FLASH Effects: Focus on *in-Vitro* Studies

Manuela Buonanno
Assistant Professor of Radiation Oncology
Columbia University Irving Medical Center

FLASH effect

Radiation delivered at high dose rates ensure similar tumor control as radiation delivered at "conventional" dose rates but with reduced normal tissue toxicity

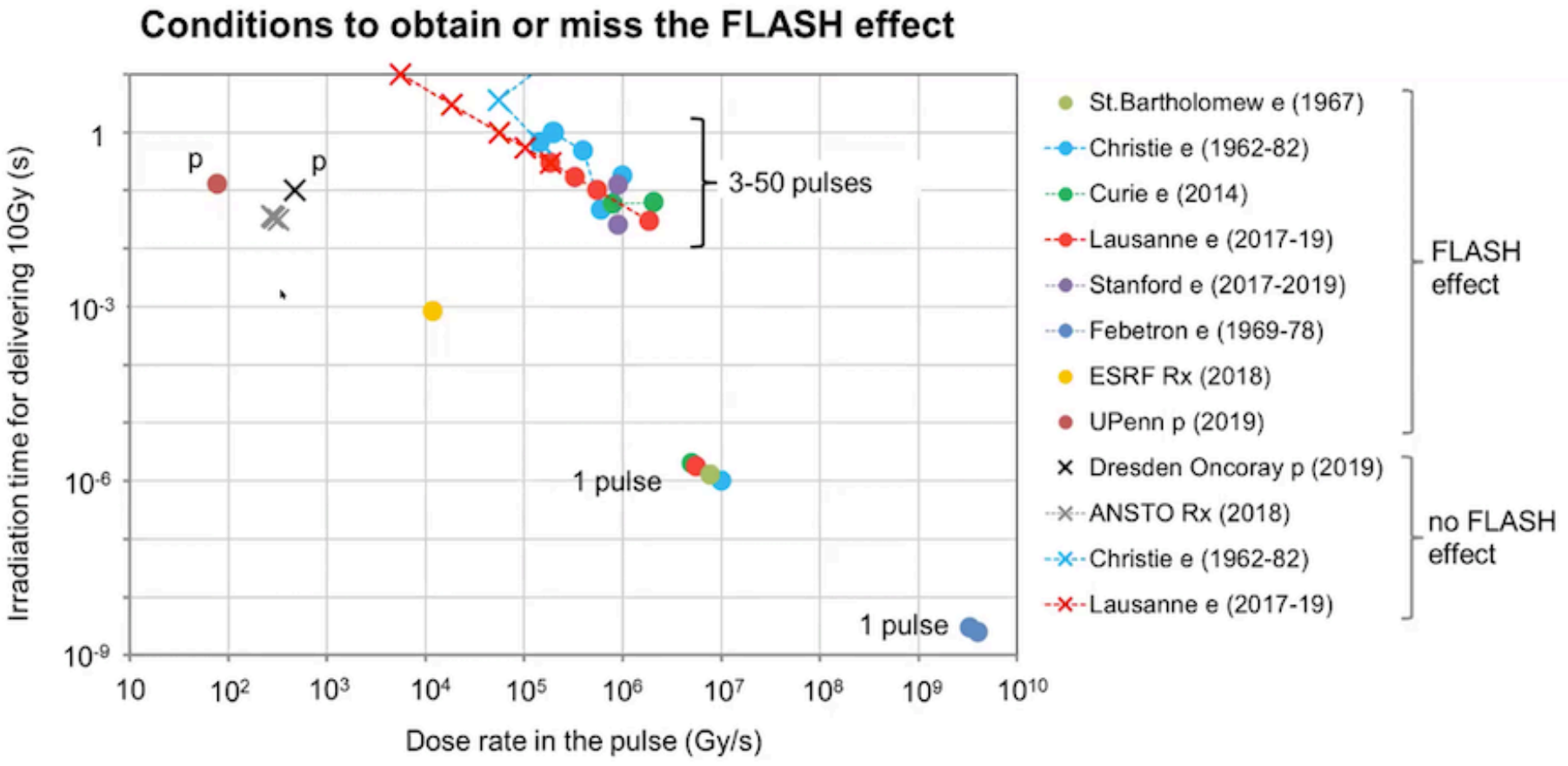
A biological effect strictly characterized by physics parameters

	Conventional	FLASH
Dose rates	1-2 Gy/min (0.02-0.03 Gy/s)	> 100 Gy/s
Dose delivery time	min	µs to ms
Dose per fraction	~ 2 Gy	≥ 10 Gy

For pulsed beams:

- Mean dose rate *vs.* instantaneous dose rate (dose rate in the pulse)
- Time to deliver the dose
- Frequency or time between two pulses

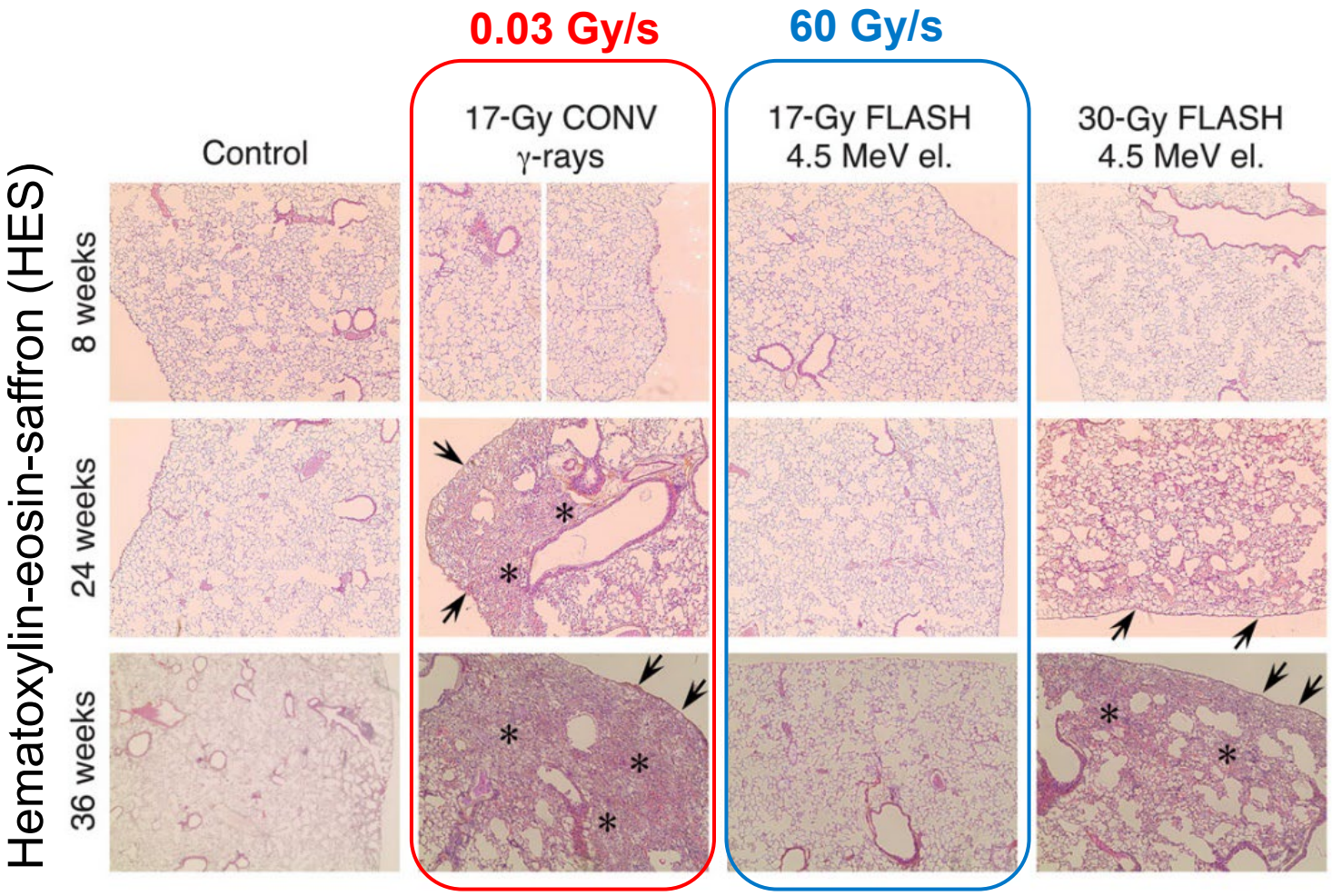
Dewey DL, Boag JW. Nature. 1959;183(4673):1450-1451.



Adapted from Vozenin MC 2021 RRS Herman D. Suit plenary lecture

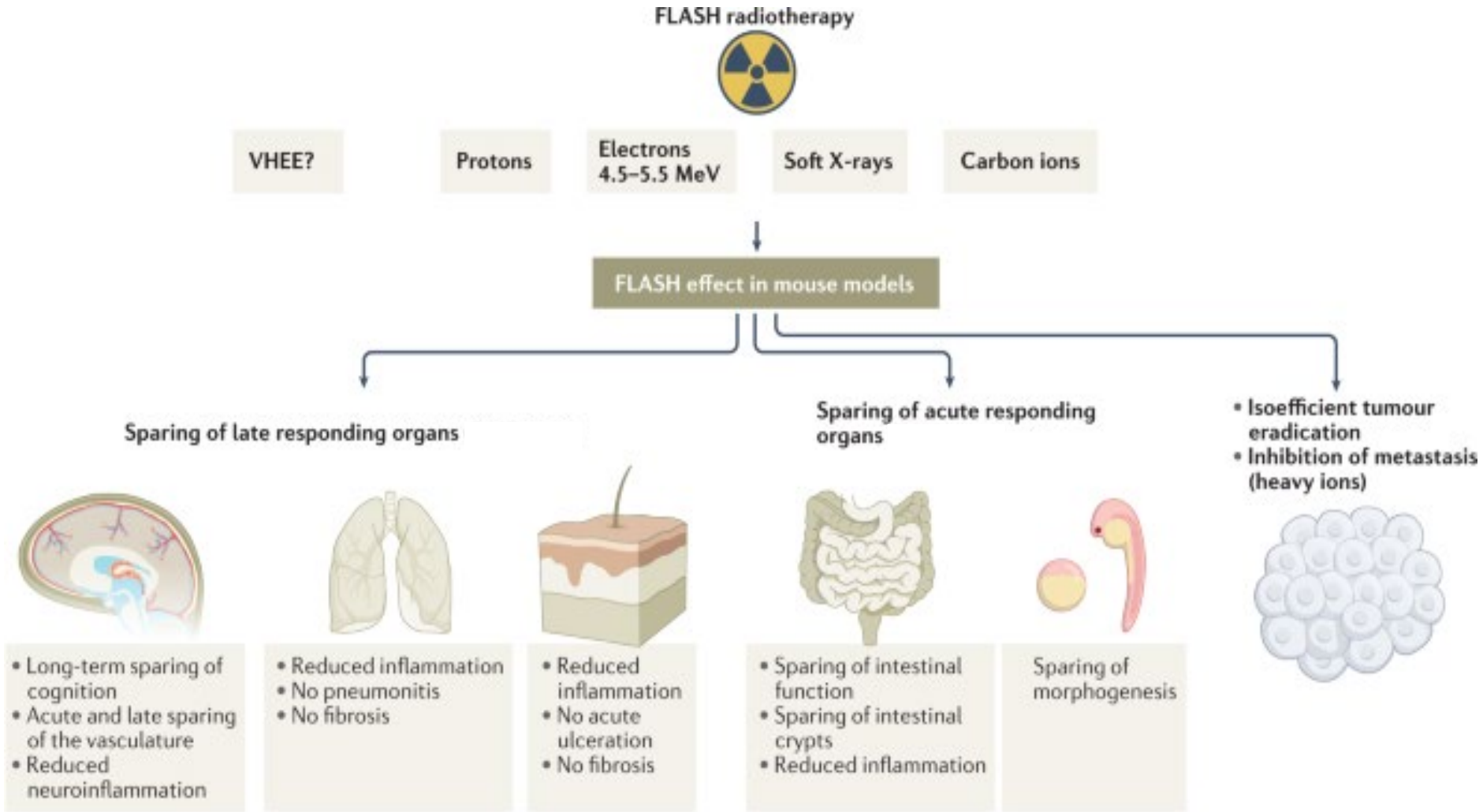
Normal tissue sparing - Lung

- C57BL/6J mice
- bilateral thorax exposure
- single fraction



Arrows -> patches of subpleural fibrosis
Asterisks -> intraparenchymal fibrosis

Favaudon V et al. (2014) Sci Transl Med 6: 245



Adapted from Vozenin, MC., Bourhis, J. & Durante, M., Nat Rev Clin Oncol 19, 791–803 (2022)

Normal tissue FLASH sparing effects have been observed in different species and organs

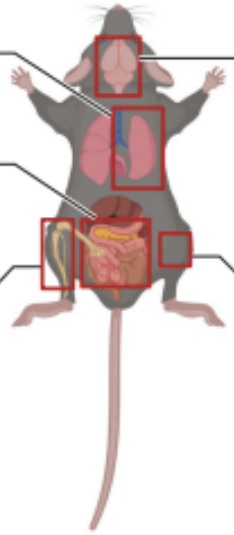
Canine
Konradsson et al. (2021)

Feline
Vozenin et al. (2019a)

Mouse lung
Favaudon et al. (2014)
Fouillade et al. (2020)

Mouse gut
Levy et al. (2020)
Velalopoulou et al. (2021)
M.M. Kim et al. (2021)

Human hematopoiesis
Chabi et al. (2021)



Mouse brain
Montay-Gruel et al. (2017)
Montay-Gruel et al. (2018)
Montay-Gruel et al. (2019)
Simmons et al. (2019)
Allen et al. (2020)
Montay-Gruel et al. (2020)
Alaghband et al. (2020)

Mouse skin
Field & Bewley (1974)
Inada et al. (1980)
Hendry et al. (1982)
Soto et al. (2020)
Cunningham et al. (2021)
Velalopoulou et al. (2021)
Sorensen et al. (2022)

Miniature pig skin
Vozenin et al. (2019a)

Zebrafish embryo
Montay-Gruel et al. (2019)
Vozenin et al. (2019b)
Kacem et al. (2022)
Karsch et al. (2022)

Are tumors spared as well?

The response of >23 tumor types have been compared using FLASH- & CONV-RT

- Syngeneic or xenograft tumors implanted subcutaneously or orthotopically in immune-competent or immune-compromised mouse models.
- Spontaneous tumors in larger vertebrates (dog and cat).

Exceptions

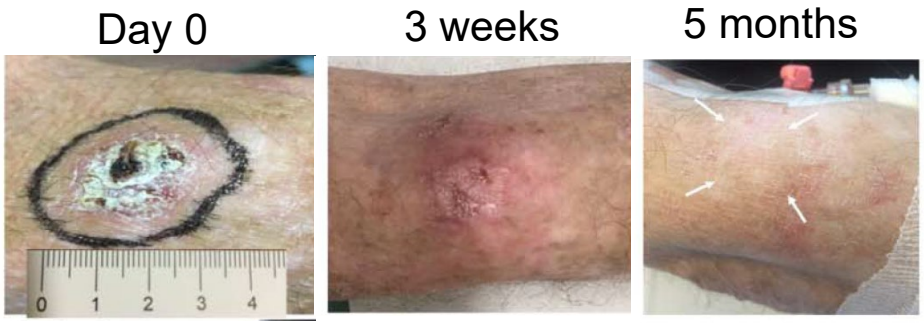
Lewis lung carcinoma and LM8 osteosarcoma
FLASH-RT was shown to be **slightly more effective** than CONV-RT in slowing the growth of tumors (B.W. Loo Jr. et al., unpublished data) and in preventing metastasis when delivered with carbon ions (Tinganelli et al. 2022).

Not effective in all human T cell acute lymphoblastic leukemias (T-ALL) grafted into immunocompromised mice as patient-derived xenografts (Chabi et al. 2021). Following bone marrow transplantation/reconstitution, mice were given 4 Gy TBI FLASH-RT or CONV-RT; one T-ALL was more responsive to CONV-RT.

In humans

The first patient with skin metastases from melanoma had one 3.5-cm diameter tumor treated with 15 Gy electron FLASH delivered in 90 ms.

- Complete tumor response at 36 days
- No recurrence after 5 months



Bourhis J, et al. Radiother Oncol. 2019;139:18-22

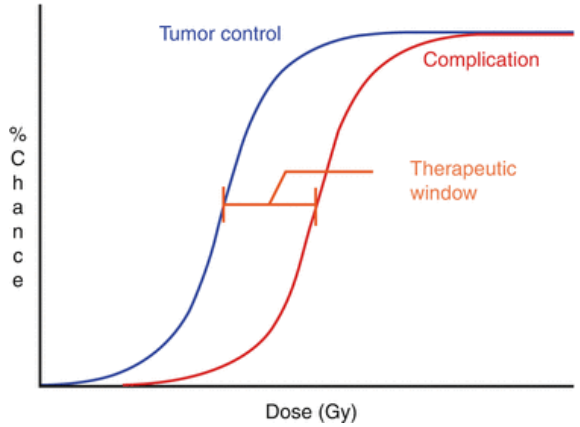
Varian and the Cincinnati Children's/UC Health Proton Therapy Center trial of patients enrolled in the FAST-01 or **F**e**A**sibility **S**tudy of FLASH Radiotherapy for the palliative **T**reatment of symptomatic bone metastases

- 10 patients underwent FLASH radiotherapy (8Gy at 60 Gy/sec) at 12 metastatic sites.
- Transient pain flares occurred in 4 of the 12 treated sites (33%). In 8 of the 12 sites (67%) patients reported pain relief, and in 6 of the 12 sites (50%) patients reported a complete response (no pain).
- Phase 2 has started.

Mascia AE, et al. JAMA Oncol. 2023;9(1):62–69

Clinical advantages

- Increase of the therapeutic index
- Reduction of dose fractions
- Reduced treatment time, overall course, and costs

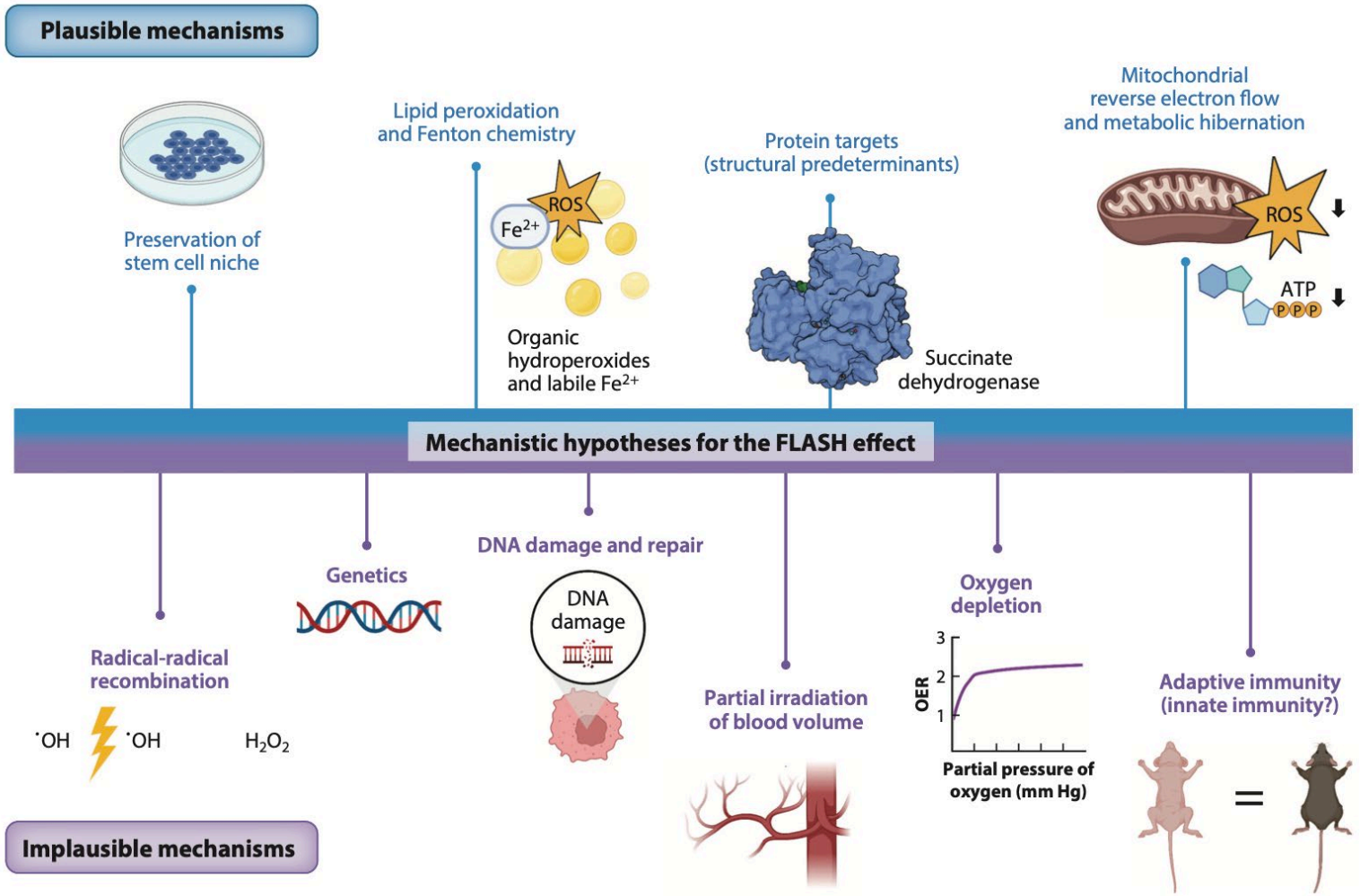


Chang, D.S et al, (2014). Therapeutic Ratio. In: Basic Radiotherapy Physics and Biology. Springer, Cham.

Ongoing challenges

- Beam parametrization and standardization
- Type of radiation that can elicit FLASH effects
- Fractionation
- Dose rate effects on tumor volume
- Dose rate effects depending on the organ
- Late effects
- Unidentified molecular mechanisms

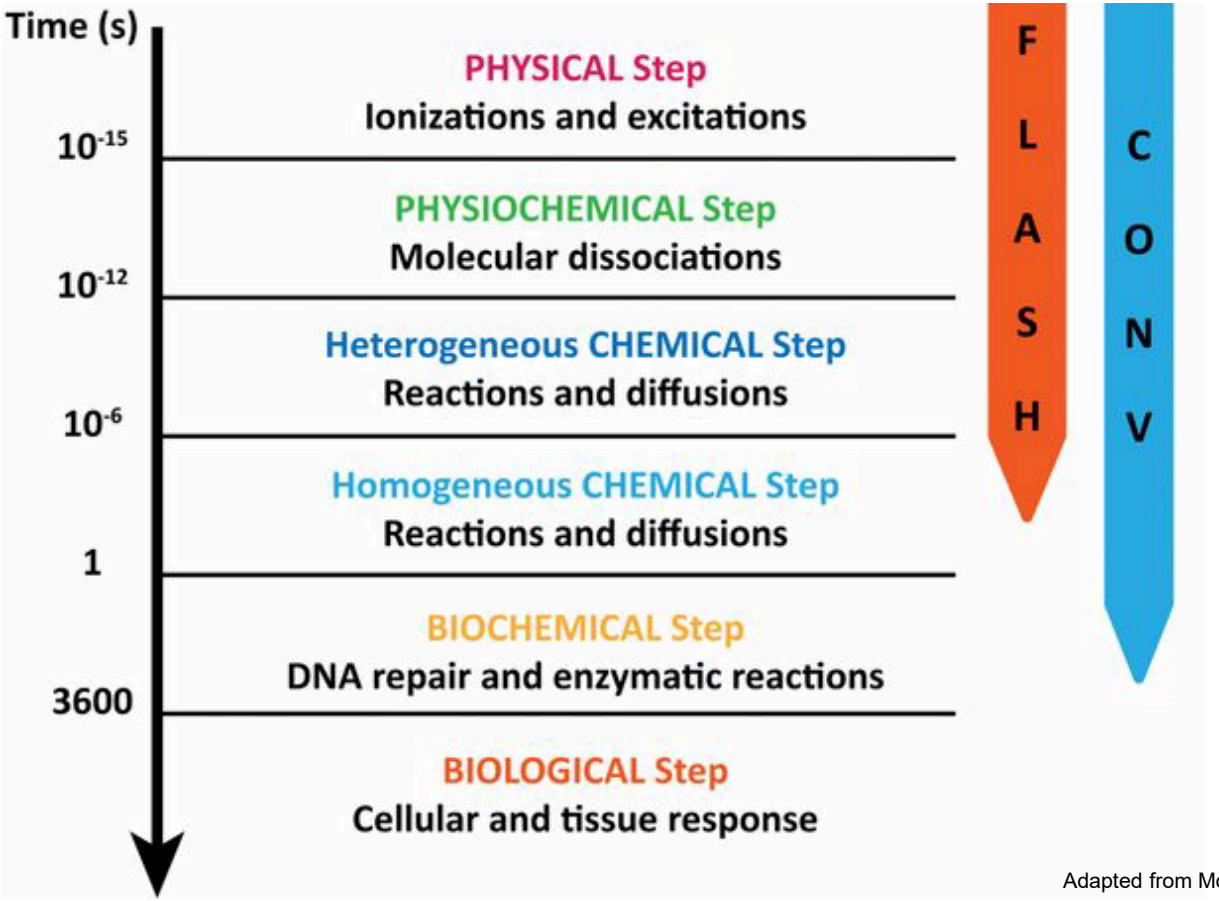
Mechanism(s) for the FLASH Sparing Effects



Distinctive molecular mechanisms still unknown.
How can FLASH-RT discriminate between normal and tumor tissue?
Are the intrinsic determinants at cellular or tissue level?

Limoli CL and Vozenin MC Annual Review of Cancer Biology 2023 7:1, 1-21

What are the mechanisms for the FLASH sparing effects?



Adapted from Montay-Gruel P. et al. PNAS 2019

The yields of different radical species and how they diffuse, react, recombine, and form new radical products during FLASH vs. CONV could influence the biological responses downstream.

In vitro studies

In vivo investigation are essential, but a pinch of reductionism is still needed:

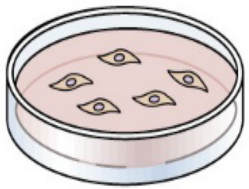
- Basic studies with the simplest conditions (normal cells and their cancer counterparts) are useful to investigate molecular mechanisms so that treatment protocols can be refined.
- Findings in normoxia can be used as benchmark.
- What is the major player in the redox metabolism is under debate.

Still unknown

- Molecular mechanisms leading to the FLASH effect
- Precise experimental conditions (i.e. beam parameters, oxygen level in cells) required to elicit the effect
- What type of radiation does elicit the effect?

Pre-clinical studies

Cells



3-D tissues

Small animals

At the Radiological Research Accelerator Facility (RARAF)

Proton FLASH

5 MeV protons, LET ~10 keV/μm
Dose rate up to 1 kGy/sec (limited by pulse length)
Geometry:
- < 1-cm diameter
- < 300-μm thick

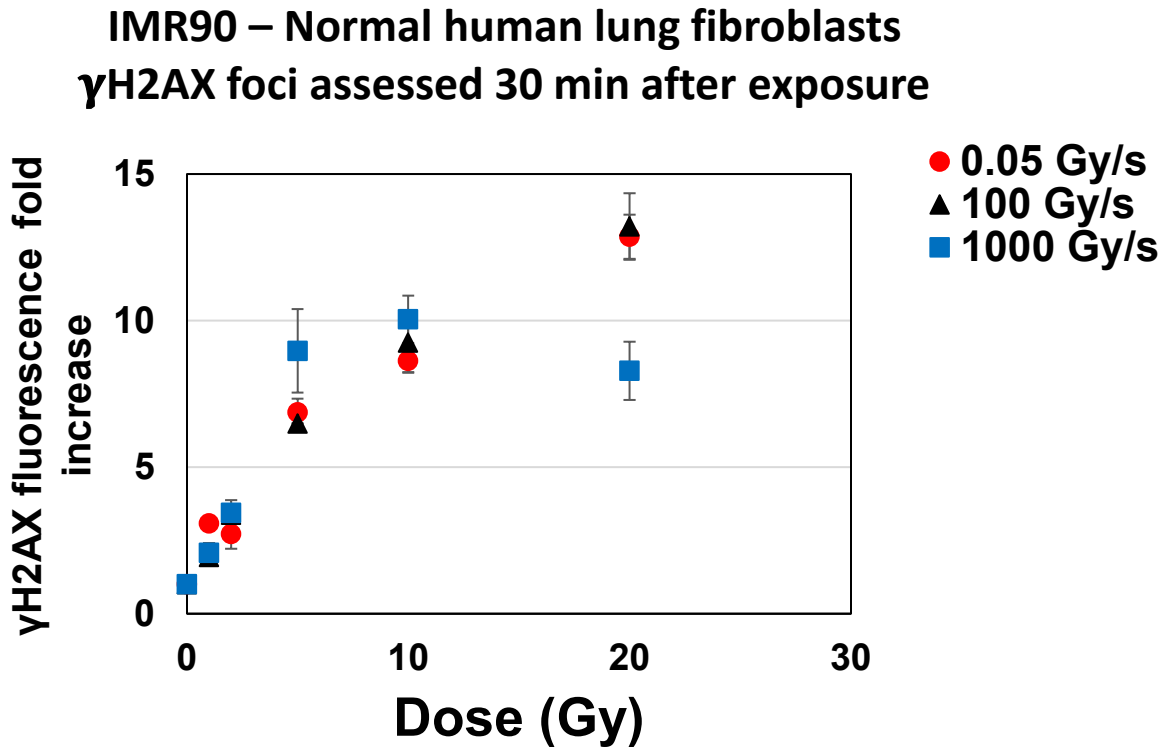
Electron FLASH

Cornell University donated a decommissioned Linac
6-9 MeV electrons
Dose rate ~200 Gy/sec
Will allow hemi-mouse irradiations:
- 4-cm field size
- >2-cm thick

Acute Effects

Clonogenic survival: The dose rate of 4.5 MeV protons does not affect survival of normal and cancer cells under normoxic conditions.

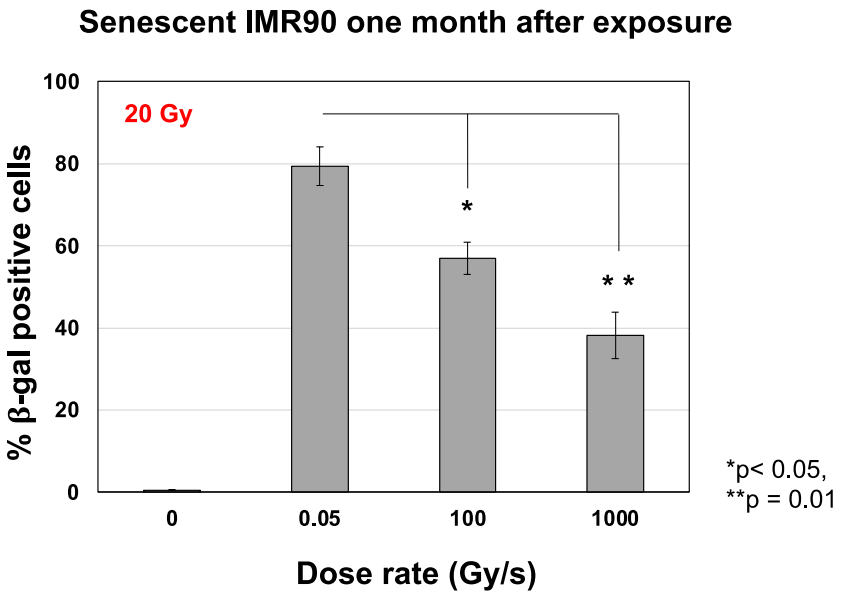
DNA damage: The effects might be more evident at relatively higher doses.



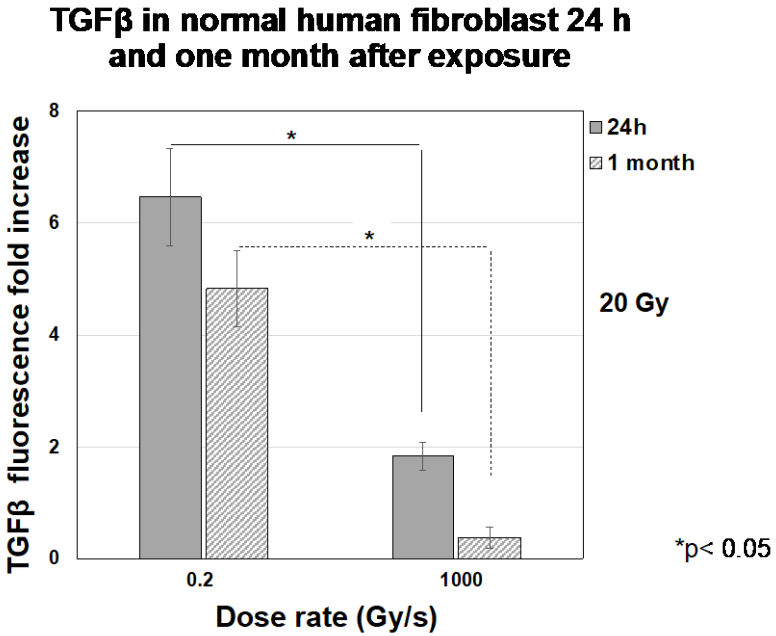
Buonanno M, et al. Radiother Oncol. 2019;139:51-5
Grilj V, et al. Radiation Research. 2020;194(6):646-55

Long-term effects: Radox metabolism

Normal cells, 20 Gy protons, 0.05, 100, 1000 Gy/s



Cell senescence - a potential mechanistic link between radiation-induced oxidative stress and prolonged tissue injury

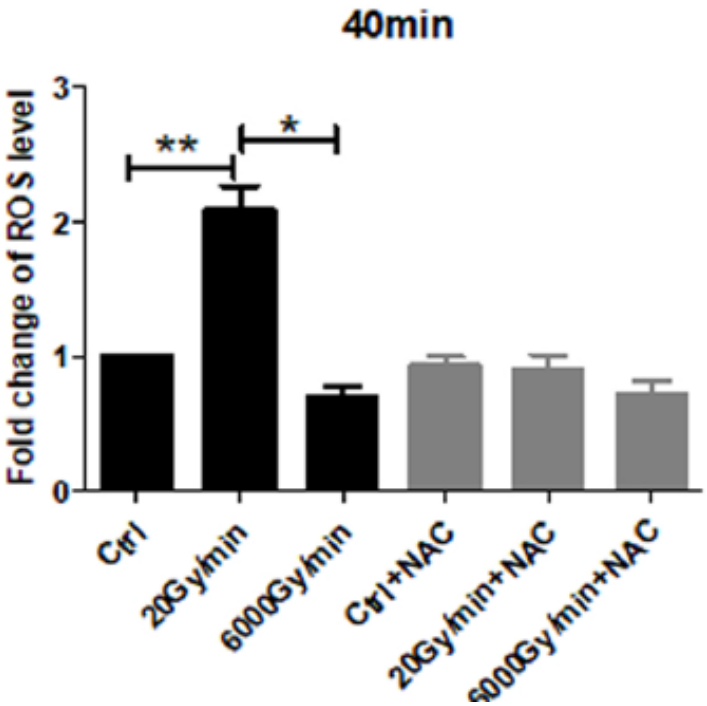


Senescent cells release pro-inflammatory molecules;
TGFβ1 is one of the major player in modulating such signals

Buonanno M, et al. Radiother Oncol. 2019;139:51-5

Redox metabolism: Mitochondria

Normal cells, 15 Gy protons, 20 vs. 6000 Gy/min

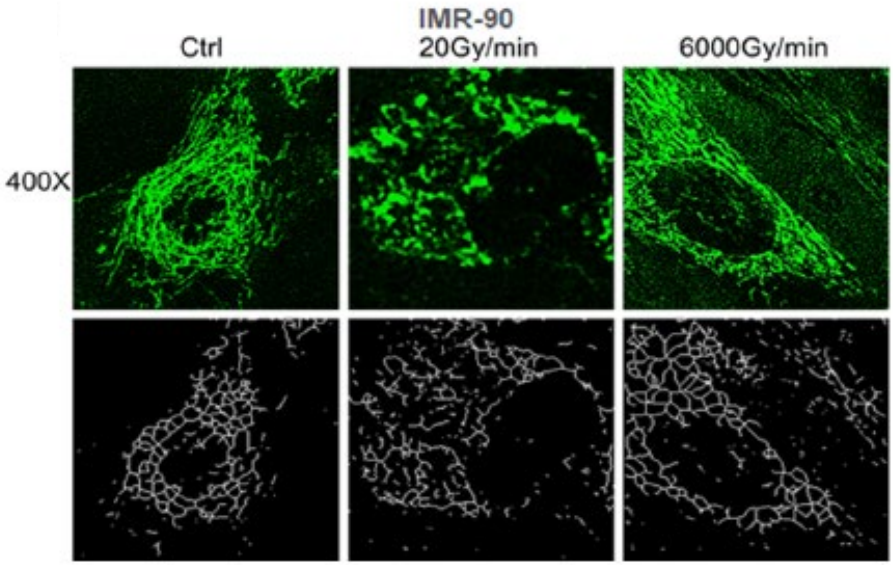


- CM-H_2DCFDA general ROS indicator
- N-Acetyl-L-cysteine (NAC) - ROS inhibitor

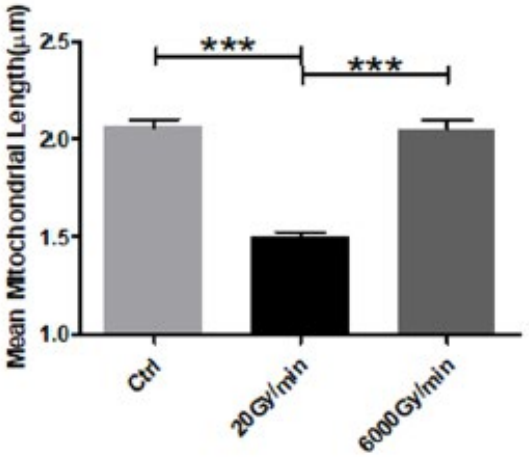
Guo Z, et al. Radiat Res. 2022;197(6):569-82

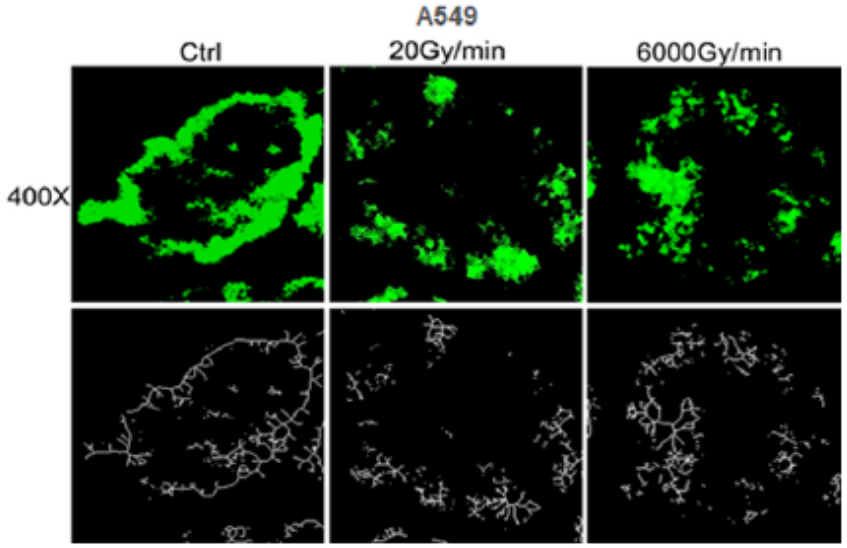
Mitochondria structure

15 Gy protons, 20 vs. 6000 Gy/min - Mitotracker green

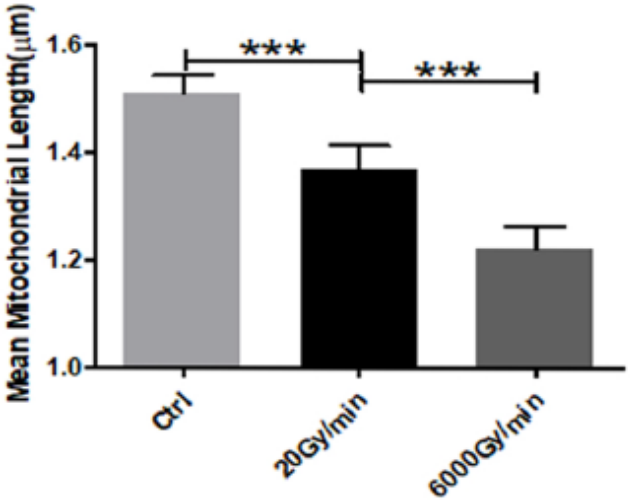


Normal cells



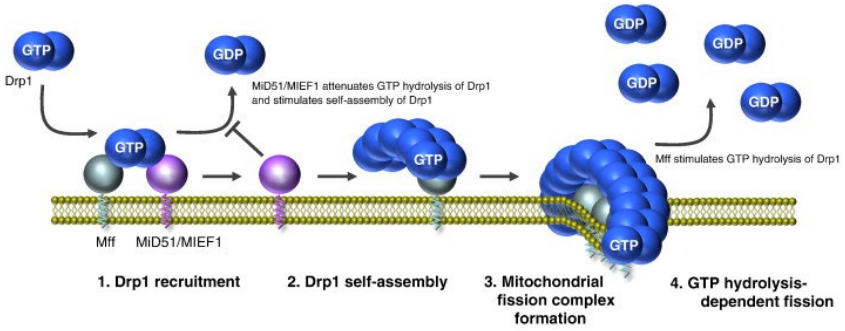


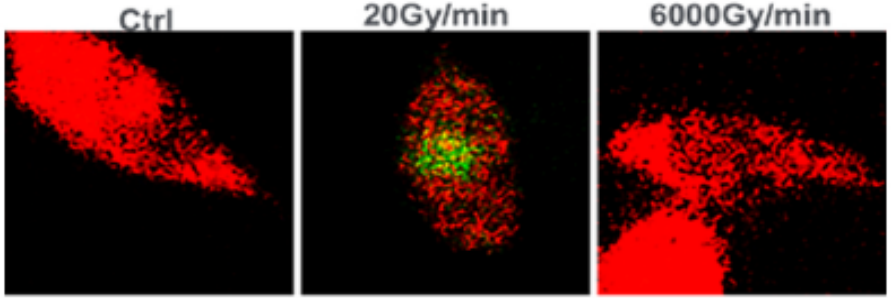
Cancer cells



Mitochondria dynamics – Drp1

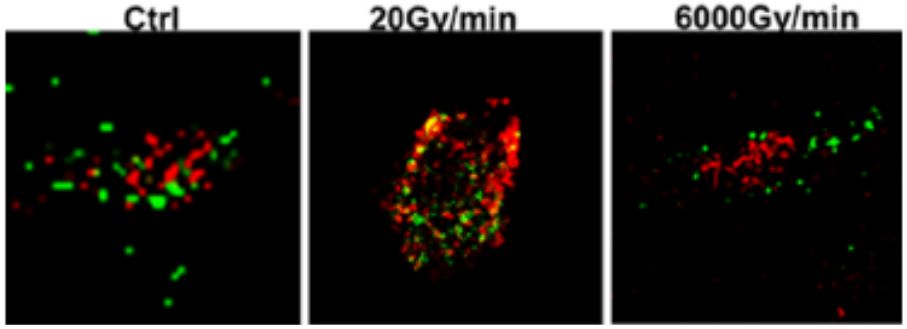
- Mitochondrial maintain their shape, size, and distribution through coordinated cycles of fission and fusion.
- The fission protein dynamin-related protein 1 (Drp1) is a GTPase that upon activation translocate from the cytoplasm to mitochondria.
- It stabilizes p53 and is required for p53 translocation to the mitochondria under oxidative stress.





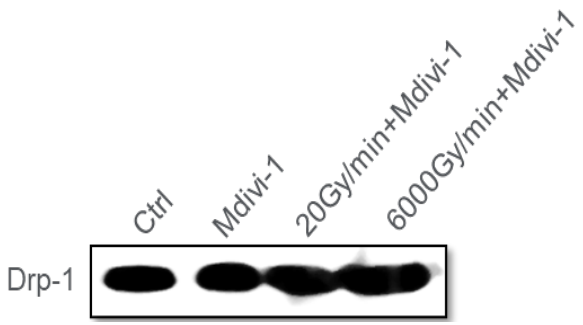
Normal cells, 15 Gy protons
20 vs 6000 Gy/min

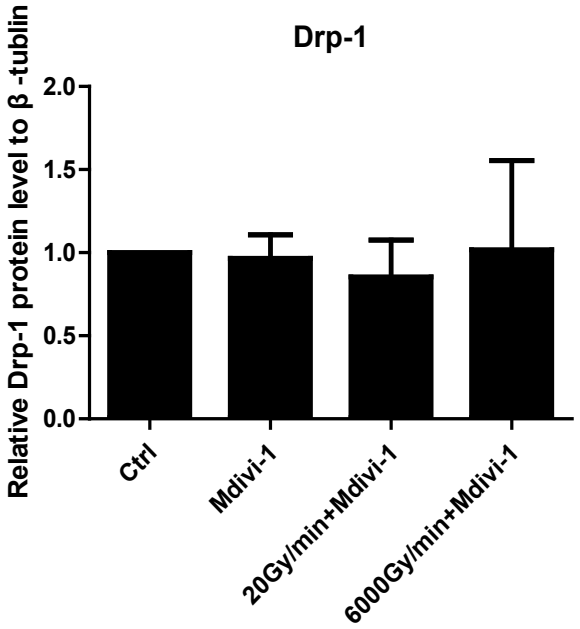
Expression of Drp1
Mitotracker red & Alexa488/anti-Drp1



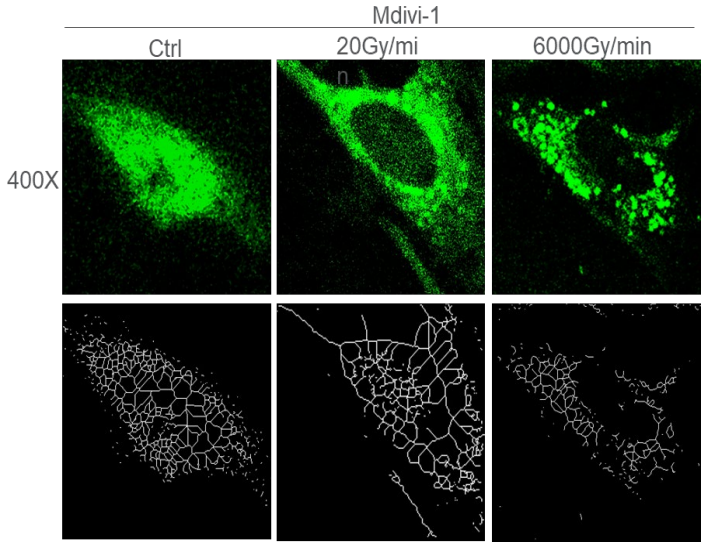
Drp-1/p53 co-localization
Alexa488/anti-Drp1 & Alexa555/anti-p53

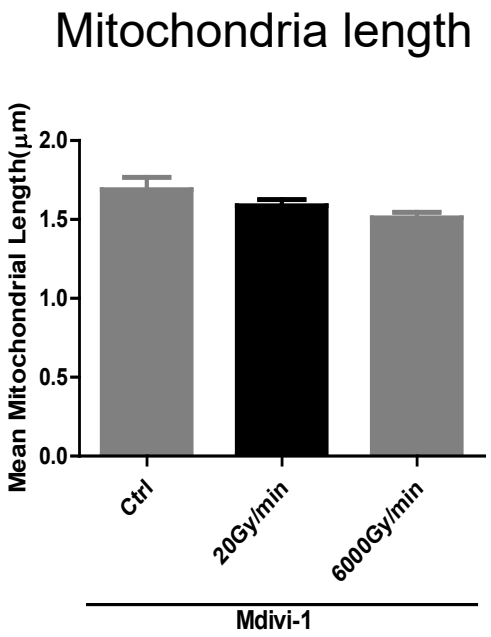
Inhibition of Drp1 by Mdivi-1





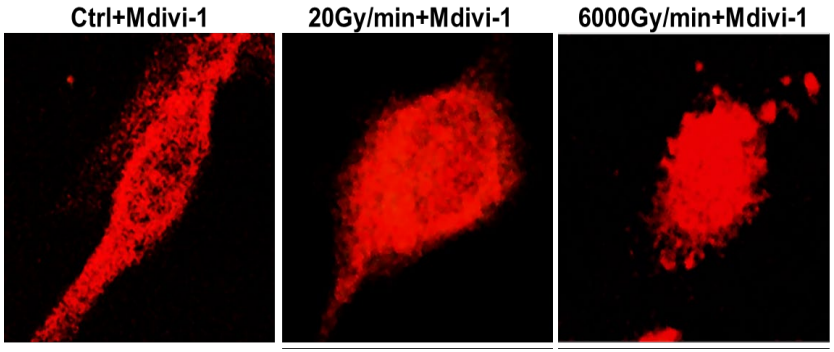
Inhibition of Drp1 by Mdivi-1





Expression of Drp1

Mitotracker red & Alexa488/anti-Drp1

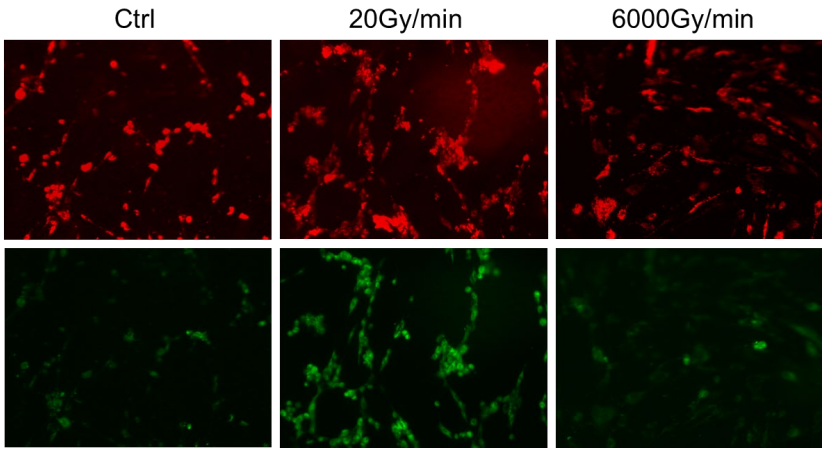


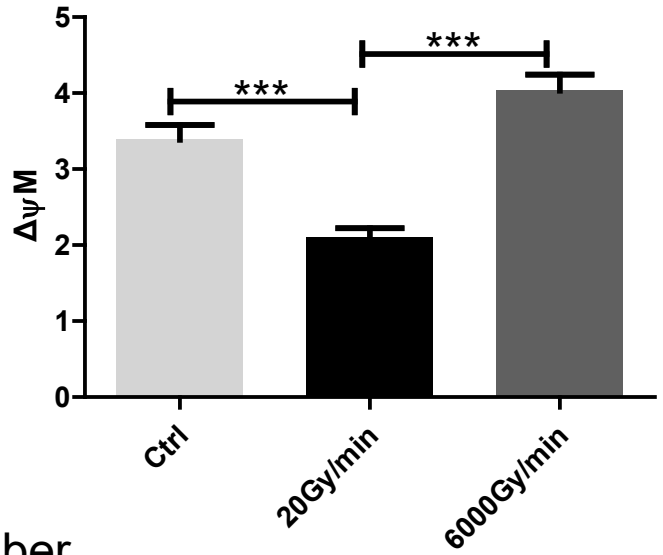
Mitochondria functions 1

Mitochondria membrane potential (JC-1)

J-aggregates in mitochondrial matrix –> red fluorescence

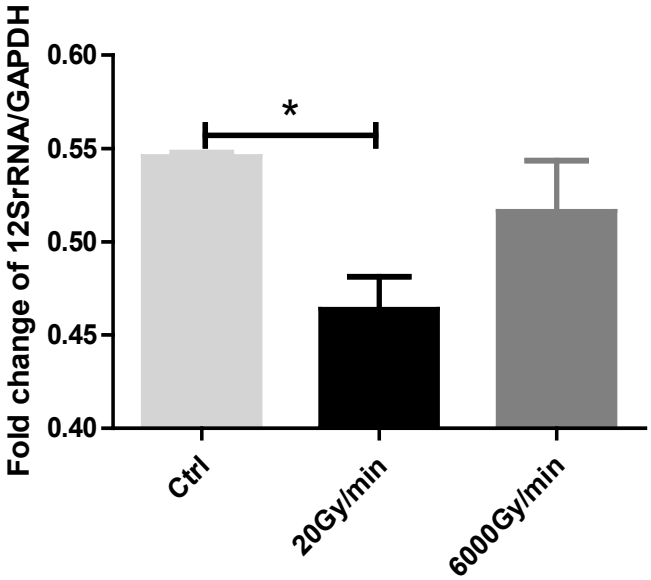
JC-1 monomers do not aggregate in the matrix and fluoresce green



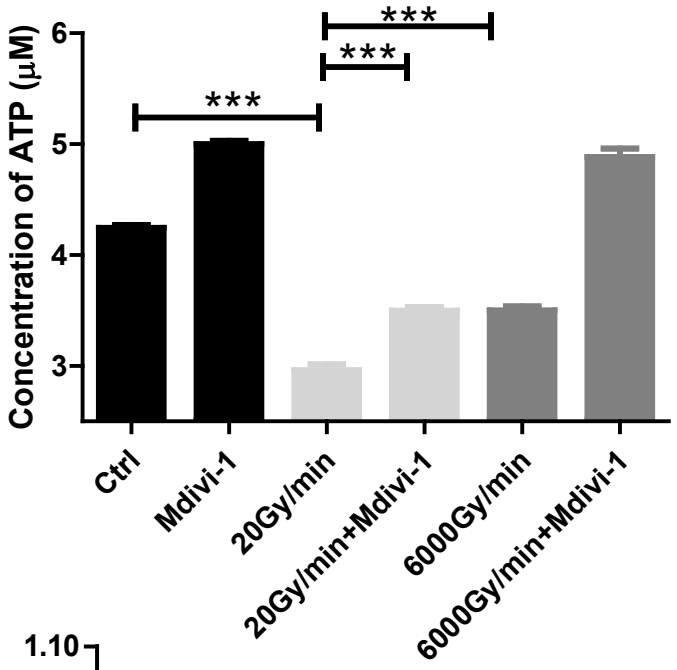


Mitochondria copy number

(RT-PCR ratio mtDNA/nDNA or 12SrRNA/18SrRNA)



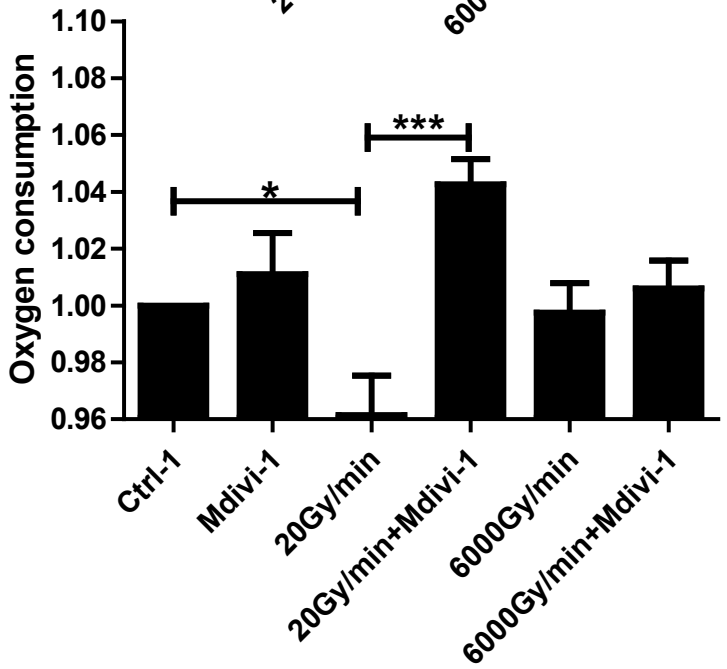
Mitochondria functions 2



Mdivi-1, inhibitor of Drp1

Cellular ATP

Luciferase enzyme/luciferin luminescence

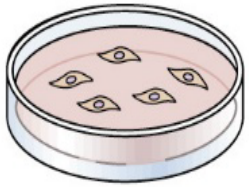


Extracellular oxygen consumption

Fluorescence ratio 380/650 nm

Pre-clinical studies

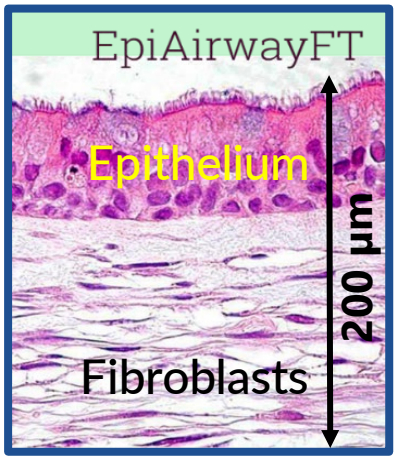
Cells

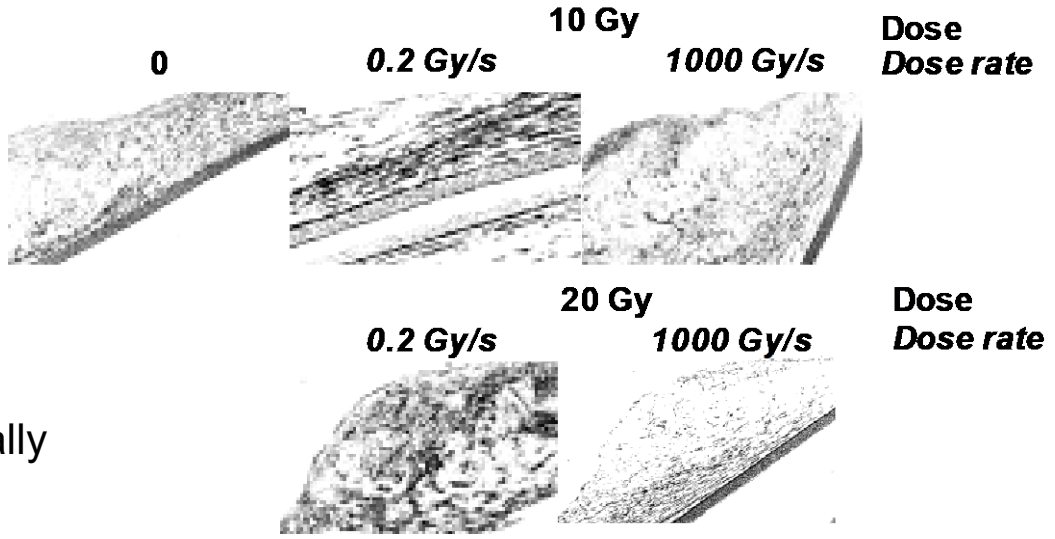


3-D tissues

Small animals

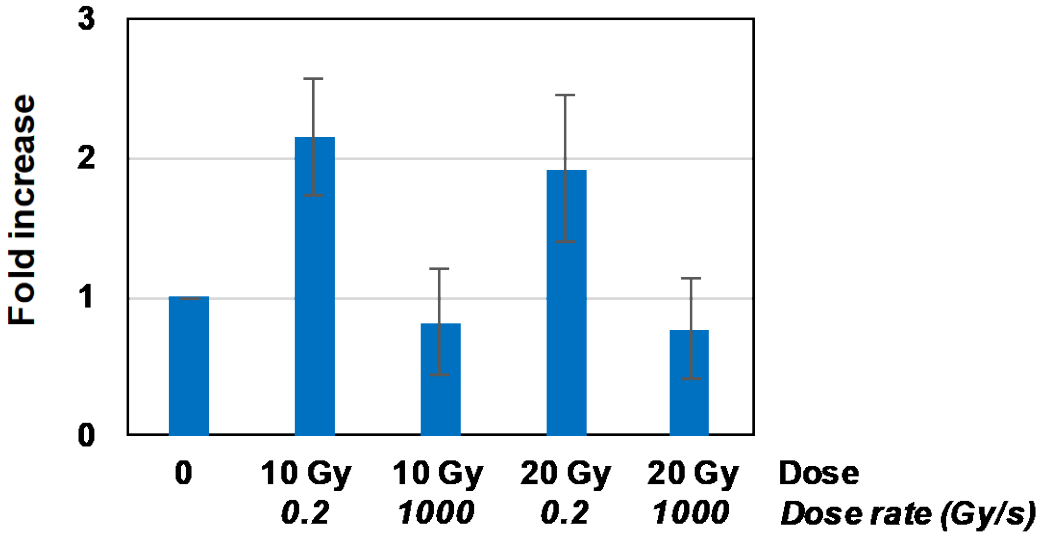
Redox metabolism: Expression of carbonic anhydrase IX in 3-D tissues





- Carbonic anhydrase IX is membrane-bound enzyme generally associated with hypoxia in many tumor cell lines.
- In normal tissues, it is involved in maintaining optimal pH for cell survival and it is directly linked to radiation-induced activation of TGFβ.

An increase in dose rate resulted in the reduction of CAIX expression 3 months after irradiation



In cells exposed to protons under ambient oxygen tension, CONV, but not FLASH:

Inflammatory responses

- Induced senescence
- TGFb expression
- ROS
- CAIX expression 3 months after irradiation

Mitochondria

- Damage to structure (shape, size, copy number)
- Damage to functions (ATP release, MMP, oxygen consumption)
- Increased Drp1 expression/translocation to mitochondria, but not the phosphorylated form

Cell death

- *Induced necrosis (as opposed to autophagy and apoptosis)*

Compared to CONV-, proton FLASH-RT seems to preserve redox functions in normal cells

Probable Mechanism(s) for the Sparing Effects ?

Transient hypoxia?

Tumors

They may not be able to cope with the increase in FLASH-induced free radicals;

Due to more iron, Fenton-based reactions may sustain free radical chain reactions.

Healthy tissue

Limited cytokine activation including TGFβ and less inflammatory reaction.

However:

No oxygen depletion (Vozenin team showed it with Fricke solution and OxyLite monitor in aqueous solutions & Oxyphor 2P probe in vitro and in vivo). It requires very high doses (e.g., 100 Gy).

Difference in radiolysis yields TBD

Reduced DNA damage and senescent cells

Chromatin remodeling mediated by poly (ADP-ribose) polymerase

Stem cell protection

Reduction of fraction of circulating blood cell irradiated and sparing of the immune system (under debate)

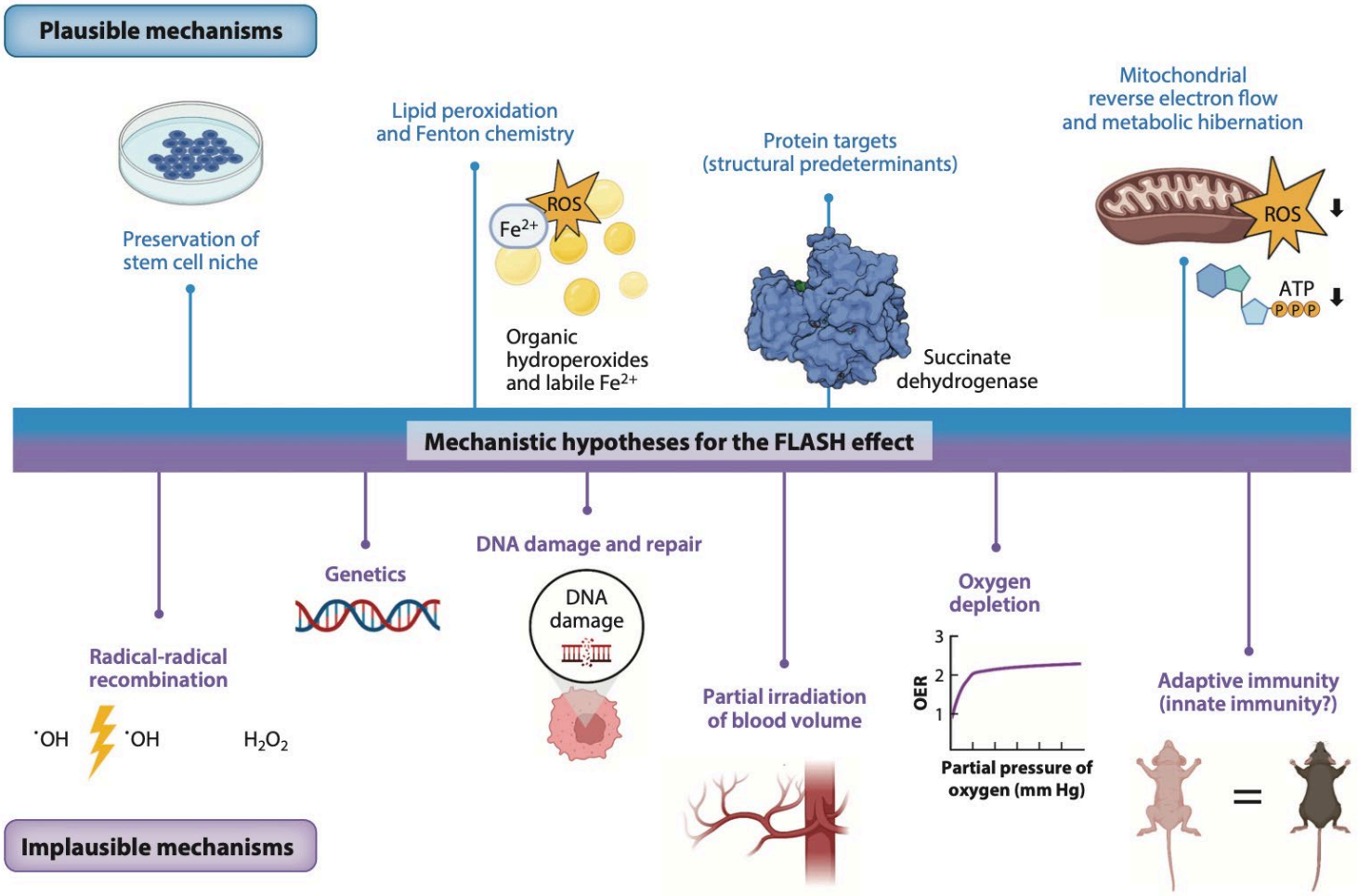
Proteins, lipids

Metabolism (low metabolic activity/hibernation)

Mitochondria

…

Mechanism(s) for the FLASH Sparing Effects



Distinctive molecular mechanisms still unknown

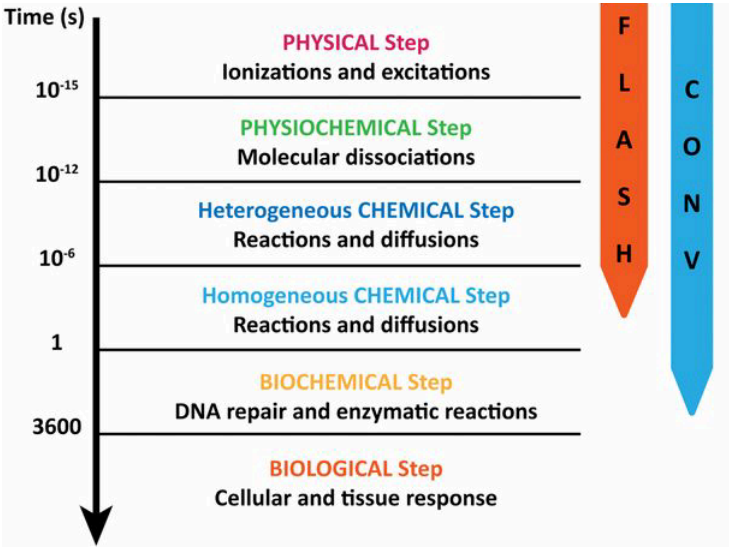
Pre-clinical studies are needed to inform clinical approaches

What's next in our lab

Grant application in collaboration with the Université de Sherbrooke, Canada

Mechanistic studies of the chemical and biological effects of ultra-high dose-rate proton radiation

1. Simulation & modeling: Jean-Paul Jay-Gerin
2. Chemical changes in DNA: Richard Wagner
3. Biological experiments: Manuela Buonanno, Guy Garty, & Ed Azzam



Ongoing collaboration with Weil Cornell University

Mechanistic studies of the chemical and biological effects of ultra-high dose-rate radiation using patient derived organoids

- Normal and breast tissue organoids derived from the same patient studied separately or as a single organoid containing both tissue types to better mimic the real tumor/normal tissue microenvironment.

Thank you for your attention

Thanks to

- NCI 1UO1CA236554
- Pilot grant from the Department of Radiation Oncology Columbia University Irving Medical Center (CUIMC)
- Dr. Guy Garty and the physicists at RARAF

- Radiation Oncology departments at CUIMC and Weil Cornell Medicine