***P-21: Gabor Andocs, Nora Meggyeshazi, Y. Okamoto, Lajos Balogh, Oliver Szasz (2012) Bystander effect of Oncothermia***

# Bystander effect of Oncothermia


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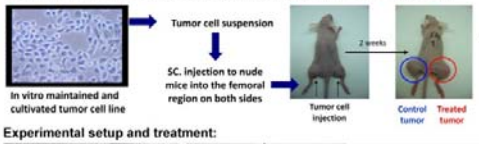
## Introduction

**Oncothermia** (OTM) is an electro-hyperthermia modality, a long time (since 1989) applied method in oncology,[1] with great clinical success [2]. OTM changes the paradigm of hyperthermia by targeted microscopic heat-liberation at the membrane of the malignant cells. This method creates inhomogeneous heating, microscopic temperature differences far from thermal equilibrium. The tumor destruction efficacy and the role of temperature independent effects of the OTM was proven earlier by laboratory research, and presented elsewhere [3],[4].
**Bystander effect** (abscopal effect) means that a local tumor treatment can affect the behavior of the far distant metastases. It was first discovered by radio-oncologists and remained higly controversial topic until recent years. [5],[6]. Intensive research is conducting to reveal the immunbiological basis [7],[8],[9] and mechanism of action of this effect [10] and using the benefits in the regular oncological practice. Objective is showing the newest results of oncothermia in research bystander effect.

## Methods

**Animal model:**
HT29 human colorectal carcinoma cell line derived xenograft tumor model in nude mouse.

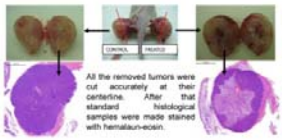



**Experimental setup and treatment:**


A single shot 30 min oncothermia treatment was done, reaching maximum 41-42°C intratumoral temperature, using the LabEHY system (Oncotherm Ltd.), under precise tumor temperature control using fluoroptic temperature measurement system (Lumasense m3300).

**Study design:**
Time course study was performed. After a single shot treatment, sampling was made after 0, 1, 4, 8, 14, 24, 48, 72, 120, 168, 216 hours. 3 mice were sacrificed at each time point, keeping 5 sham treated animals.

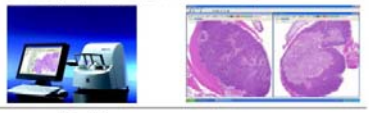
**Tumor sample processing I.:**
24 h later the single-treatment animals were sacrificed and both the control and treated tumors were removed and studied in pairs.




**Tumor sample processing II.:**
Due to the extremely high number of the tumor samples, tissue microarray (TMA) technology was used to perform accurate immono-histochemical reactions on many samples in one block.

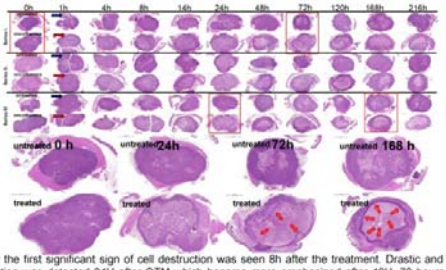

**Immunohistochemistry (IHCH):**
The following reactions and IHCH analysis were performed on the TMA samples: TUNEL (Invitrogen); TRAIL (DR5), HSP70 (Cell Signaling); Myeloperoxidase (Sigma); CD3 (Dako), CD4 (ABDSerotech).

**Digital microscopy analysis:**
All histological slides were digitalized using Panoramic Slide Scanner (3D HisTech) and special software was used for imaging and evaluation.



## Results

### 1. Histomorphological changes:




Morfologically the first significant sign of cell destruction was seen 8h after the treatment. Drastic and selective tumor-destruction was detected 24H after OTM which became more emphasized after 48H. 72 hours after the treatment a significant leucocyte infiltration (marked with red arrows) appeared around the destructed tumor tissue and reached its maximum 168 hours after the treatment.


References:
[1] Szasz A. (2007) **Hyperthermia, a modality in the wings.** J Cancer Res Ther. 3:56-60.
[2] Szasz A, Szasz O. (2010) **Oncothermia. Principles and Practices.** Springer Verlag. (http://www.springer.com/biomed/cancer/book/978-90-481-9497-1 Schungermeier) Heidelberg, Dordrecht
[3] Andocs G, Szasz O, Szasz A. (2009) **Oncothermia treatment of cancer: from the laboratory to clinic.** Electromagn Biol Med. 28(2):148-60.
[4] Andocs G, Renner H, Balogh L, Fonyad L, Jakab C, Szasz A. (2009) **Strong synergy of heat and modulated electromagnetic field in tumor cell killing.** Strahlenther Onkol. Feb;185(2):120-6.
[5] Silva C, Formenti, Sandra Demaria. **Systemic effects of local radiotherapy.** Lancet Oncol 2009; 10: 718–26
[6] William F. Morgan, Marianne B. Sowa, **Non-targeted bystander effects induced by ionizing radiation.** Mutation Research 616 (2007) 159–164
[7] Robert FA Mallin, Andreas Lundqvist,Solveig H. Ware, Arne van Boron, Rolf Kiessling,Hans-Gustaf Ljunggren, **Heat-shock proteins as activators of the innate immune system.** TRENDS in Immunology Vol.23 No.3 March 2002
[8] Stephan R. SCHOTTA, Heike NAVE, Firouzeh HOMAND, Kerstin SCHUTZ, Reinhard PABST, Elisabeth M. JAFFEE, **Apoptotic but not necrotic tumor cell vaccines induce a potent immune response in vivo.** Int. J. Cancer: 103, 205–211 (2003)
[9] Oliver Kepp, Antoine Tesniere, Frederic Schlemmee,Mickael Michaud, Laura Senovilla,Laurence Zitvogel, Guido Kroemer, **Immunogenic cell death modalities and their impact on cancer treatment,** Apoptosis (2009) 14:364–375
[10] Akhilesh D. Garg, Dominika Nowis, Jakub Golab, Peter Vandenabeele, Dmitri V. Krysko, Patrizia Agostinis, **Immunogenic cell death, DAMPs and anticancer therapeutics: An emerging amalgamation,** Biochimica et Biophysica Acta 1805 (2010) 53–71



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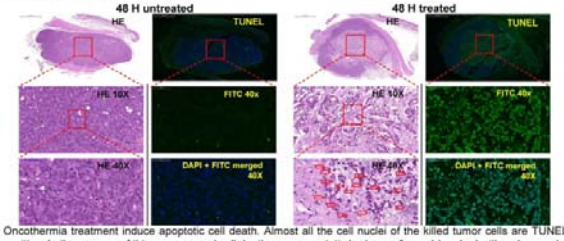
**In memoriam Reka Szasz**

### 2. Hallmarks of immunogenic cancer cell death

**2.1 Apoptotic body formation**

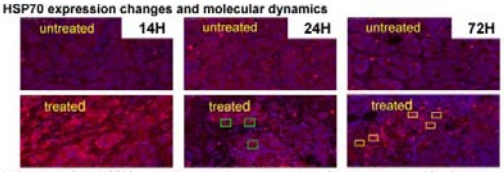



Oncothermia treatment induce apoptotic cell death. Almost all the cell nuclei of the killed tumor cells are TUNEL positive. In the process of this programmed cell death many apoptotic body was formed (marked with red arrows).

**2.2. TRAIL (DR5) expression**


TRAIL (DR5) is a higly immunogenic cell surface receptor. Expression was increased in the treated side 8H after the treatment and became more emphasized after 14H.

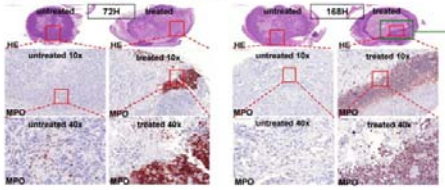
**2.3 HSP70 expression changes and molecular dynamics**



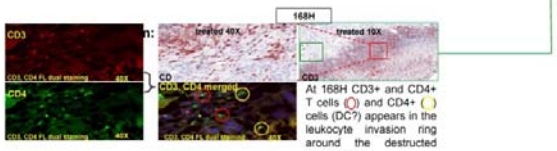

Definite increase of the HSP70 expression was observed 14 hours after the treatment. After 24 hours, unusual molecular dynamic changes of the increased amount of HSP70 can be visible: intracellular condensation ( ) and relocalization to cell membrane. After 72 hours the membrane relocalization of the HSP70 became more emphasized, especially in the region of the leukocyte invasion. ( )

### 3. Strong local immune reaction

**3.1 Myeloperoxidase (MPO) detection:**




The leukocyte invasion ring what appears at 72H and became very characteristic at 168H around the destructed tumor area, contains high number of MPO positve cells ( neutrophiles, macrophages)




At 168H CD3+ and CD4+ T cells ( ) and CD4+ ( ) cells (DC?) appears in the leukocyte invasion ring around the destructed tumor tissue.

## Conclusions

1. **Oncothermia can induce programmed cell death which create many apoptotic bodies**
2. **Oncothermia induced cell death is higly immunogenic, showing all the key molecular pattern dynamic changes what is characteristic of immunogenic tumor cell death**
3. **Oncothermia treatment can induce strong and very unusual immun reaction at the site of the treatment**
4. **The local antitumor immune reaction can be systemic, if the host has an intact immune system, and this process can control the distant metastases by bystander effect, making possible the systemic control of the malignant disease with local treatment.**

**(The intensive research is in progress on immunocompetent models!)**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