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Scientific Article

Preclinical Ultra-High Dose Rate (FLASH) Proton Radiation Therapy System for Small Animal Studies

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Purpose: Animal studies with ultrahigh dose-rate radiation therapy (FLASH, >40 Gy/s) preferentially spare normal tissues without sacrificing antitumor efficacy compared with conventional dose-rate radiation therapy (CONV). At the University of Washington, we developed a cyclotron-generated preclinical scattered proton beam with FLASH dose rates. We present the technical details of our FLASH radiation system and preliminary biologic results from whole pelvis radiation.

Methods and Materials: A Scanditronix MC50 compact cyclotron beamline has been modified to produce a 48.7 MeV proton beam at dose rates between 0.1 and 150 Gy/s. The system produces a 6 cm diameter scattered proton beam (flat to ± 3%) at the target location. Female C57BL/6 mice 5 to 6 weeks old were used for all experiments. To study normal tissue effects in the distal colon, mice were irradiated using the entrance region of the proton beam to the whole pelvis, 18.5 Gy at different dose rates: control, CONV (0.6-1 Gy/s) and FLASH (50-80 Gy/s). Survival was monitored daily and EdU (5-ethynyl-2′-deoxyuridine) staining was performed at 24- and 96-hours postradiation. Cleaved caspase-3 staining was performed 24-hours postradiation. To study tumor control, allograft B16F10 tumors were implanted in the right flank and received 18 Gy CONV or FLASH proton radiation. Tumor growth and survival were monitored.

Results: After 18.5 Gy whole pelvis radiation, survival was 100% in the control group, 0% in the CONV group, and 44% in the FLASH group ($P < .01$). EdU staining showed cell proliferation was significantly higher in the FLASH versus CONV group at both 24-hours and 96-hours postradiation in the distal colon, although both radiation groups showed decreased proliferation compared with controls ($P < .05$). Lower cleaved caspase-3 staining was seen in the FLASH versus conventional group postradiation ($P < .05$). Comparable flank tumor control was observed in the CONV and FLASH groups.

Conclusions: We present our preclinical FLASH proton radiation system and biologic results showing improved survival after whole pelvis radiation, with equivalent tumor control.

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Research data are stored in an institutional repository and will be shared upon request to the corresponding author.

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Introduction

Radiation therapy (RT) is one of the major methods for cancer treatment, with more than half of cancer patients receiving radiation at some time point. Advanced radiation techniques have been developed in the past decades to more precisely target the tumor and reduce normal tissue toxicities.[1] In recent years, there has been tremendous interest in ultrahigh dose-rate radiation (FLASH, with dose rate >40 Gy/s) for cancer treatment due to the observed "FLASH effect" in multiple preclinical studies—a phenomenon that preferentially spares normal tissues without sacrificing antitumor efficacy compared with conventional dose-rate radiation (CONV).[2] The FLASH effect has been reported in several in vivo models spanning multiple body sites and normal tissues including the brain, lungs, and intestinal tract.[3-9]

Conventional x-ray RT remains one of the major therapies for cancer, but current clinical x-ray radiation facilities cannot produce ultrahigh-dose-rates in excess of the 40 Gy/s required for FLASH radiation. However, many clinically used proton accelerators can be upgraded to produce ultrahigh-dose-rate radiation for patient treatment. Interest in FLASH proton RT has increased in recent years and published studies have shown promising results.[10-13]

Although studies have been published to demonstrate the capability of FLASH proton radiation in reducing normal tissue toxicities and improve overall survival while preserving tumor control capability, more studies are needed to understand the mechanism of FLASH proton RT, and to support translation of the technique to routine clinical application.[14,15] Additional research is needed to understand both the technical requirements needed to both produce and confirm the FLASH effect (preferential normal tissue sparing) and investigate the biologic underpinnings of this effect. In this article, we present our institution's preclinical proton FLASH radiation system, which has been used to conduct investigations of FLASH radiation in the mouse pelvis. Our system uses a 48.7 MeV cyclotron-generated preclinical proton beam with a continuous slowing down range of about 18 mm in water for mice studies. Our preclinical system produces ultrahigh-dose-rate radiation under conditions that are distinct from all other published literature, adding to the scientific community's ability to explore the technical requirements to produce the FLASH biologic effect. Compared with FLASH radiation to the abdomen as published by other groups,[10] radiation to the mouse pelvis can produce a survival difference between the FLASH and conventional dose rate arms in <15 days, versus around 20 to 25 days for abdominal radiation.

We present preclinical mouse pelvic radiation studies demonstrating that our ultrahigh-dose-rate proton radiation system produces better survival after FLASH radiation versus conventional dose rate radiation, and this improved survival is associated with normal tissue sparing in the distal colon, with equivalent tumor control under the conditions studied. We also present a new biologic endpoint for FLASH experiments with a relatively fast (<15 days) and easy to measure readout (survival versus death). This will facilitate future experiments exploring the technical requirements to generate the FLASH biologic effect, including experiments testing dose rates, dose thresholds, beam pauses, and other technical parameters.

Methods and Materials

Proton system design and beam delivery

The University of Washington hosts a Scanditronix MC50 compact cyclotron that generates a 50.5 MeV (peak energy) proton beam of 2 cm diameter directed to center on a fully stopping 5.6 mm diameter graphite collimator and a 0.9 mm thick graphite scatterer. The scattered proton beam then travels 20 m down a 6.3 cm diameter evacuated beampipe and exits through a Kapton window. This results in a 6 cm diameter scattered proton beam (flat to $\pm$ 3%) at the target location 1 m downstream from the Kapton window (Fig. 1) that has a beam energy 48.7 MeV. More details regarding this system can be found in prior publications.[16,17] All mice were irradiated in the entrance region of the 48.7 MeV beam. Reported dosimetry is based on a constant relative biologic effectiveness = 1.0 (high-energy entrance plateau region of the beam). The FLASH dose rate ranged from 50 to 80 Gy/s and the CONV dose rate ranged from 0.6 to 1 Gy/s.

Proton beam dosimetry

Absolute dose was measured using the Advanced Markus chamber (PTW) in a small water tank connected to a factory calibrated Keithley 6517B electrometer (Keithley). The Advanced Markus chamber was cross calibrated at our clinical proton facility, Fred Hutchinson Proton Center, using a NIST traceable PPC05 chamber (IBA Dosimetry).

During irradiation, dose is monitored using a PTW 60019 microDiamond detector (PTW) positioned at the edge of the beam, connected to a Pyramid I128 Electrometer (Pyramid), which can turn the beam off once the desired dose is reached (Fig. 1). The diamond detector is calibrated daily against the Advanced Markus chamber. The ratio between the readings from the Advanced Markus chamber and the microDiamond detector was measured at different dose rates and the dose per nC of the microDiamond detector was determined.

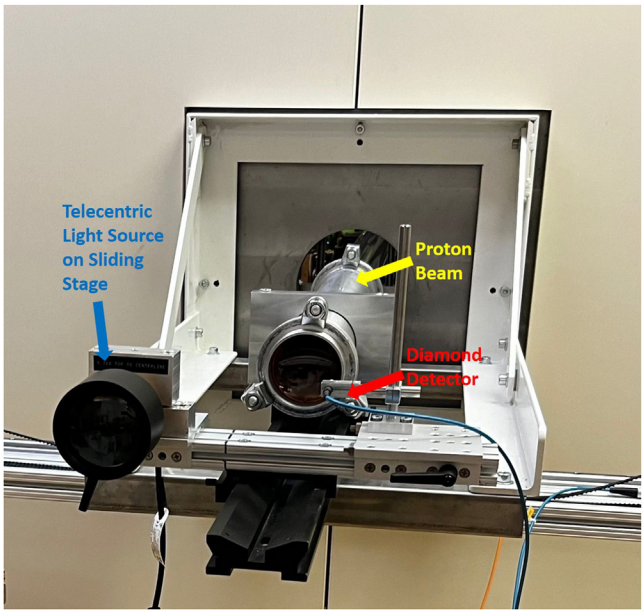

Figure 1 Proton beam exit window, microDiamond dose detector, and telecentric light source.

For each day of radiation, Gafchromic EBT3 film (Ashland) was used to verify dose delivered by placing a 3 × 3 cm piece of film in both the radiation entrance and exit side of the mouse. Uniform CONV and FLASH beams were produced and verified with EBT3 film measurements (Fig. 2). Pelvis tissue thickness was approximately 6 mm in our experimental setup (Fig. 3), and the depth dose gradient was approximately 20% between entrance to exit in the mice. All reported doses in this study are the entrance dose unless otherwise stated. A graphic user interface allowed investigators to set the dose, deliver beam, and record dose, dose rate, and delivery time.

The percent-depth-dose (PDD) curve of the beam is shown in Fig. 2B, as measured using the Exradin A11 ion chamber. In our experimental setup (Fig. 3), the thickness of the mouse body is 6 mm, therefore using the PDD curve, the ratio of the exit dose to the entrance dose is 1.17, which is consistent with measured film data. The reported dose rate is at the entrance of the mouse, and the dose rate at the exit of the mouse will be ∼1.17 times of the reported dose rate.

Animal studies

All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee. A 6-axis Mecademic Meca500 robotic arm wirelessly controlled by a Microsoft Xbox One controller acted as the mouse support and positioning assembly, with a 3-dimensional printed mouse bed attached to the robot as the end effector (Fig. 3A). The scattered proton beam is collimated to match the target area with variable jaw collimators constructed from custom graphite blocks, PanaVise Model 376 vise heads, and 80/20 framing profiles and motion accessories (Fig. 3A-B). The mouse is aligned against a laser produced coordinate system defining the coronal, sagittal, and transverse planes. The final proton field shape on target is verified with a telecentric light field produced by an Advanced Illumination 625 nm High Intensity Coaxial Spotlight coupled with an Edmund Optics 60 mm Telecentric Backlight Illuminator lens (Fig. 3B). The light source is centered on the beam axis for verification and slides out of the way before irradiation.

C57BL/6 female mice 5 to 6 weeks old (The Jackson Laboratory) were used for studies. Ketamine/xylazine (87.5 mg/kg ketamine, 2.5 mg/kg xylazine) was used to anesthetize mice for procedures. Typically, 6 to 8 mice were assigned per treatment arm: (1) control (which is treated with sham radiation); (2) conventional dose rate radiation (CONV); and (3) ultrahigh-dose-rate radiation (FLASH). Experiments were conducted to study both normal tissue effects of radiation and tumor control effects.

To study survival after whole pelvis radiation with both CONV and FLASH dose rates, 18.5 Gy was given to a field size that was 1.5 cm tall (along the long axis of the mouse) and 6 cm wide (to cover the entire width of the mouse plus about 1.5 cm extra each side because the mouse width was usually 3 cm). Each mouse is aligned against a laser produced coordinate system defining the coronal, sagittal, and transverse planes, centered between the pair of lower nipples on the mouse. Reproducibility of the mouse positioning is demonstrated by the dermal depigmentation visible 1-month postradiation, in 2 different mice as shown in Fig. 3C.

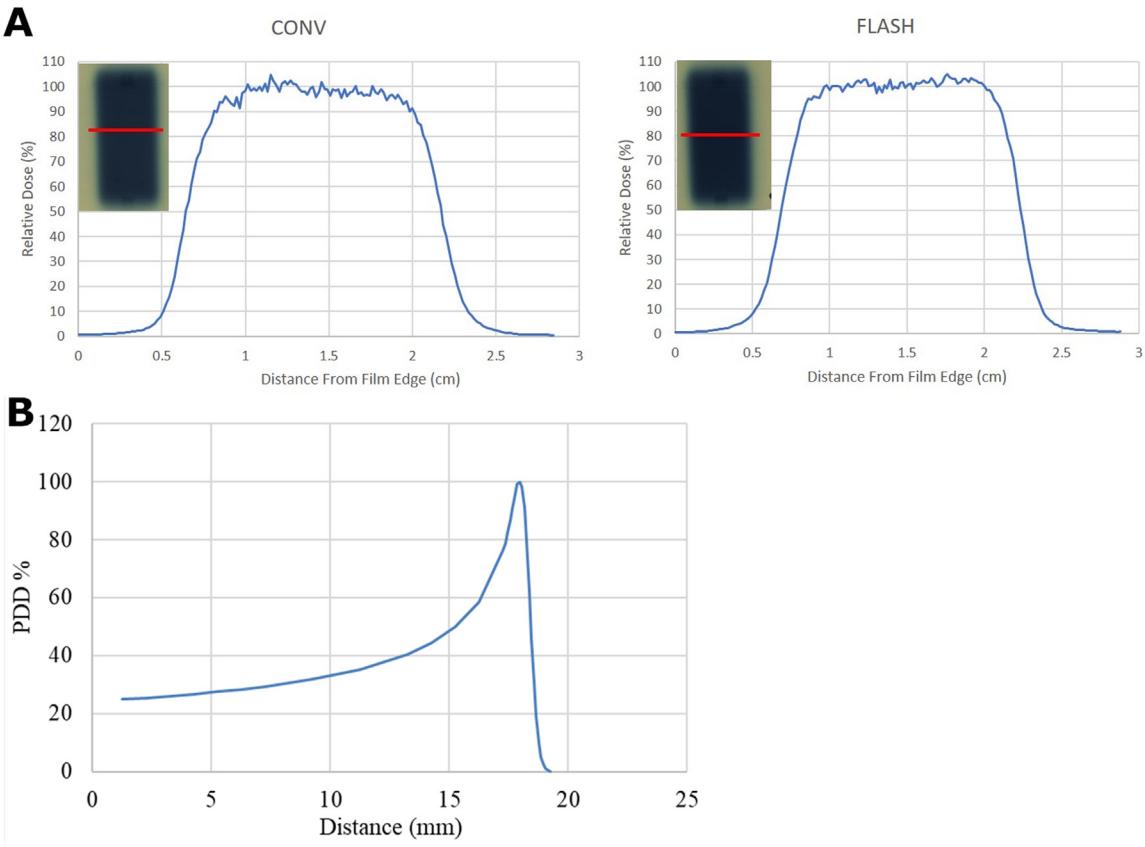

Figure 2 (A) Example proton beam profiles at the beam entrance side of mouse for a 1.5 cm × 3 cm collimated field using EBT3 film. (B) Percent-depth-dose curve of the beam. In our experimental setup (Fig. 3), the thickness of the mouse body is 6 mm, therefore using the Percent-depth-dose curve, the ratio of the exit dose to the entrance dose is 1.17. The reported dose rate is at the entrance of the mouse, so the dose rate at the exit of the mouse will be ~1.17 times of the reported dose rate. *Abbreviations*: CONV = conventional dose-rate radiation therapy; PDD = percent-depth-dose.

Flank tumor experiments

For flank tumor experiments, B16 cells at 10^5 cells/flank in 200 μL phosphate buffer saline were injected into right mouse flanks. On day 14 postimplantation, mice were treated with sham radiation (control group), CONV, or FLASH radiation to 18 Gy. Radiation was given if tumor size was >100 mm^3. Tumor size was tracked post-radiation. Mice were checked frequently and euthanized upon onset of severe morbidity, including hunched posture, tumor volume >3000 mm^3, social withdrawal, relative immobility, or apparent weight loss >30%.

Histology

Distal colon tissues from whole pelvis radiation groups were collected for histologic evaluation (1 cm length of tissue collected starting from 3 mm above the anus). Hematoxylin and eosin cleaved caspase 3 (C-CASP3), and EdU were performed. Prior publications have shown that after abdominal radiation to the proximal intestines, FLASH radiation better preserves the proliferation of intestinal crypts,[10,11,18] and produces less apoptosis[18]; we wanted to explore whether the same effects can be seen in the distal colon tissues. Colon tissue was prepared in cross-section format.

For EdU (5-ethynyl-2′-deoxyuridine) assays, at 24 and 96 hours post radiation, approximately 25 mg/kg (0.5 mg in a 20 g mouse) of 5-ethynyl-20-deoxyuridine (EdU; Invitrogen Cat# A10044) in phosphate buffer saline was injected intraperitoneally into the mice 3 to 4 hours before euthanasia. EdU is incorporated into newly synthesized DNA and is a measure of cell proliferation. EdU was detected according to the manufacturer's instructions. Number of EdU + cells/crypt were assessed by counting at least 100 crypts per mouse section. For each mouse, 3 cross section slides were analyzed. Gamma-H2AX staining was used to confirm the collected colon tissue was irradiated on all samples.

For C-CASP3, tissue sections were incubated in C-CASP3 antibody (1:300; Cell Signaling Technology) at 4°C overnight in a humidified chamber. Biotinylated secondary antibody, ABC kit and DAB substrate were employed to develop the signal. Visual assessment of ten nonoverlapping × 40 fields were performed per animal.

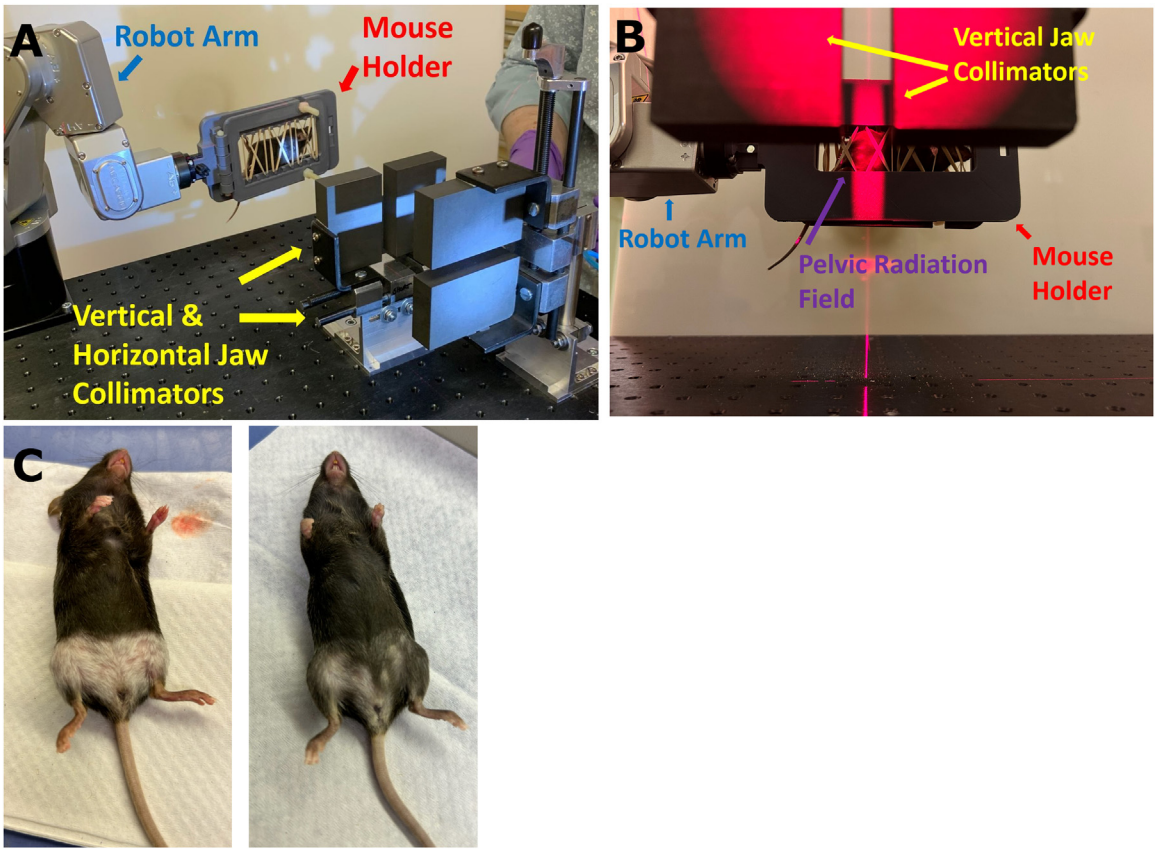

Figure 3 (A) Mouse positioning robot, jaw collimators, and laser coordinate system. (B) For whole pelvis radiation, each mouse is aligned against a laser produced coordinate system defining the coronal, sagittal, and transverse planes, centered between the pair of lower nipples on the mouse. This figure shows the robot arm holding the mouse holder, the lasers centered between the mouse lower nipples, and vertical collimators shaping the radiation field size to 1.5 cm in the long-axis of the mouse. (C) Dermal depigmentation visible 1-month postradiation, in 2 different mice irradiated in the FLASH arm, showing reproducible radiation fields across the animals.

For gamma-H2AX staining to confirm tissues were irradiated, tissue sections were incubated with mouse mAb for Phospho-Histone H2A.X (Ser139, 9F3, Abcam). Visualization of antibody binding was performed using the Histofine Simple Stain Kit (Nichirei Corp.) and 3,3′-diaminobenzidine. Quantification of gamma-H2AX staining was not performed, but visual inspection of slides was done to confirm tissue sections were irradiated. Microscopy was performed on a Nikon NiE upright fluorescent microscope.

Survival

After whole pelvis irradiation to 18.5 Gy, mice were monitored daily for survival for 1 month postradiation. Death was defined as either an animal found dead in the cage or met criteria for humane euthanasia per Institutional Animal Care and Use Committee guidelines such as weight loss >30%, hunched posture, social withdrawal, or relative immobility.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism software. Survival was compared between groups using log-rank Mantel-Cox test. Histologic results (EdU, C-CASP3) were presented as means ± standard error of the mean. Statistical significance was calculated with 2 tailed Student's *t* test for comparison between 2 groups. $P < .05$ was considered significant.

Results

Survival after whole pelvis radiation

For mice treated with whole pelvis radiation to 18.5 Gy in one fraction, survival at 21 days post irradiation was 100% for the control group, 0% for the CONV group, and 44% for the FLASH group (Fig. 4A). The differences were statistically significant with $P < .001$ for control versus

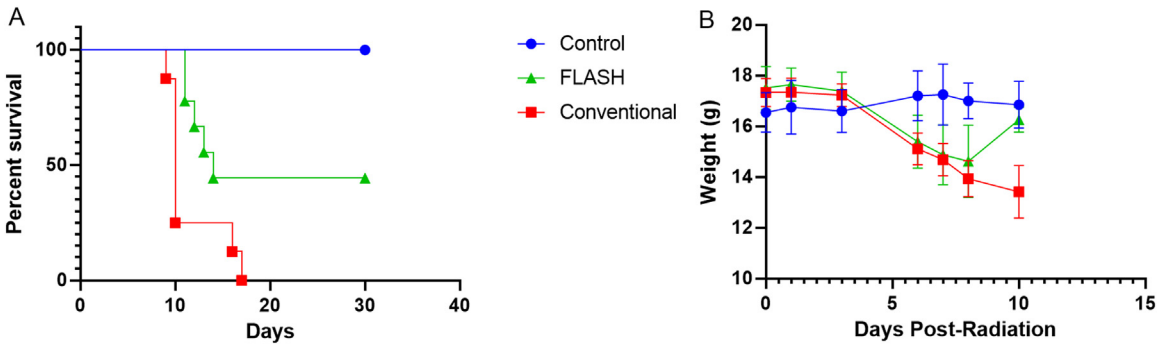

Figure 4 (A) Survival after whole pelvis radiation to 18.5 Gy. Survival was 100% for the control group (0 Gy radiation), 0% for the conventional dose rate group, and 44% for the FLASH dose rate group (N = 6 for control group, N = 8 for conventional group, N = 9 for FLASH group). $P < .001$ for control versus conventional groups, $P = .035$ for control versus FLASH groups, and $P = .017$ for conventional versus FLASH groups. (B) Mice weight after radiation. Both FLASH and conventional dose-rate radiation therapy irradiated mice lose weight after radiation, but the FLASH mice recover from the weight loss faster than the conventional dose-rate radiation therapy mice. Weight loss data cuts off at day-10 postradiation because deaths occur starting at that point, which would skew the weight data for the remaining live mice. Error bars represent standard deviation. All Experiment repeated at least in triplicate with similar differences between groups.

conventional groups, $P = .035$ for control versus FLASH groups, and $P = .017$ for conventional versus FLASH groups. This improved survival in the FLASH arm is associated with decreased weight loss in the FLASH arm, as shown in Fig. 4B. Both FLASH and CONV irradiated mice lose weight after radiation, but the FLASH mice recover from the weight loss faster than the CONV mice. Weight loss data cuts off at day-10 postradiation because deaths occur starting at that point, which would skew the weight data for the remaining live mice. All experiments repeated in at least triplicate with similar differences between groups (Fig. E1 shows results from 4 additional sets of experiments).

EdU staining

After pelvis radiation, EdU staining in distal colon tissue showed cell proliferation was significantly higher in the FLASH versus CONV group at both 24- and 96-hours postradiation, although both radiation groups showed decreased proliferation compared with the control group (Fig. 5A-B). All comparisons between groups were significantly different with $P < .05$ as shown in the Figure. Sample EdU assay images can be found in Fig. E2. Results from 2 additional independent experiments are shown in Fig. E3.

Cleaved caspase-3

At 24-hours post pelvis irradiation, lower cleaved caspase-3 IHC staining was seen in the FLASH group versus conventional group (Fig. 5C) indistal colon tissue, although both irradiated groups showed a higher level of C-CASP3 staining compared with the control group. All comparisons between groups were significantly different with $P < .05$ as shown in the figure. $P < .001$ for control versus conventional groups and control versus FLASH groups, and $P = .013$ for conventional versus FLASH groups. Sample C-CASP3 assay images can be found in Fig. E2. Results from 2 additional independent experiments are shown in Fig. E3.

Tumor control

After 18 Gy of CONV and FLASH radiation to B-16 flank tumors on day 14-post implantation, tumor size was similar between FLASH and CONV radiation groups, and both radiation groups showed slower tumor growth than the control group (Fig. 6). Results from an additional independent experiment is shown in Fig. E4.

Discussion

In this article, we present our preclinical FLASH proton radiation system and biologic results showing normal tissue sparing with equivalent tumor control. With rigorous dosimetry, we show that after equivalent radiation dose to the whole pelvis, there is superior survival after FLASH radiation compared with conventional dose rate radiation. This survival difference is measurable within 15 days after irradiation, providing an easy-to-measure and fast biologic endpoint for future experiments testing whether varying treatment parameters (such as fraction dose, dose rate, beam pauses, etc) will affect the FLASH survival advantage.

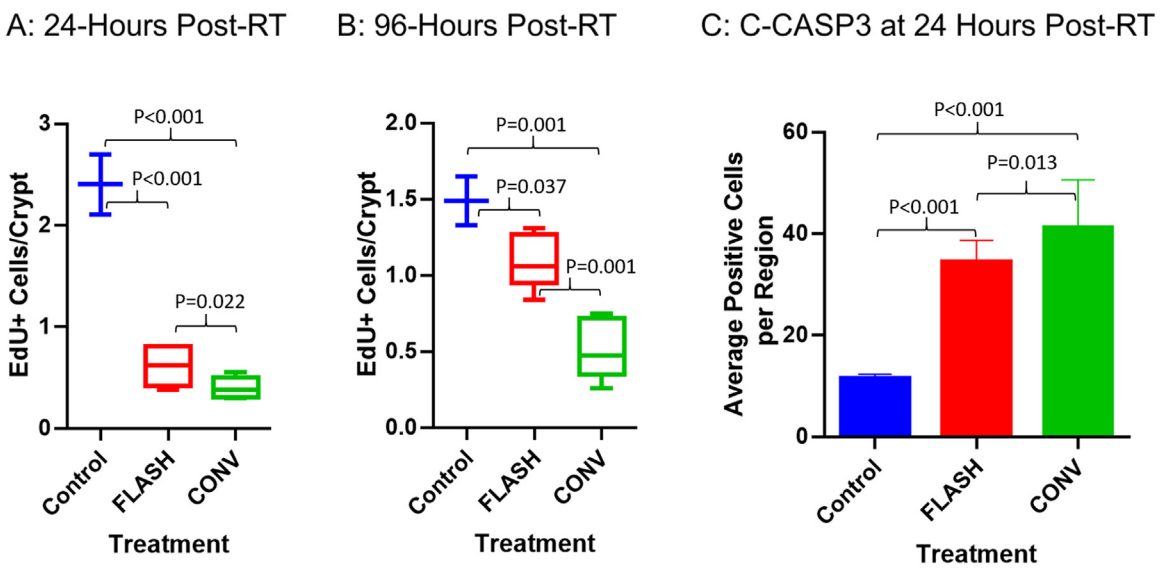

Figure 5 EdU staining at 24 hours (A) and 96 hours (B) post radiation to the pelvis. More EdU positive cells were seen in the FLASH group than conventional dose-rate radiation therapy group at both time points, with better recovery in the FLASH group by 96 hours post radiation (N = 6 for control group, N = 6 for conventional dose-rate radiation therapy group, N = 7 for FLASH group). (C) Cleaved caspase-3 IHC staining at 24 hours postradiation. Lower number of C-CASP3 positive cells were seen in the FLASH group versus conventional group, although both irradiated groups showed a higher level of C-CASP3 staining compared with the control group. Experiment repeated at least in triplicate with similar differences between groups.

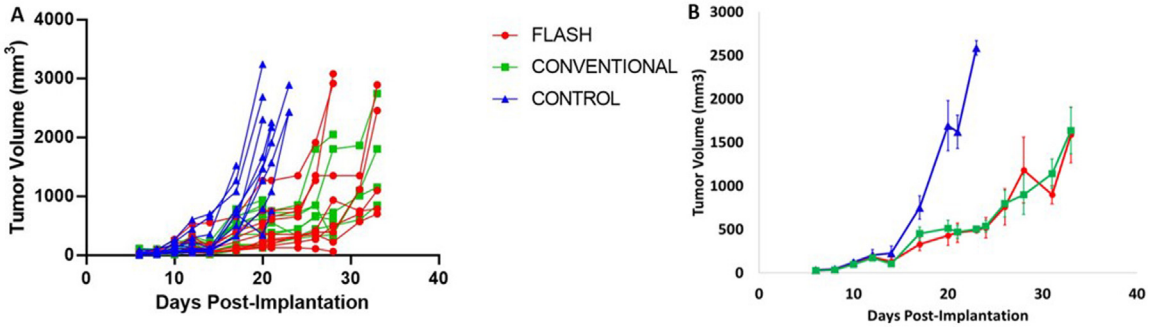

Figure 6 Flank tumor control experiments. On day-14 postimplantation with B16 cells, mice received 18 Gy to the flank tumor. (A) Individual tumor sizes are shown. N = 10 in all 3 groups for 30 mice total. Tumor size was tracked and showed improvement over the control arm for both radiation groups, with no significant difference between the FLASH and conventional dose-rate radiation therapy radiation groups. (B) Average tumor sizes per group are shown. Error bars represent standard error of the mean. Experiment repeated at least in triplicate with similar results.

The improved survival after FLASH irradiation to the pelvis is associated with less weight loss in the FLASH irradiated mice, less apoptosis after 24 hours in the FLASH versus CONV group as shown by cleaved Caspase-3 staining in the distal colon, and more cellular proliferation in the FLASH versus CONV group as shown by EdU staining at 24- and 96-hours postradiation. These results in the distal colon confirm prior publications from Stanford and the University of Pennsylvania that primarily radiated the abdomen and more proximal intestinal tissues.[10,11,18] We also show equivalent flank tumor control with FLASH and CONV radiation. With our unique FLASH proton radiation platform, we plan to engage in future studies to better understand the mechanism behind the FLASH biologic effects, as well as the physical/beam parameters required to see the FLASH effect.

Of note, the temporal microstructure of the proton beam used in this study is relatively constant. In other words, there is not a pulse structure like there is for linac-based electron and photon beams or some proton systems. Emerging data suggest that both the field dose rate

as well as the dose-rate in the pulse may contribute to the FLASH effect.[19] Therefore, our system and beamline offers one additional window into such effects.

There has been growing interest in FLASH radiation since the study in 2014 by Favaudon et al showed it is possible to spare normal tissue from radiation toxicity without compromising tumor control with ultrahigh-dose-rate radiation.[3] Most published studies so far were carried out using electron RT: the study by Favaudon et al used conventional gamma rays (^{137}Cs) and FLASH electrons (4.5 MeV) to irradiate the whole lung in mice. The results showed that lung fibrosis started to develop at 8 weeks after 17 Gy CONV RT but not FLASH RT, while 30 Gy FLASH RT was required to induce significant fibrosis. Their study also showed that FLASH RT was as efficient as CONV RT in controlling xenografted human breast and head & neck tumors as well as syngeneic orthotopic lung tumors. Aside from mice, the FLASH effect has also been observed in higher animal models: minipigs and cats.[5] Severe late skin fibronecrosis was only observed with CONV RT not FLASH RT after a single dose of 28 to 34 Gy electron radiation in the minipig. Six cat patients with nasal squamous cell carcinoma were treated with a single dose (range, 25-41 Gy) of electron FLASH RT. All cats responded well with only mild dermatitis/mucositis and no late-stage toxicities. They saw 100% local control at 6 months post radiation,[5] although a later trial showed unexpected late toxicities with FLASH at these high dose single fraction regimens.[20]

Electron radiation has low tissue penetration, and current RT electron system do not have beam shaping systems that allow fast and complex beam modulation, thus limiting the wide application of electron FLASH RT for patient treatment. Proton RT, on the other hand, provides a wide range of penetration. If FLASH dose rates can be achieved with a Bragg peak, this may further facilitate the clinical implementation of FLASH radiation. Research and development of proton FLASH RT has drawn more attention in recent years. The study by Diffenderfer et al used a clinical 230 MeV proton accelerator and double-scattered protons to deliver a FLASH beam.[11] Mice were irradiated in the entrance part of the beam to the upper abdomen, which is different from the present study, which irradiated the lower pelvis. The study showed that 15 Gy FLASH proton RT (78 Gy/s) can significantly reduce the loss of the proliferating intestinal crypt cells compared with COVN proton RT (0.9 Gy/s) for whole abdomen treatment. In the same study, the authors used a xenograft pancreatic tumor model and found similar tumor control between FLASH RT and COVN RT. A recent study used the spread-out Bragg peak to generate FLASH beam and found out that spread-out Bragg peak FLASH has the same normal tissue sparing and tumor control effects as does entrance FLASH.[10] Cunningham et al used a clinical pencil beam scanning technique for FLASH and discovered better normal tissue sparing and same tumor control compared with CONV proton radiation.[13]

Not all studies on ultrahigh-dose-rate radiation have reported a biologic advantage. For example, Zhang et al in 2020 published that using the proton platform at Massachusetts General Hospital, more mice survived partial abdomen radiation in the FLASH proton group than the COVN group.[12] However, the same group published in 2023 with a substantially larger sample size,[21] and found that all endpoints, including the survival fraction of mice, the surviving proliferating crypt cells, and the counts of circulating lymphocytes showed no FLASH-induced tissue-sparing effect at any dose level. The exact cause of this change in results is unclear. The authors concluded that more studies from institutions with different ultrahigh-dose-rate radiation systems are needed, to study differences in radiation parameters and help determine the conditions needed to generate the FLASH effect.

Despite the increasing body of literature on FLASH radiation, further investigations and interinstitutional comparisons are required to confirm the results and understand the technical parameters required to induce the FLASH effect clinically, as well as understand the biologic mechanism behind the FLASH effect.[19,22,23]

Conclusion

We present our preclinical ultrahigh-dose-rate (FLASH) proton radiation system and biologic results showing normal tissue sparing with equivalent tumor control. Future experiments will test the technological parameters needed to produce the FLASH effect in a clinical proton therapy system, as well as its biologic mechanisms.

Disclosures

Juergen Meyer receives research funding from the Germany Academic Exchange Service. Ramesh Rengan receives consulting fees from AstraZeneca, Gilead Sciences, IBA. Food/beverages from Elekta, Siemens, ViewRay. Jing Zeng receives research funding and consulting/honoraria fees from AstraZeneca, Gilead Sciences, IBA, Research funding from NIH RCA258997A and Kuni Foundation. All other authors have no disclosures.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.adro.2023.101425.

References

1. Garibaldi C, Jereczek-Fossa BA, Marvaso G, et al. Recent advances in radiation oncology. *Ecancermedicalscience*. 2017;11:785.
2. Lv Y, Lv Y, Wang Z, et al. FLASH radiotherapy: A promising new method for radiotherapy. *Oncol Lett*. 2022;24:419.
3. Favaudon V, Caplier L, Monceau V, et al. Ultrahigh dose-rate FLASH irradiation increases the differential response between normal and tumor tissue in mice. *Sci Transl Med*. 2014;6:245ra93.
4. Montay-Gruel P, Petersson K, Jaccard M, et al. Irradiation in a flash: Unique sparing of memory in mice after whole brain irradiation with dose rates above 100 Gy/s. *Radiother Oncol*. 2017;124:365-369.
5. Vozenin MC, De Fornel P, Petersson K, et al. The advantage of FLASH radiotherapy confirmed in mini-pig and cat-cancer patients. *Clin Cancer Res*. 2019;25:35-42.
6. Saade G, Bogaerts E, Chiavassa S, et al. Ultrahigh-dose-rate proton irradiation elicits reduced toxicity in zebrafish embryos. *Adv Radiat Oncol*. 2023;8: 101124.
7. Allen BD, Alaghband Y, Kramar EA, et al. Elucidating the neurological mechanism of the FLASH effect in juvenile mice exposed to hypofractionated radiotherapy. *Neuro Oncol*. 2023;25: 927-939.
8. Williams MT, Sugimoto C, Regan SL, et al. Cognitive and behavioral effects of whole brain conventional or high dose rate (FLASH) proton irradiation in a neonatal Sprague Dawley rat model. *PloS One*. 2022;17: e0274007.
9. Konradsson E, Liljedahl E, Gustafsson E, et al. Comparable long-term tumor control for hypofractionated FLASH versus conventional radiation therapy in an immunocompetent rat glioma model. *Adv Radiat Oncol*. 2022;7: 101011.
10. Kim MM, Verginadis II, Goia D, et al. Comparison of FLASH proton entrance and the spread-out Bragg Peak dose regions in the sparing of mouse intestinal crypts and in a pancreatic tumor model. *Cancers*. 2021;13:4244.
11. Diffenderfer ES, Verginadis II, Kim MM, et al. Design, implementation, and in vivo validation of a novel proton FLASH radiation therapy system. *Int J Radiat Oncol Biol Phys*. 2020;106:440-448.
12. Zhang Q, Cascio E, Li C, et al. FLASH investigations using protons: Design of delivery system, preclinical setup and confirmation of FLASH effect with protons in animal systems. *Radiat Res*. 2020;194:656-664.
13. Cunningham S, McCauley S, Vairamani K, et al. FLASH proton pencil beam scanning irradiation minimizes radiation-induced leg contracture and skin toxicity in mice. *Cancers*. 2021;13:1012.
14. Hughes JR, Parsons JL. FLASH radiotherapy: Current knowledge and future insights using proton-beam therapy. *Int J Mol Sci*. 2020;21:6492.
15. Diffenderfer ES, Sorensen BS, Mazal A, Carlson DJ. The current status of preclinical proton FLASH radiation and future directions. *Med Phys*. 2022;49:2039-2054.
16. Ford E, Emery R, Huff D, et al. An image-guided precision proton radiation platform for preclinical in vivo research. *Phys Med Biol*. 2017;62:43-58.
17. Meyer J, Stewart RD, Smith D, et al. Biological and dosimetric characterisation of spatially fractionated proton minibeams. *Phys Med Biol*. 2017;62:9260-9281.
18. Levy K, Natarajan S, Wang J, et al. Abdominal FLASH irradiation reduces radiation-induced gastrointestinal toxicity for the treatment of ovarian cancer in mice. *Sci Rep*. 2020;10:21600.
19. Bourhis J, Montay-Gruel P, Goncalves Jorge P, et al. Clinical translation of FLASH radiotherapy: Why and how? *Radiother Oncol*. 2019;139:11-17.
20. Rohrer Bley C, Wolf F, Goncalves Jorge P, et al. Dose- and volume-limiting late toxicity of FLASH radiotherapy in cats with squamous cell carcinoma of the nasal planum and in mini pigs. *Clin Cancer Res*. 2022;28:3814-3823.
21. Zhang Q, Gerweck LE, Cascio E, et al. Absence of tissue-sparing effects in partial proton FLASH irradiation in murine intestine. *Cancers*. 2023;15:2269.
22. Adrian G, Konradsson E, Lempart M, Back S, Ceberg C, Petersson K. The FLASH effect depends on oxygen concentration. *Br J Radiol*. 2019;93: 20190702.
23. Esplen N, Mendonca MS, Bazalova-Carter M. Physics and biology of ultrahigh dose-rate (FLASH) radiotherapy: A topical review. *Phys Med Biol*. 2020;65:23TR03.