functional and pathological changes in healthy eyes following FLASH or conventional (CONV) dose rate irradiation.

Materials/Methods: An immobilization device was designed to enable focal irradiation to a single mouse eye using a FLASH-capable rotating anode x-ray tube. A 5 mm diameter lead collimator was manufactured to encompass the 3 mm target of the entire eye. Dose and dose rate measurements were performed with calibrated radiographic EBT3 films. Cone-beam CTs were acquired of four C57BL6J mice in immobilization to confirm setup reproducibility, quantified by the mean Hausdorff distance between bone segmentations. Healthy 8-week-old C57BL6J mice were irradiated with 150 kVp x-rays to doses of 21 Gy or 34 Gy at FLASH (right eye) and CONV (left eye) dose rates, respectively. Both eyes were irradiated to limit the influence of varying baseline vision between animals in our analysis. Visual acuity was assessed in 3 mice per dose level using scotopic electroretinography (ERG) up to 2 months post irradiation. Histopathological changes were assessed through H&E staining of harvested eyes.

Results: Mouse setup in our immobilization device was highly reproducible, with a mean Hausdorff distance of 0.34 ± 0.10 mm. Measured dose rates within the field were 67.0 ± 1.9 Gy/s and 1.2 ± 0.1 Gy/s at FLASH and CONV settings, respectively. ERGs revealed that FLASH-irradiated eyes at 21 Gy retained visual function up to 2 months post irradiation, while 21 Gy CONV induced blindness at all sampled time points with more severe surrounding skin effects. At 34 Gy, all eyes were blinded within 1 week, with comparable skin toxicities in the irradiated areas regardless of dose rate. Pathological assessment showed a loss of the photoreceptor layer of the retina from CONV irradiation, which remained intact in FLASH-treated mice.

Conclusion: We have developed a novel platform to study x-ray FLASH effects from ocular irradiation in mice. Functional ERG assay revealed a preservation of visual function from 21 Gy at FLASH dose rates that was not present from 21 Gy CONV. Differential damages between FLASH and CONV irradiations were confirmed through histopathology. This first demonstration of FLASH normal tissue sparing effects in a mouse eye model presents a unique and promising translation opportunity for clinical FLASH treatment. Further studies are ongoing to explore the long-term effects of FLASH radiation on vision and its efficacy on intraocular tumors.

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Enhanced Radiation-Sparing Effects of Ultra-High Dose Rate Proton Radiation (FLASH-RT) in a Human Induced Pluripotent Stem Cell-Derived Cerebral Organoid Model

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Purpose/Objective(s): Superior normal tissue sparing with similar antitumoral effectiveness has been observed in preclinical animal models following delivery of radiation at ultra-high dose rates (FLASH-RT), relative to conventional dose rates (CONV-RT). Clinical application of FLASH-RT could reduce devastating radiation-related side effects for patients with aggressive brain tumors such as glioblastoma; however, the FLASH effect must be established in representative models of human systems before its implementation in the clinic. Our objective is to measure relevant response outcomes to proton FLASH-RT using advanced cerebral organoid (CO) models of normal and diseased human brain tissue, correspondingly generated with human induced pluripotent stem cells and patient-derived glioma stem-like cells (GSCs).

Materials/Methods: Mature COs were irradiated to 9 Gy using conventional (CONV, 0.2 Gy/s) and FLASH (100 Gy/s) dose rates using a proton beam and prepared for histological analysis at 30 days post-irradiation. Spatial distribution of hypoxia was visualized in COs by incubating for 2 hours with pimonidazole prior to preparation for histologic analysis. Image analysis was performed with QuPath. Lactate dehydrogenase (LDH) release, a marker of cell death, was sequentially quantified. COs implanted with GSCs expressing a luciferase reporter were co-cultured for 14 days and irradiated as above. Tumor cell proliferation was tracked via luciferase expression.

Results: At 2- and 4-days post-irradiation, there was an increase in LDH release for CONV versus FLASH-radiated COs ($p<0.01$, $p<0.01$ respectively) which normalized after 9 days. At 30 days, when specific cell populations in FLASH-radiated COs were histologically compared to CONV-radiated COs, there were higher numbers of proliferating neural progenitor cells (SOX2+/Ki67+, $p<0.01$), mature neurons (NeuN+, $p=0.03$), and deep cortical surface layer neurons (SATB2+, $p=0.01$). The spatial distribution of neural progenitor cells and mature neurons overlapped with hypoxic regions of the CO (pimonidazole+). A trend towards lower expression of activated microglia (IBA1+/CD68+) in FLASH-irradiated COs compared to CONV-radiated COs was noted ($p=0.12$). A 48.6% versus 51.2% reduction in tumor proliferation in FLASH versus CONV-irradiated GSC-laden-COs was observed at 4d post-irradiation ($p=0.98$).

Conclusion: We report preliminary findings of normal tissue toxicity reduction and iso-effective tumor response in a human-derived brain model following proton FLASH-RT. Potential protective effects following FLASH-RT to be further validated include preservation of specific cell populations within hypoxic niches including progenitor and differentiated neurons with a corresponding reduction in chronic neuroinflammation.

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The Immune Response and Intestinal Injury after X-Ray FLASH Irradiation in Murine Breast Cancer Transplanted Models

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Purpose/Objective(s): The present study investigated the anti-tumor effect, immune response, and intestinal injury in breast cancer-bearing mice after ultra-high dose rate radiotherapy (FLASH-RT) and conventional dose rate radiotherapy (CONV-RT).

Materials/Methods: Six-week-old female C57BL/6 mice were inoculated subcutaneously with Py8119 and Py230 breast tumor cells in the inguinal mammary gland, and received 10 Gy abdominal 6 MeV X-ray FLASH-RT (>100 Gy/s) or CONV-RT (0.2 Gy/s) 15 days after tumor inoculation. EBT3 radiographic films were used to confirm the dose. Tumor samples were obtained 2 weeks post-irradiation (PI) in Py8119 tumor-bearing mice, spleen tissues were collected 4 weeks PI in Py230 tumor-bearing mice. Flow cytometry (FC) was performed with tumor and spleen tissues and immunohistochemistry (IHC) staining was performed in tumor tissues to