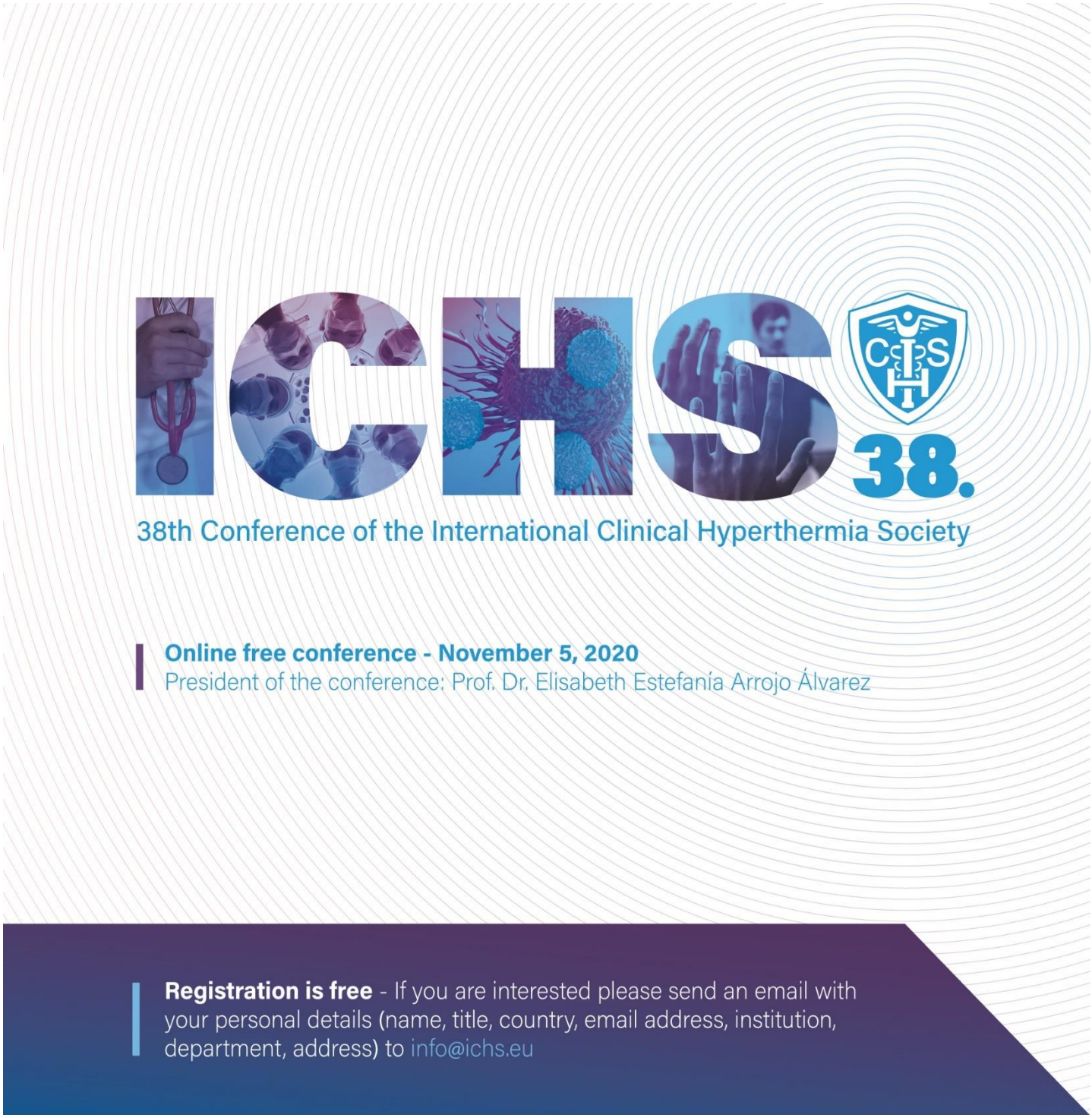
ABSTRACTS & PROGRAM



“Difficulties are the only thing that have the power to make us grow in skills …”

In these current times when we believed we were so advanced as to think that pandemics were a thing of the past, or of underdeveloped countries; where the great world powers, a few months ago, even showed a certain pride, believing themselves invincible to what was devastating their neighbors … Times that force us to rethink our priorities as a society. Times in which we prove how the rich and the poor are equal in their condition of finite humans … Times, as Darwin would say, of evolution for survival, we should stop to reflect upon such a strong impact like the one we are suffering.

Stephen Hawking said that "The worst enemy of knowledge is not ignorance; it is the illusion of knowledge." Unfortunately, we have seen and are seeing, how the illusion of knowledge in the global measures in the face of the COVID19 pandemic, which have been affirmed as safe, with subsequent disastrous results, have caused dramatic consequences. And this shows, how without investigation, without real knowledge, we will not get the weapons we need to protect our physical and mental integrity. We cannot suppose, we need “to know".

I hope that this pandemic situation improves and disappears as soon as possible, although very tough months are still ahead. But my greatest wish after this, is that this terrible situation, at least helps us to grow as human beings with values. May all this serve to put on the table what really matters. That once and for all, the scientific world can be supported in its research to improve and protect our being, our health. Research will always have an important component of personal and professional sacrifice of the researchers, but at least, that it stops being a heroic task, in which getting involved makes those people who put all their talent and effort in their great investigations ending many times exhausted, desolated, ruined, and even despised.

All my respects to all the brilliant people that exist in our world in various disciplines. But hopefully one day, we will see mass celebratory meetings with society in the street, each time we win a great "scientific match", each time a researcher finds something great which will improve our lives.

Having said that, and as human beings have great resilience, I am sure that all of this will make us stronger. In fact, we are already getting stronger. As highly adaptable beings, we are making an immense effort to carry on with our lives in the middle of the chaos. The scientific world continues to fight even harder against difficulties. We have seen our research suspended or delayed, but here we are, at this 38th Conference of the International Clinical Hyperthermia Society, to continue blazing a trail on the hard and thorny path of cancer, opening new paths that will lead us to a better destination. I do not know if one day humanity will be able to eradicate cancer, but I do know that it will cease to be one of the main causes of death, thanks to the efforts of so many researchers. Thanks to all the people who hold out your hand, sharing your experience, knowledge and dedication on the hard path of scientific knowledge.

And one last thought, this time addressed to our world leaders. People with COVID-19 without treatment may die. People with cancer, without treatment, will die for sure. Let's not try to fix a problem creating a bigger one ... "Difficulties often prepare an ordinary person for an extraordinary destiny" (C.S. Lewis). Courage colleagues.

Elisabeth Arrojo, MD, PhD Radiation oncologist, President of the ICHS

PROGRAM

Time zone: GMT +2, CET (Central European Time)

Starts	Ends	Topic	Presenter
9:00	**9:10**	**Opening**	**Prof. Elisabeth Arrojo**
9:00	**10:40**	**I. session: clinical presentations**	
9:00	9:15	Integrating loco-regional hyperthermia into the current oncology practice: A SWOT and TOWS analysis	Prof.Stephan Bodis
9:15	9:20	discussion	
9:20	9:35	Pediatric application of modulated electrohyperthermia (mEHT)	Prof. Sun-Young Lee
9:35	9:40	discussion	
9:40	9:55	Modulated electro-hyperthermia (mEHT) in monotherapy for painful bone metastases. A new promising indication?	Prof. Elisabeth Arrojo
9:55	10:00	discussion	
10:00	10:15	Four case reports on complex high risk sarcoma cases treated with modulated electro-hyperthermia	Dr. Carrie Minnaar
10:15	10:20	discussion	
10:20	10:35	Modulated electro-hyperthermic treatment in the therapy of inoperable pancreatic cancer patients - a single center case-control study	Dr. Marcell Szász
10:35	10:40	discussion	
10:40	**11:10**	**Coffee break with posters (Gertrúd Forika, Lea Danics, Csaba Schvarcz)**	
11:10	**12:40**	**II. session: non-clinical presentations**	
11:10	11:25	A biophysical framework to analyze (pre-)clinical data on non-thermal effects	Prof. Peter Wust
11:25	11:30	discussion	
11:30	11:45	Potential enhancement of host immunity and anti-tumor efficacy of nanoscale curcumin and resveratrol in colorectal cancers by modulated electro- hyperthermia	Dr. Yu-Shan Wang
11:45	11:50	discussion	
11:50	12:05	Modulated electro-hyperthermia and combined primary, immortalized NK-cell therapy in human A2058 xenograft model	Dr. Tamás Vancsik
12:05	12:10	discussion	
12:10	12:25	Where to go from here?	Prof. András Szász
12:25	12:30	discussion	
12:30	**12:40**	**Closing remarks**	**Prof. Elisabeth Arrojo**

ABSTRACTS

The abstracts are listed based on the program

Integrating loco-regional hyperthermia into the current oncology practice: A SWOT and TOWS analysis

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Moderate hyperthermia at temperatures between 39 and 45°C is a multifaceted therapeutic modality. It is a potent radiosensitizer, interacts favorably with a host of chemotherapeutic agents and with RT enforces immunomodulation akin to “in situ tumor vaccination.” By sensitizing hypoxic tumor cells and inhibiting repair of radiotherapy-induced DNA damage, the properties of hyperthermia delivered with photons provides a tumor-selective therapeutic advantage analogous to high LET neutrons, but without normal tissue toxicity. Furthermore, the high LET attributes of hyperthermia thermoradiobiologically enhance low LET protons; thus, proton thermoradiotherapy mimics 12C ion therapy. Hyperthermia with radiotherapy and/or chemotherapy substantially improves therapeutic outcomes without enhancing normal tissue morbidities yielding level I evidence as reported in several randomized clinical trials, systematic reviews and meta-analyses for various tumor sites. Further, hyperthermia along with immune check point inhibitors and DNA damage repair inhibitors could further augment the therapeutic efficacy resulting in synthetic lethality. Besides technological advancements in hyperthermia delivery, complemented by hyperthermia treatment planning, its integration with radiotherapy treatment plans, online thermometry and adherence to quality assurance guidelines have all ensured safe and effective delivery of hyperthermia to the target region. Additionally, hyperthermia induced by magnetic nanoparticles coupled to selective payloads provides a comprehensive tumor-specific theranostic modality akin to “magic (nano)bullets.” To get a realistic overview of the strength (S), weakness (W), opportunities (O) and threats (T) of hyperthermia, a SWOT analysis has been undertaken. Additionally, a TOWS analysis categorizes future strategies to facilitate further integration of hyperthermia with the current treatment modalities. These could gainfully accomplish a safe, versatile and cost-effective enhancement of the existing therapeutic armamentarium to improve outcomes in clinical oncology.

Keywords: hyperthermia, radiation therapy, chemotherapy, immunotherapy, radiosensitizer, hyperthermia treatment planning, SWOT analysis, clinical trials

Pediatric application of modulated electro-hyperthermia (mEHT)

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Introduction: Pediatric oncology has numerous tasks that are difficult to handle with conventional therapies. Most of the cancers that are common in adults differ from those seen in children and adolescents. In the last three decades, mortality from pediatric cancers has been cut in half, which is a remarkable result, but many cases are difficult and challenging and often do not have conventional protocols.

Method: I will show several cases successfully treated with a new treatment modality: modulated electro-hyperthermia (mEHT, trade name of oncothermia). When conventional therapies were not effective enough, mEHT was applied as a complementary treatment to existing protocols. We followed the case through various imaging facilities (CT, MRI, PET) as well as laboratory controls and measurement of tumor markers.

Results: I have collected 8 characteristic cases, 5 boys and 3 girls, with severe neoplasms. The age of the children ranged from 1 to 16 years. Treated tumors cover a wide spectrum of cases, including neuroblastoma, brain stem cell tumor, germ cell tumor, B cell lymphoma, Hodgkin lymphoma, and desmoplastic small cell tumor. The children received intensive pretreatments, underwent surgery when possible, and were given appropriate adjuvant chemotherapy and radiation therapy. At the end of a long follow-up (various periods of time), three children have no evidence of disease, one has stable disease, and one died. Two children were transferred to another hospital, with no data available on their current condition. The children tolerated the treatments well, no notable adverse effects were observed.

Discussion: I will show the details of the cases in my presentation. The main guide we followed in therapy was to focus on the child rather than looking at the tumors only. This complex approach was in harmony with our general philosophy: change from tumor-oriented to patient-oriented methods.

Conclusion: These case reports show the feasibility of applying mEHT to pediatric tumors in the cases studied, of children from 1 year up to 16 years.

Modulated electro-hyperthermia (mEHT) in monotherapy for painful bone metastases. A new promising indication?

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[2] ***Beatriz Suarez, MD;*** *University Hospital Central de Asturias, Asturias, Spain.*
[3] ***Diego Arribas;*** *Medical Institute of Advanced Oncology (INMOA), Madrid, Spain.*
[4] ***Amaya Serrano;*** *Medical Institute of Advanced Oncology (INMOA), Madrid, Spain.*

Introduction: Painful bone metastases (PBM) have a great negative impact in patient's quality of life. Pain drugs for PBM are usually not enough and have serious side effects. Up to date, radiotherapy is the most effective treatment for PBM, but has some important limitations (toxicity, dose limits…).

Material and methods: We prospectively included 10 patients with different types of primary active tumors with PBM and tested mEHT as an "analgesic" treatment for PBM. 9 patients had solid tumors and 1 had a multiple myeloma with only one vertebral body affected. All patients had pain which was not responding to systemic and/or analgesic treatment.

Table 1. Patient characteristics and results

	*Sex	Primary Tumor	Systemic Treatment	**mEHT NO bone / Progression		mEHT Bone / Pain Response	
1	F	GYN	YES	UTEROUS	YES	FEMUR	**YES**
2	F	BREAST	YES	BREAST	NO	VERTEBRA	**YES**
3	M	MYELOMA	NO	NO	-	VERTEBRA	**YES**
4	F	BREAST	YES	LIVER	YES	HIP	**YES**
5	F	BREAST	YES	BRAIN	YES	VERTEBRA	NO
6	F	BREAST	NO	NO	-	VERTEBRA	NO
7	F	LUNG	YES	NO	-	RIBS	**YES**
8	F	SARCOMA	YES	NO	-	HIP	**YES**
9	F	BREAST	NO	NO	-	HIP	**YES**
10	F	BREAST	YES	NO	-	VERTEBRA	**YES**

**SEX: F: Female, M: Male.*
***Patients treated with mEHT at other "no bone" sites with tumor and evidence of progression at those sites after mEHT treatment.*

Results: All patients with solid tumors had stage IV (AJCC) and the patient with myeloma had stage III (ISS). All patients received between 5 and 12 mEHT treatments at PBM sites. Seven patients were under systemic treatment. 80% of the patients had significant pain response to mEHT treatment.

Three patients had radiotherapy scheduled and after mEHT treatment, did not need to receive it. Patient's pain response to mEHT was not related to systemic tumor response. Despite tumor progression at other sites treated with mEHT, mEHT was very effective on pain control for treated PBM. It's remarkable, that the patient with the no solid tumor (myeloma), had a significant pain response after mEHT treatment in monotherapy.

Table 1. Patient characteristics and results

*SEX: F: Female, M: Male. **Patients treated with mEHT at other "no bone" sites with tumor and evidence of progression at those sites after mEHT treatment.

Conclusions: mEHT can be a very safe and effective treatment for PBM as a combined treatment, but also in monotherapy. Contrary to the common belief that mEHT does not works in hematological tumors, mEHT may have a role also in no solid tumors as multiple myeloma. These findings open a very interesting path of research.

Four case reports on complex high risk sarcoma cases treated with modulated electro-hyperthermia

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[2] *University of the Witwatersrand; Radiation Sciences*

Introduction: Radiative hyperthermia (HT) for the treatment of sarcomas has been applied in combination with chemotherapy, showing improved local control and survival [1,2]. Only two small studies have assessed HT plus radiotherapy (RT) [3,4] and few have assessed mEHT for the management of sarcomas.

Objectives: To determine if there is sufficient motivation for the addition of mEHT, as a more affordable and practical HT solution, in the management of sarcomas, in the absence of any further options.

Material and methods: We present four interesting and complex cases of local recurrences in a previously irradiated region treated with modulated electro-hyperthermia (mEHT) locally, combined with chemotherapy or RT, at our facility.

Results:
Patient 1: 42yr old female with a synovial sarcoma of the heart valve treated with a heart transplant and chronic immunosuppressant medication; mediastinum and pulmonary nodules developed 5yrs later. Treatment: 30x2Gy fractions + mEHT to the mediastinum. Inactive pacemaker present, outside the treatment field. Patient is stable at 18months post treatment and is enjoying an excellent quality of life.

Patient 2: 68yr old male with a local recurrence of a myxoid liposarcoma in the right thigh (30cm) 1yr after excision and RT. Treatment mEHT twice weekly for 19mnths (12 of which chemotherapy was administered 3wkly). Tumour shrunk significantly and remained stable until the patient died of septicaemia from a wound infection on his foot.

Patient 3: 52yr old woman with an RAS 5yrs after treatment for a SCC of the left maxilla sinus. Prescribed RT+mEHT. Treatment was tolerate well but the tumour did not respond.

Patient 4: Male patient with a sarcoma in the right shoulder, treated initially with surgery, followed by several local recurrences treated with either surgery, external beam radiation, or brachytherapy, over five years. Prescribed external beam radiation combined with mEHT.

Conclusions: mEHT could be considered when no further options are available as a safe adjunct to treatments. There is motivation for the design of a trial investigating RT+mEHT for the management of high risk sarcomas, especially in cases in which patients have been previously irradiated.

References:

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Modulated electro-hyperthermic treatment in the therapy of inoperable pancreatic cancer patients - a single center case-control study

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Introduction: Inoperable pancreatic cancer poses a challenge as it is often a therapy resistant tumor which bears a poor prognosis. Hyperthermic treatments aim to break this resistance and facilitate oncotherapies.

Objective: To analyze the benefit of concomitant mEHT for inoperable pancreatic cancer patients to form basis for further investigation.

Materials and methods: We present a retrospective single-center case-control study including 78 inoperable pancreatic cancer patients. The case group comprised 39 patients receiving first mEHT treatment at Semmelweis University Cancer Centre between 2016 September and 2019 November and underwent at least 19 mEHT treatment sessions. All pancreatic cancer diagnosis was confirmed during routine diagnostic protocol by histological examination between 2014.12.26 and 2019.10.17. Data collection was closed on 2020.01.31. The time elapsed between the date of diagnosis and death was as overall survival (OS).

Results: In first step each case-patient was individually matched to a control-patient by age, sex and chemotherapy administration during mEHT treatment. To reach higher similarity in overall status of the case and control patients also presence or absence of distal metastases and emerging ascites were taken in count as matching criteria by generating case-control pairs.

Of note, a trend in difference was found in overall survival of patients in case-control pairs matched for age, sex and chemotherapy receiving during mEHT treatment favoring mEHT ($p=0.0704$). Overall survival of inoperable pancreatic cancer patients with or without distant metastasis in both case and control groups was analyzed, metastatic disease resulting in significantly higher OS ($p=0.022$). Overall survival with or without the presence of ascites in both case and control showed a trend favoring mEHT treatment as well ($p=0.0611$). Elapsed time between diagnosis and start of mEHT treatment did not significantly influence overall survival.

Discussion: In our series of inoperable pancreatic cancer patients treated with mEHT applied as concomitant therapy, we have detected a significant improvement in overall survival, especially in metastatic setting. To further analyze the biological background for this treatment response, we have

concluded analyses investigating the tumor-host immunological interface and quality of life, and we have developed the protocol for a randomized clinical trial for this patient group.

A biophysical framework to analyze (pre-)clinical data on non-thermal effects

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Introduction: The existence of non-thermal effects of electromagnetic fields (EMF) is controversially discussed since decades. Earlier investigations did not identify evidences of for any risks of radiofrequency (RF) EMF at safety levels and denied the existence of non-thermal effects.

Objectives: Careful review of available preclinical and clinical data upon non-thermal effects, which are classified and analyzed by a novel membrane model

Material and Methods: Recently, preclinical studies ascertained additional cytotoxic effects, if RF-hyperthermia (HT) is applied at 13.56 or 27 MHz in comparison to water bath (WB)-HT at the same temperature. These effects can be further enhanced by amplitude modulation (AM) in the Hz to kHz-range. Preclinical data are confirmed by clinical studies and observations, in particular if EMF applications with AM are considered.

Results: A subtle analysis of preclinical and clinical data of WB-HT and conventional HT reveals numerous hints that non-thermal effects exist. A critical evaluation of all available empirical data provides sufficient evidence for non-thermal effects EMF, which have the potential to improve oncologic treatments.

In the next step, plausible biophysical and electrophysiological models are evaluated to decipher these non-thermal effects. Nanoheating of protein clusters in lipid rafts has been postulated, but needs excessive levels of local power not consistent with physical assessments. Basis for novel theories are models of ion channels, which function like rectifiers and low pass filter. It can be deduced that AM-RF induces ion fluxes and membrane vibrations at specific resonance frequencies. This model can explain non-thermal cytotoxic effects via ion disequilibrium (especially regarding Ca2+) and/or resonances with hole formation in the membrane, if AM-RF radiates for some time perpendicular to the membrane comprising a given density of ion channels.

Conclusions: Non-thermal effects induced by AM-RF are very probable. We recommend further evaluations. Higher effectiveness of AM-RF in tumors can occur because of their specific tumor

environment, cancer-specific ion channels (channelomes) and membrane elasticities differing from normal tissues with increasing malignancy. Suitable oncological applications can lead to significant improvements.

Potential enhancement of host immunity and anti-tumor efficacy of nanoscale curcumin and resveratrol in colorectal cancers by modulated electro-hyperthermia

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Background: Modulated electro-hyperthermia (mEHT) is a form of hyperthermia used in cancer treatment. mEHT has demonstrated the ability to activate host immunity by inducing the release of heat shock proteins, triggering apoptosis, and destroying the integrity of cell membranes to enhance cellular uptake of chemo-drugs in tumor cells. Both curcumin and resveratrol are phytochemicals that function as effective antioxidants, immune activators, and potential inhibitors of tumor development. However, poor bioavailability is a major obstacle for use in clinical cancer treatment.

Methods: This purpose of this study was to investigate whether mEHT can increase anti-cancer efficacy of nanosized curcumin and resveratrol in in vitro and in vivo models. The in vitro study included cell proliferation assay, cell cycle, and apoptosis analysis. Serum concentration was analyzed for the absorption of curcumin and resveratrol in SD rat model. The in vivo CT26/BALB/c animal tumor model was used for validating the safety, tumor growth curve, and immune cell infiltration within tumor tissues after combined mEHT/curcumin/resveratrol treatment.

Results: The results indicate co-treatment of mEHT with nano-curcumin and resveratrol significantly induced cell cycle arrest and apoptosis of CT26 cells. The serum concentrations of curcumin and resveratrol were significantly elevated when mEHT was applied. The combination also inhibited the growth of CT26 colon cancer by inducing apoptosis and HSP70 expression of tumor cells while recruiting CD3+ T-cells and F4/80+ macrophages.

Conclusions: The results of this study have suggested that this natural, non-toxic compound can be an effective anti-tumor strategy for clinical cancer therapy. mEHT can enable cellular uptake of potential anti-tumor materials and create a favorable tumor microenvironment for an immunological chain reaction that improves the success of combined treatments of curcumin and resveratrol.

Keywords: Modulated electro-hyperthermia (mEHT), curcumin, resveratrol, nanosized, apoptosis, tumor microenvironment

Modulated electro-hyperthermia and combined primary, immortalized NK-cell therapy in human A2058 xenograft model

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Introduction: Earlier we showed that modulated electro-hyperthermia (mEHT) promoted the expression and release of the potential immunogenic damage associated molecular pattern proteins and it reduced MHC-I and melan-A levels in B16F10 melanoma cells. The number of cytotoxic T cells were moderately reduced, the amount of NK cells was unchanged. NK cells could effectively recognize and kill cells which lack MHC-I. Here we tested the effect mEHT on tumor growth and tumor microenvironment with respect to infiltration and cytotoxicity of NK cells in A2058 human melanoma xenograft model in vivo.

Material and methods: A2058 melanoma cells were inoculated into both flanks of BALB/C NOD/SCID immunocompromised mice. After two weeks, 30-min 42°C mEHT was applied on the right-side tumors. One day after mEHT treatment, primary human NK-cells or the NK92MI NK-cell line labeled with fluorescent dye were injected subcutaneously above the lumbar region of the spine. NK-cell distribution was measured by in vivo fluorescent imaging. Tumor size was monitored using ultrasonic caliper. Tumor damage, growth arrest, heat stress and apoptosis related markers were assessed with immunohistochemistry. NK-attracting CXCL mRNA expression was determined after in vitro mEHT treatement of A2058 cells.

Results: mEHT induced significant tumor growth inhibition. Heatshock and apoptotic tumor cell death was proven by the significant elevation of relative dead tumor area, γH2AX, p53 and cleaved caspase-3 and hsp70 positive areas, accompanied by MMP-2 expression.In vivo, both the primary NK- and NK92MI-cells accumulated into the mEHT-treated side and further enhanced the damaging effect. Significant elevation of CXCL-11 mRNA level,was induced by in vitro treatment while the CXCL-9, and -10 dropped.

Conclusion: Our result show that mEHT can induce p53-mdiated caspase-dependent apoptosis in an A2058 melanoma xenograft model. Furthermore, mEHT treatment may provide a favorable micro-environment for the attraction and invasion of NK-cells, possibly by inducing CXCL-11 expression and promoting MMP-2 production of solid xenografts.

This study was funded by NKFIH-NVKP_16-1-2016-0042.

Where to go from here?

Andras Szasz

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Introduction: Hyperthermic oncology has great achievements but also raises questions about the basic mechanisms and clinical applications of the method. Despite its long history, the debates about its possibilities are intensely vivid, the pros and cons strongly and rigidly polarize professionals and develop a barrier to wide application and approval by medical and governmental associations.

Method: It is a relevant requirement to clarify at least the most challenging questions about the basics from my long time experience in the field using my own results and considering the also widely available international literature together with professional expert's opinions.

Results: I list just a few sensitive challenges for questions that come up as standard in current oncology practice in relation to hyperthermia:

- What are the basic mechanisms behind successes and failures?
- Should local or systemic treatment be preferred?
- What is the optimal temperature?
- What is the dose that defines the treatments?
- What about monotherapy?
- Why is it mainly applied to locally advanced and non-metastatic cases?
- How is it related to emerging physical therapies?
- How is hyperthermia involved in the newer concepts of immunotherapy?
- Why is hyperthermia not widely accepted by the oncology community?

Discussion: The challenge is obvious. We have more and more proven details on the challenge that heat alone is not effective enough to solve the problems of cancer and its development, due to the various complex physiological feedback mechanisms in humans. Probably the heating provides hot environment to the tumor, which promotes molecular and physiological processes. This way hyperthermia in cooperation with applied complementary treatments influences the malignancy, eliminates the cancer cells and tries to restore healthy functions. The application of bioelectromagnetic effects could guide changing activities from general tumor destruction to complex regulated and controlled reactions to achieve curative goals.

Conclusion: My presentation would like to make decisive proposals on these hot topics and connected challenges and show what the necessary steps are to move forward.

POSTER ABSTRACTS in the coffee break

Apoptotic response and DNA damage of the radioresistant Panc1 pancreas adenocarcinoma to combined modulated electro-hyperthermia and radiotherapy

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The pancreas ductal adenocarcinomas (PDAC) have a poor prognosis, due to the high resistance to standard therapies. Modulated electro-hyperthermia (mEHT) generated by 13.56 MHz capacitive radiofrequency can induce direct tumor damage and promote chemo- and radiotherapy. In this study, we tested the effect of mEHT either alone or in combination with radiotherapy using an in vitro model of Panc1, radioresistant PDAC cell line. A single mEHT shot of 60 min induced ~50% loss of viable cells and morphological signs of apoptosis including chromatin condensation, nuclear shrinkage and apoptotic bodies. The mEHT treatment related effects were more expressive when the cells were pretreated with 2Gy radiotherapy. Treatment related apoptosis was confirmed by a significantly elevated number of annexin V single-positive and cleaved/activated caspase-3 positive tumor cells, as well as sub-G1-phase tumor cell fractions. mEHT and mEHT+radioterapy caused the moderate accumulation of H2AX positive nuclear foci, indicating DNA double-strand breaks and upregulation of the cyclin dependent kinase inhibitor p21waf1 besides the downregulation of Akt signaling. A clonogenic assay revealed a tumor progenitor/stem cell loss too. In conclusion, mEHT treatment can contribute to tumor growth inhibition and apoptosis induction and resolves radioresistance of Panc1 PDAC cells.

Modulated electro-hyperthermia in combination with heat shock response inhibitors significantly increase tumor cell death

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

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Breast cancer is one of the most frequent cancer types among women worldwide. Triple-negative breast cancer (TNBC) is a highly aggressive type with very poor survival due to the lack of targeted therapy. Here we tested the efficiency of mEHT treatment alone and in combination with heat shock response (HSR) inhibitors in the 4T1 mouse TNBC isograft model. Tumors were treated with ergonomic pole electrode and LabEHY 200 device at 0.7 ± 0.3 W for 30 min every 48 h. Tumor growth was followed by IVIS, caliper, and ultrasound. Tumor destruction histology and molecular changes using immunohistochemistry and RT-qPCR were also revealed. In vivo, mEHT treatment transitionally elevated Hsp70 expression in surviving cells indicating heat shock-related cell stress, while IVIS fluorescence showed a significant reduction of viable tumor cell numbers. Treated tumor centers displayed significant microscopic tumor damage with prominent signs of apoptosis, and major upregulation of cleaved/activated caspase-3-positive tumor cells. Serial sampling demonstrated substantial elevation of heat shock (Hsp70) response 12h after the treatment which was exhausted by 24h after treatment. Heat shock inhibitors Quercetin or KRIBB11 could synergistically amplify mEHT-induced tumor apoptosis in vitro. In conclusion, modulated electro-hyperthermia exerted a protective heat shock response as a clear sign of tumor cell stress. Exhaustion of the HSR manifested in caspase-dependent apoptotic tumor cell death and tissue damage of triple-negative breast cancer after mEHT monotherapy. Combined therapy with HSR inhibitors synergistically increased the effect of mEHT, which finding has great translational potential.

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Modulated electro hyperthermia inhibits tumor progression in a triple negative mouse breast cancer model

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Introduction: Effective therapy of triple-negative breast cancer (TNBC) has not yet been achieved. Modulated electro-hyperthermia (mEHT) is a novel therapeutic option, based on the selective heating and energy transfer to the tumor tissue by electromagnetic field.

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

Method: 4T07 cells were inoculated orthotopically in female BALB/c mice. Tumor growth was monitored by caliper and ultrasound (Phillips Sonos 5500). Treatments started 7 days after inoculation and were repeated 3 or 5 times, on every other day. Tumor samples were taken 24 hours after last treatment for histology and molecular biology processes. Tumor destruction rate was assigned on H&E and cleaved caspase-3 stained sections, while HSP70, Ki67, CD3 and MPO expression were analyzed on immunohistochemical sections digitally (CaseViewer Software – 3DHistech). Circulating immune cells (CD4+, CD8+ lymphocytes, granulocytes, MDSCs) were analyzed with flow cytometry.

Results: mEHT caused 6.1 fold higher HSP70 elevation in the tumor tissue, compared to the sham group ($p<0.001$). Tumor size significantly decreased (tumor weight sham: 288.3±58.1 mg vs mEHT: 85.3±21.3 mg, $p<0.05$) with the elevation of tissue destruction and reduction of Ki67 positive nuclei number (sham: 2823.4±211.9 pcs/mm2 vs mEHT: 1736.7±315.3 pcs/mm2, $p<0.05$) in treated tumors. mEHT optimized the systemic immune-response with the elevation of lymphocytes and reduction of granulocytosis and the number of MDSCs.

Conclusion: Our findings suggest, that repeated mEHT could reduce tumor growth with heat-shock-mediated tissue destruction and impaired cell proliferation and could optimize systemic immune-response. Thus, mEHT could be a possible alternative adjuvant therapeutic strategy for TNBC cancer patients.

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