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

Lea Danics[1], Csaba Schvarcz[1], Zita Zolcsak[1], Zoltan Benyo[1], Tamas Kaucsar[1], Peter Hamar[1]
[1] Institute of Clinical Experimental Research, Semmelweis University, Budapest, Hungary

Presented at 36th ICHS, Budapest, 2018

Cite this article as:
Danics L. (2018): Modulated electro hyperthermia inhibits tumor progression in a triple negative mouse breast cancer model; Oncothermia Journal 24: 442-454
www.oncothermia-journal.com/journal/2018/Modulated_electro_hyperthermia_inhibits_tumor.pdf

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

Lea Danics[1], Csaba Schvarcz[1], Zita Zolcsak[1], Zoltan Benyo[1], Tamás Kaucsar[1], Peter Hamar[1]
[1] Institute of Clinical Experimental Research, Semmelweis University, Budapest

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 the highly selective heating of the tumor tissue by a 13.56 MHz radiofrequency current induced electric field.

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

Methods
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 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 / mm2 count was significantly lower (sham: 2823.4±211.9 pcs/mm2 vs mEHT: 1736.7±315.3 pcs/mm2, $p<0.05$) and the p21 positive nucleus / mm2 count showed increasing tendency (sham: 127.0±25.3 pcs/mm2 vs mEHT: 242.2±78.2 pcs/mm2, p = 0,073) in the mEHT treated group, compared to the sham group.

Conclusion
Our findings suggest, that repeated mEHT could lower tumor cell proliferation by promoting cell cycle arrest in vivo. Thus, mEHT could be a possible alternative adjuvant therapeutic strategy for TNBC cancer patients. We plan next generation sequencing to elucidate the biological mechanism behind the effects of mEHT.

NVKP_16-1-2016-0042

Modulated electro hyperthermia inhibits tumor progression 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]

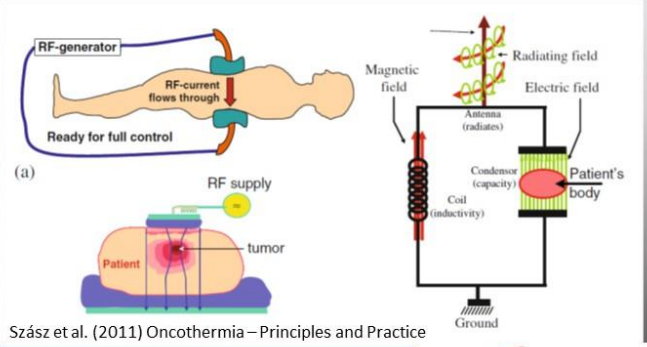
[1] Institute of Clinical Experimental Research

36th Conference of the International Clinical Hyperthermia Society
2018. Budapest, September 28-29.

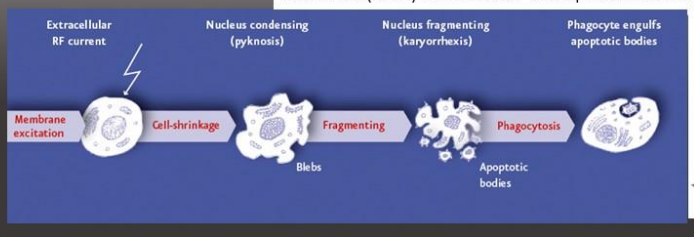
NVKP-16-1-2016-0042 project

Modulated electrohyperthermia

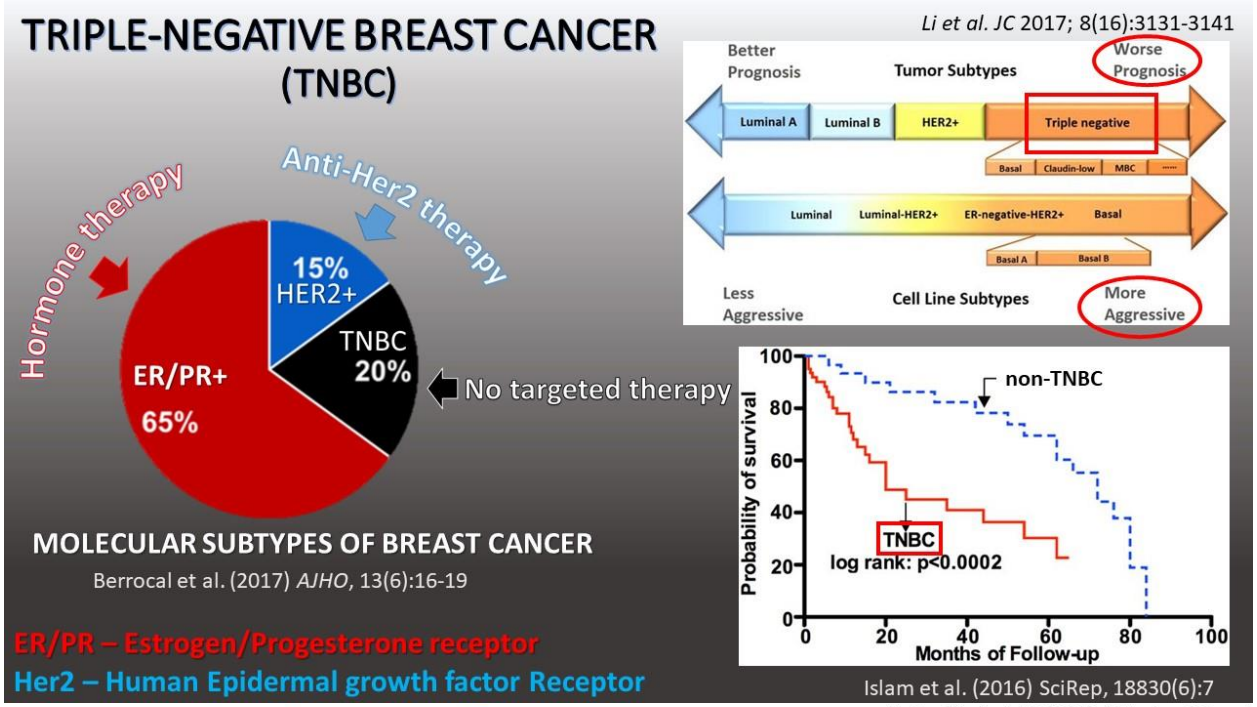
Highly-selective heating of the tumor



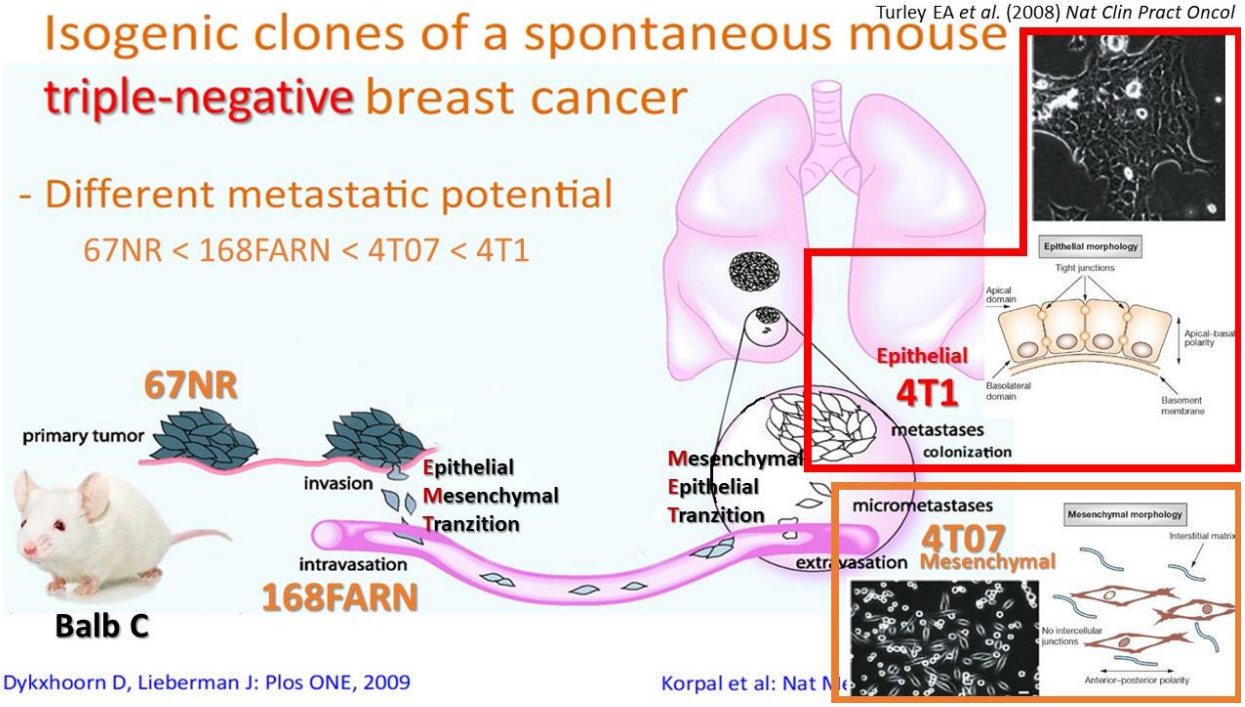
Szász et al. (2011) Oncothermia – Principles and Practice



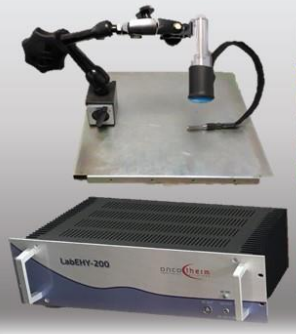
www.oncotherm.org

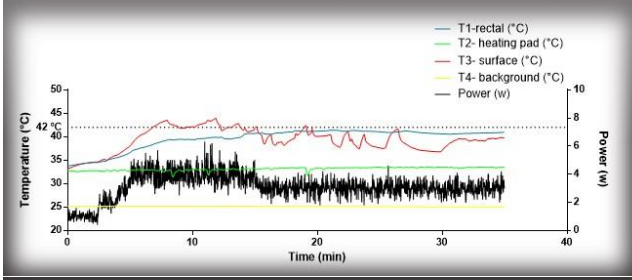


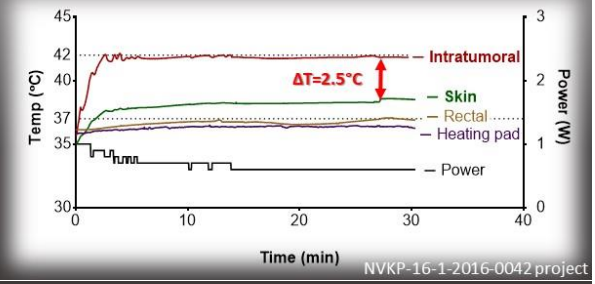
Turley EA *et al.* (2008) *Nat Clin Pract Oncol*



TREATMENT OPTIMIZATION



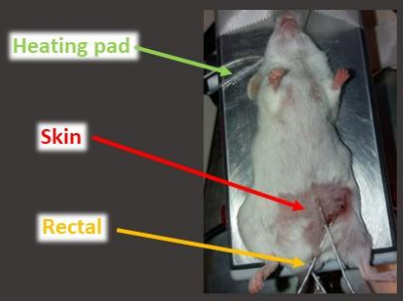


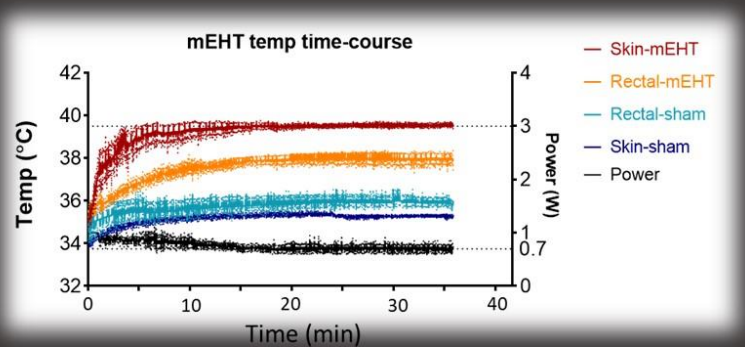


NVKP-16-1-2016-0042 project

Treatment settings:

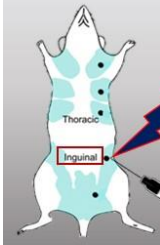
Heating pad temperature	37-38 °C
Skin temerature	40 °C
Rectal temperature	37-38 °C
Power	0.7±0.3 W
Time	35 min





ONE-TREATMENT PROTOCOL

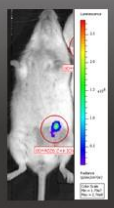
Female Balb/C mice (N = 14)



1X modulated electrohyperthermia
LabEHY-200
40°C 30min

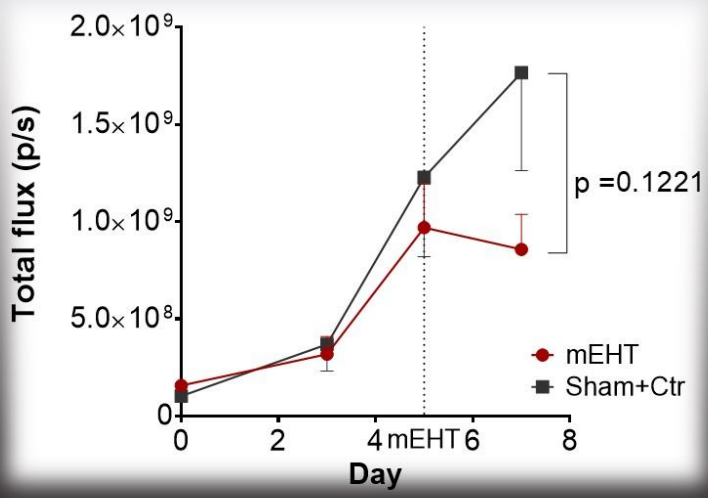
10^6 **4T1**-GFP-mCherry-**Luciferase**
TNBC cells / 100 µl **PBS:Matrigel**

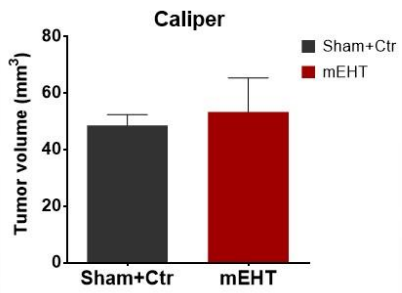
Day after inoculation	0	1	2	3	4	5	6	7
4T1 cell inoculation	x							
IVIS				x		x		x
Ultrasound				x		x		x
Caliper				x	x	x	x	x
mEHT						x		
Harvest								x

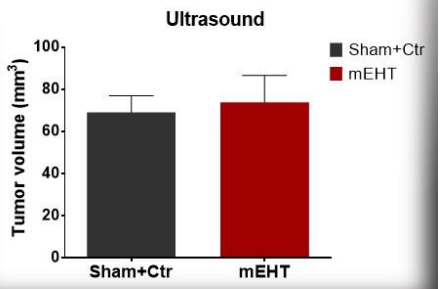


NVKP-16-1-2016-0042 project

RESULTS – one treatment

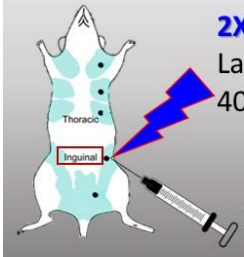




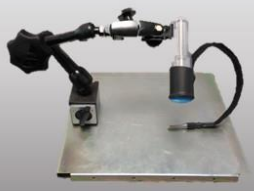


TWO-TREATMENT PROTOCOL

Female Balb/C mice (N = 12)

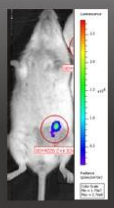


2X modulated electrohyperthermia
LabEHY-200
40°C 30min



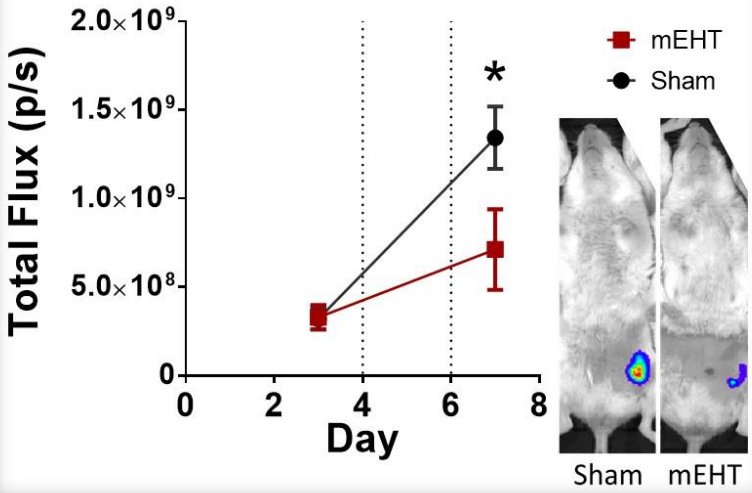
10^6 **4T1**-GFP-mCherry-**Luciferase**
TNBC cells / 50 µl **PBS:Matrigel**

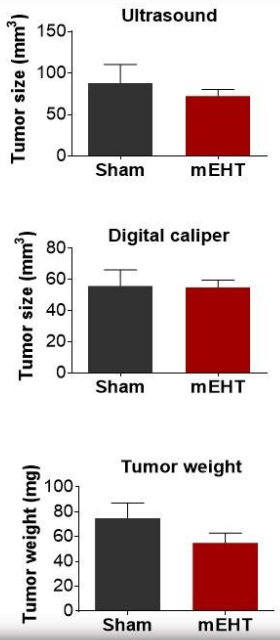
Day after inoculation	0	1	2	3	4	5	6	7
4T1 cell inoculation	x							
IVIS				x				x
Ultrasound								x
Caliper								x
mEHT					x		x	
Harvest								x

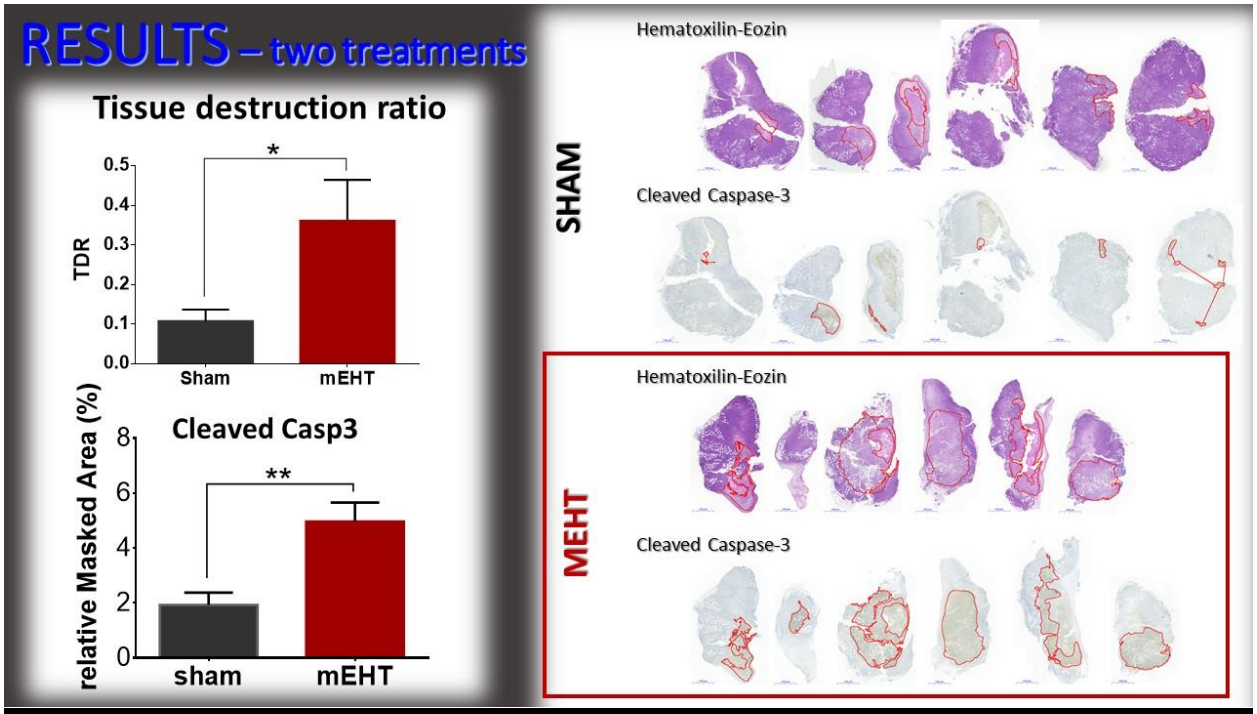


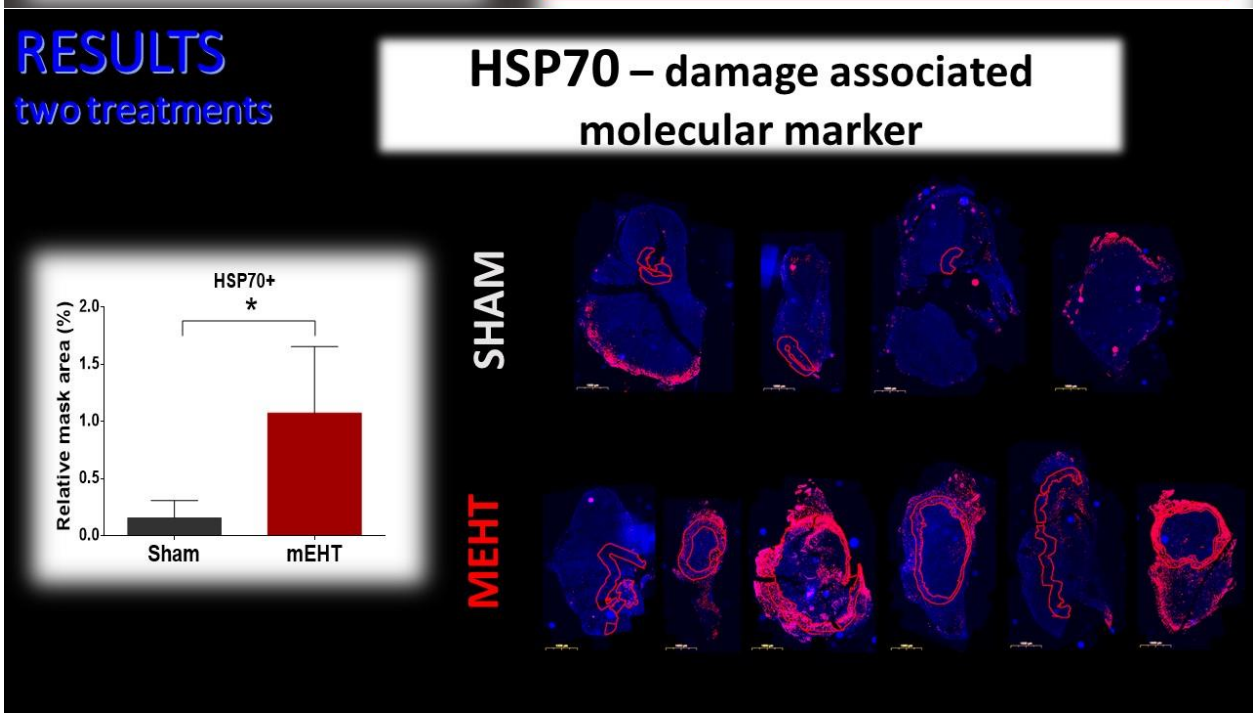
NVKP-16-1-2016-0042 project

RESULTS – two treatments

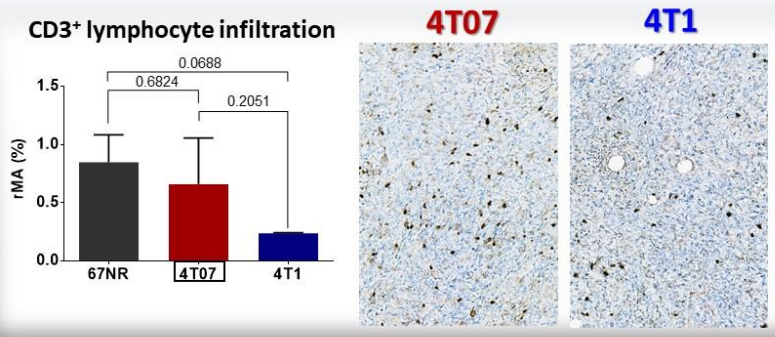


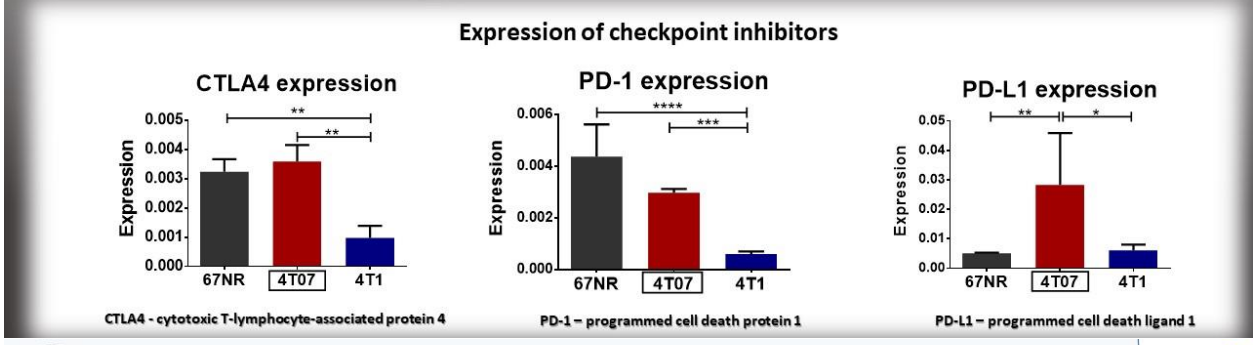



RESULTS – two treatments
Tissue destruction ratio
TDR
0.5
0.4
0.3
0.2
0.1
0.0
*
Sham
mEHT
Cleaved Casp3
relative Masked Area (%)
8
6
4
2
0
**
sham
mEHT
SHAM
Hematoxilin-Eozin
Cleaved Caspase-3
MEHT
Hematoxilin-Eozin
Cleaved Caspase-3


RESULTS
two treatments
HSP70 – damage associated molecular marker
HSP70+
Relative mask area (%)
2.0
1.5
1.0
0.5
0.0
*
Sham
mEHT
SHAM
MEHT

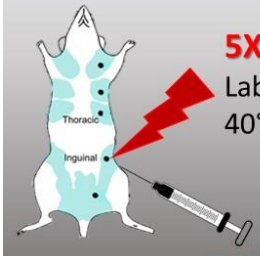
Immune profile of TNBC isografts





FIVE-TREATMENT PROTOCOL

Female Balb/C mice (N = 18)



5X modulated electrohyperthermia

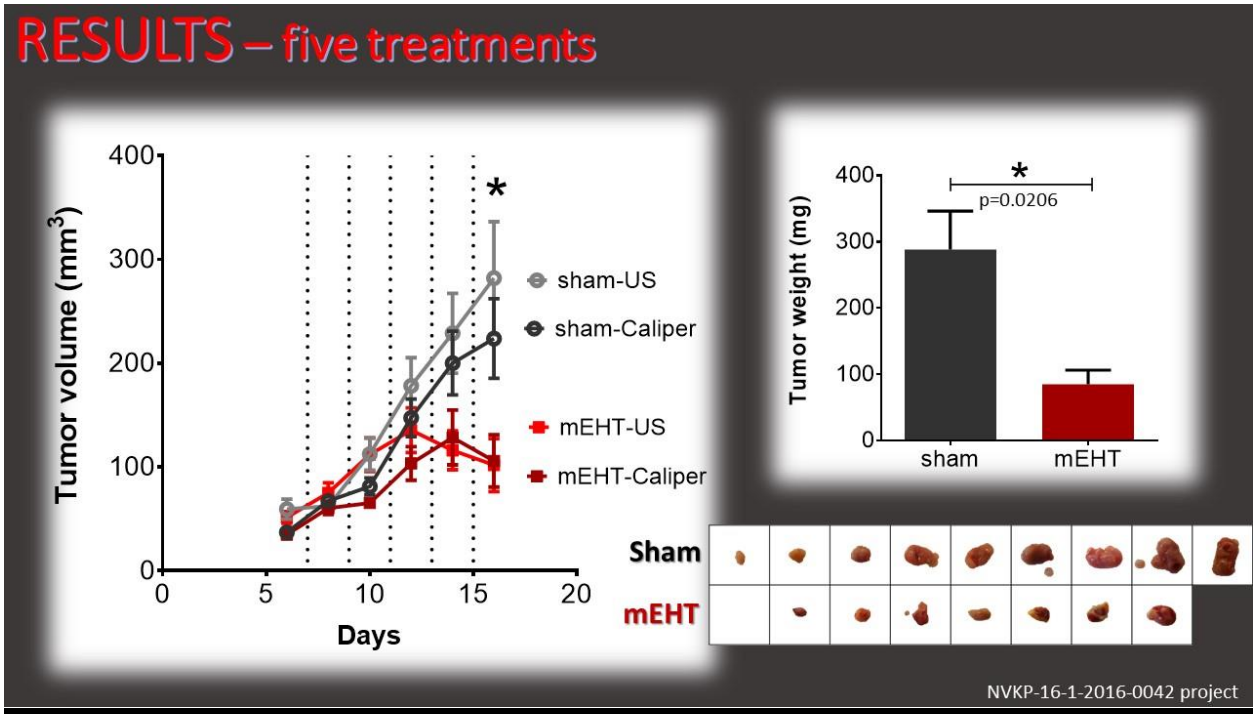
LabEHY-200

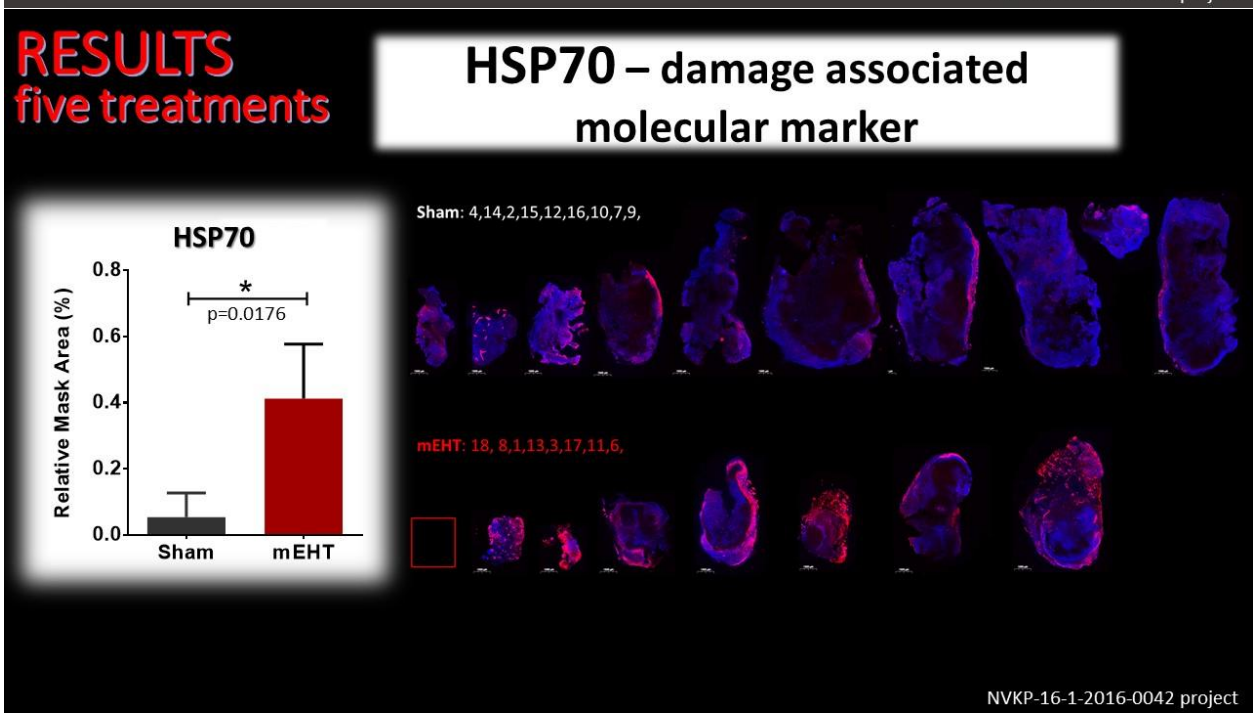
40°C 30min

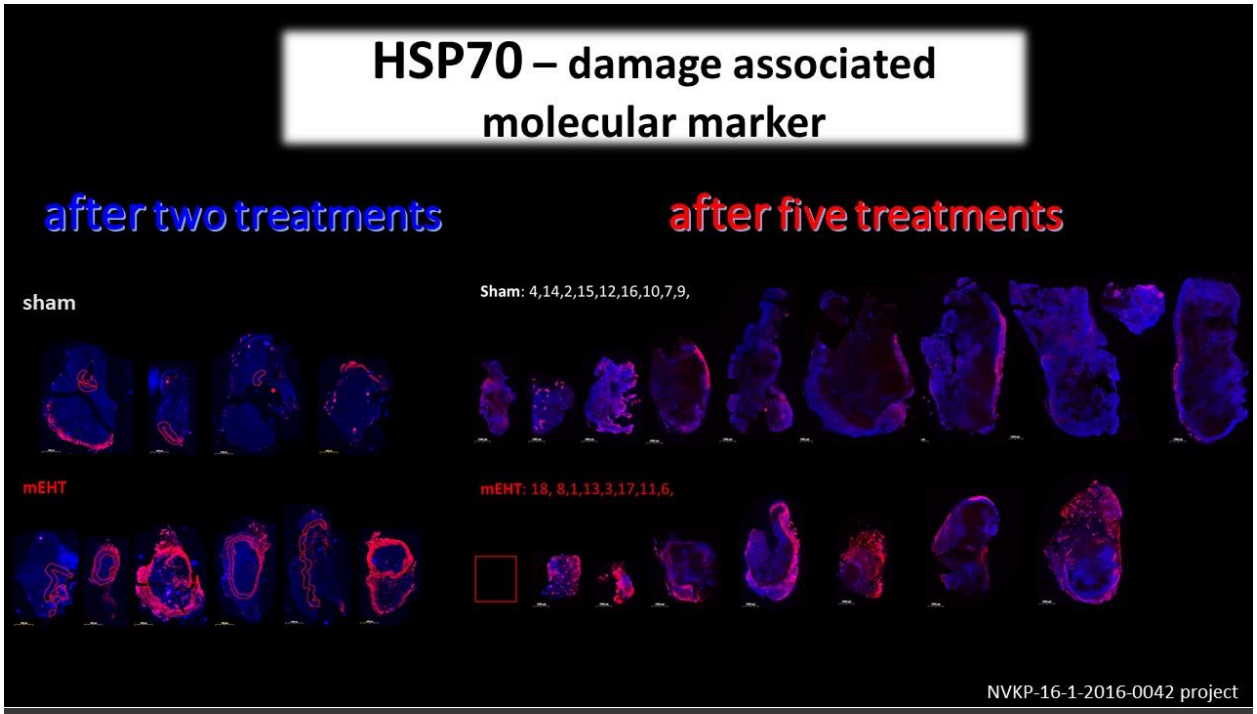
10^6 **4T07** TNBC cells / 50 µl PBS

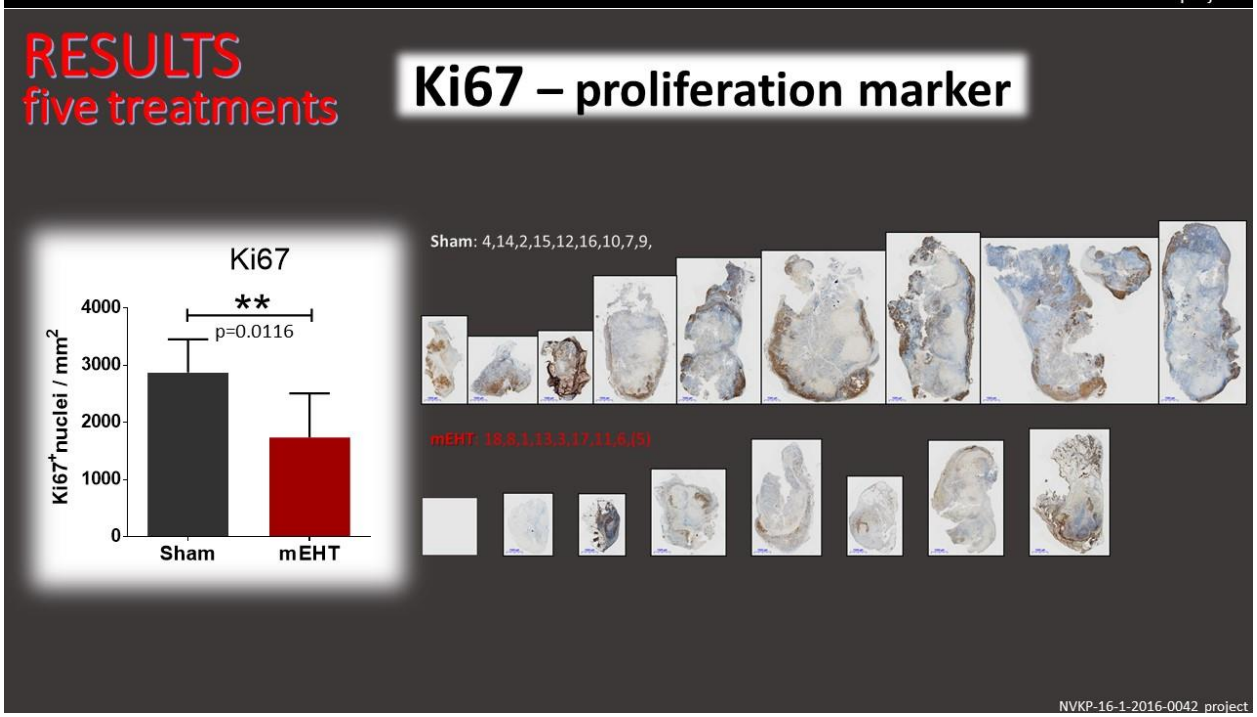
Day after inoculation	-1	0	6	7	8	9	10	11	12	13	14	15	16
4T07 cell inoculation		x											
Tumor size (US, caliper)			x		x		x		x		x		x
mEHT				x		x		x		x		x	
Harvest													x

NVKP-16-1-2016-0042 project


RESULTS – five treatments
Tumor volume (mm3)
400
300
200
100
0
0
5
10
15
20
Days
*
sham-US
sham-Caliper
mEHT-US
mEHT-Caliper
Tumor weight (mg)
400
300
200
100
0
*
p=0.0206
sham
mEHT
Sham
mEHT
NVKP-16-1-2016-0042 project

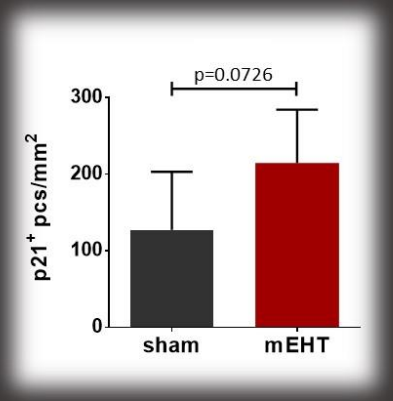

RESULTS
five treatments
HSP70 – damage associated molecular marker
HSP70
Relative Mask Area (%)
0.8
0.6
0.4
0.2
0.0
*
p=0.0176
Sham
mEHT
Sham: 4,14,2,15,12,16,10,7,9,
mEHT: 18, 8,1,13,3,17,11,6,
NVKP-16-1-2016-0042 project

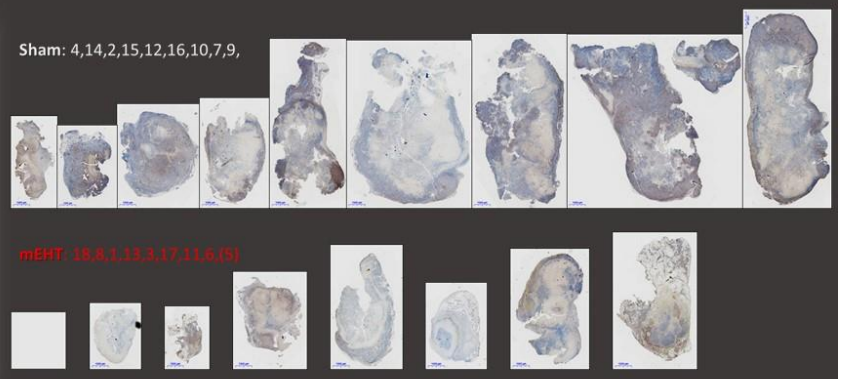

HSP70 – damage associated molecular marker
after two treatments
after five treatments
sham
Sham: 4,14,2,15,12,16,10,7,9,
mEHT
mEHT: 18, 8,1,13,3,17,11,6,
NVKP-16-1-2016-0042 project


RESULTS
five treatments
Ki67 – proliferation marker
Ki67
**
p=0.0116
Ki67+nuclei / mm2
4000
3000
2000
1000
0
Sham
mEHT
Sham: 4,14,2,15,12,16,10,7,9,
mEHT: 18,8,1,13,3,17,11,6,(5)
NVKP-16-1-2016-0042 project

RESULTS
five treatments

p21 - common cyclin-dependent kinase inhibitor





NVKP-16-1-2016-0042 project

Summary

Short-term effects

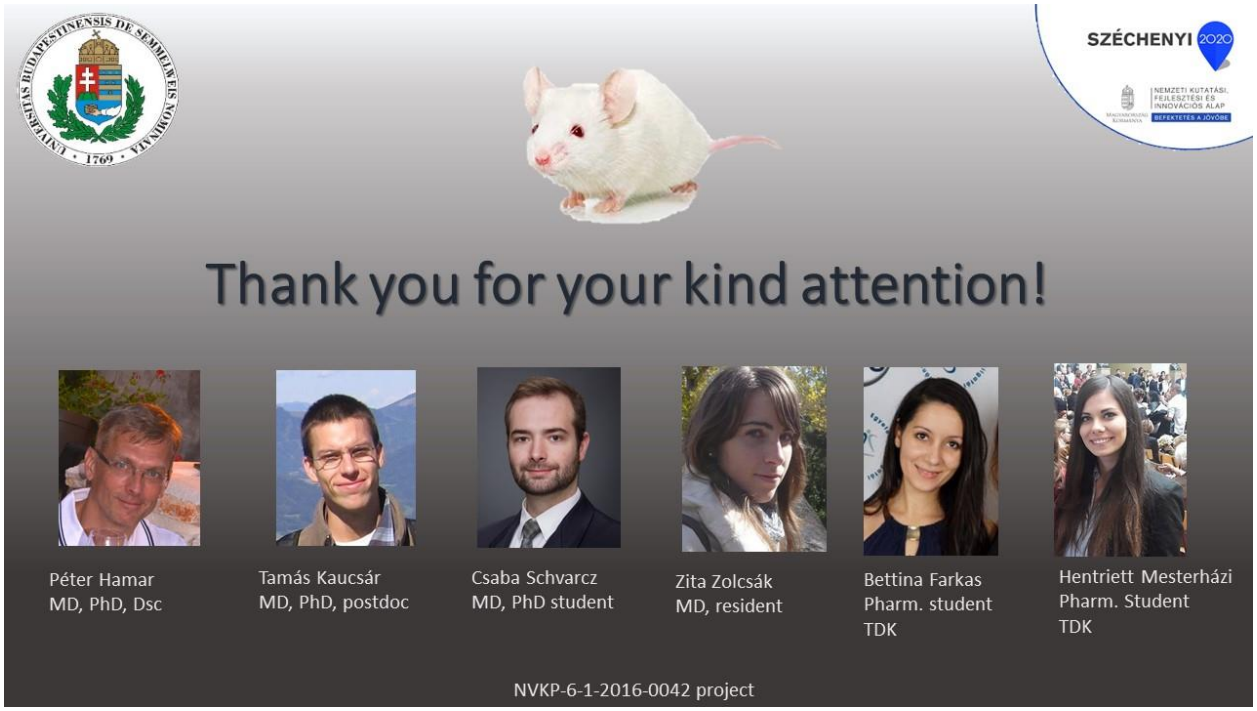
- **tissue damage** (TDR, cCasp3)
- tumor **cell death** (IVIS)
- **no reduction in** tumor **size** with traditional methods (US, caliper) but with IVIS and TDR (weight and volume)

Long-term effects

- **decrease** tumor cell **proliferation** (Ki67)
- **Inhibit tumor growth** (weight and volume)

heat-shock (Hsp70)

NVKP-16-1-2016-0042 project


SZÉCHENYI 2020
Thank you for your kind attention!
Péter Hamar
MD, PhD, Dsc
Tamás Kaucsár
MD, PhD, postdoc
Csaba Schvarcz
MD, PhD student
Zita Zolcsák
MD, resident
Bettina Farkas
Pharm. student
TDK
Hentriett Mesterházi
Pharm. Student
TDK
NVKP-6-1-2016-0042 project