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Normal tissue and tumor response to FLASH-RT
Biological mechanisms
Disclosures

Collaborative Research project with PSI-Varian (CH)
Advisory Board IBA
Research project ROCHE pharma
Learning objectives

- Become familiar with the research strategies and the preclinical models
- Compare tumor response to CONV and FLASH-RT
- Identify the clinically relevant issues Identify the relevant biological mechanisms
- Identify the needs and limitations
Enhancing the therapeutic ratio: a balance between tumour control and toxicity
What are the tools to improve the therapeutic ratio

Biology

Technology
Fractionation and Enhanced precision

1930-1970
Target volume

1970-1990
2D planning

1990-2000
3D Conformal

2016
Stereo-RT

High Precision
FLASH radiotherapy
Irradiation at ultra high dose rate

Very fast delivery of the dose
Shift from minute of exposure to milli- and even micro-second

eRT6 Oriantron PBM/Alcen
Electron beam, 5.5 MeV energy
Pulsed beam
THE FLASH EFFECT is a biological effect

**Normal tissue sparing**
FLASH-RT does not induce Normal tissue toxicity
When CONV-RT does

**And FLASH-RT is equally able to eradicate tumors compared to CONV-RT**

**Electron**

**X-ray-synchrotron**

**Proton**
Cunningham et al., *Cancers*, 2021 (PBS).

**Electron**

**X -ray synchrotron**

**Proton**

**Electron**
FLASH-RT enhances the therapeutic window
Explored 40 years ago... it was abandoned
Why?


Tumor and Normal tissue response should be investigated in parallel and *in vivo* models should be used.
Normal tissue response
Normal tissue toxicity cascade?

Where does FLASH make a difference?

DNA damage

Cell death

Vascular damage

Inflammatory response

Stem Cells and Progenitors depletion

Organ function alteration

Pierre Montay-Gruel, PhD
Differential DNA damage *in vivo*

**A**

![Images of gH2AX with labels: NI, CONV, FLASH](Image)

**B**

![Bar graphs showing percentage of affected nuclei](Image)

**C**

![Immunofluorescence images with labels: γH2AX, Lysozyme, DAPI](Image)

**D**

![Immunofluorescence images with labels: CD31, γH2AX, DAPI](Image)

**E**

![Graph showing γH2AX-positive CSC cell](Image)

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**Intrapulse DR > 10^6 Gy/s**

_Fouillade et al. 2020_

**Normal cells** in tumor- LLC

**Intrapulse DR > 10^5 Gy/s**

_Levy et al. 2020_

_“Normal cells” in tumor- LLC_

**Kim et al. 2020**
Differential cell death *in vivo*

**Favaudon et al. 2014**

**Intrapulse DR > 10^6 Gy/s**

**A**

**Senescence**

CONV

FLASH

**B**

**NI**

CONV

FLASH

**C**

**D**

**Fouillade et al. 2020**

**Intrapulse DR > 10^6 Gy/s**

**Levy et al. 2020**

**TUNEL**

**Intrapulse DR > 10^5 Gy/s**

**TUNEL (SVZ) 24 Hours**

**TUNEL (DG) 24 Hours**

**Intrapulse DR > 10^6 Gy/s**

**Allen et al. 2020**
Differential effect on Stem cells and progenitors *in vivo*

Montay-Gruel et al. 2017

Fouillade et al. 2020

Diffenderfer et al. 2020

Levy et al. 2017

Intrapulse DR > $10^6$ Gy/s

Montay-Gruel et al. 2017

Intrapulse DR > $10^5$ Gy/s

Chabi et al. 2020

Intrapulse DR > $10^6$ Gy/s

Fouillade et al. 2020
Differential effect on the vascular system

Intrapulse DR > $10^6$ Gy/s

Allen et al. 2020

+ work on the tumor vasculature, Kim et al. 2021
Differential inflammatory response

Montay-Gruel et al. 2020

Intrapulse DR > 10^6 Gy/s

Favaudon et al. 2014

Intrapulse DR > 10^6 Gy/s

Simmons et al. 2019

Intrapulse DR > 10^5 Gy/s

Cunningham et al. 2021

Intrapulse DR > 10^5 Gy/s

Velalopoulou et al. 2021
Organ outcome and function

Brain function

Cognition

Montay-Gruel et al. 2017
Montay-Gruel et al. 2018
Montay-Gruel et al. 2019
Simmons et al. 2019
Alaghband et al. 2020

Intrapulse DR > 10^6 Gy/s

Alaghband et al. 2020

Intrapulse DR > 10^5 Gy/s

Levy et al. 2017

Intrapulse DR > 10^5 Gy/s

Velalopoulou et al. 2021

80 Gy/s
Diffenderfer et al. 2020

45 Gy

69 – 124 Gy/s

Diffraction pattern

H&E

Trichrome

NR

SR

FR

% regenerated crypts

P = .0002

P < .0001

P = .0107

P = .0001

P = .0014

Average muscle layer thickness (μm)

Days post-TAI

Days post radiation

Lymphedema Score

Control 0 Gy
CONV 14 Gy
FLASH 14 Gy
Normal tissue toxicity cascade?

DNA damage
- Less γH2AX and 53BP1

Cell death
- Less apoptosis and senescence

Vascular damage
- Preservation endothelial cells and vascular integrity

Inflammatory response
- Less expression of pro-inflammatory molecules
- Less activation of innate immune cells

Stem Cells and Progenitors depletion
- Better pool preservation and proliferation

Organ function alteration
- Function preservation
Tumor response
With Electron beam- from simple SubQ model to orthotopic and GEMMs

**SubQ breast and H&N cancer (immunocompromised mice)**
60 Gy/s (2Fx HBCx and 1 Fx for HEp)

![Graphs showing tumor volume over time for HBCx-12A and HEp-2](image)

Favaudon et al, STM, 2014

In immunodeficient mice

**SubQ GBM models (immunocompromised mice)**

**SubQ LLC model (immunocompetent mice)**
Kim et al. IJROBP, 2021
Orthotopic ovarian cancer (ID8):
216 Gy/s, 2 Gy/pulse

Levy et al, Sc Rep, 2020

In immunocompetent mice

Orthotopic GBM
Transgenic GBM
GFAP-HRas<sup>V12</sup>; GFAP-CRE; p53<sup>flox/wt</sup>
8.3x10<sup>5</sup> Gy/s

**Limoli et al., Book review: The Modern Technology of Radiation Oncology—a Compendium for Medical Physicists and Radiation Oncologists (Volume 4) edited by Jacob Van Dyk**

In immunocompetent mice
With Proton beam- double scattered beam

SubQ Pancreatic cancer  M641905
78 Gy/s +/-9

SubQ (30Gy) and GEMM (12 Gy) (immunocompetent
69-124 Gy/s

Diffenderfer et al. IJROBP, 2020

Velalopoulou et al. Cancer Res, 2021
With Proton beam-pencil beam scanning

SubQ MOC cells immunologically cold vs hot
62 Gy/s average and 207 Gy/s in the spot

Cunningham et al. Cancers, 2021
FLASH-RT can be fractionated

Hypofractionated FLASH-RT as an Effective Treatment against Glioblastoma that Reduces Neurocognitive Side Effects in Mice


News FLASH-RT: To Treat GBM and Spare Cognition, Fraction Size and Total Dose Matter

Christina C. Huang and Marc S. Mendonca
Human Tumors
All tumors are not equally sensitive to FLASH-RT

Human T-ALL with different susceptibility profile to FLASH-RT

5000 cells M T-ALL

BM analysis, 20 days post-RT
Survival of mice

Non irradiated
4Gy FLASH
4Gy CONV

BM analysis

4 weeks

BRgc<sup>+</sup> mice

4Gy FLASH
4Gy CONV

5 weeks

0 5 10 15 20

0 50 100 80 100 120 140

Percent survival

P=0.025
P=0.01

0 5 10 15 20

0 50 100 100 150 200 250

Percent survival

P=0.07
P=0.0139

M106 PDX/T-ALL

M114 PDX/T-ALL

M108 PDX/T-ALL

Human T-ALL with different susceptibility profile to FLASH-RT

Chabi et al, IJROBP, 2020
FLASH does not activate classical radiobiological response in Normal tissues

**CONV**

0.1 Gy/s

- Neurocognitive decline
  - Neuron spine loss
  - Apoptosis
  - Reduced neurogenesis

- Loss of vascular Integrity
  - BBB disruption
  - Loss of vascular density

- Neuroinflammation
  - Microglial activation
  - Astroglisis

**FLASH**

>100 Gy/s

- Neurocognitive sparing
- Maintain vascular integrity
- Less neuroinflammation
FLASH eradicates tumors

CONV
0.1 Gy/s

FLASH
>100 Gy/s

Reactive Astrocyte (GFAP+)
Astrocyte
Activated microglial (CD68+)
Resting microglial
Neuron
Degenerating Neuron
Neural progenitor cells
Brain vessels
Tumor cell
Neutrophil
Lymphocyte

Tumor control isoefficacy

Rv in Kacem et al., IJRB, 2021
At the biology level

What is known about the FLASH effect

FLASH-RT spares normal tissue and is equally able to eradicate tumors compared to CONV-RT
Using TGD assay (no TCD50 assay has been published)

- Using electron, photon and proton beams
  - In pre-clinical mouse model
    - Small volume
  - Single dose and hypofractionated regimen
What is currently being explored

- Modality of cell death
- Immune component
  - Metabolism
  - DNA repair
  - Cell signaling
FLASH could be an unique tool to explore the fundamental difference between normal tissues and tumors.
At the physics level

The higher the better

The shorter the better

Adapted from Montay-Gruel P et al., CCR, 2020.