Ultra-high dose rate radiation (FLASH) vs. conventional radiotherapy: Normal tissue toxicity and long-term survival in cGAS-/- and C57BL/6 mice

Banita Thakur[1], **Adel Zaid I. Mutahar**[1], Stavros Melemenidis[2], Suparna Dutt [2], Lucy Whitemore[1], Kerriann M. Casey[3], Kathleen C Horst[2], Edward Graves[2], Michael F Clarke[4], Dr. Lingyin Li[5], Billy Loo[2], Frederick M Dirbas[1]; 1-Department of General Surgery, 2-Department of Radiation Oncology, 3- Department of Comparative Medicine, 4-Stem cell Biology and Regenerative Medicine; 5- Department and ChEM-H Institute; United States of America.

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Abstract

Conventional radiotherapy (CONV RT) effectively controls tumors but often damages normal tissue. Ultra-high dose rate radiotherapy (FLASH RT) may reduce such toxicity. To investigate the role of cGAS-STING pathway in these effects, wild-type and cGAS double knockout (cGAS-/- KO) mice received a single 30 Gy dose of FLASH or CONV RT and were monitored for up to 6 months. C57BL/6 CONV RT mice developed early severe skin toxicity, while FLASH caused minimal damage. cGAS-/- mice showed markedly reduced toxicity with CONV as well as FLASH. Both cGAS loss and FLASH mitigated radiation-induced injury, supporting FLASH RT as a promising, less-toxic alternative to CONV treatment.

Introduction

Radiotherapy is central to cancer treatment, used before or after surgery to reduce or eliminate residual tumor cells. While effective, CONV RT (≤0.03 Gy/s) can cause significant short and long-term side effects that can compromise quality of life [1–3]. Ultra-high dose-rate radiotherapy (FLASH RT > 40 Gy/s) is an emerging approach that is gaining attention for achieving comparable tumor control while sparing normal tissue [4, 5]. RT-induced DNA damage has been shown to activate the cGAS-cGAMP-STING pathway, driving inflammation and tissue damage. Although the precise mechanism of the FLASH effect remains unclear, its reduced normal tissue toxicity suggests that modulation of this pathway may play a role. To test this, we compared long term tissue toxicity and survival (up to six months) following FLASH and CONV RT in C57BL/6 and cGAS-/- mice

Methods and Materials

- C57BL/6 and cGAS-/- mice (6–8 weeks) were irradiated on the left chest with 30 Gy FLASH vs CONV. using a TrueBeam electron linac
- FLASH was delivered with 12.8 MeV electrons (>100 Gy/s, mean ~180 Gy/s; pulse width 3.6 µs, 180 Hz, ~1 Gy/pulse, 30 pulses = 30 Gy). Conventional RT used 11.9 MeV electrons (~0.1 Gy/s). Dosimetry was verified using EBT-XD film and an ionization chamber.
- The mice were monitored regularly over several weeks for skin cracking, hair loss & depigmentation.
- Vetericyn was applied to help with visible skin irritation. Observations focused on how quickly side effects on skin appeared, their severity, and short and long-term healing.
- The skin toxicity was calculated with a graded scoring system with 1 for no injury to 7 for ulceration.

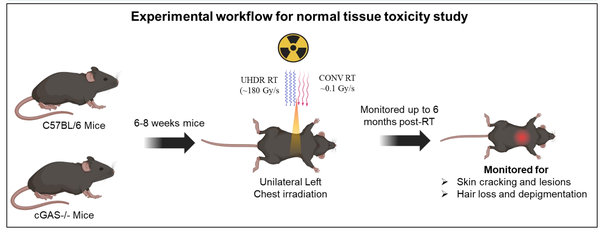


Figure 1: Schematic representation of the experimental design to assess skin toxicity after FLASH and CONV RT: Wild-type (C57BL/6) and cGAS-/- mice received a single 30 Gy dose of either FLASH or CONV irradiation to the left thoracic region. Mice were monitored for up to 6 months post-RT for skin cracking, lesions, hair loss, and depigmentation to evaluate normal tissue response.

Results

- C57BL/6 wild type mice treated with CONV demonstrated, skin cracking, hair loss, and hair depigmentation within the first few weeks after radiation (Score 4) (Figure 2 & 3).
- Effects were most noticeable early but gradually improved (aside from hair depigmentation) with signs of healing by the end of the 6-month observation period.
- In cGAS-/- mice treated with CONV, side effects were milder with slight hair loss & mild skin changes (Score 2).
- In contrast, mice treated with FLASH showed no skin damage in C57BL/6 as well as cGAS-/- groups (Score 0).
- All mice remained stable and healthy throughout the study with none requiring euthanasia.
- These findings confirm that FLASH mitigates normal tissue toxicity vs CONV.
- There was again no skin toxicity in the cGAS-/- mice with FLASH and less skin toxicity with CONV.

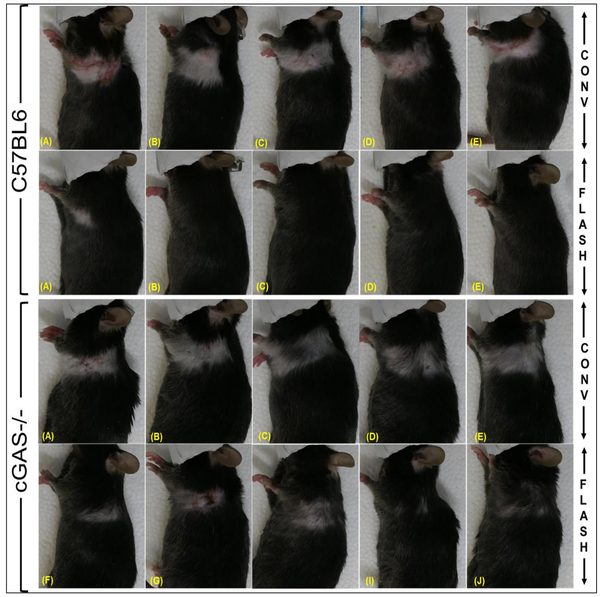


Figure 2: Pictures showing skin toxicity 45 days post-radiation in C57BL/6 and cGAS-/- mice after Unilateral Left Chest (CONV or FLASH) RT: C57BL/6 CONV mice displayed severe early skin toxicity, while the FLASH group showed no visible damage. In cGAS-/- mice, CONV RT caused only mild lesions, and FLASH treatment further reduced toxicity compared to their respective CONV controls.

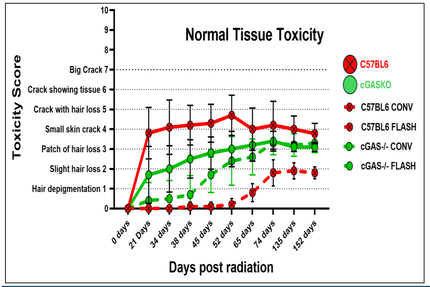


Figure 3: Long-term skin toxicity following CONV and FLASH RT in C57BL/6 and cGAS-KO mice: CONV-treated C57BL/6 mice showed early, severe toxicity, while FLASH caused delayed, mild effects. cGAS-KO mice also had reduced toxicity in both groups.

Discussion

- Our findings demonstrate that FLASH RT significantly reduces radiation-induced skin toxicity compared with CONV RT.
- The protective effect was observed in both wild-type and cGAS-/- mice, suggesting that cGAS signaling contributes to radiation-induced inflammation and tissue injury.
- The absence of toxicity in FLASH-treated groups indicates that normal tissue sparing by FLASH may occur independently or downstream of cGAS activation.
- These results support further investigation into the molecular basis of the FLASH effect and its potential to improve the therapeutic window of RT.

Conclusions

- Inactivation of the cGAS pathway is associated with less early radiation-related inflammation and tissue damage.
- FLASH RT resulted in much lower or no skin toxicity in both wild type and cGAS-/- mice.
- These findings support FLASH RT as a promising, less toxic alternative to CONV RT.
- The lack of significant toxicity with FLASH in both wild type and cGAS-/- mice suggests that further investigation is needed at higher doses to determine if the FLASH effect is mediated by cGAS-cGAMP-STING pathway.

Contact



Banita Verma, PhD
Stanford University
banita@stanford.edu

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Frederick M. Dirbas, MD
Principal Investigator

Dirbas Lab



Banita Verma
Post Doctoral Researcher



Adel Zaid Mutahar
Post Doctoral Researcher

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