Elevated apoptosis and tumor stem cell destruction in a radioresistant pancreatic adenocarcinoma cell line when radiotherapy is combined with modulated electro-hyperthermia

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Objective: Malignant exocrine tumors of the pancreas are among the worst to respond to oncotherapy. Despite sophisticated guidelines and new targeted therapies, the 5-year survival rate of patients with pancreatic adenocarcinomas is under 10%. The most critical factor responsible for this
is the high resistance of the tumor cells to the available chemo- or radiotherapies.

Modulated electro-hyperthermia (mEHT) is a complementary non-invasive cancer treatment using impedance-coupled radiofrequency to generate selective heat of <42°C causing cell stress and destruction in malignant tissue. In this study, we tested the combination of radiotherapy with mEHT in a radioresistant pancreatic adenocarcinoma cell line Panc1.

Methods: Panc1 adherent cells grown on coverslips were used to create 3 parallels in 4 groups: control (C); mEHT treated for 60 min (mEHT); irradiated with 2 Gy using ^{137}Cs (R), and combination treatment: irradiation followed by the same dose of mEHT (mEHT+R). 24 hours after treatments we observed the cells morphology, the proportion of apoptotic and necrotic (AnnexinV/propidium iodide positive) cells, the ALDH+ tumor stem cell (CSC), the colony forming capacity of cells, the H2Axγ positivity and the calreticulin presence in the cells. For quantitative and semiquantitative analysis we used: hematoxylin-eosin staining, flow cytometry and immunocytochemistry.

Results: Visible morphological changes were observed after 24 hours in the treated groups: an elevated number of apoptotic bodies and cell number loss. Compared to the control group, the apoptotic ratio was the highest in the mEHT+R group and significant elevation was measured also in the mEHT group. ALDH+ tumor stem cells decreased significantly after mEHT and mEHT+R treated groups compared to the control. As it was expected the irradiated group showed the same amount
of CSC cells as the control group (due to well-known radioresistance of the cell line). The CSCs colony
forming capacity was also significantly lower in the mEHT and mEHT+R group compared to the control group. Furthermore, H2Axγ and calreticulin positive cell fractions, indicating DNA double strand-brakes and ER-stress, respectively, were also significantly increased in the mEHT and the mEHT+R treated groups.

Conclusion: mEHT treatment alone can lead to massive apoptosis in Panc1 cells by inducing cell
stress and DNA double-strand break. Irradiation alone caused some necrosis but without major effect on CSCs. The combined treatment significantly improved the efficacy of radiotherapy resulting in major apoptosis and reduction of CSCs despite of the inherent radioresistance of Pan1.

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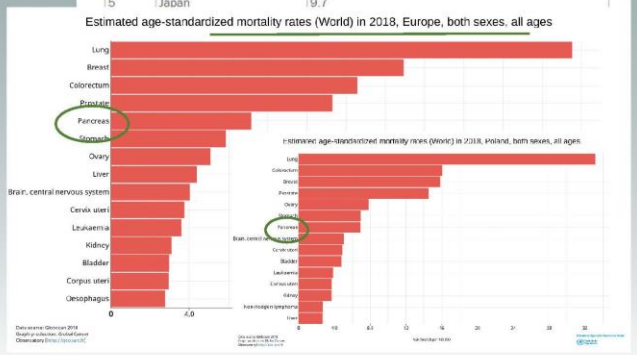
Pancreas adenocarcinomas - statistics

- Hungary had the highest rate of pancreatic cancer in 2018 age-standardised rate per 100,000 (https://www.wcrf.org/dietandcancer/cancer-trends/pancreatic-cancer-statistics)
- Pancreatic cancer is the 5th common cause of death in Europe (https://gco.iarc.fr/today/home)
- The 5 year survival rate is under 10% (https://seer.cancer.gov/statfacts/html/pancreas.html)

Pancreatic cancer rates: both sexes

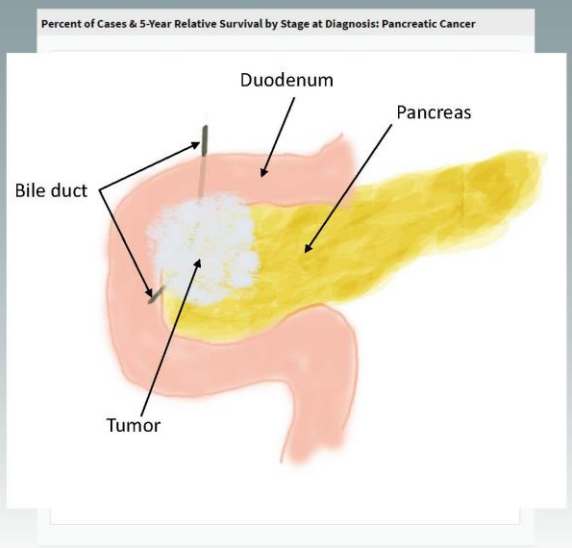
Hungary had the highest rate of pancreatic cancer in 2018, followed by Uruguay.

Rank	Country	Age-standardised rate per 100,000
1	Hungary	10.8
2	Uruguay	10.7
3	Moldova	10.5
3	Latvia	10.3
5	Japan	9.7



Pancreas adenocarcinomas - treatment

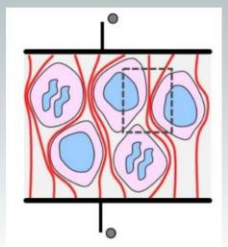
- The cancer stages at diagnosis are not promising [1]
- Just 10% of patients diagnosed with pancreatic cancer in England during 2013-2014 had surgery to remove their primary tumour, as part of their primary cancer treatment [4]
- Commonly used chemotherapy drugs:
  - Paclitaxel
  - 5FU (5 Fluorouracil)
  - Gemcitabine Hydrochloride
  - Irinotecan
- Combination: FOLFIRINOX
- Targeted therapy: Erlotinib (Tyrosine Kinase Inhibitor)
- Radiotherapy
- Radiochemotherapy

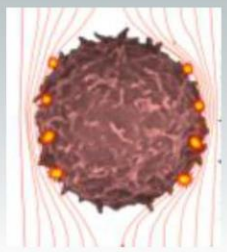


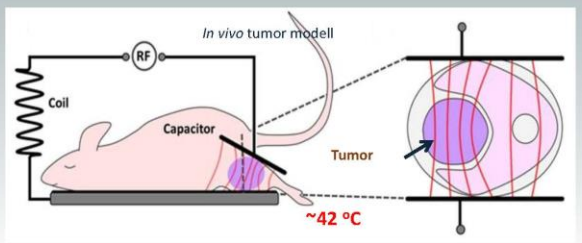
(4) National Cancer Registration & Analysis Service and Cancer Research UK: "Chemotherapy, Radiotherapy and Tumour Resections in England: 2013-2014"

Modulated electro-hyperthermia

- Regional – deep tissue hyperthermia
- Complementer therapy to radio- or chemotherapy
- Non invasive
- 13.56 MHz radiofrequency -> electric field=> 42°C heat
- Selective: eleveated glycolysis, ion concentration and conductibility (Warburg efect)



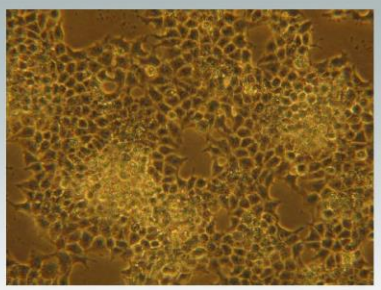




Material and methods

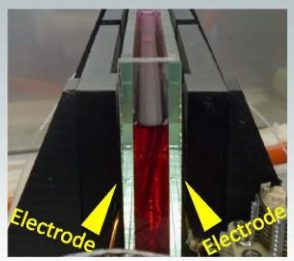
Cell line: Panc1

- Isolated from a ductal adenocarcinoma, 56-year-old male in 1975
- Epithelial morphology
- High tumorigenicity

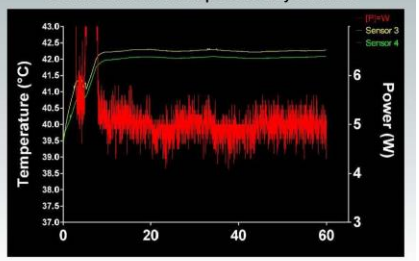


Radiotherapy

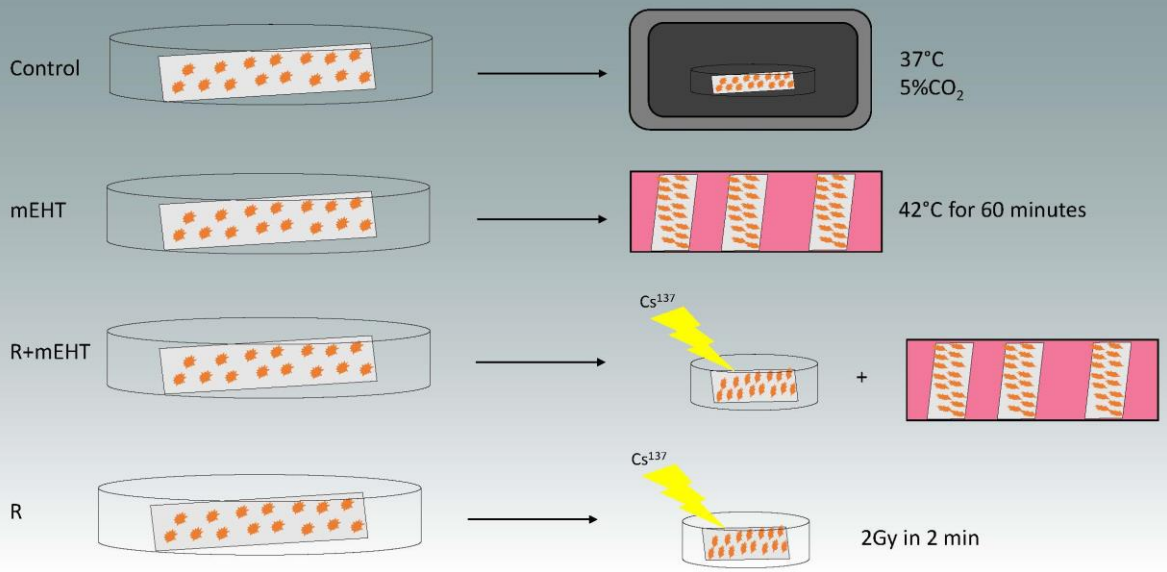
mEHT treatment

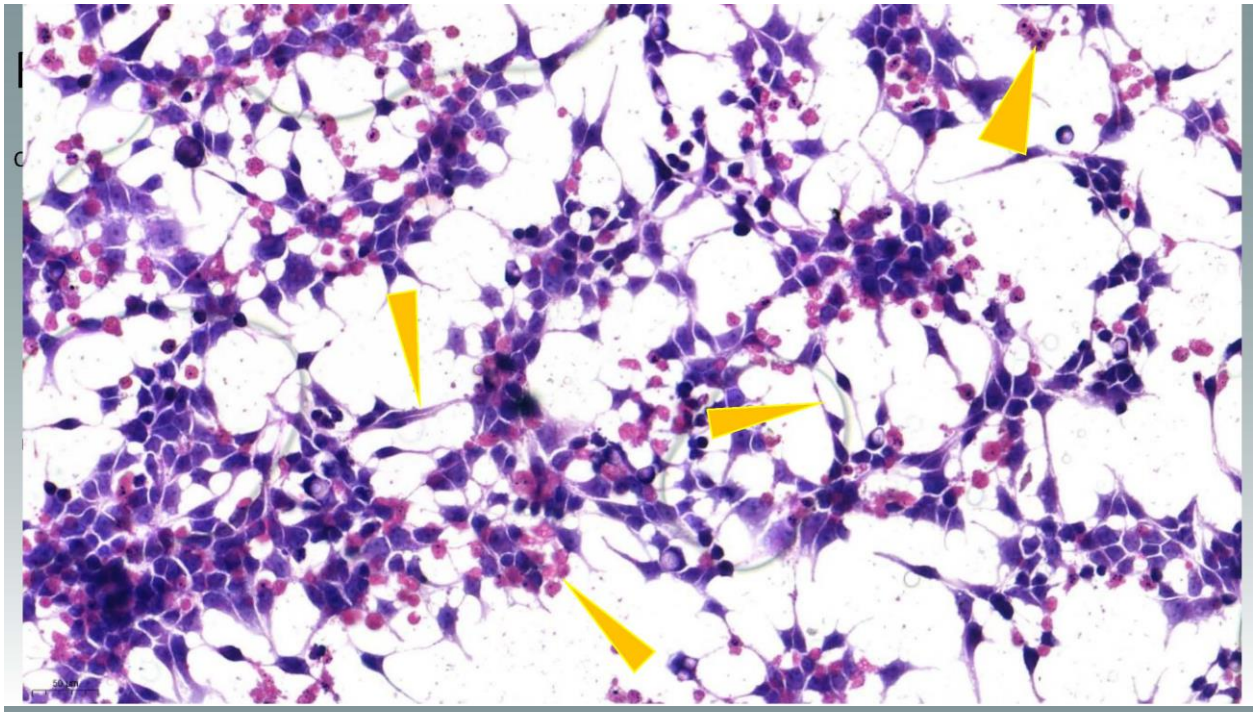


Heat controll with power adjustment



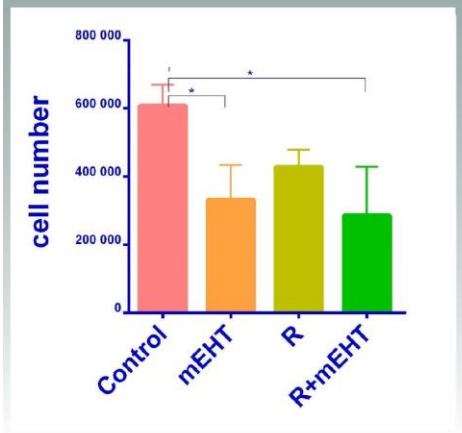
Methods: treatment protocol



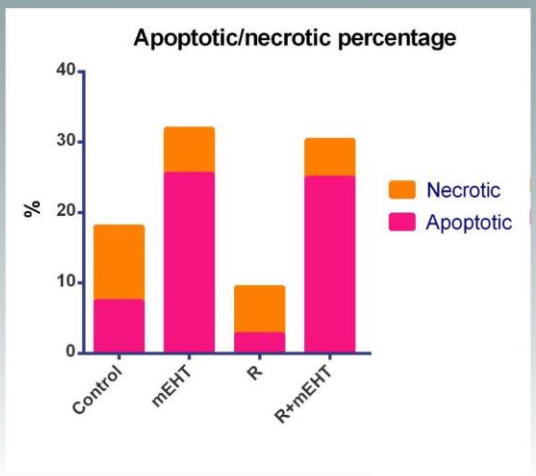


Results: apoptosis/necrosis

Cell numbers counted with trypan blue dye after treatments



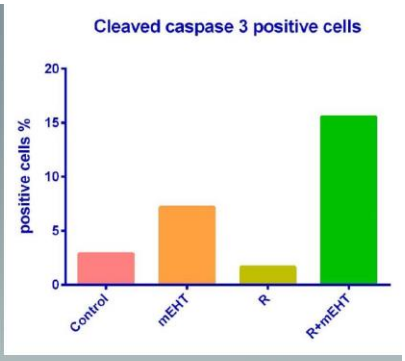
Apoptotic/necrotic rate analysed with flow cytomerty. Used staining: Annexin V and propidium iodide

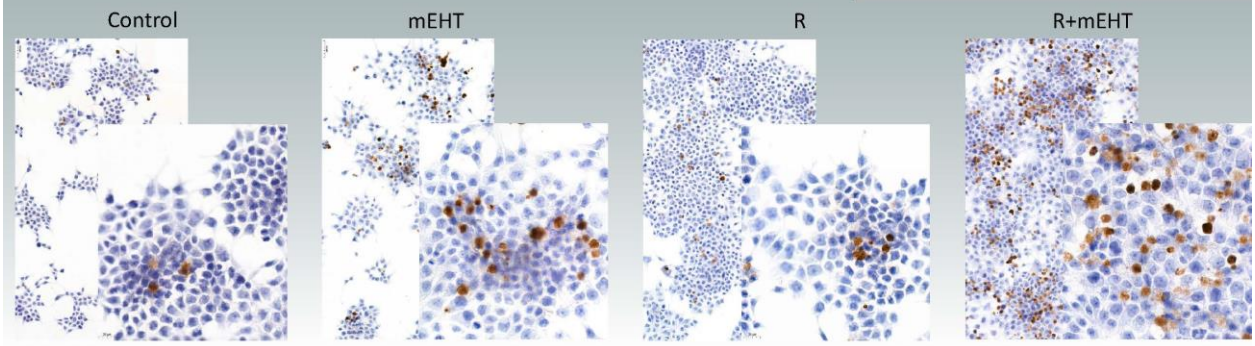


Results: apoptosis

Cleaved caspase 3 positivity

Presumably the apoptosis is caspase 3 dependent. The immunocytochemistry reaction showed many positive nuclei in the mEHT treated groups.

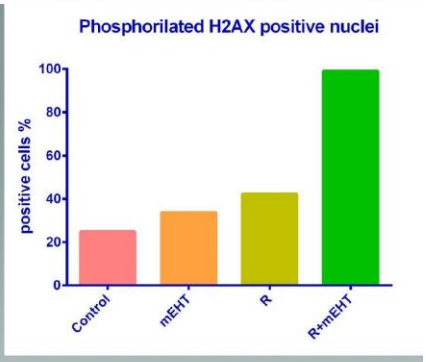


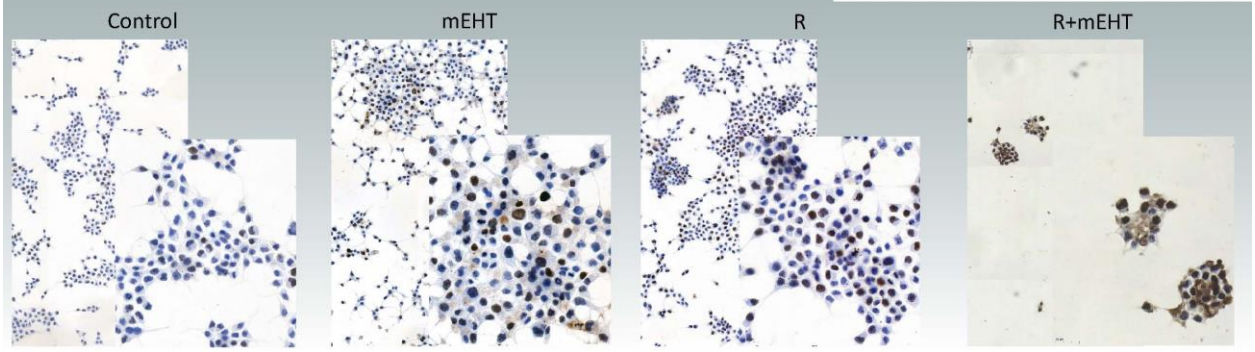


Results: DNA double strand breaks

Phosphorilated H2AX positivity

The phosphorilated H2AX is a marker of DNA double strand breaks

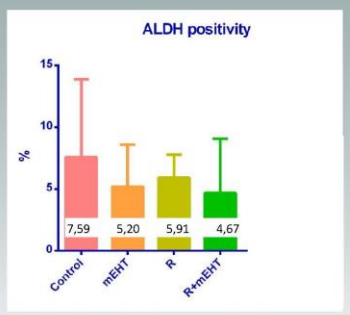




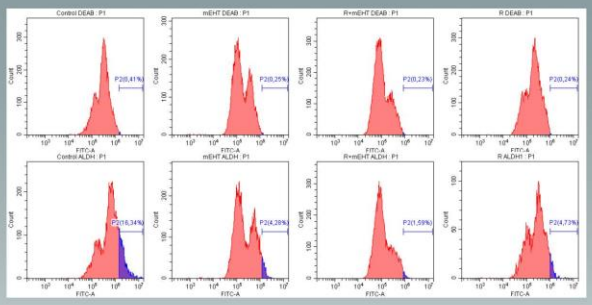
Results: tumor progenitor cells

ALDH+

ALDH – aldehyde dehydrogenase is highly expressed by tumor progenitor cells

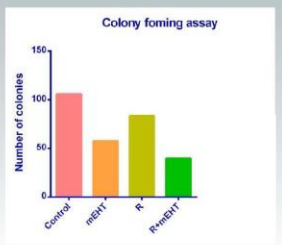


Percentage of ALDH positive cells →



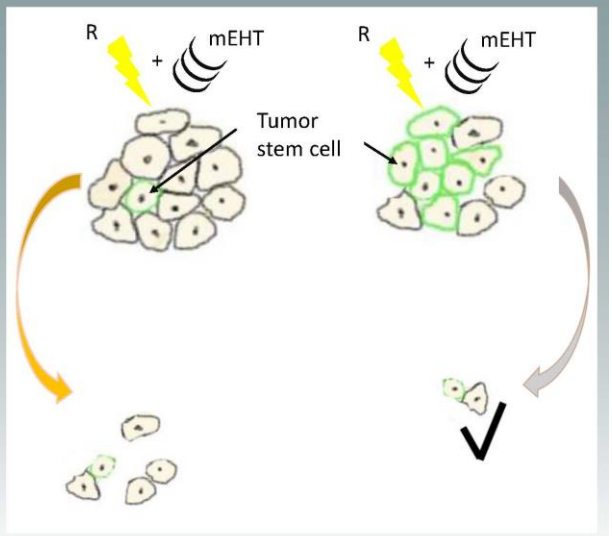
Colony forming capacity

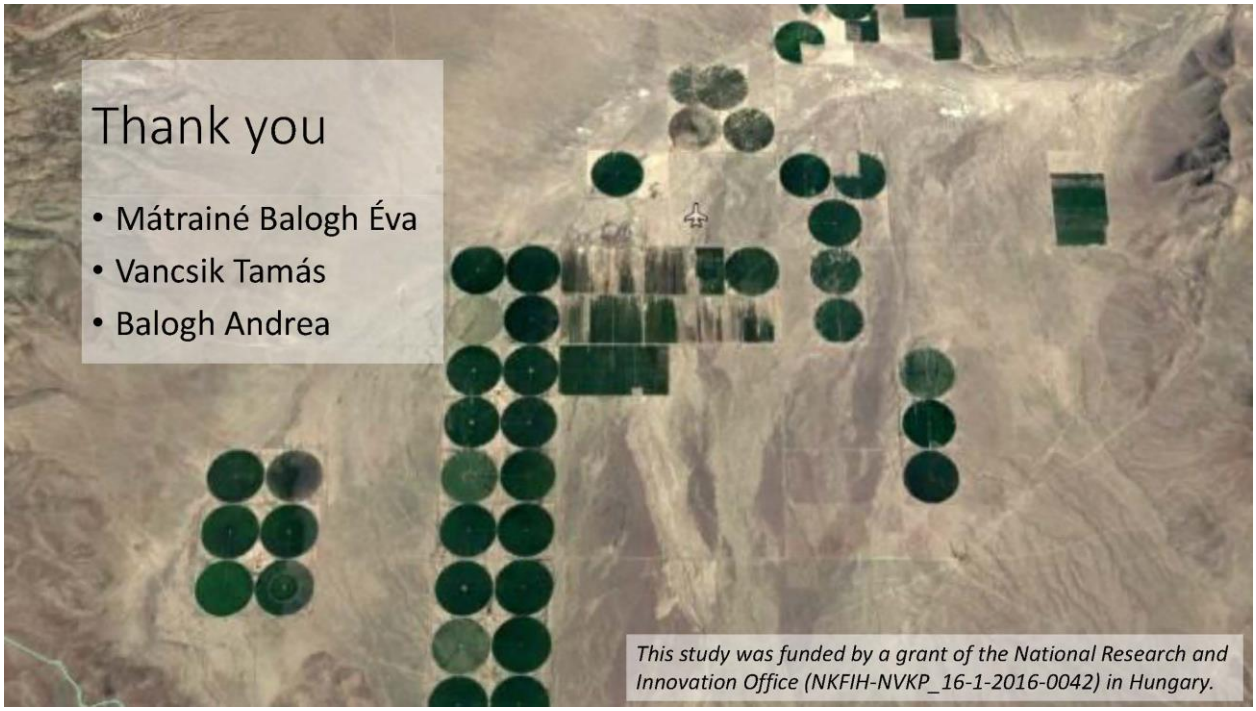
Tumor progenitor cells has the capacity to form colonies



Conclusion

- 60 minutes mEHT can lead to a massive apoptosis
- Combined with radiotherapy, mEHT potentiate the effectivity of the treatment
- Tumor stem cells are sensitive for mEHT or for combined treatment despite of their inherent radioresistance
- The mEHT treatment leads to caspase dependent apoptosis
- Presumably the mEHT has negative effect on DNA repair




Thank you
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• Balogh Andrea
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