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Ultra-high dose rate electron beams and the FLASH effect: From preclinical evidence to a new radiotherapy paradigm

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Abstract

In their seminal paper from 2014, Fauvadon et al. coined the term FLASH irradiation to describe ultra-high-dose rate irradiation with dose rates greater than 40 Gy/s, which results in delivery times of fractions of a second. The experiments presented in that paper were performed with a high-dose-per-pulse 4.5 MeV electron beam, and the results served as the basis for the modern-day field of FLASH radiation therapy (RT). In this article, we review the studies that have been published after those early experiments, demonstrating the robust effects of FLASH RT on normal tissue sparing in preclinical models. We also outline the various irradiation parameters that have been used. Although the robustness of the biological response has been established, the mechanisms behind the FLASH effect are currently under investigation in a number of laboratories. However, differences in the magnitude of the FLASH effect between experiments in different labs have been reported. Reasons for these differences even within the same animal model are currently unknown, but likely has to do with the marked differences in irradiation parameter settings used. Here, we show that these parameters are often not reported, which complicates large multistudy comparisons. For this reason, we propose a new standard for beam parameter reporting and discuss a systematic path to the clinical translation of FLASH RT.

KEYWORDS
biological effects, electron FLASH, FLASH effect, dosimetry, reporting system

1 | FLASH TERMINOLOGY AND CRITICAL BEAM PARAMETERS

The early definition of FLASH irradiation (mean dose rates ($\bar{D}$) ≥ 40 Gy/s) has served as a good starting point for studying the dose rates at which the "FLASH effect" (normal tissue sparing with isoeffective tumor control after FLASH compared to conventional [CONV] irradiation) could be expected.[1] Although FLASH irradiation has been shown to evoke strong, reproducible responses across many different organ systems (e.g., brain, lungs, gastrointestinal [GI] tract, skin) across multiple species,[2] variations in the magnitude of the FLASH effect has been reported between studies, and others have shown that ultra-high dose rate irradiation has either no or detrimental effects

compared to CONV irradiation.[3–9] Although these studies involved different types of radiation (X-rays, protons, and electrons), the common denominator was low mean dose rate, and two of the three studies used $\bar{\dot{D}} \leq 40$ Gy/s. Whether this could explain the lack of an observed FLASH effect is unknown at this time. However, understanding the intrinsic physical differences that exist between these positive and negative experiments should provide a better understanding of the FLASH effect.

A likely factor contributing to the variations in the magnitude of the FALSH effect between different studies could stem from inconsistencies in the physical radiation beam and fractionation parameters. Indeed, more recent findings suggest that the definition of $\bar{\dot{D}} > 40$ Gy/s to achieve the FLASH effect is overly simplified, and the FLASH effect may also depend on other aspects of the radiation beam, potentially related to the total dose delivered, radiation source (e.g., electrons, protons, heavy particles, photons), irradiated volume, overall delivery time, and pulse-related factors such as dose and dose rate per pulse, as well as the frequency of the pulse delivery.[10]

To date, the most detailed characterizations of how the FLASH effect depends on the physical aspects of the radiation beam have been completed with mouse models of whole-brain irradiation. In one such series of experiments, the absorbed dose, pulse duration, and pulse frequency were kept constant (10 Gy, 1.8 μs, and 100 Hz, respectively), and the dose per pulse—and thus the mean dose rate, instantaneous dose rate, and irradiation duration—were varied.[11] A sigmoidal response in neurocognitive performance (the endpoint in this model) was noted in which a dose-rate–dependent increase in memory was seen at $\bar{\dot{D}} > 18.5$ Gy/s (instantaneous dose rate ($\dot{D}_p$): 1.0E5 Gy/s), with a plateau reached after $\bar{\dot{D}}$:100 Gy/s ($\dot{D}_p$: 5.6E5 Gy/s). In another study of mouse whole-brain irradiation, FLASH ($\bar{\dot{D}}$: 200–300 Gy/s, dose per pulse (D_p): 1.75 Gy, $\dot{D}_p$: 8.75E5 Gy/s) irradiation was found to produce less toxicity than CONV irradiation even when delivered with the same dose per pulse and number of pulses as FLASH (D_p: 1.75 Gy, 18 pulses) but over a longer total time (0.1 vs. 240 s), suggesting the importance of overall delivery time.[12] In 2019, a meta-analysis of available data on FLASH radiotherapy (RT) from in vivo models revealed the importance of overall irradiation time and dose rate within each pulse[13]; this work was updated in 2021.[14] How other physical characteristics of the radiation beam could affect the robustness and optimization of the FLASH effect remain unknown.

Although most of the data investigating the FLASH effect to date have been obtained with pulsed electron beams, robust comparisons among studies require certain parameters to be defined,[15] such as beam energy, beam structure, total dose, mean dose rate, instantaneous (intra-pulse) dose rate, pulse repetition frequency, dose per pulse, pulse width, duration of exposure, field size, percentage depth dose, dose profiles, and irradiated volume (summarized in Table 1). Also crucial are specific definitions of where these parameters are defined, and what the dose gradient is across the irradiated volume of interest. For example, in mouse irradiations, the dose gradient for electron irradiations can be >15%/cm depending on the tissue type traversed by the beam, beam energy, field size, and the source-to-surface (SSD) distance.[16] For this reason, it is important to have a common definition of how and where the dose and dose parameters are defined, and that the dosimetry is performed in a geometry that is representative of the experimental setup.

To date, different studies have used different definitions and approaches for dose determination. For a better understanding of the irradiation setup, and to facilitate for a broader comparison of the irradiation parameters used both temporally and spatially while still considering the limitations in hardware and software available, we suggest the following set to be at a minimum reported in terms of dose parameter: The dose and dose parameters should be defined to a dose specification point (DSP) at the center of the irradiated volume of interest. If a highly irregular volume is considered, then a representative DSP in this volume should be defined and used. The reporting of the dose parameters should be accompanied by the coordinates of the DSP as well as dose profile measurements along the lateral and axial directions, centered on the DSP. These dose profiles should be taken in a geometry that closely resembles the experimental setup if determined experimentally. If it is not feasible to obtain the dose profiles, then at least one more dose point, in addition to the central point, should be defined along the central axis of each beam used to facilitate evaluations of the dose gradient across the volume of interest. In the published literature, the entrance dose has been used extensively to define the delivered dose. However, the relationship between surface dose and dose at depth will depend on the type of radiation, the energy, and the SSD. Nevertheless, in the absence of a full percentage depth dose, it is recommended that the entrance dose also be defined along with the dose at the center of the irradiated volume of interest.

For multi-beam deliveries, the parameters in Table 1 should be reported for each individual beam and as a composite for each fraction. In addition, the time between each beam delivery needs to be reported to allow determination of the overall treatment time for the entire fraction.

Carefully documented and clearly defined experimental conditions are essential for ensuring reproducibility and hence results that can be compared among studies of FLASH RT, which in turn is needed for the safe and

TABLE 1 Reportable physical parameters of the electron FLASH beam

Irradiation parameters	Unit of measure	Comments
Beam energy	MeV	
Total absorbed dose	Gy	Defined to a dose specification point (DSP) at the center of the irradiated volume of interest. The coordinates of this point in relation to the geometry of interest must also be reported.
Fractionation schedule		Dose per fraction, number of fractions, and time between fractions
Mean dose rate per fraction ($\bar{D}$)	Gy/s	
Instantaneous dose rate ($\dot{D}_p$)	Gy/s	Dose rate within one pulse. Also reported is the variation in instantaneous dose rates within the pulse train delivered.
Pulse frequency	Hz	
Dose per pulse (D_p)	Gy	Mean dose per pulse and dose per pulse variation within the pulse train delivered
Pulse width	S	At full width at half maximum (FWHM)
Duration of exposure	S	Total irradiation time = number of pulses * pulse width + (number of pulses – 1) * pulse separation
Beam field size		Field shape and area irradiated, defined at the surface of the irradiated geometry. For example, AxB [mm^2] for square fields, or diameter [mm] of circular fields.
Dose profiles	%	Dose profiles taken along the lateral and axial directions in the coordinate system are defined and described by the researcher. The profiles are centered on and normalized to the DSP. Alternatively, two point-doses along the central axis should be reported (DSP + surface dose) in case no dose profiles are supplied.
Irradiated volume	mm^3	Volume receiving 10% or more of prescription dose

reliable transfer of FLASH RT strategies into the clinic.[15] This detailed reporting of the irradiation parameters is needed to elucidate the contributions of the physical beam parameters to the FLASH effect, because pooling of data from different studies will be required. Although many of the parameters presented in Table 1 can be derived from one another, explicit definitions of each will minimize downstream errors. Notably, these parameters reflect only the physical parameters of the beam; biological parameters, described in the following sections, are equally important.

1.1 | Measuring physical beam parameters

Measuring many of the physical parameters in Table 1 would require the implementation of detectors and dosimetry protocols that are not routinely used at present. Many of the characteristics of an ideal radiation detector are, however, shared by both CONV and FLASH radiation beams, and include tissue equivalence, energy independence, and nonperturbing qualities. Other characteristics of increasing importance in FLASH beams are dose–rate independence, in terms of both mean and instantaneous dose rate, and the temporal resolution of the detector.[2,13] The D_p and $\dot{D}_p$ for electron beams can be on the order of 20 Gy and 10^{12} Gy/s, respectively, which pose a challenge when conventional detectors and dosimetry protocols are used due to, for example, saturation effects and loss of signal due to the rapid energy deposition in the detectors and dosimeters.[17,18] Related to the temporal resolution of the detector, the ability to resolve individual pulses is needed to allow dose monitoring and beam control. In addition to the short time scale of resolving individual pulses, real-time (as opposed to passive) dose monitoring also becomes increasingly important in the development of the new control systems needed to translate FLASH-capable machines into clinical use.

In relation to dose–rate dependence is also the dynamic range of the detector. Ideally, the dynamic range should cover both the lower and upper limits of both CONV- and FLASH irradiation to facilitate the use of a single detector for both types of irradiation without having to resort to excessive corrections to the readings. Spatial resolution is also vital, particularly in preclinical models that involve the use of extremely small fields; such irradiations fall into the realm of "small-field dosimetry" owing to the loss of the lateral charged particle equilibrium.[19] Volume averaging becomes a concern in small fields, as larger detectors would be exposed to different dose rates across different parts of the detector volume.

An in-depth review of the available detector systems and their applicability in FLASH dosimetry is beyond the scope of this review; interested readers are referred to another review by Ashraf et al.[18] However, certain detector systems are worth mentioning because of their universal use in FLASH dosimetry. Gafchromic film is extensively used as a dosimetry system in FLASH-related studies and due to its dose–rate independence is often used as the reference which to compare other detector systems to.[20,21] Gafchromic film is extensively

used as a dosimetry system in FLASH-related studies and due to its dose–rate independence is often used as the reference to which other detector systems are compared against. Gafchromic film has been extensively characterized in high dose rate beams and has been found to be independent of dose rates up to 9E12 Gy/s.[20,21] However, Gafchromic film's major drawback is that it is limited by delays in the readout; ideally, the film should not be analyzed until 24 h after the irradiation to allow the rate of polymerization to stabilize.[18] Other passive dosimeters heavily used in FLASH dosimetry include alanine and thermoluminescent dosimeters (TLDs).[22,23] These detectors share many of the characteristics of Gafchromic film but are limited to point-dose measurements. Nevertheless, alanine and TLDs have been shown to be independent of dose rates of >1.5E9 Gy/s and are very useful for the characterization of FLASH beams in in vivo dosimetry.[20,24]

Ion chambers, which are an integral part of normal clinical operations in CONV RT, are challenging to use with FLASH beams. As a large amount of energy is transferred within a single pulse to the collection volume of the chamber, the collection efficiency of ion chambers is reduced by recombination of the generated ion pairs before being collected by the electrodes. Models that are typically used to account for recombination are not applicable to FLASH dose rates.[18] However, Petersson et al.[25] attempted to circumvent this limitation. With their detailed characterization of the Advanced Marcus chamber and with the use of Gafchromic film as the standard, this group developed a logistic function for ion recombination correction that can be used successfully in FLASH beam lines.

Perhaps the most promising detectors for use in FLASH beam dosimetry are scintillator- and Cherenkov-based detectors. Organic scintillator detectors are tissue equivalent, energy- and dose-rate independent, and allow measurement of integrated dose in real time as well as temporal resolution of individual linac pulses.[26–28] Inorganic scintillator detectors have similar characteristics, but their use of high atomic number materials precludes their tissue equivalence and energy independence. However, because these detectors are generally more radiation resistant, and have higher light output and easier corrections for the stem-effect than organic scintillators, they remain attractive for FLASH beam dosimetry. For FLASH dosimetry, the signal generated would be several orders of magnitude higher than that generated in CONV dose-rate dosimetry. For this reason, the active volume could be made smaller and allow easier filtering of signals pertaining to stem-effect features, even with organic scintillators. Archer et al. recently demonstrated the successful implementation of a 10 μm thick BC-400 film plastic scintillator coupled to a 1 mm diameter optical fiber in a synchrotron X-ray beam, which showed excellent performance at dose rates of up to 4435 Gy/s.[29] Two- and three-dimensional measurements with luminescent technology have also been successfully implemented.[30–34]

2 | FLASH: PRECLINICAL INVESTIGATIONS USING ELECTRON BEAMS

2.1 | Normal tissues

The FLASH effect, as noted above, is defined by the preservation of normal tissues simultaneous with antitumor efficacy equivalent to that of CONV RT at the same dose level.[1,12,13,15,22,23,35–39] To date, the FLASH effect has been characterized in several in vivo models, primarily wild-type mice, and in several organ systems, as summarized in Table 2. These organs include either the so-called acute-responding organs (gut, hematopoietic system)[36,40] as well as late-responding organs (brain, lung, skin),[1,12,13,15,22,23,35,37,38,41,42] thereby suggesting that FLASH RT modifies a common initial event that can control the development of both acute and delayed toxicity. The fundamental physicochemical mechanisms underlying the FLASH effect are currently under investigation; one hypothesis implicating transient local oxygen depletion was first proposed nearly 40 years ago.[43,44] However, direct measurements of such depletion in vivo are difficult to obtain,[45,46] and so most of the evidence to date has been collected via indirect measurements. As one example, reductions in hydrogen peroxide (H_2O_2) in FLASH-irradiated water have been reported.[35] This result, considered with the lack of radioprotection triggered by antioxidants in FLASH-irradiated zebrafish embryos,[13,35] suggests that FLASH-RT can modify the initial rate or production of reactive oxygen species (ROS) in tissues, which in turn can affect the entire downstream biological cascade. Some important biological mechanisms, downstream to the ROS-mediated events, known to be involved in the FLASH effect on normal tissues include reduction of DNA damage (lung),[41] apoptosis (lung and brain),[1,12] and pro-inflammatory consequences (brain)[12,47] (Table 2).

2.2 | Tumors

To date, data derived from single-beam, single-dose studies provide convincing evidence that FLASH RT is isoefficient relative to CONV irradiation at controlling tumor growth rates. This observation has been reported in various xenograft models (breast, prostate, lung, glioblastoma [GBM])[1] and in orthotopic tumor models (lung, GBM)[13,36,48] as well as in transgenic mice.[36,48] More recently, patient-derived xenograft models of T-cell acute lymphoblastic leukemia have shown tumor subtype–specific susceptibility to FLASH RT.[40] Because fractionated RT regimens are the standard of care for the treatment of solid tumors, it is also important to

TABLE 2 In vivo studies of electron beams to investigate FLASH effects in normal and cancerous tissues (in order of publication year)

In vivo studies				Irradiation parameters						
Reference	**Model/ species**	**Target site or organ**	**Biological endpoint**	**Total dose (Gy)**	**Beam energy (MeV)**	**Delivery time (s)**	**Mean dose rate, $\bar{D}$ (Gy/s)**	**Instantaneous dose rate, $\dot{D}_p$ (Gy/s)**	**Number of pulses**	**Pulse rate (Hz)**
56	Rat	Skin and hind feet	Early skin reactions; late deformities	20–35	7	2.5×10^{-1}–5×10^{-1}	67–80	Not specified	Not specified	Not specified
57	Mouse (ICR)	Skin (Right hind leg)	Early skin reactions	30–50	8	12 - 20	2.5	$>1.6 \times 10^{4}$	Not specified	23
43	Mouse (C57Bl/6J)	Tail skin	Skin necrosis (ND50); skin regeneration; epithelial integrity	30, 50	10	1.8×10^{-1}–2.9	17–170	1×10^{5}–1×10^{6}	Not specified	50
1	Mouse (C57Bl/6J; Swiss Nude)	Lung	Lung fibrosis, tumor kill, growth delay	17–30	4.5	$> 1 \times 10^{-1}$	40–60	1×10^{6}	4–6	100–150
11	Mouse (C57Bl/6J)	Whole brain and hippocampi	Behavior (NOR); cell proliferation (BrdU)	10	6	1.8×10^{-6}–0.3	33–5.6×10^{6}	1.9×10^{5}–5.6×10^{6}	1–10	100
10	Zebrafish embryo	Embryos	Fish length	5–12	6	1.8×10^{-6}	$>2.7 \times 10^{6}$	$> 4.4 \times 10^{6}$	1	Single pulse
35	Zebrafish embryo	Embryos	Fish length	8	6	1.8×10^{-6}	4.4×10^{6}	4.4×10^{6}	1	Single pulse
23	Mini pig	Skin (dorsal)	Fibronecrotic lesions	28, 31, 34	6	0,1	>280	1.8×10^{6}	10	100
23	Cat[a]	Nose Squamous cell carcinoma	Skin macroscopic complete response	25–41	4.5 and 6	$> 9.0 \times 10^{-2}$	>277	0.5×10^{6}–1.8×10^{6}	9–10	100
12	Mouse (C57Bl/6J)	Whole brain	Dendritic spines (hippocampal neurons), neuroinflam-mation, brain cytokines	30	16–20	1.0×10^{-1}–1.6×10^{-1}	200, 300	8.75×10^{5}	18	106, 180

(Continues)

TABLE 2 (Continued)

In vivo studies				Irradiation parameters						
Reference	**Model/ species**	**Target site or organ**	**Biological endpoint**	**Total dose (Gy)**	**Beam energy (MeV)**	**Delivery time (s)**	**Mean dose rate, $\bar{D}$ (Gy/s)**	**Instantaneous dose rate, $\dot{D}_p$ (Gy/s)**	**Number of pulses**	**Pulse rate (Hz)**
35	Mouse (C57Bl/6J)	Whole brain	Cognition, anxiety, neuron structure, neuroinflammation, gliosis	10–14	6	1.8×10^{-6}	5.6×10^{6} –7.8×10^{6}	5.6×10^{6} –7.8×10^{6}	1	Single pulse
58	Human Patient $CD30^{+}$ T-cell cutaneous lymphoma	Cancerous and normal skin	Tumor response, normal skin health	15	6	9.0×10^{-2}	158	1.5×10^{6}	10	100
5,59	Mouse (C57Bl/6J, BALB/C^{-})	Thoracic, splenic, abdominal	Lymphopenia parameters, survival	10–16	20	2.6×10^{-1}–4.9×10^{-1}	32.6, 38.8	Not specified	Not specified	180
41	Mouse (C57BL/6J, $Terc^{-/-}$)	Lung	DNA damage markers, cell proliferation, senescence, RNA-seq (inflammation genes)	17	4.5	3.3×10^{-2}–1.1×10^{-1}	135–600	8×10^{5}–3.2×10^{6}	5–11	100–150
40	Mouse NSG (PDX T-ALL model)	Total body irradiation Patient T-ALL IV injection	Hematopoiesis, survival, gene expression array (cancer markers)	4	6	2×10^{-2}	200	7.4×10^{5}	3	100
48	Mouse (C57Bl/6J; Swiss Nude)	Hole brain and hemi brain	Tumor (GBM) growth, survival, behavior (NOR)	10, 14, 25, 2×7, 4×3.5	6	1.0×10^{-2}–1.8×10^{-6}	2.5×10^{3}–7.8×10^{6}	1.9×10^{6}–7.8×10^{6}	1-2	100
60	Juvenile mouse (C57Bl/6J)	Whole brain	Cognition, neurogenesis, neuroinflammation	8	6	1.8×10^{-6}	4.4×10^{6}	4.4×10^{6}	1	Single pulse
61	Mouse (C57Bl/6J)	Whole brain	Astrogliosis, complement activation, TLR4	10	6	1.8×10^{-6}	5.6×10^{6}	5.6×10^{6}	1	Single pulse

(Continues)

TABLE 2 (Continued)

In vivo studies				Irradiation parameters						
Reference	Model/ species	Target site or organ	Biological endpoint	Total dose (Gy)	Beam energy (MeV)	Delivery time (s)	Mean dose rate, $\bar{D}$ (Gy/s)	Instantaneous dose rate, $\dot{D}_p$ (Gy/s)	Number of pulses	Pulse rate (Hz)
62	Mouse (C57Bl/6J)	Whole brain	Brain microvasculature	10, 25	6	10^{-2}–1.8×10^{-6}	2.5×10^{3}–1.8×10^{6}	5.6×10^{6} –6.9×10^{6}	1–2	100
39	Mouse (C57Bl/6J)	Subcutaneous Lewis lung carcinoma	Tumor vascular morphology, ROS, immune cell infiltration, DNA damage	14	16	3.9×10^{-2}	352	4.3×10^{5}	8	100
38	Mouse (C57Bl/6J)	Hemi-thorax	Skin health, survival	10–40	16	5.5×10^{-2}–2.2×10^{-1}	180	4.0×10^{5}	5–20	90
36	Mouse (C57Bl/6J)	Abdominal	GI syndrome, survival, crypt cell regeneration, epithelial integrity, tumor growth	14–16	16	6.5×10^{-2}–7.4×10^{-2}	216	4.0×10^{5}	7–8	108

[a]Veterinary patient study.

determine whether dose fractionation would preserve the FLASH effect. To address this directly, several hypo-fractionated regimens (3.5 Gy x 4, 7 Gy x 2, and 10 Gy x 3) were found to produce no distinguishable differences in overall survival and tumor growth delay after FLASH versus CONV irradiation.[14] Notably, large single-dose regimens (a single 10 Gy fraction) and hypo-fractionated regimens (doses of $\geq$ 7 Gy per fraction) could still spare normal tissues without affecting antitumor effectiveness.

The difference in the reactions of normal tissues versus tumors to FLASH RT provides a unique opportunity for the field of radiation oncology to enhance tumor control safely and more effectively. Moreover, as radiation oncology shifts to using more hypo-fractionated regimens to treat a variety of tumor types, the capability to dose-escalate with FLASH RT facilitates these approaches and minimizes the number of patient visits, which will ultimately reduce the cost of healthcare.

3 | HIGH-THROUGHPUT MODEL SYSTEMS FOR INVESTIGATING BEAM PARAMETERS CRUCIAL FOR ACHIEVING THE MAXIMUM FLASH EFFECT

The ideal biological system for investigating how physical beam parameters influence the FLASH effect would have a fast readout, high throughput, and robust and reproducible responses. The standard approach to fulfilling these requirements has historically been through detailed in vitro studies, primarily clonogenic assays.[49] However, because the FLASH effect is defined in terms of sparing of normal tissues after a dose delivered at ultra-high dose rate relative to CONV dose rates, then by definition, the FLASH effect should be evaluated in vivo. Indeed, testing proposed hypotheses regarding the mechanisms of FLASH, with the likelihood that the FLASH effect is a combination of several effects, makes it crucial that beam validation is done in vivo before undertaking any mechanistic studies in vitro. However, in vivo studies have some major drawbacks relative to in vitro studies including general animal experimentation ethics and handling issues, increased complexity of the response, and longer readout time for the results. The realization that the FLASH effect is related to specific irradiation parameters, and that different organ systems are likely to require their own optimized set of parameters, underscores the need to find robust, high-throughput, and reproducible in vivo systems to properly evaluate how the numerous possible combinations of beam parameters can maximize the FLASH effect.

The potential of high-throughput screening of the FLASH effect in an in vivo system is perhaps best illustrated with the zebrafish model, in which embryos are exposed to radiation after fertilization and then assessed for viability and morphologic abnormalities. The benefits of this model include ease of handling, rapid development, and ease of visualizing major organs.[50] The relevance and responsiveness of early-stage zebrafish embryos (4 h after fertilization) to ultra-high dose rate irradiation was validated by using an eRT6 electron beam[51] (Figure 1) and subsequently used to evaluate FLASH RT in combination with the ROS scavenger amifostine (single pulse delivery, $\bar{\dot{D}} = \dot{D}_p : 5.6 - 7.8\text{E}6\ \text{Gy/s},\ D_p : 10 - 14\ \text{Gy}$).[35] On the other hand, Beyreuther et al.[4] failed to observe any difference in survival or morphologic integrity when zebrafish embryos at a later developmental stage (24 h after fertilization) were exposed to a proton beam line at the Proton Therapy University of Dresden. Only reductions in pericardial edema after the delivery of 23 Gy with FLASH ($\bar{\dot{D}}$: 100 Gy/s, $\dot{D}_p$: 0.5E3 Gy/s) compared to CONV irradiation ($\bar{\dot{D}}$: 0.08 Gy/s, $\dot{D}_p$: 0.4 Gy/s) were reported.[4] More recently, the same group using the same model succeeded in protecting zebrafish embryo development by using a research electron beam line (Electron Beam of high Brilliance and low Emittance[52]) operating at a higher dose rate (CONV: $\bar{\dot{D}}$: 0.11 Gy/s (continuous delivery); FLASH: $\bar{\dot{D}}$: 10E5 Gy/s, D_p: 1.8E-2 Gy $\dot{D}_p$: 10E9 Gy/s).[53] The significance of these discrepant results is under investigation but may be related to the mean and instantaneous dose rates used and the nature of the beam.

Another system with the capability of high-throughput readout that has been extensively described in the literature is the Withers–Elkind crypt assay, also known as the microcolony assay. This assay, first described in 1970,[54] monitors the regeneration of intestinal crypts after irradiation. In brief, mice are killed 3.5 days after irradiation of the GU tract; segments of the jejunum are removed and processed by routine histologic techniques; and transverse sections are cut and stained with hematoxylin and eosin. The numbers of regenerating crypts are counted and presented as regenerating crypts per circumference. Regenerative crypts are generally scored visually; Withers and Elkind defined objective criteria for regenerative crypts as "10 or more cells, each with a prominent nucleus and little cytoplasm, lying close together and appearing crowded." Levy et al. used this assay to study the different effects of total abdominal irradiation given by FLASH versus CONV irradiation.[36] FLASH irradiation ($\bar{\dot{D}}$: 216 Gy/s, D_p : 2 Gy, $\dot{D}_p$: 4E5 Gy/s) induced significantly less intestinal injury (increased survival and decreased death of crypt base columnar cells) in healthy mice compared to CONV-irradiated mice. Moreover, FLASH irradiation increased sparing of normal tissue while retaining tumor control after total abdominal irradiation in the ovarian cancer model ID8 compared to CONV-irradiated mice.

Notably, however, zebrafish embryos and the microcolony assay both involve the use of highly proliferative

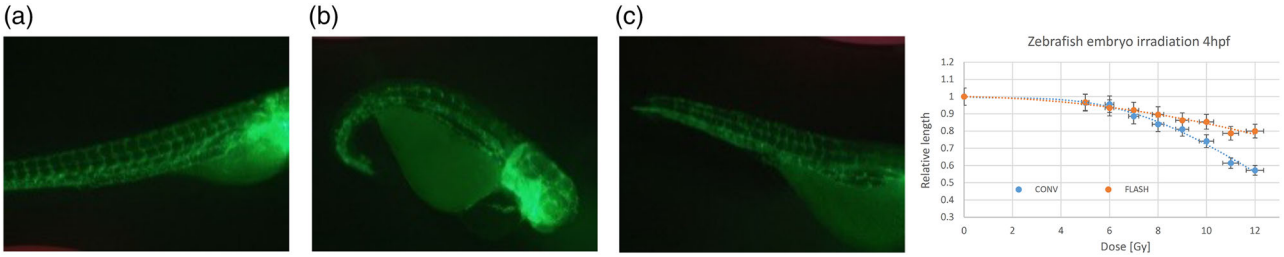

FIGURE 1 Zebrafish embryos irradiated with the eRT6 electron beam FLASH did not show developmental retardation. At 4 h after fertilization, zebrafish embryos were irradiated and their development was analyzed 5 days later. Below: Normal development of zebrafish embryos (a) was altered following conventional (CONV) irradiation (b) but not after FLASH irradiation. (c) Right panel: Dose–response curve after CONV and FLASH radiation therapy (RT). Mean standard deviation and Mann–Whitney test (at 10 Gy, $p < 0.01$; at 11–12 Gy, $p < 0.001$; $n = 20$ embryos/group)

cells that model the response of the acute responding organ well, but they are not relevant for investigating delayed response, suggesting that the best model with which to validate the FLASH effect at this time is still mice.

Although cell culture has been widely used for radiobiology studies over the years, in vitro experimentation to validate the FLASH effect does not substitute for in vivo functional validation. For instance, past[43,44] and present studies[55] have shown that in vitro, radioprotection was not observed under atmospheric conditions (21% O_2) but was observed in hypoxic conditions at doses around 20 Gy. Given the very low surviving fraction obtained at such high doses (<0.001 in hypoxic environment),[55] the relevance of these in vitro studies with such high doses for observing the FLASH effect is unclear. More globally, the relevance of 2D cultures to investigate radiation response to FLASH RT can be questioned for many reasons. For example, the use of FLASH can induce radioprotection in tumor cell lines irradiated in vitro, whereas FLASH RT does not protect tumors in vivo[1,36,48] (see Section 2.2). Under standard clonogenic conditions (doses of 2–6 Gy and atmospheric dioxygen tension), no differential effects have been noted thus far between normal and tumor cells exposed to FLASH RT in vitro (Figure 2).

Recently, 3D culture models such as spheroids, organoids, or even more complex organ-on-a-chip systems have emerged in radiobiology, and their relevance for studying the FLASH effect has yet to be explored.[42] Some potential advantages are obvious, such as the opportunity to investigate the effects of FLASH RT on human cells rather than rodent cells. The microenvironment in such models is also complex, and the paracrine signals between the various cellular compartments are maintained (e.g., crosstalk between vasculature and cell types as well as circulating blood and immune cells). Other advantages of such models are the ease of modeling several physiobiological equations, such as the diffusion and metabolism of oxygen within the spheroids; its depletion through reactions involving radiation-induced radicals; and the increase in radio-resistance using the classical models of oxygen enhancement ratio and linear-quadratic response. Subsequently, these models can be readily verified by evaluating growth after irradiation. While spheroids provide an innovative model to study FLASH radiation therapy, both computationally and experimentally, it should be noted that the relevance to in vivo tumors is unclear, thus results from such studies need to be evaluated carefully and placed into context.

4 | PATH TO CLINICAL TRANSLATION: MODELS RELEVANT TO CLINICAL QUESTIONS

Scaling FLASH technology from conditions and geometries that work for rodents to those that would be applicable to human patients is a substantial challenge. For example, exploring the effects of volume and conformality would require experiments with large animals. To this end, pigs have been used in radiopathology and radiooncology for decades,[63–65,66] and a previous study has shown that pigs are suitable for investigating normal tissue responses to FLASH RT as well as being useful for comparing normal skin response to CONV RT versus FLASH RT.[23] However, investigations of tumor response ideally require model systems in which cancers arise spontaneously. Such model systems may already be available through veterinary practice. Indeed, RT has become an essential part of cancer treatment in animals[23,67]; the advances in high-precision treatment delivery and multimodal imaging used for human patients are increasingly being translated to veterinary practice, thereby providing opportunities to use domesticated animals with spontaneously arising tumors to explore the potential benefits of FLASH RT.[23,67] Radiation effects have been well studied in cats and dogs[68–70]; these animals could serve as models for testing the safety of FLASH RT.[23] As one example, a phase III randomized trial of FLASH RT versus CONV RT for cats with squamous cell carcinoma of the nasal planum is currently ongoing at the CHUV (Lausanne University

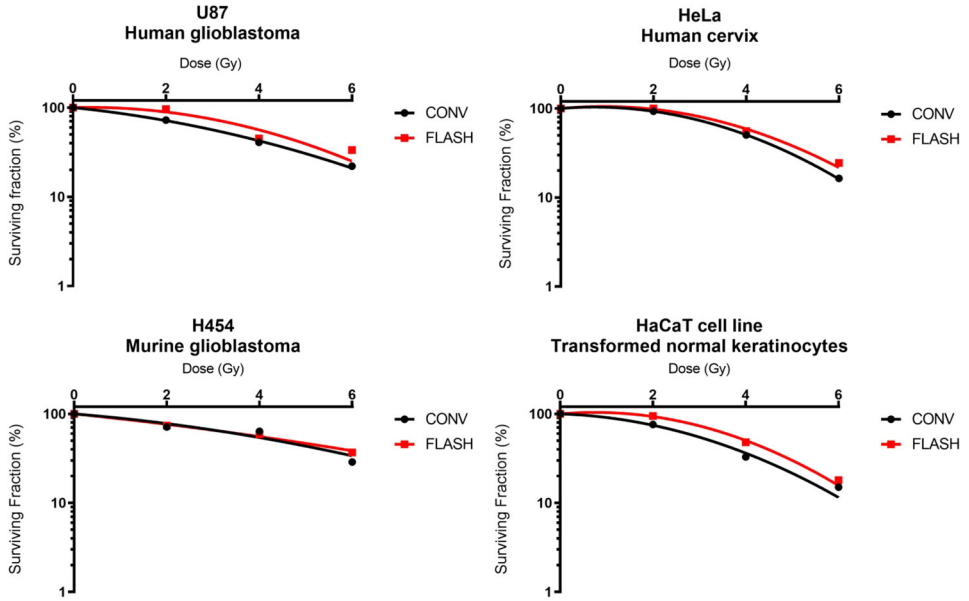

FIGURE 2 Clonogenic assays after irradiation with the eRT6 electron beam. Three tumor cell lines (human GBM U87, human cervix HeLa, murine GBM H454) and one normal cell line (HaCat) were irradiated with FLASH and CONV RT. No differences in clonogenic survival were measured between FLASH and CONV irradiation in cancer cell lines and one normal cell line (HaCat)

Hospital) in collaboration with Prof C. Rohrer-Bley at the University of Zurich. The aim of this trial is to compare tumor control and short-term and long-term toxicity between standard-of-care CONV RT (4.8 Gy x 10) and a single 30 Gy dose of FLASH RT. Other feasibility experiments in dogs with osteosarcoma and sarcoma are underway, one with proton-FLASH at the University of Pennsylvania and another at the University of Lund with electron-FLASH.[71] Unfortunately, these trials do not include a control condition in which RT is given at CONV dose rates.

In summary, using FLASH RT to treat companion animals with cancer may be important not only as an intermediate step toward applying this technology to human patients but also could be beneficial for the animals as well. For example, the ability to deliver FLASH RT in hypo-fractionated regimens—and even in single fractions—would be expected to enhance the pet patient's quality of life during treatment (because of the need for fewer anesthesia sessions) and after treatment (tumor control without normal-tissue toxicity). FLASH RT may also make RT more affordable and reduce the workload at veterinary clinics. Finally, trials of FLASH RT in companion animals can also be useful for designing clinical workflows for future studies with patients.

5 | TECHNOLOGICAL PERSPECTIVES ON FUTURE DEVELOPMENTS

Cancer is predicted to be the leading cause of death worldwide, with about 30.2 million newly diagnosed cases and 16.3 million related deaths per year by the year 2040.[72] These numbers underscore the need for innovative treatment modalities against cancer and warrant ways of meeting the associated major challenges for 21st-century health care. In that context, the tantalizing possibility that the FLASH effect crosses tissues and species, and the magnitude of the benefit observed in various preclinical studies, highlights the need to define and promote its clinical application.[2,10,13,73,74] Typically, the speed at which FLASH RT is delivered may be sufficient to circumvent problems with organ motion during treatment in the setting of real-time imaging, which is otherwise an important consideration in CONV RT. However, the required parameters for clinical application need to be carefully defined and tested in proof-of-safety trials before FLASH RT can be widely applied in clinical settings.

From a technological point of view, only a few systems exist at present that can operate at the ultra-high dose rates associated with the FLASH effect. Although most FLASH studies to date have been performed with experimental 4–6 MeV electron devices[13,15] or modified clinical linacs,[16,75,76] the use of synchrotron X-rays for FLASH RT has also been studied,[77] as has the use of proton beams.[4,78,79] In 2021, the CHUV/Lausanne University Hospital team plans to begin using two electron beams of about 9 MeV (Mobetron/IntraOp and FLASHknife/PMB-Alcen) to treat superficial skin cancers and for intraoperative treatment of other types of cancer.[80] This pragmatic approach is expected to provide the proof of principle as to a FLASH RT benefit in humans before the development of devices

for treating deep-seated tumors. Another approach being developed as a collaboration between CHUV and CERN, the European Council for Nuclear Research in Geneva (https://cerncourier.com/a/adapting-clic-tech-for-flash-therapy), aims to produce a very high energy electron (VHEE)-FLASH device that can deliver high doses at high dose rates to relatively large volumes and deep-seated tumors.[81–84] VHEE beams offer both the penetration needed and the penumbra that is practical for deep-seated tumors.[81,85,86] VHEE beams also have the advantage of increased uniform dose at high-density boundaries compared to photon beams, and compared to protons, they allow for easy electromagnetic scanning. The potential disadvantages of using VHEE beams depend on the proposed technique, but with the currently proposed systems, there is added complexity and cost compared to conventional clinical technology, which will limit the widespread availability of this technology in the near future.

6 | CONCLUSION

The field of FLASH RT is still in its infancy and the true potential of this novel treatment strategy is still to be determined. In order for us to determine the true potential of FLASH RT, we need to understand what constitutes FLASH irradiation in terms of the physical beam parameters needed to induce the FLASH effect. We also need to understand what effects the manipulation of the physical beam parameters have on the magnitude of the FLASH effect. This knowledge can then be used to elucidate the underlying biological mechanism(s) of the FLASH effect and allow for an optimization of FLASH RT in terms of normal tissue sparing to critical organ systems. The first step to get to this point is for the community to agree upon a common reporting system of the critical beam parameters used to allow for transparency and retrospective studies of all aspects of the beam delivery and experimental setup. In this review, we are proposing a set of parameters to be reported in future studies within the field of FLASH RT. This list constitutes the first step to a common reporting system and can be easily adapted also to other irradiation types beyond electron irradiations. This reporting system will likely need to be modified as the field moves forward, and more advanced delivery systems are developed. However, if adopted, it would constitute the first step toward a new standard of beam parameter reporting and facilitate the robust and safe translation of this technology to the clinical setting.

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CONFLICT OF INTEREST

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