

# The FLASH effect: when extreme dose rates reshape radiobiology

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UNIVERSITÀ DI PISA



## Congresso del Dipartimento di Fisica

**Pisa 27 maggio 2026**

AULA MAGNA F.LLI PONTECORVO

Area Pontecorvo, Edificio E

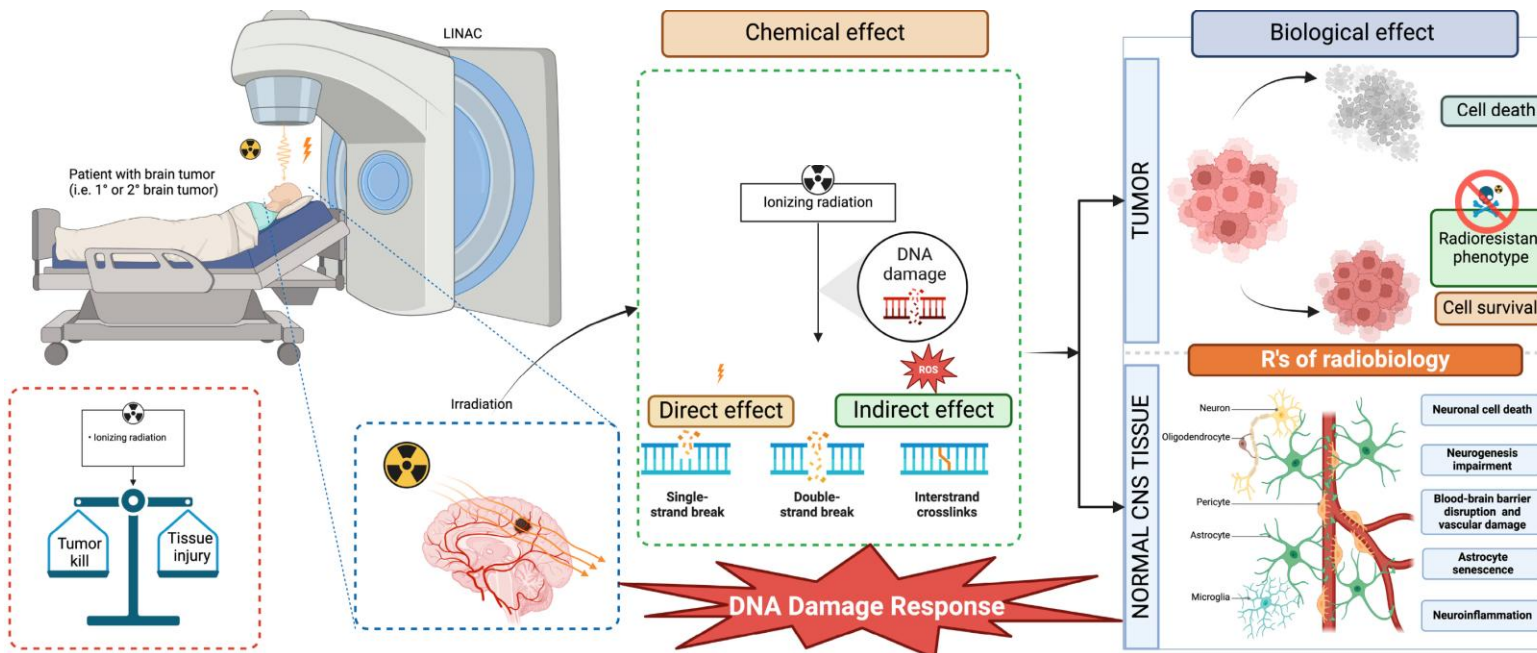
Largo Bruno Pontecorvo, 3 - Pisa

1. Basics of conventional radiotherapy: why do we fractionate in the clinics?
2. FLASH-RT: what is it? And why does it seem such a big deal?
3. How is our Department contributing to the field of FLASH-RT research?

# Conventional external beam radiotherapy

- Local treatment of tumors with beams of ionizing radiation
- **Particles:** photons (>90%), electrons, protons, carbon ions, ...
- Ionizing radiation causes irreparable DNA damage, inducing cell death

- Usually combined with **surgery** and/or **chemotherapy**
- **Fractionation:** a treatment **schedule** (Mon-Fri), for a few weeks
- The keyword is **selectivity**, i.e., to simultaneously
  1. **Maximize** effects on **tumors**
  2. **Minimize** damage to surrounding **normal tissue**
- Selectivity depends on
  - Our ability to **conform** the delivery (nr of beams, angles, energy, etc)
  - the **biological** response to irradiation

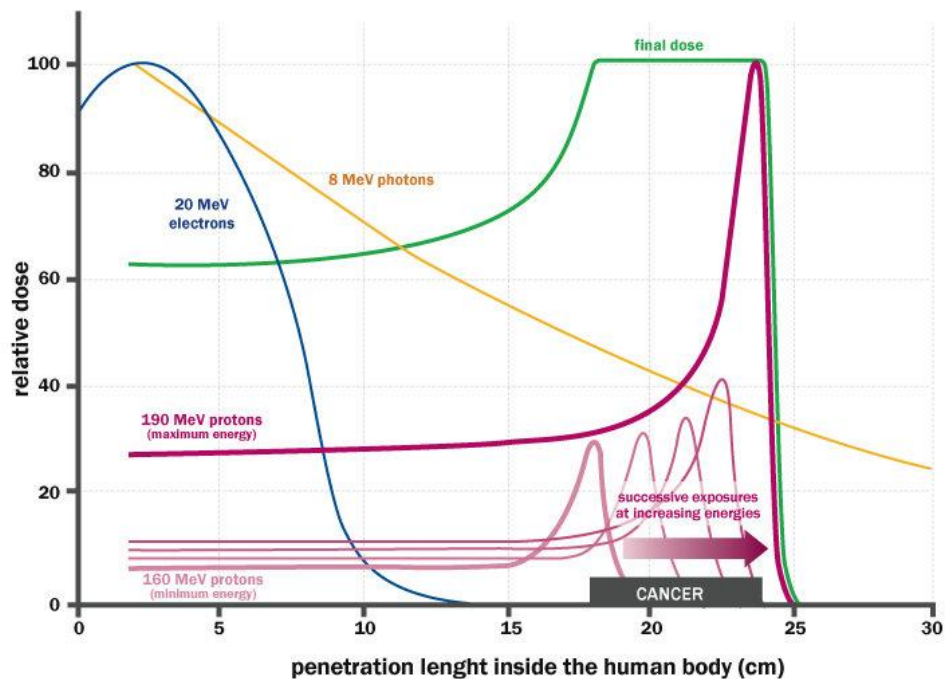


<https://www.mdpi.com/2673-4087/5/4/34>

# Conventional external beam radiotherapy

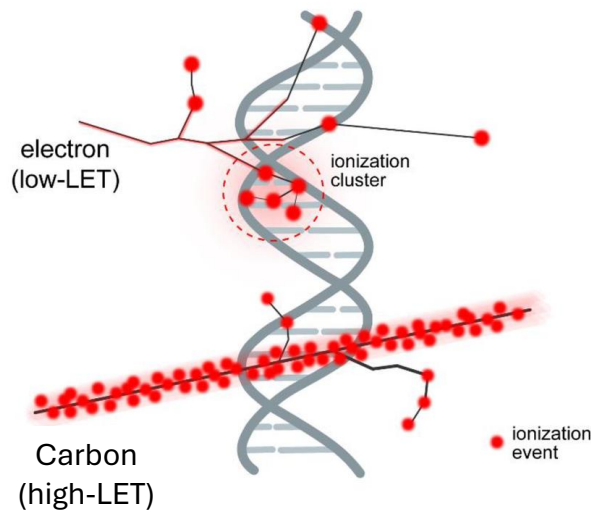
## Physical dose

$$D = \frac{dE}{dm} \left[ Gy = \frac{J}{kg} \right]$$

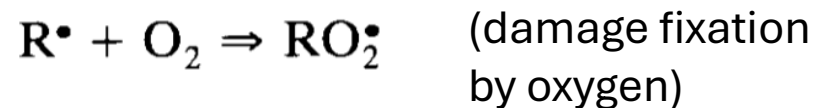
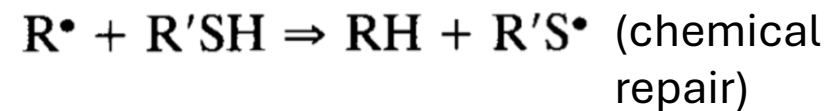


The **biological** response is modulated by several factors, including:

**LET**  
(ionization density)



**Oxygen**



## TrueBeam (photons)

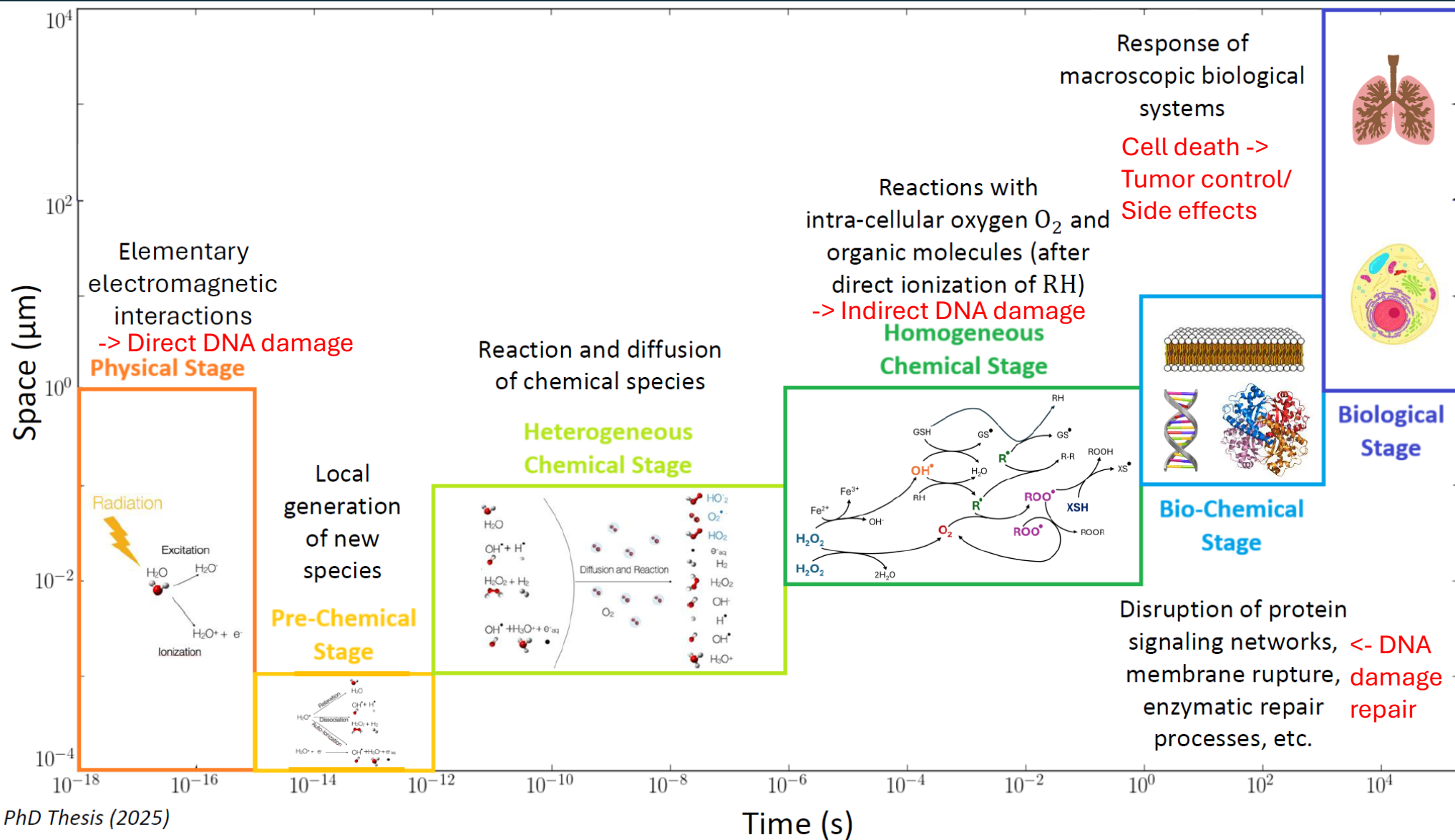


## CNAO (protons and C ions)



➔ Much more demanding!

# Radiobiological damage in a nutshell



M. Battestini, PhD Thesis (2025)

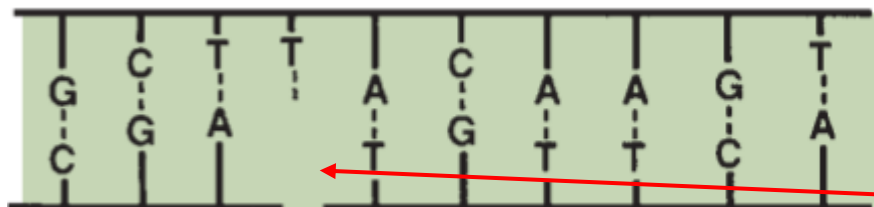
Esther Ciarrocchi - May 27th, 2026

# Why do we fractionate in the clinics?

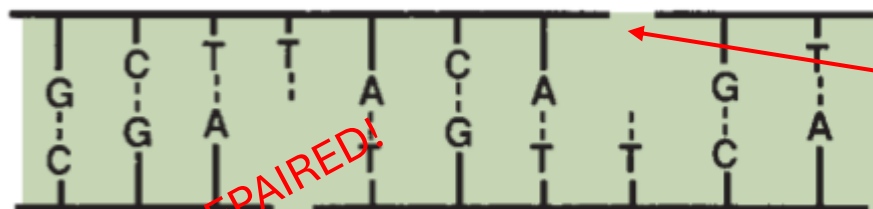
## The 4 R's of radiobiology

- ✓ **Repair.** There is cellular recovery (repair of DNA damage) during the few hours after exposure, especially in normal cells

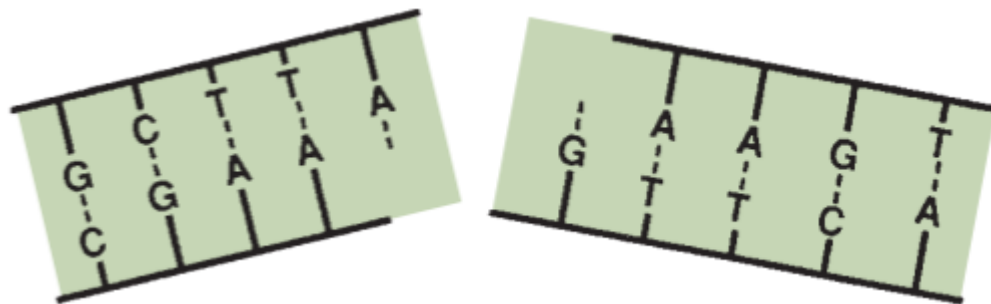
DNA molecule



Fraction #1 -> 1 Single Strand Break (SSB): **non-lethal**

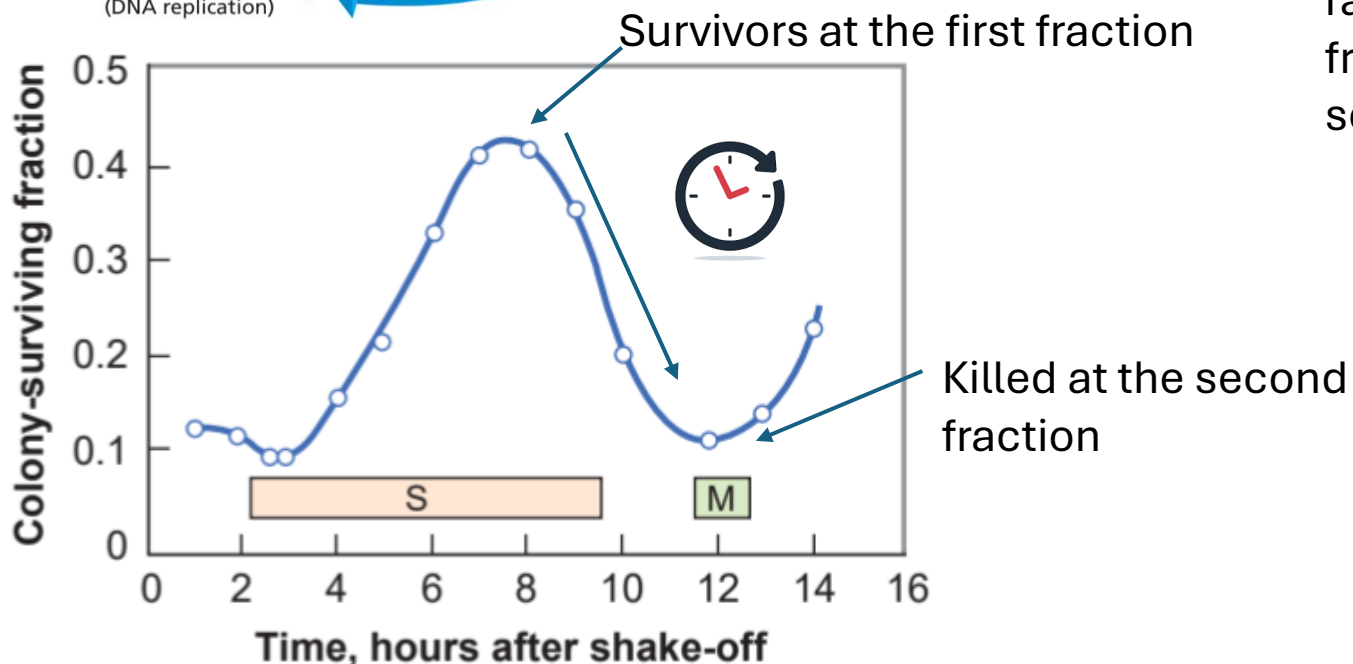
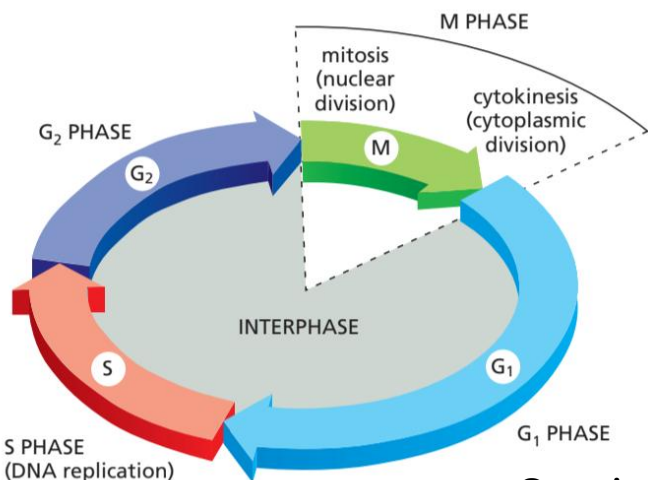


Fraction #2 -> 1 SSB



Both in one fraction -> 2 nearby SSBs = 1 **DSB**  
-> Potentially **lethal** damage

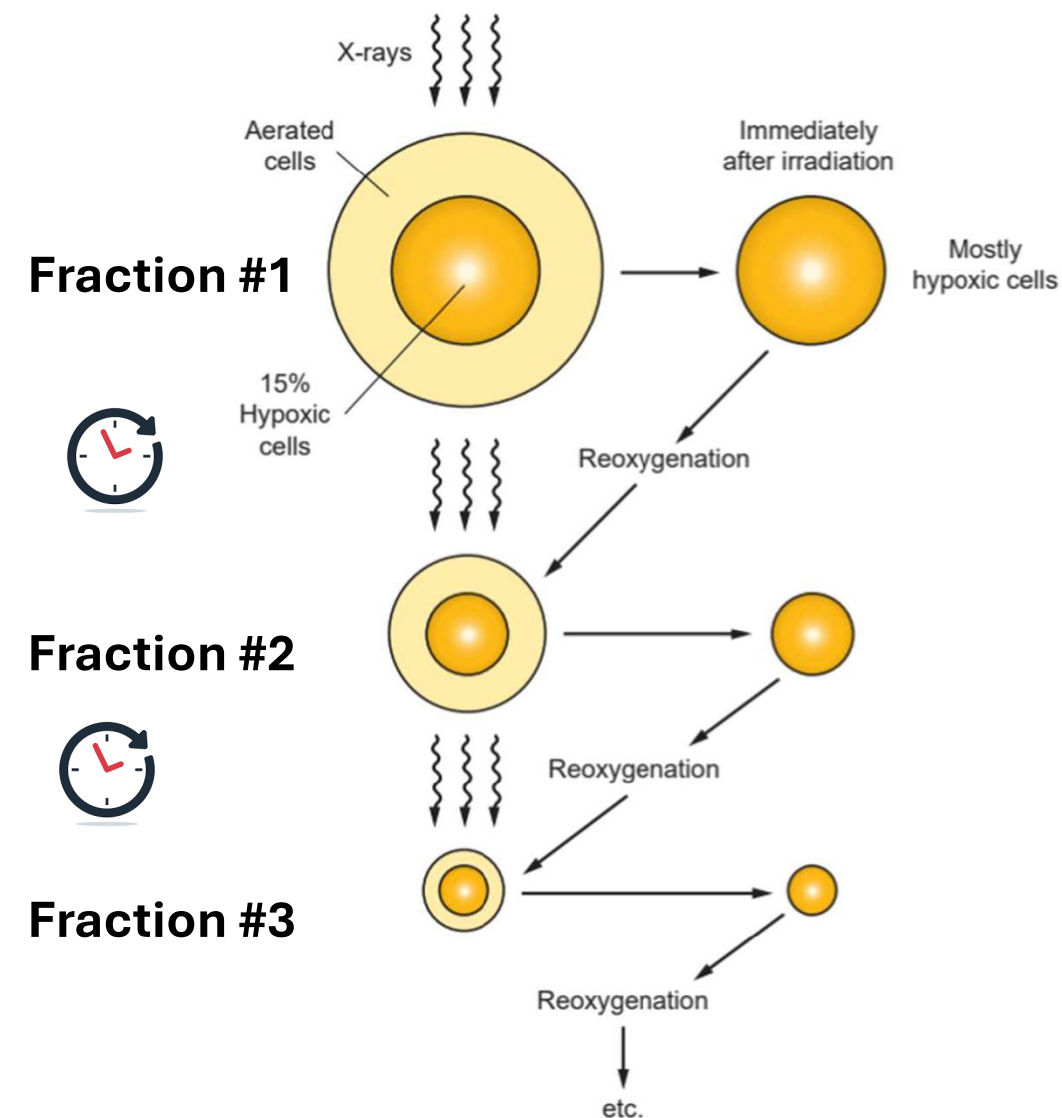
# Why do we fractionate in the clinics?



## The 4 R's of radiobiology

- ✓ **Repair.** There is cellular recovery (repair of DNA damage) during the few hours after exposure, especially in normal cells
- ✓ **Reassortment.** Tumor cells that were in a radioresistant phase of the cell cycle at the first fraction, can be in a radiosensitive phase at the second fraction

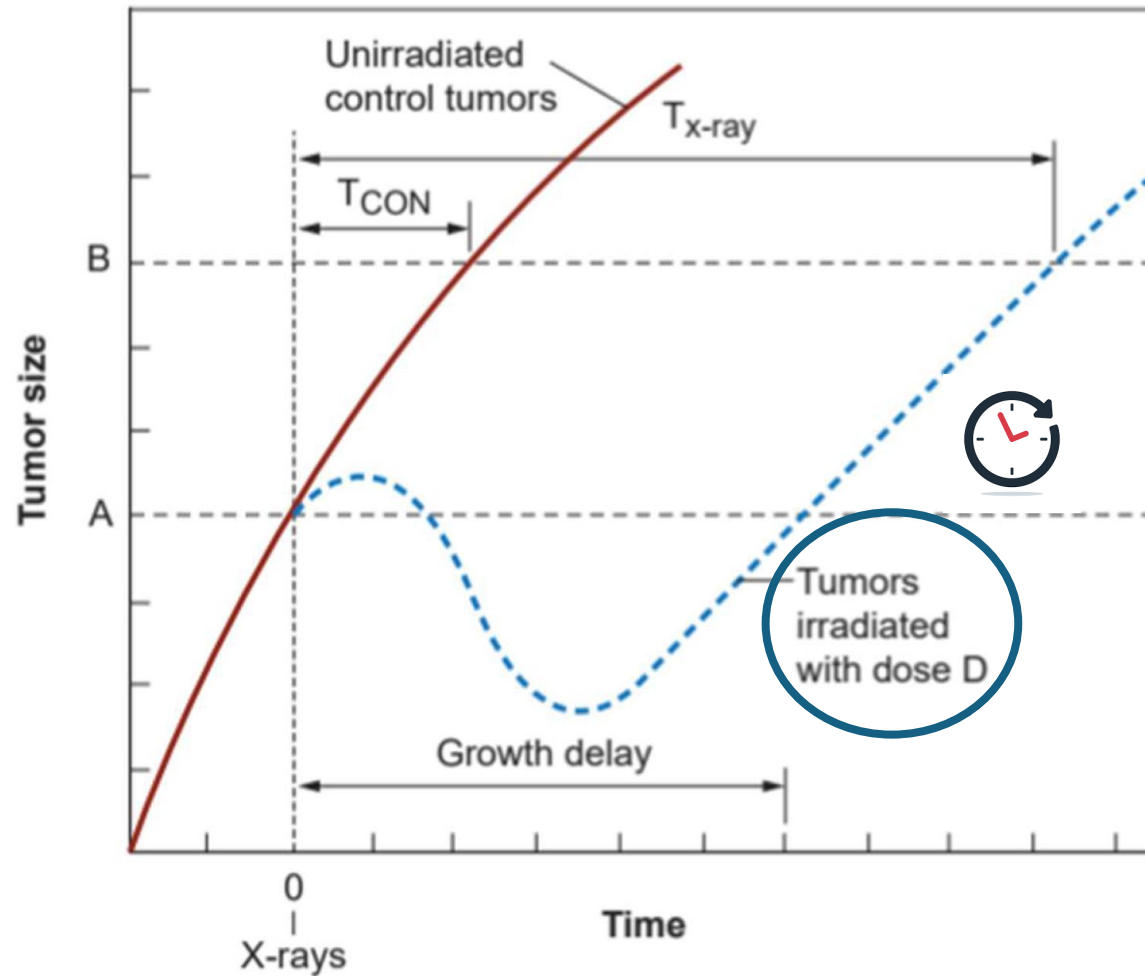
# Why do we fractionate in the clinics?



## The 4 R's of radiobiology

- ✓ **Repair.** There is cellular recovery (repair of DNA damage) during the few hours after exposure, especially in normal cells
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- ✓ **Reoxygenation.** Central, hypoxic tumor cells reoxygenate as the tumor shrinks

# Why do we fractionate in the clinics?



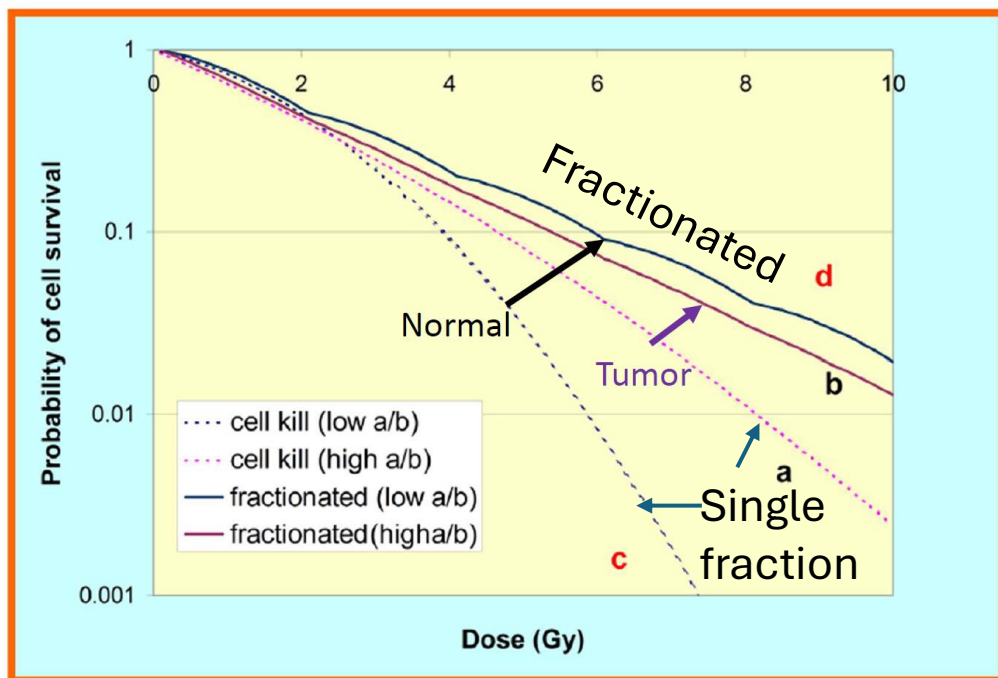
## The 4 R's of radiobiology

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- ✓ **Reoxygenation.** Central, hypoxic tumor cells reoxygenate as the tumor shrinks
- ✗ **Repopulation.** Survived tumor cells repopulate in between fractions

# Why do we fractionate in the clinics?



## The effect of fractionation



[www-pub.iaea.org/MTCD/publications/PDF/TCS-55\\_CD/PDF/presentations/03%20Altered%20fractionation.pdf](http://www-pub.iaea.org/MTCD/publications/PDF/TCS-55_CD/PDF/presentations/03%20Altered%20fractionation.pdf)

Single fraction:  $S = e^{-(\alpha D + \beta D^2)}$

Fractionation ( $D = nd$ )  $\rightarrow S = \left( e^{-\alpha d - \beta d^2} \right)^n = e^{-(\alpha D + \beta n d D)}$

## The 4 R's of radiobiology

- ✓ **Repair.** There is cellular recovery (repair of DNA damage) during the few hours after exposure, especially in normal cells
  - ✓ **Reassortment.** Tumor cells that were in a radioresistant phase of the cell cycle at the first fraction, can be in a radiosensitive phase at the second fraction
  - ✓ **Reoxygenation.** Central, hypoxic tumor cells reoxygenate as the tumor shrinks
  - ✗ **Repopulation.** Survived tumor cells repopulate in between fractions
- <- Normal tissues tend to benefit more than tumor cells from fractionation (because of the lower a/b ratio)



# Why do we fractionate in the clinics?

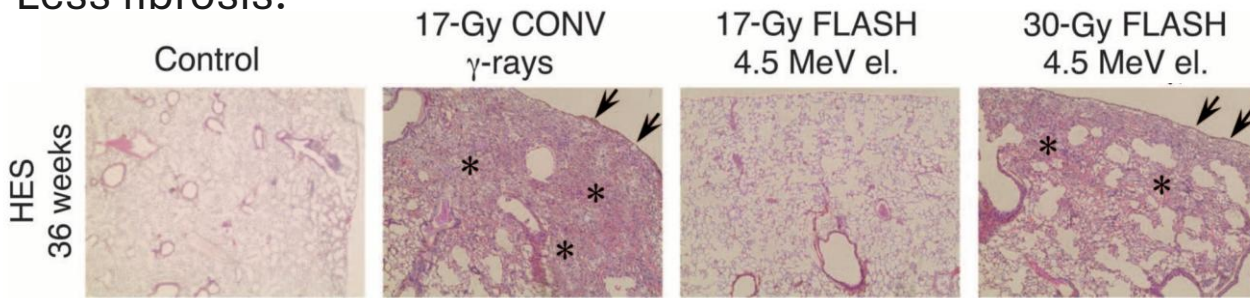
Decades of radiobiology experiments and clinical practice have consistently demonstrated that fractionation is the best approach to achieve selectivity...



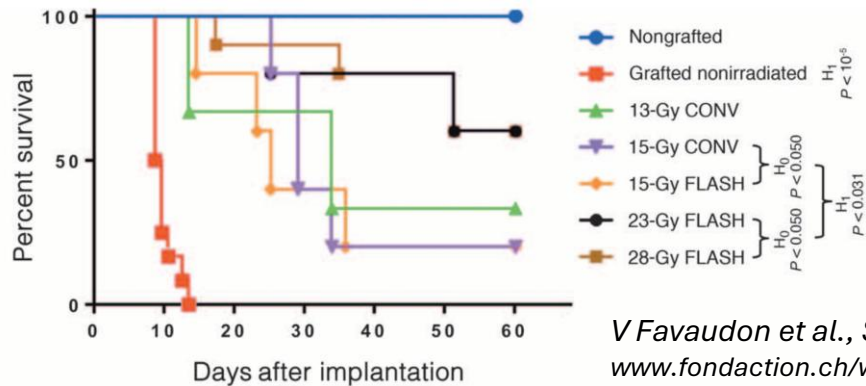
Conventional fractionation 35 fractions, 2 Gy M-F, Total 66-70 Gy, 7 weeks

# ... Until the FLASH effect

Less fibrosis:



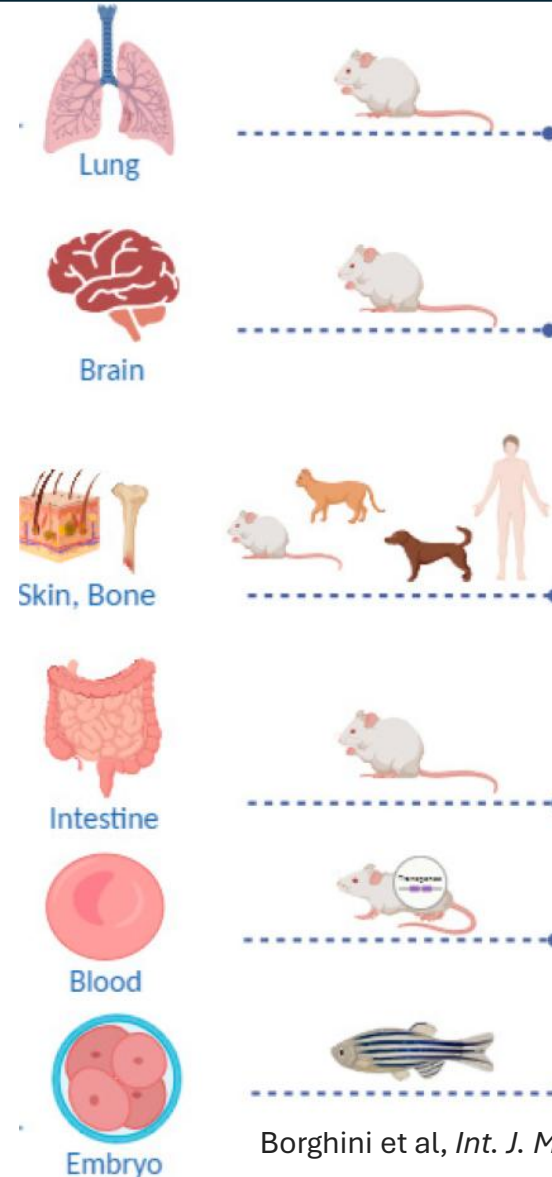
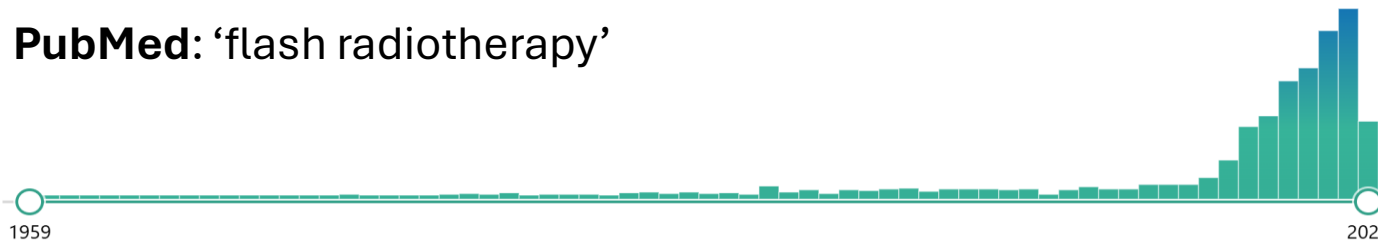
Same tumor control:



V Favaudon et al., *Sci. Transl. Med.* **6**,245ra93-245ra93(2014)  
[www.fondaction.ch/wp-content/uploads/2014/10/245ra93.full\\_.pdf](http://www.fondaction.ch/wp-content/uploads/2014/10/245ra93.full_.pdf)

RESULTS BY YEAR 1,208 results Page 1 of 25

PubMed: 'flash radiotherapy'



Borghini et al, *Int. J. Mol. Sci.* **2024**, 25, 2546.

# The FLASH effect

## PHYSICS

Ultra-high dose rates (UHDR)



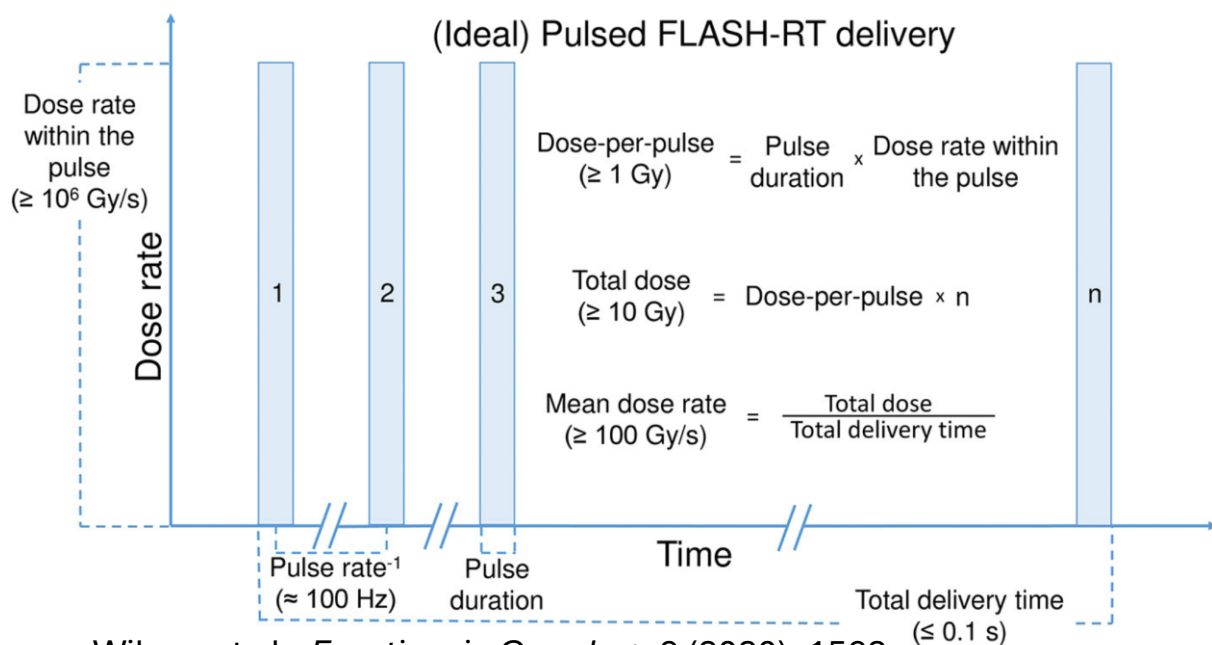
## BIOLOGY

Same tumor control + Less normal tissue toxicity

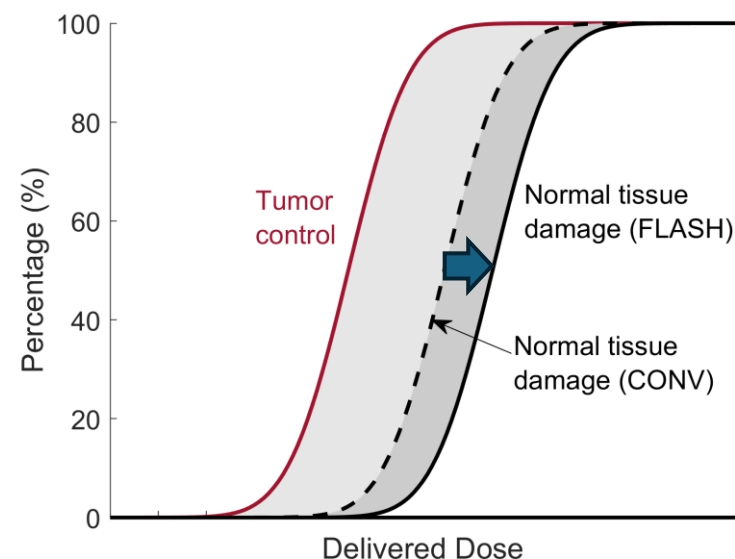
What does UHDR mean?

1. Dose rates: X(100-1000)
2. Times: /(100-1000)
3. One-few fractions vs 30 fractions

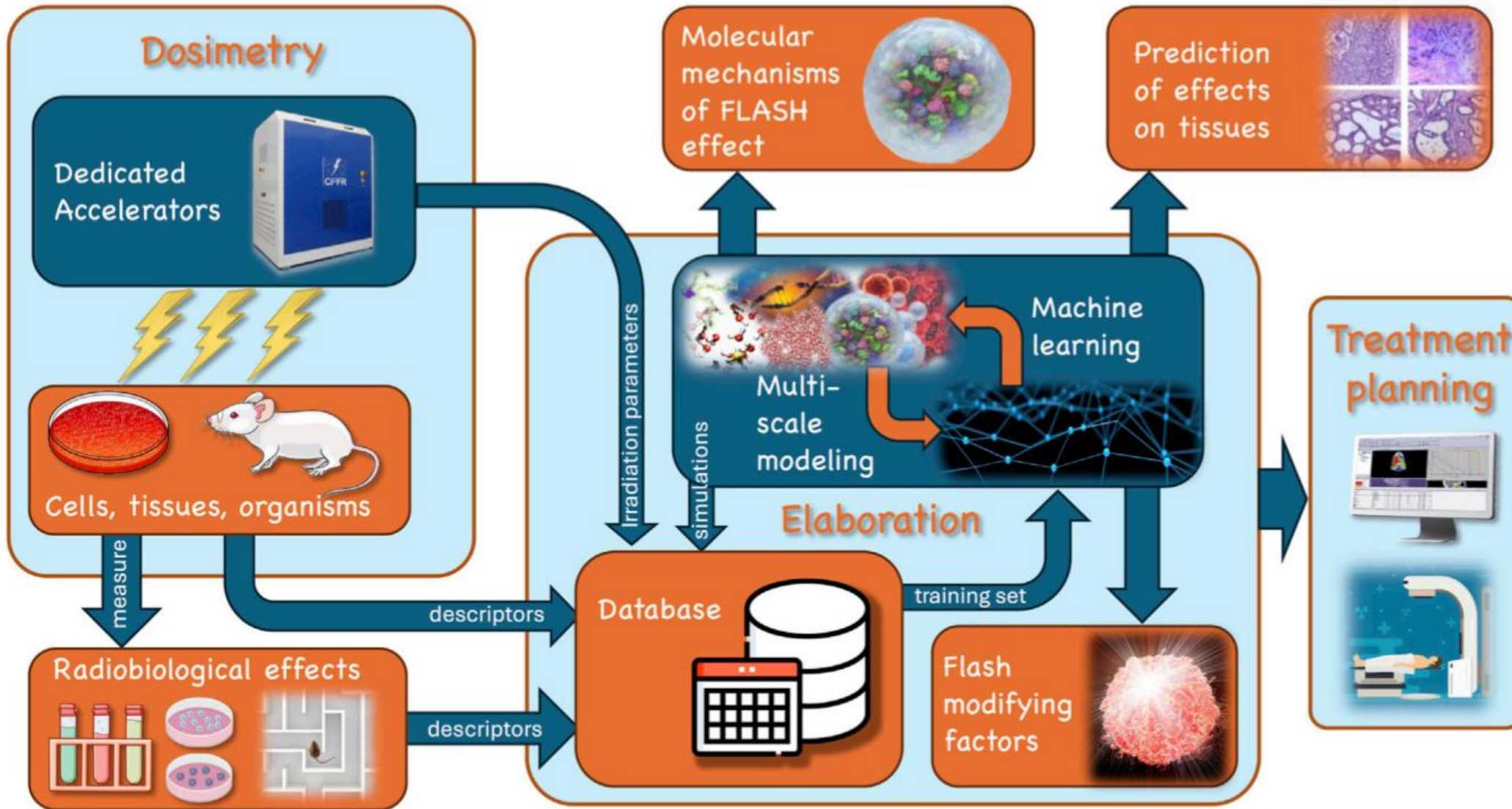
- It can be exploited in two ways
  1. To **increase** the dose to **radioresistant** tumors with the **same damage** to normal tissue
  2. To **reduce** normal tissue **toxicity** and still have the **same effect** on tumors



Wilson et al., *Frontiers in Oncology* 9 (2020): 1563.

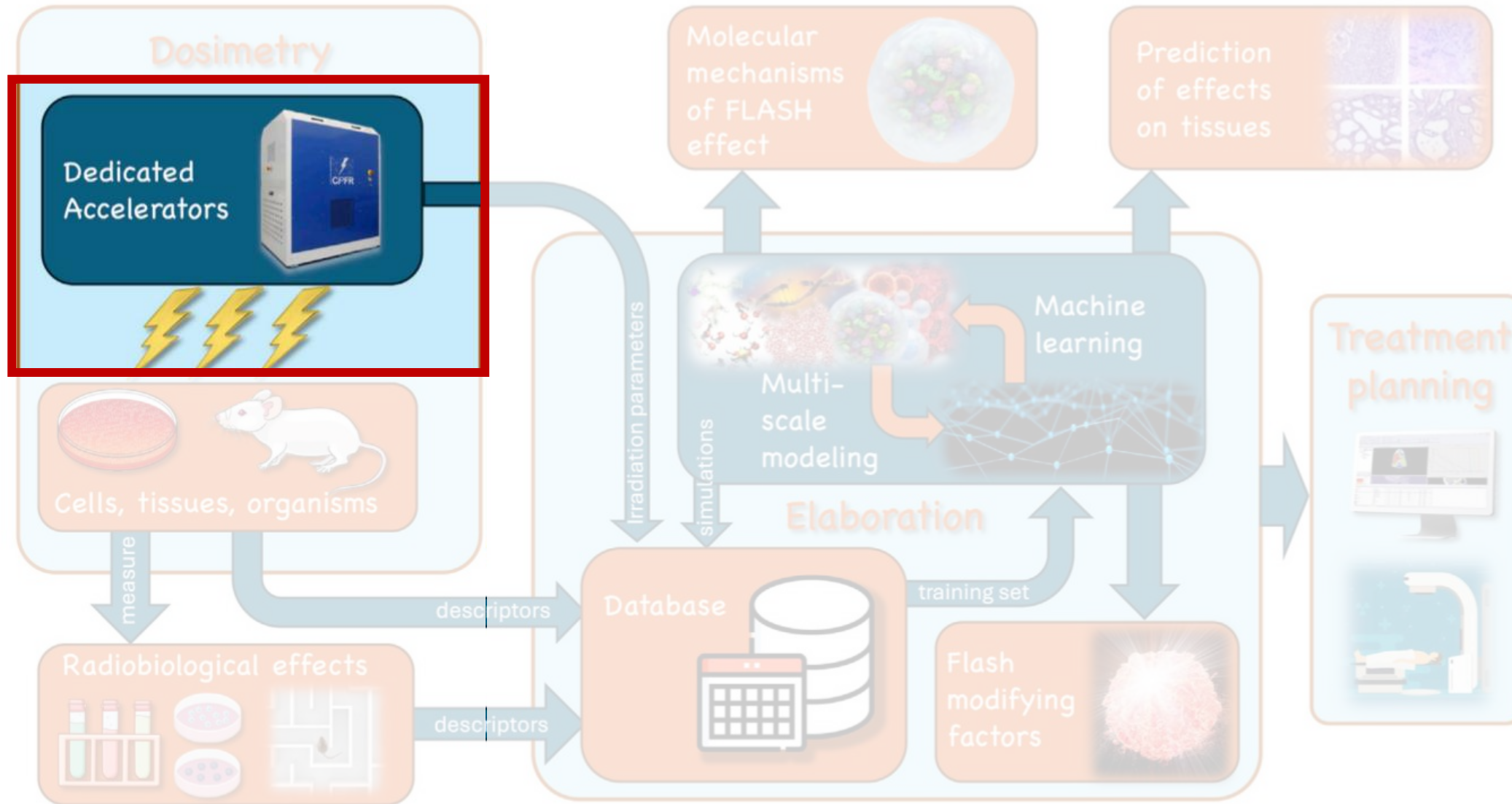


# FLASH research



Di Martino F, Scifoni E, Patera V, Montay-Gruel P, Romano F, Darafsheh A and Tozzini V (2024), Editorial: Multidisciplinary approaches to the FLASH radiotherapy. *Front. Phys.* 12:1439081. doi: 10.3389/fphy.2024.1439081

# FLASH research @DF-UNIFI



Di Martino F, Scifoni E, Patera V, Montay-Gruel P, Romano F, Darafsheh A and Tozzini V (2024), Editorial: Multidisciplinary approaches to the FLASH radiotherapy. *Front. Phys.* 12:1439081. doi: 10.3389/fphy.2024.1439081

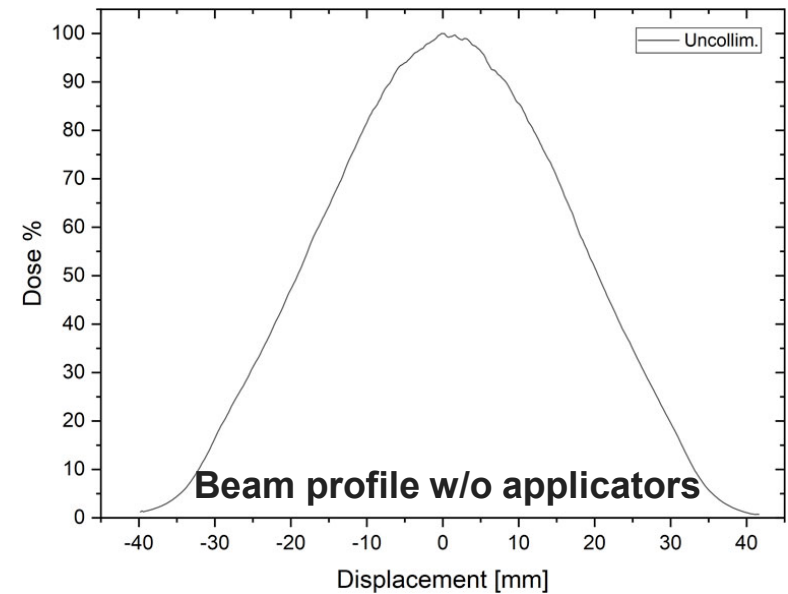
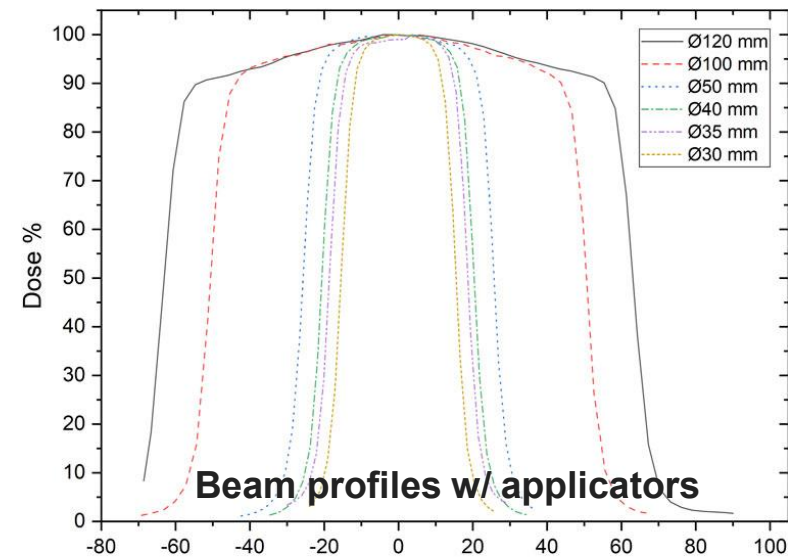
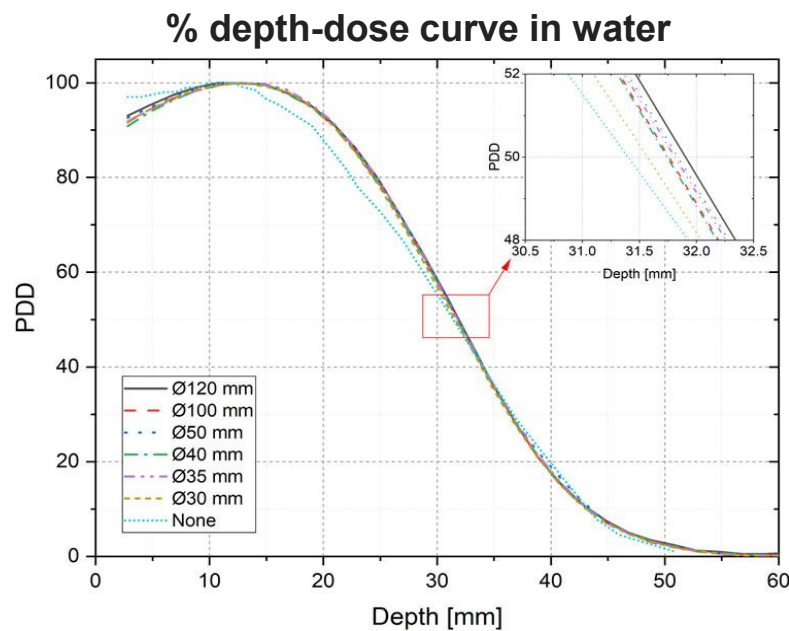
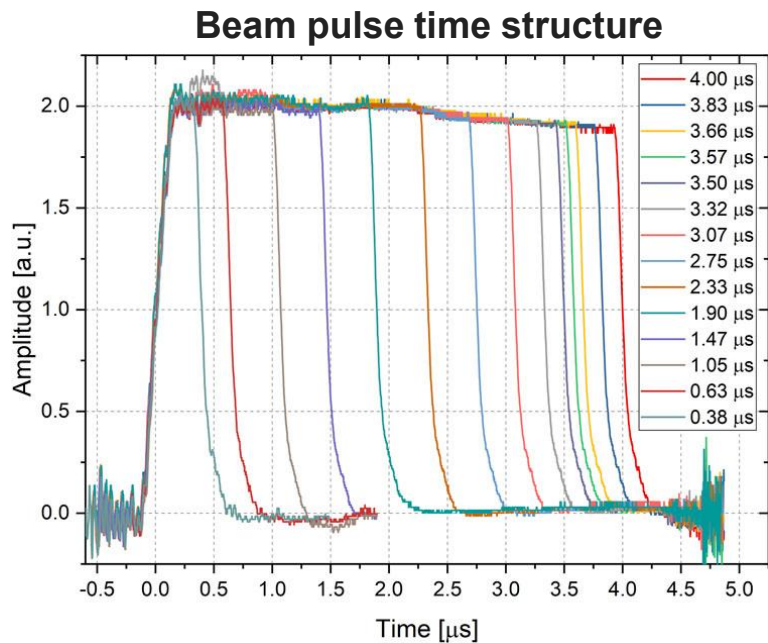
# The CPFR@CISUP

- Centro Pisano per lo studio multidisciplinare della Flash Radiotherapy (CPFR)
- One of many facilities run by the Center for Instrumentation Sharing of the University of Pisa (CISUP)
- September 2021: Installation and commissioning
- June 2022: Start of research activities
- 7-9 MeV electrons
- Beam pulse length 0.5-4 us
- PRF 1-245 Hz
- Triode gun to switch **CONV** <-> **FLASH**
- Three irradiation **directions**: horizontal (detector testing), vertical (upward – in vitro studies), -30° wrt horizontal (in vivo studies)
- Integrated current **monitoring** system
- Simulation in the **Geant4** repository



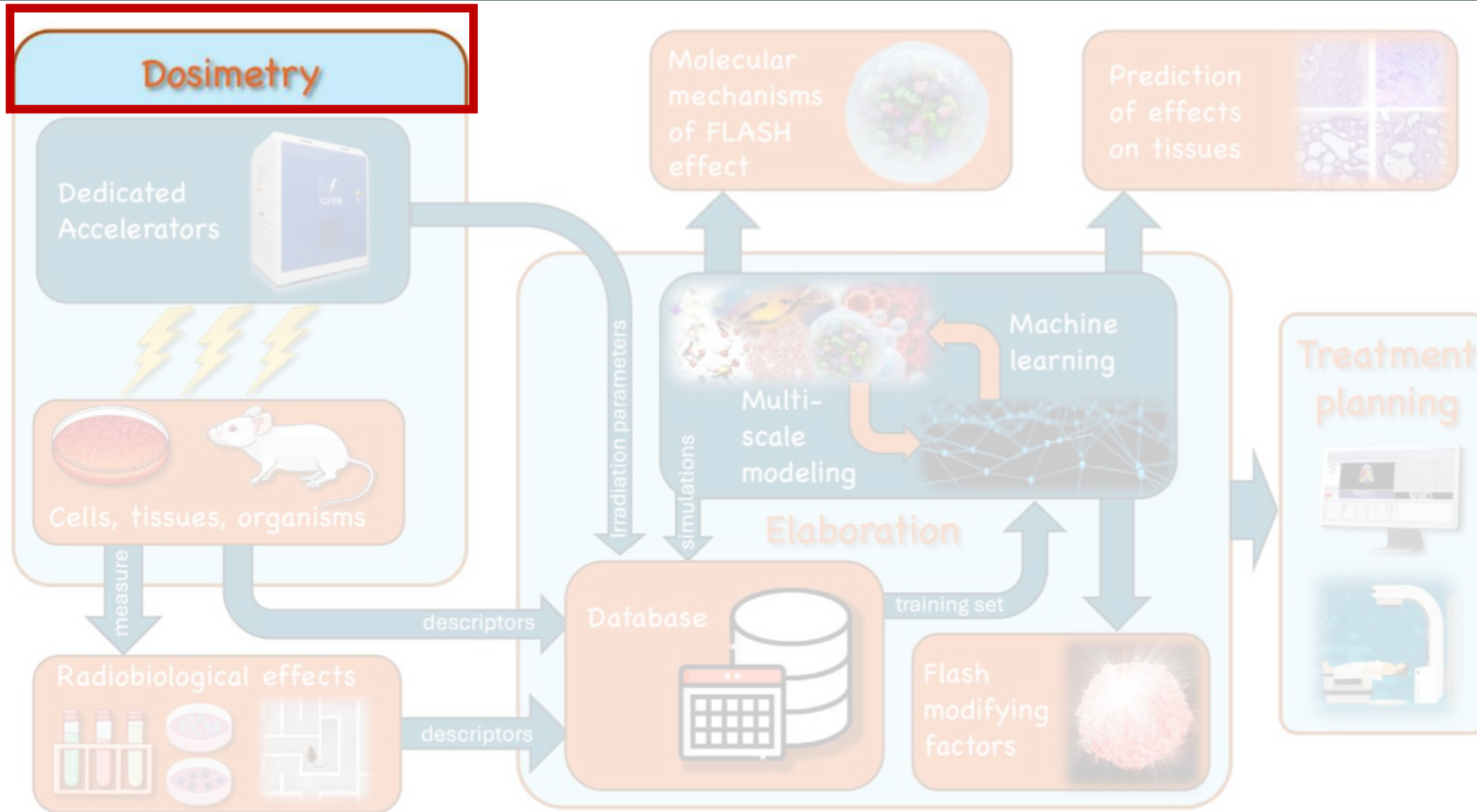
<https://cisup.unipi.it/electron-flash-therapy>





Di Martino, F., et al. *Frontiers in Physics* 11 (2023): 1268310.

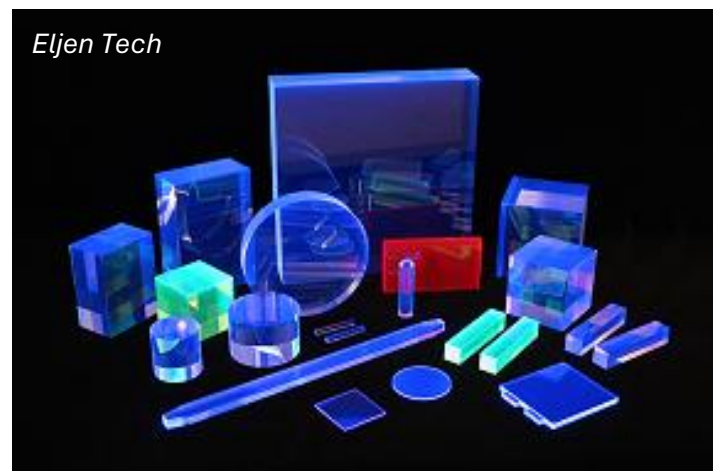
# FLASH research @DF-UNIFI



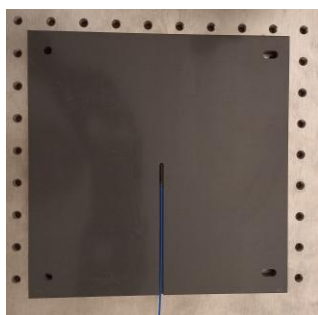
Di Martino F, Scifoni E, Patera V, Montay-Gruel P, Romano F, Darafsheh A and Tozzini V (2024), Editorial: Multidisciplinary approaches to the FLASH radiotherapy. *Front. Phys.* 12:1439081. doi: 10.3389/fphy.2024.1439081

# Plastic-scintillator-based dosimetry for FLASH-RT

- Conventional dosimeters are non-linear in UHDR
- Why plastic scintillators?
  - **Fast** time response
  - High **spatial** resolution
  - **Water** and **tissue** equivalence
  - **Cost**-effectiveness
  - Reusability



Point, fiber detectors

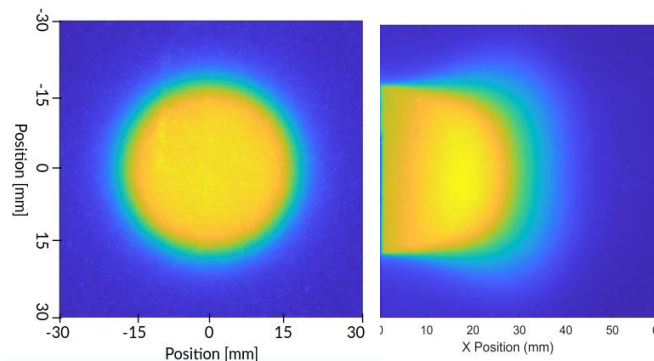


Ciarrocchi et al. *Physica Medica* 121 (2024): 103360.

1D array of fiber detectors

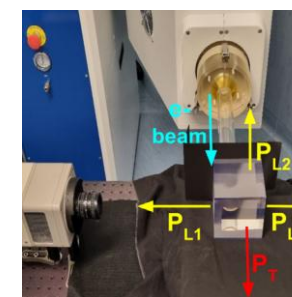


2D imaging

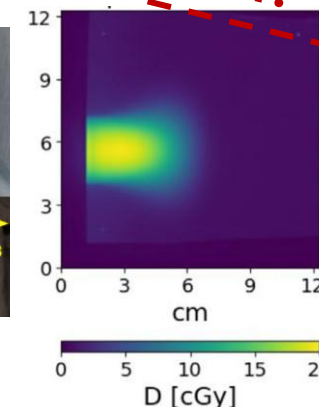


Morrocchi et al. *Medical Physics* 52.8 (2025): e18033.

3D dosimetry



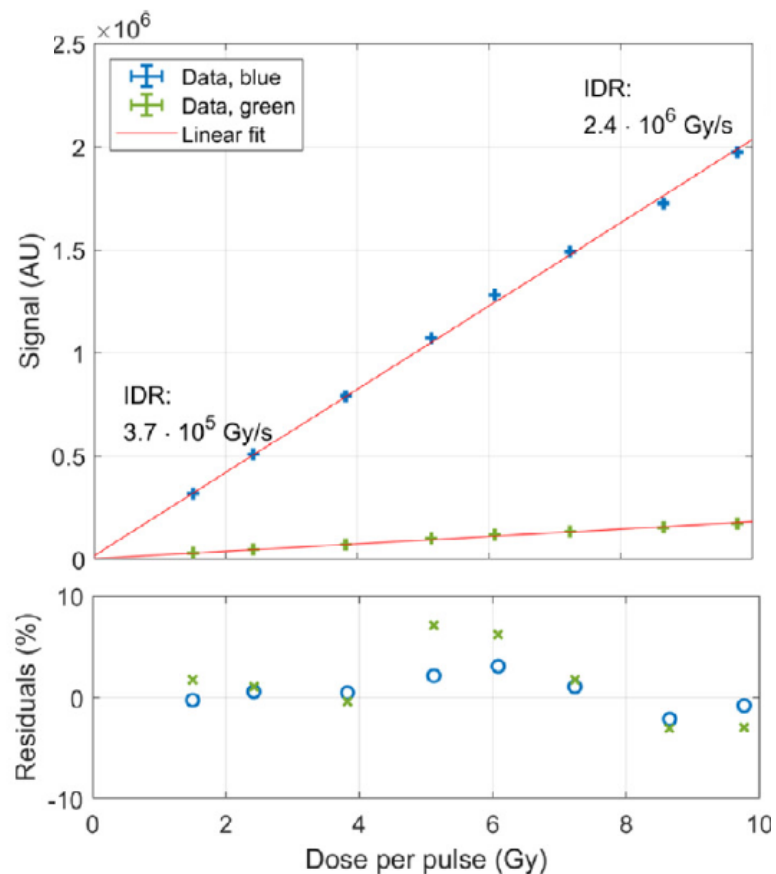
Ravera et al, *NIM-A 1069* (2024): 169910



Go see her poster!

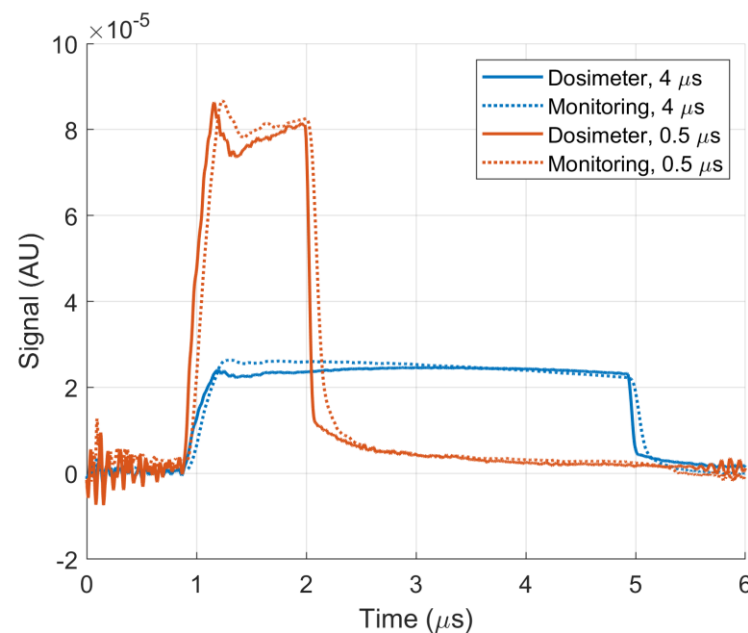
# Plastic-scintillator-based dosimetry for FLASH-RT

## Linearity of point detectors in UHDR



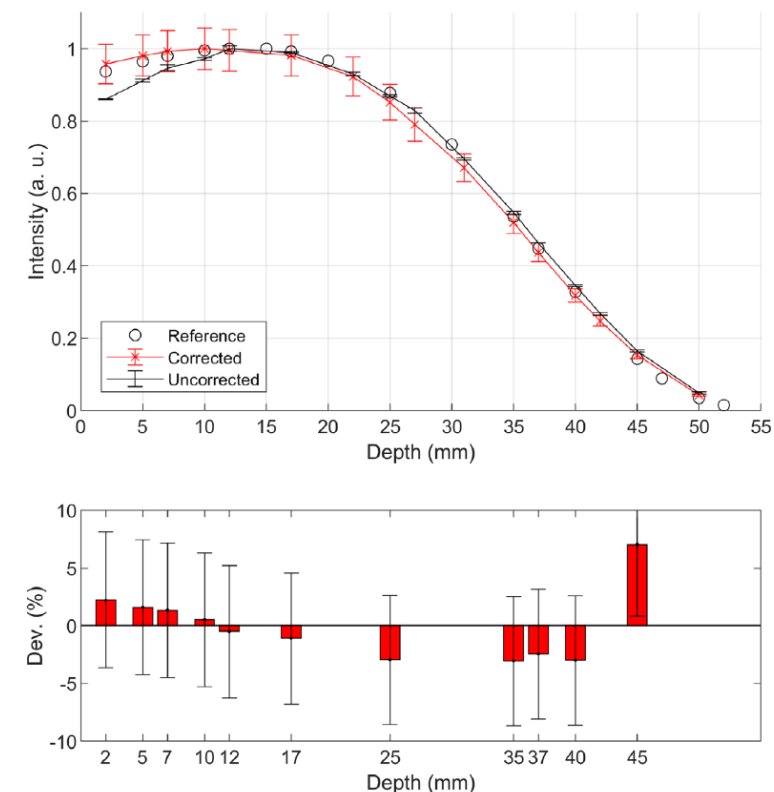
Ciarrocchi et al, 2024, *Physica Medica* 121 (2024): 103360

## Capability to monitor the dose pulse time structure



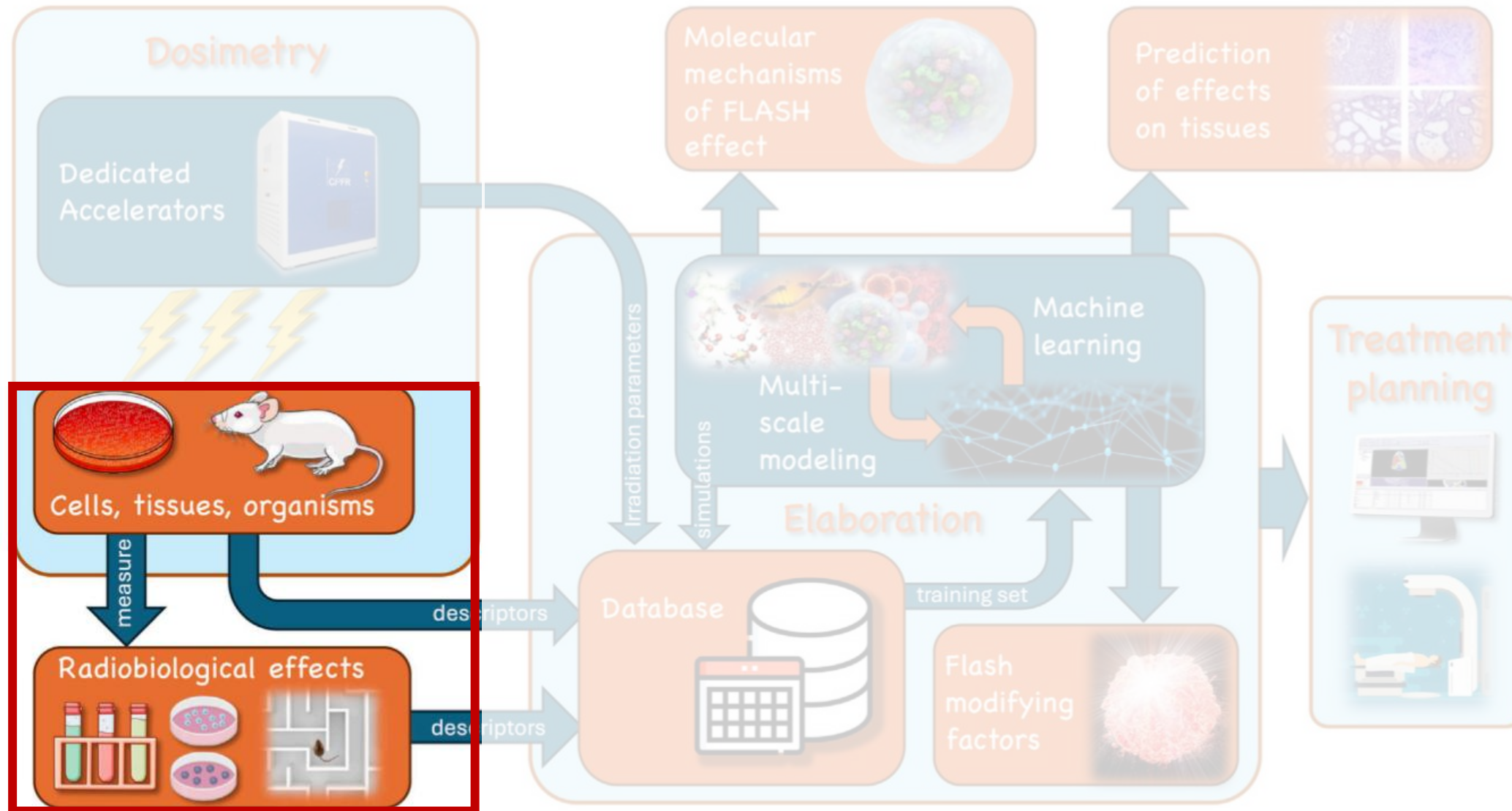
Adapted from Ciarrocchi et al, 2024, *Physica Medica* 121 (2024): 103360

## Capability to measure the depth-dose curve in water



Morrocchi et al., *Medical Physics* 52.8 (2025): e18033

# FLASH research @DF-UNIFI



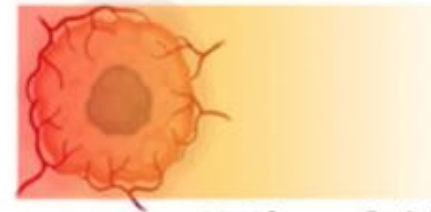
Di Martino F, Scifoni E, Patera V, Montay-Gruel P, Romano F, Darafsheh A and Tozzini V (2024), Editorial: Multidisciplinary approaches to the FLASH radiotherapy. *Front. Phys.* 12:1439081. doi: 10.3389/fphy.2024.1439081

# Super-resolution for radiobiology of the FLASH effect

- *Biological models*
- *Biophysical tools*

*...biology-driven optimization  
of radiotherapy*

Conventional-RT



Uniform field

Minibeam-RT (MBRT)



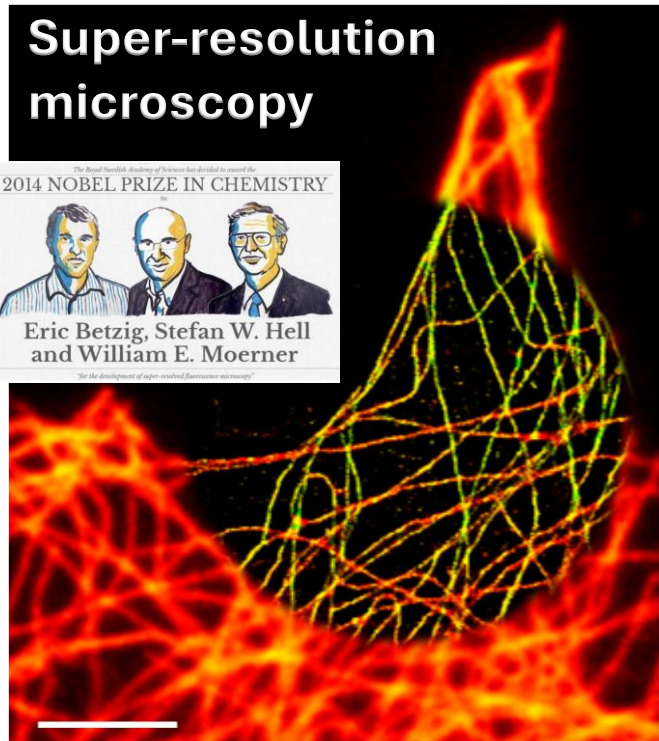
>100  $\mu\text{m}$  wide beams

Super-Resolution  
imaging - DNA damage

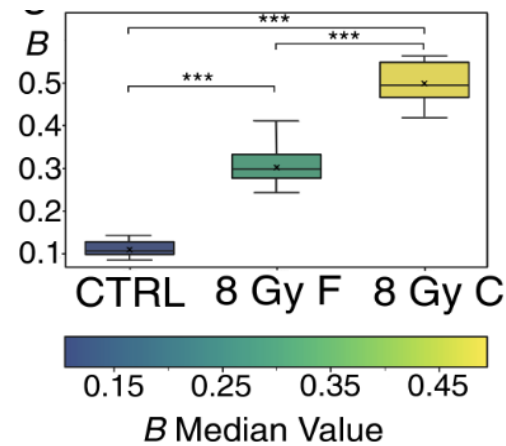
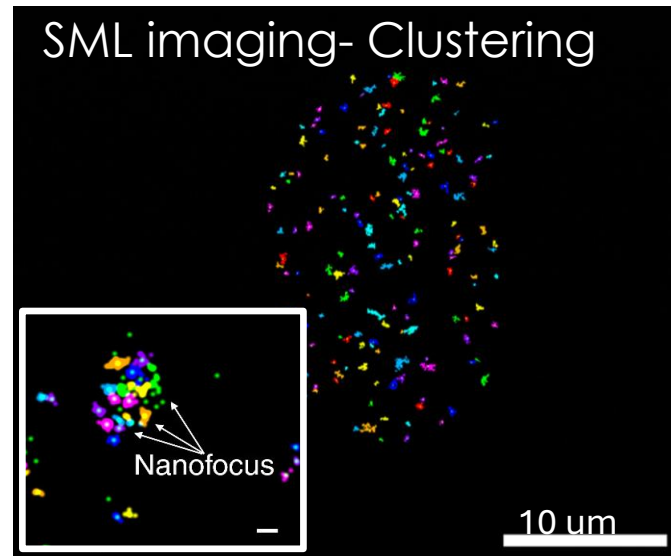
Francesca Cella Znacchi, Simone Capaccioli  
Dipartimento di Farmacia: E. Da Pozzo, Satia Caroti

# Super-resolution for radiobiology of the FLASH effect

**THE ROLE OF SR MICROSCOPY IN THE BIOMEDICAL FIELD:** Super-resolution fluorescence microscopy visualizes nanoscale features in biological systems (reaching 10-20nm resolution).

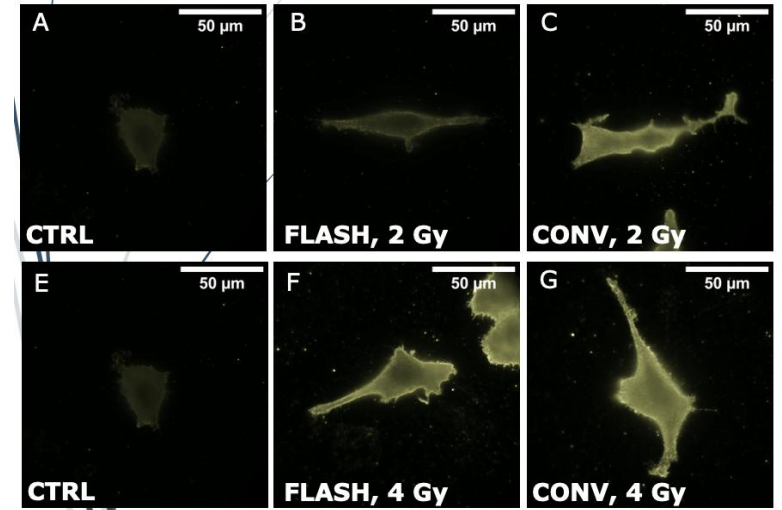


We need a super-resolution method for DNA damage estimation that also considers nanoscale rearrangements...

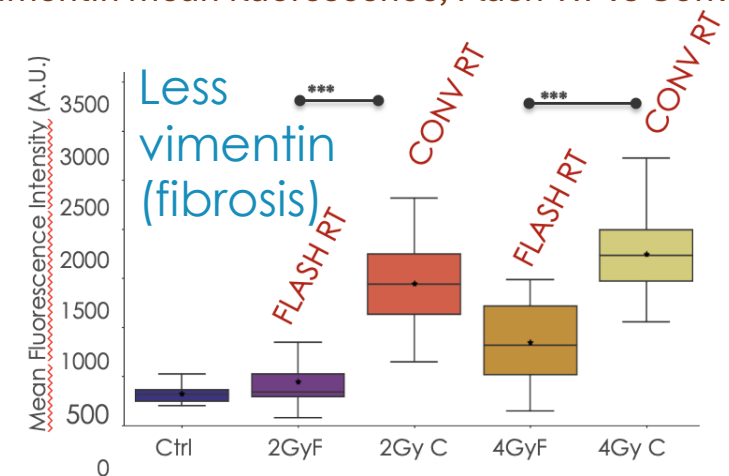


Less DNA damage in FLASH vs CONV

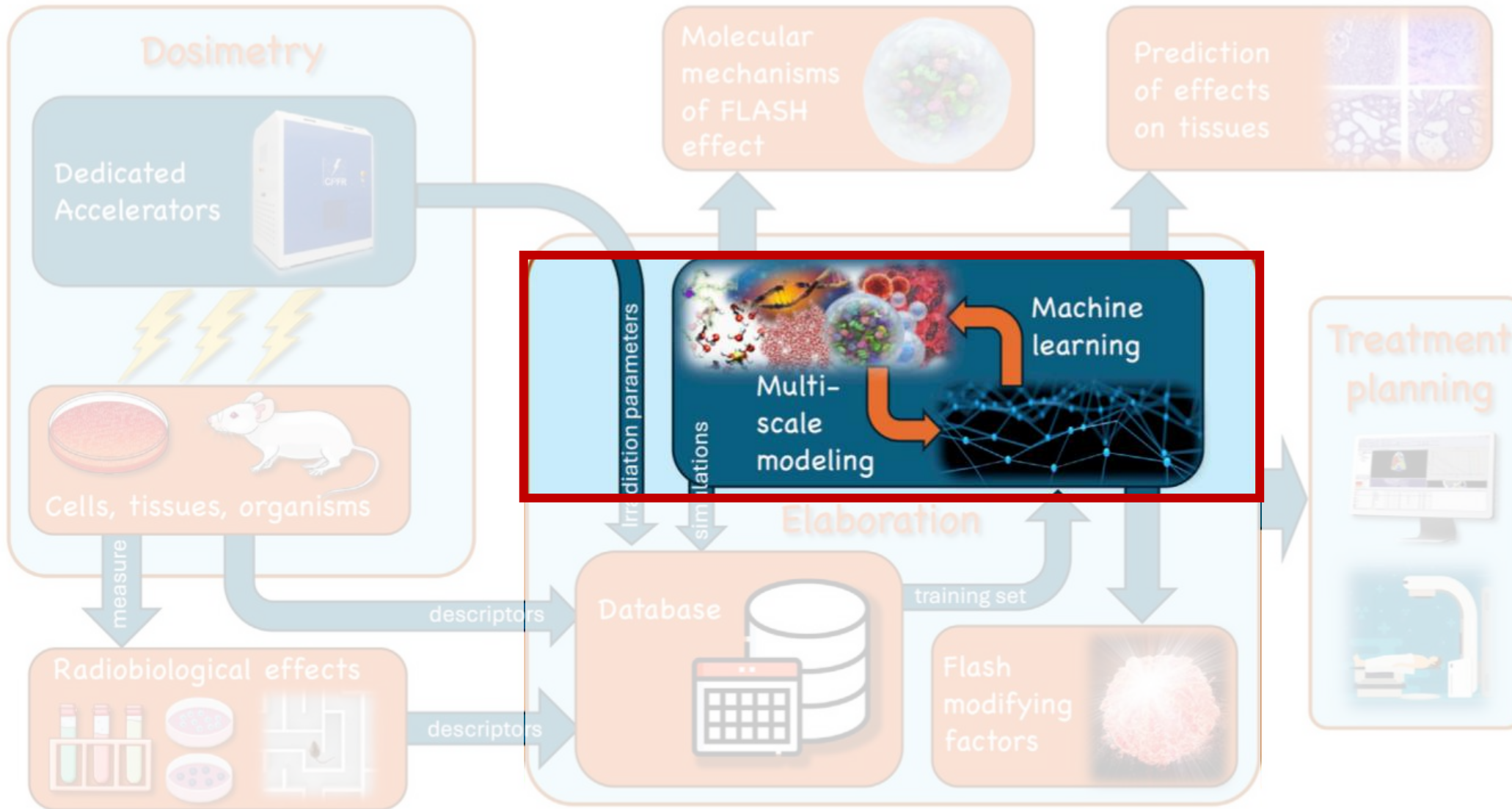
Evaluation of radio-induced side effects: vimentin expression



Healthy lung cell line (16HBE)  
Vimentin mean fluorescence, Flash-RT vs Conv-RT



# FLASH research @DF-UNIFI



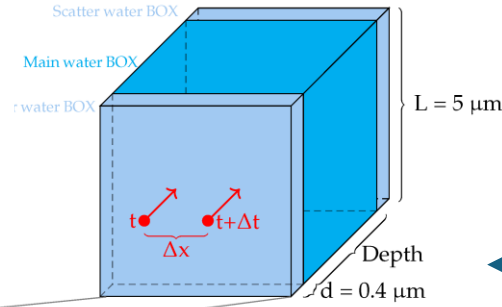
Di Martino F, Scifoni E, Patera V, Montay-Gruel P, Romano F, Darafsheh A and Tozzini V (2024), Editorial: Multidisciplinary approaches to the FLASH radiotherapy. *Front. Phys.* 12:1439081. doi: 10.3389/fphy.2024.1439081

# Multi-scale simulations of radiobiological effects

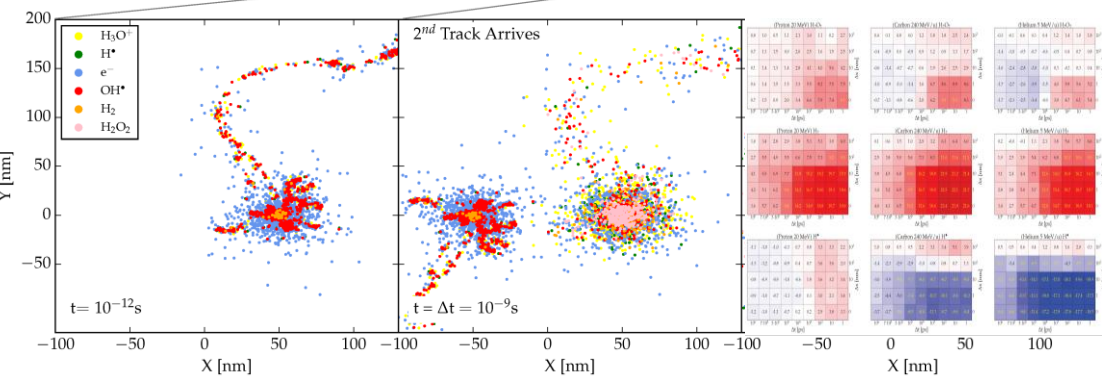
Combining **Reaction Diffusion Monte Carlo (MC)** with **reactive molecular dynamics (MD)** returns a complete view of the physical/chemical phases up to DNA damage

## MC track representation reveals

- Time-space vicinity effects due to UHDR
- Non-linearities due to [O]

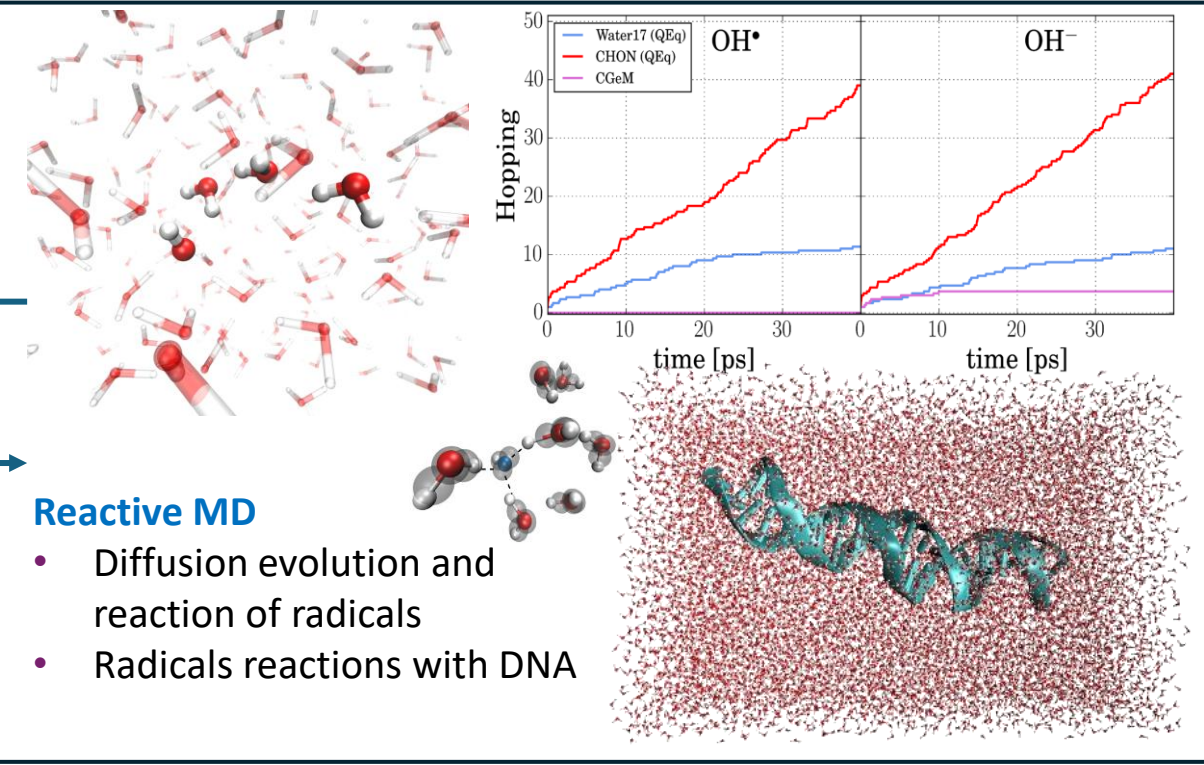


Projection on XY plan (beam eye)



Parameters (D, k)

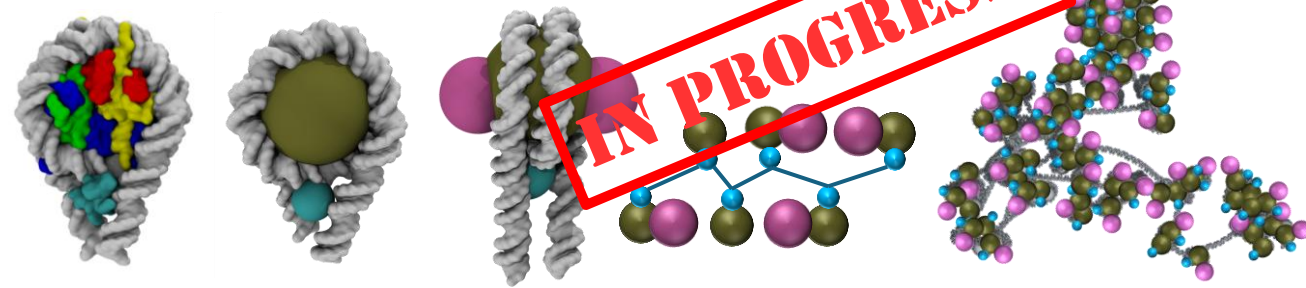
Particles distributions



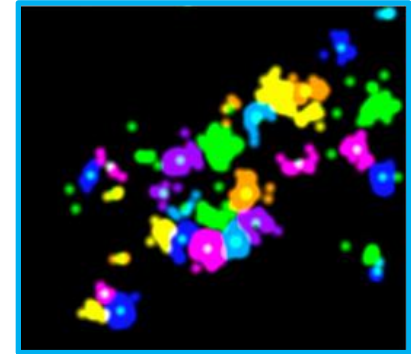
## Reactive MD

- Diffusion evolution and reaction of radicals
- Radicals reactions with DNA

## Coarse grained models

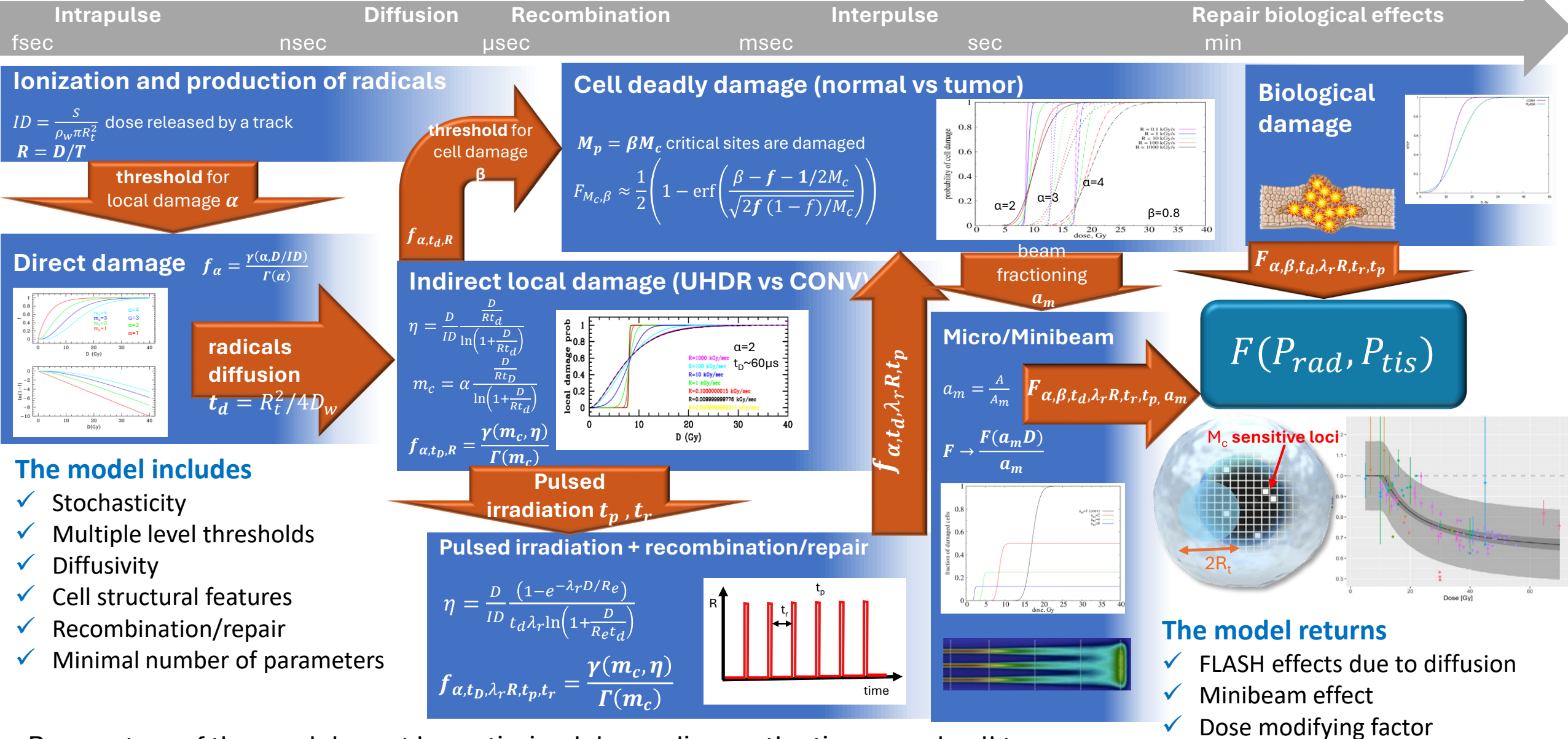


- Build low-resolution models
- Simulate chromatin
- Compare and interpret super-resolution images

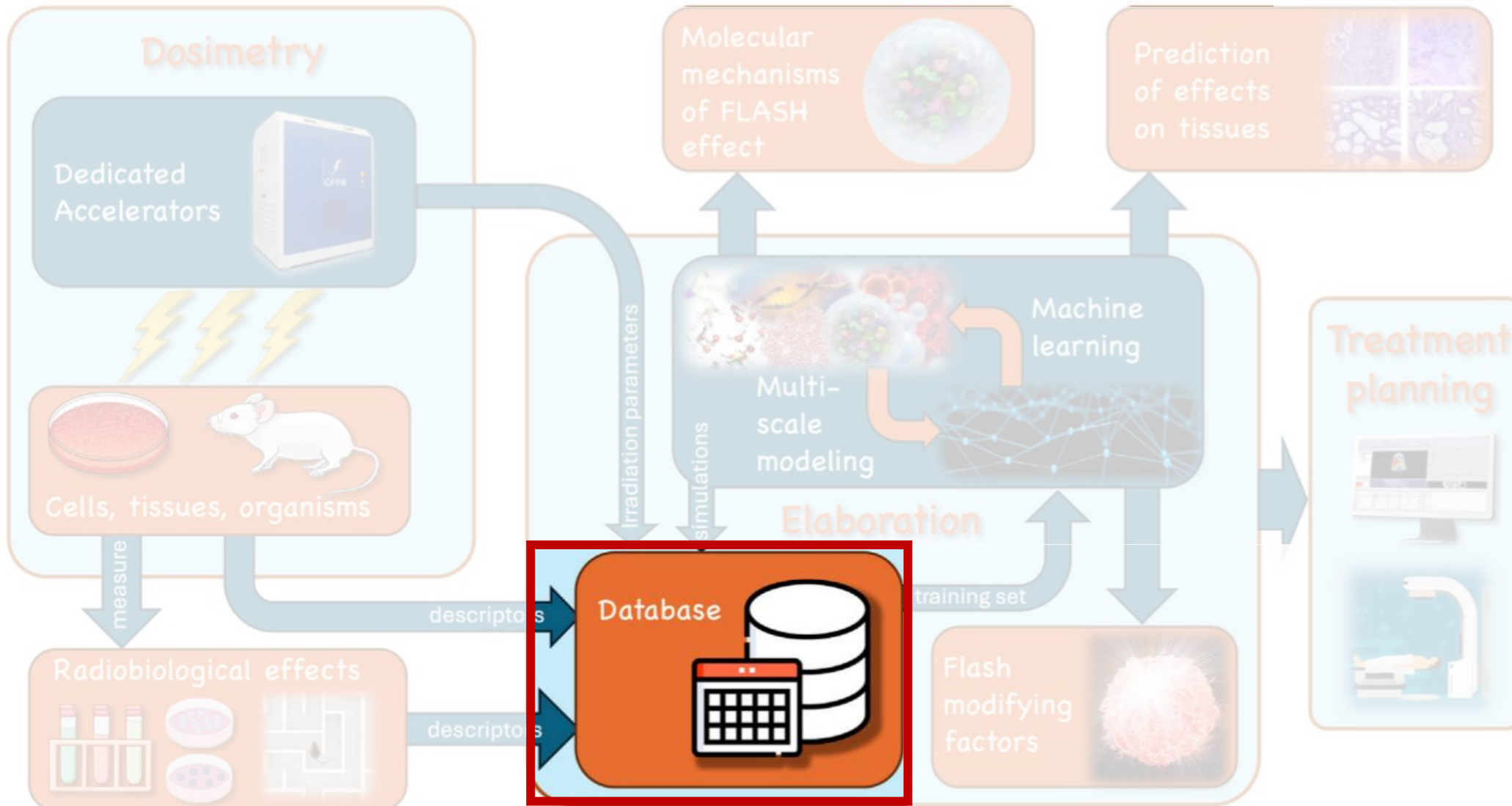


# A multi-stage empirical model for quantification of dose modifying factors in FLASH and MiniBeam

Bodrenko, Tozzini

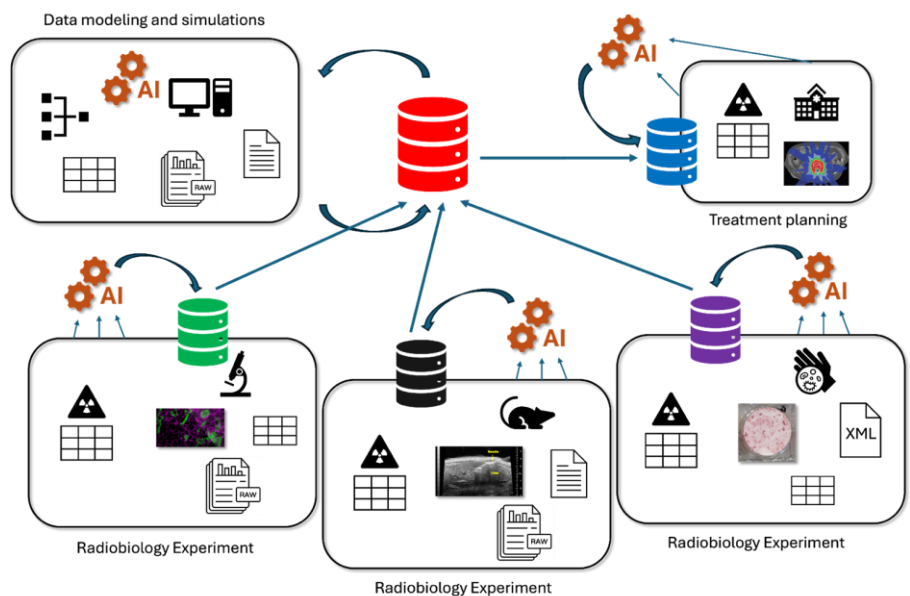


# FLASH research



Di Martino F, Scifoni E, Patera V, Montay-Gruel P, Romano F, Darafsheh A and Tozzini V (2024), Editorial: Multidisciplinary approaches to the FLASH radiotherapy. *Front. Phys.* 12:1439081. doi: 10.3389/fphy.2024.1439081

## CONNECTING DATA TO UNDERSTAND THE FLASH EFFECT MECHANISMS



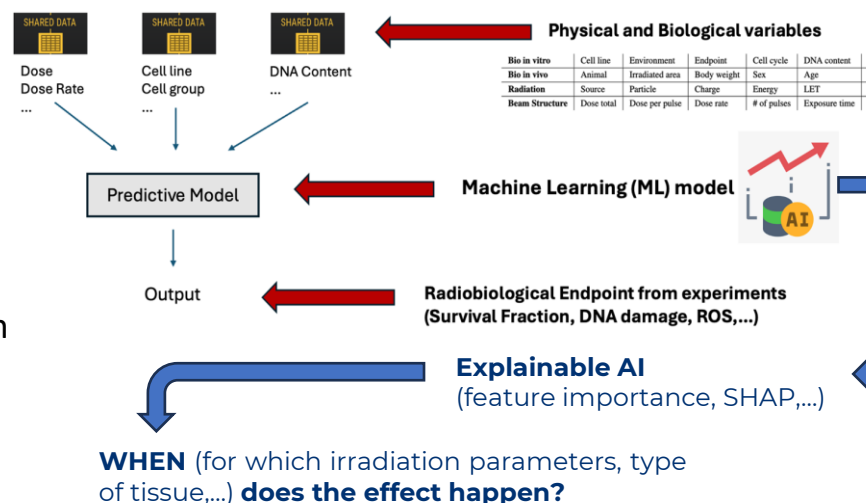
A **custom IT platform** has been designed and deployed to collect, link and harmonize heterogeneous **preclinical data** coming from different experiments within the THE-Spoke 1 project. This can facilitate **multivariate analyses** and enable the community to explore the parameter space systematically.

XNAT

Subject	M/F	Irradiation	mouseId	age	strain	initialWeight	cage	code	group	tumorType	tumorCa
THE_InVivo_Group2_001	F	1	F7 Niente Sx Mezzo	8.0	C57BL/6	21.3	SM	tumor	ocular_melanoma	50000.0	
THE_InVivo_Group2_002	F	1	F8 Niente Dx Naso	8.0	C57BL/6	20.5	DN	tumor	ocular_melanoma	50000.0	
THE_InVivo_Group2_003	F	1	F9 Niente Dx Mezzo	8.0	C57BL/6	19.2	DM	tumor	ocular_melanoma	50000.0	
THE_InVivo_Group2_004	F	1	F11 Niente Sx Naso	8.0	C57BL/6	20.5	SN	tumor	ocular_melanoma	50000.0	
THE_InVivo_Group2_005	F	1	F12 Puntina Sx Tempo	8.0	C57BL/6	19.1	ST	tumor	ocular_melanoma	50000.0	
THE_InVivo_Group2_006	M	1	irm-51	15.0	C57BL/6J	27.17	IRM5	IRM5_TgDx	tumor	gbm_gi261	38000.0
THE_InVivo_Group2_007	F	1	irf-51	15.0	C57BL/6J	23.3	IRF5	IRF5_p.Striscia	tumor	gbm_gi261	38000.0
THE_InVivo_Group2_008	M	1	irm-Sbis1	12.0	C57BL/6J	24.15	IRM5 Bis	IRM5Bis_Puntona	tumor	gbm_gi261	38000.0
THE_InVivo_Group2_009	F	1	irf-Sbis1	12.0	C57BL/6J	20.41	IRF5 Bis	IRF5Bis_P.Striscia	tumor	gbm_gi261	38000.0
THE_InVivo_Group2_010	M	1	irm-52	15.0	C57BL/6J	25.49	IRM5	IRM5_p.Sbr	tumor	gbm_gi261	38000.0
THE_InVivo_Group2_011	F	1	irf-52	15.0	C57BL/6J	21.6	IRF5	IRF5_Puntina	tumor	gbm_gi261	38000.0
THE_InVivo_Group2_012	M	1	irm-Sbis2	12.0	C57BL/6J	26.0	IRM5 Bis	IRM5Bis_TgDx	tumor	gbm_gi261	38000.0
THE_InVivo_Group2_013	F	1	irf-Sbis2	12.0	C57BL/6J	21.31	IRF5Bis	IRF5Bis_Puntonissima	tumor	gbm_gi261	38000.0
THE_InVivo_Group2_014	U	1	Flash-1	3.0	C57BL/6J	12.26	Rosso-1	naive			
THE_InVivo_Group2_015	U	1	Flash-2	3.0	C57BL/6J	13.84	Rosso-2	naive			
THE_InVivo_Group2_016	U	1	Flash-3	3.0	C57BL/6J	13.06	Rosso-3	naive			
THE_InVivo_Group2_017	U	1	Verde1	3.0	C57BL/6J	13.39	Verde-1	naive			
THE_InVivo_Group2_018	U	1	Verde2	3.0	C57BL/6J	12.86	Verde-2	naive			
THE_InVivo_Group2_019	U	1	Verde3	3.0	C57BL/6J	13.0	Verde-3	naive			

In collaboration with **FLASH\_IT Cascade Call project**

The platform links **diverse raw data in heterogeneous formats** from different experimental domains (beam and dosimetric parameters, biological parameters describing the irradiated sample, the experiment, and the endpoints, and simulation data).



By connecting raw preclinical data, the platform preserves the granularity needed to fit mechanistic models and train **ML algorithms**, aimed at identifying the most influential parameters in the FLASH effect through explainability tools.

**WHEN** (for which irradiation parameters, type of tissue,...) **does the effect happen?**

# So, how far are we from clinical FLASH-RT?



Based on my notes of the talk by Michael J Taylor (University of Manchester, UK)  
@ the FRPT2024 opening session

- The FLASH effect has been observed in many **subjects**, with many types of **particles**
- So why isn't it the clinical standard already?
  - Doctors want the 'gold standard', i.e., the **randomized controlled trial**
  - Dose and dose rate need to have a consensus: >10Gy? > 40 Gy/s?
  - Dose rates need to be measured AND guaranteed
  - Which dose rate is the one that matters?
  - The **FLASH effect** depends on the **biological system** too, not only on the **physical beam parameters**
- ...Maybe... if we understood the **mechanism** behind the FLASH effect, we could use it in the clinic ...
- But we take **paracetamol** almost every day without knowing completely how it works!
- Yes, but **we know the dose that works without being toxic**
- To use FLASH-RT, we need to have the same information



# Thank you for your attention!

## DF

Giusi Bisogni  
Simone Capaccioli  
Francesca Cella Znacchi  
Evelina Fantacci  
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## Other depts

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Fabiola Paiar

## AOUP

Fabio Di Martino  
Stefania Linsalata

... and many MSc students, PhD students, medical physics residents, research associates, ...

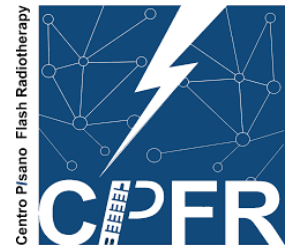
## Acknowledgments

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FLASH Radiotherapy with high  
Dose-rate particle beams



Funding Grant

AOO "CLE" - Prot.: 0104712/2021 del 06/09/2021 -  
Convenzioni - 385/2021

**FONDAZIONE PISA**



# Backup slides

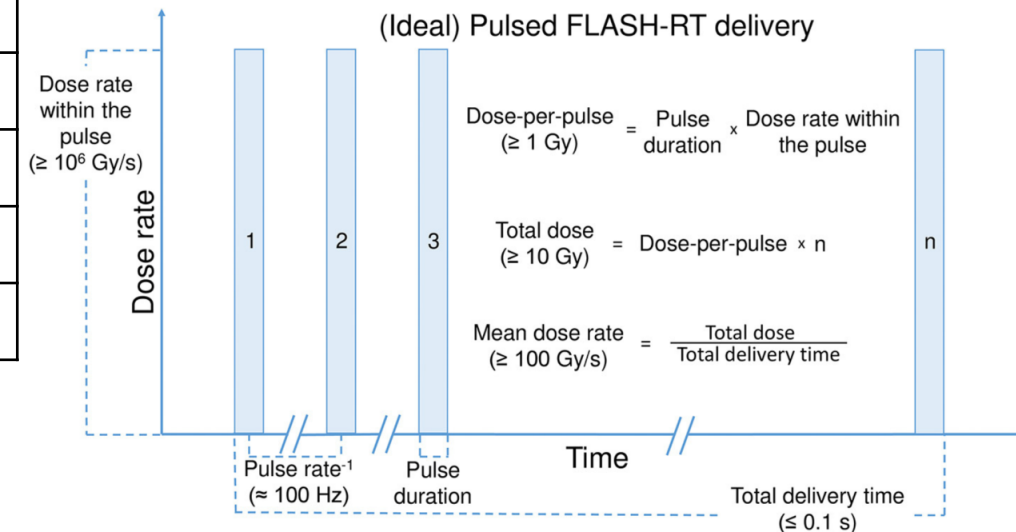
# UHDR beam parameters for low energy e-

Parameter	Description	FLASH value	CONV value
$D$	Total dose	10 Gy	10 Gy
$T$	<b>Total irradiation time</b>	<b>100 ms</b>	<b>5 min</b>
$\langle \dot{D} \rangle$	<b>Average dose rate</b>	<b>100 Gy/s</b>	<b>2 Gy/min</b>
$IDR$	Instantaneous/intra-pulse dose rate	1e+6 Gy/s	150 Gy/s
$t_p$	Pulse length	2 us	2 us
$DPP$	Dose per pulse	2 Gy	0.3 mGy
$n_p$	Number of pulses	5	~30000
$PRF$	Pulse repetition freq.	50 Hz	50 Hz

> 10 Gy?

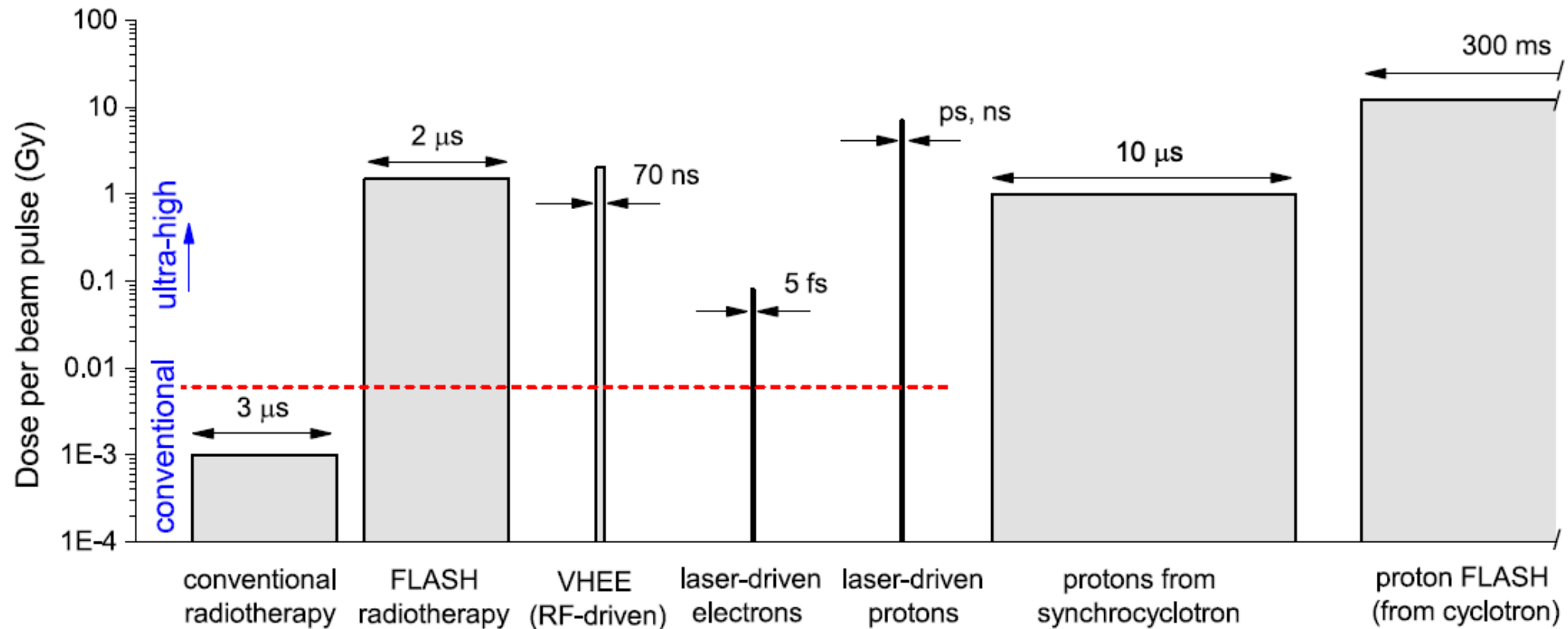
<200 ms?

>40 Gy/s?



Wilson et al, 2020, Ultra-high dose rate (FLASH) radiotherapy: silver bullet or fool's gold?

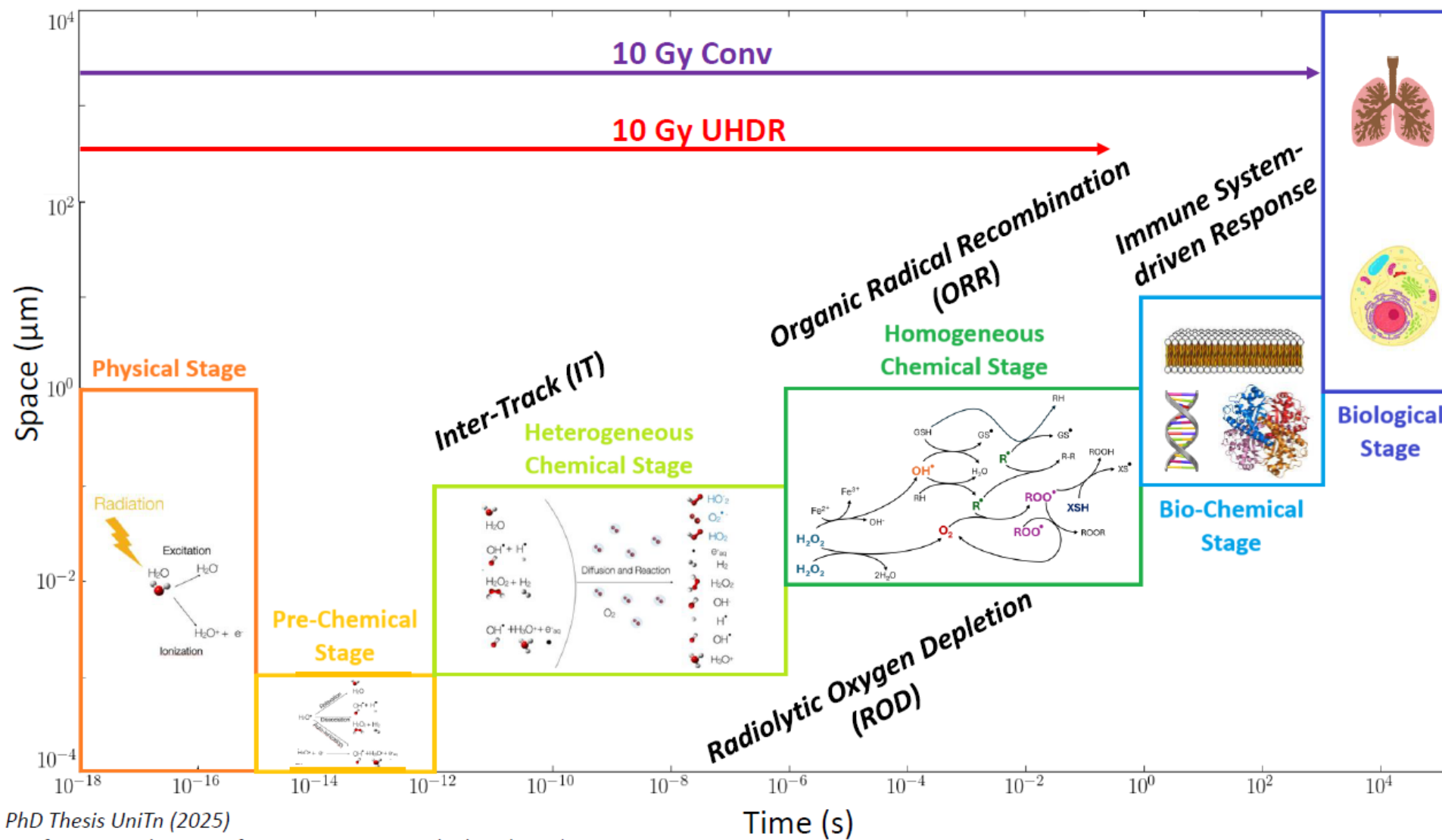
# UHDR beams



The European Joint Research Project UHDpulse – Metrology for advanced radiotherapy using particle beams with ultra-high pulse dose rates

Large range of pulse length and structure

# Hypotheses for the FLASH effect



Battestini, PhD Thesis UniTn (2025)

modified starting from U. Weber, E. Scifoni, M. Durante, Med. Phys. (2022)

19/05/2025

Marco Battestini

