Radiotherapy and Oncology 190 (2024) 109953

Contents lists available at ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

Original Article

Dosimetric and biologic intercomparison between electron and proton FLASH beams

A Almeida [a,1], M Togno [b,1], P Ballesteros-Zebadua [a,f,1], J Franco-Perez [a,f], R Geyer [c], R Schaefer [b], B Petit [a], V Grilj [e], D Meer [b], S Safai [b], T Lomax [b], DC Weber [b,c,d], C Bailat [e,2], S Psoroulas [b,2], Marie-Catherine Vozenin [a,g,*,2]

[a] Laboratory of Radiation Oncology/Radiation Oncology Service/Department of Oncology/CHUV, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland
[b] Center for Proton Therapy, Paul Scherrer Institute, 5323, Villigen, Switzerland
[c] Department of Radiation Oncology, Inselspital, Bern University Hospital, University of Bern, Switzerland
[d] Department of Radiation Oncology, University Hospital of Zurich, Switzerland
[e] Institute of Radiation Physics (IRA)/CHUV, Lausanne University Hospital, Lausanne, Switzerland
[f] Instituto Nacional de Neurología y Neurocirugía MVS, Mexico City, Mexico
[g] Radiotherapy and Radiobiology sector, Radiation Therapy service, University hospital of Geneva, Geneva, Switzerland

ARTICLE INFO

Keywords:
FLASH
Intercomparison
Dosimetry
Neurocognition
Tumor response

ABSTRACT

Background and purpose: The FLASH effect has been validated in different preclinical experiments with electrons (eFLASH) and protons (pFLASH) operating at an average dose rate above 40 Gy/s. However, no systematic intercomparison of the FLASH effect produced by eFLASH *vs*. pFLASH has yet been performed and constitutes the aim of the present study.

Materials and methods: The electron eRT6/Oriatron/CHUV/5.5 MeV and proton Gantry1/PSI/170 MeV were used to deliver conventional (0.1 Gy/s eCONV and pCONV) and FLASH (≥110 Gy/s eFLASH and pFLASH) dose rates. Protons were delivered in transmission. Dosimetric and biologic intercomparisons were performed using previously validated dosimetric approaches and experimental murine models.

Results: The difference between the average absorbed dose measured at Gantry 1 with PSI reference dosimeters and with CHUV/IRA dosimeters was −1.9 % (0.1 Gy/s) and + 2.5 % (110 Gy/s). The neurocognitive capacity of eFLASH and pFLASH irradiated mice was indistinguishable from the control, while both eCONV and pCONV irradiated cohorts showed cognitive decrements. Complete tumor response was obtained after an ablative dose of 20 Gy delivered with the two beams at CONV and FLASH dose rates. Tumor rejection upon rechallenge indicates that anti-tumor immunity was activated independently of the beam-type and the dose-rate.

Conclusion: Despite major differences in the temporal microstructure of proton and electron beams, this study shows that dosimetric standards can be established. Normal brain protection and tumor control were produced by the two beams. More specifically, normal brain protection was achieved when a single dose of 10 Gy was delivered in 90 ms or less, suggesting that the most important physical parameter driving the FLASH sparing effect might be the mean dose rate. In addition, a systemic anti-tumor immunological memory response was observed in mice exposed to high ablative dose of electron and proton delivered at CONV and FLASH dose rate.

Introduction

FLASH has recently emerged as an innovative and transformative strategy in radiation oncology thanks to its ability to enhance the differential response between normal tissue and tumors. In several animal species and various organs, and at doses that are normally toxic when administered with conventional dose rate (CONV), FLASH retains the antitumor efficacy but spares normal tissues, a beneficial effect

* Corresponding author.
E-mail addresses: marie-catherine.vozenin@chuv.ch, marie-catherine.vozenin@hcuge.ch (M.-C. Vozenin).
[1] First authors with equal contribution.
[2] Last authors with equal contribution.

https://doi.org/10.1016/j.radonc.2023.109953
Received 12 April 2023; Received in revised form 7 October 2023; Accepted 11 October 2023
Available online 13 October 2023

named the "FLASH effect" [1].

So far, most of the pre-clinical studies available were performed with experimental electron beams of low energy [2,3], with limited application to small animals and/or superficial tumors. Therefore, demonstrating the FLASH effect with beams able to treat deep-seated tumors is required for clinical translation. Recently, clinical proton beams have been optimized to operate at an average dose rate above 40 Gy/s [4] and the FLASH effect has been validated in preclinical murine models using gut [4,5] and skin [6]. Normal tissue sparing was also described using proton-FLASH-PBS [7] and in the Bragg peak [8,9]. Although the temporal structures of electron and proton FLASH (eFLASH and pFLASH) beams are different, both have been shown to trigger the FLASH effect in mice.

Despite many positive findings, some publications failed to reproduce the FLASH sparing effect using proton on zebrafish embryos [10] or electron at 35 Gy/s on mice [11]. A recent study conducted in our laboratories found that pCONV and pFLASH generated similar protection of zebrafish embryos morphogenesis and were equal to that of eFLASH [12]. Additionally, a single patient study with eFLASH showed comparable treatment efficacy and adverse events to that of CONV dose rates [13], whilst a nonrandomized trial with pFLASH irradiation showed comparable treatment efficacy and adverse events to that of previously published standard proton therapy results [14]. These results emphasize the need to better understand the experimental conditions (such as the physical and biological parameters, geometry, organ, volume) required to produce the FLASH effect in clinical settings. Extensive understanding of the FLASH conditions should promote its safe and reliable transfer into clinic.

This study was designed to compare electrons and protons delivered at FLASH and CONV dose rates, both dosimetrically and biologically. First, we showed that a dosimetric consensus strategy recently developed for eFLASH [15] is applicable to proton beams. Second, using the model of late tissue damages developed for eFLASH [16–18], we validated the capability of Gantry 1 proton beam to achieve the FLASH sparing effect at 110 Gy/s. Finally, a complete tumor response associated with systemic anti-tumor immunity were obtained with a single ablative dose of 20 Gy, independently of the beam and the dose rates.

Material and method

Irradiation devices

Irradiation was performed using.

1) The Oriatron 6e (eRT6; PMB-Alcen, Peynier, France), a 5.5 MeV electron beam linear accelerator (LINAC) described previously [19] and extensively validated to produce the FLASH effect [1]. The prototype was operated at 0.1 Gy/s for CONV and at $\geq$ 110 Gy/s for FLASH. The beam parameters used in this study are shown in Table 1.
2) The PSI Comet is an isochronous cyclotron that delivers a quasi-continuous 250 MeV proton beam, with pulse frequency of 72.85 MHz and pulse length of 0.8 ns [20]. The beam is transported with ~ 85 % efficiency to Gantry1. Range shifters are inserted in the nozzle to scatter the single pencil proton beam and lower its energy to 170 MeV. The scattered beam is collimated downstream of the nozzle to deliver uniform dose distribution at 0.1 Gy/s for CONV and 110 Gy/s for FLASH in transmission mode. Additional irradiation parameters used in this study are included in Table 2.

Dosimetric intercomparison

We adopted the comparison scheme developed and validated by the CHUV/Institute of Radiation Physics (CHUV/IRA, Lausanne) to cross validate the PSI Gantry 1 beam and verify the dosimetric compatibility with the experiments performed at CHUV-eRT6. The comparison scheme seeks to establish consensus in the measured absorbed dose between different facilities operating FLASH dose rate beams. The methodology is detailed in Jorge et al. [15] and relies on a cuboid phantom ($25 \times 25 \times 32$ mm^3) made of acrylic (PMMA, $\rho = 1.19$ g·cm^{-3}) that can be mailed to the audited institute. The phantom (supplementary Figure 2) has a 5 mm (diameter) by 10.4 mm (length) central cylindrical cavity to simultaneously house three TLDs, two alanine pellets, and six laser-cut EBT3 Gafchromic films (Ashland, Bridgewater, US). After irradiation, the phantoms are sent back to IRA for dose readout.

The mailed phantoms were irradiated in the same conditions as for the pre-clinical experiments. A graphite applicator ($13.0 \times 13.0 \times 2.5$ cm^3) with 17 mm diameter aperture was used at CHUV-eRT6. Additional details on the measurements with cuboid phantoms and on reference dosimetry at CHUV-eRT6 are reported in recently published work [2,15,19]. The standard uncertainty ($k = 1$) on the absorbed dose measurements using the passive dosimeters in the cuboid phantoms was evaluated at 4 % for TLDs, 3 % for alanine, and 4 % for the laser-cut EBT3 Gafchromic films.

A 6 cm thick copper collimator with 17 mm diameter aperture was used at PSI-Gantry1. Five PMMA phantoms were mailed to PSI and four of them were irradiated behind the collimator, with the rectangular face orthogonal to the main beam axis. The non-irradiated phantom served as a background monitor. Beam quality correction factors were determined experimentally to correct the dose measured with the passive detectors in the cuboid phantoms. We report additional details on the PSI Gantry1 irradiation field and setup in sections 1.1 and 1.2 of the supplementary material as well as on the determination of beam quality correction factors in section 1.3.

Additionally, we used the cuboid phantoms with passive dosimeters provided by CHUV/IRA to benchmark the reference dosimeters typically used at PSI gGantry1, i.e. EBT3 Gafchromic films and a synthetic single-crystal microDiamond (PTW, Freiburg, Germany). Both dosimeters are cross-calibrated in CONV dose rate proton beams to a reference chamber traceable to the Swiss primary standard laboratory METAS. EBT3

Table 2
Gantry 1 beam parameters.

Mode	Prescribed Dose (Gy)	Average Dose Rate (Gy/s)	Beam current (nA)	Treatment time (s)
pCONV	10	0.1	0.66	100
	20	0.1	0.66	200
pFLASH	10	110	750	0.09
	20	110	750	0.18

Table 1
eRT6 beam parameters.

Mode	Prescribed Dose (Gy)	Average Dose Rate (Gy/s)	Frequency (Hz)	Pulse width (μs)	Number of pulses	Treatment time (s)
eCONV	10	0.1	10	1	≈600	≈60
	20	0.1	10	1	≈1500	≈150
eFLASH	10	$5.5 \cdot 10^6$ *	na	1.8	1	$1.8 \cdot 10^{-6}$
	10	110	100	1.8	10	0.09
	20	2000	100	1.8	2	0.01

* When one pulse is delivered, instantaneous dose rate = average dose rate.

Gafchromic films were calibrated at the same proton energy (170 MeV) used in the biological experiments. The dosimeters' responses at different dose rates were previously characterized by Togno et al. [21]. In this study, we corrected the response of the microDiamond at 110 Gy/s by 1.2 %. The overall combined uncertainty on absorbed dose to water in proton beams was 2.5 % ($k = 1$) and 1.9 % ($k = 1$) for EBT3 Gafchromic films and PTW microDiamond, respectively. This estimated uncertainty applies to both FLASH and CONV conditions.

In preparation for the mice experiments, the prescribed field dose of 10 Gy was measured with the PSI EBT3 Gafchromic films and microdiamond, as well as with an Advanced Markus ion chamber (PTW, Freiburg, Germany) calibrated at METAS. The uncertainty associated to the dose measured with the Advanced Markus ion chamber amounts to 2.2 % ($k = 1$). No dose rate correction was applied to the Advanced Markus reading.

Biologic intercomparison

To enable biological comparison, irradiation settings were defined to be similar between the two beams. The prescription dose for mice irradiations was determined by surface dose measurements behind the 17 mm diameter applicator. Animal experiments were approved by the Swiss (VD3603) ethics Committees for Animal Experimentation and performed within institutional guidelines.

Normal brain response

Whole brain irradiations (WBIs)

Female C57BL/6J mice (n = 10–12 animals per group) were purchased from Charles River laboratories at the age of eight weeks. WBI were performed under isoflurane anaesthesia. The mouse head was positioned behind and in contact with the 17 mm diameter applicator to irradiate the whole encephalon region while limiting the dose to the eyes, the mouth, and the rest of the body.

Novel object recognition testing

Neurocognitive impairments are typically found after treatments with CONV dose rate.

To determine the effects of FLASH and CONV using electron and proton beam irradiation on cognitive function, tumor-free animals were used to avoid confounding factors caused by tumor growth. Whole brain irradiation was performed using a single dose of 10 Gy delivered with FLASH (≥110 Gy/s) or CONV (0.1 Gy/s), with eRT6 or Gantry1 parameters described in tables 1 and 2. Novel Object Recognition (NOR) tests were performed 2 months post-RT, which is a time when alteration of hippocampal and frontal cortical learning and memory are stabilized. The NOR test was performed as previously described [16] to validate the sparing effect of pFLASH. It involved a sequence of habituation (no objects), familiarization (2 identical objects) and a final test in which one of the prior objects is switched with a different one. Animals tend to explore the novel object and successful performance on this task relies on intact perirhinal cortex function [22].

Tumor response

Primary tumor irradiation

Female C57BL/6J mice (n = 4–6 animals per group) were purchased from Charles River laboratories at the age of eight weeks and used for subcutaneous implantation with 5 million murine Glioblastoma (GBM) GL261 cells (Seligman, 1939) in the left flank. When tumor volume reached 80–100 mm^3, they were locally irradiated with a single dose of 20 Gy using the 17 mm in diameter applicator by stretching the skin and tumor over the applicator. Tumor growth was monitored by caliper measurement three times a week, and the volume was calculated with the formula of an oblate ellipsoid: $V = (a \times b^2)/2$, where a and b are the minor and major axes of the tumor.

Tumor rechallenge

GL261 cells are known to be highly aggressive but moderately immunogenic and radiosensitive *in vitro* as 2 Gy is already sufficient to decrease the surviving fraction by half [23]. To evaluate the potential of irradiation to generate *in situ* vaccine and T cell memory response, animals with a stable complete response for over 140 days post-RT were rechallenged with 5×10^6 cells implanted in the opposite right flank. Tumor growth was monitored by caliper measurement.

Statistical analyses

Statistical analyses were performed using GraphPad Prism (version 9.1).

The normality of groups was assessed using the Shapiro-Wilk test. For NOR evaluation one-way ANOVA was used to determine the significance between all groups using Tuckey's multiple comparison test. For tumor response, P values were estimated from Kruskal-Wallis test using Dunn's multiple comparison test. Results were expressed as mean ± SEM. All analyses considered a value of $P \leq 0.05$ to be statistically significant.

Results

Dose measurements

The results of the dosimetric comparison are shown in Fig. 1. Two PMMA phantoms were irradiated using pFLASH and pCONV. Fig. 1**A** shows, for the detectors loaded into the PMMA phantoms, the Co-60 reference values of absorbed dose to water provided by CHUV/IRA. Thus, the values represent the absorbed dose to water that should be delivered in a Co-60 calibration beam to induce the same signal as measured in the Gantry1 proton beam. To provide consistency with the dose measured with PSI detectors, beam quality correction factors ($k_{Q,Q_0}^{detector}$) are necessary. Using the same approach as Palmans et al. [24], we experimentally determined beam quality correction factors by cross-calibration against a reference ion-chamber in a proton beam at PSI Gantry 2. The details are included in section 1.3 of the supplementary material. The estimated correction factors are 1.00, 1.12 and 0.98 for Alanine, TLDs and laser-cut EBT3 Gafchromic films, respectively. The uncertainties associated to the correction factors are in the (2.4 – 4.7) % range with the uncertainty of the TLDs being affected mostly by measurement reproducibility. After correction with experimental $k_{Q,Q_0}^{detector}$ factors, the dose measured with Alanine and TLD detectors agrees within the standard uncertainty ($k = 1$) with the dose measured at PSI with EBT3 Gafchromic films and a microdiamond detector (Fig. 1**B**). Laser-cut EBT3 Gafchromic films in the PMMA phantoms underestimate by 11.1 % the average of the doses measured with Alanine and TLDs at CONV dose rate. At FLASH dose rate the difference amounts to −11.8 %. These results are consistent with previously reported data [15]. The relative bias between the average dose measured with the PSI detector and the average dose measured in the audit phantoms was −1.9 % (0.1 Gy/s) and +2.5 % (110 Gy/s).

Doses measured with PSI dosimeters (i.e. microDiamond, EBT3 Gafchromic films and Advanced Markus) in preparation of the biological experiments are shown in Fig. 2. The detectors were irradiated sequentially, hence the readings were corrected for beam output fluctuations (<1.5 %) between different deliveries at different dose rates. The dose measured with the three dosimeters agrees well within the experimental uncertainties, for both FLASH and CONV. The relative bias, i.e. the percentage difference between measured and prescribed dose, is in the range (-1.4 – 0) % for all detectors and dose rates.

Neurocognition is preserved with 10 Gy eFLASH and pFLASH

Animals (n = 10–12) WBI with 10 Gy pFLASH were subjected to the

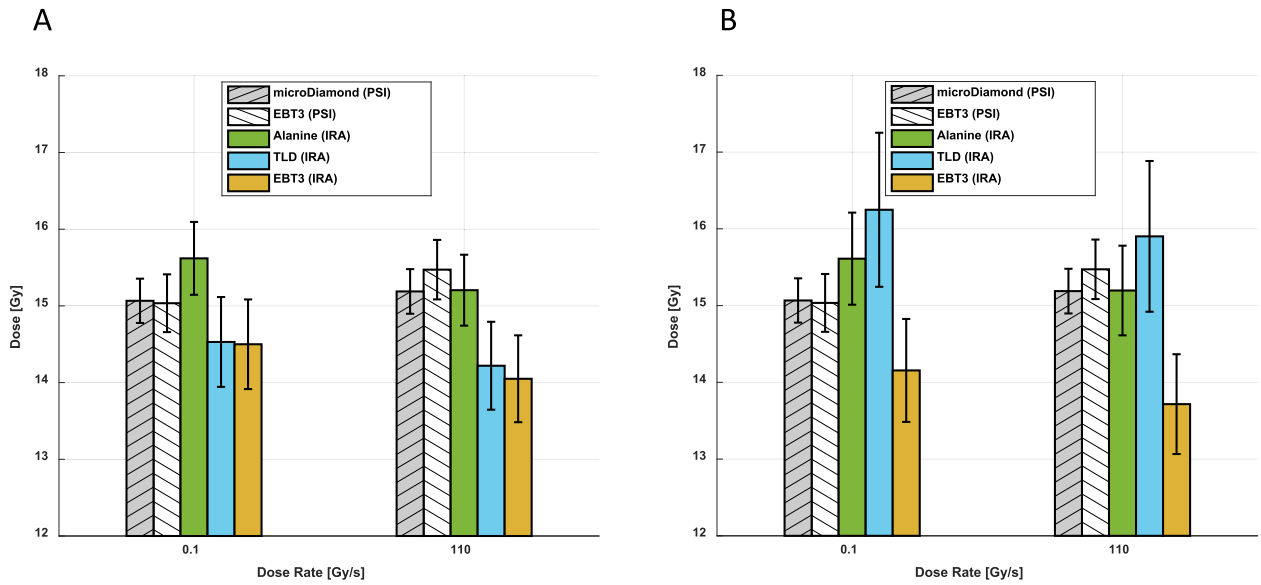

Fig. 1. Results of the dosimetric comparison between PSI and CHUV/IRA dosimeters, at dose rates of 0.1 Gy/s and 110 Gy/s. The dose measured with CHUV/IRA phantom dosimeters (Alanine, TLD, EBT3 IRA) is reported as Co-60 absorbed dose to water (A) and with experimentally determined corrections for beam quality applied (B). Error bars represent the combined standard uncertainty ($k = 1$).

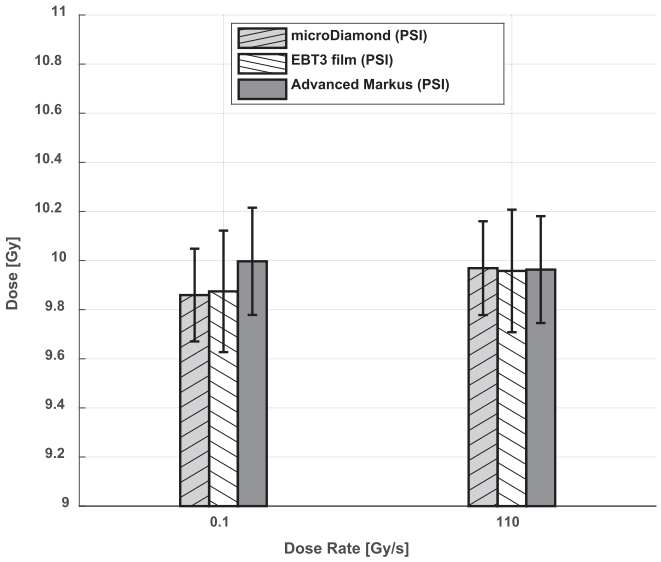

Fig. 2. Results of field dose measurements (target dose 10 Gy) using PSI dosimeters, at dose rates of 0.1 Gy/s and 110 Gy/s. Error bars represent the combined standard uncertainty ($k = 1$).

NOR test 2 months post-irradiation (Fig. 3), and found to be statistically indistinguishable from controls, whereas CONV irradiated cohorts exhibited a reduction in their recognition ratio. As expected, cognitive protection after eFLASH using either 10^7 Gy/s or 110 Gy/s was obtained with the eRT6, whereas eCONV caused cognitive decrement. Importantly, mice from all cohorts which were subjected to spontaneous exploration tasks exhibited normal motor function.

Complete and long-term anti-tumor response is beam-type and dose-rate independent using an ablative dose of 20 Gy

Five groups of C57BL/6J mice (n = 4–6) subcutaneously implanted with GL261 murine GBM model were irradiated (or not, control) with a single dose of 20 Gy FLASH or CONV using either electron (CHUV/eRT6) or proton (PSIGantry 1) beams. All tumor-bearing animals showed a complete and long-term response after irradiation, irrespective of the beam and dose rate used (Fig. 4**A and C**). No tumor relapse occurred in the irradiated cohorts >140 days post-irradiation.

Radiation-induced in situ vaccination is beam-type and dose-rate independent using an ablative dose of 20 Gy

Since a long-term cure was achieved in all irradiated animals, we also evaluated the possible occurrence of a radiation-induced memory response. 140 days after the complete response **(**Fig. 4**B and D)**, mice were rechallenged with GBM GL261 tumors engrafted on the opposite flank. While tumor growth occurred in 100 % naïve control animals, the rechallenge experiments resulted in spontaneous tumor rejection for all modalities with 100 % efficacy, indicating that the radiation-induced T cell memory response was long-lasting, beam type and dose rate independent at a single dose of 20 Gy.

Discussion

This paper is the first to report a systematic and longitudinal comparison of the FLASH capability of electron and proton beams, encompassing dosimetry to biological models. It marks the first successful report of the FLASH effect utilizing the Gantry1 proton beam at PSI and does not reveal significant differences between electron and proton beams at the dosimetric and biological level despite major differences in the temporal structure between electron and proton beams. Dosimetric standards can be established and used for subsequent radiobiological evaluation investigating the FLASH effect. At the biological level, electron and proton beams, when delivering FLASH in 90 ms or less, spared neurocognitive functions. A complete tumor response associated with sustained T memory response when subjected to an ablative dose was also observed.

Accurate determination of the dose is fundamental to conduct comprehensive research on the FLASH effect, but it remains challenging at ultra-high dose rate. At PSI Gantry 1, various detectors have undergone testing at dose rate reaching several kGy/s [25,26]. To prepare for the biological investigations, we verified that the measured field dose was reproducible and consistent between EBT3 Gafchromic films, Advanced Markus ion-chambers and synthetic microDiamonds. Based on the results of this work, and taking in consideration previous

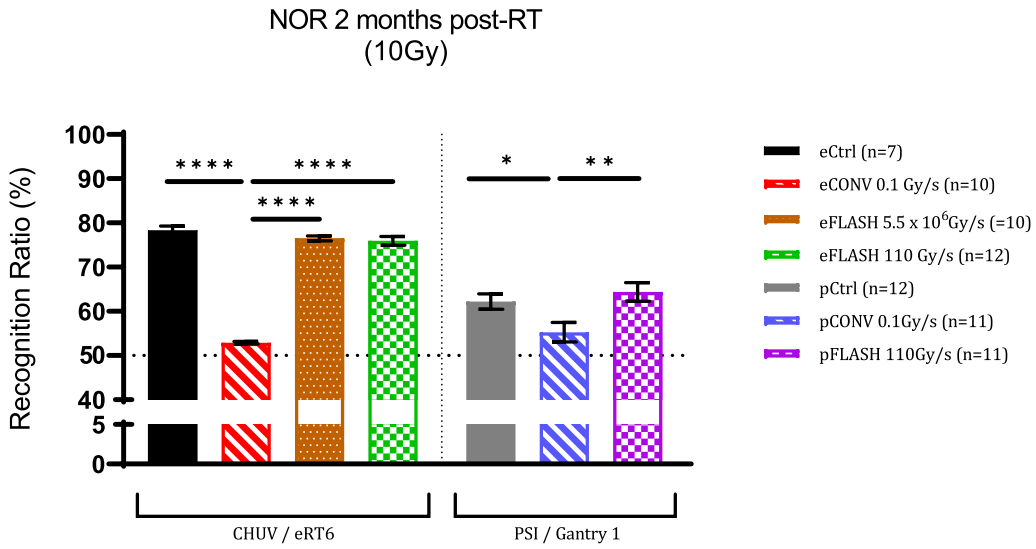

Fig. 3. Novel Object Recognition (NOR) Test: Animals exposed to 10 Gy with both eFLASH and pFLASH, have statistically indistinguishable recognition ratios relative to controls indicating a preference for the novel object, whereas mice irradiated with eCONV and pCONV showed impairment compared to controls. Mean ± SEM (n = 10–12 per group); p-values were compared against CONV and derived from One-way ANOVA followed by Tukey's correction for multiple comparisons. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

published studies [21], the microDiamond stands out as an efficient and practical dosimeter for accurately measuring the absorbed dose when ultra-high dose rate quasi-continuous proton beam is used. Furthermore, in contrast to Gafchromic films, the microDiamond dosimeter exhibits independence from the proton beam energy within the clinically-relevant range [27]. On the other hand, while the Advanced Markus ion-chamber was tested up to 110 Gy/s, its ion-collection efficiency at higher dose rates would necessitate further exploration and investigation.

Currently, no primary standard exists for proton beams, whether they are used at CONV or FLASH dose rates. In the present study, we have expanded upon a comparison scheme previously utilized with MeV electron beams [15] to establish a dosimetric consensus and cross-validate the Gantry1 and eRT6 irradiation beams. To ensure consistency between the reported doses by PSI and CHUV/IRA, we experimentally determined beam quality correction factors through the cross-calibration of CHUV/IRA detectors in proton beams. While the reproducibility of the measurements varied in the (0.9 – 3.8) % range for different detectors, the measured correction factors for Alanine (1.00 ± 0.02), TLDs (1.12 ± 0.05) and laser-cut EBT3 Gafchromic films (0.98 ± 0.02) were found to be compatible with values reported in the literature [24,28–30]. Clearly, a more precise determination of these correction factors is needed to further improve the accuracy of the measured dose to water in proton beams. PSI EBT3 Gafchromic films and microDiamond agree with CHUV/IRA dosimeters (alanine, TLDs) within the standard uncertainty ($k = 1$) of the measurements, therefore proving the dosimetric consistency of PSI and CHUV irradiation fields. CHUV/IRA laser-cut EBT3 Gafchromic films underestimate by 11 % the average dose measured by the other dosimeters in the cuboid phantoms. This discrepancy could be due to the energy spectrum of the beam reaching the detectors, which is composed of primary and secondary contributions. These can be different in water, during calibration, than in the phantom, essentially in air. Moreover, the energy dependence of Gafchromic films is believed to be dose dependent under 1 Gy. Such effects are difficult to deconvolute and require additional experimental investigations as well as simulation tools, but we estimated the uncertainties accordingly.

The majority of recent publications reporting the FLASH capability of novel beams have used the gastro-intestinal syndrome [5,31] which is an ideal model to investigate acute response but irrelevant for delayed toxicities which still remain the main concern in the field of radiation oncology. Similarly, our recent results show that delayed toxicities will remain of concern with FLASH especially if high single doses are used. In the study conducted on domestic cat patients with squamous cell carcimoma, while acute toxicity was minor, late osteoradionecrosis occurred in 3 out of 7 cats treated with 30 Gy in 3 pulses at 1500 Gy/s [32]. Given these considerations, our objective was to validate Gantry1's FLASH capability using a well-known model of late-responding tissue, the brain. We operated Gantry1 at its maximum dose rate to ensure uniform dose and dose-rate coverage of the brain at 110 Gy/s, which was also applied at eRT6 (at 110 Gy/s and 1 single pulse). Results from the NOR tests conducted at both Gantry1 and eRT6 indicated preservation of the neurocognition when a dose rate of 110 Gy/s was used. It should be noted that cognitive scores varied between the two experimental groups (electron and proton), which is an inherent limitation of the NOR test as discussed in Drayson et al. [22]. Nevertheless, these relative findings are consistent with our previous experiments on dose rate escalation [16] and support the notion that the FLASH-sparing effect can be replicated in the brain of mice at an average dose rate of 100 Gy/s or more, regardless of the beam type. While further studies are ongoing to determine a dose-modifying factor at Gantry1 and explore the FLASH-sparing effect in a dose escalation study, our findings indicate that one of the most critical physical parameter for inducing the FLASH-sparing effect is the overall time of irradiation exposure in other words the average dose rate.

In addition, while our recent study in ZF embryos did not show any difference between pFLASH and pCONV [12], the present study shows that protons from Gantry1 are able to preserve neurocognition after FLASH as compared to CONV. These studies suggest subtle biological differences triggered by proton and electron beams contingent to the specific biological target. They suggest that ZF embryos at early stage of their development may be more sensitive to the nature of the beam than mice and might require specific conditions as already described [10,33], and/or a higher dose rate (above 10^6 Gy/s) to reveal the FLASH-sparing effect as observed in mouse tissues (around 100 Gy/s).

At the tumor level, the present study shows that curative potential and long-term anti-tumor immunity generated by irradiation is also possible at FLASH dose rates. 20 Gy delivered either with pCONV and pFLASH and eCONV and eFLASH controlled GL261 subcutaneous tumors and induced a T cell memory response that triggered rejection of secondary tumors. These results are consistent with a previous report performed with eFLASH [34] and are confirmed here with pFLASH

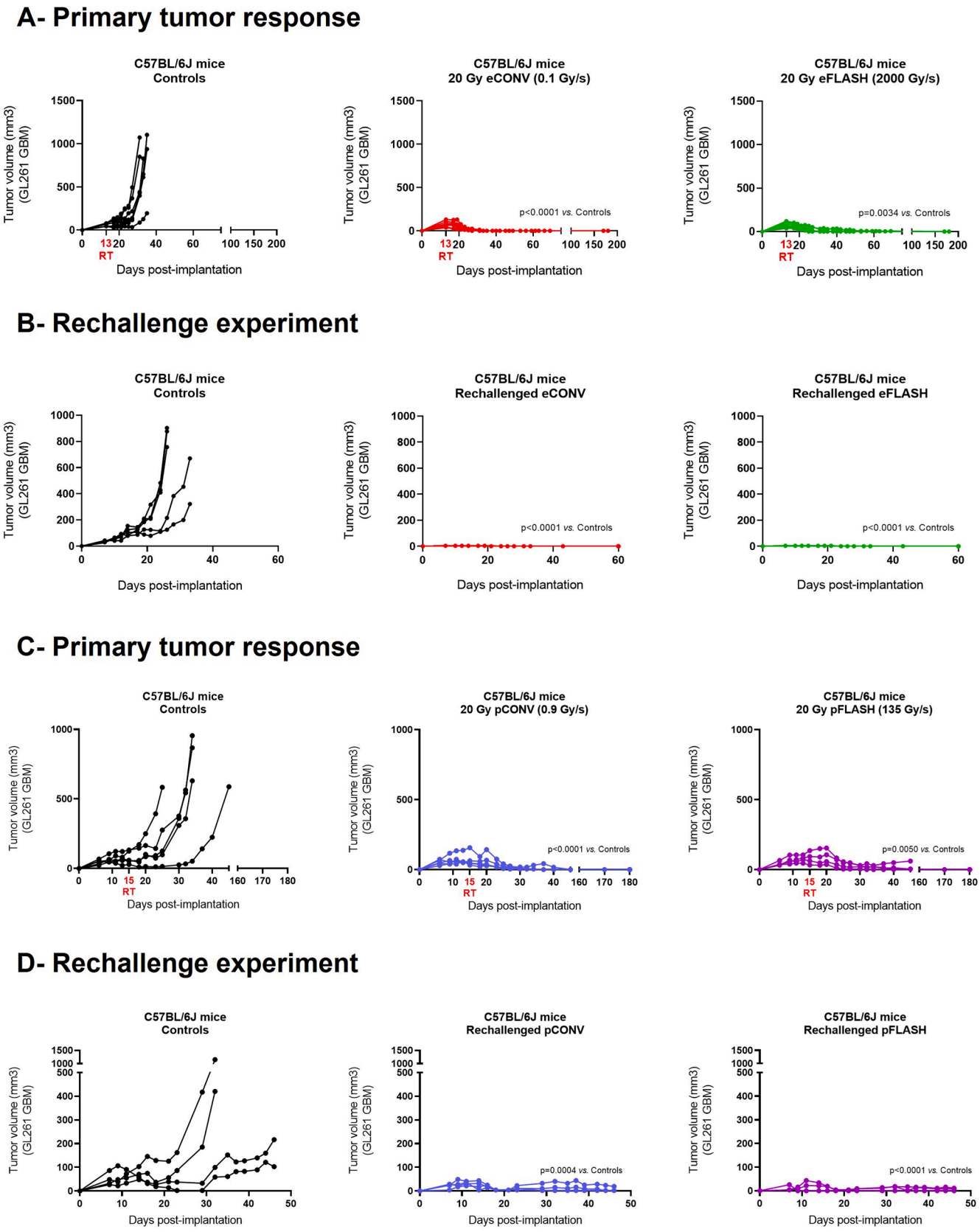

Fig. 4. GL261 glioblastoma (GBM) tumors were irradiated at 20 Gy with e/pFLASH or e/pCONV after subcutaneous engraftment into immunocompetent C57BL/6J female mice (A and C). Cured immunocompetent C57BL/6J female mice were rechallenged with 5×10^6 cells implanted in the opposite flank (B and D). Tumor growth delay was followed by caliper measurement 3 times per week. Results are given in individual values. Statistical analysis of tumor growth curves was performed using Mann-Whitney test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

beams. In addition and consistently with the recent study by Iturri et al. using an orthotopic glioma rat model [35], our study does not indicate occurrence of a FLASH-specific immune response in tumors.

One limitation of this study lies in the use of independent models to assess normal tissue and tumor response. However, this approach was employed deliberately to avoid confounding factors, particularly since brain tumors could potentially impact cognition. Consequently, we evaluated the normal brain response at a dose of 10 Gy which biological equivalent dose (BED) is similar to that of prophylactic whole-brain radiation therapy (WBRT), known to cause long-term cognitive effects. In contrast, brain tumors were injected subcutaneously and irradiated with a high dose of 20 Gy that induced complete response, while enabling a longer follow-up compared to the orthotopic model. Thus, doses were adapted accordingly for each selected model. Our study effectively demonstrates that tumor control and normal tissue sparing can be achieved successfully with pFLASH modalities.

In summary, this study presents a comprehensive strategy for validating novel FLASH beams, encompassing dosimetric and biological endpoints. It also suggests that a dose rate of 110 Gy/s delivered in 90 ms could be sufficient to produce the FLASH effect in mice for both proton and electron beams.

CRediT authorship contribution statement

A Almeida: Conceptualization, Formal analysis, Investigation, Writing – original draft, Visualization. **M Togno:** Conceptualization, Formal analysis, Investigation, Writing – original draft, Visualization. **P Ballesteros-Zebadua:** Conceptualization, Formal analysis, Investigation, Writing – original draft, Visualization, Funding acquisition. **J Franco-Perez:** Investigation, Validation, Writing – review & editing. **R Geyer:** Validation, Writing – review & editing. **R Schaefer:** Investigation, Validation, Writing – review & editing. **B Petit:** Investigation, Validation, Data curation. **V Grilj:** Investigation, Writing – review & editing. **D Meer:** Methodology, Writing – review & editing, Supervision. **S Safai:** Methodology, Writing – review & editing, Supervision. **T Lomax:** Conceptualization, Methodology, Writing – review & editing, Supervision. **DC Weber:** Conceptualization, Resources, Writing – review & editing, Supervision. **C Bailat:** Conceptualization, Methodology, Formal analysis, Writing – review & editing. **S Psoroulas:** Conceptualization, Methodology, Investigation, Writing – original draft, Supervision, Project administration. **Marie-Catherine Vozenin:** Conceptualization, Investigation, Resources, Data curation, Writing – original draft, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Vozenin reports financial support was provided by Varian Medical Systems Inc. Vozenin reports a relationship with Varian Medical Systems Inc that includes: funding grants. the support also apply to D Weber, T Lomax, D Meer, S Psoroulas.

Acknowledgment

Funding was provided by National Institutes of Health grant P01CA244091-01 (to MCV supporting AA, VG); Swiss National Science Foundation grant Spirit IZSTZ0_198747/1 (to MCV and PBZ supporting JFC) and MAGIC - FNS CRS II5_186369 (to MCV and supporting VG); and CONACYT for supporting PBZ sabbatical in Switzerland. We also thank the Lausanne core facilities including the Animal facility, In vivo imaging facility, Mouse pathology facility at Epalinges.

Disclosure

PSI team (DW, TL, DM, SP) and MCV have received and receive research funds from Varian, a Healthineers company.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.radonc.2023.109953.

References

[1] Vozenin M-C, Bourhis J, Durante M. Towards clinical translation of FLASH radiotherapy. Nat Rev Clin Oncol 2022;19:791–803.
[2] Jorge PG, Jaccard M, Petersson K, Gondré M, Durán MT, Desorgher L, et al. Dosimetric and preparation procedures for irradiating biological models with pulsed electron beam at ultra-high dose-rate. Radiother Oncol 2019;139:34–9.
[3] Simmons DA, Lartey FM, Schüler E, Rafat M, King G, Kim A, et al. Reduced cognitive deficits after FLASH irradiation of whole mouse brain are associated with less hippocampal dendritic spine loss and neuroinflammation. Radiother Oncol 2019;139:4–10.
[4] Diffenderfer ES, Verginadis II, Kim MM, Shoniyozov K, Velalopoulou A, Goia D, et al. Design, implementation, and in vivo validation of a novel proton FLASH radiation therapy system. Int J Radiat Oncol Biol Phys 2020;106:440–8.
[5] Velalopoulou A, Karagounis IV, Cramer GM, Kim MM, Skoufos G, Goia D, et al. FLASH proton radiotherapy spares normal epithelial and mesenchymal tissues while preserving sarcoma response. Cancer Res 2021;81:4808–21.
[6] Singers Sørensen B, Krzysztof Sitarz M, Ankjærgaard 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.
[7] S. Cunningham, S. McCauley, K. Vairamani, J. Speth, S. Girdhani, E. Abel, R. A. Sharma, J. P. Perentesis, S. I. Wells, A. Mascia, M. Sertorio, FLASH Proton Pencil Beam Scanning Irradiation Minimizes Radiation-Induced Leg Contracture and Skin Toxicity in Mice. *Cancers (Basel)* **13**, 1012 (2021).
[8] Kim Y-E, Gwak S-H, Hong B-J, Oh J-M, Choi H-S, Kim MS, et al. Effects of ultra-high doserate FLASH irradiation on the tumor microenvironment in Lewis lung carcinoma: Role of myosin light chain. Int J Radiat Oncol Biol Phys 2021;109:1440–53.
[9] Evans T, Cooley J, Wagner M, Yu T, Zwart T. Demonstration of the FLASH effect within the spread-out bragg peak after abdominal irradiation of mice. Int J Part Ther 2022;8:68–75.
[10] Beyreuther E, Brand M, Hans S, Hideghéty K, Karsch L, Leßmann E, et al. Feasibility of proton FLASH effect tested by zebrafish embryo irradiation. Radiother Oncol 2019;139:46–50.
[11] Venkatesulu BP, Sharma A, Pollard-Larkin JM, Sadagopan R, Symons J, Neri S, et al. Ultra high dose rate (35 Gy/sec) radiation does not spare the normal tissue in cardiac and splenic models of lymphopenia and gastrointestinal syndrome. Sci Rep 2019;9:17180.
[12] Kacem H, Psoroulas S, Boivin G, Folkerts M, Grilj V, Lomax T, et al. Comparing radiolytic production of H2O2 and development of Zebrafish embryos after ultra high dose rate exposure with electron and transmission proton beams. Radiother Oncol 2022;175:197–202.
[13] Gaide O, Herrera F, Jeanneret Sozzi W, Gonçalves Jorge P, Kinj R, Bailat C, et al. Comparison of ultra-high versus conventional dose rate radiotherapy in a patient with cutaneous lymphoma. Radiother Oncol 2022;174:87–91.
[14] Mascia AE, Daugherty EC, Zhang Y, Lee E, Xiao Z, Sertorio M, et al. Proton FLASH radiotherapy for the treatment of symptomatic bone metastases: The FAST-01 nonrandomized trial. JAMA Oncol 2023;9:62–9.
[15] Jorge PG, Melemenidis S, Grilj V, Buchillier T, Manjappa R, Viswanathan V, et al. Design and validation of a dosimetric comparison scheme tailored for ultra-high dose-rate electron beams to support multicenter FLASH preclinical studies. Radiother Oncol 2022;175:203–9.
[16] Montay-Gruel P, Petersson K, Jaccard M, Boivin G, Germond J-F, 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.
[17] Montay-Gruel P, Acharya MM, Petersson K, Alikhani L, Yakkala C, Allen BD, et al. Long-term neurocognitive benefits of FLASH radiotherapy driven by reduced reactive oxygen species. PNAS 2019;116:10943–51.
[18] Montay-Gruel P, Acharya MM, Jorge PG, Petit B, Petridis IG, Fuchs P, et al. Hypofractionated FLASH-RT as an effective treatment against glioblastoma that reduces neurocognitive side effects in mice. Clin Cancer Res 2021;27:775–84.
[19] Jaccard M, Durán MT, Petersson K, Germond JF, Liger P, Vozenin MC, et al. High dose-per-pulse electron beam dosimetry: Commissioning of the Oriatron eRT6 prototype linear accelerator for preclinical use: Commissioning. Med Phys 2018;45:863–74.
[20] Nesteruk KP, Togno M, Grossmann M, Lomax AJ, Weber DC, Schippers JM, et al. Commissioning of a clinical pencil beam scanning proton therapy unit for ultra-high dose rates (FLASH). Med Phys 2021;48:4017–26.
[21] Togno M, Nesteruk KP, Schäfer R, Psoroulas S, Meer D, Grossmann M, et al. Ultra-high dose rate dosimetry for pre-clinical experiments with mm-small proton fields. Phys Med 2022;104:101–11.
[22] O. G. G. Drayson, M.-C. Vozenin, C. L. Limoli, A Rigorous Behavioral Testing Platform for the Assessment of Radiation-Induced Neurological Outcomes. *Methods in Cell Biology* (In press).

[23] Szatmári T, Lumniczky K, Désaknai S, Trajcevski S, Hídvégi EJ, Hamada H, et al. Detailed characterization of the mouse glioma 261 tumor model for experimental glioblastoma therapy. Cancer Sci 2006;97:546–53.
[24] H. Palmans, A. Carlino, C. Gouldstone, P. Sharpe, CROSS CALIBRATION OF ALANINE FOR SCANNED PROTON BEAMS. .
[25] Christensen JB, Togno M, Nesteruk KP, Psoroulas S, Meer D, Weber DC, et al. Al2O3: C optically stimulated luminescence dosimeters (OSLDs) for ultra-high dose rate proton dosimetry. Phys Med Biol 2021;66:085003.
[26] Motta S, Christensen JB, Togno M, Schäfer R, Safai S, Lomax AJ, et al. Characterization of LiF:Mg, Ti thermoluminescence detectors in low-LET proton beams at ultra-high dose rates. Phys Med Biol 2023;68:045017.
[27] Gomà C, Marinelli M, Safai S, Verona-Rinati G, Würfel J. The role of a microDiamond detector in the dosimetry of proton pencil beams. Z Med Phys 2016; 26:88–94.
[28] Kry SF, Alvarez P, Cygler JE, DeWerd LA, Howell RM, Meeks S, et al. AAPM TG 191: Clinical use of luminescent dosimeters: TLDs and OSLDs. Med Phys 2020;47: e19–51.
[29] Chen Y-S, Wu S-W, Huang H-C, Chen H-H. Absolute dose measurement and energy dependence of LiF dosimeters in proton therapy beam dosimetry. Therap Radiol Oncol 2022;6. https://doi.org/10.21037/tro-22-16.
[30] Smith BR, Pankuch M, Hammer CG, DeWerd LA, Culberson WS. LET response variability of Gafchromic EBT3 film from a Co calibration in clinical proton beam qualities. Med Phys 2019;46:2716–28.
[31] Ruan J-L, 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;111:1250–61.
[32] C. Rohrer Bley, F. Wolf, P. Gonçalves Jorge, V. Grilj, I. Petridis, B. Petit, T. T. Böhlen, R. Moeckli, C. Limoli, J. Bourhis, V. Meier, M.-C. Vozenin, 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* **28**, 3814–3823 (2022).
[33] Karsch L, Pawelke J, Brand M, Hans S, Hideghéty K, Jansen J, et al. Beam pulse structure and dose rate as determinants for the flash effect observed in zebrafish embryo. Radiother Oncol 2022;173:49–54.
[34] Liljedahl E, Konradsson E, Gustafsson E, Jonsson KF, Olofsson JK, Ceberg C, et al. Long-term anti-tumor effects following both conventional radiotherapy and FLASH in fully immunocompetent animals with glioblastoma. Sci Rep 2022;12:12285.
[35] Iturri L, Bertho A, Lamirault C, Juchaux M, Gilbert C, Espenon J, et al. Proton FLASH radiation therapy and immune infiltration: Evaluation in an orthotopic glioma rat model. Int J Radiat Oncol Biol Phys 2022. https://doi.org/10.1016/j.ijrobp.2022.12.018.