, that repeated modulated electro-hyperthermia could inhibit tumor cell proliferation by promoting cell cycle arrest in vivo. Thus, mEHT could be a possible alternative adjuvant therapeutic strategy for TNBC cancer patients.
NVKP_16-1-2016-0042

Data of the presenter
E-mail address: danics.lea@med.semmelweis-univ.hu ; danicslea@gmail.com
oral presentation

Repeated treatment with modulated electro-hyperthermia inhibits tumor growth in a triple negative mouse breast cancer model

Lea Danics[1], Csaba Schvarcz[1], Zita Zolcsák[1], Zoltán Benyó[1], Tamás Kaucsár[1], Péter Hamar[1]
[1] Institute of Clinical Experimental Research, Semmelweis University, Budapest

Introduction

Triple negative breast cancer

- 15% of breast cancer
- Highly aggressive
- High mortality
- Poor clinical outcome
- No targeted therapy

Modulated electro-hyperthermia (mEHT)

- Non-invasive therapeutic option
- Electromagnetic field generated by amplitude modulated 13.56 MHz radiofrequency

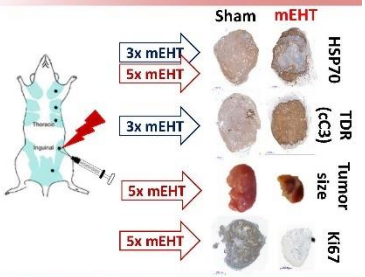
Discussion

3x repeated mEHT treatment induced

- Heat shock response
- Considerable tissue destruction
- Inhibition of tumor growth (4T1)

5x repeated mEHT treatment induced

- Heat shock response
- Decrease of cell proliferation
- Inhibition f tumor growth (4T07)



Methods

Inoculation

10^6 4T1 or 4T07 cells in 50ul PBS
♀ Balb/c mice
4th mammary gland

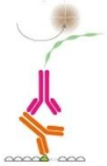
Treatments

LAB-EHY 200
Pole electrode

Measurements

Ultrasound
Digital caliper

Immunohisto-chemistry



Hsp70
cC3

Experimental protocol with 3 treatments

Day after inoculation	0	6	7	8	9	10	11	12
4T07 cell inoculation	x							
Tumor size (US, caliper)		x		X		x		x
mEHT			x		x		x	
Harvest								x

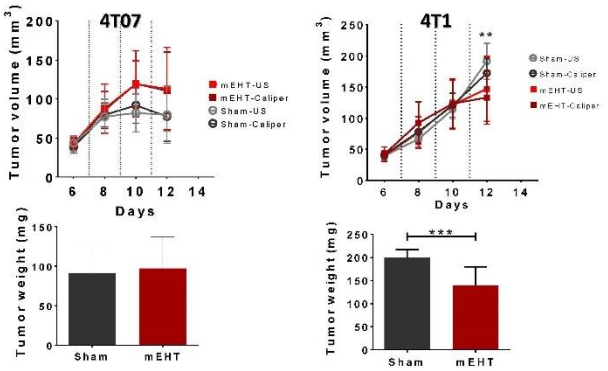
Experimental protocol with 5 treatments

Day after inoculation	0	6	7	8	9	10	11	12	13	14	15	16
4T07 cell inoculation	x											
Tumor size (US, caliper)		x		x		x		x		x		x
mEHT			x		x		x		x		x	
Harvest												x

Results

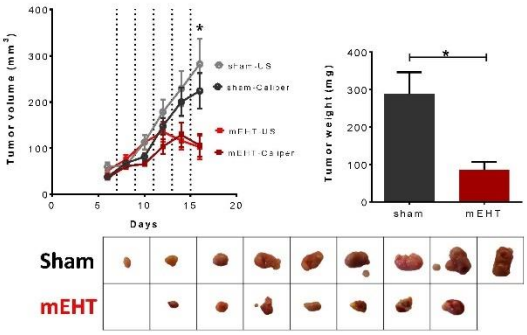
Three treatments

Tumor size changes after 3 mEHT treatments

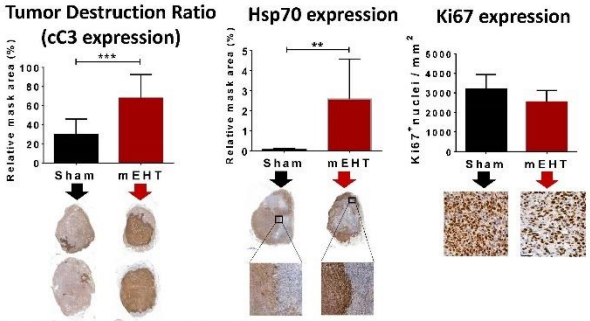


Five treatments

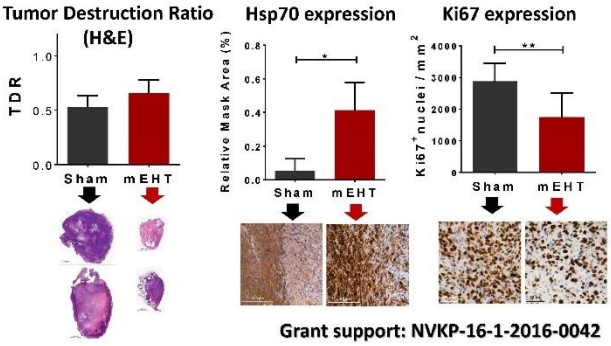
Tumor size changes after 5 mEHT treatments



Molecular changes after 3 mEHT treatments



Molecular changes after 5 mEHT treatments



ESHO 2019, Warsaw, 22-24 May 2019

Grant support: NVKP-16-1-2016-0042

Elevated apoptosis and tumor stem cell destruction in a radioresistant pancreatic adenocarcinoma cell line when radiotherapy is combined with modulated electro-hyperthermia

Gertrud Forika[1], Andrea Balogh[2], Tamás Vancsik[1], Zoltán Benyó[2], Tibor Krenács[1]

[1]Semmelweis University, 1st Department of Pathology and Experimental Cancer Research, Budapest, Hungary

[2]Semmelweis University, Institute of Clinical Experimental Research, Budapest, Hungary

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https://oncotherm.com/sites/oncotherm/files/2019-07/Elevated_apoptosis_and_tumor_stem_cell.pdf

Objective: Malignant exocrine tumors of the pancreas are among the worst to respond to oncotherapy. Despite sophisticated guidelines and new targeted therapies, the 5-year survival rate of patients with pancreatic adenocarcinomas is under 10%. The most critical factor responsible for this
is the high resistance of the tumor cells to the available chemo- or radiotherapies.

Modulated electro-hyperthermia (mEHT) is a complementary non-invasive cancer treatment using impedance-coupled radiofrequency to generate selective heat of <42°C causing cell stress and destruction in malignant tissue. In this study, we tested the combination of radiotherapy with mEHT in a radioresistant pancreatic adenocarcinoma cell line Panc1.

Methods: Panc1 adherent cells grown on coverslips were used to create 3 parallels in 4 groups: control (C); mEHT treated for 60 min (mEHT); irradiated with 2 Gy using ^{137}Cs (R), and combination treatment: irradiation followed by the same dose of mEHT (mEHT+R). 24 hours after treatments we observed the cells morphology, the proportion of apoptotic and necrotic (AnnexinV/propidium iodide positive) cells, the ALDH+ tumor stem cell (CSC), the colony forming capacity of cells, the H2Axγ positivity and the calreticulin presence in the cells. For quantitative and semiquantitative analysis we used: hematoxylin-eosin staining, flow cytometry and immunocytochemistry.

Results: Visible morphological changes were observed after 24 hours in the treated groups: an elevated number of apoptotic bodies and cell number loss. Compared to the control group, the apoptotic ratio was the highest in the mEHT+R group and significant elevation was measured also in the mEHT group. ALDH+ tumor stem cells decreased significantly after mEHT and mEHT+R treated groups compared to the control. As it was expected the irradiated group showed the same amount
of CSC cells as the control group (due to well-known radioresistance of the cell line). The CSCs colony
forming capacity was also significantly lower in the mEHT and mEHT+R group compared to the control group. Furthermore, H2Axγ and calreticulin positive cell fractions, indicating DNA double strand-brakes and ER-stress, respectively, were also significantly increased in the mEHT and the mEHT+R treated groups.

Conclusion: mEHT treatment alone can lead to massive apoptosis in Panc1 cells by inducing cell
stress and DNA double-strand break. Irradiation alone caused some necrosis but without major effect on CSCs. The combined treatment significantly improved the efficacy of radiotherapy resulting in major apoptosis and reduction of CSCs despite of the inherent radioresistance of Pan1.

This study was funded by a grant of the National Research and Innovation Office (NKFIH- NVKP_16-1-2016-0042) in Hungary.

Elevated apoptosis and tumor stem cell destruction in a radioresistant pancreatic adenocarcinoma cell line when radiotherapy is combined with modulated electro-hyperthermia

Presenter: Fórika Gertrúd

Supervisor: Krenács Tibor

Semmelweis University, 1st Department of Pathology and Experimental Cancer Research, Budapest, Hungary

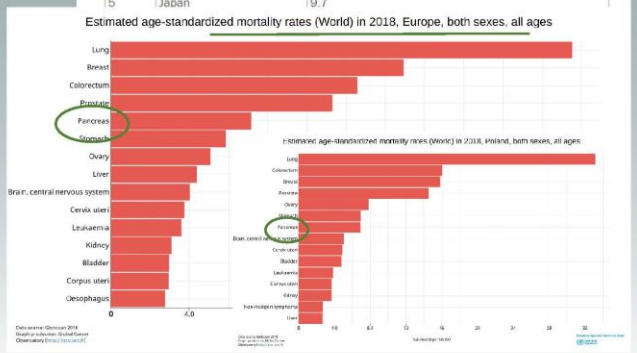
Pancreas adenocarcinomas - statistics

- Hungary had the highest rate of pancreatic cancer in 2018 age-standardised rate per 100,000 (https://www.wcrf.org/dietandcancer/cancer-trends/pancreatic-cancer-statistics)
- Pancreatic cancer is the 5th common cause of death in Europe (https://gco.iarc.fr/today/home)
- The 5 year survival rate is under 10% (https://seer.cancer.gov/statfacts/html/pancreas.html)

Pancreatic cancer rates: both sexes

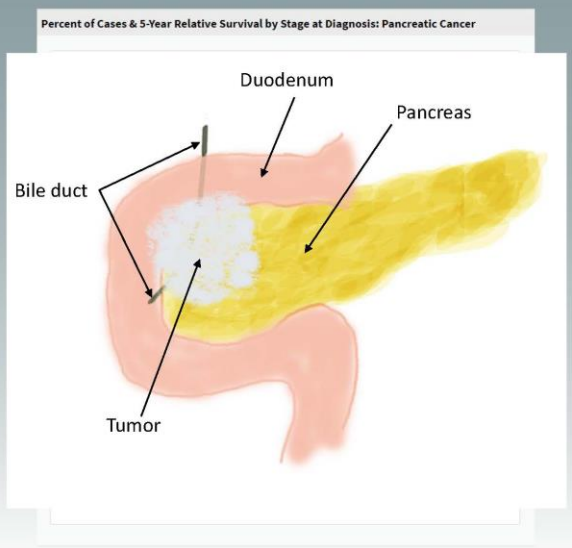
Hungary had the highest rate of pancreatic cancer in 2018, followed by Uruguay.

Rank	Country	Age-standardised rate per 100,000
1	Hungary	10.8
2	Uruguay	10.7
3	Moldova	10.5
3	Latvia	10.3
5	Japan	9.7



Pancreas adenocarcinomas - treatment

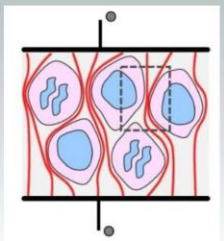
- The cancer stages at diagnosis are not promising [1]
- Just 10% of patients diagnosed with pancreatic cancer in England during 2013-2014 had surgery to remove their primary tumour, as part of their primary cancer treatment [4]
- Commonly used chemotherapy drugs:
  - Paclitaxel
  - 5FU (5 Fluorouracil)
  - Gemcitabine Hydrochloride
  - Irinotecan
- Combination: FOLFIRINOX
- Targeted therapy: Erlotinib (Tyrosine Kinase Inhibitor)
- Radiotherapy
- Radiochemotherapy

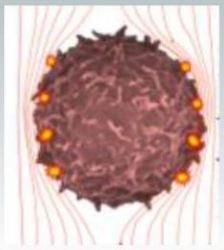


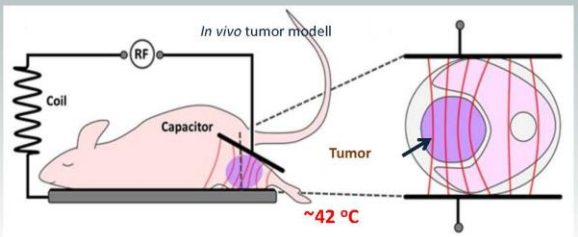
(4) National Cancer Registration & Analysis Service and Cancer Research UK: "Chemotherapy, Radiotherapy and Tumour Resections in England: 2013-2014"

Modulated electro-hyperthermia

- Regional – deep tissue hyperthermia
- Complementer therapy to radio- or chemotherapy
- Non invasive
- 13.56 MHz radiofrequency -> electric field=> 42°C heat
- Selective: eleveated glycolysis, ion concentration and conductibility (Warburg efect)







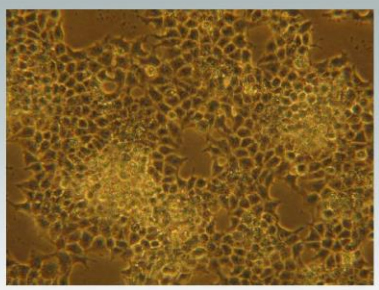
Material and methods

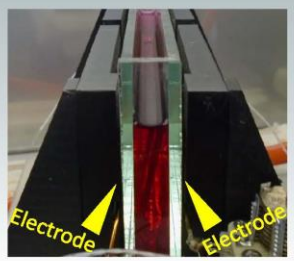
Cell line: Panc1

Isolated from a ductal adenocarcinoma,
56-year-old male in 1975
Epithelial morphology
High tumorigenicy

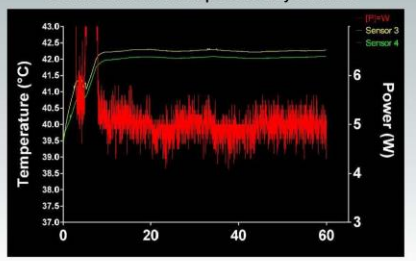
Radiotherapy

mEHT treatment

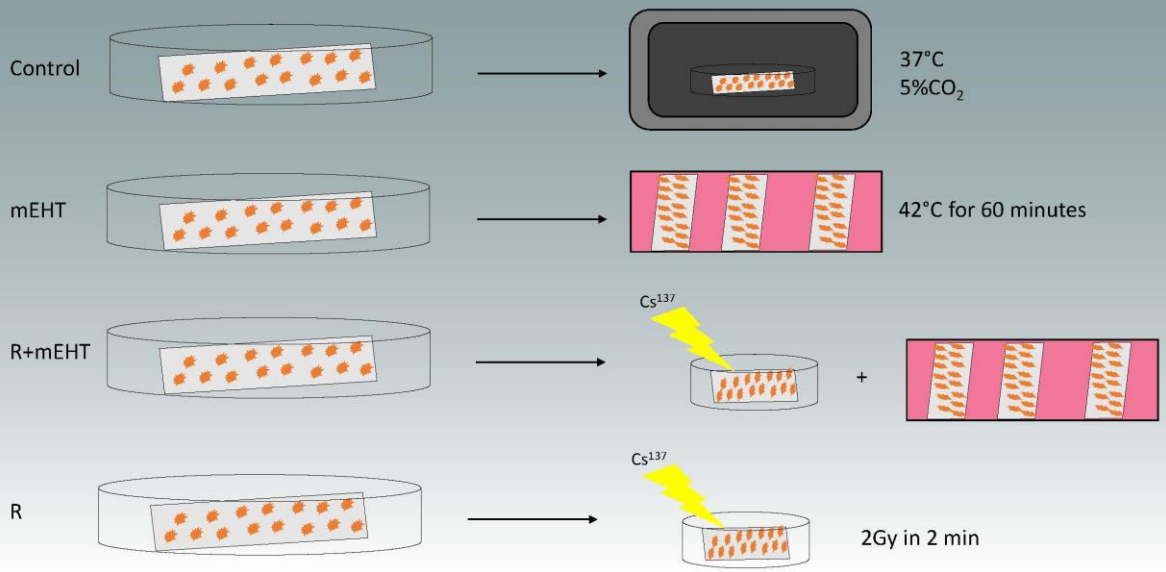


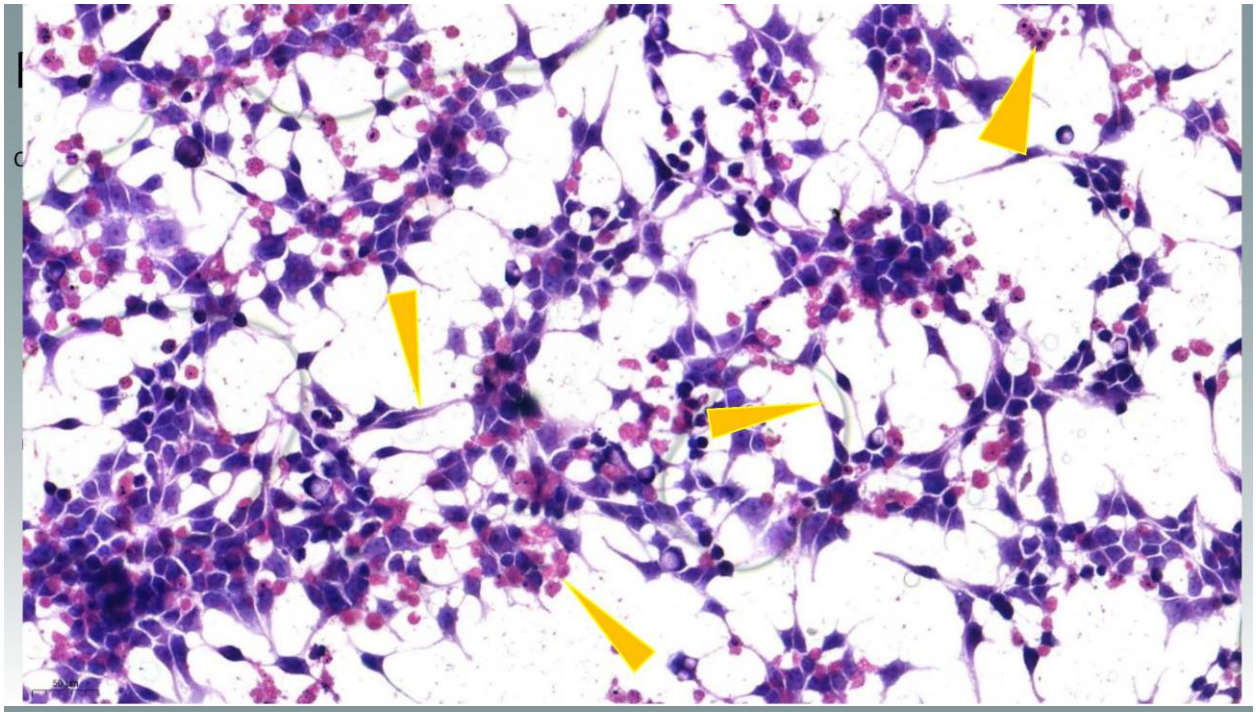


Heat controll with power adjustment



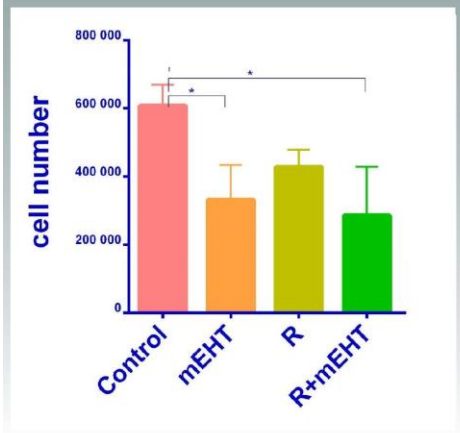
Methods: treatment protocol



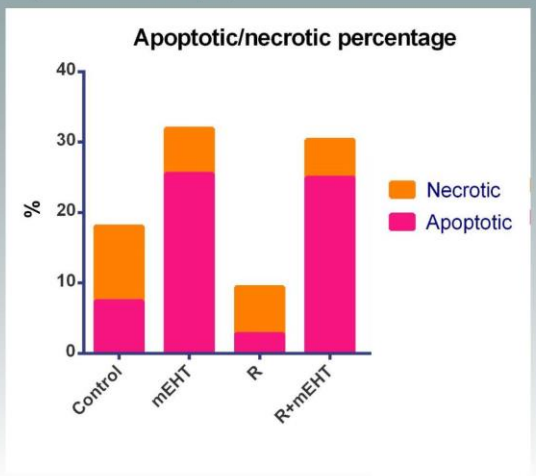


Results: apoptosis/necrosis

Cell numbers counted with trypan blue dye after treatments



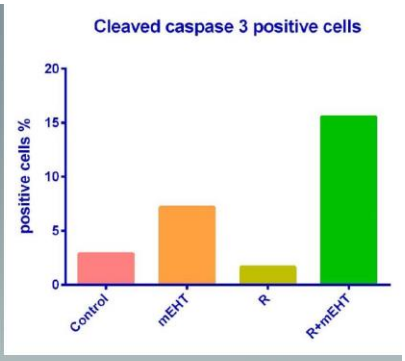
Apoptotic/necrotic rate analysed with flow cytomerty. Used staining: Annexin V and propidium iodide

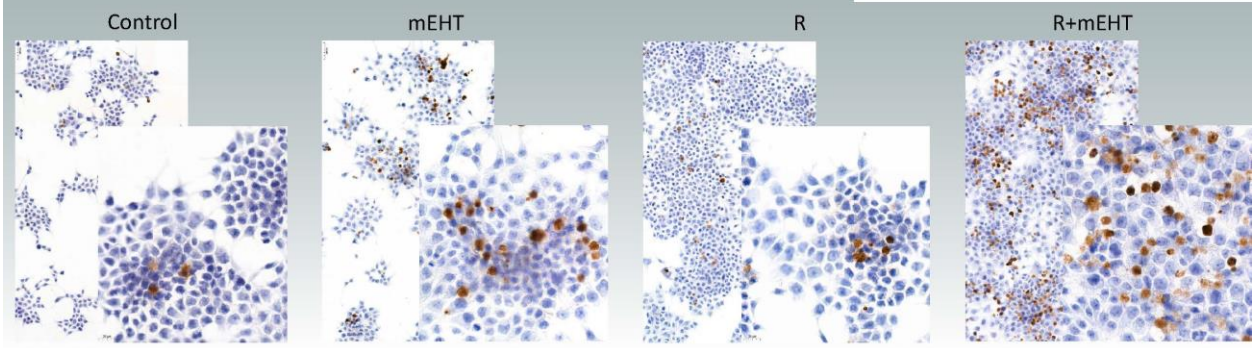


Results: apoptosis

Cleaved caspase 3 positivity

Presumably the apoptosis is caspase 3 dependent. The immunocytochemistry reaction showed many positive nuclei in the mEHT treated groups.

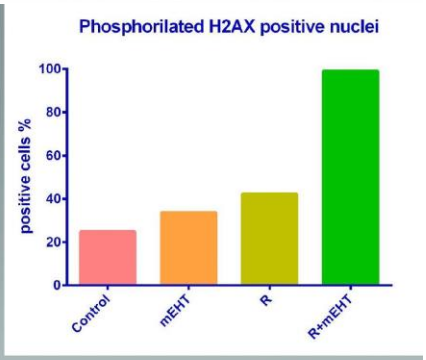


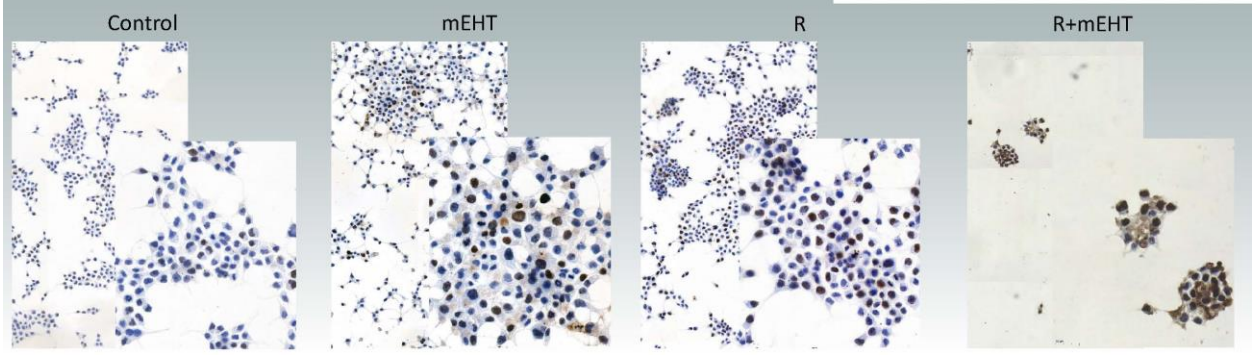


Results: DNA double strand breaks

Phosphorilated H2AX positivity

The phosphorilated H2AX is a marker of DNA double strand breaks

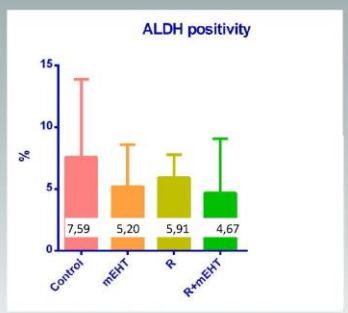




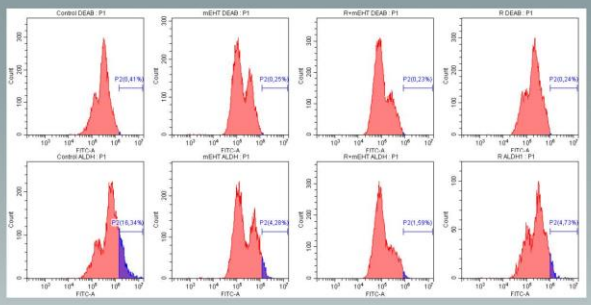
Results: tumor progenitor cells

ALDH+

ALDH – aldehyde dehydrogenase is highly expressed by tumor progenitor cells

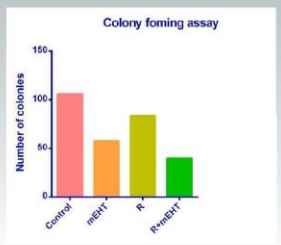


Percentage of ALDH positive cells →



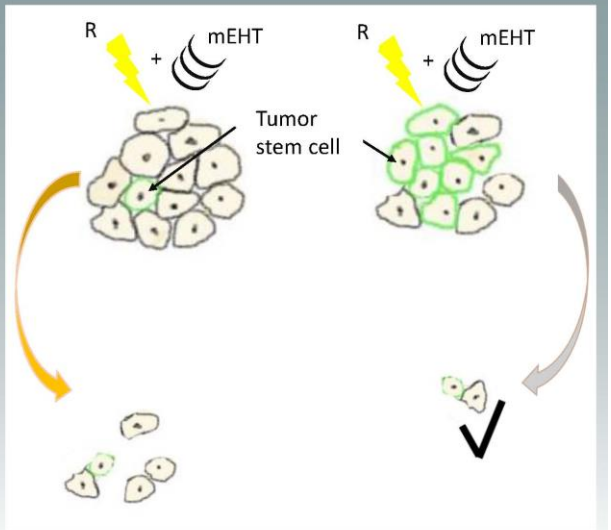
Colony forming capacity

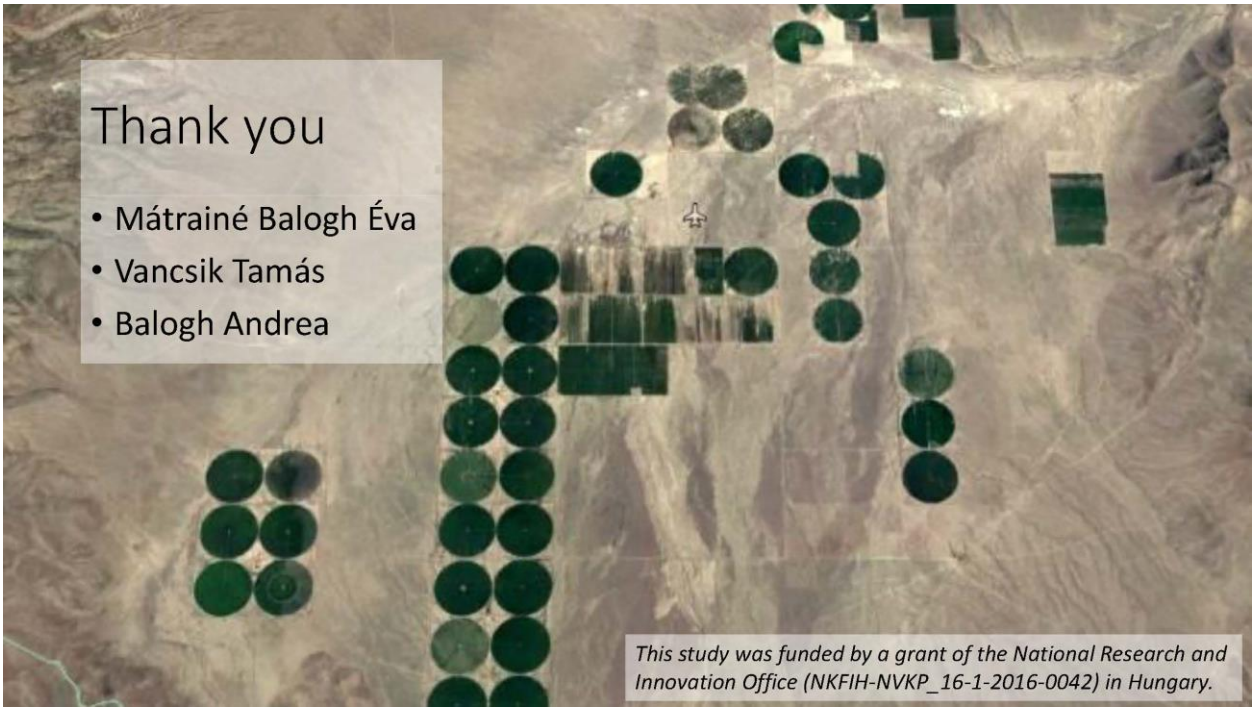
Tumor progenitor cells has the capacity to form colonies



Conclusion

- 60 minutes mEHT can lead to a massive apoptosis
- Combined with radiotherapy, mEHT potentiate the effectivity of the treatment
- Tumor stem cells are sensitive for mEHT or for combined treatment despite of their inherent radioresistance
- The mEHT treatment leads to caspase dependent apoptosis
- Presumably the mEHT has negative effect on DNA repair




Thank you
• Mátrainé Balogh Éva
• Vancsik Tamás
• Balogh Andrea
This study was funded by a grant of the National Research and Innovation Office (NKFIH-NVKP_16-1-2016-0042) in Hungary.

Modulated Electro-Hyperthermia As Additional Treatment Option In Oncology - Feasibility Study On Quality-Of-Life, Imaging And Biomarkers To Support Treatment Planning

Karaszi Adam, Szasz A. Marcell, Garay Tamas, Borbenyi Erika, Lorant Gergo, Szentmartoni Gyongyver, Huszti Zsolt, Dank Magdolna

Cancer Center, Semmelweis University, Budapest, Hungary

Presented at the 33rd ESHO,Warsaw

Cite this article as:
Karaszi A. et al. (2019): Modulated Electro-Hyperthermia As Additional Treatment Option In Oncology - Feasibility Study On Quality-Of-Life, Imaging And Biomarkers To Support Treatment Planning, Oncothermia Journal 26:99-101
https://oncotherm.com/sites/oncotherm/files/2019-07/Modulated_ElectroHyperthermia_As_Additional_Treatment.pdf

Background: Hyperthermia is composed of a wide variety of treatment methods which ranges from physiotherapy to oncology and can have various physical and technical background. Modulated electro-hyperthermia (mEHT) is a new kind of hyperthermia in oncology.

Methods and Aim : We designed a thorough investigation on multiple aspects of mEHT cancer therapy. We present the design of our study. Our objective in this presentation is to summarize our experience on the first 20 patients enrolled in our study investigating imaging by PET/CT scans, blood-based markers of the immune system, quality-of-life questionnaires and patient outcomes.

Results : The essence of the clinical study design will be presented and the cohort will be reported with primary and metastatic tumors treated with mEHT (grouped into carcinomas of organ systems, and sarcomas of bone and soft tissues), FACS, QoL and radiographic findings in patient cohorts will be shown as starting points of this investigation.

Conclusion : Based on clinical studies, the method mEHT is a feasible hyperthermia technology for oncological applications. Concomitant utilization of capacitive hyperthermia is now supported by the outcome data and we look forward to adjust the treatment protocol and establish predictive markers from blood, tissue and other clinicopathological parameters.

Funding
KNN121510 and NVKP_16-1-2016-0042 by the National Research, Development and Innovation Office of Hungary.

Modulated electro-hyperthermia as additional treatment option in oncology : feasibility study on quality-of-life, imaging and biomarkers to support treatment planning

Adam Karaszi, Marcell A Szasz, Tamas Garay, Erika Borbenyi, Gergo Lorant, Gyongyver Szentmartoni, Zsolt Huszti, Magdolna Dank

Cancer Center, Semmelweis University, Budapest, Hungary

Background

Hyperthermia is composed of a wide variety of treatment methods which ranges from physiotherapy to oncology and can have various physical and technical background. Modulated electro-hyperthermia (mEHT) is a current development among the conventional heating methods applied in oncology. mEHT as a complementary treatment has gained support in the treatment of cancer patients as supplementary method besides the standard treatment or for those who exhausted conventional treatment.

Aim and Methods

We designed a thorough investigation on multiple aspects of mEHT cancer therapy. We present the design of our study. Our objective in this presentation is to summarize our experience on the first 30 patients enrolled in our study investigating imaging by PET/CT scans, blood-based markers of the immune system, quality-of-life questionnaires and patient outcomes.

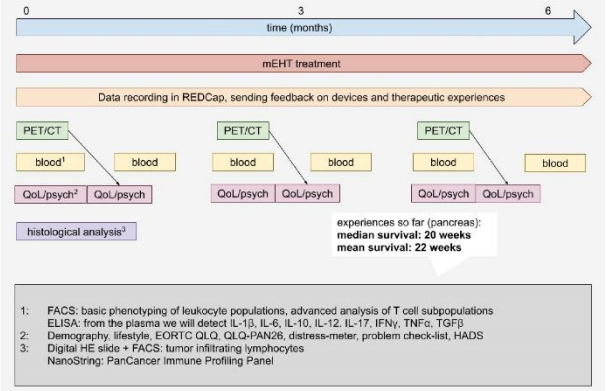


Figure 1. Study design

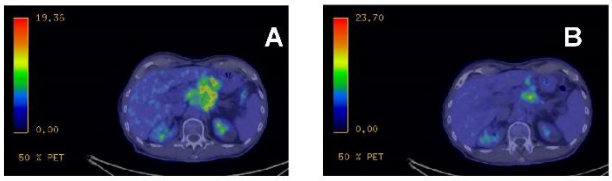


Figure 2. Pre (A) and post treatment (B) PET/CT scans

Effect of treatment is followed by multiple PET/CT scans. In this case the first scan (A) in January 2019 showed avid FDG-uptake in the primary pancreatic tumor (7,5 cm max diameter, SUVmax: 9,6). The post treatment scan (B) in April 2019 showed moderate regression with decreased FDG-uptake and decreased size in the pancreatic lesion (3,6 cm max diameter, SUVmax: 8,3).

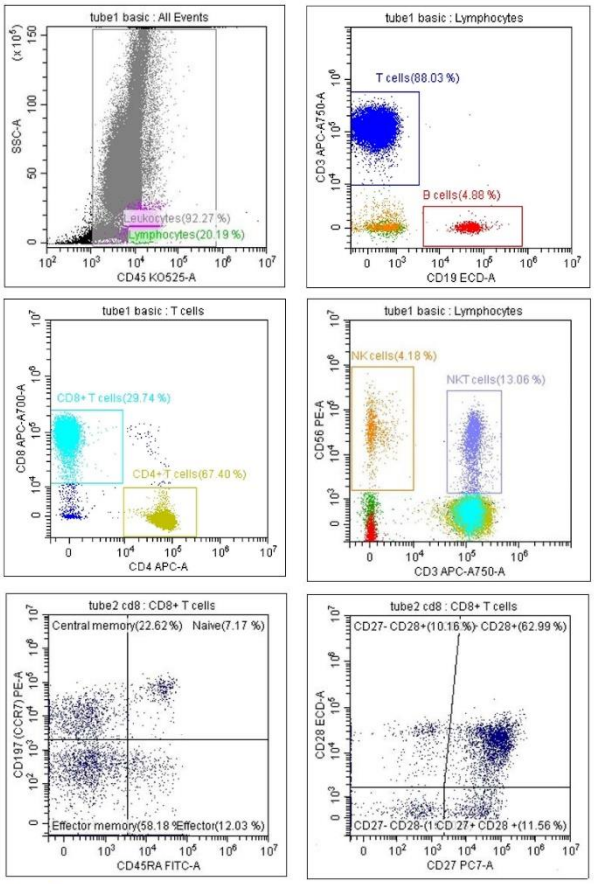


Figure 3. Flow cytometry immunophenotyping

Results

The essence of the clinical study design will be presented and the cohort will be reported with primary and metastatic tumors treated with mEHT (grouped into carcinomas of organ systems, and sarcomas of bone and soft tissues), FACS, QoL and radiographic findings in patient cohorts will be shown as starting points of this investigation.

Conclusions

Based on clinical studies, the method mEHT is a feasible hyperthermia technology for oncological applications. Concomitant utilization of capacitive hyperthermia is now supported by the outcome data and we look forward to adjust the treatment protocol and establish predictive markers from blood, tissue and other clinicopathological parameters.

Acknowlegements

The study was supported by the NVKP_16-1-2016-0042 grant of the National Research, Development and Innovation Office of Hungary.

Effects of modulated electro-hyperthermia on disease control and 3 year disease-free survival in locally advanced cervical cancer patients: Preliminary results

Carrie Anne Minnaar[1]; Ans Baeyens [2]; Jeffrey Allan Kotzen[2]

[1] University of the Witwatersrand, Johannesburg, South Africa

[2] Ghent University, Ghent, Belgium

[3] Wits University Donald Gordon Medical Centre, Johannesburg, South Africa

Presented at the 33rd ESHO,Warsaw

Cite this article as:
Minnaar C.A. et al. (2019): Effects of modulated electro-hyperthermia on disease control and 3 year disease-free survival in locally advanced cervical cancer patients: Preliminary results, Oncothermia Journal 26:102-113
https://oncotherm.com/sites/oncotherm/files/2019-07/Effects_of_modulated_electrohyperthermia.pdf

Introduction: Modulated electro-hyperthermia (mEHT) proposes an alternative, feasible, method to the conventional thermo-monitoring techniques currently applied in HT. Literature also suggests that mEHT induces immunological processes which can be harnessed for the management of malignancies. For these reasons mEHT was selected for investigation in a resource-constrained setting with high-risk patients in South Africa. We present preliminary results on the effects of mEHT in our ongoing phase III randomised controlled trial on disease control disease-free survival (DFS) in locally advanced cervical cancer (LACC) patients.

Methods: Participants were randomised to receive mEHT with/without chemoradiotherapy (CRT). mEHT was administered 2/week using 13.56MHz capacitive heating (planned power of 130W; 55 minutes) before external beam radiation. Disease control and disease pattern were assessed by 18F-FGD PET/CT at six months post-treatment. Participants were followed up every three months in the first year and every six months thereafter. Regression models were used to assess DFS.

Results: 165 participants have reached two years post-treatment (mEHT=68; Control=97), of whom 66 mEHT and 94 Control group participants were eligible for six month local DFS analysis. Six month local DFS was significantly higher in the mEHT group: 25(38%) than in the control group: 20(21%) (p=0.21). In participants eligible for analysis at two years, two year DFS was significantly higher in the mEHT group (52% (33 out of 64)) than in the Control Group (34% (31 out of 91)) (HR: 1.60; p=0.033). In the participants who have reached three years post-treatment, three year DFS is also significantly higher in the mEHT group: 58% (18 out of 31) versus 35% (16 out of 46) (HR: 1.97; p=0.042). A subgroup analysis of participants with extra-pelvic disease visualised on pre-treatment scans showed complete metabolic response of all disease was higher in the mEHT group (13 out of 54; (24.1%)) than in the Control Group (3 out of 54 (5.6%)) (p=0.013).

Conclusion: Preliminary results continue to show a positive trend in local control and disease free survival benefit in the participants treated with mEHT combined with CRT versus participants treated with CRT alone. The novel finding of a significant reduction in disease outside the radiation field in the mEHT Group suggests the potentiation of the abscopal effect by mEHT and future studies should be designed to investigate this further.

ESHO 2019 33RD ANNUAL MEETING OF THE EUROPEAN SOCIETY FOR HYPERTHERMIC ONCOLOGY

Effects of modulated electro-hyperthermia on disease control and 3 year disease-free survival in locally advanced cervical cancer patients: Preliminary results

C.A. Minnaar[1]; A. Baeyens [2]; J.A. Kotzen[3];

1) University of the Witwatersrand, Radiation Sciences: Radiobiology, Johannesburg, South Africa.

2) Ghent University, Basic Medical Sciences, Ghent, Belgium.

3) Wits University Donald Gordon Medical Centre, Radiation Oncology, Johannesburg, South Africa.

22-24 May 2019
WARSAW

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Disclosures

The authors confirm that they have no financial disclosures which may result in a conflict of interest

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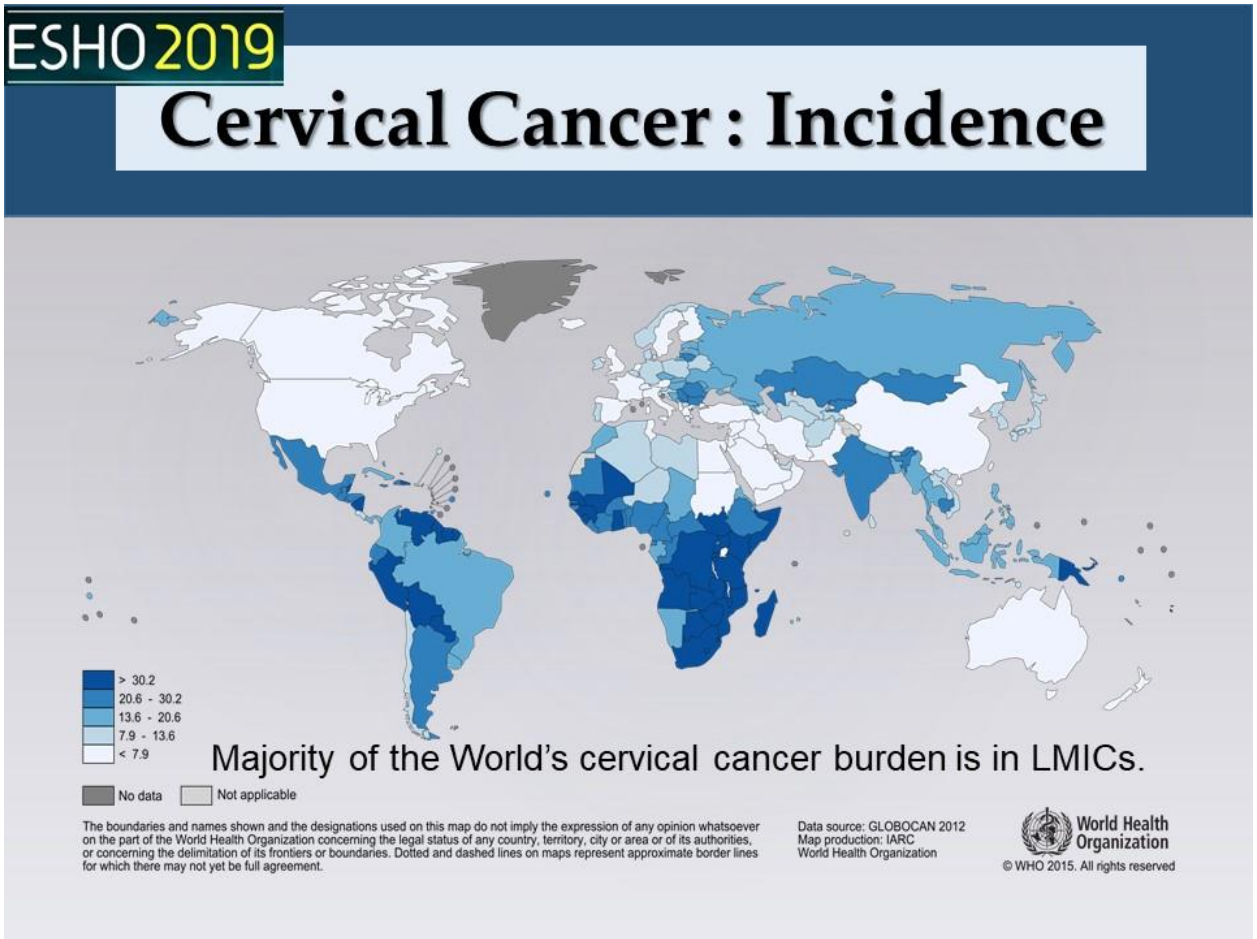
ESHO 2019
Cervical Cancer : Incidence
> 30.2
20.6 - 30.2
13.6 - 20.6
7.9 - 13.6
< 7.9
Majority of the World's cervical cancer burden is in LMICs.
No data
Not applicable
The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.
Data source: GLOBOCAN 2012
Map production: IARC
World Health Organization
World Health Organization
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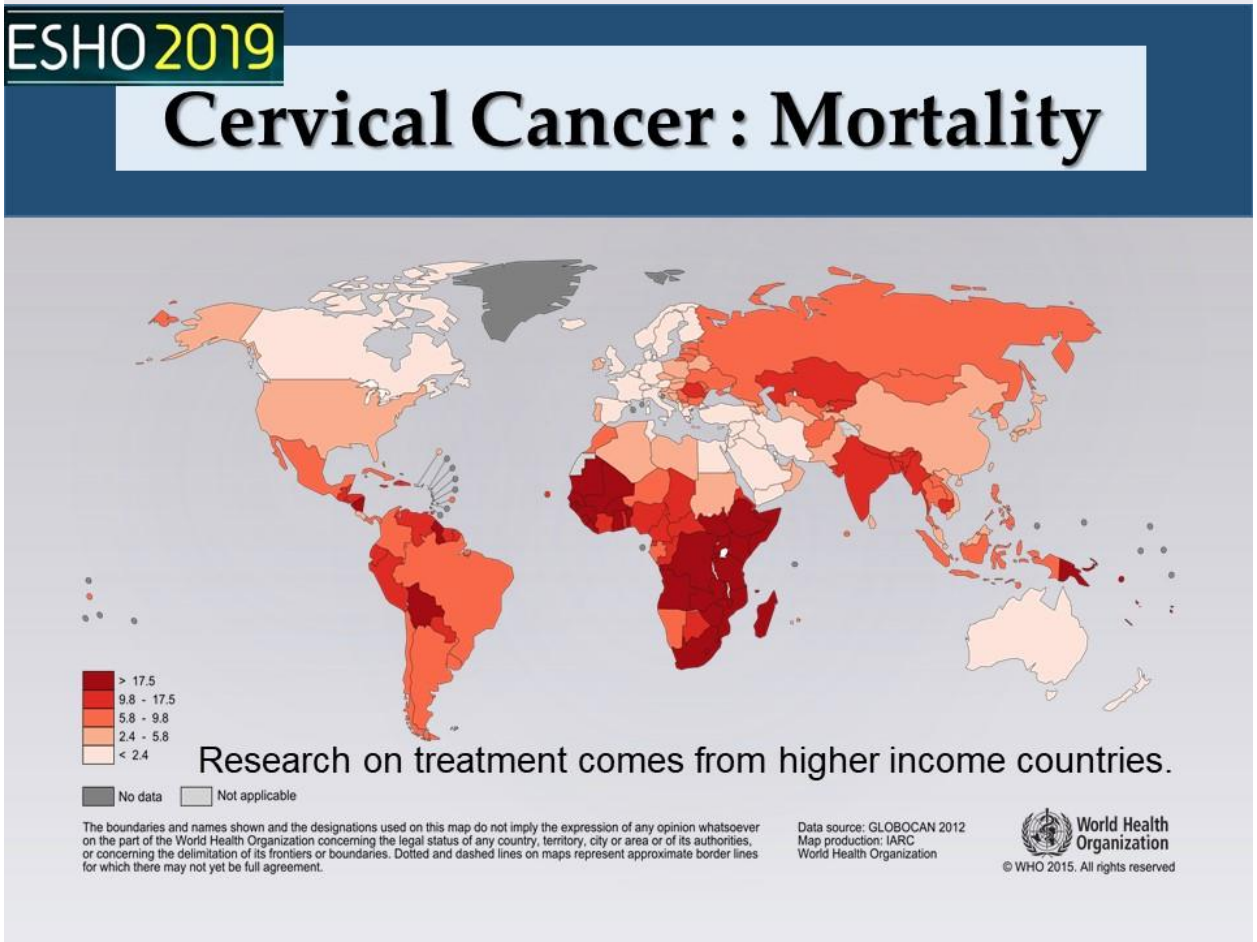
ESHO 2019
Cervical Cancer : Mortality
> 17.5
9.8 - 17.5
5.8 - 9.8
2.4 - 5.8
< 2.4
Research on treatment comes from higher income countries.
No data
Not applicable
The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.
Data source: GLOBOCAN 2012
Map production: IARC
World Health Organization
World Health Organization
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ESHO 2019

Introduction

85% cervical cancer diagnoses and

87% ca cx related death occur in LMICs.

Out of 54 countries in Africa:

28 countries do not have radiation equipment

Only 20 have access to Brachytherapy(BT)

Where RT is available – often low energy (Cobalt60)

No imaging techniques available =

2D planning and no image guided BT.

International protocols cannot be applied.

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Introduction

- Modulated electro-hyperthermia (mEHT) uses capacitive heating with RF of 13.56MHz.
- Proposes a dose concept which relies on energy rather than the measurement of temperature.
- Temperature is calculated by the device using forwarded/absorbed power, based on phantom measurements.
- Therefore treatments can safely be given without invasive thermometry.
- Literature suggests that mEHT induces immunological processes.

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Rationale

The proposal that the technique does not require temp. monitoring is what attracted us given our setting.

Lee et al (2018) confirmed Temp. ↑ using mEHT on ca cx patients

Is the dose concept effective?

- First HT study on HIV-positive patients.
- First HT study in a resource constrained setting.
- First HT study from Africa.
- First phase III study investigating the efficacy of the mEHT method of dose calculation.

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Aim

To present preliminary results on three year survival rates from the ongoing phase III randomised controlled trial on local disease control in locally advanced cervical cancer patients treated with mEHT and chemoradiotherapy

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Methods

Criteria

- FIGO stage IIb to IIIb cervical cancer
- HIV-positive and –negative
- CD4 count >200cells/mL OR on ARVs> 6 months
- Bilateral hydronephrosis / creatinine clearance <60mL/min excluded

Randomisation

- CRT +/- mEHT using online RedCap randomization tool
- Stratum: HIV status; Age; Stage

LDC measured at 6 months using 18F-FDG PET/CT

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Methods

Treatment

- 50Gy EBRT (25 fractions)
- 3 fractions of 8Gy HDR BT
- Cisplatin (planned 2 doses of 80mg/m2)

mEHT group:

- 2 x mEHT / week
- 13.56MHz
- Minimum of 55 minutes
- Planned power of 130W
- Planned calculated temperature of 42.5ºC
- Step up heating
- Immediately before EBRT

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Results

mEHT		Control	
HIV- 53%	HIV+ 47%	HIV- 46%	HIV+ 54%
RT Mean: 81.7 Gy Cisplatin: (mean 1.3) 15% had 0 dose, 39% had 1 dose, 46% had 2 doses Mean 8.9 mEHT treatments 86% had 8 or more mEHT		**RT Mean: 82.2Gy** Cisplatin: (mean: 1.2) 18% had 0 dose, 43% had 1 dose, 39% had 2 doses	

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Results

Two years post-treatment (mEHT=68; Control=97)

Eligible for 6 month Local disease-free survival

mEHT: 25 out of 66 (38%)

Control: 20 out of 94 (21%)

(p=0.21)

Complete results accepted for publication in PlosOne

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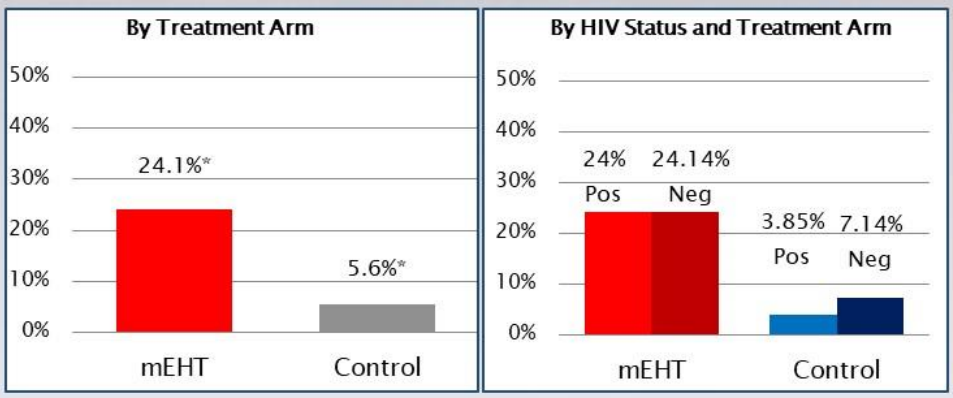
Results

Analysis of extra-pelvic lymph node response

mEHT: 13 out of 54; [24.1%] Control: 3 out of 54 [5.6%] *(p=0.013).*

Participants with ALL Disease Resolved



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Results

TOXICITY

Evaluated toxicity for the following systems based on CTCAE 4.0

- Dermatological
- Urogenital
- Upper GI
- Lower GI
- Hematological

mEHT specific toxicity:

- Grade I Adipose tissue burns: 9.5%
- Grade I surface burns 2.0%
- Pain: 8.6%

A multivariate analysis showed that HIV status; BMI and KJs were not predictive of adverse events associated with mEHT treatments.

QoL: pain, social and emotional functioning improved in mEHT group

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Conclusion

- Improved rates of LDC at six months in patients treated with mEHT.
- Preliminary results continue to show a positive trend in disease-free survival benefit at three years.
- The novel finding of a significant reduction in disease outside the radiation field in the mEHT Group suggests the potentiation of the abscopal effect by mEHT and future studies should be designed to investigate this further.

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Future prospects

The ethics committee has extended the survival follow up period of the current study to five years.

Further trials on diseases common in LMICs are either underway/planned

Studies have been designed to investigate the addition of mEHT to radiotherapy plus Immunotherapies

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Acknowledgements

- Funding was obtained from the National Research Foundation of South Africa.
- 18F-FDG was supplied at a discounted rate by Radioisotopes SOC Ltd.
- HT device was supplied for the research purpose by Oncotherm GmbH.
- We thank all our colleagues in the radiation oncology and nuclear medicine department.
- Thank you to all of the participants of the study whose fierce bravery was indeed an inspiration to us all.

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THE EUROPEAN SOCIETY
FOR HYPERTHERMIC ONCOLOGY
22-24 May 2019
WARSAW

Thank You

Short term treatment with modulated electro-hyperthermia in triple negative breast cancer bearing mice

Csaba Schvarcz[1], Lea Danics[1], Tamás Kaucsár[1], Tamás Vancsik[2], Tibor Krenács[2], Zoltán Benyó[1], Péter Hamar[1]

[1] Institute of Clinical Experimental Research, Semmelweis University, Budapest, Hungary
[2] 1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary

Presented at the 33rd ESHO,Warsaw

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Introduction: The effective therapy of triple-negative breast cancer (TNBC) has not yet been achieved. Modulated electro-hyperthermia (mEHT) is a novel adjuvant antitumor therapeutic approach, based on the highly selective effects on tumor cells by a 13.56 MHz radiofrequency current promoted electric field.

Aims: Our aim was to investigate the effects of mEHT on a triple-negative mammary carcinoma bearing mouse model.

Method: 4T1 cells transfected with firefly luciferase were inoculated orthotopically in immunocompetent female Balb/c mice. The isografts were treated with mEHT on day 3 and 5 after inoculation. Treatments were performed with 1.0±0.5W power to reach 40°C of the skin above the tumor, treatment length was 30 minutes. Tumor growth was measured in vivo by IVIS Lumia system (Perkin Elmer), digital caliper and ultrasound (Phillips Sonos 5500) before and after treatments. Mice were euthanized on day 7 after inoculation and the tumors were harvested and processed for histology. The ratio of the damaged area compared to the whole tumor area (Tissue Damage Ratio, TDR) was evaluated on H&E stained sections, while HSP70 was analyzed on immunohistochemical staining with HistoQuant module of CaseViewer Software (3DHistech).

Results: There was a significant decrease in tumor growth measured by IVIS in the mEHT treated group (mEHT: 7.1*108 p/s vs sham: 13.5*108 p/s, $p<0.05$), which couldn't be detected by the less sensitive caliper (mEHT: 51.17±4.86 mm3 vs sham: 52.36±10.25 mm3, $p=0.93$) and ultrasound (mEHT: 73.29±22.18 mm3 vs sham: 74.87±7.95 mm3, $p=0.52$) measurements. Furthermore, the tumor damage ratio (fold change: 4.1, $p<0.05$) and the HSP70 positive signal around the damaged area (fold change: 12.9, $p<0.05$) was significantly higher in the mEHT treated group compared to the sham group.

Conclusion: Our findings suggest, that modulated electro-hyperthermia could be a possible alternative adjuvant therapeutic strategy for TNBC cancer patients. We plan next generation sequencing to elucidate the molecular mechanisms behind the effects of mEHT.
NVKP_16-1-2016-0042

Data of the presenter
E-mail address: schvarcz.csaba@med.semmelweis-univ.hu ; csaba.schvarcz@gmail.com
oral presentation

Short term treatment with modulated electro-hyperthermia in triple negative breast cancer bearing mice

Csaba Schvarcz, Lea Danics, Zita Zolcsák, Zoltán Benyó, Tamás Kaucsár, Péter Hamar
Institute of Clinical Experimental Research, Semmelweis University, Budapest

Introduction

Triple negative breast cancer:

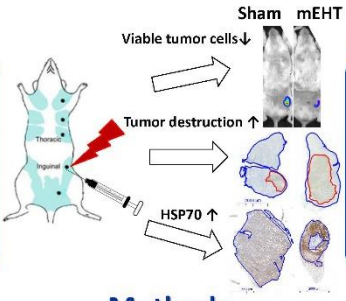
- 15% of breast cancer
- Highly agressive
- High mortality

poor clinical outcome

- No targeted therapy

Modulated electro-hypertermia (mEHT)

- Non-invasive therapeutic option
- Electromagnetic field generated by amplitude modulated 13.56 MHz radiofrequency.



Discussion

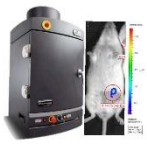
2x mEHT treatment induced:

- Decrease in the number of viable tumor cells (IVIS)
- Significant damage in the tumor tissue (TDR)
- Heat shock response (HSP70)

mEHT: a new, adjuvant treatment for TNBC patients.

Methods

4T1 cells (10^6/50 µl Matrigel:PBS)
♀ Balb/c mice
4th mammary gland



Fluorescent, viable cells
In Vivo Imaging System (IVIS)

Treatments
Pole electrode
Lab-EHY200

Tumor size measurement
Ultrasound
Digital caliper

Immunohisto-chemistry
HSP70, cC3

Day after inoculation	0	3	5	7
4T1 cell inoculation	x			
IVIS	x	x	x	x
Tumor size (US, caliper)		x	x	x
mEHT			x	
Harvest				x

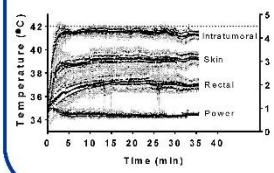
Experimental protocol, 1 treatment

Day after inoculation	0	3	4	6	7
4T1 cell inoculation	x				
IVIS		x			x
Tumor size (US, caliper)					x
mEHT			x	x	
Harvest					x

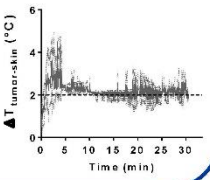
Experimental protocol, 2 treatments

Results

Temperatures of the tumor, skin and rectum

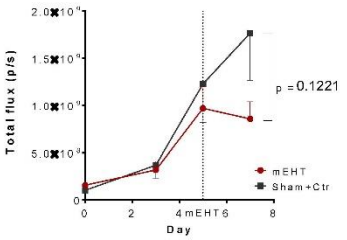


Temperature gradient btwn skin and tumor

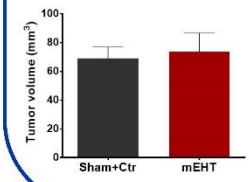


One treatment

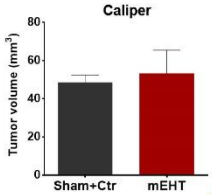
Number of viable cells measured by IVIS



Ultrasound

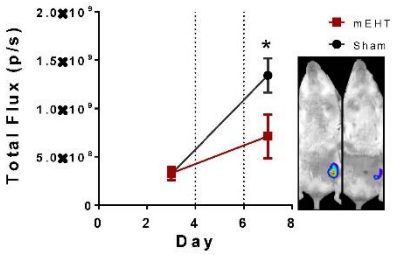


Caliper



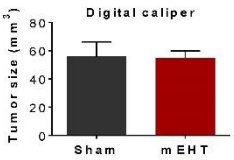
Two treatments

Viable cells measured by IVIS

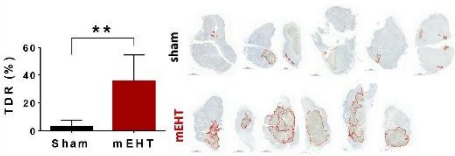


Tumor size and weight

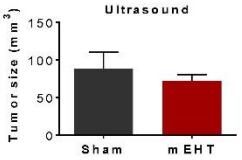
Digital caliper



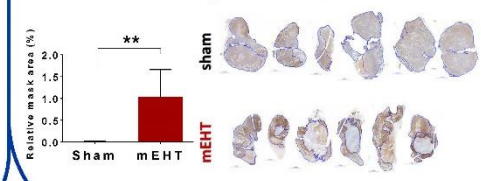
Tumor destruction (cC3, immunohisto)



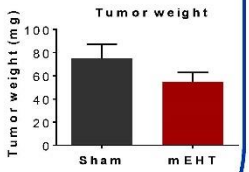
Ultrasound



Heat shock response (HSP70, immunohisto)



Tumor weight



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Modulated electro-hyperthermia promotes doxorubicin cytotoxicity in a C26 colorectal carcinoma cell line model

Tamas Vancsik[1], Eva Kiss[1], Gertrud Forika[1], Andrea Balogh[2], Tibor Krenacs[1]

[1]1st Department of Pathology and Experimental Cancer Research,
[2]Institute of Clinical Experimental Research, Semmelweis University, Budapest, Hungary

Presented at the 33rd ESHO,Warsaw

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https://oncotherm.com/sites/oncotherm/files/2019-07/Modulated_electrohyperthermia_promotes_doxorubicin_cytotoxicity.pdf

Objective: Modulated electro-hyperthermia (mEHT), a non-invasive complementary treatment to radio- or chemotherapy, can also induce a selective tumor damage by itself based on cell stress and heat shock at ~42°C. Here we studied the molecular background of mEHT tumor destruction and its combination with doxorubicin treatment using an *in vitro* model.

Methods: Coverslip cultures of C26 mouse colorectal adenocarcinoma cell were treated with mEHT at 42°C (2x60 min with 120 min breaks) either alone or in combination with the topoisomerase inhibitor and DNA-intercalating 1 M doxorubicin (mEHT+Dox). Post-treatment stress response, cell death, apoptosis and proliferation related markers were detected using immunocytochemistry; complemented with resazurin viability assay, qPCR, flow-cytometry and clonogenic assay compared to non- (Ctrl) and doxorubicin (Dox)-treated control cultures.

Result: Modulated EHT induced the significant release of hsp70 and calreticulin proteins 24 h after treatment and reduced the tumor stem-cell related colonies 10-days post-treatment. Early (1-3h) after the significant decrease of the anti-apoptotic XIAP, BCL-2 and BCL-XL, and the elevation of the pro-apoptotic BAX and PUMA mRNA levels was detected. P21 transcripts were also significantly increased between the 1-9th h. From 24 to 48 h the progressive reduction of cell viability was seen accompanied by the occurrence of cleaved-caspase-3 positive tumor cells which was further augmented in combination with Dox. In line with this, mEHT caused major apoptotic cell death, which was significantly enhanced after combined mEHT+Dox treatment, while Dox alone dominantly caused necrosis. After 24h the nuclear phospho-p53(Ser15) protein levels were also significantly increased in all treated groups, while phospho-Akt(Ser473) levels were reduced but only in the mEHT and mEHT+Dox groups.

Conclusion: mEHT induced cell stress caused caspase-dependent programmed cell-death and inhibition of tumor-cell proliferation, possibly linked to p53 activated p21^{waf1} upregulation and the concomitant reduction of active Akt protein, which could normally inhibit p53 functions. This mEHT induced mechanism could potentiate the cytotoxic effect doxorubicin in C26 colorectal cancer cells.

This study was funded by a grant of the National Research and Innovation Office (NKFIH-NVKP_16-1-2016-0042) in Hungary.

Modulated electro-hyperthermia promotes doxorubicin cytotoxicity in a C26 colorectal carcinoma cell line model

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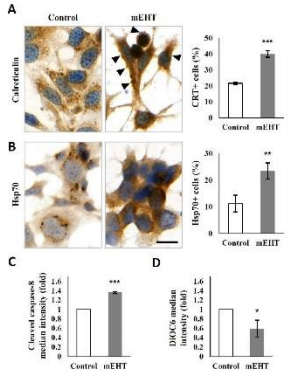
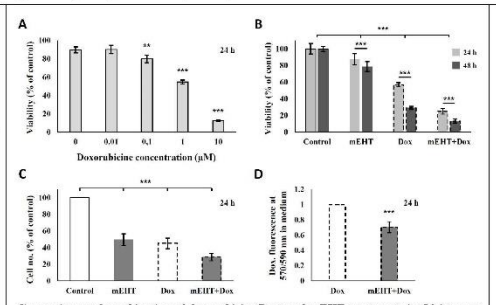
Introduction: Modulated electro-hyperthermia (mEHT, tradename: oncothermia), a non-invasive complementary treatment to radio- or chemotherapy, can induce a selective tumor damage by itself based on cell stress and heat shock at ~42°C. Here we studied the molecular background of mEHT tumor destruction and its combination with doxorubicin treatment using an *in vitro* model.

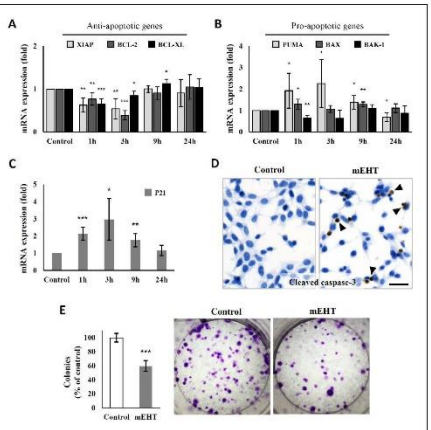
Materials and methods: Coverslip cultures of C26 mouse colorectal adenocarcinoma cell line were treated with mEHT at 42°C (2x60 min with 120 min breaks) either alone or in combination with the topoisomerase inhibitor and DNA-intercalating 1 µM doxorubicin (mEHT+Dox). Post-treatment stress response, cell death, apoptosis and proliferation related markers were detected using immunocytochemistry, complemented with resazurin viability assay, qPCR, flow-cytometry and clonogenic assay compared to non- (Control) and doxorubicin (Dox)-treated control cultures.



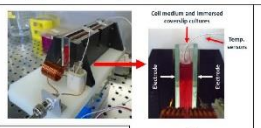
Signs of significant cell stress in C26 tumor cells 24 hours after mEHT treatment. Cytosolic release and cell membrane translocation of calreticulin with positive membrane blebs (arrowheads) (**A**). Elevated cytoplasmic Hsp70 reaction released from paranuclear vesicles (**B**). Scale bar: 20 µm. Significant increase of cleaved caspase-8 levels (**C**) and reduction of DiOC6 uptake by mitochondrial membranes (**D**) measured using flow cytometry indicate the induction of both the intrinsic and the extrinsic programmed cell death pathways, respectively. *p<0.05; **p<0.01; ***p<0.001.



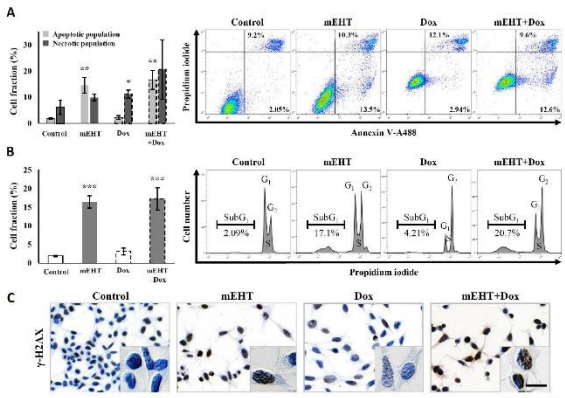
Comparison and combination of doxorubicin (Dox) and mEHT treatments in C26 tumor cell cultures. 1 µM Dox, reducing cell viability (resazurin assay) by ~40%, was chosen for further investigations (**A**). Progressively and significantly reduced tumor cell viability (**B**) and numbers (**C**) 24 and 48 h after mEHT, Dox and combined (mEHT+Dox) treatments. Enhanced effect of the combined treatment might be due to the reduced Dox concentration (elevated Dox uptake) in the culture supernatants (**D**). **p<0.01; ***p<0.001.



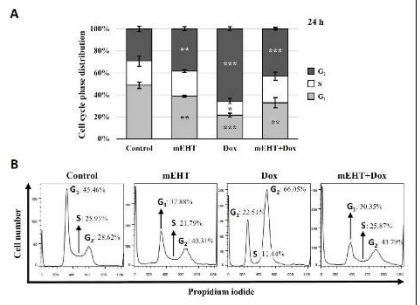
Expression of apoptosis regulation related genes in C26 tumor cells after mEHT treatment. Significant reduction of the anti-apoptotic XIAP, BCL-2, BCL-XL mRNA levels 1- and 3-hours post-treatment (**A**). Elevated pro-apoptotic PUMA mRNA levels at 1, 3 and 9 hours, and BAX levels at 1 and 9 hours after mEHT (**B**). Similarly increased temporal pattern of P21 mRNA levels to that of PUMA (**C**). In line with the apoptosis-promoting mRNA profile, cleaved caspase-3 protein expression (arrowheads) was significantly elevated 24 h after treatment as tested with immunocytochemistry (**D**). Scale bar: 100 µm. Significant reduction of colony-forming tumor progenitor-cell populations 10 days after mEHT treatment (**E**). *p<0.05; **p<0.01; ***p<0.001.



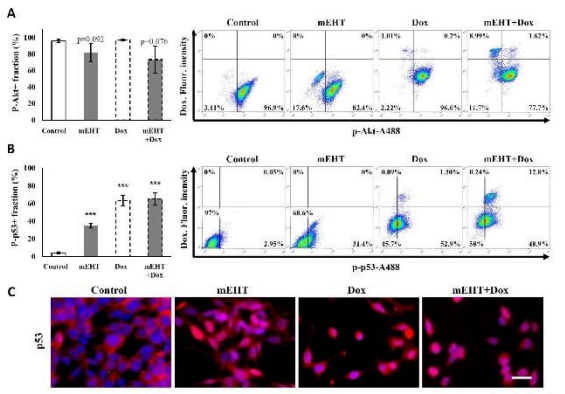
The *in vitro* applicator includes two plan-parallel copper condenser plates as the electrodes. Between the electrodes a medium containing chamber stands where the coverslip cultures and the thermo-sensors were put.



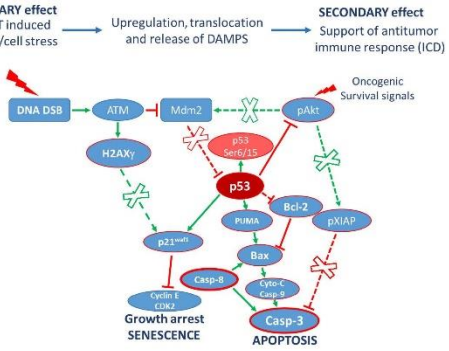
Comparison of the ratio of cell and DNA damage 24h after treating cultured C26 tumor cells. Significantly elevated apoptotic cell fractions after mEHT and necrotic cell fractions after Dox treatments and their additive, merged effect after combination (mEHT+Dox) therapy (**A**). Significant increase in subG$_1$ phase cell fractions both after mEHT and combined treatments refer to the apoptosis-related DNA damage (**B**), where DNA double strand breaks were indicated by upregulated H2AXγ granular positivity (brown) in cell nuclei using immunocytochemistry (**C**). Scale bar is 100 µM on the larger FOVs. *p<0.05; **p<0.01; ***p<0.001.



Summary graph (**A**) of flow cytometry analysis (**B**) of the treatment related cell cycle fractions. Significant but less G$_2$-phase cell cycle arrest seen 24 h after mEHT than after Dox treatment, where the sizes of G$_1$ and G$_2$ populations in the combined (mEHT+Dox) group show about the averages of the single treatments. *p<0.05, **p<0.01 and ***p<0.001.



Comparison of phospho-Akt and -p53 levels 24h after treating cultured C26 tumor cells. Reduced phospho-Akt^{Ser473} kinase positive cell populations after mEHT and mEHT+Dox treatments (**A**). Significantly elevated post-treatment phospho-p53^{Ser15} levels showing the same highest values after Dox and the combined treatments (**B**). Increased cytoplasmic to nuclear relocalization of phospho-p53^{Ser15} indicated p53 activation (**C**). Scale bar: 100 µM. ***p<0.001.



Conclusion: Here we introduce an *in vitro* mEHT treatment model of C26 mouse colorectal cancer useful for feasibility studies of the molecular background of combining mEHT with other treatment modalities, starting with Dox chemotherapy. Our results show that mEHT monotherapy can induce irreversible cell stress both through caspase-dependent apoptosis and p21^{wafl} mediated cell cycle arrest, which are likely to be driven by p53 activation. Elevated phospho-p53^{Ser15} and reduced phospho-Akt^{Ser473} are known to promote p53 escape from Mdm2 control. In combination, mEHT seems to promote the uptake and significantly potentiate tumor destruction and control by Dox through merging efficient apoptosis, necrosis and cell cycle arrest induction by mEHT and Dox.

As a validation of this *in vitro* model the release of damage associated molecular pattern (DAMP) signals from damaged cells were also observed like earlier in our *in vivo* studies on C26 tumor allografts. DAMPs may promote the accumulation of T-cells and antigen presenting cells contributing to a secondary immune-mediated tumor cell death. This model can serve for pilot testing of mEHT combinations prior to comprehensive investigations of allografted C26 cells using immune competent animals.

This study was funded by a grant of the National Research and Innovation Office (NKFIH-NVKP_16-1-2016-0042) in Hungary.

Can we monitor immunogenic cell death (ICD) induced with modulated electrohypertheria and oncolytivc virus injections?

Stefaan Van Gool, Jennifer Makalowski, Oliver Feyen, Andrea Neinhuis, Wilfried Stuecker
Immun-Onkologisches Zentrum Köln

Presented at the 33rd ESHO,Warsaw

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https://oncotherm.com/sites/oncotherm/files/2019-07/Can_we_monitor_immunogenic_cell_death.pdf

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vangoolstefaan@gmail.com

Immun-Onkologisches Zentrum Köln

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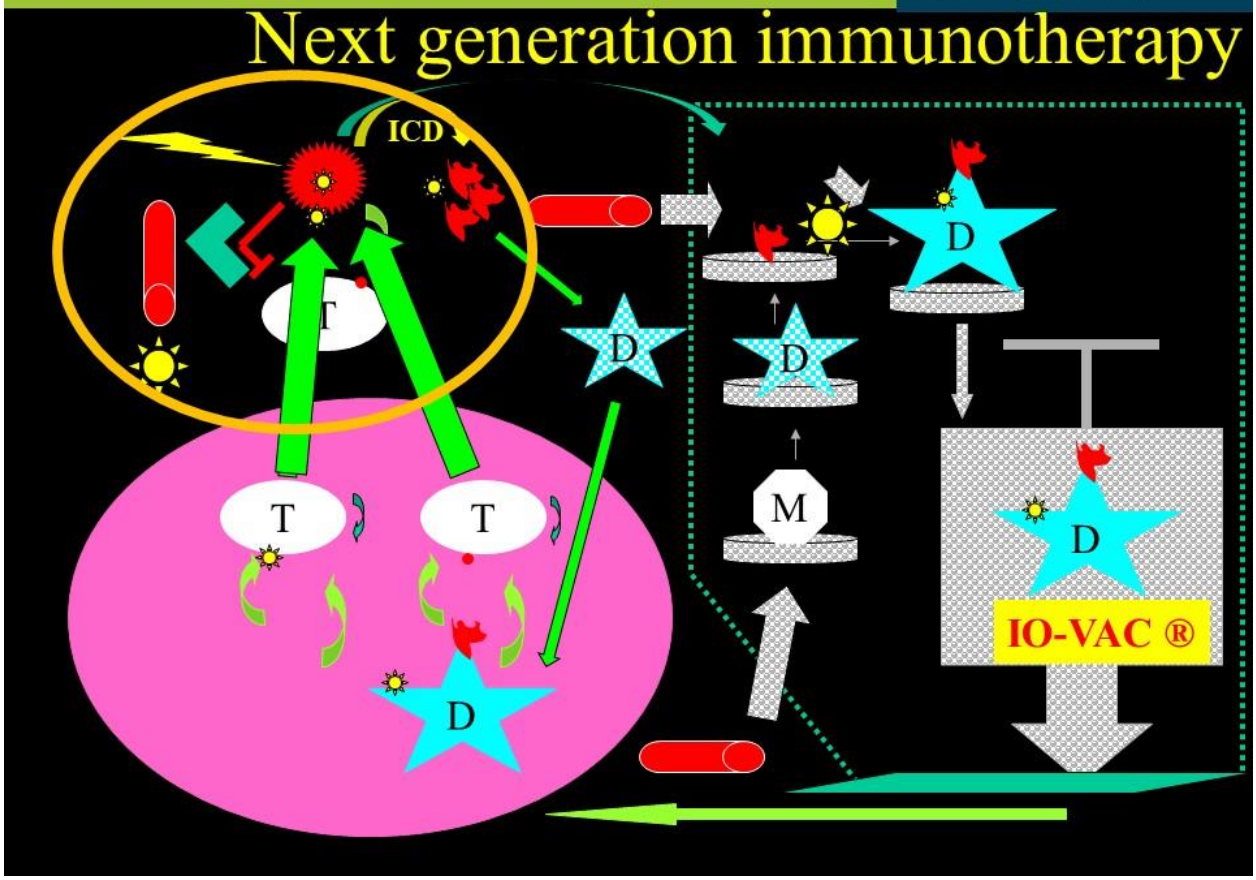
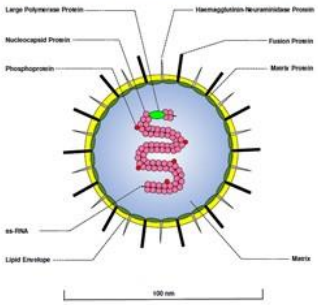
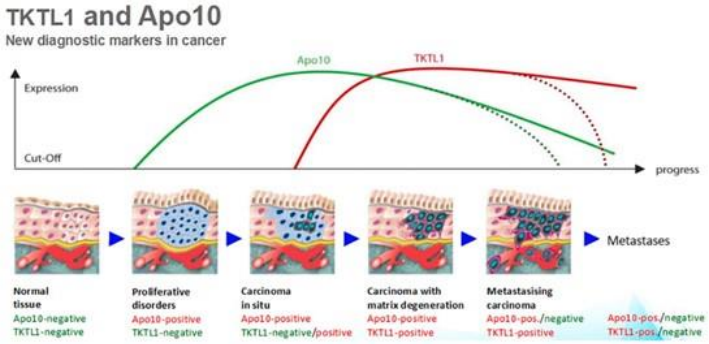
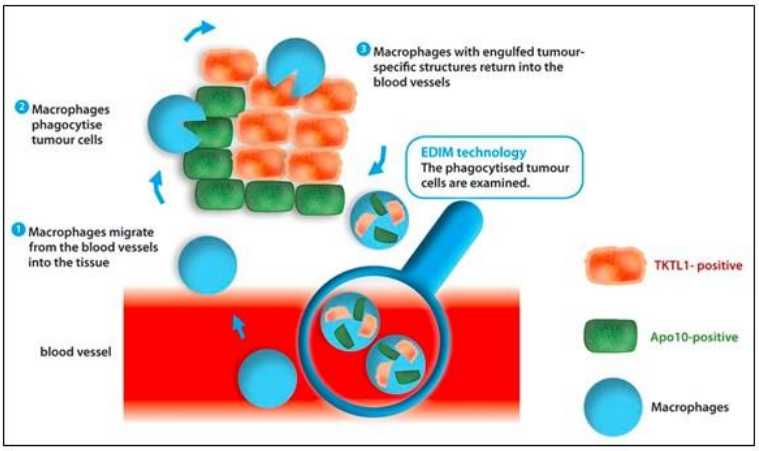
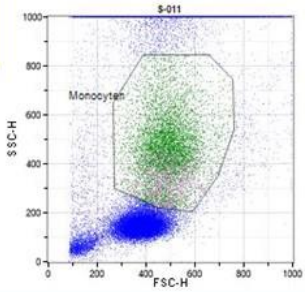


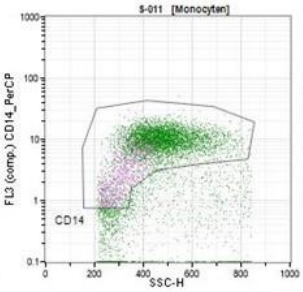


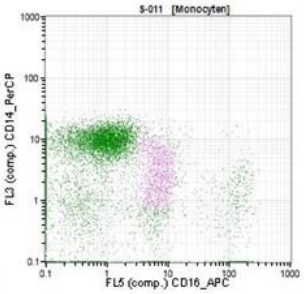


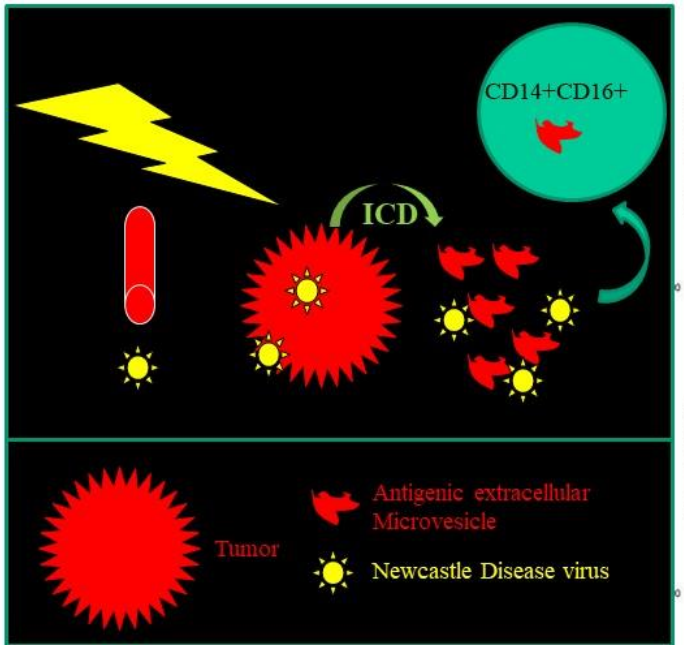


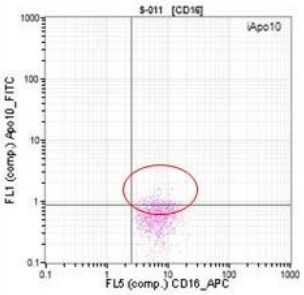
IMMUN-ONKOLOGISCHES ZENTRUM KÖLN

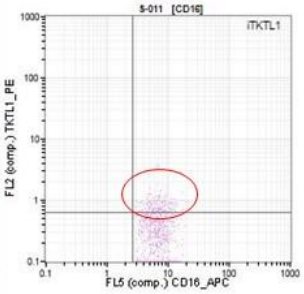








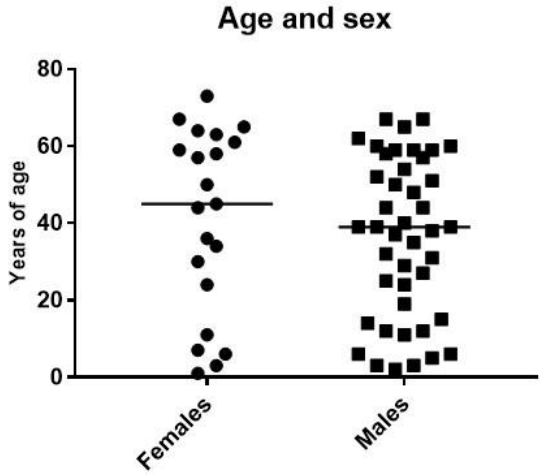


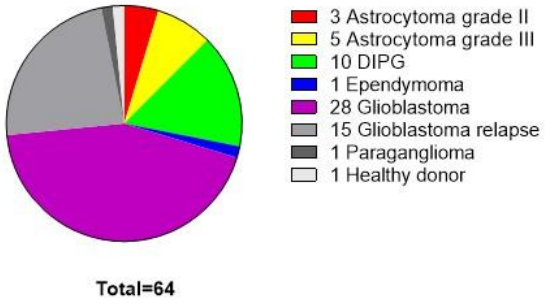


Update 15/01/2019

IOZK | IMMUN-ONKOLOGISCHES ZENTRUM KÖLN

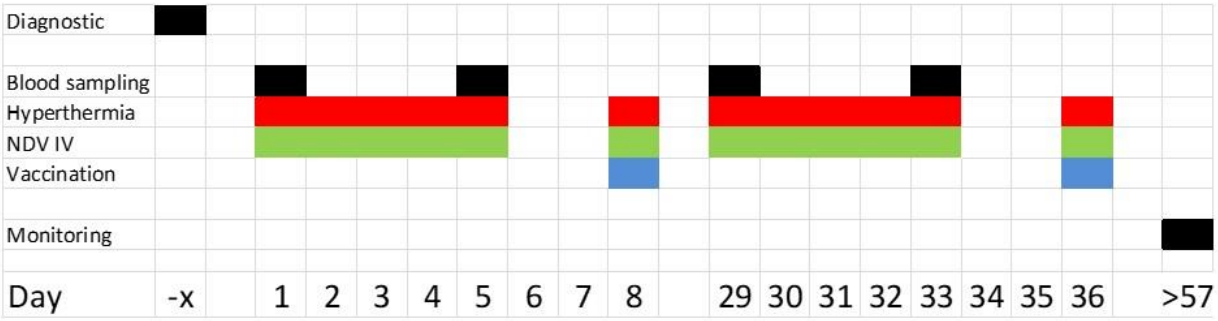
PanTum Tests using the EDIM platform
During treatment





IOZK | IMMUN-ONKOLOGISCHES ZENTRUM KÖLN

Vaccination cycles



Update 15/01/2019

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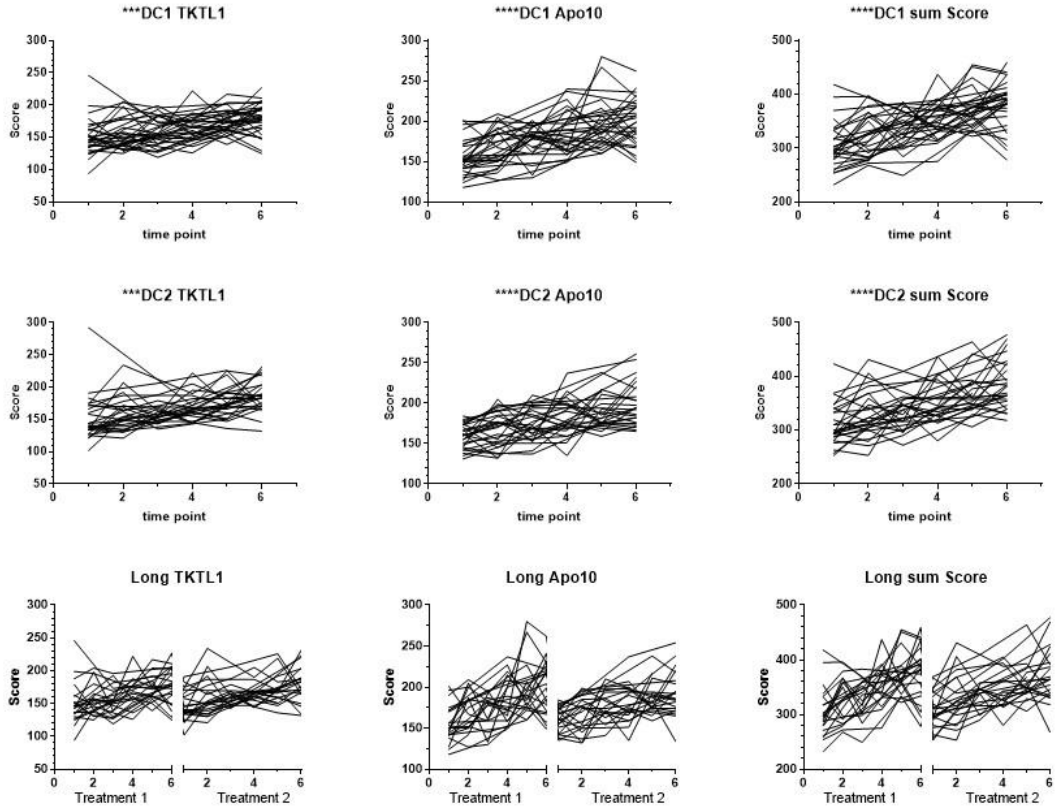

IOZK | IMMUN-ONKOLOGISCHES ZENTRUM KÖLN

Conclusion

- Instant monitoring of ICD with **PanTum-Detect**
  - PanTum-Detect is positive at time of immuno-oncologic evaluation
  - PanTum-Detect increases significantly upon 5 day ICD treatment
  - PanTum-Detect can reflect response to overall treatment

Symposium & Fortbildung am IOZK: Immuntherapie in der Neuroonkologie, Fr. 06.09.2019

-> Inauguration of 8 new clean-room spots
-> Inauguration of EU project INOVENT

Modulated electrohyperthermia as part of immunogenic cell death treatment in pediatric neuro-oncology

Stefaan W Van Gool, Jennifer Makalowsky , Volker Schirrmacher , Wilfried Stuecker

Immun-Onkologische Zentrum Köln

Presented at the 33rd ESHO,Warsaw

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Van Gool W.S. (2019): Modulated electrohyperthermia as part of immunogenic cell death treatment in pediatric neuro-oncology, Oncothermia Journal 26:126-127
https://oncotherm.com/sites/oncotherm/files/2019-07/Modulated_electrohyperthermia_as_part_of_immunogenic_cell.pdf

Modulated electrohyperthermia as part of immunogenic cell death treatment in pediatric neuro-oncology

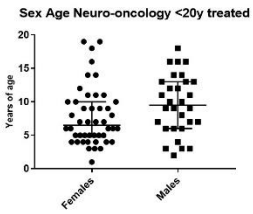
Stefaan W Van Gool, Jennifer Makalowsky , Volker Schirrmacher , Wilfried Stuecker

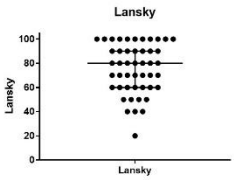
Immun-Onkologische Zentrum Köln, www.iozk.de

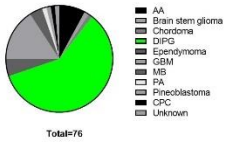
Introduction. The induction of immunogenic cell death (ICD) becomes an important treatment methodology in cancer. Convincing data demonstrate that modulated electrohyperthermia (mEHT) contribute to ICD. No data have been reported about the use of mEHT in a large pediatric brain cancer population.

Patients. We retrospectively analyzed a series of 76 children treated at IOZK with multimodal immunotherapy consisting of the combination of Newcastle Disease Virus (NDV) injections with mEHT and autologous dendritic cell vaccines ***IO-VAC®***.

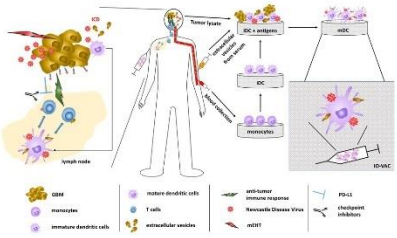
Patient characteristics







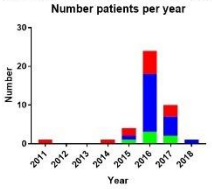
The principle of *IO-VAC®*. Full vaccination cycles are administered with three weeks interval. Each full vaccination cycle consists of NDV and mEHT administrations at days 1 to 5 and at day 8. An intradermal injection of autologous mature dendritic cells (DCs) loaded with autologous tumor antigens is administrated at day 8. Immature DCs are differentiated *ex vivo* out of adherent peripheral blood monocytes in the presence of 800 U/ml IL-4 and 1000 U/ml GM-CSF. DCs are loaded at day 5 with autologous tumor antigens, obtained via tumor lysate or obtained from serum after induction of tumor-derived antigenic extracellular microvesicles, induced via ICD by mEHT and NDV. DC maturation is induced with NDV (10^5 infectious particles per 10^6 DCs) and the cytokine cocktail 1000 U/ml IL-6, 1100 U/ml TNF-a and 1900 U/ml IL-1b. GMP-approved culture medium and cytokines are purchased from Cellgenix (Freiburg, Germany). ***IO-VAC® is an approved medicinal product by the German authorities (DE-NW-04-MIA-2015-0033).***



Results. There were 46 females and 30 males with a median age of 6.5 resp. 9.5 years (range 1-19y resp. 2-18y). Diagnoses were anaplastic astrocytoma (6), Brain stem glioma (1), chordoma (1), DIPG (45), Ependymoma (4), GBM (12), medulloblastoma (3), pilocytic astrocytoma (1), pineoblastoma (1), choroid plexus carcinoma (1), unspecified (1). In total 811 mEHT sessions were given. Median number of mEHT sessions per child were 15, ranging up to 53. In all cases, mEHT doses were 40 Watt during 40 minutes. Treatments were feasible without sedation. There were no intervention-related side effects. NDV injections were associated to the mEHT sessions. Multimodal immunotherapy included the DC vaccinations with ***IO-VAC®***. In total 77 ***IO-VAC®*** vaccines were produced, and in median 2 (range 0-4) ***IO-VAC®*** vaccines were given per child. The use of PanTum Detect tests (TKTL1 and Apo10) seemed reliable to monitor on daily basis the response to ICD treatment and over the treatment course. Median overall survival of 28 DIPG children treated with multimodal immunotherapy as part of their first line treatment showed a median overall survival of 14 months. Remarkable long-term remissions were also observed in a child with relapsed pineoblastoma and a child with metastasized Myc-amplified medulloblastoma group 3.

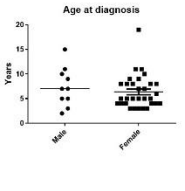
Data on 41 children with DIPG treated with multimodal immunotherapy

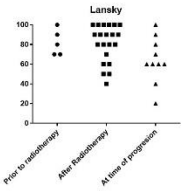
The patient group is splitted into a subgroup treated with immunotherapy prior to radiotherapy, a second subgroup treated with immunotherapy after radiotherapy, and a third subgroup treated with immunotherapy at the time of progressive disease. Depicted are number of patients treated per year; Age and sex distribution, and Lansky score.

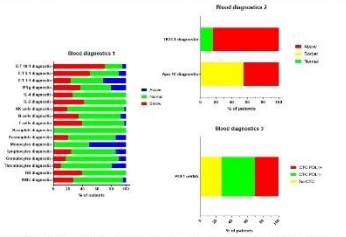


Relapse
After radiotherapy
Before radiotherapy

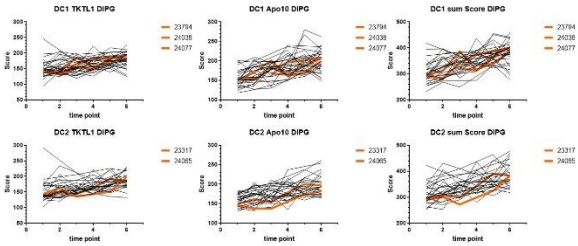
8/41 Biopsy
13/41 ctDNA+
(3 Biopsy+)
8/41 ctDNA-
(1 Biopsy+)



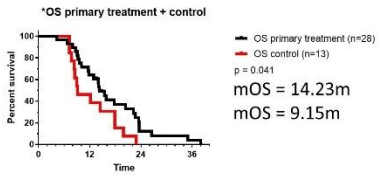




Blood values at time of start of immunotherapy. Left: immune parameters. Right top: TKTL1 and Apo10 score. Right bottom: mRNA expression for PDL1 on circulating tumor cells when detected.



Intracellular expression of TKTL1 (left), Apo10 (middle) and sum of both (right) in CD14+CD16+ gated peripheral blood white blood cells, measured during first (DC1) or second (DC2) vaccination cycle. Measurements are performed at day 1/2/3/4/5/8 of each cycle. From day 1 to day 5, NDV injections and mEHT treatments are given. Each line represents the data of a neuro-oncologic patient, most adults with GBM. The colored lines represent available data from DIPG children treated with DC vaccination cycles.



Overall survival of children treated with multimodal immunotherapy in the context of first line treatment (black) , or at time of first progression after first line treatment (red). The latter serves as control to the former taking into account the selection biases of patients coming to IOZK for treatment. OS is always calculated from the time of first diagnosis. The figure suggests that multimodal immunotherapy as part of first line treatment gives better overall survival outcome as compared to patients with similar disease and family profile who were treated at time of progressive disease.

Conclusion. Modulated electrohyperthermia (mEHT) for children with brain cancer as part of multimodal immunotherapy is feasible and safe. Response to treatment could be monitored. Dosing of mEHT is now expanded to 60 Watt. First experiences demonstrate clinical feasibility. Multimodal immunotherapy contributed to improved tumor control and overall survival.

Physical potentials of radiofrequency hyperthermia with amplitude modulation

P. Wust[1], P. Ghadjar[1], J. Nadobny[1], M. Beck[1], D. Kaul[1], L. Winter[2], S. Zschaeck[1]

[1]Charité Universitätsmedizin Berlin, Department of Radiation Oncology, Augustenburger Platz 1, 13353 Berlin, Germany
[2]Physikalisch-Technische Bundesanstalt, Abbestr. 2-12, 10587 Berlin

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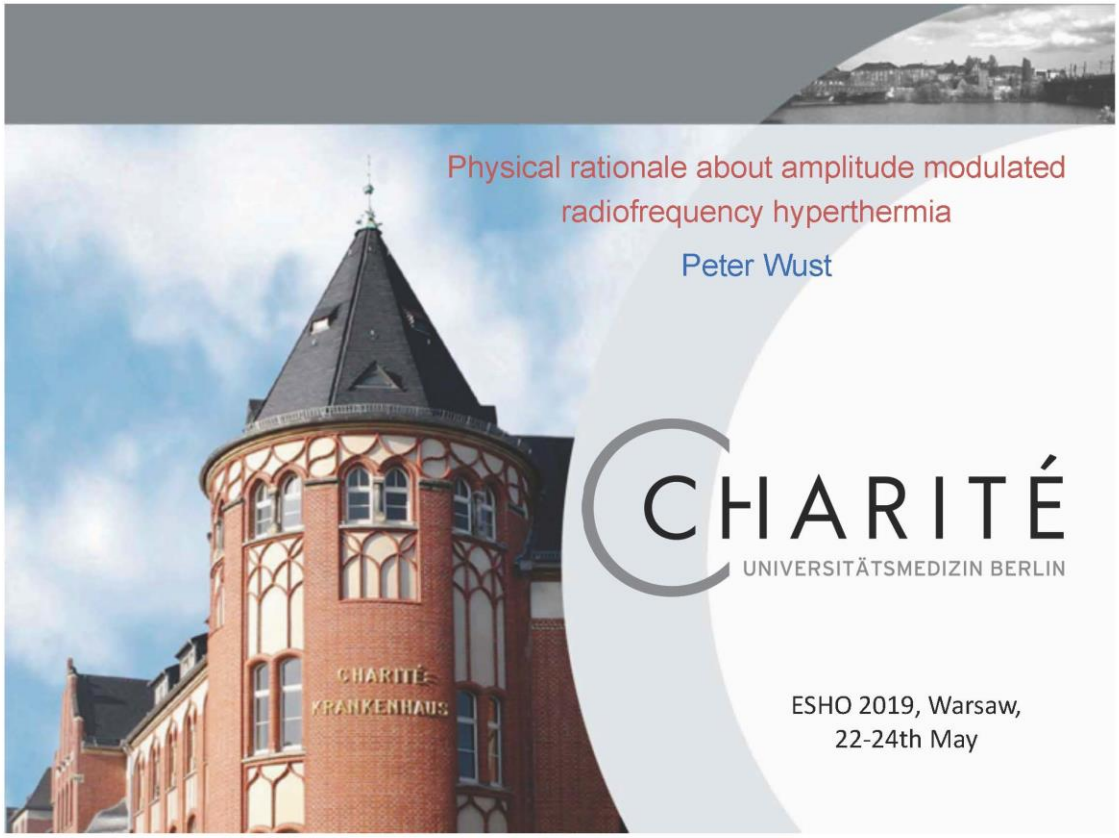
https://oncotherm.com/sites/oncotherm/files/2019-07/Physical_potencials_of_radiofrequency.pdf

Introduction: Preclinical studies and some clinical observations indicate that modulated electromagnetic hyperthermia (mEHT), which employs a radiofrequency (RF) carrier (13.56 MHz) with amplitude modulation (AM) at very low frequencies (VLF, <100kHz), enhances the effect of conventional mono-frequent RF hyperthermia. We conducted a physical evaluation to explore potential benefits of VLF modulation.

Methods: We reviewed the electrical behavior of cell suspensions, normal tissues and tumors at VLF (table). Then, we conceptualized microscopic models upon tumors and their microenvironment and analyzed the mechanism of power dissipation (conductive versus dielectric) specifically in tumors and normal tissues (muscle, organs, fat). We outline that cell suspensions as well as suitable tumors can ensure demodulation if the time constant RC of the equivalent circuit diagram of the tumor (resistivity R, capacitance C) and cycle duration $T_c=1/\Omega$ of the carrier at frequency Ω satisfy the relationship $RC \sim 2T_c$. R and C of different tissues (including tumor architectures) are estimated. Using this framework, we analytically solved the temperature/diffusion equation and calculated the magnitude of modulation-dependent thermal and non-thermal effects.

Results: We substantiate that additional VLF modulation can increase the specific absorption rate (SAR) in the extracellular fluid of tumors (necrosis formation) by a factor of 10 or even higher as compared to conventional mono-frequent RF hyperthermia. Such SAR-peaks can induce effective or even thermoablative temperatures (hot spots) in necrotic areas of millimeter to centimeter size typically disseminated over tumors. A relationship between SAR peak value [W/kg] and required minimum size of necrosis [mm] for relevant temperature increases (>43-44°C) can be derived (figure). In addition, we recognized that VLF modulation would have an electro-chemical effect at biological membranes in an extracellular medium caused by alternating ion currents. In particular, movements of the ions through the membrane can impair/destroy the disparate ion concentrations between vital cells and the surrounding extracellular fluid and can principally lead to cell death.

Conclusion: Our physical analysis suggests that RF hyperthermia with additional VLF-AM modulation is more effective against tumors than conventional RF hyperthermia due to thermal and non-thermal effects. Therefore mEHT should be further evaluated in prospective clinical trials.

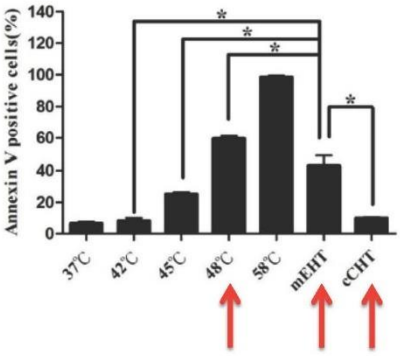


Present status of RF hyperthermia

Perfusion [ml/100g/min]	5	10	20
			Difficult-to-heat
SAR = 15 W/kg	42 °C	39.8 °C	38.6 °C
SAR = 30 W/kg	46.5 °C	42 °C	39.8 °C
SAR = 60 W/kg	55.5 °C	46.5 °C	42 °C
	Easy-to-heat		

Preclinical studies

In tumor cell suspensions differences of 6 °C were found comparing waterbath/infrared or RF-heating with mEHT at 13.56 MHz



Andocs et al 2009,
Yang et al 2016:

mEHT at 42 °C similar to 48 °C waterbath for cells, superior to RF-CHT at 42°C

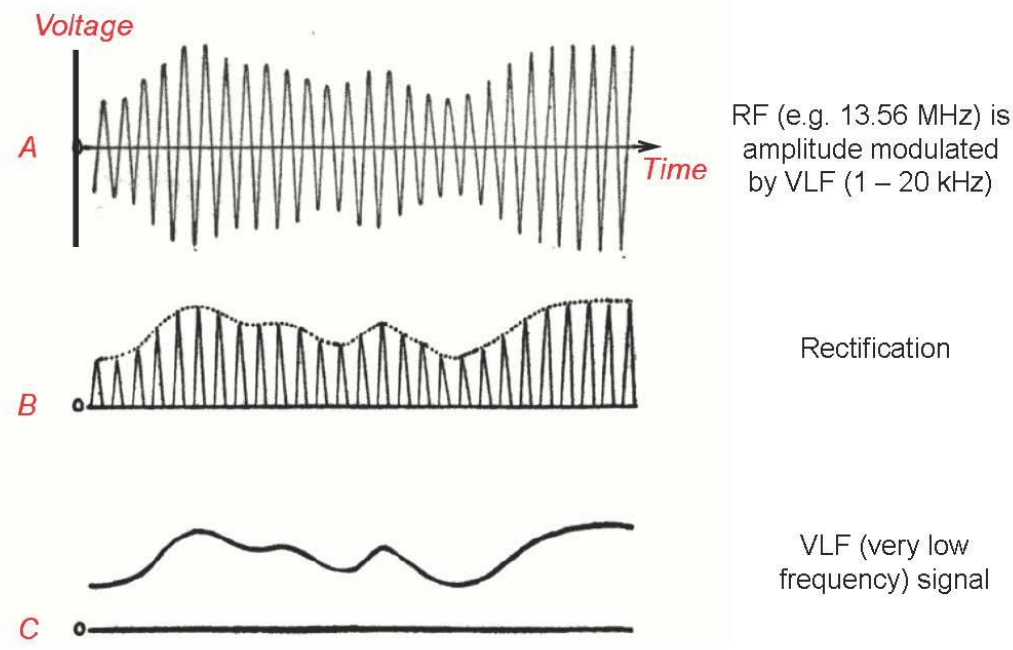
Clinical observations

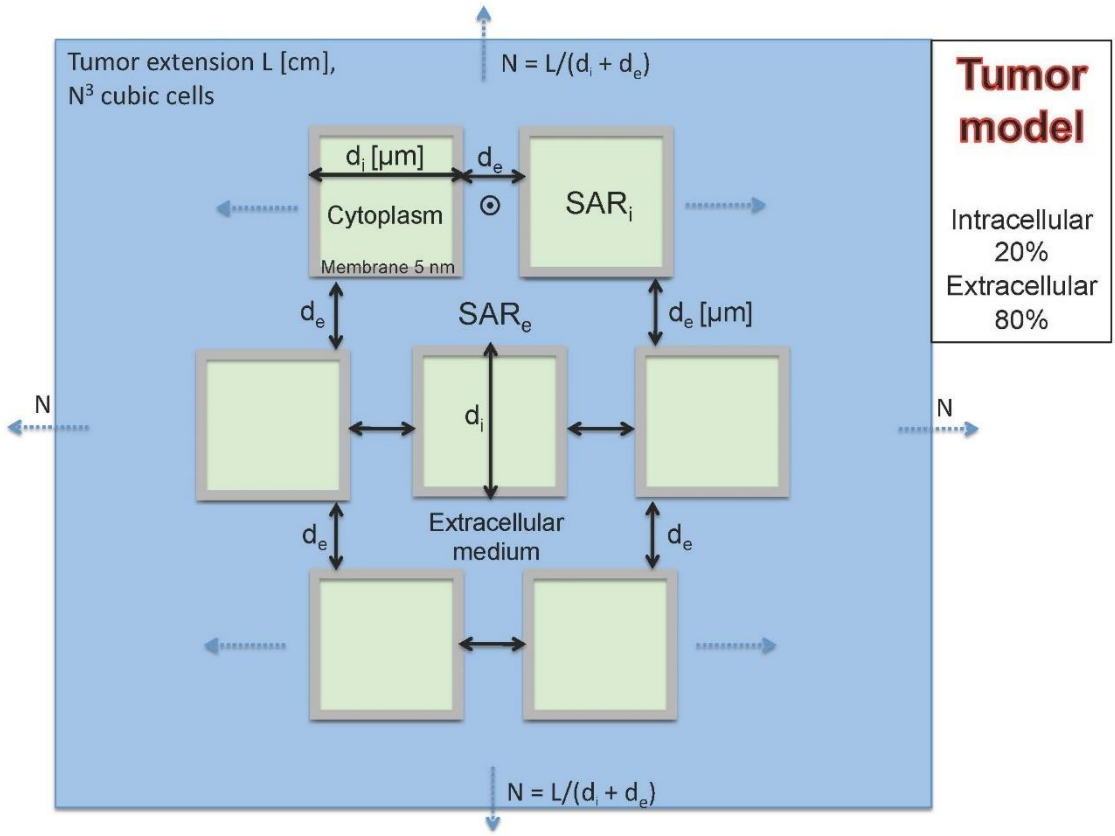
Responses (PR, CR) after mEHT as mono-therapy (brain tumors, liver metastases)

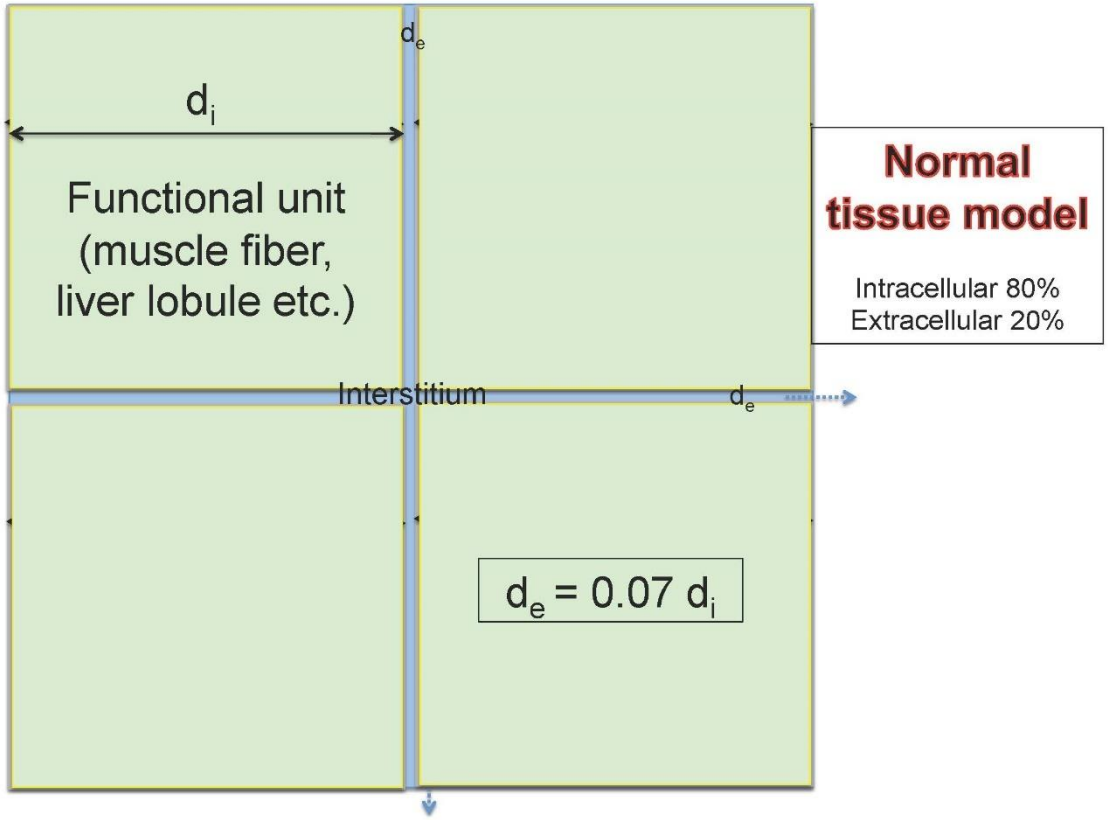
Fiorentini et al 2018:
Recurrent glioblastoma/astrocytoma after standard treatment
40 – 150 W mEHT mono-therapy (no chemotherapy),
CR 3/50, PR 14/50, SD 14/50, PD 17/50
Objective remissions in one third of the patients

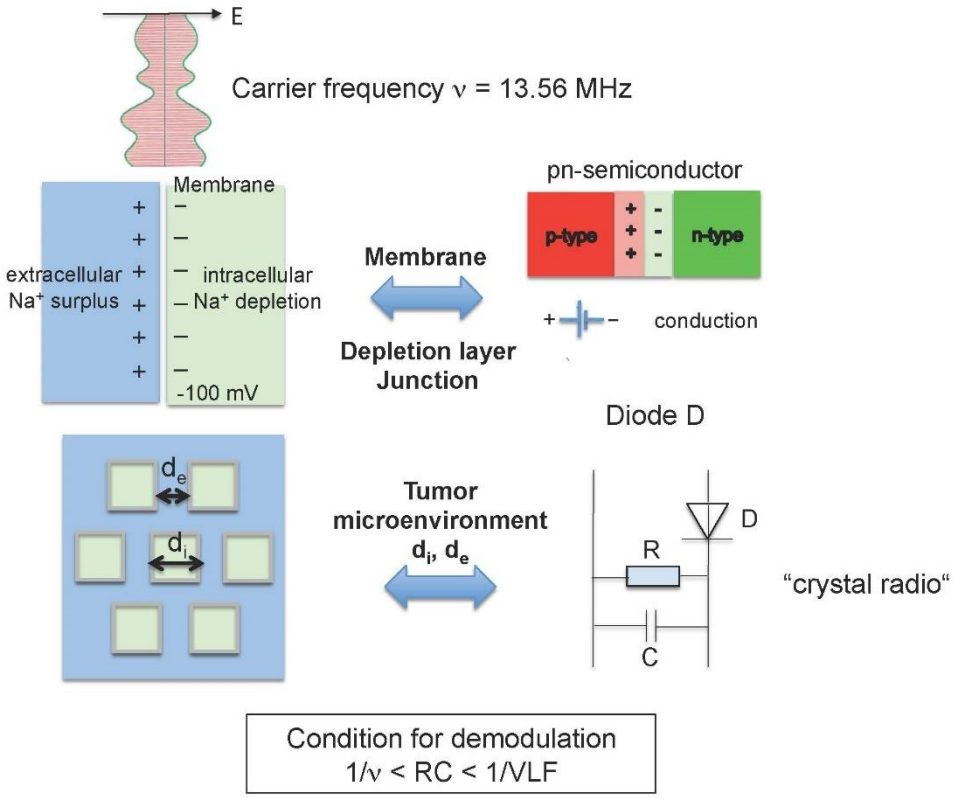
Minnaar et al 2019 (this congress):
Phase III study upon cervical cancer using 130 W
RCT ± mEHT
Improved clinical endpoints

Is there a physical explanation for higher efficacy in tumors, if we use AM-modulated RF?






d_e
d_i
Functional unit
(muscle fiber,
liver lobule etc.)
Normal
tissue model
Intracellular 80%
Extracellular 20%
Interstitium
d_e
$d_e = 0.07\ d_i$


E
Carrier frequency ν = 13.56 MHz
Membrane
extracellular
Na⁺ surplus
intracellular
Na⁺ depletion
-100 mV
Membrane
Depletion layer
Junction
pn-semiconductor
p-type
n-type
conduction
Diode D
d_e
d_i
Tumor
microenvironment
d_i, d_e
D
R
C
"crystal radio"
Condition for demodulation
1/ν < RC < 1/VLF

κ = 0.6 W/m/°C	Thermal conductivity (tumor)
ρ = 1.0 kg/l	Density (water)
c = 4,000 Ws/kg/°C	Heat capacitance (tumor)
σ_e = 1.2 S/m	Extracellular medium conductivity (DC – 100 MHz)
σ_i = 0.3 S/m	Cytoplasm conductivity (DC – 100 MHz)
$\sigma_m = 3\times10^{-7}$ S/m	Membrane conductivity (< 1 MHz)
$c_m = \varepsilon_m/d = 0.9\times10^{-2}$ F/m² ≅ 1 µF/cm²	Membrane capacitance $\varepsilon_m = 4.4 \times 10^{-11}$ As/Vm, d = 5 nm (membrane)
$\varepsilon_{r/e}$ = 72.5	Extracellular medium relative permittivity (DC – 100 MHz)
$\varepsilon_{r/i}$ = 72.5	Cytoplasm relative permittivity (DC – 100 MHz)
$\varepsilon_{r/m}$= 5	Membrane relative permittivity (< 10 MHz)
$\mu = 4.6 \times 10^{-8}\ m^2V^{-1}s^{-1}$	Mobility of Na^+ ions in water
$\mu = 6.75 \times 10^{-8}\ m^2V^{-1}s^{-1}$	Mobility of K^+ ions in water
$\mu = 6.85 \times 10^{-8}\ m^2V^{-1}s^{-1}$	Mobility of Cl^- ions in water
E = 100 – 300 V/m	E-field for SAR = 10 – 60 W/kg E = 200 V/m for SAR = 25 W/kg
$v = \mu \times E$	Drift velocity [m/s]
$\tau = d^2\ \rho\ c/\kappa$	Relaxation time for hot spot of extension d τ = 10 s for d = 1 mm $\tau = 10^{-1}$ s for d = 100 µm $\tau = 10^{-3}$ s for d = 10 µm $\tau = 10^{-9}$ s for d = 10 nm

Data from Kotnik, Miklavcic 2000

Foster, Schwan 1989

Capacitance in the tumor (d_e, d_i [µm])

$$C_{Tm} = N \times C_{cell} = L[cm] \times d_i^2/(d_i + d_e) \times 0.05\ nF$$

Resistance in the tumor (d_e, d_i [µm])

$$R_{Tm} = (L/F) \times \sigma = (1/L[cm]) \times 100\ ohm$$

Time constant (with d [µm])

$$\mathbf{RC = d_i^2/(d_i + d_e) \times 0.05 \times 10^{-7}\ s}$$

Note:
RC is independent of tumor size L and is therefore a **local parameter** characterizing the *microenvironment* and *electrical behavior* in this part of the tumor.
Thus in every part of the tumor *demodulation* occurs, if the condition is met

$$1/f_{carrier} < RC < 1/f_{mod}$$

Inspecting the condition for demodulation in an *envelope detector* (crystal radio, detector receiver)

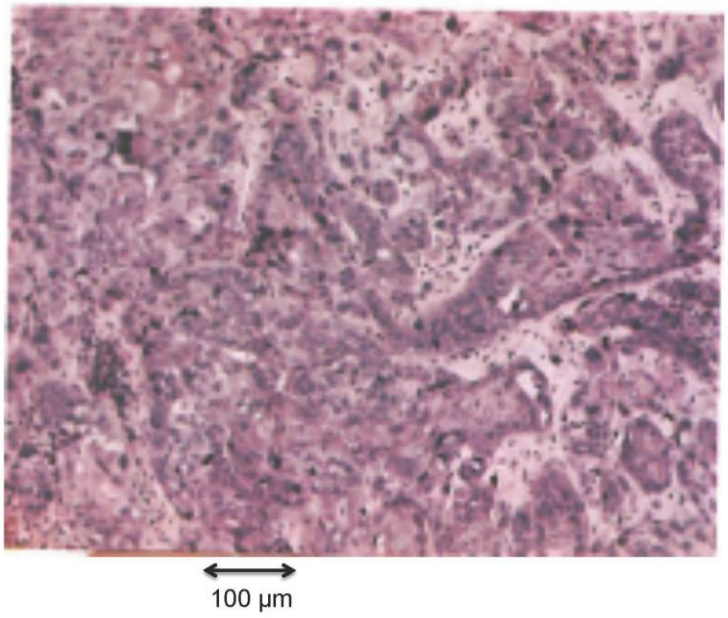
$$1/f_{carrier} < RC < 1/f_{mod}$$

E.g. $f_{carrier}$= 10 MHz, f_{mod}= 10 kHz
10^{-7} s < RC < 10^{-4} s

According to the inequation larger $f_{carrier}$ might be even better.

It is seen that for small d_i (10 – 20 µm) and large d_e RF towards 100 MHz is a suitable carrier frequency.

A squamous cell carcinoma is composed of cell clusters of 10 - 100 µm size or larger with chaotic structure



The extracellular space is a conducting medium and the current path is narrowed, which locally increases the SAR

Characteristics of AM-RF

Demodulation preferentially occurs in the microenvironment of tumors (and much less in normal tissues)

SAR(VLF) at *audio frequencies* (1 – 20 kHz) is deposited at the membranes (where rectification and demodulation occurs)

SAR(VLF) might be concentrated in a small volume at the membrane

Membranes are *isolators* in the VLF-range (negligible intracellular power dissipation)

Conductive power dissipation is dominant in the extracellular medium with high σ = 1.2 S/m.

Non-temperature dependent effects in the VLF-range should be considered.

Electrochemical effect at VLF caused by a drift of the ions Na^+ and Cl^- in the extracellular electrolyte solution exposed to E (with mobility μ)

deflection δ[µm] = E[V/m]/40 × VLF[Hz]

Na^+ ions are crossing the membrane for δ > some nm

Modulation frequency	Deflection for E = 200 V/m SAR = 25 W/kg	Deflection for E = 1,200 V/m SAR = 1,000 W/kg
1 Hz	5 µm (part of cell)	30 µm (cell)
10 Hz	500 nm (part of cell)	3 µm (part of cell)
100 Hz	50 nm (>> membrane)	300 nm (part of cell)
1 kHz	5 nm (membrane)	30 nm (>> membrane)
10 kHz	0.5 nm (< membrane)	3 nm (membrane)
100 kHz	0.05 nm (<< membrane)	0.3 nm (< membrane)

Conclusions:

AM-RF is an interesting approach to increase efficacy in tumors employing their typical microenvironment

It seems promising to explore the suitable frequencies for *modulation* (some kHz?) and for the *carrier* (10 – 100 MHz?)

Our physical analysis supports the view that non-temperature dependent effects might be relevant (especially electrochemical effects)

Biology is with us, but physics is against us

What if the saying were:

Physics is our friend, but we have not noticed it.

It would be unrequited love (and tragic).

Thank you for your attention

Charité – Campus Virchow Klinikum

Immune-effects with local hyperthermia

Andras Szasz

Department of Biotechnics, St. Istvan University, Hungary

Presented at the 33rd ESHO,Warsaw

Cite this article as:
Szasz A. (2019): Immune-effects with local hyperthermia, Oncothermia Journal 26:139-148
https://oncotherm.com/sites/oncotherm/files/2019-07/Immune_effects_with_local_hyperthermia.pdf

Introduction: The immune-stimuli combined to local hyperthermia could extend the local action of the treatment to systemic acting on distant micro and macro-metastases with abscopal effect. Our objective is to study the effect from local to systemic made by local, cellularly selective hyperthermia, showing the clinical evidences of the abscopal effect.

Method: Impedance controlled modulated RF radiation (mEHT, trade name: oncothermia) was used complimentary to conventional tumor-therapies. The capacitive coupled energy transfer is amplitude modulated by the homeostatic time-fractal pattern. The selected transmembrane proteins are excited developing immunogenic cell-death. The nano-selection is based on the certain biophysical differences of the malignant and healthy cells, like deviations of metabolic-processes, broken intercellular connections and rearranged pathologic pattern of malignant tissue. There were various clinical evidences proving the abscopal effect and in consequence improving the local response and the survival time combined with better quality of life.

Results: The immune action had been shown in preclinical studies showing the production of damage associated molecular pattern and showing the synergy with dendritic cell therapy, which works like vaccination, rejecting the re-challenging of the same tumor. Our talk shows the clinical evidences of the mEHT immune action. Extended number of clinical reports will be shown with check-point inhibitors for various advanced, relapsed and/or metastatic malignancies, including hepatocellular, non-small-cell lung, small-cell lung, pancreas, gastric, renal and cholangio cancer cases. The clinical cases with complimentary viral immune-stimulation to mEHT for glioblastoma, metastatic prostate, and breast as well as stage IV Wilms tumor with traditional immune stimuli will be presented. Independent talk will show the abscopal effect in a large number clinical study too [1].

Conclusion: Preclinical and clinical studies show evidences of the feasibility of mEHT for systemic cancer-specific immune reaction, when the local hyperthermia treatment extends its action to systemic, eliminating the distant metastases.

References

[1] Minnaar C. Effects of modulated electro-hyperthermia on disease control and 3 year disease-free survival in locally advanced cervical cancer patients: Preliminary results

ESHO

Immune-effects with local hyperthermia

-

Andras Szasz, Ph.D.

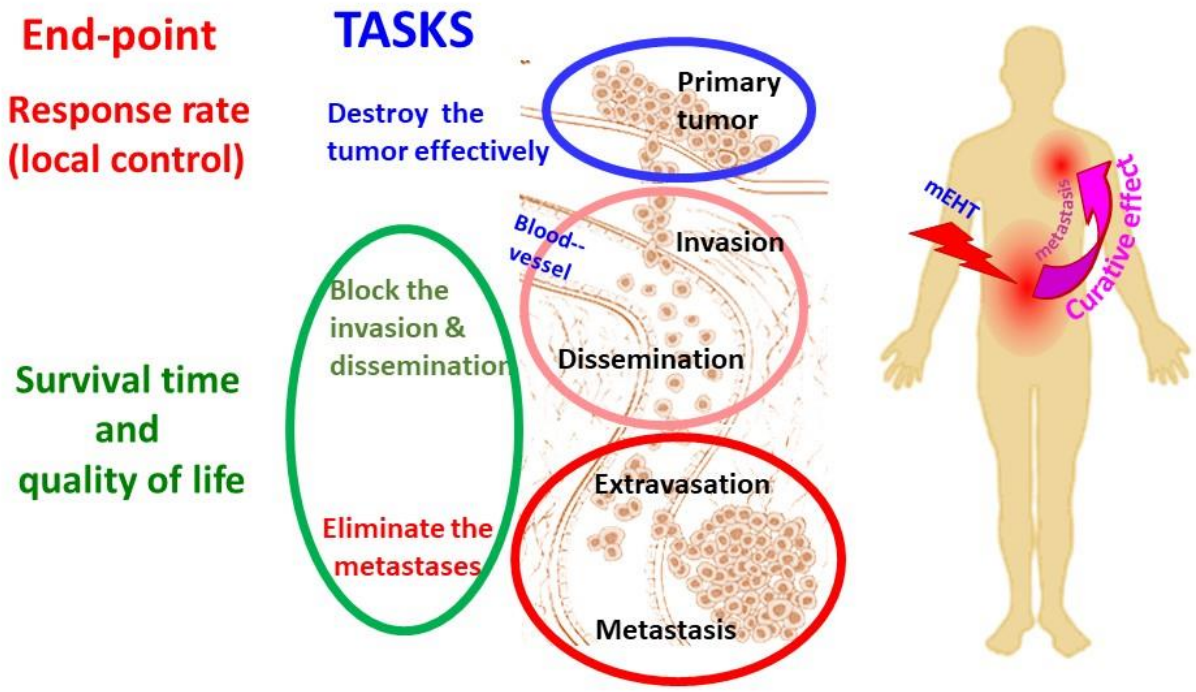
Possible conflict of interest and expertise:

- founder & CSO of company Oncotherm (1988)
- chair, professor, lectureship Biophysics at St. Istvan University
- visiting lectureship Bioelectromagnetics at Pazmany Catholic University
- visiting lectureship Fractal-physiology at Chiba University (Japan)

Outline

- ❑ **Challenge of locality**
- ❑ **Possible solution**
- ❑ **Verification**
- ❑ **Validation**

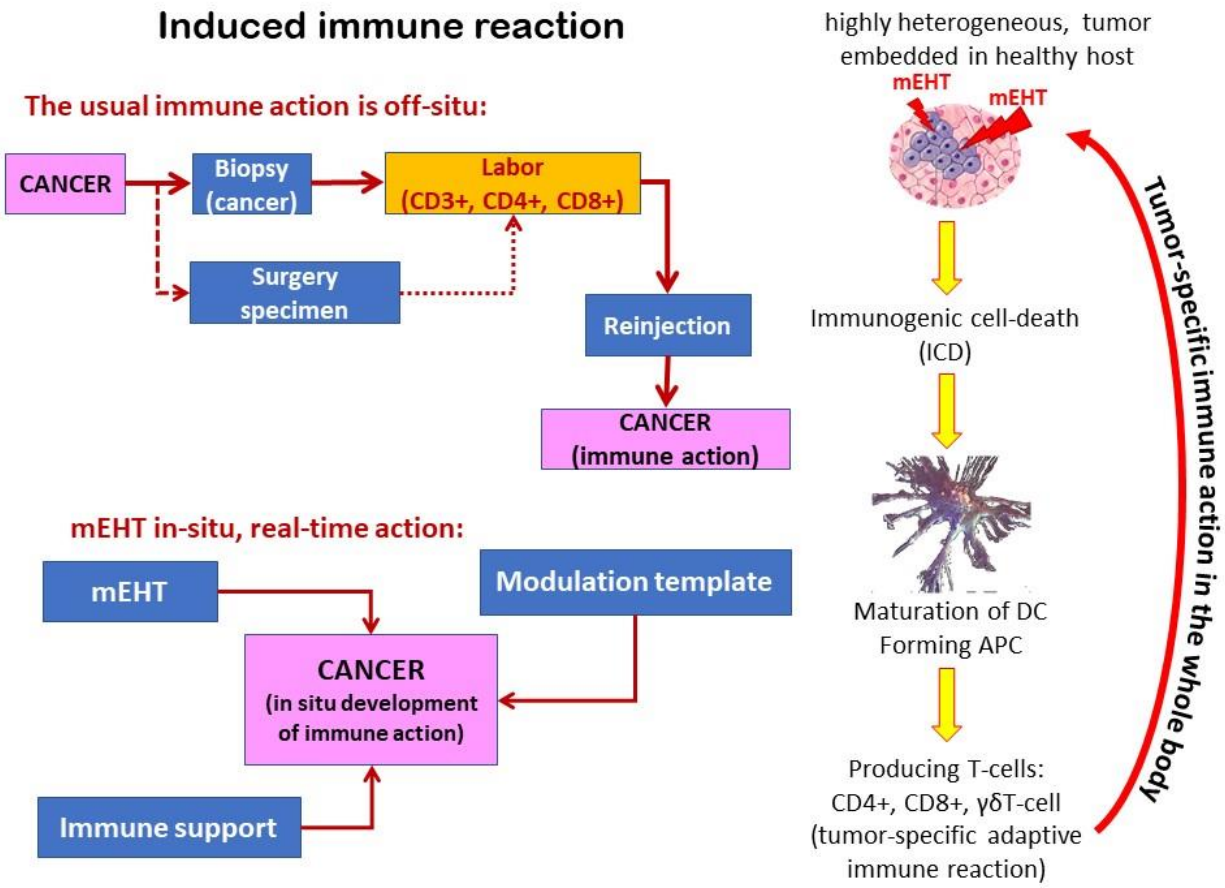
Challenge in hyperthermia – what to treat?



Goal: treat locally, act systemicly

Outline

- ❑ Challenge of locality
- ❑ Possible solution
- ❑ Verification
- ❑ Validation



Outline

- ❑ Challenge of locality
- ❑ Possible solution
- ❑ Verification
- ❑ Validation

Challenge of tumor-specific immune support (abscopal)

Apoptosis

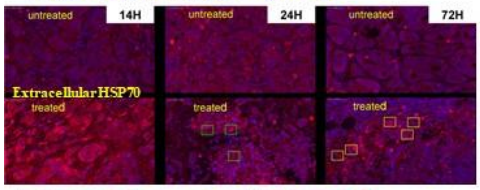
mEHT

Produce DAMP and ICD

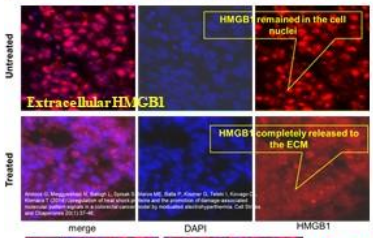
tumor-specific immune reaction, like vaccination

Andocs G, et al. (2014) Cell Stress and Chaperones 20(1):37-46

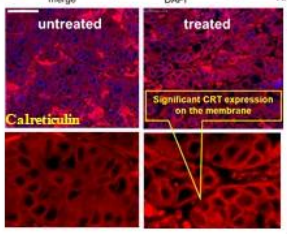
HT29 cell-line, mEHT (42°C)



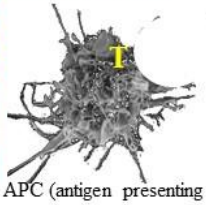
- CRT „eat me" signal
- ATP „find me" signal
- HMGB1 „danger" signal
- HSP70 "info" signal



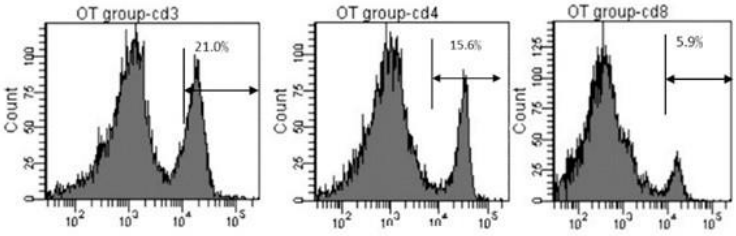
Wei Quin et.al;; Oncology Reports 32: 2373-2379, 2014



DC maturation



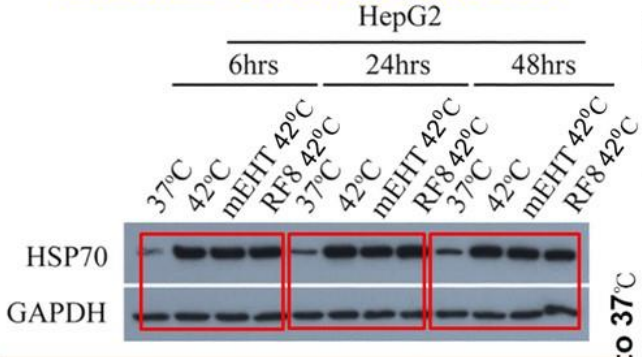
APC (antigen presenting cell)



CD3 | **CD4 T cell (helper T-cells)** | **CD8 T cell (killer T-cells)**

Comparison of the induced HSP70

Intracellular level of HSP70 expression

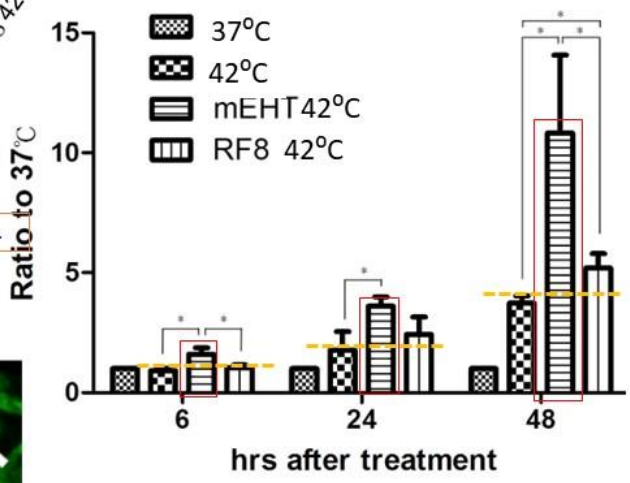


All kinds of hyperthermia could induce intracellular HSP70 expression.

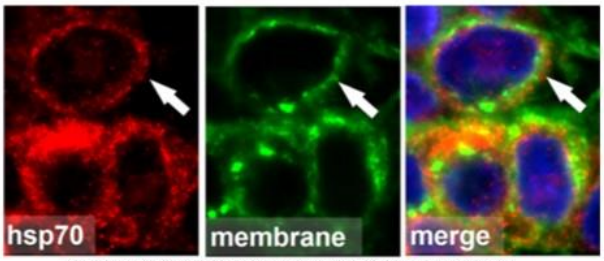
Yang K-L, et.al. (2016), Oncotarget, oi: 10.18632/oncotarget.11444

Extracellular level of HSP70 expression

Yang K-L, Huang C-C, Chi M-S, Chiang H-C, Wang Y-S, Andocs G, et.al. (2016) In vitro comparison of conventional hyperthermia and modulated electro-hyperthermia, Oncotarget, oi: 10.18632/oncotarget.11444



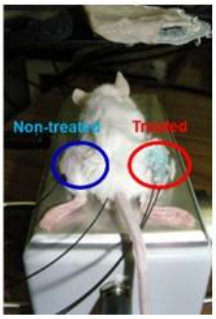
Membrane localization of HSP70 (14h)



Andocs G, et al. (2014). Cell Stress and Chaperones 20(1):37-46,

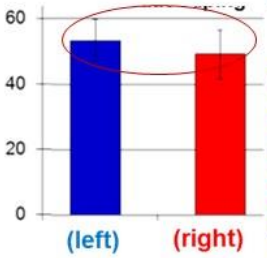
Oncothermia triggered a significantly secretion of HSP70 from cancer cells.

Abscopal effect supported by Marsdenia Tenacissima (MTE)

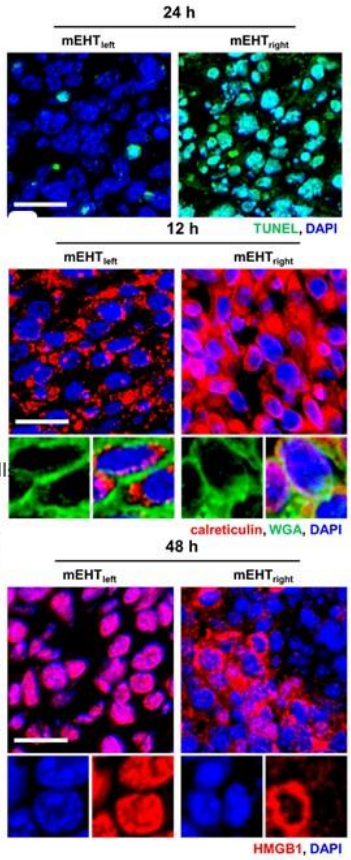


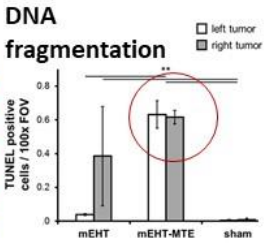
Vancsik T, et al (2018)
Journal of Cancer 9:41-53.

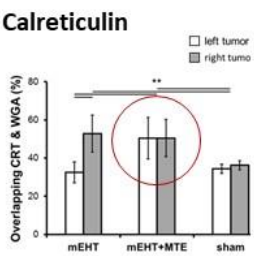
MTE + Oncothermia treated, kill the tumor systemically

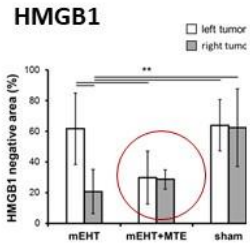


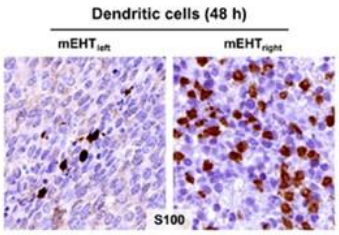
Only the right was treated!

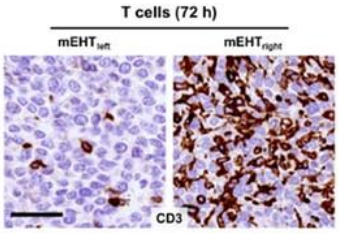


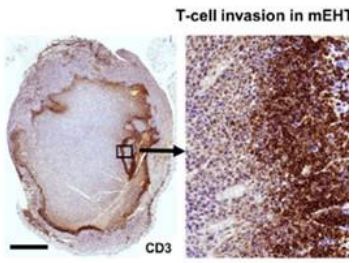












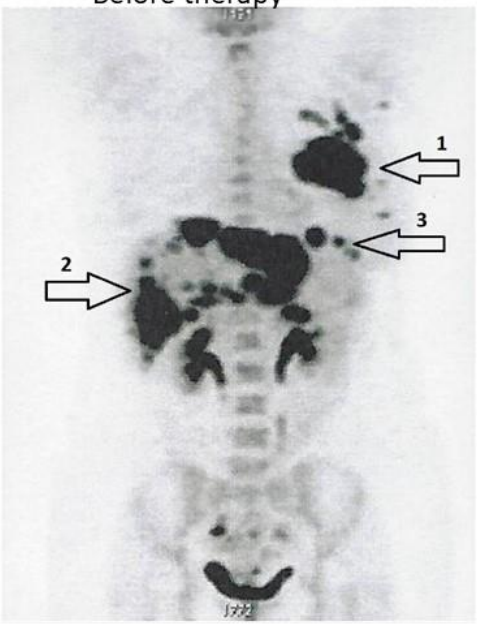
Outline

- ❑ Challenge of locality
- ❑ Possible solution
- ❑ Verification
- ❑ Validation

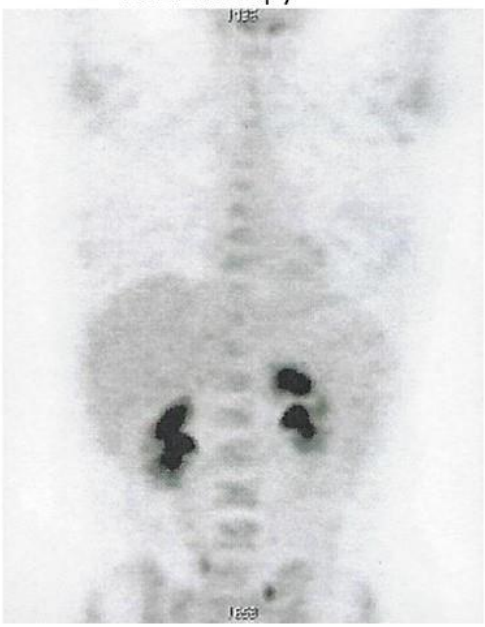
Challenge of metastases of stage IV Triple-Negative Breast Cancer

İyikesici MS, Slocum AK, Slocum A, Berkarda FB, Kalamian M, Sey\\fried TN; (2017) Efficacy of Metabolically Supported Chemotherapy Combined with Ketogenic Diet, Hyperthermia, and Hyperbaric Oxygen Therapy for Stage IV Triple-Negative Breast Cancer; Cureus 9(7): e1445. DOI 10.7759/cureus.1445

Before therapy

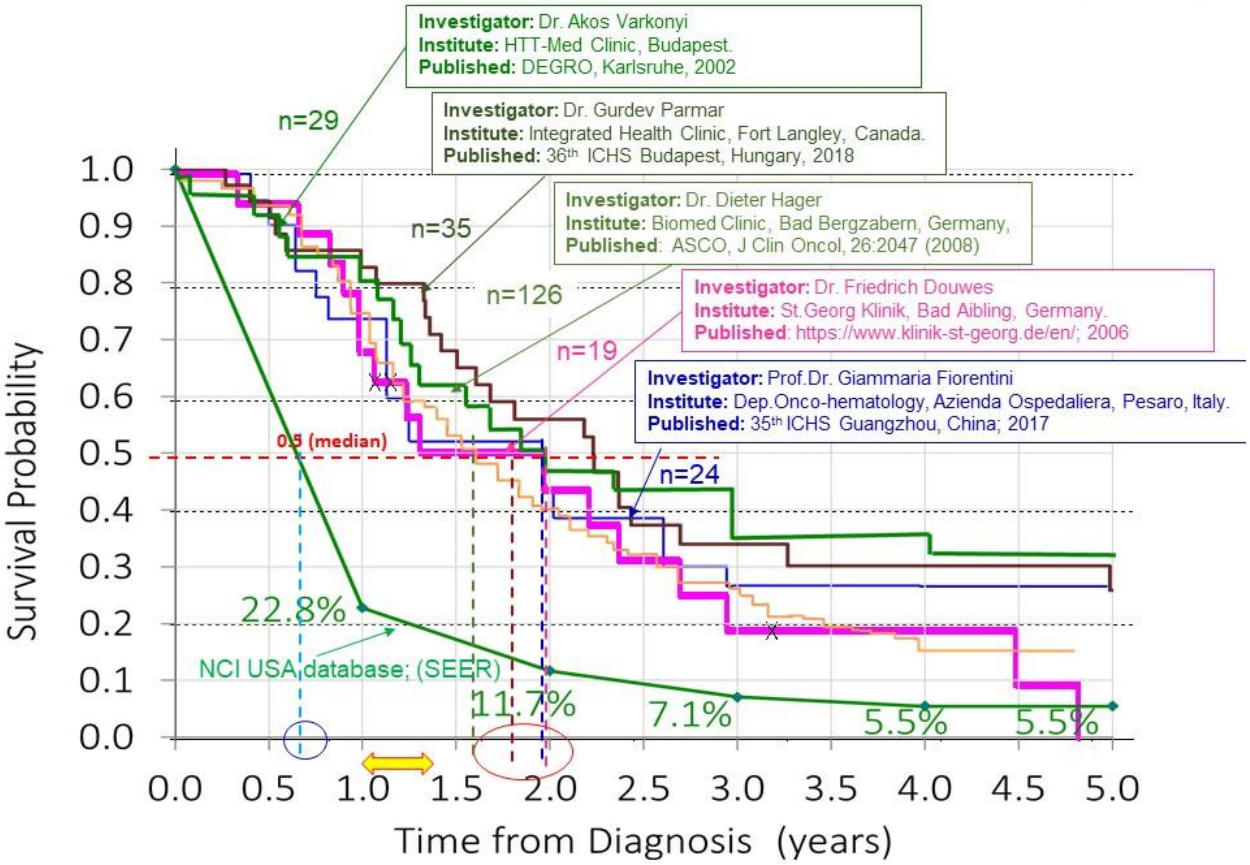


After therapy

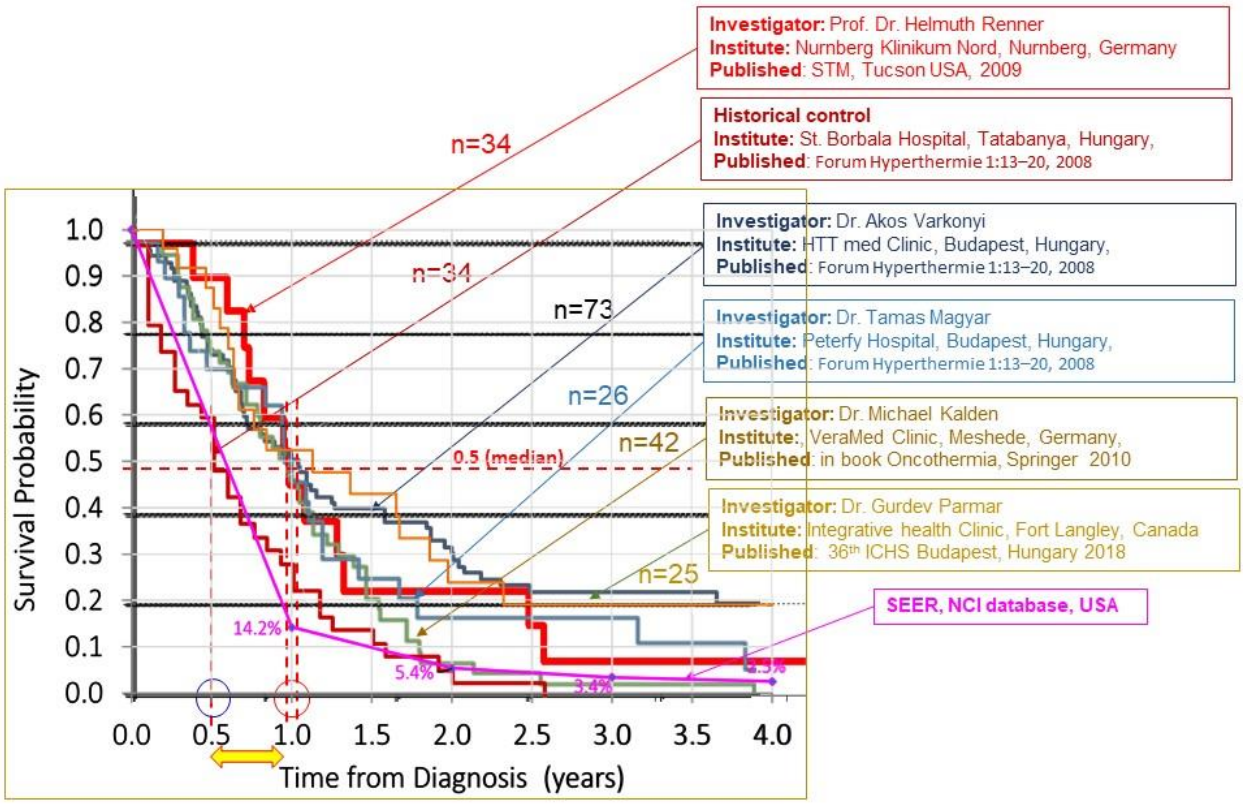


Whole body (18F)-FDG-PET-CT scan showing a 77 mm x 55 mm primary tumor in the left breast (arrow 1), multiple widespread liver masses (arrow 2), and an upper left nodular abdominal lesion (arrow 3). The metastases are so widespread, the use of arrows is nearly insufficient. Abbreviations: CT, computed tomography; FDG, fluorodeoxyglucose; PET, positron emission tomography.

Glioblastoma multiform – comparison of three survival results (N=204)



Advanced inoperable pancreas (stage IV) [N=234]



Thank you very much

Szasz Andras, PhD, professor
Szasz.Andras@gek.Szie.hu

This work was supported by the Hungarian Competitiveness and Excellence Programme grant (NVKP_16-1-2016-0042).

Molecular basis of modulated electro-hyperthermia combination with radio- and chemo-therapies

Oliver Szasz

Department of Biotechnics, St. Istvan University, Hungary

Presented at the 33rd ESHO,Warsaw

Cite this article as:
Szasz O. (2019) Molecular basis of modulated electro-hyperthermia combination with radio- and chemo-therapies: Oncothermia Journal 26:149-157
https://oncotherm.com/sites/oncotherm/files/2019-07/Molecular_basis_of_modulated_electrohyperthermia.pdf

Introduction: Hyperthermia is mostly a complimentary therapy applied together with conventional radio- and chemotherapies. Our objective is to show the molecular basis of the selectively applied hyperthermia and its interaction with radiotherapy and chemotherapy drugs.

Method: Modulated electro-hyperthermia (mEHT, trade name: oncothermia) is a complementary treatment to conventional tumor-therapies. The impedance-controlled radiofrequency current is amplitude modulated and maximized to selective targeting of the tumor-cells. Biophysical differences between the malignant and healthy cells allow the selective energy absorption in the tumor-volume. There are numerous molecular evidences proving the extreme capability of mEHT to kill the malignant cells while the healthy cells are unharmed. Additional simple compounds like Quercetin, Methotrexate, or Salicylic acid were combined with mEHT.

Results: The applied chemotherapies show synergic combination with the molecular processes of mEHT, and the radiation therapy also show synergy for cell-destruction. Significant tumor-cell death is shown by TUNEL caused by mEHT. Immunohistochemistry and apoptosis protein array proved elevated hsp70 and hsp90 expression extracellularly. The set of molecules in the measured apoptotic processes concluding to immunogenic cell-death (ICD). The abscopal effect is proven by the in-vivo experiment using an intratumoral dendritic cell (DC) injection together with the mEHT. The systemic antitumor effects are proven. The abscopal effect with mEHT works like vaccination. The ionizing radiation combined with mEHT increases the apoptosis significantly (20%) higher than in case of conventional heating, while the autophagy changes 5 times. The oxygenation of the tumor were significantly higher when mEHT was applied with radiation, than in conventional heating. All the combined application with chemo-agents had shown significantly better apoptosis than the same drugs with conventional heating did on the same temperature. The Quercetin helped the caspase independent apoptotic pathway significantly increasing the apoptosis inducing factor and the salicylic acid improved the abscopal effect of melanoma to lung. The preclinical experiments with liposomal doxorubicin also shown significant advantage of mEHT.

Conclusion: Complementary application of mEHT with chemotherapies and radiation therapy showed its significant advantages in preclinical experiments.

ESHO

Molecular basis of modulated electro-hyperthermia combination with radio- and chemo-therapies

-

Oliver Szasz, Ph.D.

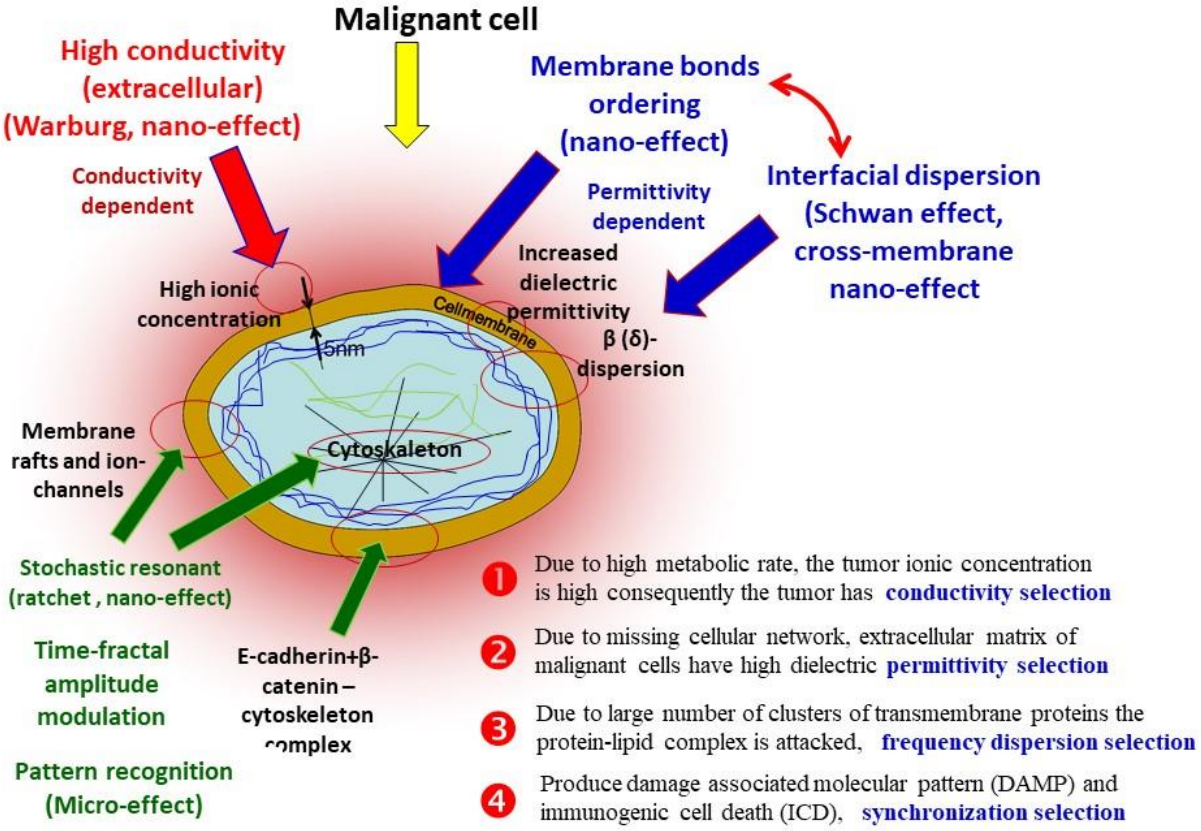
Possible conflict of interest and expertise:

- CEO of company Oncotherm (1988)
- Private docent (associate professor) at Biotechnics Department of St.Istvan University

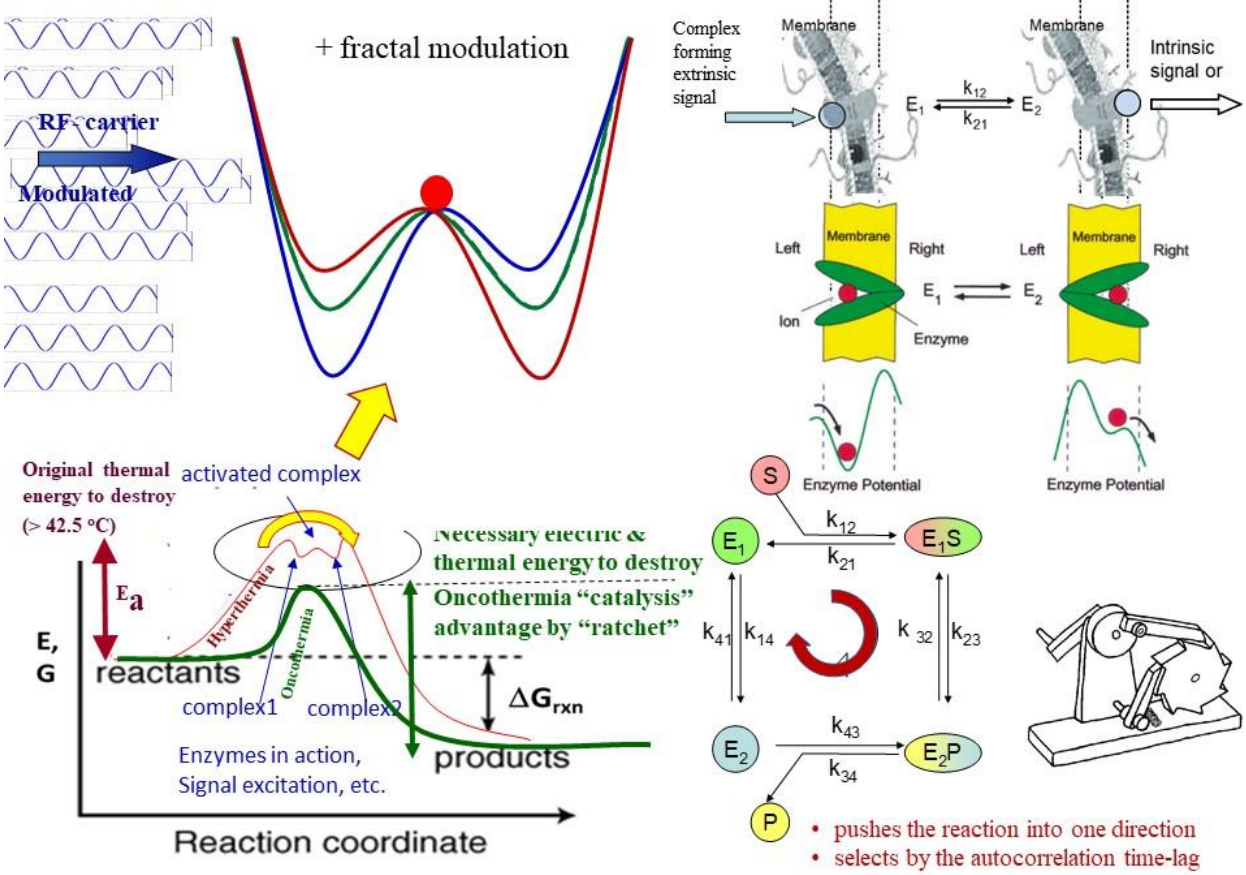
Outline

- ❑ **Cellular selection**
- ❑ **Molecular selection**
- ❑ **Complementary synergy**

Effects of modulated electrohyperthermia (mEHT)



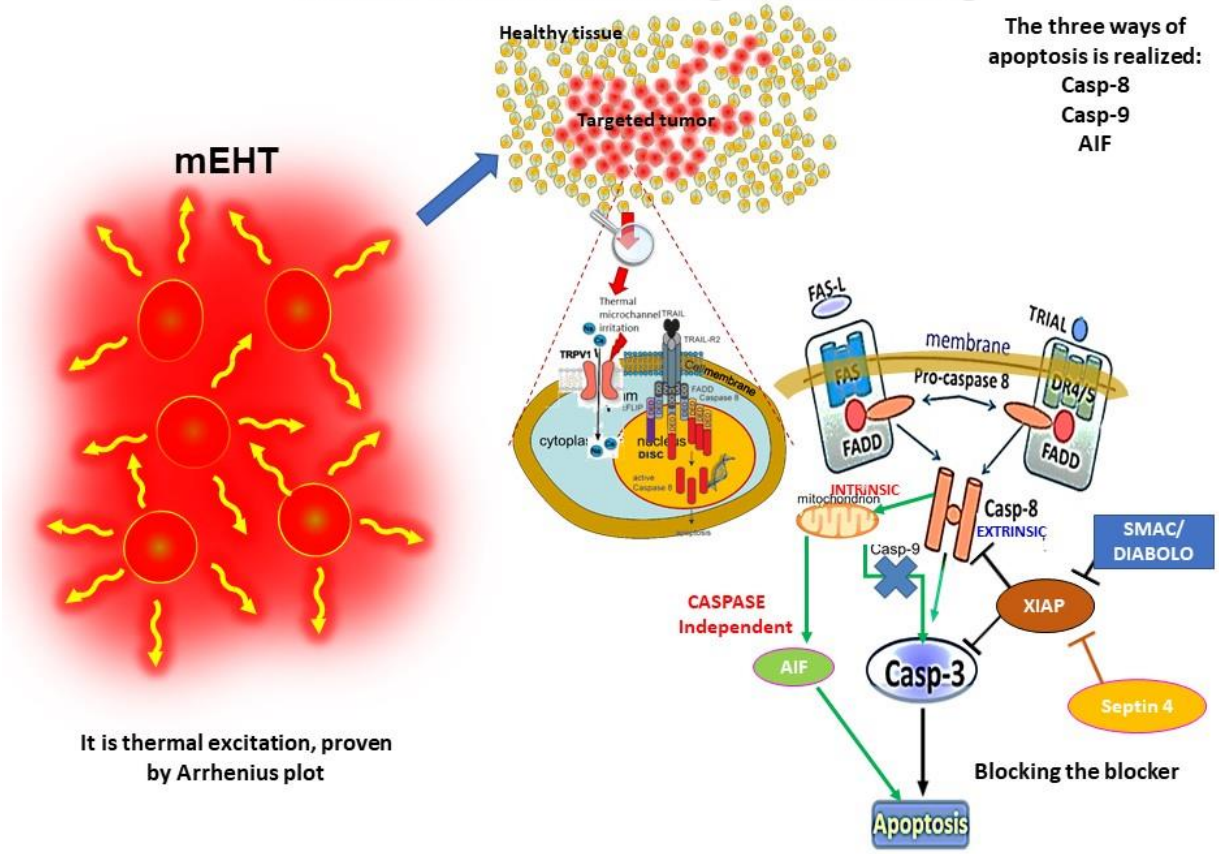
Stochastic resonant "ratchet"



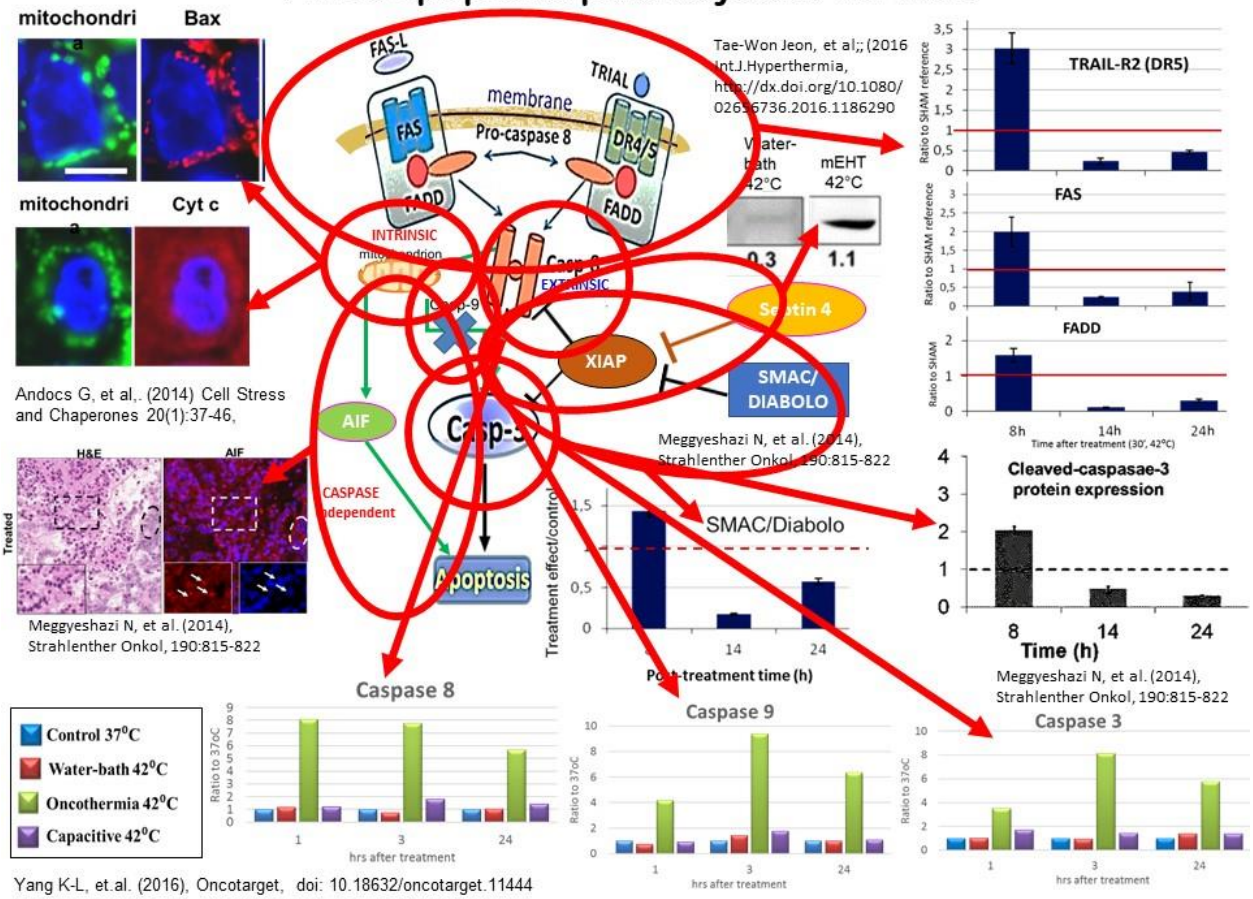
Outline

- ❑ Cellular selection
- ❑ Molecular selection
- ❑ Complementary synergy

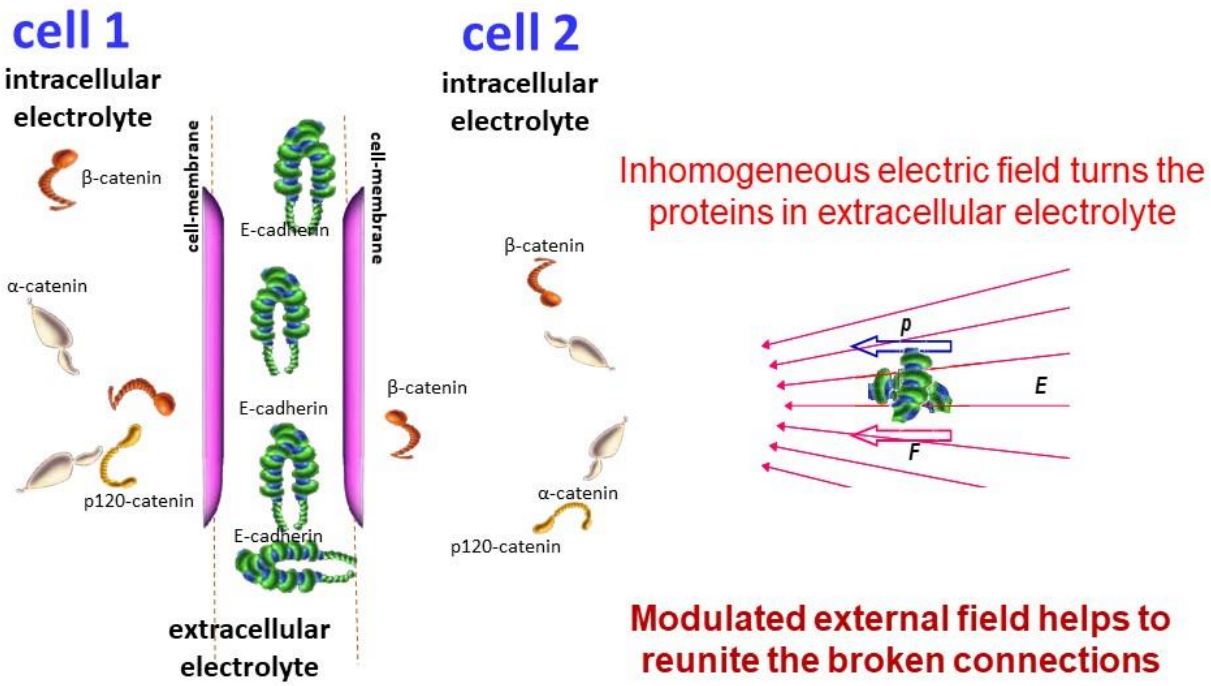
Cellular selective, heterogeneous heating



Three apoptotic pathways are verified

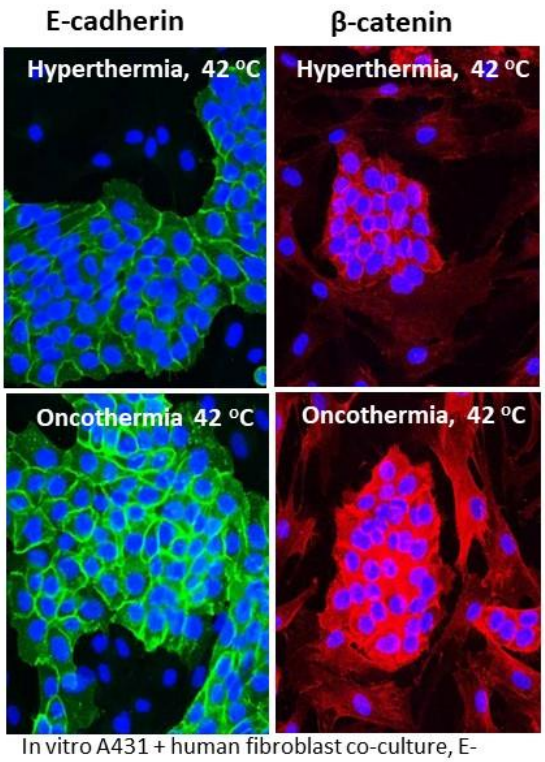


Challenges in invasion/dissemination



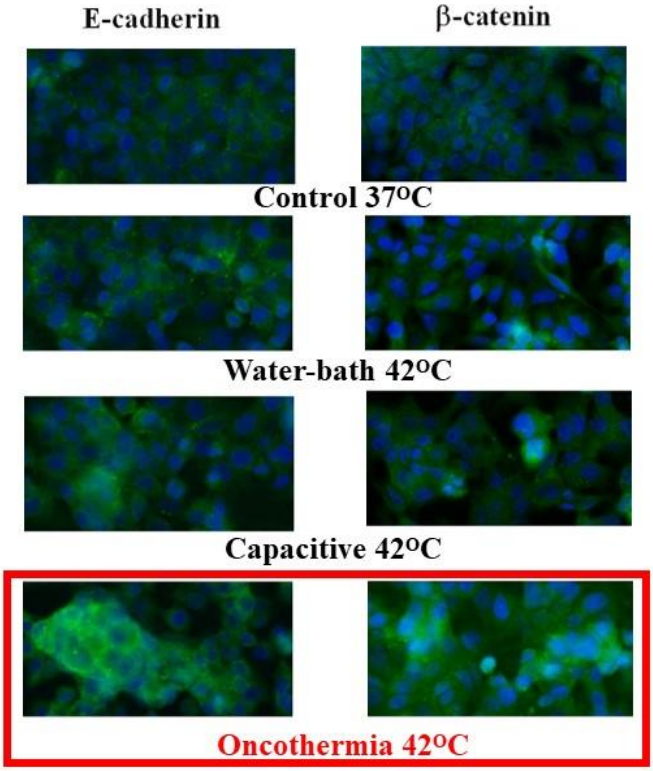
Malignant autonomy

Block the invasion and dissemination



In vitro A431 + human fibroblast co-culture, E-cadherine and β-catenin; 24h after the treatments

Andocs G, Szasz O, Szasz A (2009) Oncothermia treatment of cancer: from the laboratory to clinic. Electromagn Biol Med 28(2):148–165



Yang K-L, Huang C-C, Chi M-S, Chiang H-C, Wang Y-S, Andocs G, et.al. (2016) In vitro comparison of conventional hyperthermia and modulated electro-hyperthermia, Oncotarget, oi: 10.18632/oncotarget.11444

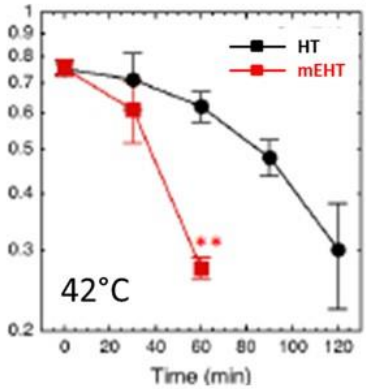
Outline

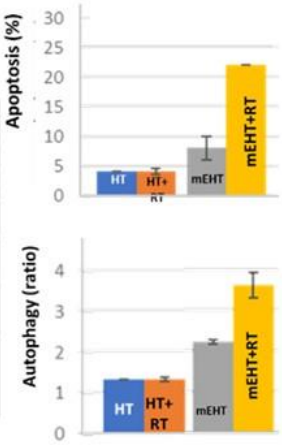
- ❑ **Cellular selection**
- ❑ **Molecular selection**
- ❑ **Complementary synergy**

Radiation with mEHT

In vitro SCCVII (SCC7), a mouse head and neck carcinoma cell line

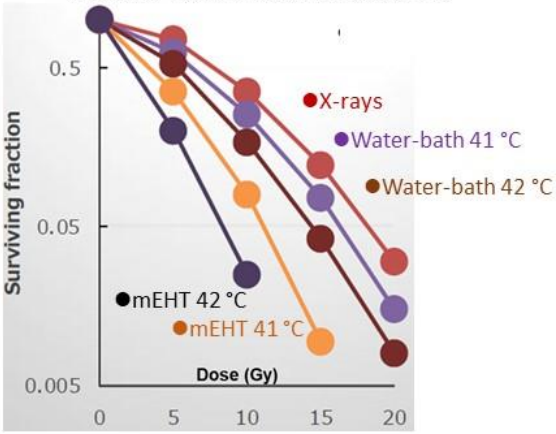
Airi OTA et al.(2017) Conf. JHA of Cancer



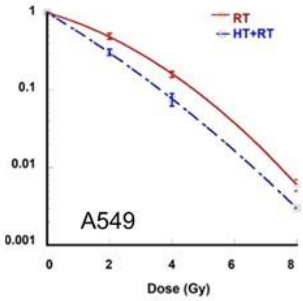


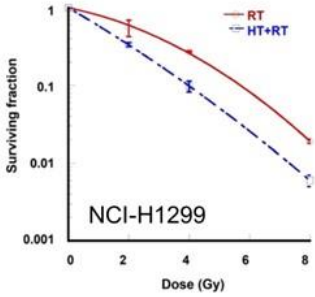
In vivo oral-cancer (SAS) cell-line

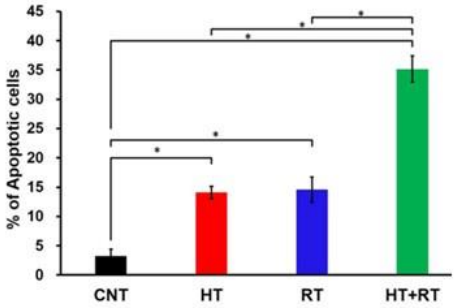
Matsumoto Y. et al. (2018), 35th Annual conference of Japanese Thermal Society, Fukui August 27-28



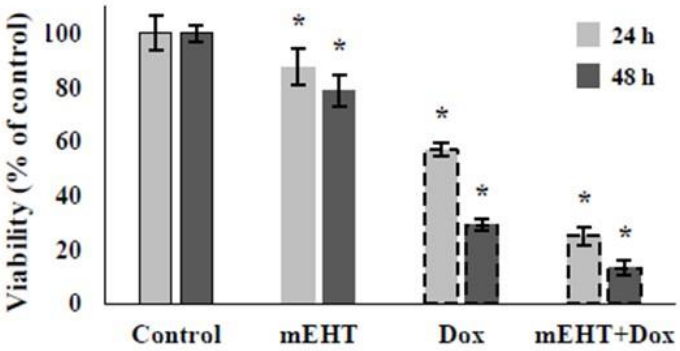
In vivo lung tumor (A549 and HCI-H1299 cell-lines; Bibin Prasad, et al; (2019); Scientific reports *9:3942;* www.nature.com/scientificreports

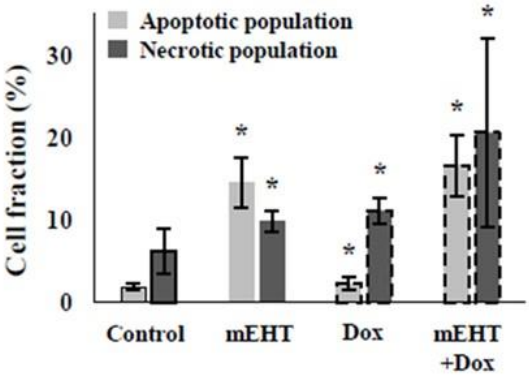






mEHT with doxorubicin chemotherapy (Poster P01)





Vancsik T et al (2019) Modulated electro-hyperthermia induced p53 driven apoptosis and cell cycle arrest additively support doxorubicin chemotherapy of colorectal cancer *in vitro,*

Thank you very much

Oliver Szasz, PhD,
associate professor

biotech@gek.szie.hu

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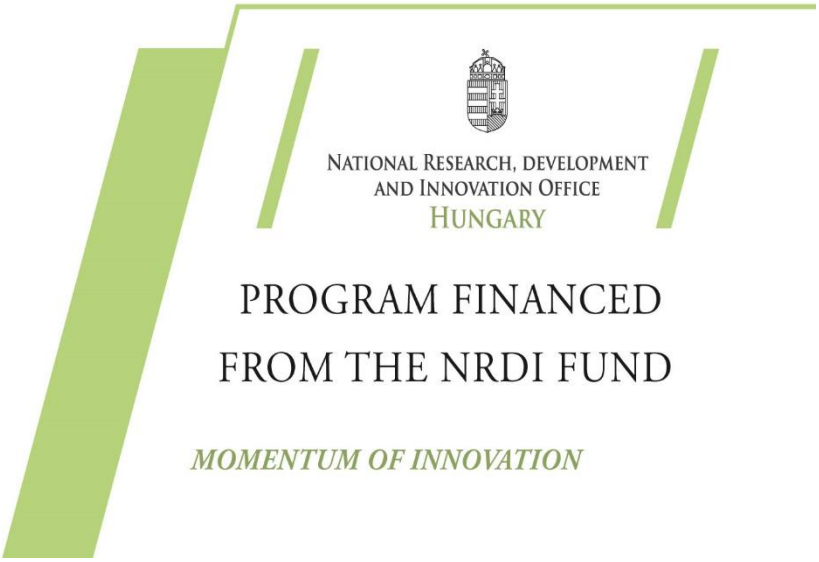

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NEW BOOK!

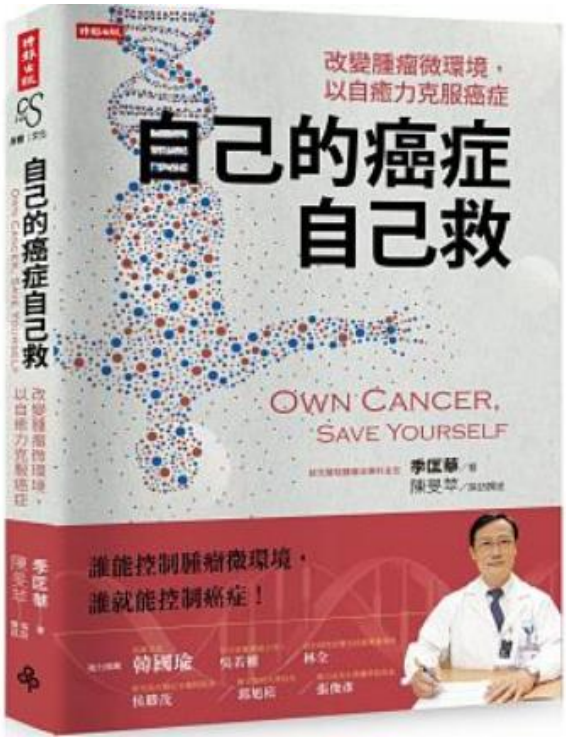
NEW BOOK!

Prof. Dr. Kwan Hwa Chi

Rescuing your own cancer: changing the microenvironment of the tumor to overcome cancer with self-healing

Authors: **Ji Kuang Hua , Chen Min Ping**

Publisher: **Times Publishing**



How to order the book?

Visit the following link:

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- **Umfassende Studienlage**

Intervall-Höhenlufttraining auf Knopfdruck

- im individuellen einstellbaren Intervall wird abwechselnd hypoxische (O_2-reduziert) und hyperoxische (30-40% O_2) Luft inhaliert.
- Hypoxie- und Hyperoxiephasen werden automatisch geregelt und eingestellt.
- Die Steuersoftware passt den Sauerstoffgehalt der eingeatmeten Luft permanent an die individuelle Sauerstoffsättigung (SPO_2) des Trainierenden an.
- Der Sauerstoffgehalt kann sehr feinfühlig (in 0,1 Schritten – entsprechend ca. 50 Metern) eingestellt werden.

Höhenverträglichkeitstest

- Vorprogrammierte Höhenverträglichkeitstests (Hypoxic-Response-Test) zur Beurteilung der individuellen Hypoxie-Sensitivität und zur Ermittlung optimaler, individueller Trainingsparameter.

Genauigkeit und Sicherheit

- **Biofeedback:** Bei Abweichungen der gewünschten Sauerstoffsättigung reguliert der Generator den O_2-Gehalt sehr feinfühlig, bis der gewünschte SPO_2-Zielwert erreicht wird.
- **Safety Cut Off:** Bei Unterschreitung einer vorgegebenen minimalen Sauerstoffsättigung (SPO_2) schaltet der Generator sofort auf Umgebungsluft (Normoxie) um.

Visualisierung und Dokumentation der Messdaten

- Echtzeit-Messung und Darstellung der Bioparameter auf dem PC-Monitor im Zeitverlauf dargestellt: O_2-Gehalt, Sauerstoffsättigung, Puls, Zeit, Phasen (Hypoxie/Normoxie).
- Dokumentation und Ausdruckmöglichkeit der einzelnen Sessions.

European Representative – The Pockwood, Corp.
Neuer Wall 80, D-20354 Hamburg
Tel. 0049 - (0)40 - 822138415
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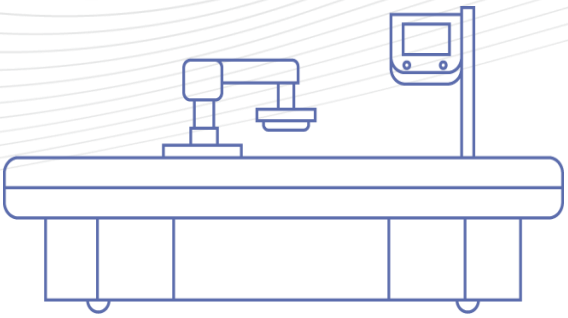
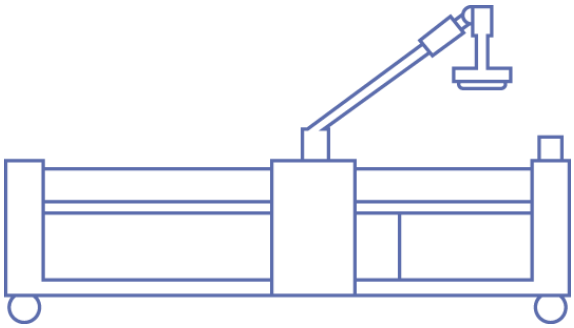
THE ONCOTHERMIA METHOD & DEVICES

- Oncothermia is based on the classical method of Hyperthermia, one of the oldest cancer treatment methods. Unlike conventional Hyperthermia, Oncothermia does more than simply warm up deep layers of tissue. It combines such warming with a modulated electric field, with a carrier frequency of 13.56 MHz, which is generated by two active electrodes.

EHY-2030

The EHY-2030 is our latest development in the treatment of loco-regional (including deep and surface) tumors. The newly designed device includes the Smart Electrode Seystem (*SES*), the plug-in Patient Management System (*PMS-100*) and a user-friendly touch screen display with full system control. The new RF generator with increased power has been developed with a new intelligently controlled step motor tuning system for rapid impedance matching to achieve faster tuning times.



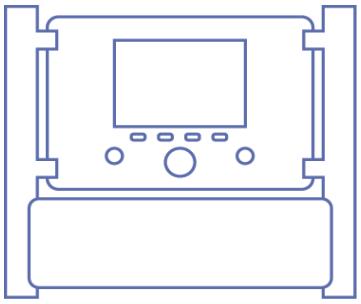


EHY-2000plus

The EHY-2000plus is a widely accepted system for loco-regional deep mEHT applications. This model has been used for treatment worldwide for more than 20 years. Popular, versatile device, applicable for a range of solid tumors and improved over the years through feedback from our doctors and experts and the requirements of patients and the people treating them. The EHY-2000plus is an easy to use and highly reliable device.

EHY-1020

The EHY-1020 is specifically designed to treat prostate diseases. Both malignant and benign tumors (BPH) can be treated using this system. It uses a catheter with built-in electronics and counter electrode. The EHY-1020 system is compact and easy to use. The method has been successfully used by our customers since 2010 with high success rates and minimal side effect.



- HUNGARY

Oncotherm Kft.
Gyár utca 2.
2040 Budaörs, Hungary

Phone (+36) 23-555-510
Fax (+36) 23-555-515

info@oncotherm.org
www.oncotherm.hu

- GERMANY

Oncotherm GmbH
Belgische Allee 9
53842 Troisdorf, Germany

Phone (+49) 2241-319920
Fax (+49) 2241-3199211

info@oncotherm.de
www.oncotherm.de

- USA

Oncotherm Ltd.
LLC, 1942 Broadway Street
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United States

Phone: (406) 225-7009
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