

Tumor specific stress and immune response induced by modulated electrohyperthermia in relation to tumor metabolic profiles

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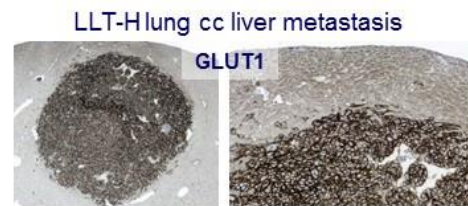
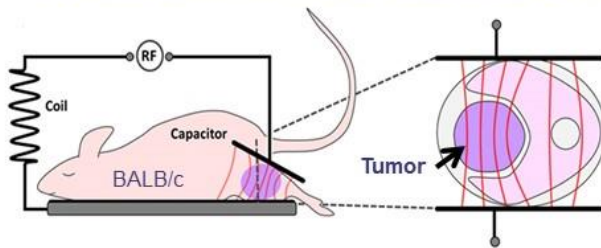
ESHO Congress
Athens, June 21-23rd 2017



mEHT of 13.65 MHz – selective tumor destruction

- Electric field can be selectively enriched in malignant tumors**

Elevated permittivity, glycolysis, lactate and ion concentration (Warburg-effect)

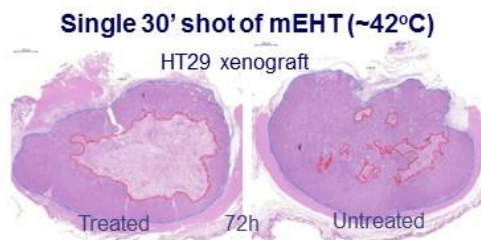
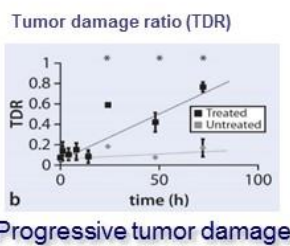


- Xenograft HT29 CRC(HepG2 HCC) immunocompromised (NUDE)
- Allograft C26 CRC(C38 CRC, LLT-H, lung ADC) immunocompetent

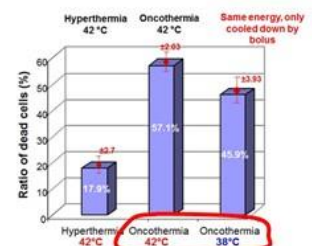
Symmetrical tumors

- right leg - mEHT treated
- left leg - control

- Programmed tumor cell death in synergy of electric field & induced heat**

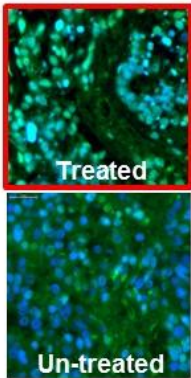
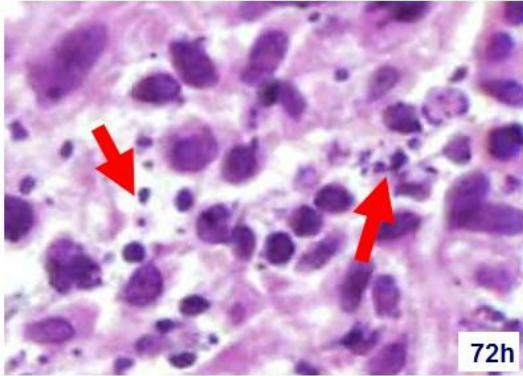


Andocs et al. *Strahlenther Onkol.* 2009, 185:120-126.

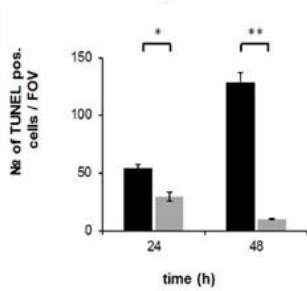


mEHT induced programmed cell death

HT29 (p53 mutant) CRC xenograft

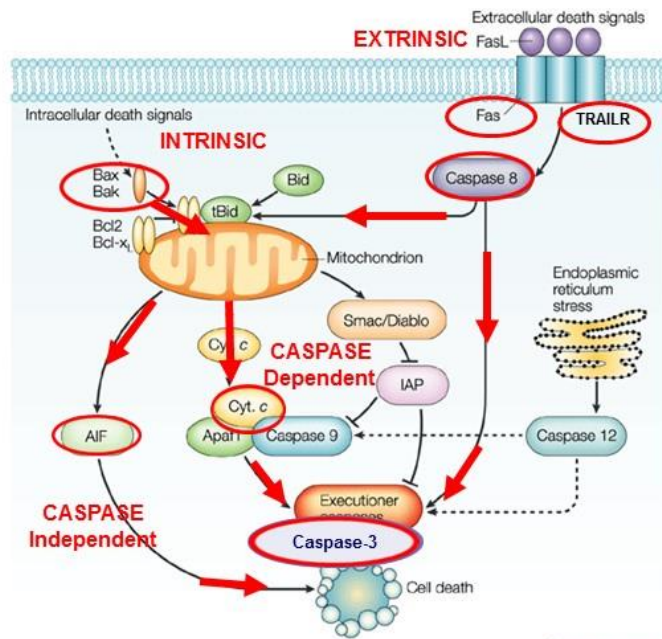


DNA fragmentation



Meggyeshazi, Andocs et al. *Strahlenther Onkol.* 2014, 190:815-822.

APOPTOSIS



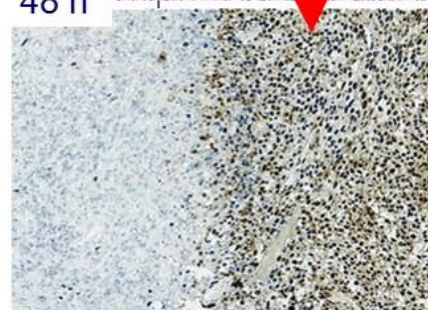
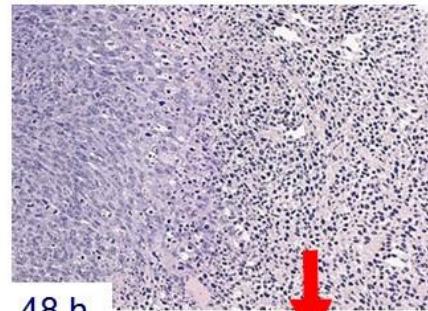
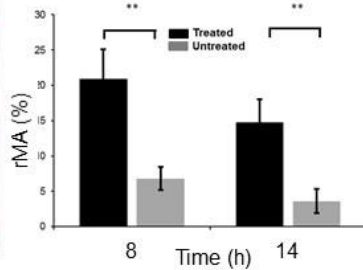
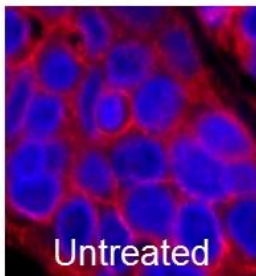
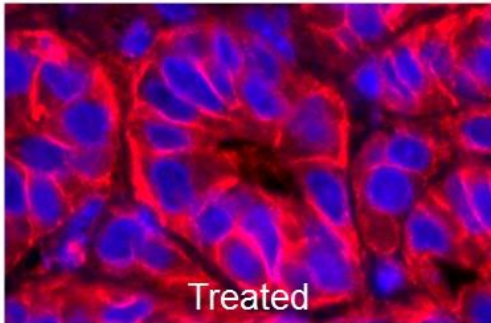
Vila-Przedborski: Apoptosis-2003

mEHT induced death receptor mediated extrinsic pathway

HT29 CRC xenograft

TRAIL-R2 (Death Receptor 5)

Cleaved-Caspase 8



Target therapy: rh-TRAIL and MAbs (HGS ETR2/ lexatumumab)

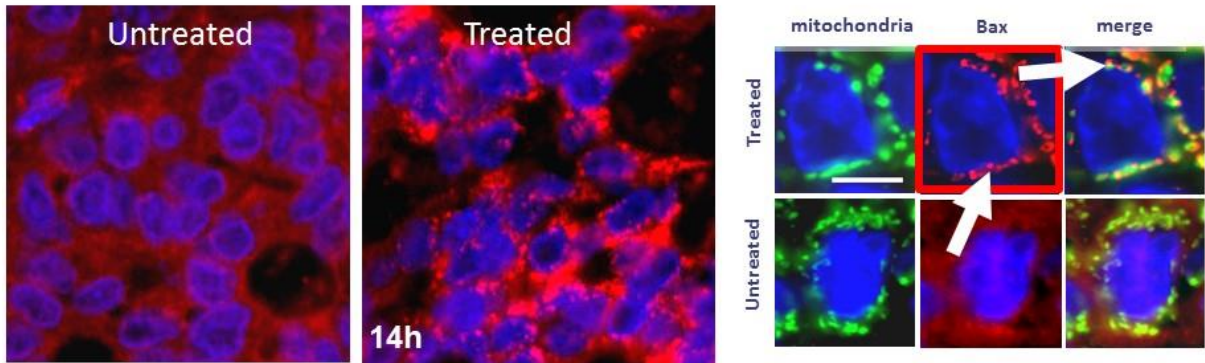
- Synergy with cytotoxic chemo- and radiation therapy in breast cancer & CRC
- Phase-I-II trials are running

Rajen. *Transl Pediatr* 2013; 2:66-69

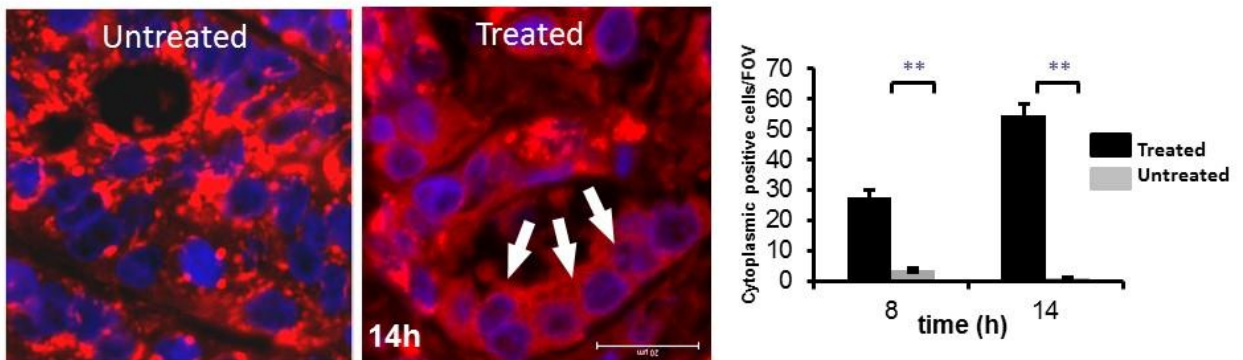
mEHT induced Caspase-dependent intrinsic pathway

HT29 CRC xenograft

Bax - mitochondrial translocation



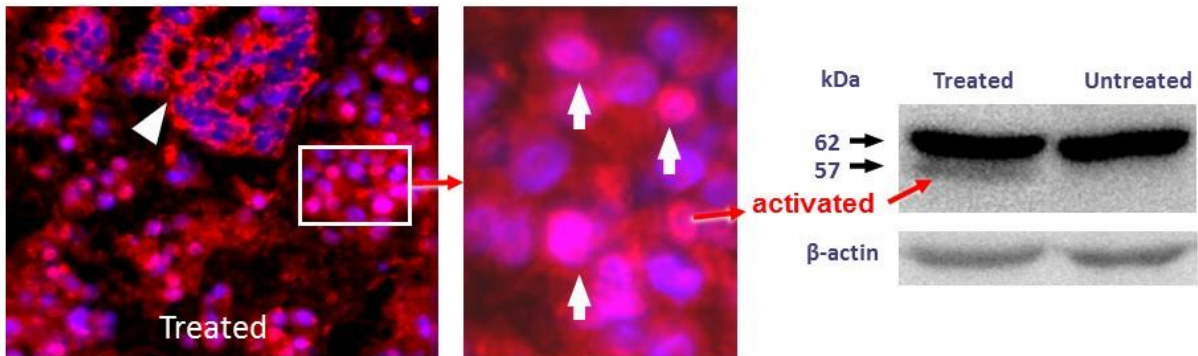
Cytochrome C - cytoplasmic release



mEHT induced Caspase dependent & independent apoptosis

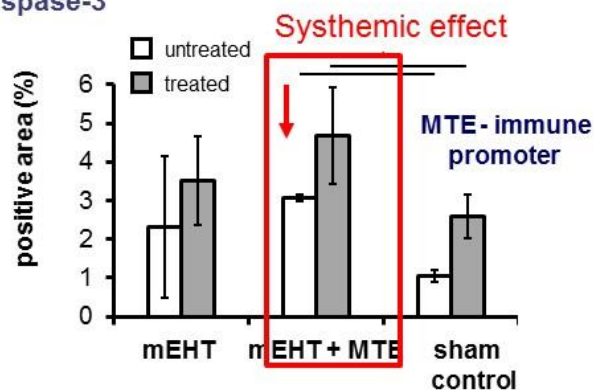
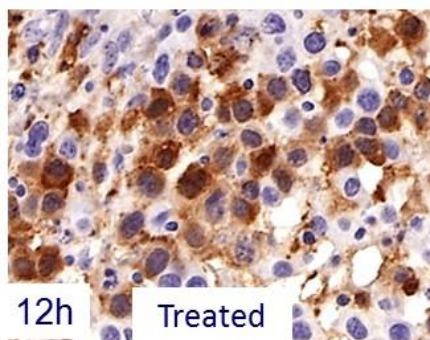
HT29 CRC

AIF (apoptosis inducing factor) translocation



C26 CRC

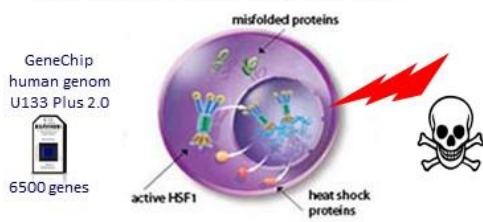
Cleaved-caspase-3



Heat shock/cell stress – chaperones – damage signaling

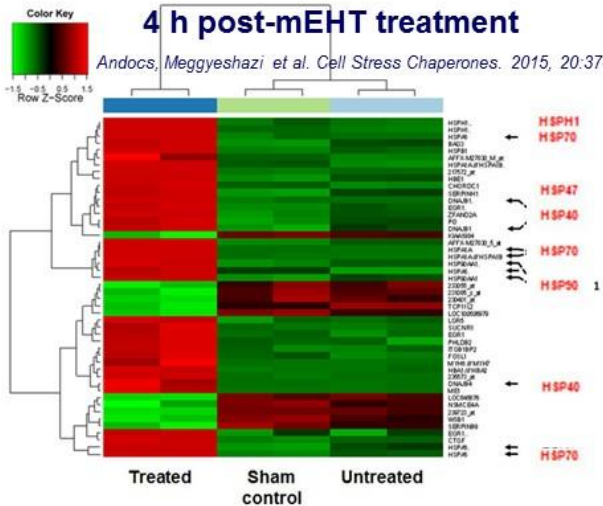
Human HT29 CRC xenograft

HEAT SHOCK + ELECTROMAGNETIC FIELD

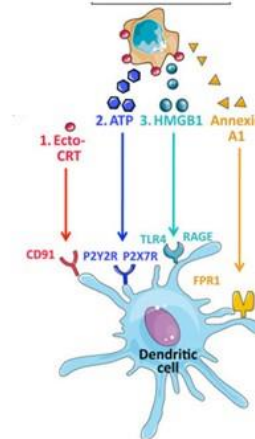


4 h post-mEHT treatment

Andocs, Meggyeshazi et al. Cell Stress Chaperones. 2015, 20:37-46.



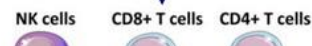
Damage associated molecular patterns (DAMP)



Hernandez et al. Oncogene. 2016, 35:5931-5941

- **Calreticulin (CRT)**
„eat me” signal
- **ATP**
„find me” signal
- **HMGB1**
„danger” signal
- **HSP70**
Granzyme B endocytosis

- DC maturation, activation
- Tumor antigen processing
- T-cell & NK-cell activation



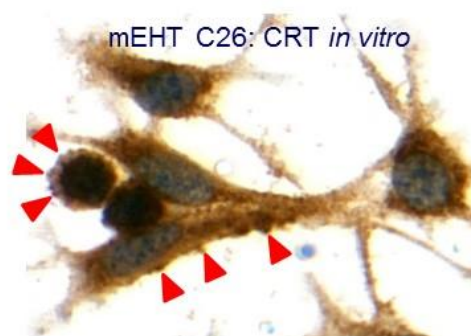
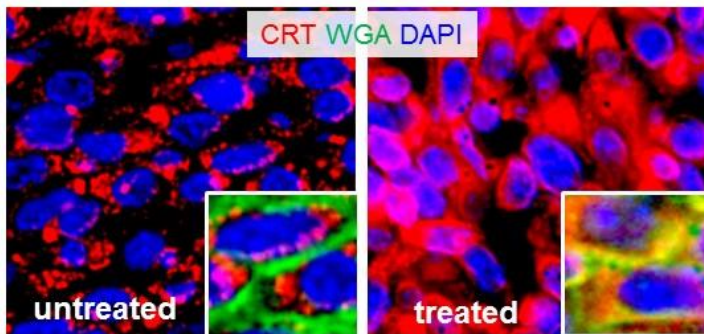
- Antracyclins
Doxorubicin
- UV or γ irradiation
- EGFR immunotherapy
- Capsaicin

- Antitumor immune response
- Immunogenic cell death (ICD)

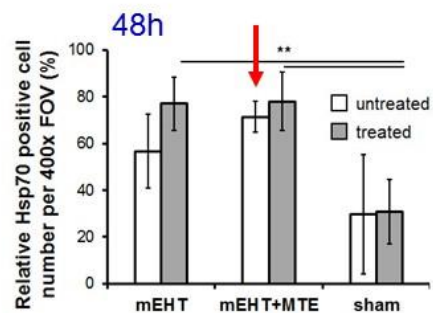
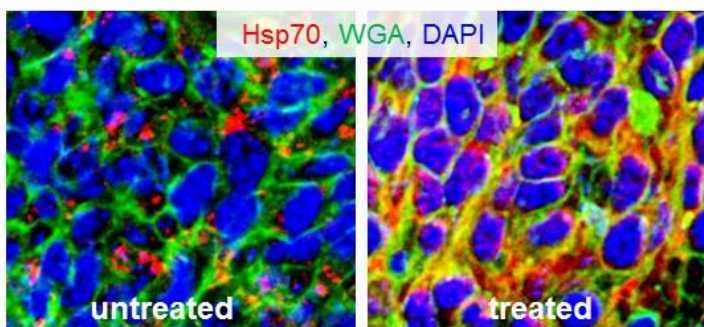
Spatiotemporal DAMP signaling – systemic effect

C26 CRC allograft

Calreticulin membrane translocation



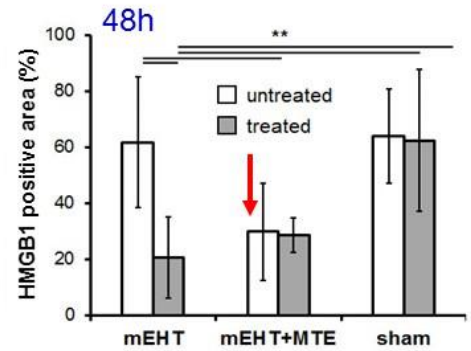
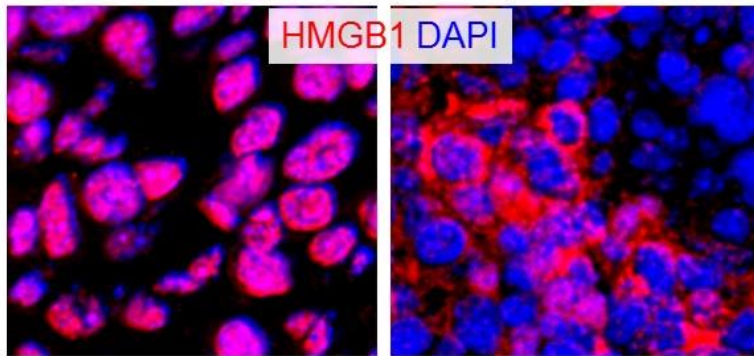
Hsp70 membrane translocation



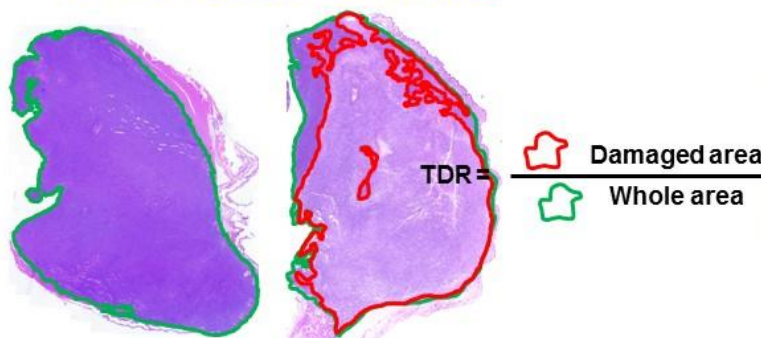
Spatiotemporal DAMP signaling – systemic effect

C26 CRC allograft

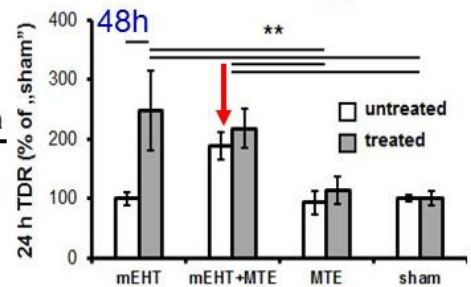
HMGB1 release – cytoplasmic & extracellular



Tumor Destruction Ratio ↑



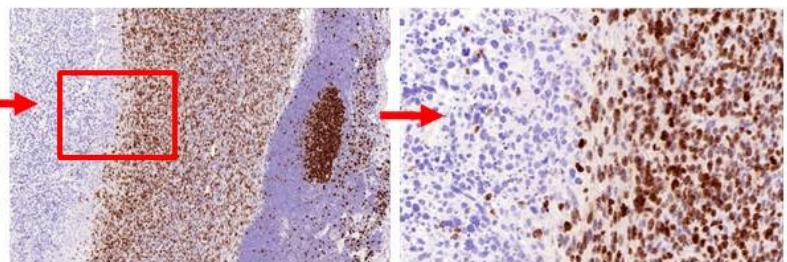
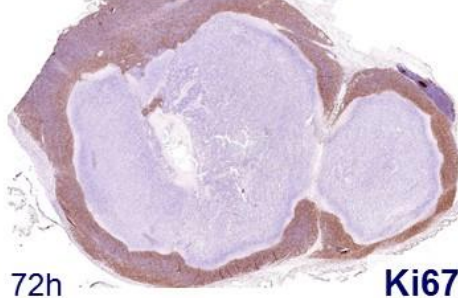
In mEHT treated & mEHT+MTE treated & in mEHT+MTE treated opposite site



Complete cell cycle arrest – apoptosis

C26 CRC proliferate ~100%

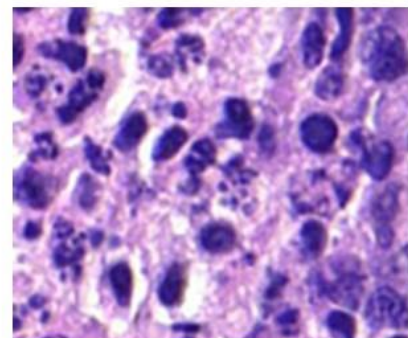
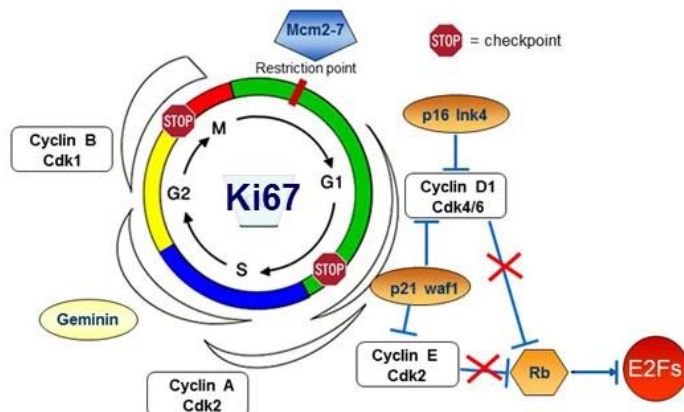
mEHT - Complete cell cycle arrest



72h

Ki67

Apoptosis vs DNA repair

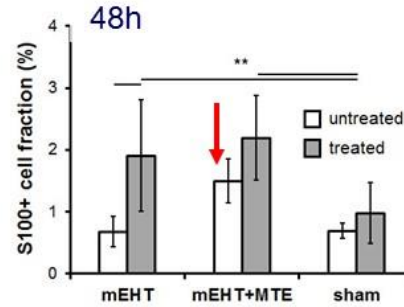
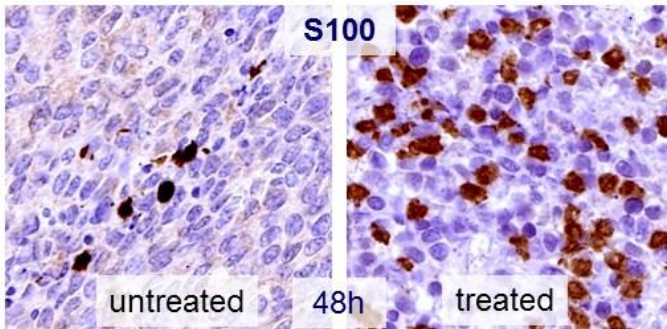


Ehrhardt et al. Cell cycle-arrested tumor cells exhibit increased sensitivity towards TRAIL-induced apoptosis *Cell Death and Disease* (2013) 4, e661

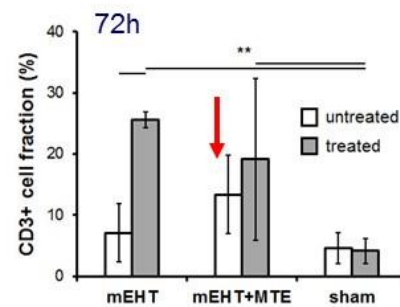
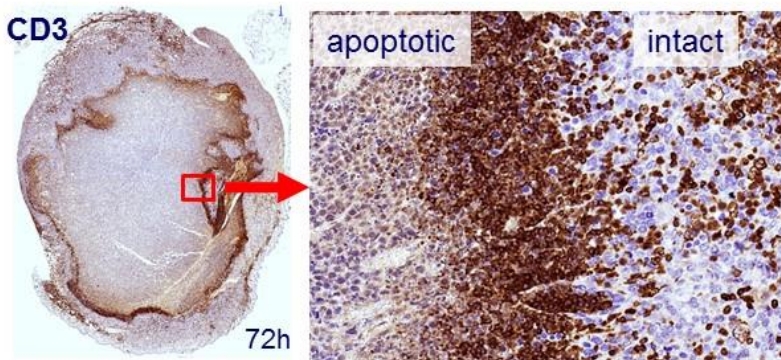
Antitumor immune response - local and systemic

C26 CRC allograft

Elevated number of antigen presenting DC (APC)



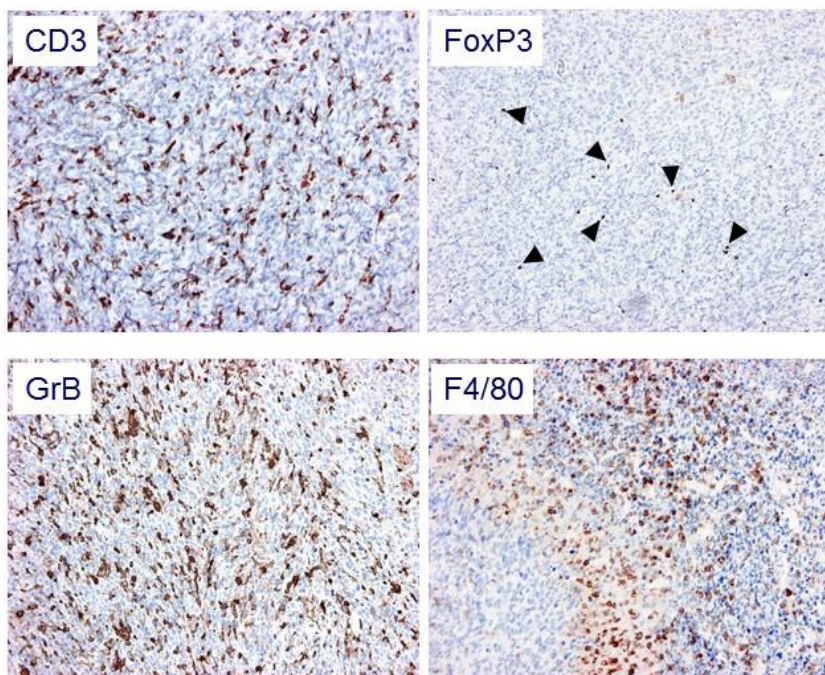
Massive T-cell infiltration



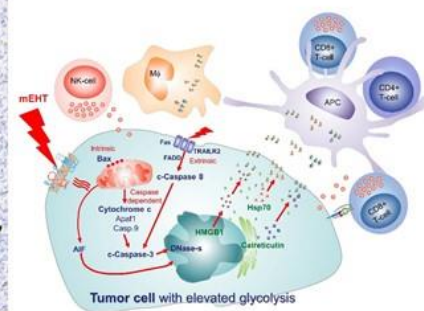
Antitumor immune response – immunogenic cell death

48 h post-mEHT

T-cell infiltration, negligible regulatory T-cells



C26 CRC allograft



Single mEHT shot

Progressive

- accumulation of immune cells &
- tumor damage

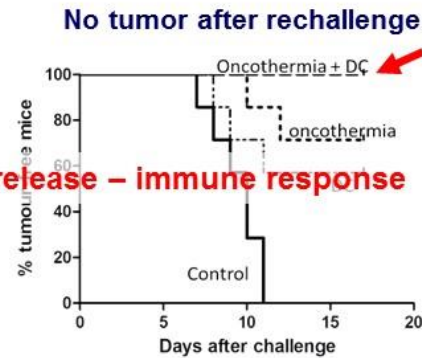
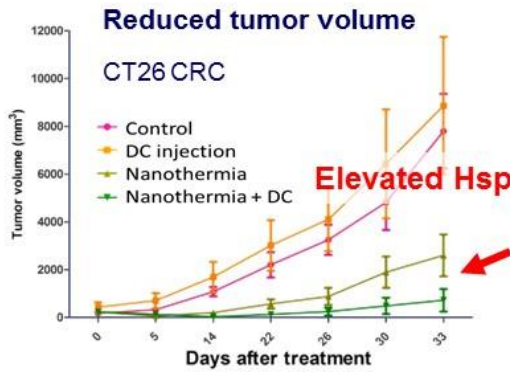
↓
Immunogenic cell death
ICD

Cytotoxic T-cells & NK cells + Macrophages

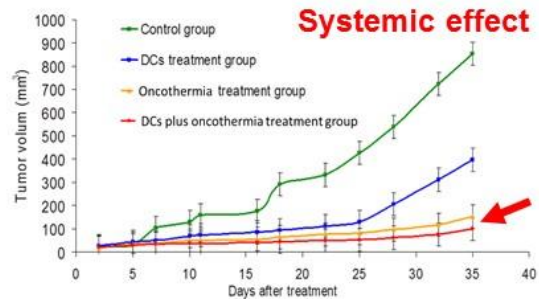
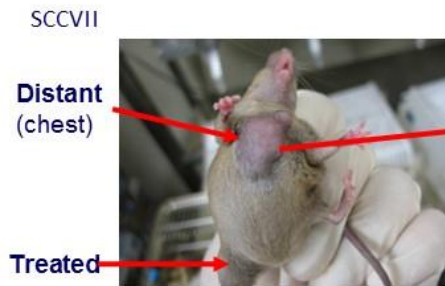
mEHT + DC therapy – protection & systemic effect

In vivo allographs

Tsang et al. BMC Cancer, 2015, 15:708



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



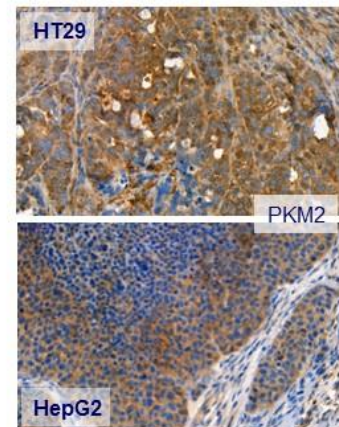
mEHT tumor selectivity – Elevated glycolysis (Warburg effect)



Glycolytic, metabolic activity

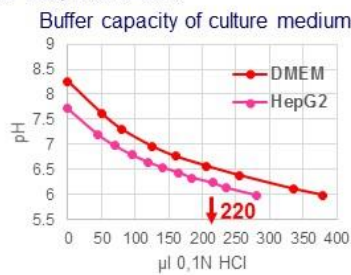
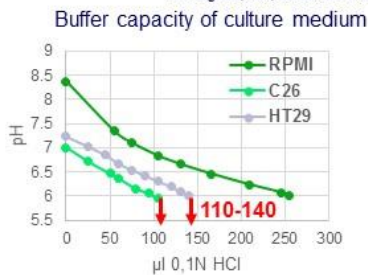


mEHT treatment efficiency

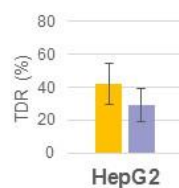
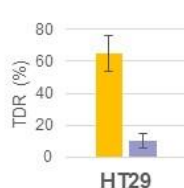
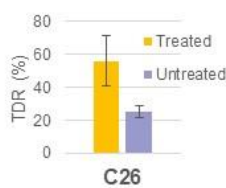


Lactate concentration ↔ buffer capacity

Yamagata, M., et al. Br J Cancer. 1998, 77:1726-1731.

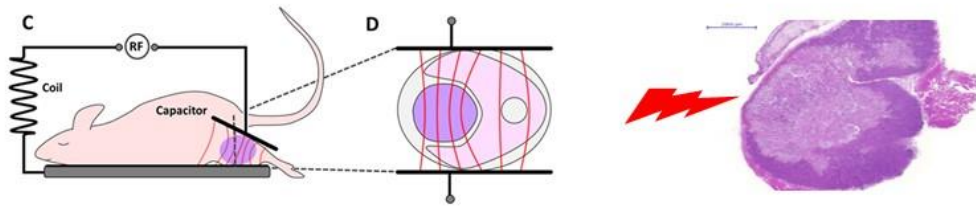


mEHT tumor destruction 24 h post-treatment



- Testing glycolytic enzyme levels *in situ* (IHC) and *in vitro* (Western blot)
- For predictive marker(s) of mEHT therapy

Conclusions: Oncothermia - *in vivo* model systems



- Can induce selective, programmed tumor cell death (apoptosis)
- Upregulation of death receptors (TRAIL-R2 & Fas)
- Both the extrinsic and the intrinsic caspase-dependent pathway
- Preferred pathway depends on genetic/epigenetic predisposition:
 - HT29 human CRC (p53 deficient) - AIF
 - C26 mouse CRC - caspase mediated
- The damage response generates DAMP signal sequence
- Progressive infiltration by APC, cytotoxic T-cells, NK-cells & macrophages
- Progressive damage – immunogenic cell death (ICD)

Acknowledgements



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Oliver Szasz & Balazs Acs

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