





Tuscany Health Ecosystem (THE)

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

First radiobiology assays with UHDR VHEE beam

Andrea Borghini

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









Tissue Sparing effect Model Preclinical and first-in-human clinical evidence [Favaudon et al. 2014] (electrons) [Favaudon et al. 2014] (electrons) [Montay-Gruel et al. 2017] (electrons) [Shukla et al. 2023] (protons) Lung [Dai et al. 2023] (X rays) [Montay-Gruel et al. 2017, 2018, 2019, 2020] (electrons, X rays) [Simmons DA et al. 2019] (electrons) [Alaghband et al. 2020] (electrons) [Liljedahl et al. 2022] (electrons) [Allen et al. 2023] (electrons) Brain [Iturri et al. 2022] (protons) **FLASH RT** Over the last few years, a growing body of studies pointed to [Velalopoulou et a. 2021] (protons) [Cunningham et al. 2021] (protons) the potential capacity of FLASH radiotherapy (FLASH RT) in [Soto et al. 2020] (electrons) different tissues using different preclinical models Zhang et al. 2023] (protons) Vozenin et al. 2019] (electrons) Skin, Bone FLASH effect Bley et al. 2022] (electrons) [Konradsson et al. 2021] (electrons) [Bourhis et al. 2019] (electrons) Successful clinical translation of FLASH RT depends on a [Gaide et al. 2022] (electrons) [Mascia et al. 2023] (protons) understanding biological of the mechanisms [Zhang et al. 2023] (electrons) underpinning the FLASH effect Intestine [Ruan et al. 2021] (protons) [Diffenderfer et al. 2020] (protons) [Chabi et al. 2021] (electrons) [Jin et al. 2020] [Cucinotta et al. 2023] Blood [Galts et al. 2024] (protons) [Vozenin et al. 2019] (electrons) [Beyreuther et al. 2019] (protons) [Karsch et al. 2022] (electrons, protons) [Pawelke et al. 2021] (electrons)

Embrvo

Borghini et al. Int J Mol Sci. 2024

•

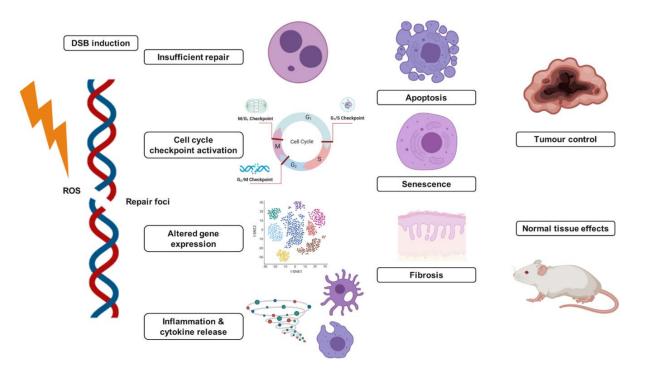
better







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



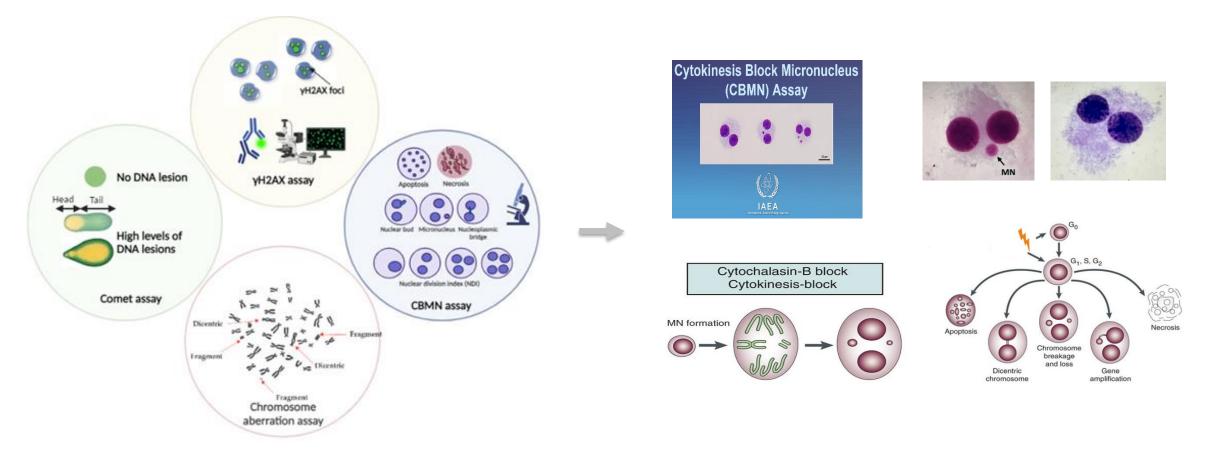
- DNA is the main target of radiation-induced damage resulting from direct ionizations or reactive oxygen species (ROS) that cause multiple DNA damage lesion
- Double-strand breaks (DSBs) are considered the most deleterious lesions that activate downstream cellular responses, including DNA repair, resulting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

Friedl et al. Med Phys. 2022

THE TUSCANY HEALTH ECOSYSTEM



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

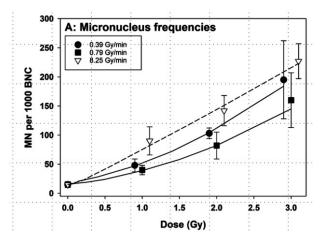


Borghini et al. Int J Mol Sci. 2024

THE TUSCANY HEALTH ECOSYSTEM



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)

600·

400

200

ſ

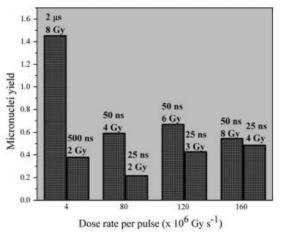
MN/1000 BN cells

1Gy

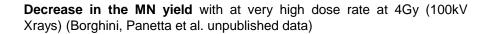
0 0.1Gy/min0.01Gy/s 0.1Gy/s 1Gy/s

4Gy

0.1Gy/min 0.01Gy/s 0.1Gy/s 1Gy/s



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)



All p values are vs. 0.1Gy/min



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

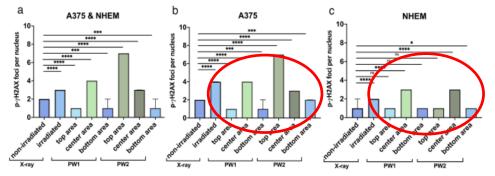
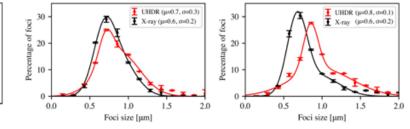


Figure 5. Comparison of "number of $p-\gamma$ -H2AX foci per nucleus" values for each tested irradiation condition. The bar graphs show the comparisons between treatments for the overall A375 and NHEM co-cultures (a), for the A375 cells in co-cultures (b), and for the NHEM cells in co-cultures (c). Sampled 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 < 0.001, ns = not significant).

AG01522 D E2 X-ray (μ=1.3, σ=0.4) I X-ray (μ=0.9, σ=0.4) cells **Ι** UHDR (μ=1.9, σ=0.7) **UHDR** (μ=1.3, σ=0.6) 60 ÷ (b) (a) ercentage 40 0-1 1-2 2-3 3-4 4-5 5-6 0-1 1-2 2-3 3-4 4-5 5-6 Foci/cell/Gy Foci/cell/Gy



AGO1522 D

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 rs (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= 10¹³ 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)

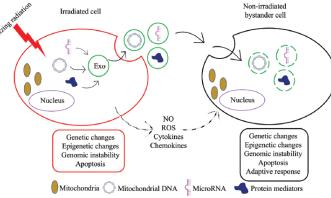
THE TUSCANY HEALTH ECOSYSTEM

E2









Targeted effect UHDR VHEE irradiation Bystander effect 500-500-400-400-MN/1000 BN Cells 300-Transfer serum/plasma in MN/1000 BN cells Irradiated whole blood Collect serum/plasma unirradiated cells 200-72h incubation 72h incubation 100with colture medium with colture medium 20_T 15-10 Collect for MN, TL, mtDNAcn analysis Collect for MN, TL, mtDNAcn analysis 10 5 0.2 0.57 0.05 0.41 1.3 0 0.05 0.2 0.41 0.57 0 **BYSTANDER EFFECTS** DIRECT EFFECTS Dose (Gy) Dose (Gy)

UHDR VHEE pulses – Preliminary results on chromosomal damage

~120-150 MeV electrons, 10¹² 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

1.3





~120-150 MeV electrons, 1012 Gy/s



~1.5 MeV electrons, 10¹² Gy/s

100 -500-400-WN/1000 BN cells 200-100-20-10-10-10-80 60 40 20 0 n 0.05 0.2 0.41 0.57 1.3 0.5 0.1 0.2 2 0 0 1 Dose (Gy) Dose (Gy)

(Andreassi et et al. Radiat Res. 2016)

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

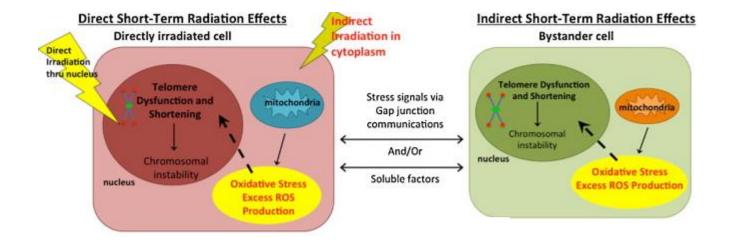
MN/1000 BN cells







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

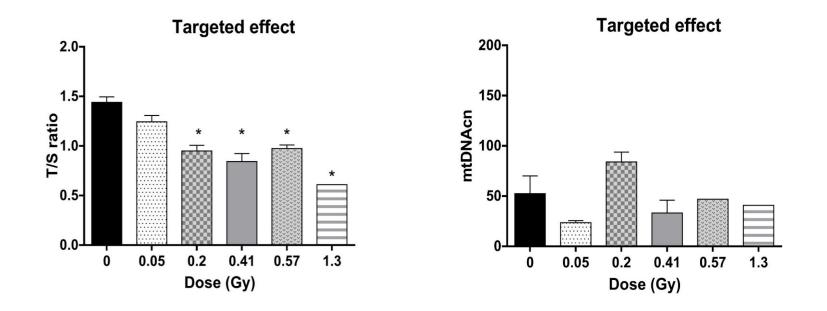
(Shim et al. Mutat Res Rev Mutat Res. 2014)

THE TUSCANY HEALTH ECOSYSTEM

9



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













MARIA GRAZIA ANDREASSI RUDINA NDREU ANTONELLA MERCURI STEFANO TURCHI PAOLA CANALE LEONIDA A. GIZZI LUCA LABATE FERNANDO BRANDI PETRA KOESTER FEDERICA BAFFIGI LORENZO FULGENTINI SIMONA PICCININI GABRIELE BANDINI MARTINA SALVADORI COSTANZA PANAINO DANIELE PALLA ALESSANDRO FREGOSI DAVID GREGOCKI SIMON VLACHOS FEDERICO AVELLA



11