Clinical and Translational Radiation Oncology 56 (2026) 101052

Contents lists available at ScienceDirect

Clinical and Translational Radiation Oncology

journal homepage: www.sciencedirect.com/journal/clinical-and-translational-radiation-oncology

Technical Note

Quantifying the flash effect and its dependence on average dose rate in vivo for 6 MeV electron and 6 MV photon beams

Salomé Paillas, Edward R.J.F. Taylor, Nathalie Lövgren, Iain D.C. Tullis, Kristoffer Petersson *

Department of Oncology, University of Oxford, Oxford OX3 7DQ, United Kingdom

ABSTRACT

This study shows that an increase in average dose rate delays the onset, and reduces the severity, of radiation induced skin toxicity in mice following hemi-thorax irradiation. The FLASH sparing effect's magnitude and dependence on dose rate appear similar following irradiations using 6 MV photon and 6 MeV electron beams.

Introduction

Previous studies have shown that average dose rate/total delivery time are key parameters for the FLASH effect [1]. This was first shown in mice following 10 Gy whole brain irradiation, using a novel object recognition test to evaluate memory function two months post irradiation. Reduced neurotoxicity was seen for dose rates ≥30 Gy/s, with further reduction with increasing average dose rates, with full sparing seen at dose rates ≥100 Gy/s [2]. How the FLASH effect varies with average dose rate has also been shown in mouse intestines. Using a Swiss-roll crypt assay, the number of crypts remaining following 11.2 Gy or 12.5 Gy whole abdominal irradiation showed significant sparing at dose rates ≥280 Gy/s and ≥310 Gy/s, respectively. Again, sparing increased with increasing average dose rates [3]. Another recent publication used a similar assay as well as survival to show significant sparing at 106 Gy/s following 17 Gy whole abdominal irradiation. That study also indicated that average dose rate is the most important temporal parameter for the FLASH effect [4]. These studies used 6 Mega electron Volt (MeV) electron beams. A similar dose rate escalation study using transmission 244–250 MeV proton beams, evaluated radiation-induced skin toxicity in mice following 39.3 Gy back limb irradiation. Here, a FLASH sparing effect was seen from 2-5.6 Gy/s, with greater sparing for further increased average dose rates [5].

Most preclinical FLASH studies, like those mentioned above, have used electron or proton beams [1]. However, the vast majority of radiotherapy treatments utilise Mega Voltage (MV) photon beams [6]. Therefore, for the future clinical translation of FLASH radiotherapy, it is important to also investigate the FLASH effect for MV photon beams. However, it is challenging to reach the ultra-high dose rates needed for FLASH with MV photon beams because of the relatively poor efficiency of production of bremsstrahlung photons [6]. In our previous work, we have shown how adding a tungsten foil as a bremsstrahlung target and reducing the source-to-surface distance (SSD) at our dedicated 6 MeV electron FLASH linear accelerator (linac) [7], allows us to also produce 6 MV photon beams usable for preclinical FLASH studies [8].

In this study, we investigate how the FLASH effect varies with average dose rate for radiation-induced skin toxicity in mice, following irradiation from 6 MeV electron and 6 MV photon beams.

Material and methods

All animal research was conducted in accordance with UK Home Office Guidelines, following the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines, and approved by the University of Oxford Animal Welfare and Ethical Review Body (AWERB), under University of Oxford project licence PP8415318. Female C57BL/6 mice (aged 8–10 weeks at time of irradiation) were purchased from Charles Rivers UK Ltd, housed in a temperature-controlled environment with a 12-hours reversed-phase light/dark cycle (lights on 07:00 h), and provided with food and water ad libitum at the Department of Biomedical Services, Radiobiology Research Institute, University of Oxford, Oxford.

For the irradiation, the mice were anesthetised using isoflurane (4 % for anaesthetic induction and 2 % for maintenance, with a total anaesthesia time of less than 10 min) supplemented with 95 % oxygen (1/1 mixture with air resulting in a mixture of approximate 60 % oxygen), and then placed upright in a mouse cradle in front of a horizontal beam

---

* Corresponding author at: Department of Oncology, University of Oxford Old Road Campus Research Building, Roosevelt Drive, Oxford OX3 7DQ, United Kingdom.

E-mail address: kristoffer.petersson@oncology.ox.ac.uk (K. Petersson).

https://doi.org/10.1016/j.ctro.2025.101052
Received 4 June 2025; Received in revised form 27 August 2025; Accepted 22 September 2025

(Fig. S1 in supplementary material) [3,7]. For the electron beam irradiations, beam collimation was achieved using a 6 mm thick brass plate, with a 20 × 20 mm^2 central aperture. Meanwhile for photon beam irradiations, beam collimation was achieved using a 50 mm thick Wood's metal layer, with a 17 × 17 mm^2 (used for the 25 and 30 Gy deliveries) or 20 × 20 mm^2 (used for the 20 Gy delivery) central aperture (Fig. S2 in supplementary material). The mice were positioned such that the collimator shielding only enabled high dose exposure of the right half of the thorax. A single dose of 20, 25, or 30 Gy was prescribed and verified with GafChromic EBT-XD film (Ashland Inc, Covington, KY, USA)

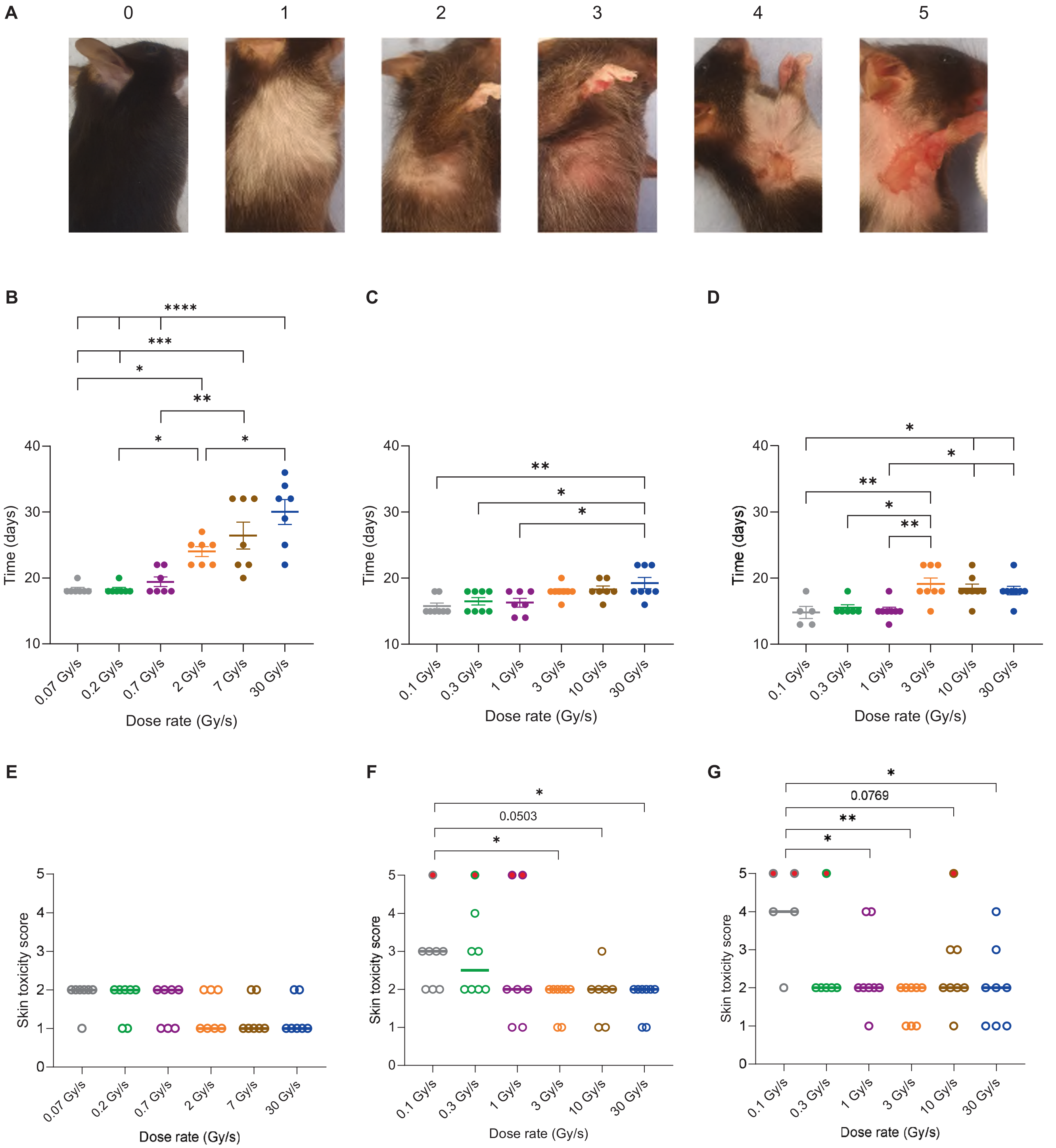

Fig. 1. Increasing the average dose rate of photon beam irradiations delays the radiotoxicity emergence and reduces skin toxicity. (A) Skin toxicity scoring scale with representative pictures. (B-D) Skin radiotoxicity emergence. Right lung of mice was irradiated with a single dose of (B) 20 Gy, (C) 25 Gy, or (D) 30 Gy at different dose rates and mice were monitored every other day to determine the time of skin toxicity onset. Mean values are shown with error bars representing ± SEM. (E-G) Macroscopic skin lesions scoring at 8 weeks post irradiation, based on the skin toxicity scoring scale, after (E) 20 Gy, (F) 25 Gy, or (G) 30 Gy photon beam irradiation. Bars represent the median and red dots indicate animals that had to be euthanised early for meeting skin toxicity euthanasia criteria (moist desquamation-ulceration). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

measurements before and after mice irradiation at the surface of a mouse phantom (Fig. S3 in supplementary material). Additional online dosimetry was performed with an Advanced Markus ionisation chamber at 1 mm depth in solid water (15 × 15 × 2.1 cm^3 RW3 slabs, PTW-Freiburg GmbH, Freiburg, Germany). The beam energy was maintained for all dose rates by slight detuning of the radiofrequency source and online monitoring of the resulting beam energy [7]. Variations in dose rate were achieved by changing the pulse amplitude (by adjusting gun heater current) and the repetition rate of the 3.4 μs wide (macro-) pulses delivered (Tables S1-S4 in supplementary material).

An ordinal scale was used to score skin toxicity (Fig. 1A): 0 = normal; 1 = depigmentation; 2 = alopecia (± depigmentation); 3 = erythema (±alopecia and depigmentation); 4 = dry desquamation; 5 = moist desquamation-ulceration. All mice were monitored every other day for 8 weeks post irradiation for development of skin toxicity within the radiation field. Mice noted to develop cutaneous lesions were monitored daily. Mice meeting euthanasia criteria (moist desquamation-ulceration or moist desquamation that did not improve after 10 days) were euthanised via cardiac exsanguination at any point the criteria were met during the study and their skin toxicity was scored at that point. All other mice were euthanised and scored for skin toxicity at the 8-week timepoint.

GraphPad Prism 10 software (GraphPad Software Inc., La Jolla, CA, USA) was used for statistical analysis. One-way ANOVA with Šídák's multiple comparisons test was used to analyse the radiotoxicity emergence data, with results presented as mean ± standard error of the mean. Mann-Whitney test was used to compare the distribution of skin toxicity scoring. The Kaplan-Meier method was performed to compare the median survival using Log-Rank test. For all tests, a *p-value* <0.05 was considered statistically significant.

Results

For 20 Gy photon beam hemi-thorax irradiations, a delay in onset of radiation-induced skin toxicity with increasing average dose rates was seen from 0.7 Gy/s, with a significant delay seen from 2 Gy/s, with a further delay at higher dose rates (Fig. 1B). Similar results with delay in onset of toxicity were seen for 25 and 30 Gy irradiations (significant for 30 Gy/s and ≥3 Gy/s, for 25 and 30 Gy, respectively), albeit with a shorter delay with increased doses (Fig. 1C-D). While mice irradiated with 20 Gy could be kept alive until the 8-week time point post irradiation, this was not the case for those that received 25 and 30 Gy. At this time, the severity of the skin toxicity showed a trend to be reduced with increasing dose rate (Fig. 1E-G). Significant sparing was seen for ≥3 Gy/s and ≥1 Gy/s for 25 and 30 Gy irradiation, respectively.

For 30 Gy electron beam hemi-thorax irradiations, a trend towards a delay in onset of skin toxicity was seen for increased average dose rates, and a significant delay seen from 10 Gy/s (Fig. 2A). The majority of mice in this cohort required euthanasia due to the severity of their skin toxicity prior to the 8-week time point post irradiation. Increasing average dose rates resulted in significantly ($p < 0.01$) better survival (Fig. 2B), with all mice irradiated at the highest average dose rate of 1,800 Gy/s surviving until the 8-week time point. The severity of the skin toxicity was reduced with increased average dose rate, with significant sparing seen at 1,800 Gy/s (Fig. 2C).

By matching the lowest and highest photon dose rates with our electron dose rates, while also evaluating a much higher electron dose rate, we were able to assess what the effect of an increased dose rate beyond the current capabilities of our photon setup would mean for the FLASH effect. Significant differences between 20, 25, and 30 Gy could be seen in delay in onset and severity of toxicity at the 8-week timepoint (Fig. 2D-E). A dose-modifying factor (DMF, defined as the dose required for a given effect in the higher dose rate group divided by the dose resulting in the same effect in the 0.1 Gy/s group [9]) for FLASH of ≈1.5 could be seen for the highest vs. lowest electron dose rate (as 30 Gy at 1,800 Gy/s showed a similar delay and severity of toxicity to 20 Gy at 0.1 Gy/s), while this DMF was reduced to ≈1.2–1.25 for 30 Gy/s (where 25 Gy at 30 Gy/s was slightly more toxic than 20 Gy at 0.1 Gy/s, while 30 Gy at 30 Gy/s showed a similar toxicity to 25 Gy at 0.1 Gy/s, Fig. 2D-E). Similarly, for the photon irradiation our results indicated a FLASH DMF for 30 Gy/s of ≈1.2–1.25 (where 25 Gy at 30 Gy/s showed a similar toxicity to 20 Gy at 0.1 Gy/s, while 30 Gy at 30 Gy/s showed slightly less toxicity than 25 Gy at 0.1 Gy/s, Fig. 1).

Discussion

While preclinical FLASH studies have mainly been performed with electron and proton beams, the vast majority of all radiotherapy treatments are delivered with MV photon beams [6]. The photon beam studies published so far have mostly used kilovoltage (kV) photon beams, e.g., with beams from large synchrotron facilities [10,11] or beams from x-ray tubes [12,13]. A limitation with such beams is their penetration ability in tissue, and for x-ray tubes in FLASH studies there is also the issue with photoelectric effect, which has a significant role in the biological response for the soft energy spectra produced [14]. FLASH studies published so far using MV photon beams have utilised large facilities [15], with the majority coming from the PARTER platform which is part of the superconducting linac installation at the Chengdu THz Free Electron Laser (CTFEL) facility [16]. Though capable of higher dose rates, these large facilities share comparable limitations to our setup; with a short SSD and limited field size. By using the same linac for ultra-high as conventional dose rates, with the same mouse setup and pulse structure for electron and photon irradiations, our setup enables a direct comparison of the FLASH effect between these modalities.

The short SSD results in a divergent beam (Fig. S4) and a large effect on dose with any variation in SSD. This sensitivity to variation in SSD was reflected by the larger spread of toxicity scores within the photon irradiated groups, meaning that even a small setup variation could result in a large variation in toxicity as exemplified by the outlier mouse with a skin toxicity score of 5 in the 10 Gy/s irradiation group in Fig. 1G. The beam divergence and wide penumbra required the use of a collimator with a smaller aperture for the 25 and 30 Gy photon beam deliveries, to limit the dose to the gastrointestinal tract, and to better match the high dose area of irradiated skin on the mouse for electron beam irradiations (Fig. S2 in supplementary material).

Dosimetry is challenging at ultra-high dose rates. However, for the dose rates used in this study, the dose-per-pulse only reached 100–150 mGy, except for the highest electron dose rate (5 Gy/pulse). So, for all but the highest electron dose rate, the ion recombination correction needed for the Advanced Markus is limited (a few %) [17], making it useful for relative online dosimetry.

In this study, we show that the time to onset of radiation-induced skin toxicity in mice following hemi-thorax irradiation is delayed, and the severity of toxicity reduced, with increasing dose rate (Figs. 1 and 2). We see similar skin toxicity profiles following 6 MV photon, and 6 MeV electron irradiations, though the differences in dose distributions (beam divergence, penumbra and depth dose shown in Figs. S4-S5) prevents a perfect correlation between prescribed doses and observed toxicity between the modalities. For example, the full width at half maximum (FWHM) of the electron beam is consistently ≈20 mm from surface to 10 mm depth in solid water, while the FWHM of the diverging photon beam increases with distance from the surface, from ≈19 and ≈22 mm at surface to ≈23.5 and ≈27.5 mm at 10 mm depth for the 17 × 17 mm^2 and 20 × 20 mm^2 collimators, respectively. Furthermore, the penumbra (if defined as the distance of 75 % to 25 % of maximum dose) at the surface of the mouse phantom was substantially different between the electron (<1 mm) and the photon beam (≈9 mm). As demonstrated previously for skin toxicity following proton beam irradiation [5], an increase in average dose rate from conventional values (0.1 Gy/s) to a few (1–10) Gy/s is enough for significant sparing. Further increase to 30 Gy/s (the highest achievable dose rate with our photon setup) shows a DMF of ≈1.2–1.25, for both photon and electron irradiation. This DMF

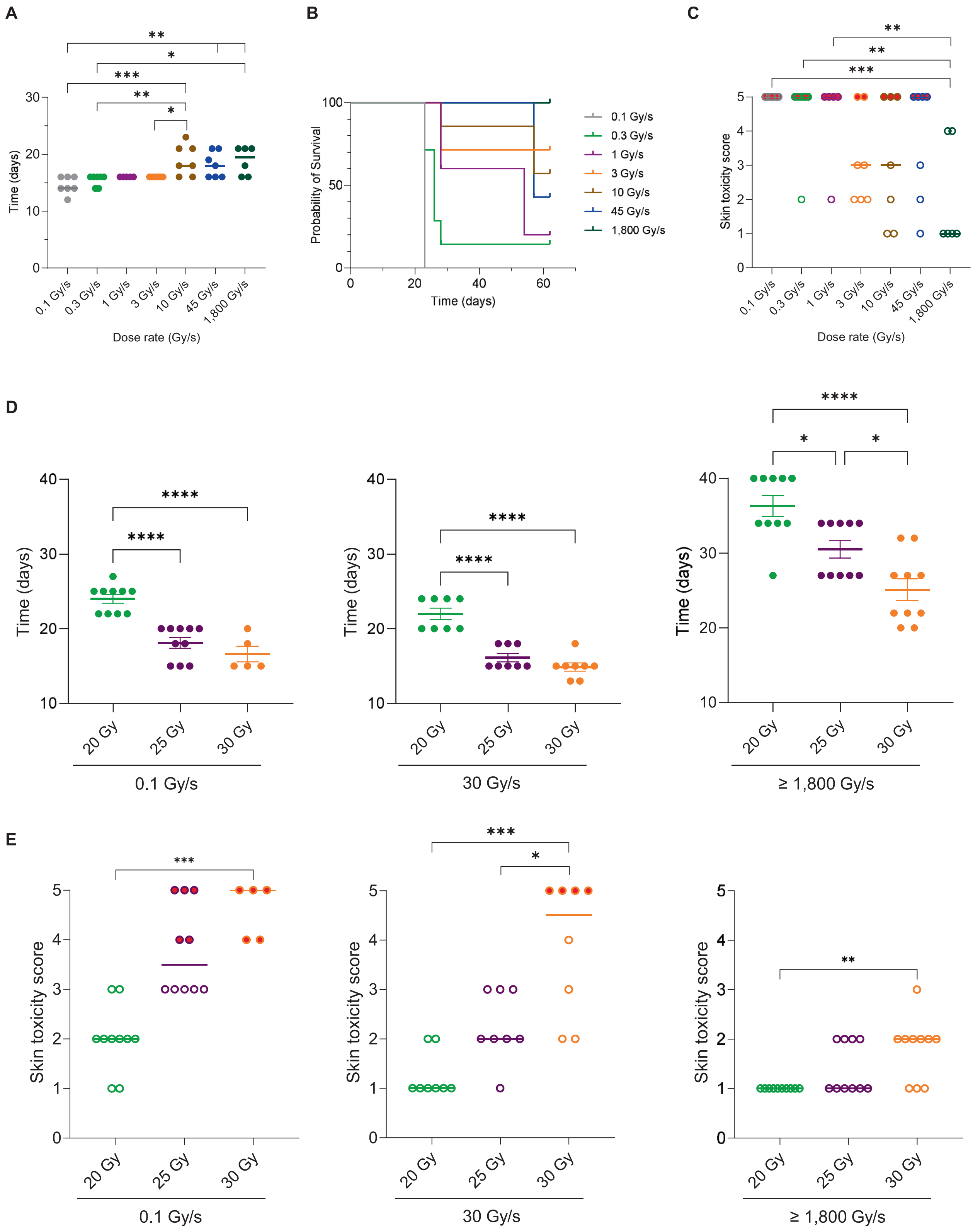

Fig. 2. Increasing the average dose rate of electron beam irradiations delays the radiotoxicity emergence and reduces skin toxicity. (A) Skin radiotoxicity emergence. Right lung of mice was irradiated with electrons with a single dose of 30 Gy at different dose rates and mice were monitored every other day to determine the time of skin toxicity onset. Mean values are shown with error bars representing ± SEM. (B) Kaplan-Meier survival curves for 30 Gy irradiation at different dose rates irradiation (n =5 for 1 Gy/s group, n =6 for 1,800 Gy/s group and n =7 for the other groups). (C) Macroscopic skin lesions scoring at 8 weeks post irradiation, based on the skin toxicity scoring scale, for 30 Gy irradiation at different dose rates. A separate experiment evaluating (D) skin radiotoxicity emergence and (E) macroscopic skin lesions scoring at 8 weeks post irradiation, for mice irradiated with electron beams in the dose range of 20 to 30 Gy in a single fraction at 0.1 Gy/s (left panel), 30 Gy/s (middle panel) or ≥1,800 Gy/s (right panel). Bars represent the median and red dots indicate animals that had to be euthanised early for meeting skin toxicity euthanasia criteria (moist desquamation-ulceration or moist desquamation that did not improve after 10 days). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was increased to ≈1.5 for our highest used electron dose rates (≥1,800 Gy/s), similar to FLASH DMF published values for skin toxicity following electron (1.45–1.54) and proton (1.44–1.58) beam irradiation [18,19]. These studies reached a DMF value of ≈1.5 while using substantially lower average dose rates than our maximum here, of around 80 Gy/s for protons and 230 Gy/s for electrons. This indicates that the DMF for skin toxicity increases with average dose rate, becomes significant at a few (1–10) Gy/s, and continues to increase with dose rate to the 80–230 Gy/s range [5,20], where it seems to saturate with limited additional benefits of using even higher average dose rates.

The FLASH sparing effect appears similar for irradiations using 6 MV photon, and 6 MeV electron beams, indicating that electron preclinical FLASH data can inform MV photon delivery. As the vast majority of all radiotherapy treatments delivered today utilises MV photon beams, it is an essential future clinical modality for FLASH radiotherapy.

Funding

Cancer Research UK—RadNet (C6078/A28736) financial support is gratefully acknowledged. We thank the Medical Research council for financial support through a Programme grant (MR/X006611/1) and for funding ERJFT and NL studentships.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The contribution of John Prentice and Kyle Hallett from the Department of Oncology Mechanical Workshop has been essential for the completion of this work. We would also like to acknowledge the contribution with mice monitoring by Hala Estephan.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ctro.2025.101052.

References

[1] Wilson JD, Hammond EM, Higgins GS, Petersson K. Ultra-high dose rate (FLASH) radiotherapy: silver bullet or fool's gold? Front Oncol 2020;9.

[2] Montay-Gruel P, Petersson K, Jaccard M, Boivin G, Germond JF, Petit B, et al. Irradiation in a flash: Unique sparing of memory in mice after whole brain irradiation with dose rates above 100Gy/s. Radiother Oncol 2017;124:365–9.

[3] Ruan JL, Lee C, Wouters S, Tullis IDC, Verslegers M, Mysara M, et al. Irradiation at ultra-high (FLASH) dose rates reduces acute normal tissue toxicity in the mouse gastrointestinal system. Int J Radiat Oncol Biol Phys 2021.

[4] Grilj V, Zayas AV, Sesink A, Devanand P, Repáraz D, Paisley R, et al. Average dose rate is the major temporal beam structure parameter for preserving murine intestines with pulsed electron FLASH-RT. Internat J Radiat Oncol Biol Phys 2025.

[5] Sørensen BS, Kanouta E, Ankjærgaard C, Kristensen L, Johansen JG, Sitarz MK, et al. Proton FLASH: impact of dose rate and split dose on acute skin toxicity in a murine model. Internat J Radiat Oncol Biol Phys 2024;120:265–75.

[6] Montay-Gruel P, Corde S, Laissue JA, Bazalova-Carter M. FLASH radiotherapy with photon beams. Med Phys 2022;49:2055–67.

[7] Berne A, Petersson K, Tullis IDC, Newman RG, Vojnovic B. Monitoring electron energies during FLASH irradiations. Phys Med Biol 2021;66:045015.

[8] Taylor ERJF, Tullis IDC, Vojnovic B, Petersson K. Megavoltage photon FLASH for preclinical experiments. Med Phys 2025.

[9] Williams JP, Brown SL, Georges GE, Hauer-Jensen M, Hill RP, Huser AK, et al. Animal models for medical countermeasures to radiation exposure. Radiat Res 2010;173:557–78.

[10] Montay-Gruel P, Bouchet A, Jaccard M, Patin D, Serduc R, Aim W, et al. X-rays can trigger the FLASH effect: Ultra-high dose-rate synchrotron light source prevents normal brain injury after whole brain irradiation in mice. Radiother Oncol 2018.

[11] Smyth LML, Donoghue JF, Ventura JA, Livingstone J, Bailey T, Day LRJ, et al. Comparative toxicity of synchrotron and conventional radiation therapy based on total and partial body irradiation in a murine model. Sci Rep 2018;8:12044.

[12] Rezaee M, Iordachita I, Wong JW. Ultrahigh dose-rate (FLASH) x-ray irradiator for pre-clinical laboratory research. Phys Med Biol 2021;66:095006.

[13] Hart A, Dudzic JP, Clarke JW, Eby J, Perlman SJ, Bazalova-Carter M. High-throughput, low-cost FLASH: irradiation of Drosophila melanogaster with low-energy X-rays using time structures spanning conventional and ultrahigh dose rates. J Radiat Res 2024;65:836–44.

[14] Arno J, Mundt M, Roeske JC, PhD TD, Chung MD, Weichselbaum Ralph R. Physical basis of radiation therapy. 6th ed: Hamilton (ON): BC Decker; 2003.

[15] Esplen N, Egoriti L, Planche T, Rädel S, Koay HW, Humphries B, et al. Dosimetric characterization of a novel UHDR megavoltage X-ray source for FLASH radiobiological experiments. Sci Rep 2024;14:822.

[16] Yang Y, Wang J, Gao F, Liu Z, Dai T, Zhang H, et al. FLASH radiotherapy using high-energy X-rays: current status of PARTER platform in FLASH research. Radiother Oncol 2024;190:109967.

[17] Petersson K, Jaccard M, Germond JF, Buchillier T, Bochud F, Bourhis J, et al. High dose-per-pulse electron beam dosimetry - a model to correct for the ion recombination in the Advanced Markus ionization chamber. Med Phys 2017;44:1157–67.

[18] Kristensen L, Rohrer S, Hoffmann L, Praestegaard LH, Ankjaergaard C, Andersen CE, et al. Electron vs proton FLASH radiation on murine skin toxicity. Radiother Oncol 2025;206:110796.

[19] Singers Sorensen B, Krzysztof Sitarz M, Ankjaergaard C, Johansen J, Andersen CE, Kanouta E, et al. In vivo validation and tissue sparing factor for acute damage of pencil beam scanning proton FLASH. Radiother Oncol 2022;167:109–15.

[20] Poulsen PR, Johansen JG, Sitarz MK, Kanouta E, Kristensen L, Grau C, et al. Oxygen enhancement ratio-weighted dose quantitatively describes acute skin toxicity variations in mice after pencil beam scanning proton FLASH irradiation with changing doses and time structures. Int J Radiat Oncol Biol Phys 2024;120:276–86.