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Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA

THE Tuscany Health Ecosystem

Tuscany Health Ecosystem (THE)

Spoke 1- Subproject 1.2: Simulations, Molecular Mechanism Validation and Radiobiological Effect Modelling

First radiobiology assays with UHDR VHEE beam

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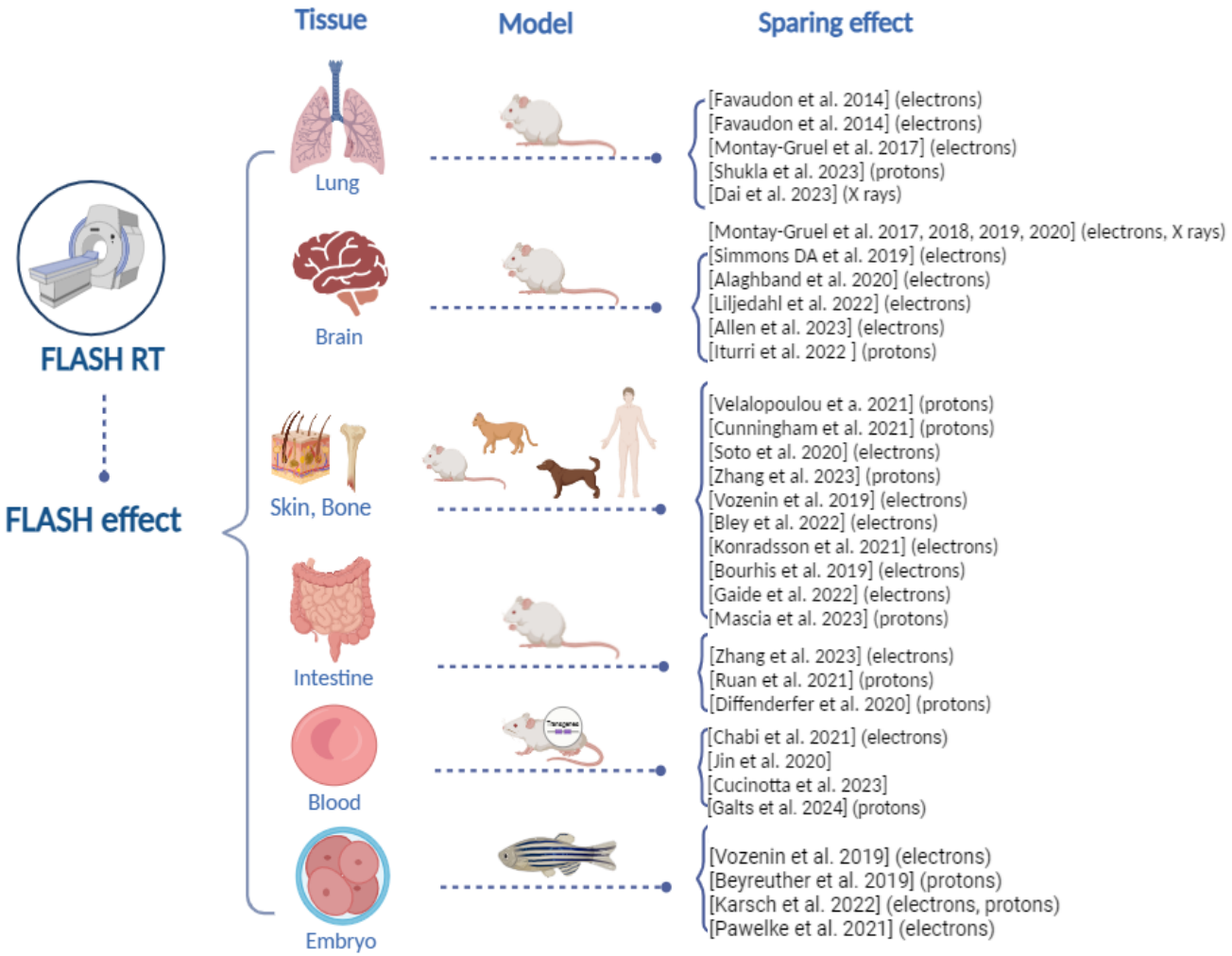
4°Incontro sull'ecosistema toscano per l'innovazione- Area della ricerca di Pisa, 12th December 2024

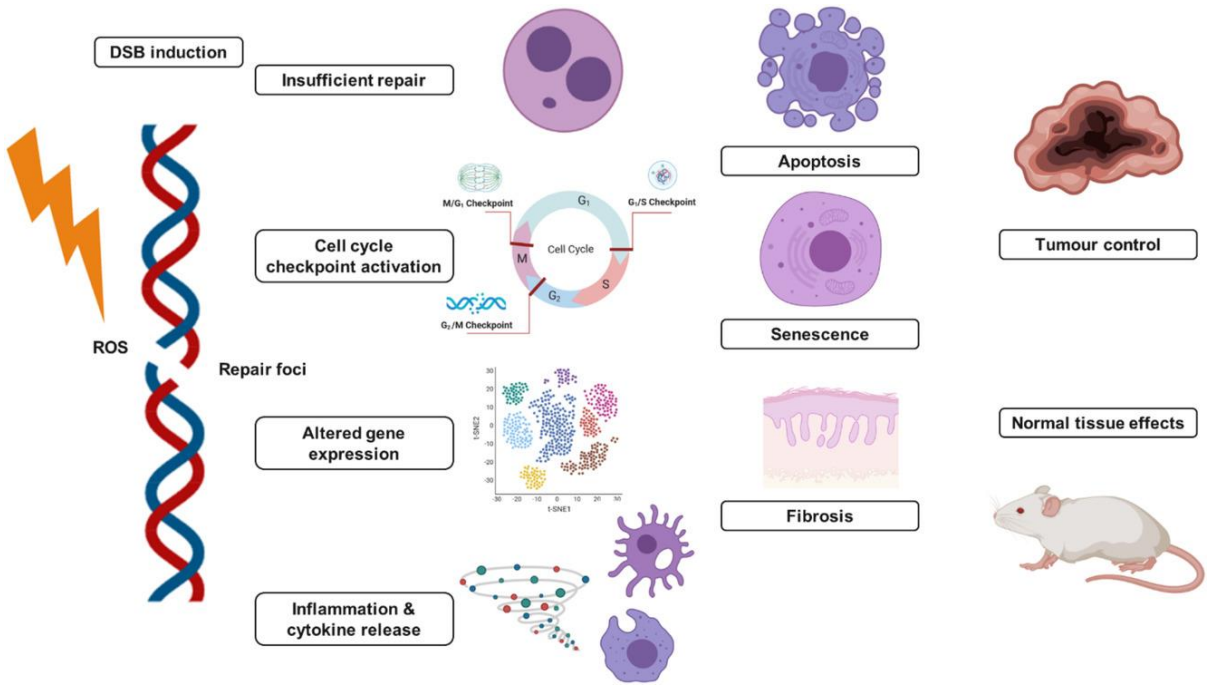
Preclinical and first-in-human clinical evidence

- Over the last few years, a growing body of studies pointed to the potential capacity of FLASH radiotherapy (FLASH RT) in different tissues using different preclinical models
- Successful clinical translation of FLASH RT depends on a better understanding of the biological mechanisms underpinning the FLASH effect



Borghini et al. Int J Mol Sci. 2024

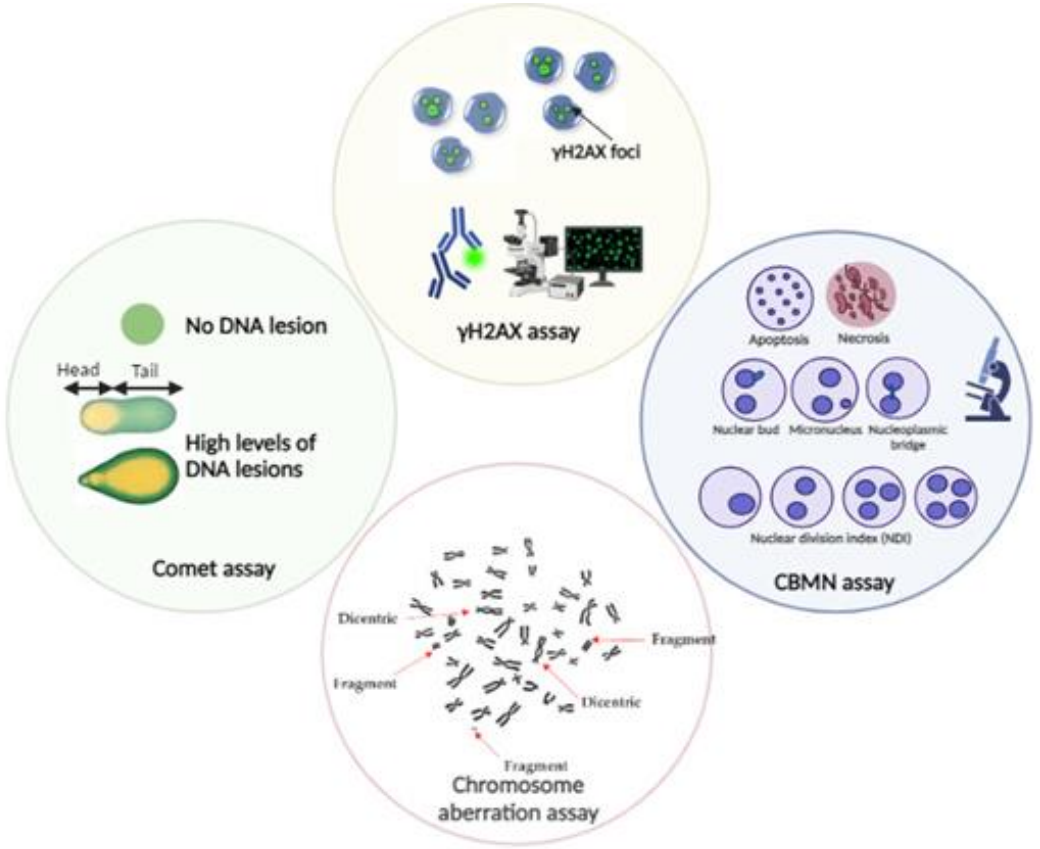
Biological mechanisms behind the FLASH effect: The role of DNA damage

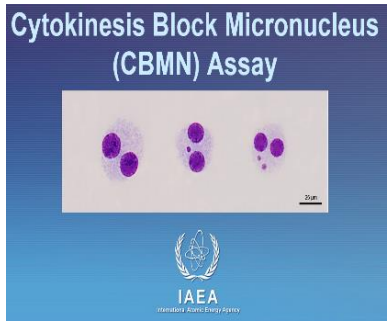


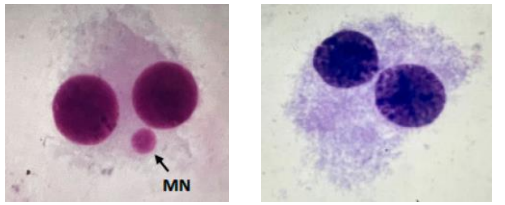
- DNA is the main target of radiation-induced damage resulting from direct ionizations or reactive oxygen species (ROS) that cause multiple DNA damage lesion
- Double-strand breaks (DSBs) are considered the most deleterious lesions that activate downstream cellular responses, including DNA repair, resulting in tumor control and normal tissue responses

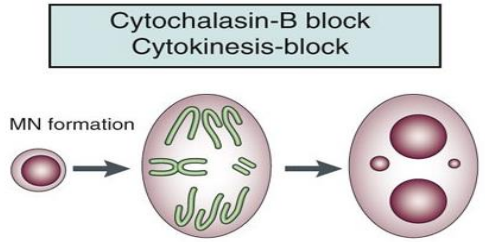
Friedl et al. Med Phys. 2022

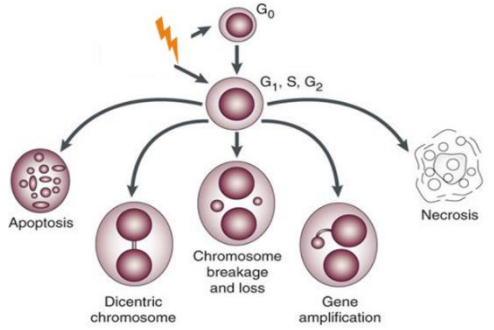
Cytokinesis-block micronucleus assay: The gold standard endpoint for radiobiological studies





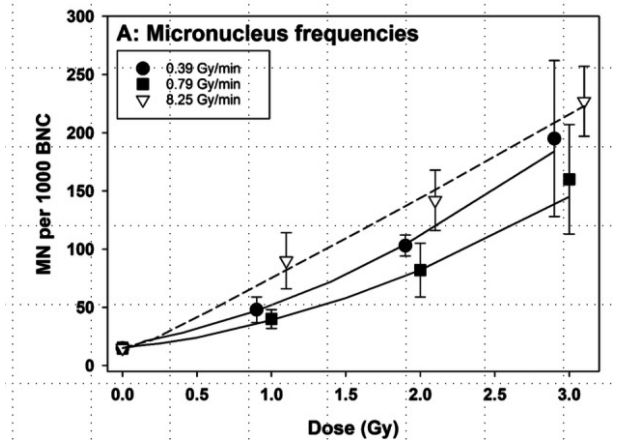




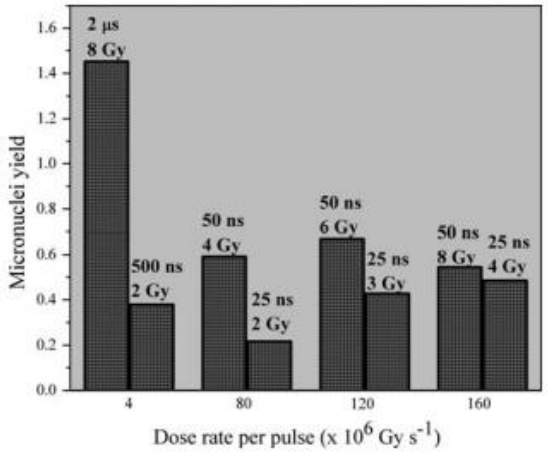


Borghini et al. Int J Mol Sci. 2024

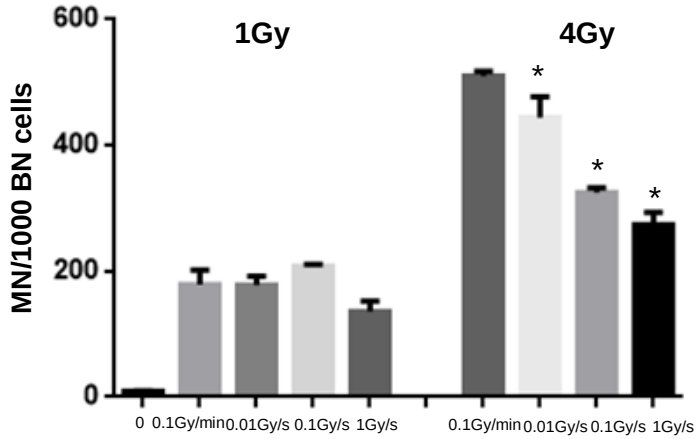
Dose rate effects on micronuclei induction in human blood lymphocytes



Low dose rates (<1 Gy/min) are less effective than high dose rates (>1 Gy/min) when lymphocytes are exposed to gamma radiation from a 137Cs source (Olofsson et al. Radiation and Environmental Biophysics. 2020)



Decrease in the MN yield at ultra-high dose-rate by using single 7 MeV electron pulses, suggesting possible radical recombination, which leads to decreased biological damage (Acharya et al. Radiat Environ Biophys. 2011)



Decrease in the MN yield with at very high dose rate at 4Gy (100kV Xrays) (Borghini, Panetta et al. unpublished data)

All p values are vs. 0.1Gy/min

UHDR VHEE - First in vitro experiments using laser-induced VHEE FLASH irradiation

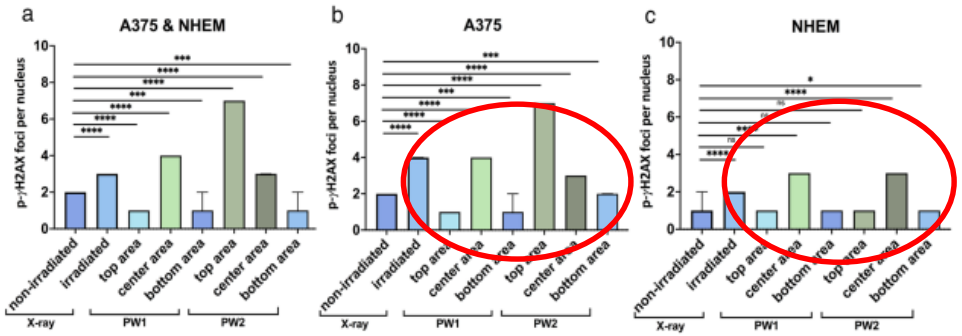


Figure 5. Comparison of "number of p-γ-H2AX foci per nucleus" values for each tested irradiation condition. The bar graphs show the comparisons between treatments for the overall A375 and NHEM co-cultures (**a**), for the A375 cells in co-cultures (**b**), and for the NHEM cells in co-cultures (**c**). Sampled areas of PW-irradiated specimens and the standard pulsed X-ray-irradiated cells were compared to non-irradiated controls. Medians of each data set and 95% confidence intervals are displayed. The statistical differences were determined using the nonparametric Mann–Whitney test, (n = 4473–10,629 analysed nuclei, $*p<0.05$, $***p<0.001$, $****p<0.0001$, *ns* = not significant).

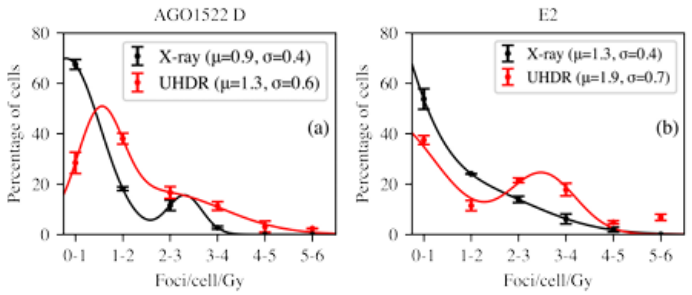


FIG. 8. **Distribution of sub-population of cells as a function of foci persisting 24 hours after irradiation** for (**a**) AG01522 D and (**b**) E2, in oxic conditions.

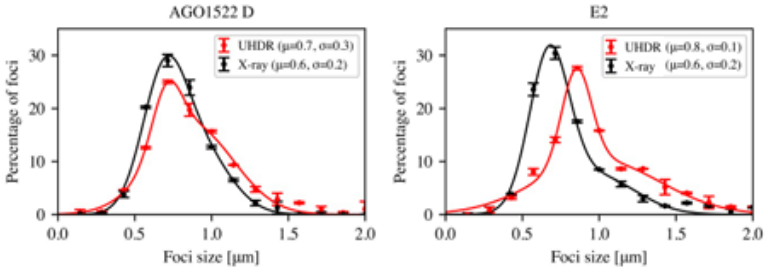


FIG. 9. **Distribution of radiation induced 53BP1 foci size 24 hours after irradiation** for (**a**) AG01522 D and (**b**) E2 cells in oxic conditions.

TAKE-HOME MESSAGE

- More DNA-damaged cell nuclei were generated by LPA electrons than by X-ray irradiation
- In certain areas, a differential response of normal cells vs. cancer cells occurred when exposed to LPA electrons; if present, this differential response could account for the FLASH effect

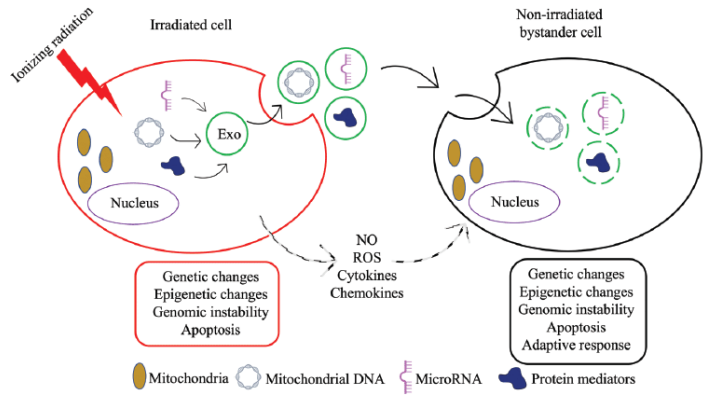
(Orobeti et al. Sci Rep. 2024)

TAKE-HOME MESSAGE

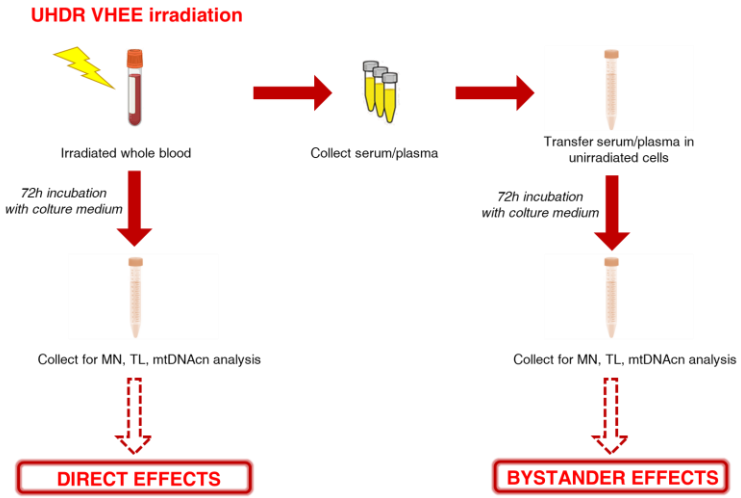
- First experimental characterization of a laser-accelerator able to deliver, in a single pulse, doses in excess of 1 Gy on timescales of tens of femtoseconds (dose rate= 10^{13} Gy/s)
- A significant decrease in survival rate for both cell lines and a reduction of the radioresistance of tumour cells
- These effects may be linked to a higher complexity of DNA damage by UHDR VHEE irradiation

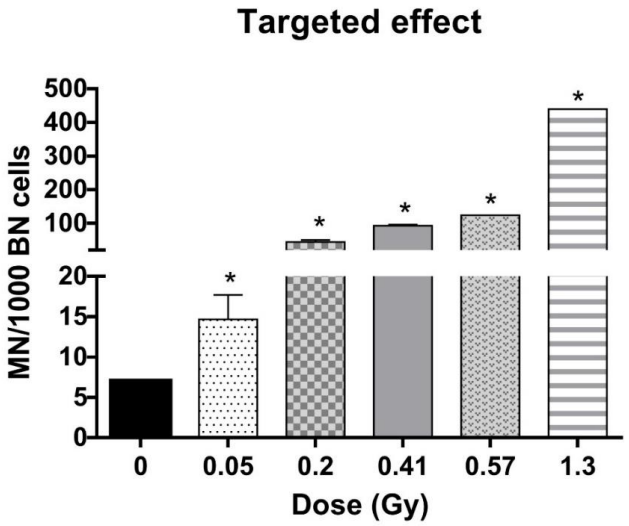
(McAnespie et al. arXiv physics. 2024)

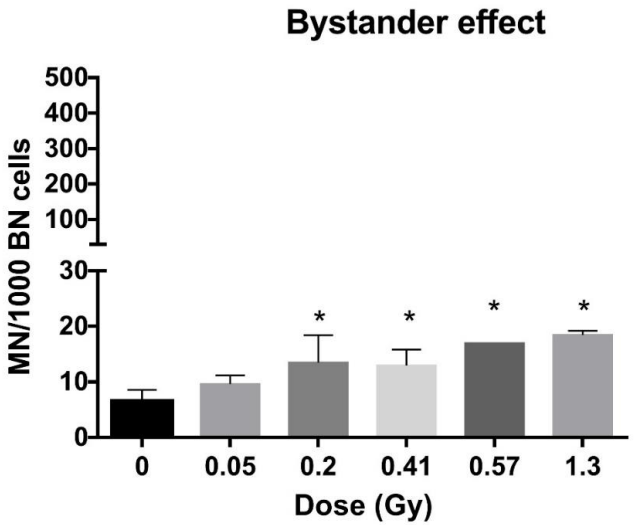
UHDR VHEE pulses – Preliminary results on chromosomal damage



~120-150 MeV electrons, 10^{12} Gy/s



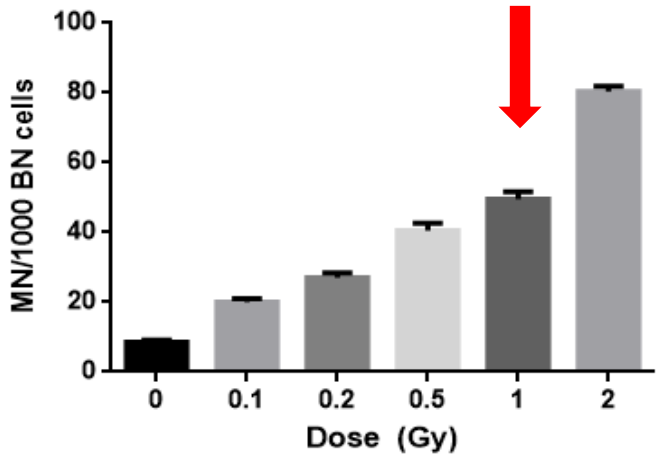




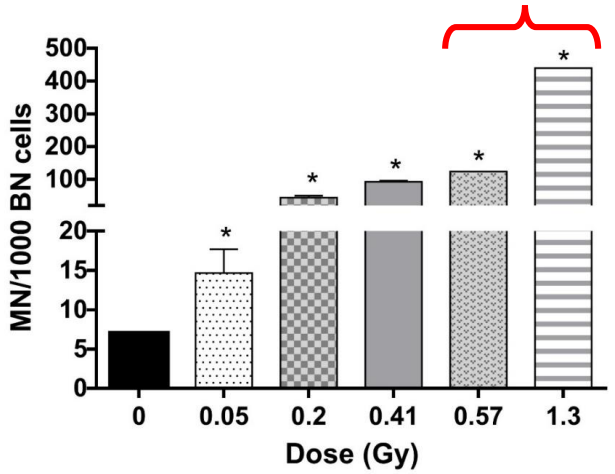
*All p<0.05 vs. control value

Our findings showed a radiobiological response as mirrored by the induction of micronuclei in blood lymphocytes

~1.5 MeV electrons, 10^{12} Gy/s



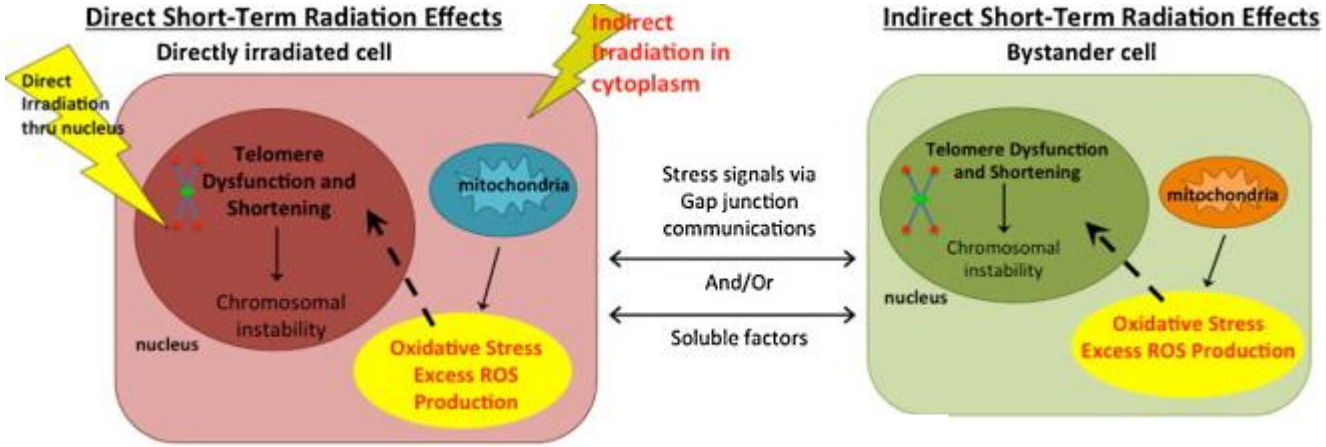
~120-150 MeV electrons, 10^{12} Gy/s



(Andreassi et et al. Radiat Res. 2016)

VHEE irradiation seems to cause more damage to DNA compared to low-energy electrons

Telomere length and mitochondrial DNA

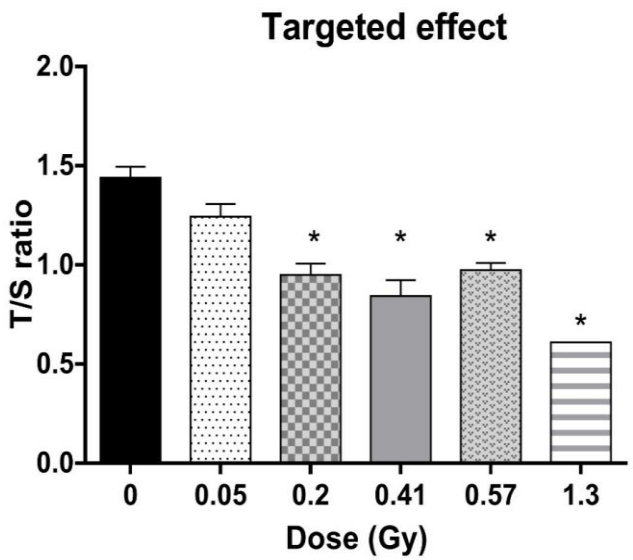


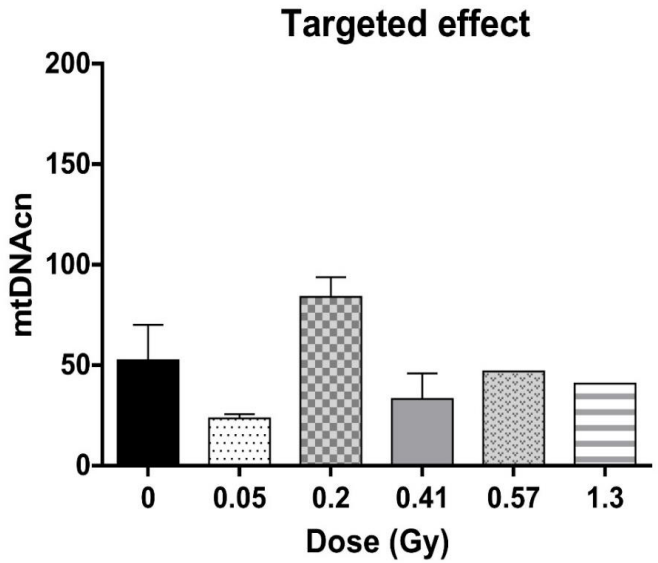
- **Telomeres have been proposed as "hallmarks of radiosensitivity"**
- Telomeres are the ending areas of chromosomes - protective «caps» that ensure the stability of chromosomes
- Telomeres are preferred targets for reactive oxygen species (ROS), which cause their progressive shortening and subsequent chromosomal instability

- **Mitochondrial DNA (mtDNA) is another target of ionizing radiation.**
- mtDNA is more susceptible to damage under exogenous and endogenous stresses due to its close proximity to the sites of oxidative phosphorylation and the deficiency of protection from histones
- The number of mitochondria varies across different cell types, ranging from just a few to hundreds and each mitochondrion may contain from 2 to 10 mtDNA copies (mtDNAcn)
- **mtDNAcn is a marker of mitochondrial function**

(Shim et al. Mutat Res Rev Mutat Res. 2014)

UHDR VHEE pulses - Telomere length and mitochondrial DNA copy number (mtDNAcn)





Our preliminary findings reveal a radiation dose-response relationship in telomere shortening induced by VHEE

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