

## **Oncothermia in laboratory**

**Dr. Nora Meggyeshazi<sup>1</sup>, Dr. Tibor Krenacs<sup>1</sup>, Dr. Gabor Andocs<sup>2</sup>, Dr. Oliver Szasz<sup>3</sup>**

- (1) 1<sup>st</sup> Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary
- (2) Department of Veterinary Clinical Medicine, Faculty of Veterinary Science, Tottori University, Tottori, Japan
- (3) Department of Biotechnics, Faculty of Engineering, St. István University, Budapest, Hungary

## Oncothermia in laboratory

### *Theoretical background: The hallmarks of cell death*

#### **When is the cell dying? (=point-of-no-return)**

- Massive caspase activation
- Mitochondrial transmembrane potential decreases (mitochondrial membrane permeabilization)
- PS appears in the outer membrane of the cytoplasm.

#### **When is the cell dead?**

- The integrity of plasma membrane is lost
- The cells is fragmented
- The surrounding cells phagocytosing the dead ones.
- Kroemer, G., et al., Classification of cell death: recommendations of the Nomenclature Committee on Cell Death. Cell Death Differ, 2005. 12 Suppl 2: p. 1463-7.
- Kroemer, G., et al., Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. Cell Death Differ, 2009. 16(1): p. 3-11.

#### **How many ways to die?**

A LOT...

... Extrinsic apoptosis, Caspase dependent intrinsic apoptosis, Caspase independent intrinsic apoptosis, Necroptosis (regulated necrosis), Autophagic cell death, Mitotic catastrophe, Netosis, Parthanatos, Pyroptosis Entosis

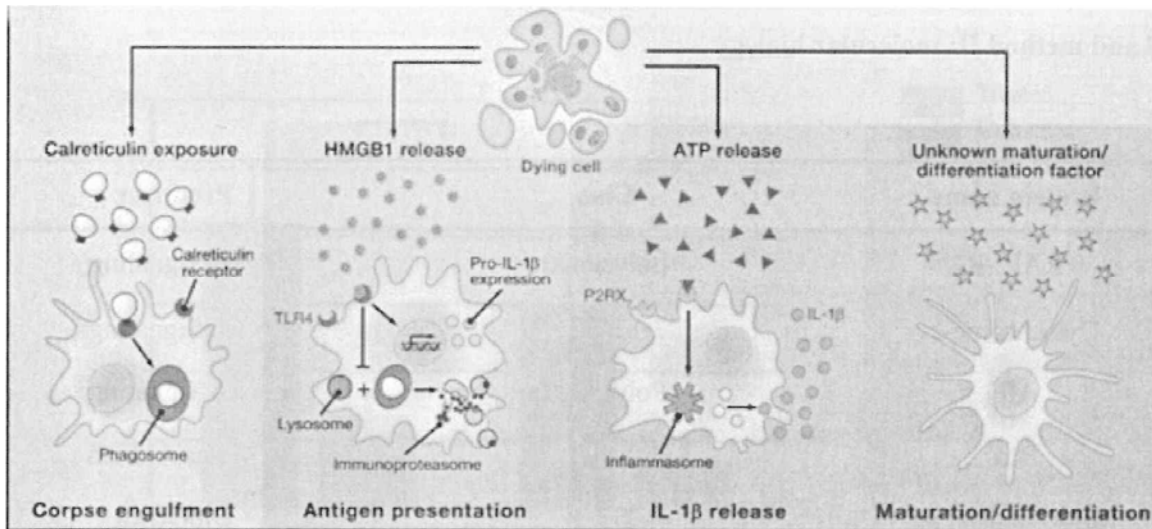
- Kroemer, G., et al., Classification of cell death: recommendations of the Nomenclature Committee on Cell Death Differ, 2005. 12 Suppl 2: p. 1463-7.

#### **BUT**

What can happen after the cell death? (possible interactions with the immune system)

- Inflammation can occur after accidental necrosis (professional phagocytes are involved)
  - No immune reaction (usually in physiological apoptosis)
  - Immunogenic cell death can occur after specific apoptosis inducers (photodynamic therapy, chemotherapy)
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- Calreticulin exposure
  - Membrane appearance of hsp70
  - ATP release
  - HMGB1 release

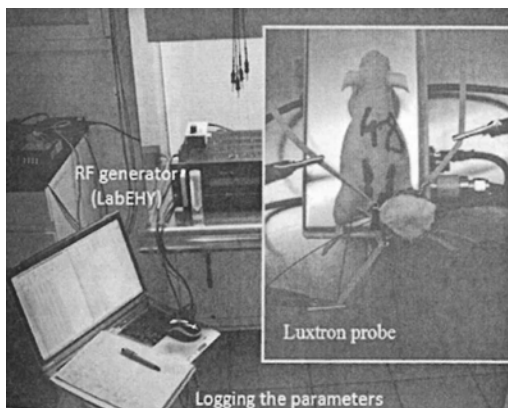
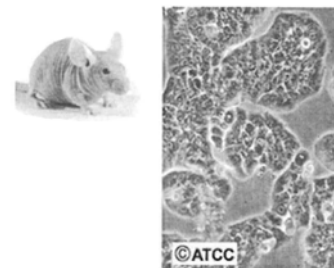
} Temporospatial pattern on the tumor cells in ICD



Kroemer et al. *Journal of Experimental Medicine* (2005 Dec 19;202(12):1691-701)

### Material and method I: the model and treatment

BALB/c (nu/nu) mice inoculated with HT29 (human colorectal adenocarcinoma) in both femoral region (3\*10<sup>6</sup> cells/0,1 ml) of 6-8 week old females



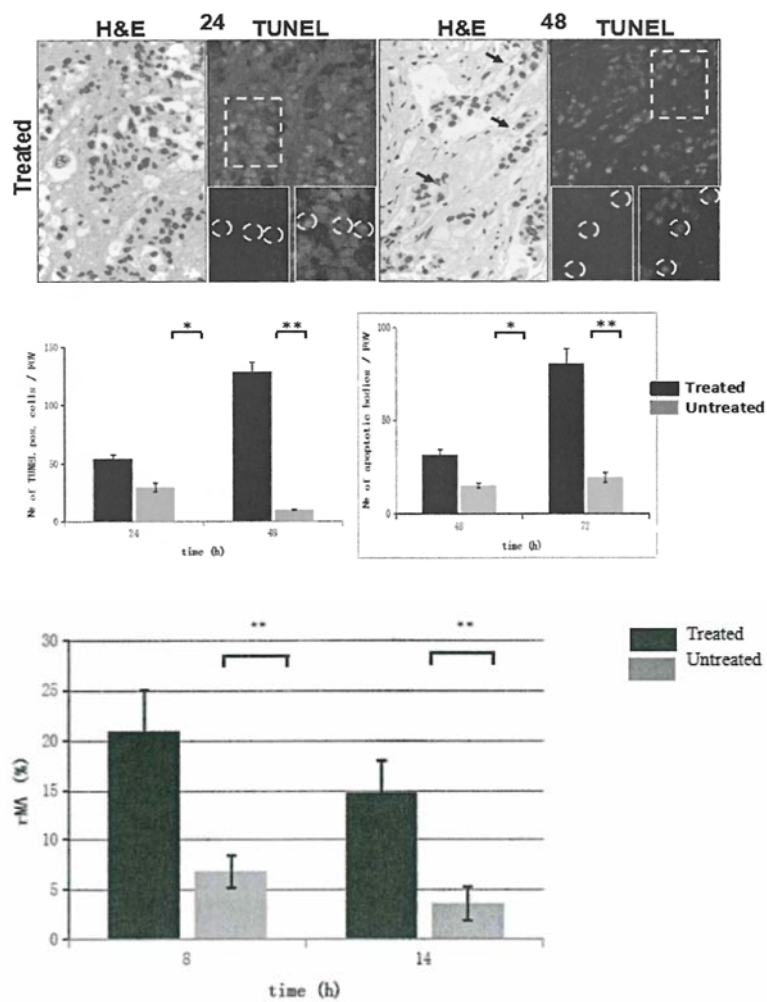
18 days later single shot treatment for (30 min), temperature between 41-42 °C. Sampling was carried out: 0, 1,4,8,14,24,48,72,120,168, 216h post-treatment

18 days later 30 min single shot treatment (treated tumor core temperature: 41-42 °C)

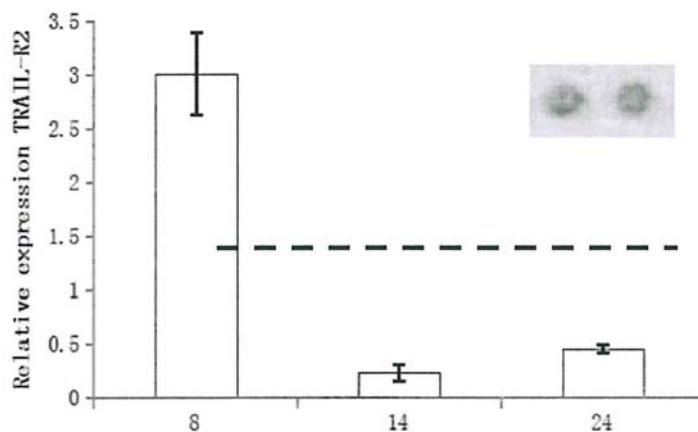
### Material and method II: molecular biology

Protein name	Clon	Producer
TRAIL-R2	polyclonal	Cell Signaling
Cytochrome-c	136F3	Cell Signaling
AIF	Polyclonal	Cell Signaling
Bax	Polyclonal	Sigma Aldrich
Mitochondrial ag	113-1	BioGenex
hsp70	polyclonal	Cell Signaling
hsp90	Polyclonal	Cell Signaling
hsp60	Polyclonal	Cell Signaling
HMGB1	Polyclonal	Cell Signaling
CRT	Polyclonal	Cell Signaling

**Results: Apoptotic bodies and DNA fragmentation**



**Untreated**

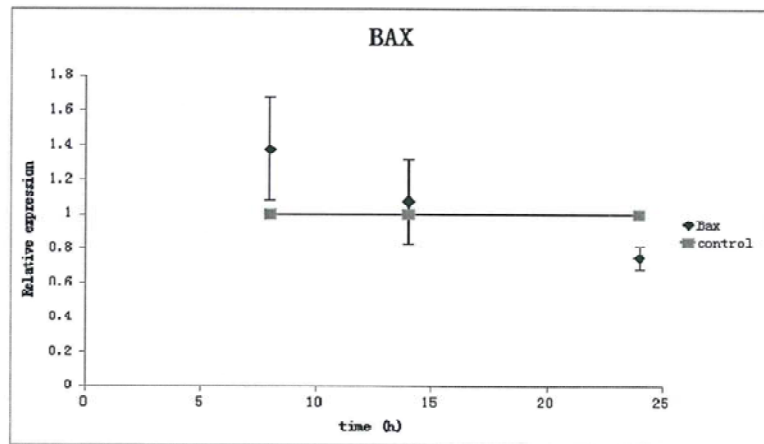
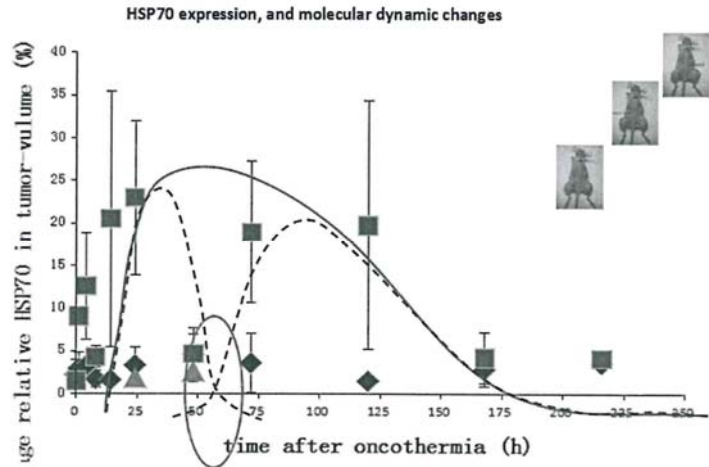


Are there signs of immunogenic cell death?

Hallmarks of ICD: CRT

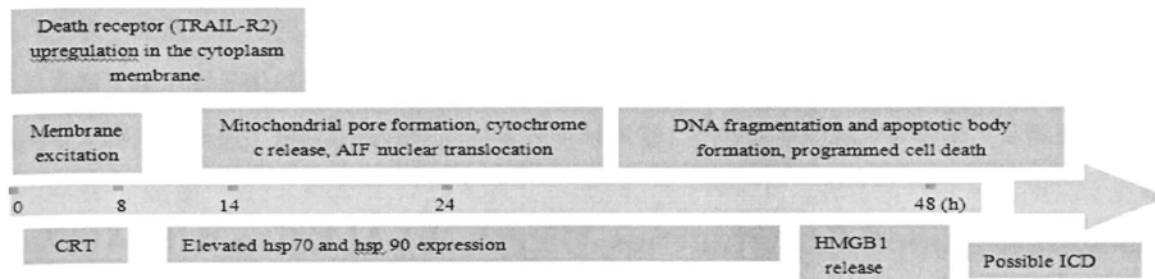
Hallmarks of ICD: membrane hsp70

## Heat shock protein 70 (HSP70)



## Hallmarks of ICD: HMGB1 release

### Summary



Oncothermia causes programmed cell death (as an obligatory event in ICD) with concomitant TRAIL-R2, calreticulin, heat shock protein upregulation and HMGB1 release from the nuclei.