The biological effects of ultra-high dose rate (FLASH) radiation on normal and cancer cells

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OBJECTIVES

- Brief overview of FLASH-radiation research
- Biological effects of FLASH radiation on normal and cancer cells
  - Previous significant findings
  - Our studies of breast/pancreatic normal and cancer cells
- Future direction

FLASH-radiation Research Overview

Radiation therapy (RT) for cancer treatment

RT has been an effective tool for cancer treatment for **>100 years since 1896**

About two-thirds of all cancer patients received RT as a part treatment

The invention of the **linear accelerator in 1950s** has began **rapid technology advances**

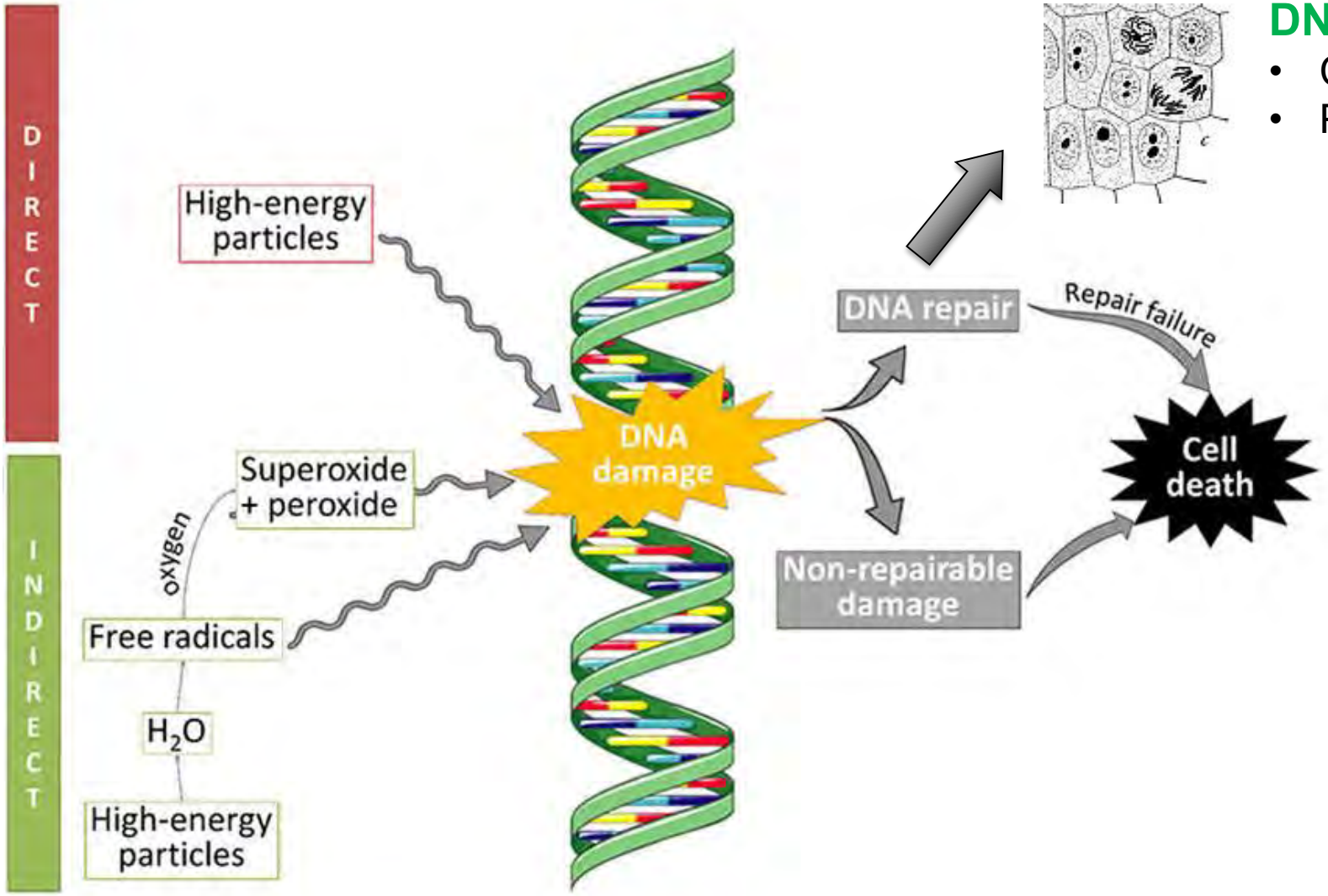
Advances in planning and treatment delivery has enabled RT more effective and precise while reducing the severity of side effects

Radiation dose-rate/dose range remains unchanged over past 5 decades

1. Radioresistance of cancer cells

2. Normal tissue injury impedes the efficacy of RT for cancer control

Irradiation (IR) causes DNA damage by both direct and indirect mechanisms, leading to cell survival or death



DNA repaired/ Cell survival

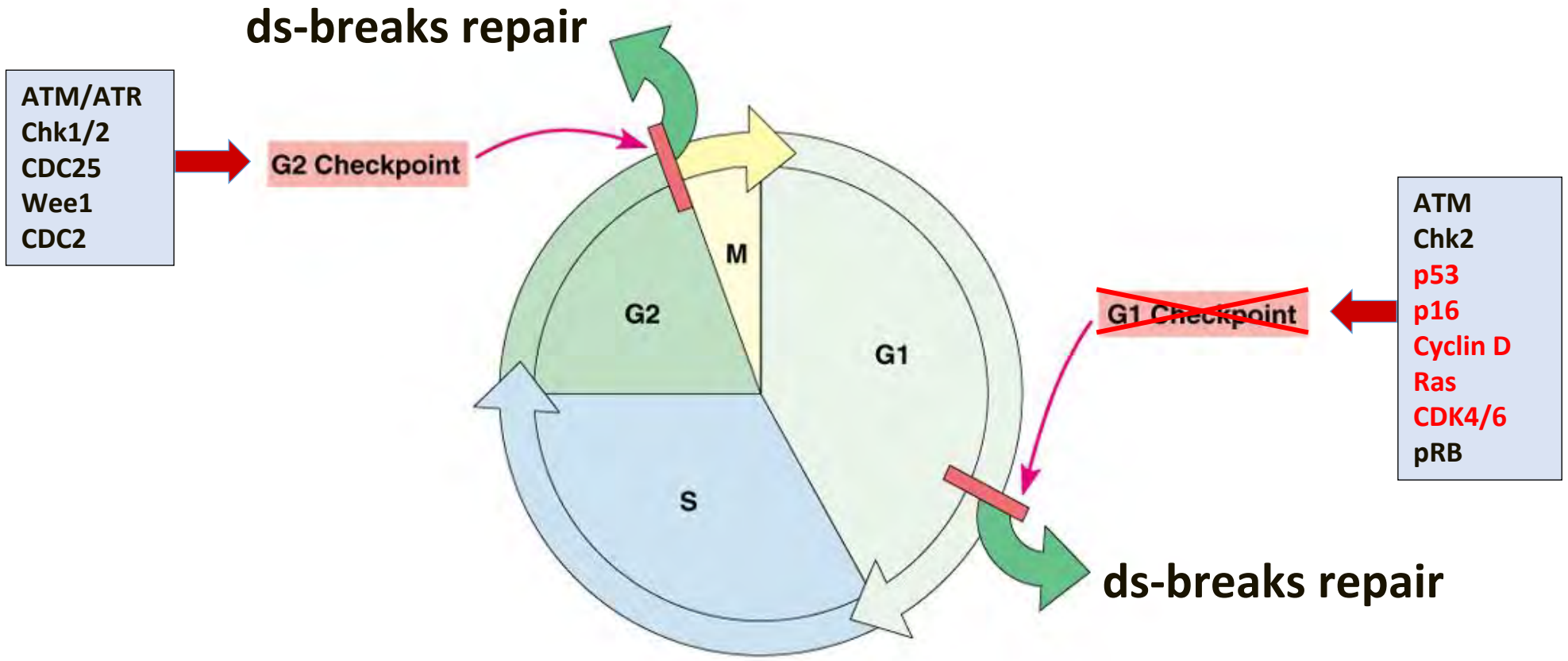
- Cell re-entering cell cycle
- Proliferation

Replicative cell death

- Apoptosis
- Necrosis
- Mitotic catastrophe
- Premature senescence

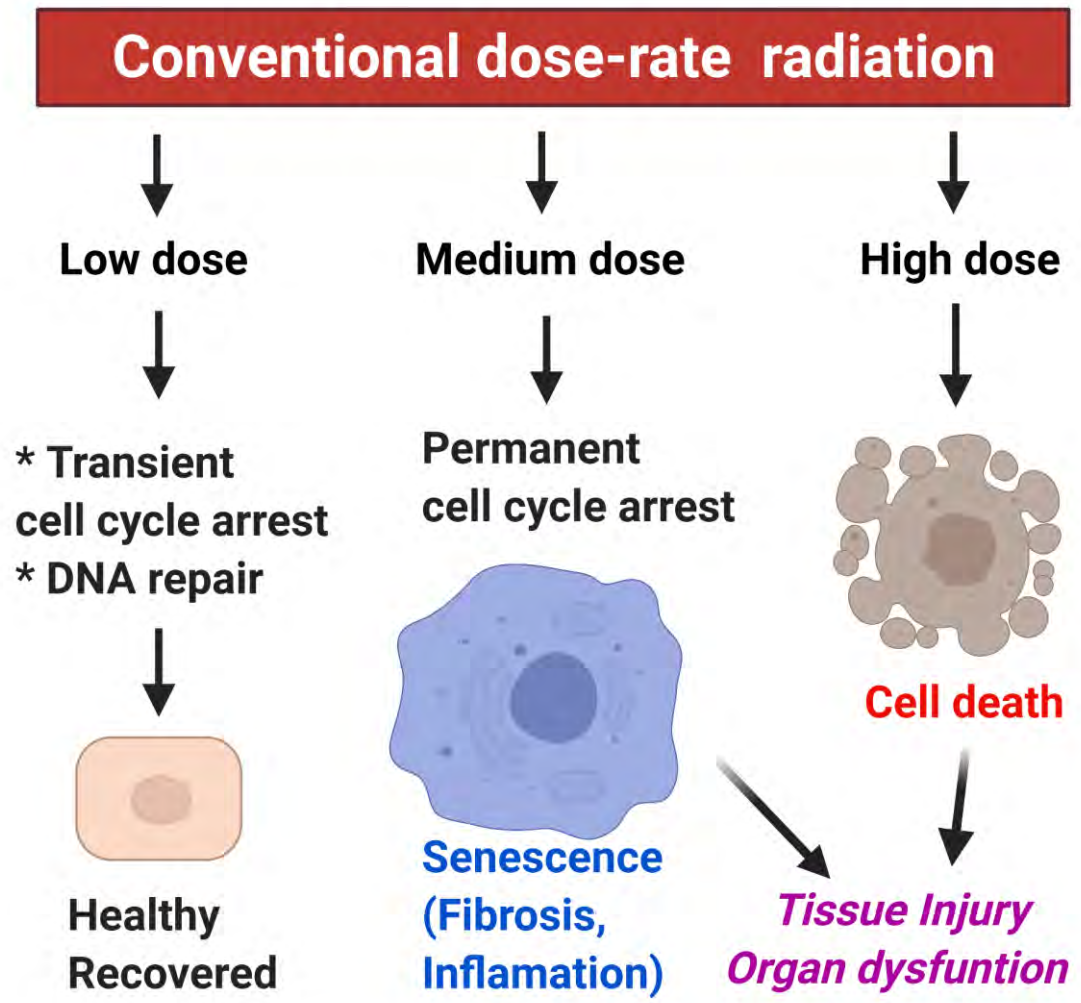
Frontiers in Pharmacology, 10:851, 2019

IR activates G1 and G2 checkpoint to repair DNA damage



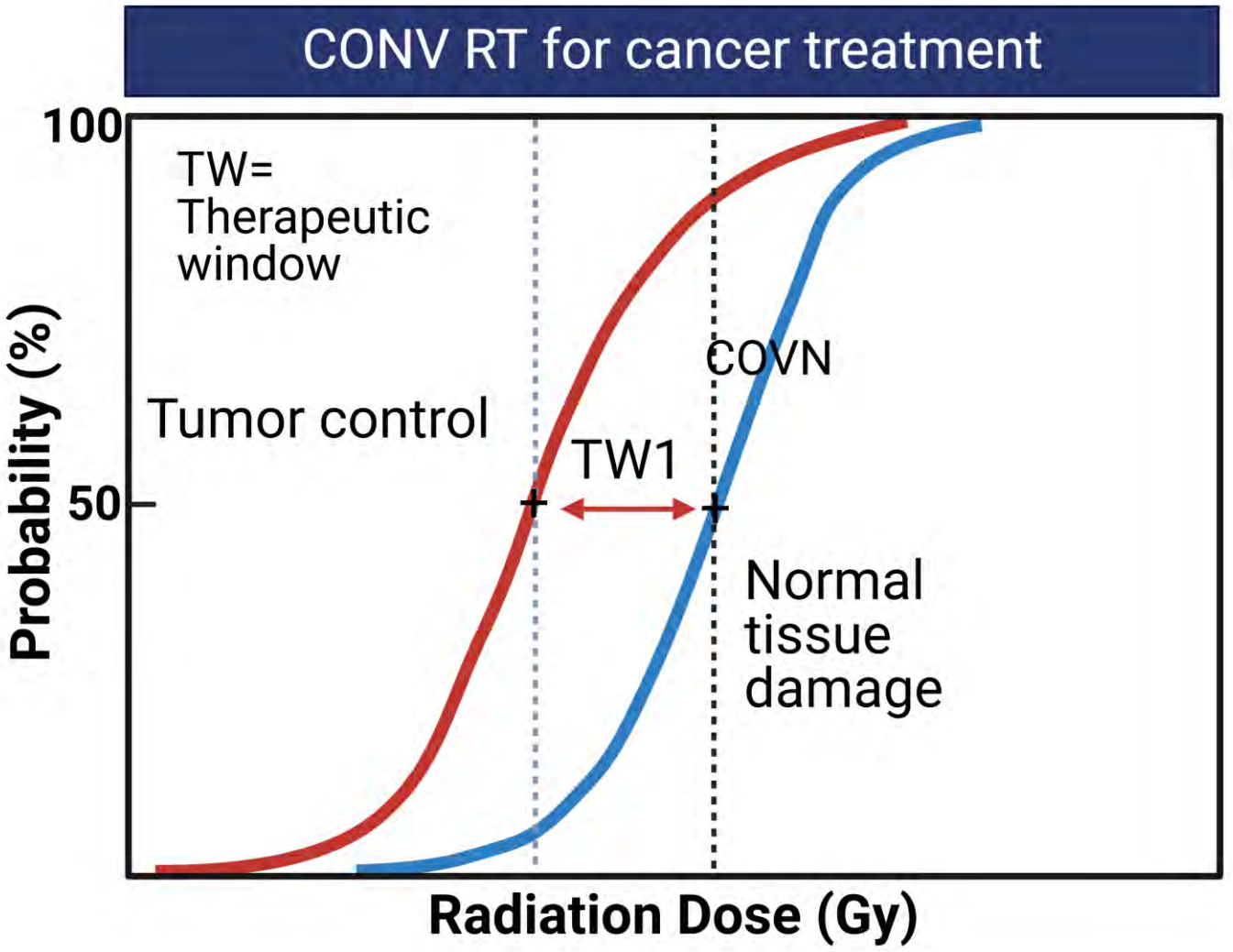
- **In normal cells**, **DNA damage activates both G1 and G2 checkpoint** to allow time for DNA repair
- **Most cancer cells are defective in the G1 checkpoint due to mutations but maintain a functional G2 checkpoint,** which promotes DNA repair thus contributing to the radioresistance of cancer cells.

Irradiation damage normal cells at high doses



Normal tissue damage limits the escalation of RT dosage to eradicate tumor cells

- Radioresistance of tumor cells
- Normal tissue injury



Ultra-high dose rate (FLASH) radiation is reported can improve normal cell survival compared to conventional (CONV) dose rate radiation

FLASH radiation

- > 40Gy/s
- Range: 40 – 5.6 x 10^6 Gy/s

CONV radiation

- ~ 0.1 Gy/s
- Range 4 – 24 Gy/min

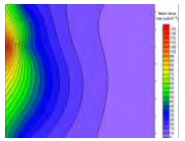
First described by Town, C.D. (Nature 1967, 215: 847–848)

First applied to tumor therapy in mice Favaudon et al, Sci Transl Med 2014, 6: 245)

First patient treatment (Bourhis et al, 2019 Radiother Oncol 139:18–2)

213/225 papers were published during 2019- 2023

Reported parameters for the observed FLASH Effects



Dose rate

- >40 Gy/s (possible)
- 100-150 Gy/s (likely)

Dose /Fraction

- > 10Gy /fraction
- No dose-limiting effect observed in animal models between 15-40 Gy

Radiation type

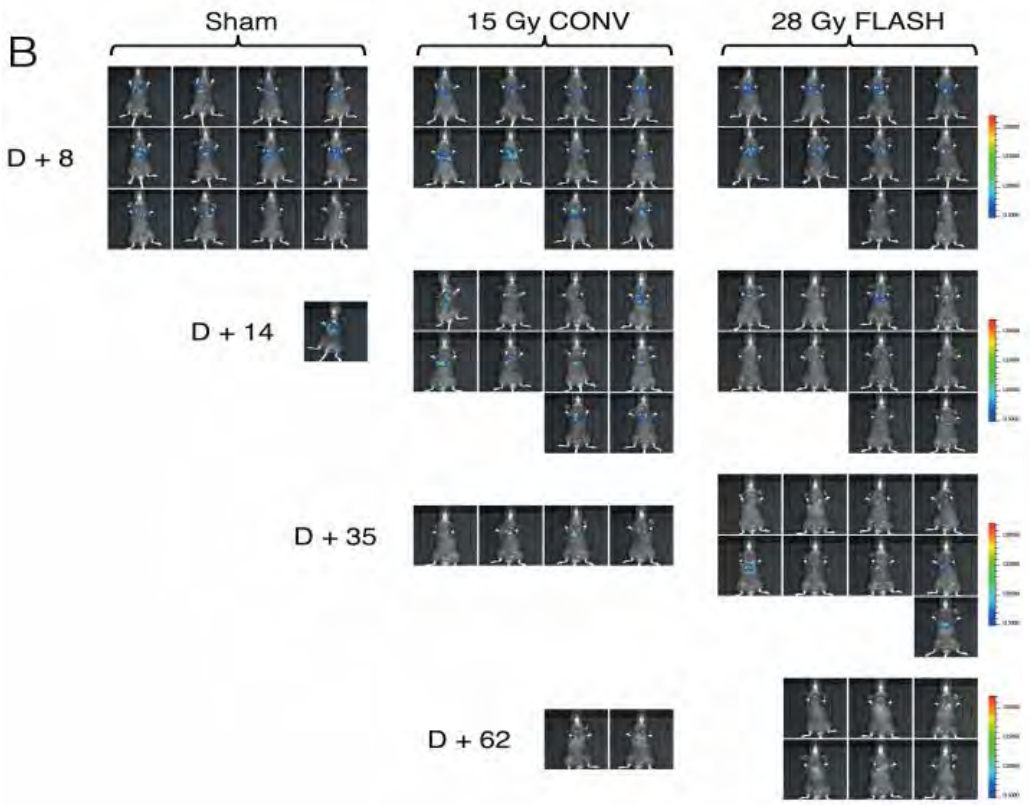
- Most studies were done with electron and proton FLASH radiation
- Fewer works were done with X-rays FLASH radiation

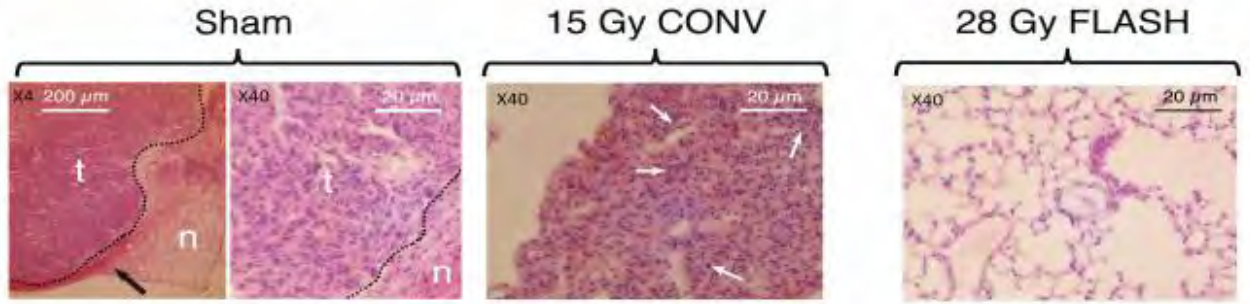
Subjects

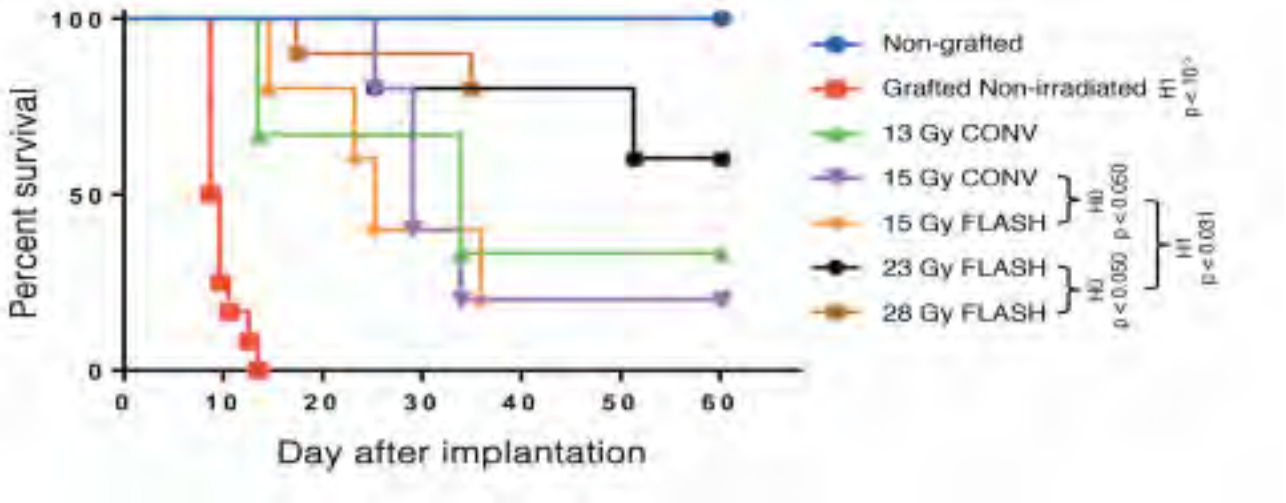
- Most studies used mouse models and a few studies used a pig model
- Ongoing research on humans including 4 clinical trials
- Biological mechanism is still gap of knowledge

Preclinical studies in tumor mice models: FLASH RT eliminates lung injury and improve survival

Tumor xenograft models: 1) HBCx-12A, triple-negative breast cancer; 2) HEp-2 Head-and-neck carcinoma





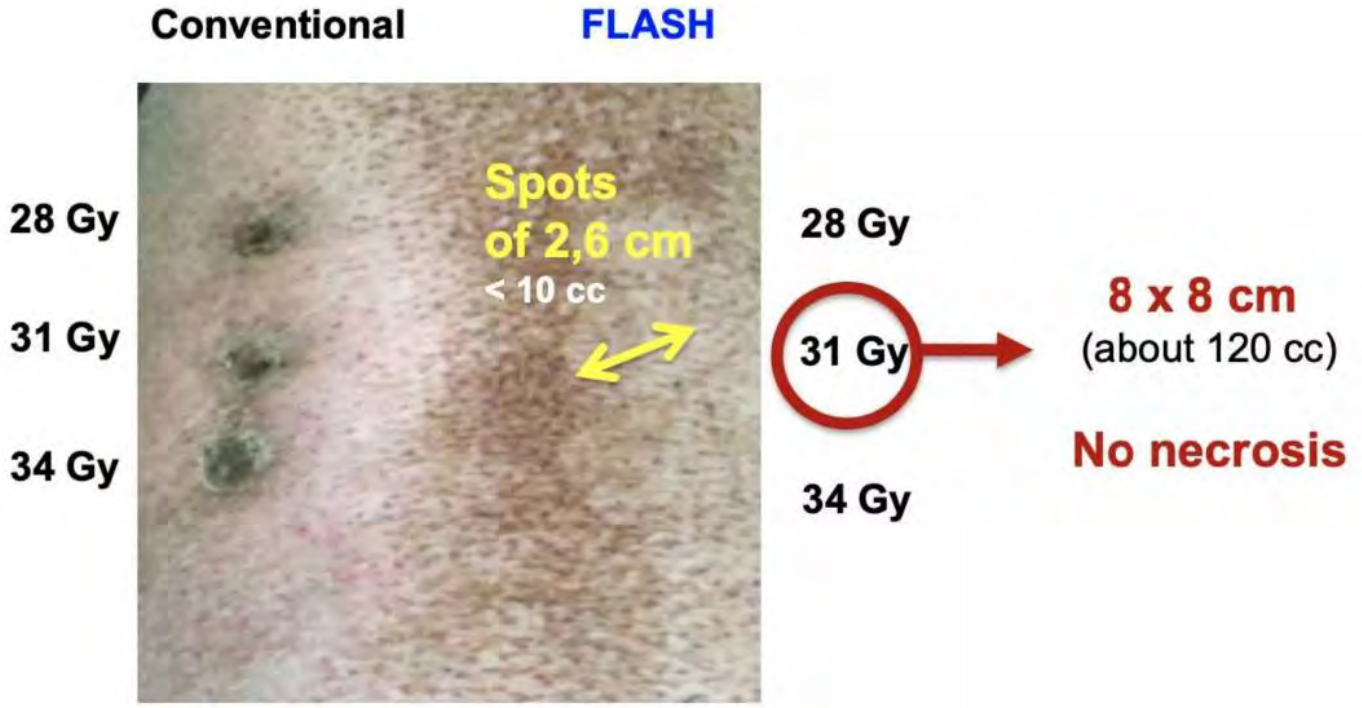


Favaudon et al, Sci Transl Med 2014, 6(245)

Preclinical Studies in a Pig Model:
Pig skin treated with FLASH RT shows no necrosis after 9 months

Pig skin was a irradiated with 6 MeV eradiation at the indicated single dosage with

- CONV IR (5Gy/min or 0.08Gy/s)
- FLASH IR (300 Gy/s)

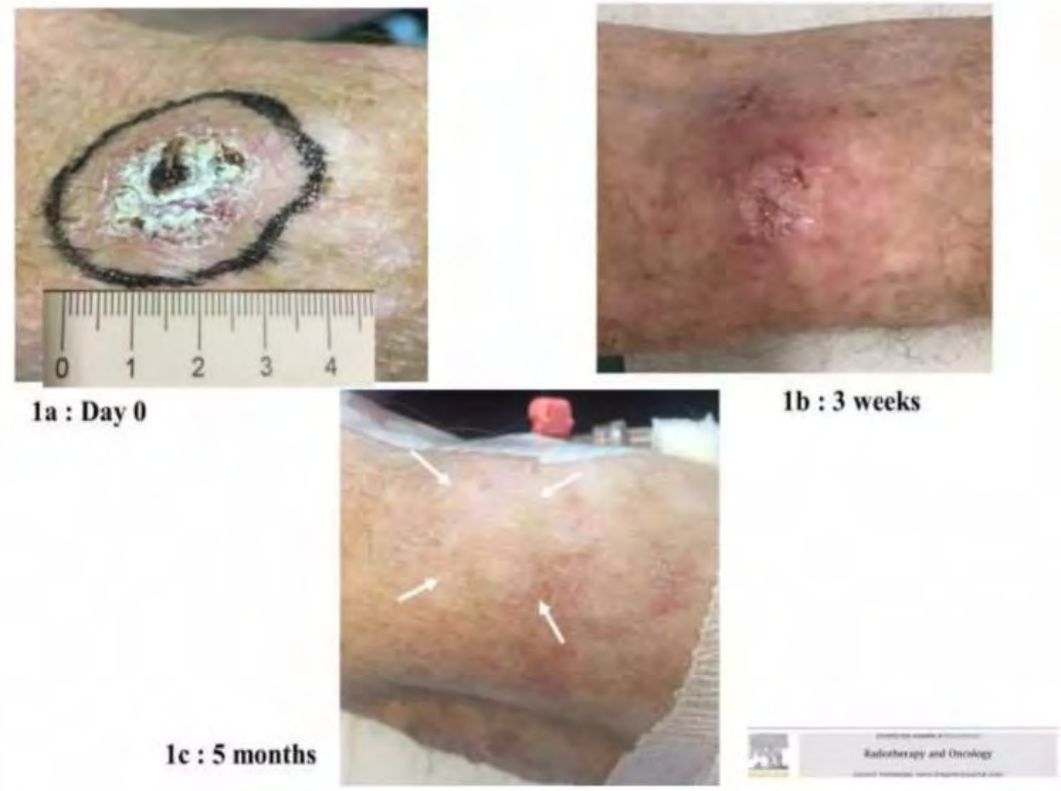


Vozenin et al, 2019 Clin Cancer Res (2019) 25 (1): 35–42

The first patient treatment in 2019: Feasibility and safety test

A patient with muti-resistant T cell Lymphoma:

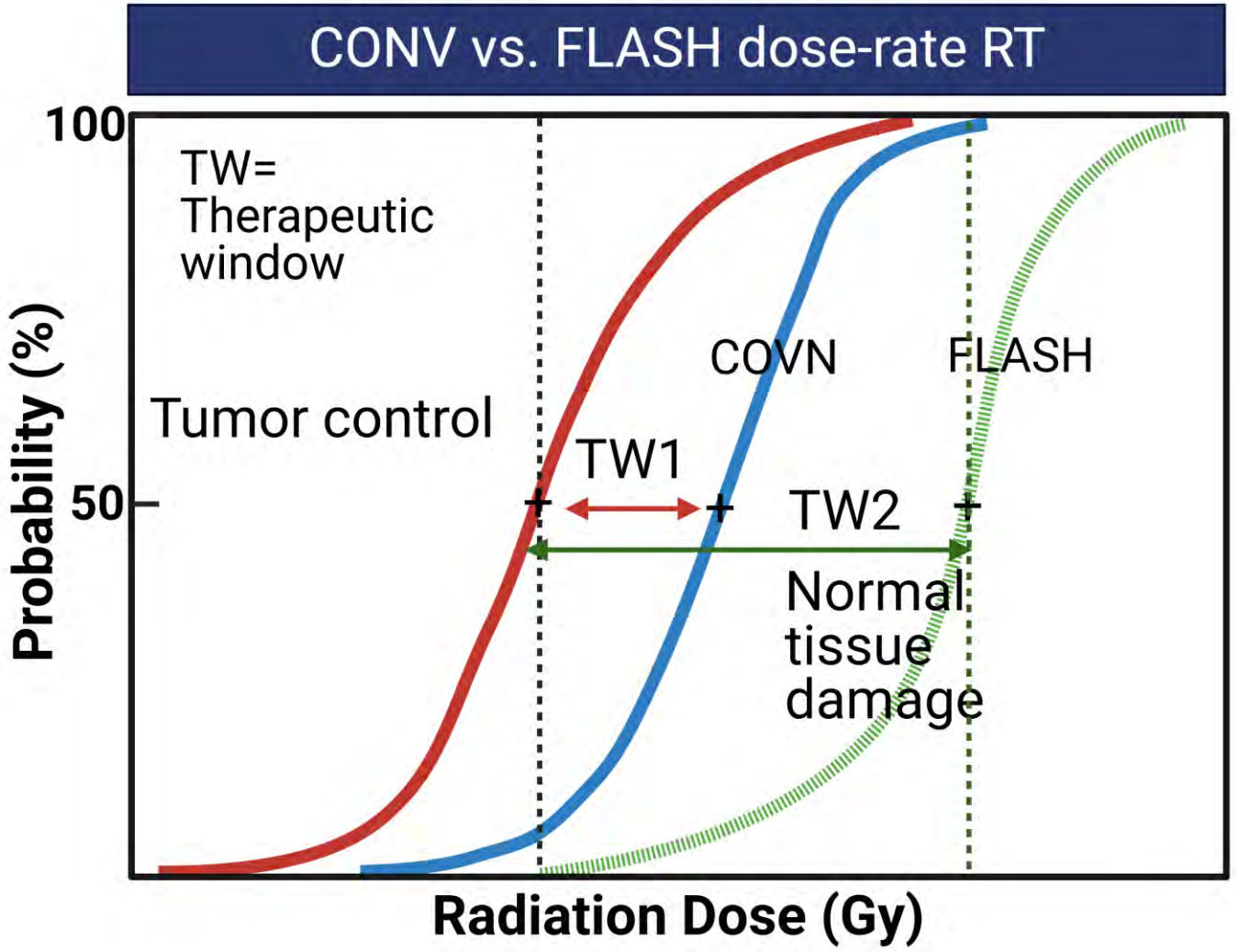
- FLASH (150 Gy/s), 15 Gy in 90 ms



Vozenin et al, 2019 Clin Cancer Res (2019) 25 (1): 35–42

The hypothesis for FLASH RT to improve cancer therapy

- FLASH RT reduces normal tissue damage while maintaining a similar tumor control as CONV RT



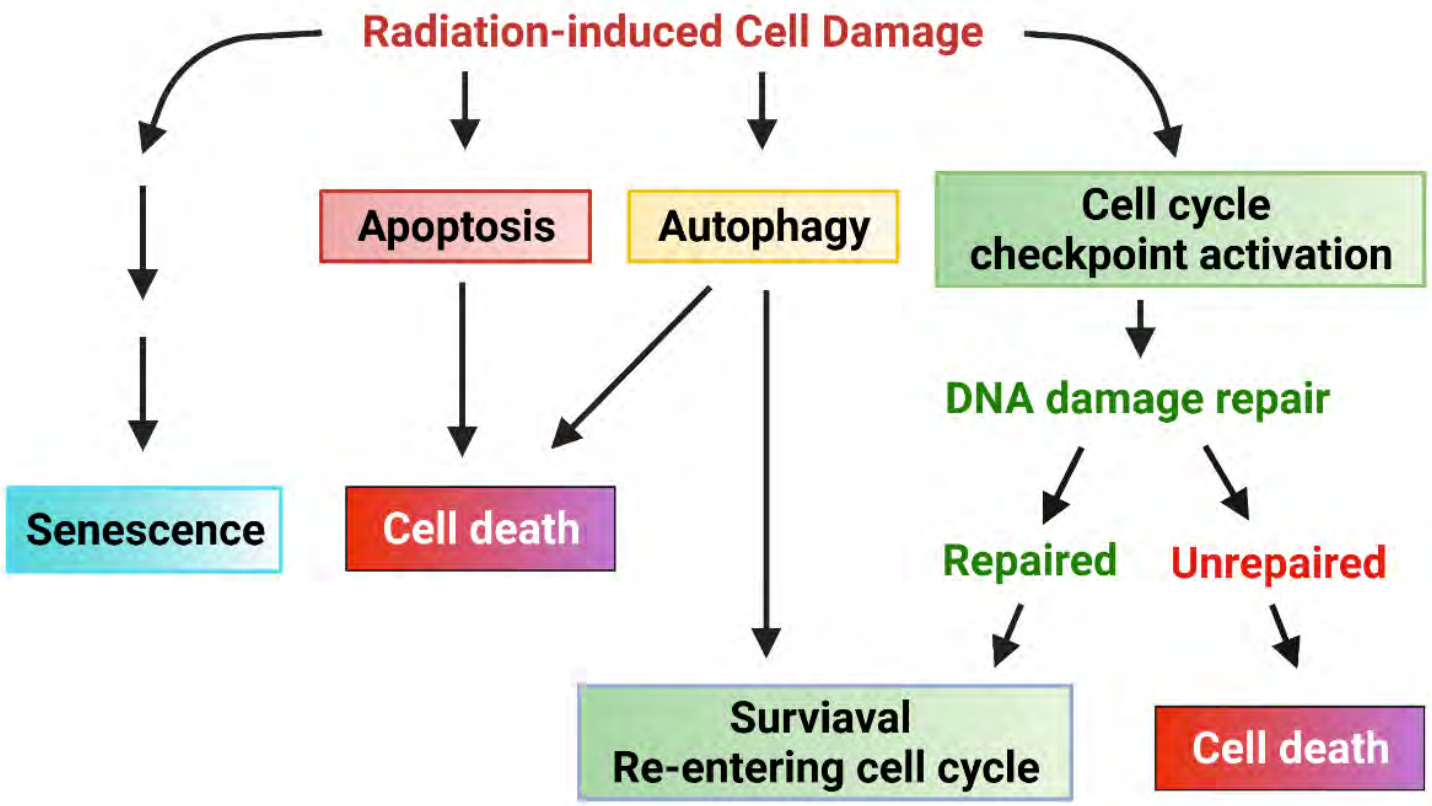
Clinical trials with FLASH radiotherapy for cancer

Titles	Sponsor	#ClinicalTrials.gov ID
FLASH Radiotherapy for Skin Cancer	Centre Hospitalier Universitaire, **Switzerland**	NCT057248750, Phase II (2023-06-26) PI: Olivier Gaide,MD/PhD
Irradiation of Melanoma in a Pulse (IMPulse)	Centre Hospitalier Universitaire Vaudois, **Switzerland**	NCT04986696, Phase I (2021-2022) PI: Lana Kandalaft, Pharm D, PhD
FLASH Radiotherapy for the Treatment of Symptomatic Bone Metastases in the Thorax	Varian. Site: Cincinnati Children's Hospital medical Center, **US**	NCT05524064, Phase I (2023-03-22) PI: John Brenmen, MD
Feasibility Study of FLASH Radiotherapy for the Treatment of Symptomatic Bone Metastases	Varian. Site: Cincinnati Children's proton therapy Center, **US**	NCT04592887 (2020-2021), Phase I PI: John Brenmen, MD

But……

what is the biological mechanism underlying the FLASH effects?

Cellular response to irradiation

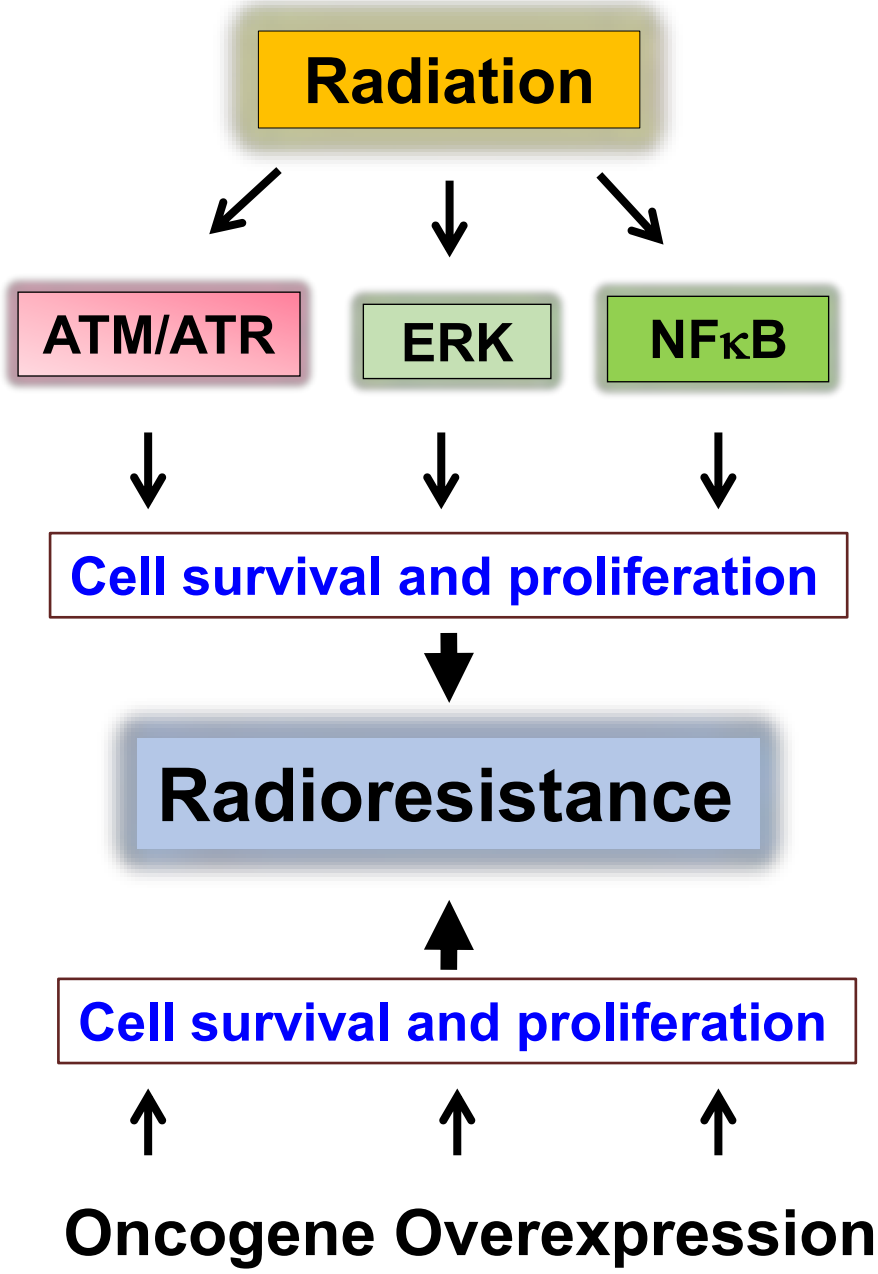


Diagnostics 2022, 12, 656

Signaling pathways promote cell survival

- **Extrinsic pathways**:
  IR-activated DNA repair pathways (e.g., ATM/ATR, ERK, NFκB)

- **Intrinsic pathways**:
  Oncogenes overexpression (e.g., **YAP/TAZ**, c-Myc, β-catenin)



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Radioresistant cancer cells express enhanced anti-apoptotic pathways

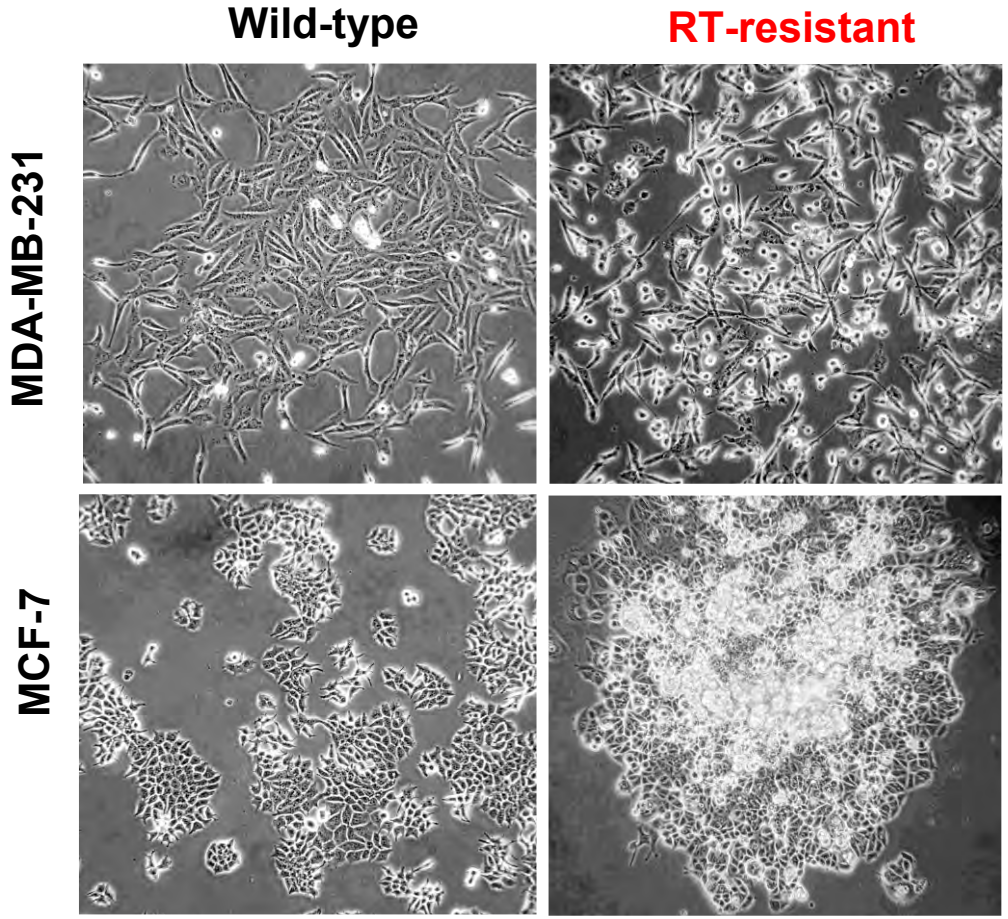
Breast cancer cells

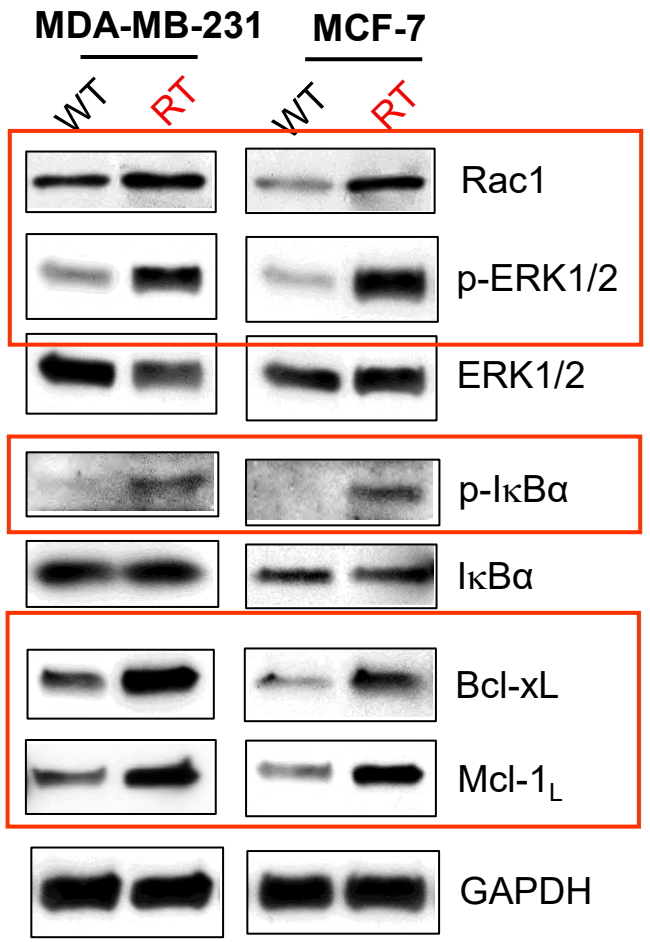
↓

2.5 Gy / fraction, total of 50 Gy

↓

Surviving cells

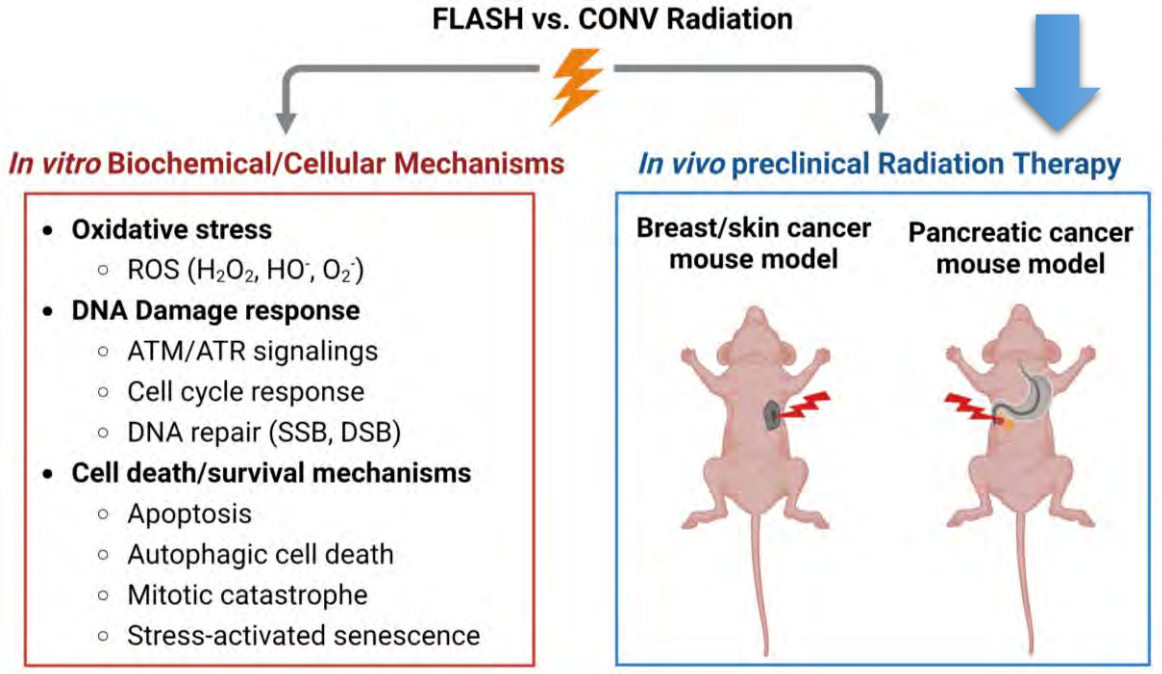




Oncogene, 35(49):6319-29, 2016

Our Strategies to define the mechanisms of the FLASH effect with *in vitro* cellular and *in vivo* mice models

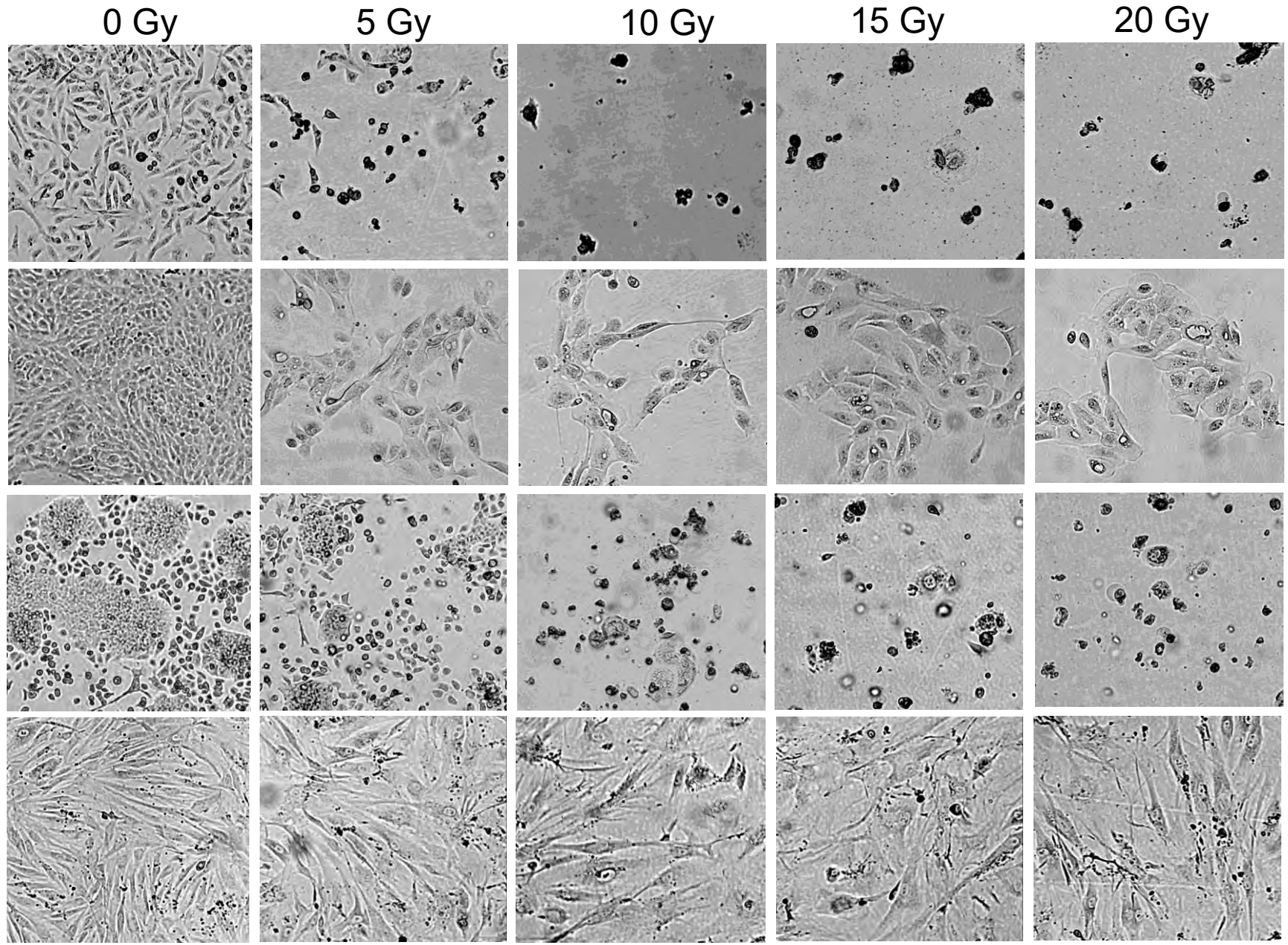
Parameters	FLASH	CONV
E-beam energy	~16 MeV	~15 MeV
Repetition rate	180 Hz	72 Hz
Dose / pulse	1.0 Gy	0.001 Gy
Average dose rate	180 Gy/s	0.067 Gy/s
Instantaneous dose rate (pulse length 5μS)	$2x10^5$ Gy/s	~ 200 Gy/s



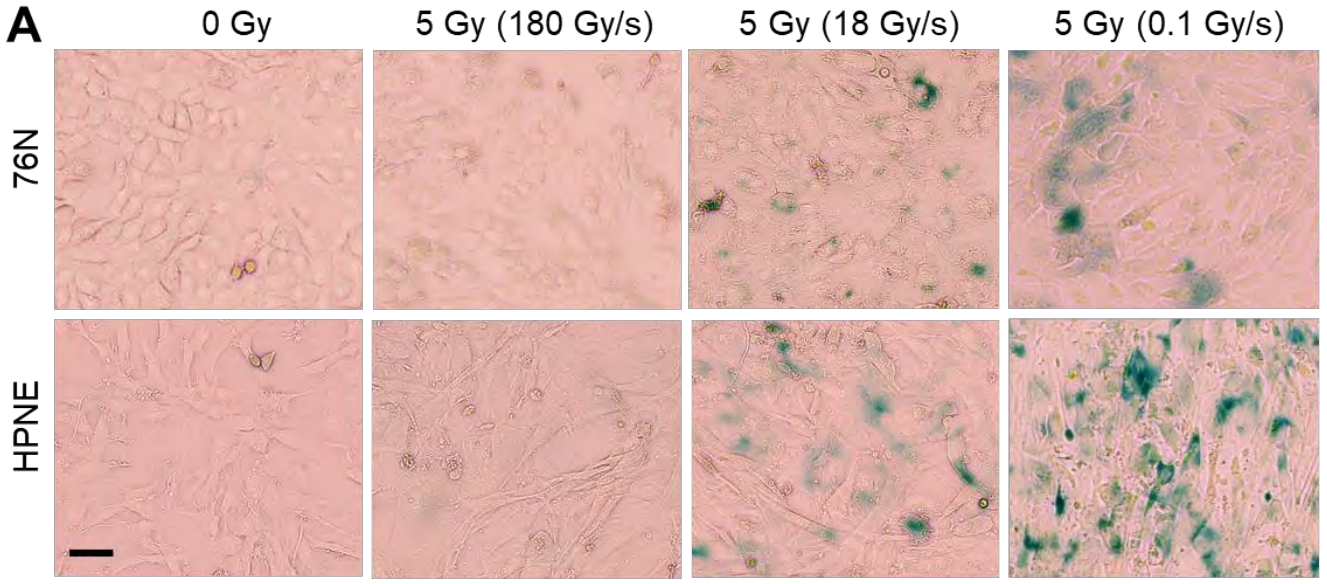
Cellular models for FLASH IR research

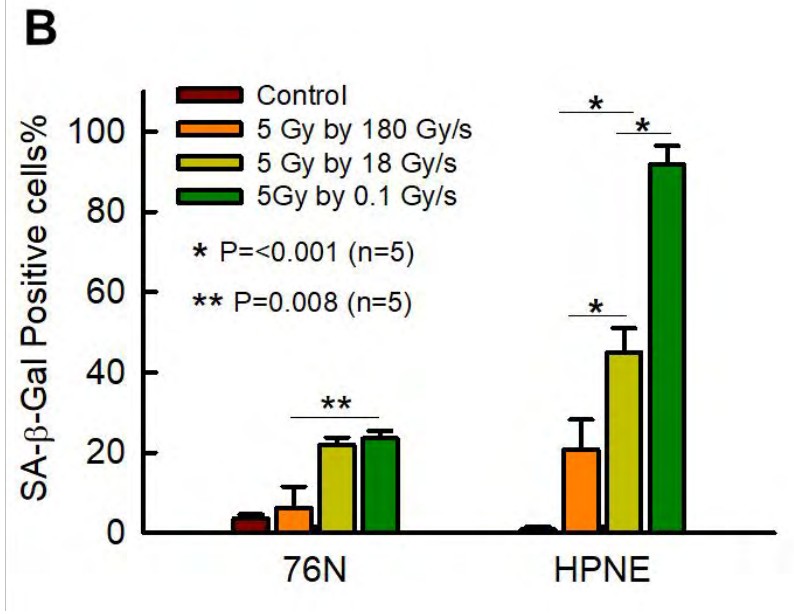
Cell Line	p53	Cell Type	Human organ
76N	Wild-type	**Normal** mammary epithelial	Breast
HPNE	Wild-type	**Normal** Pancreatic ducal	Pancreas
BJ	Wild-type	**Normal** skin fibroblast	Skin
BT549	Mutant	Triple negative breast cancer	Breast
CD18/HPAF	Mutant	Pancreatic ductal adenocarcinoma	Pancreas
A-375	Wild-type	Melanoma	Skin

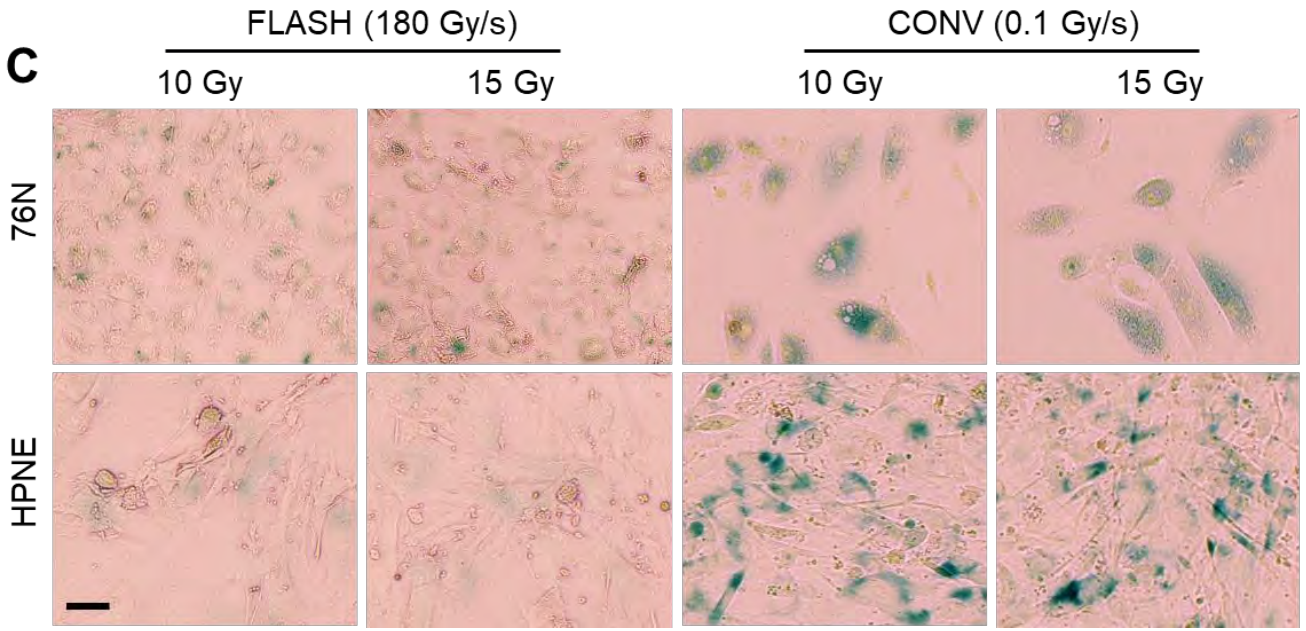
Effect of FLASH-IR on normal and cancer cells after 7 days

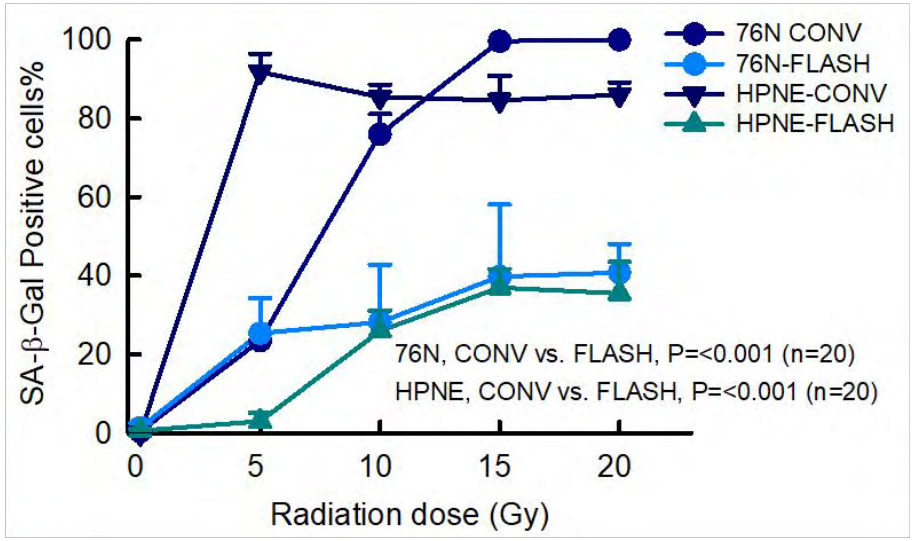


The Effect of FLASH vs. CONV IR on cell senescence



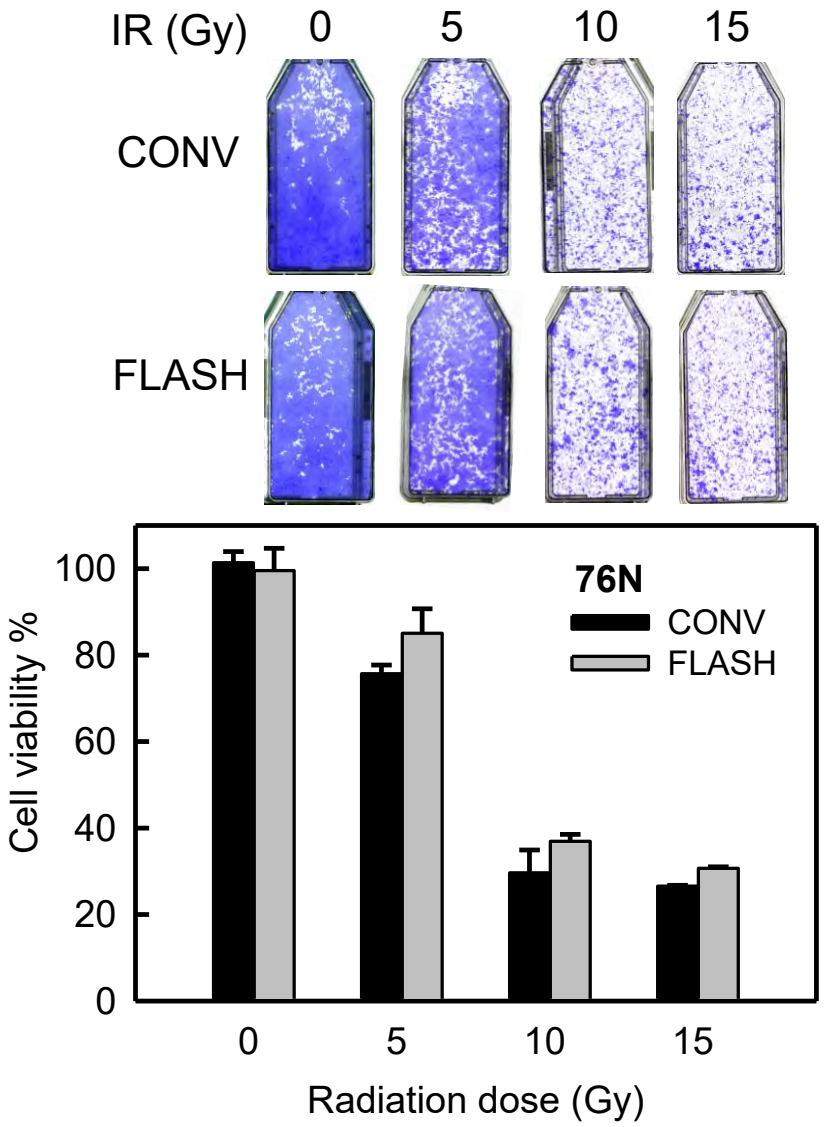




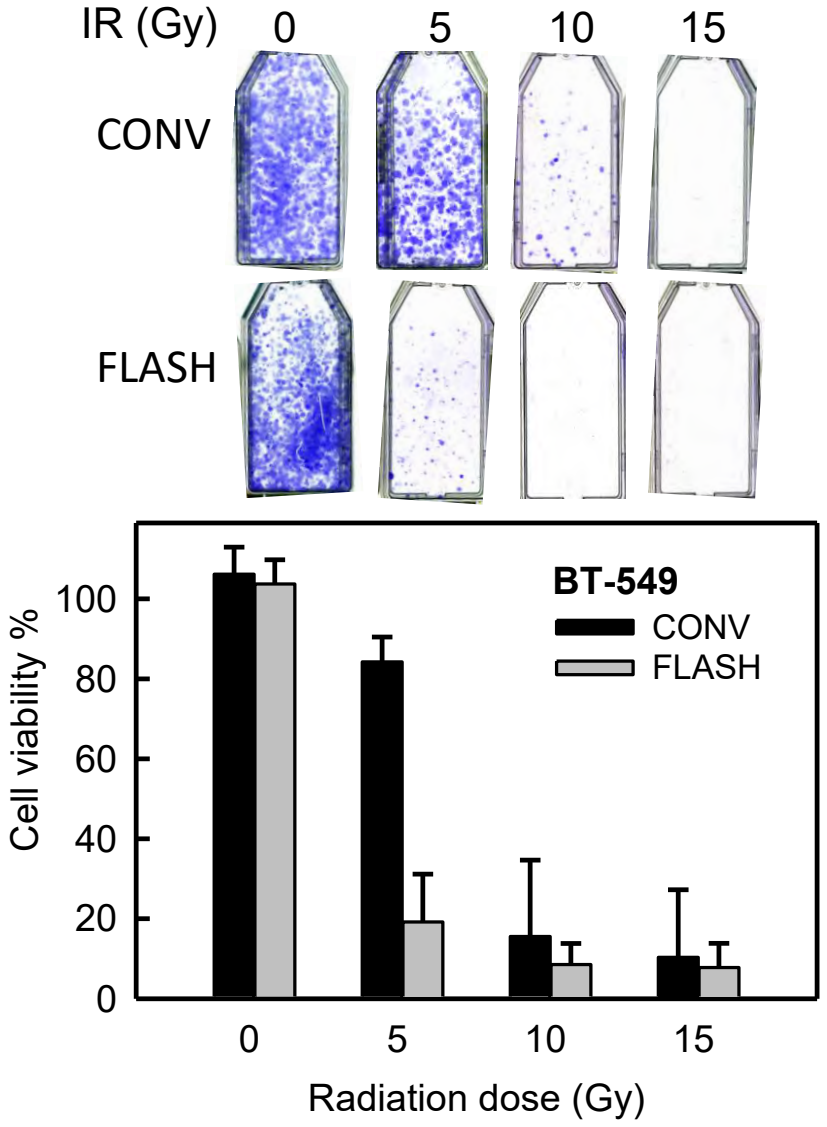


FLASH vs. CONV IR: Effect on breast normal and cancer cell survival

76N (normal mammary epithelial cells)

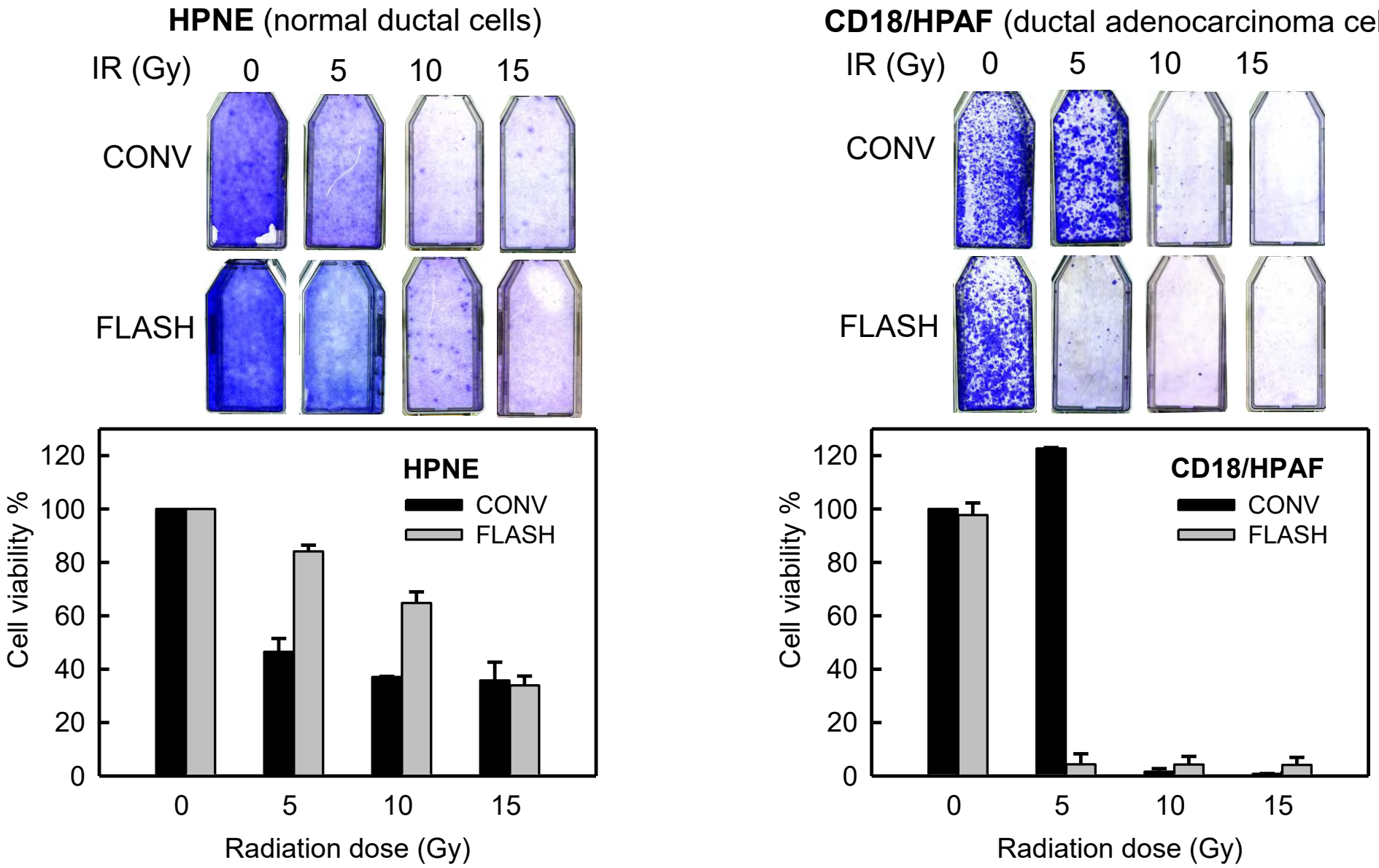


BT-549 (triple-negative breast cancer cells)



- Normoxia (~18-20% O_2); 14 days post IR

FLASH vs. CONV IR: Effect on pancreatic normal and cancer cell survival



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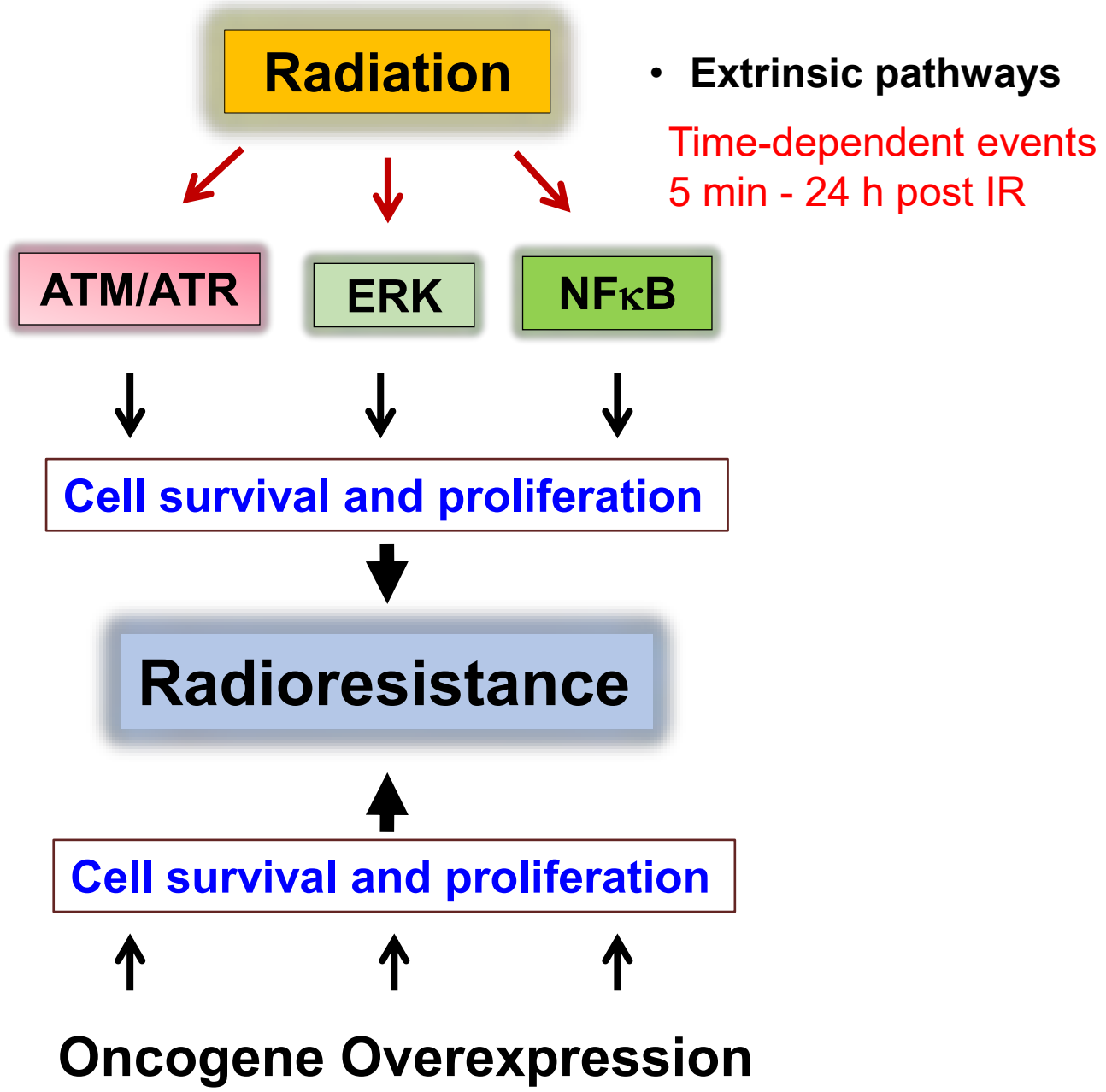

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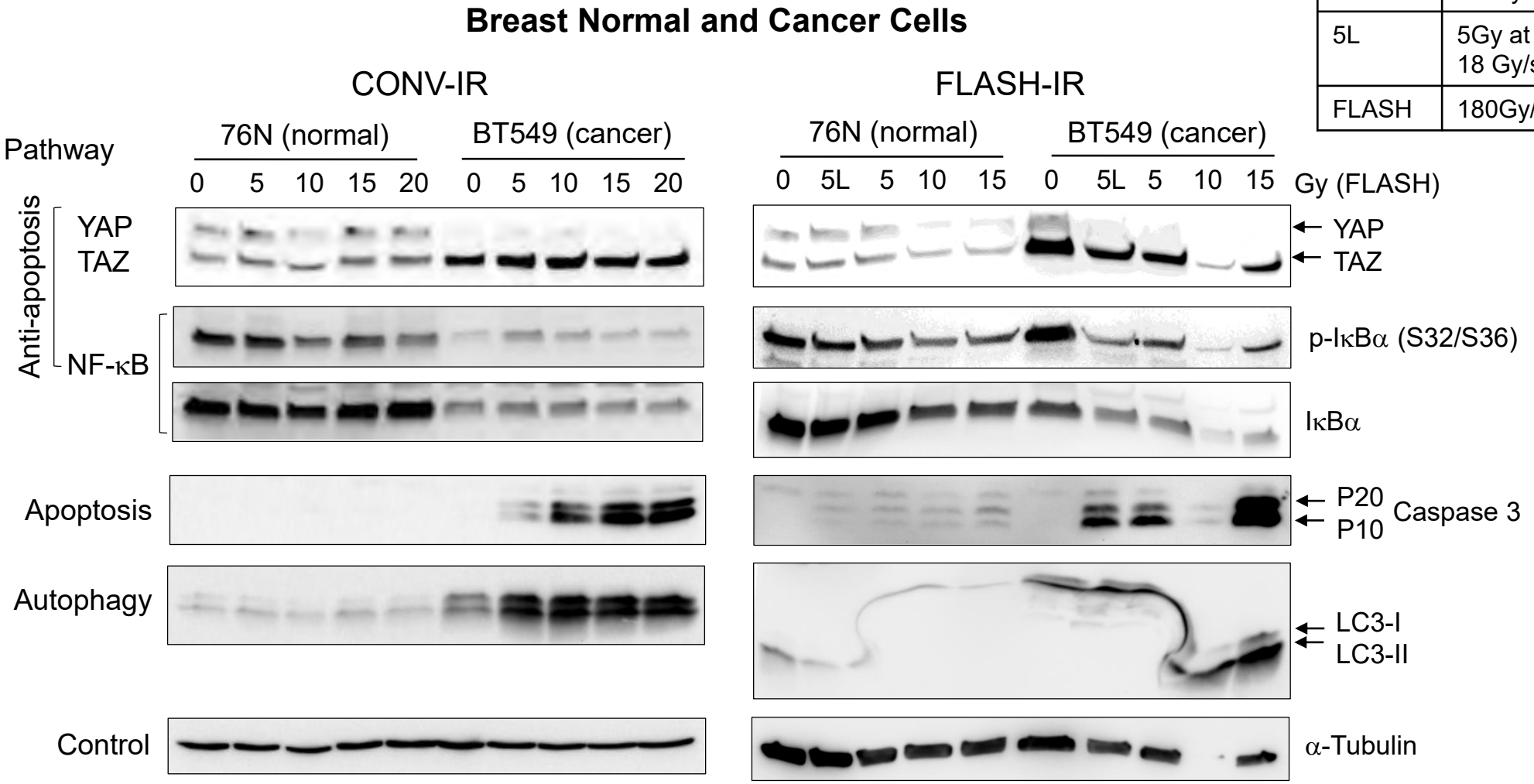
CONV vs FLASH: the effect of radiation on breast normal and cancer cells

CONV	0.1Gy/s
5L	5Gy at 18 Gy/s
FLASH	180Gy/s



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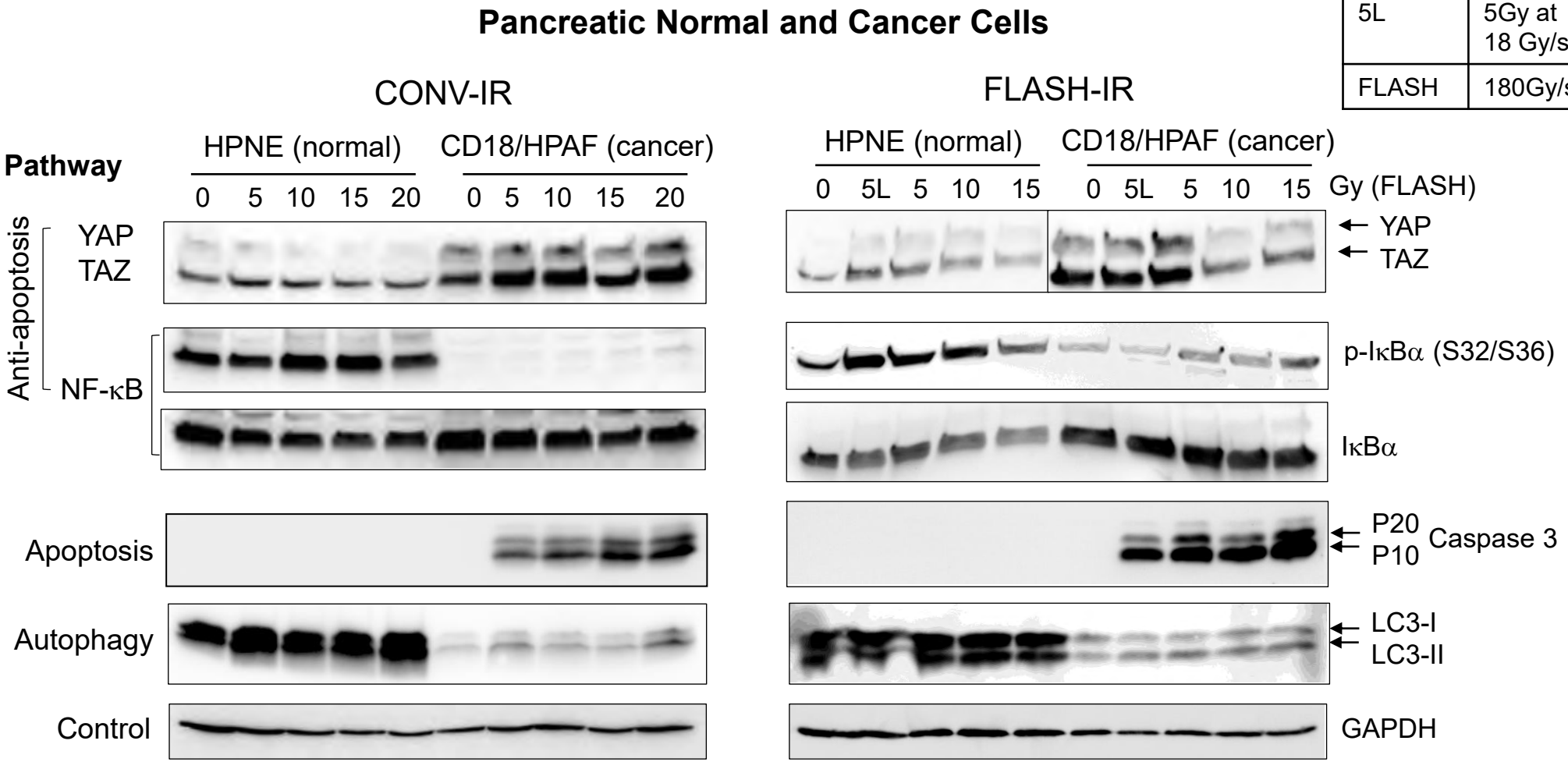

SUMMARY

- FLASH IR enhances radiotoxicity in cancer cells, which involves a decrease in the YAP/TAZ oncogene expression and an increase in apoptosis induction.
- FLASH dose rate diminishes senescence induction in normal cells compared to the CONV dose rate.
- The autophagy pathway appears not involved in the FLASH effect.

Conclusion: FLASH IR produces a better therapeutic ratio between normal and cancer cells than CONV IR

FUTURE DIRECTIONS

- Define the biochemical mechanisms of FLASH effects on normal and cancer cells
  - Define the role of YAP/TAZ in FLASH effects on cancer cells
  - Define the mechanism of the FLASH effects on normal cells with a focus on senescence induction by IR
  - Define the role DNA repair pathways in FLASH effects on normal and cancer cells
- Assess FLASH-radiotherapy for cancer treatment in preclinical mice models of Skin, breast, and pancreatic cancer
- Evaluate the efficacy of the combination therapy (Chemo-/Immuno-therapy and FLASH RT in preclinical tumor mouse models

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