# P-02 – Dr. Gabor Andocs, et al - Apoptosis induction with modulated radiofrequency (RF) hyperthermia (oncothermia) in immuno-defficient mice xenograft tumors (Review)

## Apoptosis induction effect of modulated radiofrequency (RF) hyperthermia (oncothermia) in immunodeficient mice xenograft tumors


**Andócs G.[1,2], Balogh L.[1], Meggyesházi N.[3], Jakab Cs.[4], Krenács T.[3], Szász A.[5]**

(1) National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary
(2) Department of Pharmacology and Toxicology, Faculty of Veterinary Science, St Istvan University, Budapest, Hungary
(3) 1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary
(4) Pathological Department, Faculty of Veterinary Medicine, St Istvan University, Budapest, Hungary
(5) Department of Biotechnics, Faculty of Engineering, St Istvan University, Budapest, Hungary


### Introduction – objective of the work

Oncothermia method is more than twenty years serving the medical practices. It has successful applications either as a complementary therapy with the "gold-standard" modalities either as monotherapy, when no other possibility could be applied. The specialized animal-experiments had been started five years ago intending to clarify the basic mechanisms by in vivo scientific approaches. The complexity and interdisciplinary of the in vivo experimental series requested a wide cooperative scheme of various respected and honored research institutes and university laboratories. Our objective is to summarize the results of this intensive work and show the conclusions at the recent phase of the investigations.

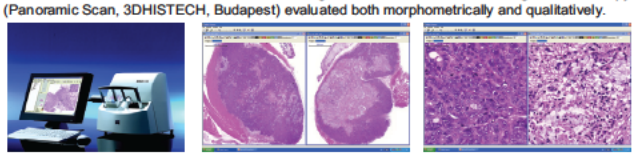
### Materials and methods

Immuno defficient nude mice (BalbC/nu/nu) were used for xenograft and allograft models with HepG2, PC3, HT29, A431, GL261 cell lines The definite amount of cell line suspension was injected to the femoral region of the 6 8 weeks old female mice and 18 20 days later the oncothermia treatment was performed, when the tumors were developed symmetrically in both sides on diameter 1 1 5 cm The single shot treatment was identically performed for all the mice on their right lesion, while the left lesion was kept as untreated individual control to reduce the inaccuracies due to the individual variability of the animals

Treatments were performed by highly specialized laboratory equipment (Lab EHY, Oncotherm), optimized on mice dimensions, taking into account the physiology of the small animals, collecting all the important technical and biological parameters [1] The impedance selection and automatic focusing which is well known in human clinical practices were applied in these experiments too [2] The temperature of the tumors was controlled by high accuracy fluoroptical system (Luxtron m3300, LumaSense)


*Tumors satisfactory for treatment in mouse (A), Treatment device LabEHY 100 (B), Capacitive coupled electrode applicator for oncothermia of mice (C), Fluoroptikal temperature measuring system (Luxtron) (D)*

Slides of TMA tissue-multiblocks of the tumors of mice sacrificed in series of 0-72 h after the single shot treatment was stained by conventional hematoxilin-eozin (H-E) as well as by immunohistochemical methods and were digitalized and studied with digital microscopy (Panoramic Scan, 3DHISTECH, Budapest) evaluated both morphometrically and qualitatively.

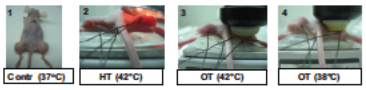


*PanoramicScan device, its sample slide and the pattern from the digital microscopy software*

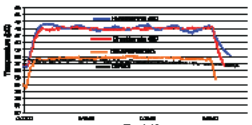
**A subsequent series of our experiments were performed:**

1. **Experimental phase:** Effect of oncothermia (single shot, 30 min, 42 C) on various tumor tissues were studied obtained from allograft and xenograft models. The investigated cell-lines were: HepG2 (human hepatocellular carcinoma), HT29 (human colorectal carcinoma) GL261 (mouse glioblastoma), A431(human epidermoid carcinoma), PC3 (human prostate carcinoma). Combined effect of chemotherapy (Mitomycin C) was studied in this phase also.

2. **Experimental phase :** Comparison of the efficacy of classical hyperthermia (HT) and of oncothermia (OT) with high number of experimental animals (four groups with 7-7 animals), using HT29 xenograft modell. We measured the effect of cell-killing independently from the temperature too. The effect was determined by digital quantitative analysis.

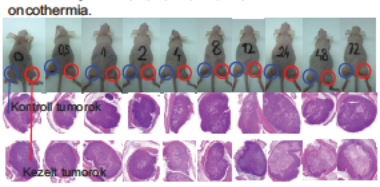



*Experimental groups **1.** Untreated control, **2.** Group of conventional infrared HT, **3.** Oncothermia treated group, **4.** The same as Group 3, but keeping the tumor on physiological temperature (This experiemnt was performed by intensive cooling of the tumor with the water bolus of the electrode, limiting the temperature increase upto 38°C*




*Representative pattern of temperature development measured in the center of the tumors during the treatments*

3. **Experimental Phase:** Effect of single shot 30 min oncothermia treatment was investigated immediately and 1, 4, 8, 12, 24, 48, 72 h after the treatment exploring the mechnaism of the oncothermia.




*Experimental animals, and the full cross section of their tumors*

*Leica Bond Max IHCH automata*

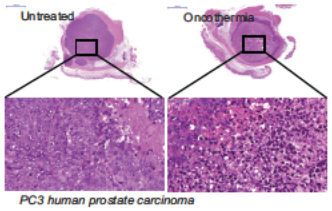
*TMA Master*

Based on morphological patterns we supposed the apoptosis is the dominant cell-killing mechanism. Based on this assumption we immunohistochemically measured the expression of p53 protein expression and also detected the apoptosis induced DNA fragmentation by TUNEL (Terminal deoxy-nucleotidyl transferase dUTP nick end labeling) The TMA slides collect 3-3 characteristic samples from every experimental tumors. The automatic immune-staining performed on the TMA blocks assured a standard antigen detection.

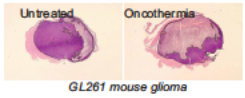
### Results

In its time development we observed the followings:

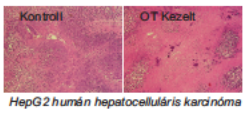
1. **A**, Oncothermia treatment made significant tumor distortion relative to the control in all the investigated tumors, irrespective its origin.




*PC3 human prostate carcinoma*

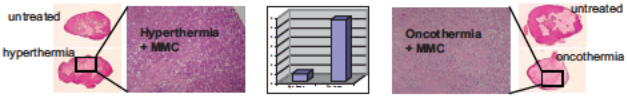



*GL261 mouse glioma*



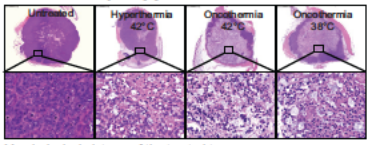

*HepG2 humán hepatocelluláris karcinóma*

1. **B**, Significant improvement of the antitumor-effect of Mitomycin-C (MMC) was observed.

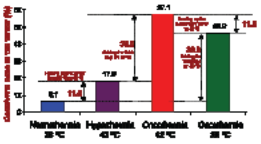



*Complementary application of MMC with hyperthermia and with oncothermia on HT29 xenograft*

2. Both the conventional hyperthermia and oncothermia have certain destruction of the malignant cells in the tumors in the studied cases, but the efficacy of oncothermia is almost three-times higher, [4].

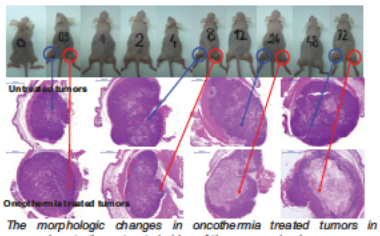



*Morphological pictures of the treated tumors*

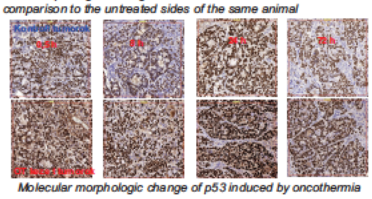


*Quantitative analysis shows the significant differences between the treatments*

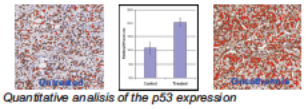
3. The documented cell-destruction is dominantly apoptotic. This is shown by the upregulation of the p53 protein, involved in the apoptotic-control, and also the certain fragmentation of DNA measured by TUNEL reaction.



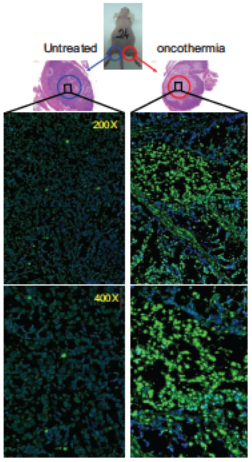

*The morphologic changes in oncothermia treated tumors in comparison to the untreated sides of the same animal*



*Molecular morphologic change of p53 induced by oncothermia*



*Quantitative analisis of the p53 expression*




*DNA fragmentation after 24 h of the oncothermia treatment, measured by TUNEL, indicates the apoptotic cell destruction by oncothermia*

### Conclusion

The applied mice models were suitable to study the effect of oncothermia on molecular level. The dominant role of apoptosis in the oncothermia cell-destruction is highly probable. Further investigations are in progress to study the mechanism of apoptotic induction and its connection with the cell-cycles as well as the role of the adherens and other cellular connections.


**References**

[1] Szasz A Szasz N Szasz O (2010) Oncothermia Principles and Practices Springer Scientific Heidelberg
[2] Szasz A (2006) Physical background and technical realization of hyperthermia In *Hyperthermia in cancer treatment: a primer* (Eds: Baronzio GF Hager ED) Springer Science pp 27 59 2006;
[3] Andocs G Szasz O Szasz A (2009 e) Oncothermia treatment of cancer: from the laboratory to clinic Electromagnetic Biology and Medicine 28:148 165
[4] Andocs G Renner H Balogh L Fonyad L Jakab Cs Szasz A (2009 d) Strong synergy of heat and modulated electromagnetic field in tumor cell killing Study of HT29 xenograft tumors in a nude mice model Radiology and Oncology (Strahlentherapie und Onkologie) 185:120 126