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REVIEW

FLASH ultra-high dose rates in radiotherapy: preclinical and radiobiological evidence

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ABSTRACT

Purpose: Flash radiotherapy (FLASH-RT) is currently being regarded as the next breakthrough in radiation treatment of cancer, delivering ultrahigh radiation doses in a very short time, and sparing normal tissues from detrimental injury. Here we review the current evidence on the preclinical findings as well as the radiobiological mechanisms underlying the FLASH effect. We also briefly examine the scenario of available technologies for delivering FLASH dose-rates for research and their implications for future clinical use.

Conclusions: Preclinical studies report that the FLASH-RT reduces radiation-induced toxicity whilst maintaining an equivalent tumor response across different animal models. However, the molecular radiobiology underlying FLASH effect is not fully understood and further experiments are necessary to understand the biological response. Future studies also includes the design of a FLASH delivery system able to produce beams appropriate for treatment of tumors with ultra-high dose rates. All these research activities will greatly benefit from a multidisciplinary collaboration across biology, physics and clinical oncology, increasing the potential of a rapid clinical translation of FLASH-RT.

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Introduction

The biological effectiveness of ionizing radiation in relation to high dose-rates was established more than 80 years ago (Spear and Grimmett 1933; Lea 1938). At increasing dose rates, the higher effectiveness is generally explained as the cell incapacity to activate the mechanisms of repair or recovery, that may lead to consecutive damaging events (Spear and Grimmett 1933; Lea 1938). Even though in vitro experiments revealed also inverse dose-rate effects, which may occur in certain regimes due to specific cell-cycle distributions (Hall and Brenner 1991; Matsuya et al. 2018), the overall advantage of high-dose rate has gained increased consensus through years, especially in the context of brachytherapy (Orton 2001; King 2002) and radiosurgery (Garau 2017).

The phenomenon of the normal tissue sparing by high dose rate irradiations was also reported and early attributed to the depletion of oxygen in tissues by the end of the 1960s (Hornsey and Alper 1966; Town 1967; Hornsey and Bewley 1971; Hendry et al. 1982). The extensive amount of radiobiological knowledge acquired through the last decades has been translated to the so-called linear-quadratic model (LQ), based on the α/β ratio for tumors (Dale 1985; van Leeuwen et al. 2018). This model is at the basis of current clinical practice of treatment fractionation, having the practical goal to allow normal tissue to get repaired (at faster rate than the tumor) between consecutive fractions.

Irradiation at ultra-high dose rates named FLASH radiotherapy (FLASH-RT) has recently attracted considerable attention as a promising tool to obtain similar tumor control compared to conventional radiotherapy while sparing healthy tissues from side effects (Bourhis, Montay-Gruel et al. 2019). FLASH-RT involves delivery of irradiation at dose-rates a thousand times higher than those used in clinical practice (e.g. 40 Gy/s vs 0.01 Gy/s) in a short time interval (<200 milliseconds). This is currently obtained in the laboratory, by custom setups developed modifying existing electron linear accelerators (LINACs), by removing the Bremsstrahlung converter into photons, which reduces considerably the final output beam power. As a result, a beam of electrons is obtained with an energy of typically 10 MeV, depending on the equipment. As a consequence, all radiobiological investigations currently focus on dose deposition with penetration depth limited to the cm scale.

The short duration of delivery of the dose has been found to noticeably reduce the adverse side effects in normal tissue, maintaining an equal efficiency for tumor control. Here, we review actual evidence on preclinical findings and the main biologically relevant mechanisms, discussing the need of further studies and suitable FLASH-capable radiotherapy instrumentation, beyond the LINAC concept, for a rapid translation into clinical settings.

Pre-clinical studies of FLASH-RT

In 2014, the reduction of toxicity in healthy tissue was described in the lungs of a well-established mouse model (C57BL/6J) after high single dose exposure delivered at

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FLASH dose rate (≥40 Gy/s, 4.5 MeV electrons), with a non-clinical Oriatron LINAC (PMB Alcen, France), compared with Cs^{137} γ-ray irradiation at conventional dose rates of ≤0.03 Gy/s (Favaudon et al. 2014). The results reported the presence of fibrosis and lung pneumonitis in all mice irradiated with conventional dose rates. Contrariwise, a complete lack of lung toxicity after similar doses at FLASH rates has been shown in mice. Indeed, the ultrafast irradiation avoided the-activation of transforming growth factor and the apoptosis of epithelial cells in bronchi (Favaudon et al. 2014). Remarkably, FLASH has been found to be efficient as conventional radiotherapy in repressing tumor growth (Favaudon et al. 2014) thus leading to an effective widening of the useful therapeutic window.

Subsequently, the different effect of FLASH-RT in tumor and normal tissues was confirmed in several animal models, including mouse, mini-pig and cat (Table 1).

In 2017, Schuler et al. developed a preclinical FLASH setup for small rodents by modifying a clinical LINAC (Varian Medical Systems, Palo Alto, CA, USA) yielding extremely high dose rates (70 and 210 Gy/s) with e^- energies of 9 MeV and 20 MeV, respectively. After abdominal

Table 1. Parameters of FLASH-RT in various experimental models.

Model	Dose delivered (Gy)	FLASH dose rate (Gy/s)	Average energy (or energy range)[a]	Device	Tissue effects	References
Mouse	16–30	≥40	4.5 MeV	LINAC (Oriatron)	Less pulmonary fibrosis, less smooth muscle and epithelial cell apopotosis, efficient in repression of tumor growth	Favaudon et al. (2014)
Mouse	10 – 22	70- 210	9–20 MeV	Modified CLINAC (Varian)	Increased survival	Schüler et al. (2017)
Mouse	10	0.1- 500	4.5 MeV and 6 MeV	LINAC (Kinetron and Oriatron)	Preservation of memory and neurogenesis	Montay-Gruel et al. (2017)
Mouse	10	100	6 MeV	LINAC (Oriatron)	Preservation of host neuronal structure and synaptic protein levels, attenuation of neuroinflammation, preservation of cognitive function	Montay-Gruel et al. (2019)
Mouse	10	37	225 KeV	Synchrotron X-rays (ID17 – ESRF)	Preservation of long-lasting memory skills, less astrogliosis, preservation of the cellular division in the hippocampus	Montay-Gruel et al. (2018)
Mouse	10–25	2500	6 MeV	LINAC (Oriatron)	Preservation of vascular parameters including blood vessel volume, the expression of eNOS, tight junction proteins and radiation-induced apoptosis	Allen et al. (2020)
Mouse	10–14(single dose); $4 \times 3.5{,}2 \times 7{,}3 \times 10$ (hyfractiond dose)	0.1–7.8×10^6	6 MeV	LINAC (Oriatron)	Reduced radiation-induced cognitive deficits in learning and memory	MMontay-Gruel et al.(2021)
Mouse	2–17	≥40	4.5 MeV	LINAC (Kinetron)	Reduced activation of pro-inflammatory genes, reduced DNA damage , radiation recovery by preserving lung progenitor cells, reduced risk of replicative senescence	Fouillade et al. (2020)
Humanized mouse	4	200	6 MeV	LINAC (Oriatron)	Reduced functional damage to blood stem cells and therapeutic effect on T cell acute lymphoblastic leukemia	Chabi et al. (2020)
Zebrafish	5–12	≥40	6 MeV	LINAC (Oriatron)	Lower morphological alterations	Vozenin et al. (2019)
Mini-pig, cat	22–41	300	4.5 MeV and 6 MeV	LINAC (Kinetron and Oriatron	Reduced pig skin toxicity in terms of depilation and skin fibrosis, no acute toxicity or mild transient erythema in cats, favorable tumor control in cats	Vozenin et al. (2019)

[a]All the cited experimental studies employ electrons, except for Montay-Gruel et al. (2018), employing X-rays at a synchrotron facility.

irradiation with doses of 10–22 Gy, an increase of survival after FLASH irradiation was observed in mice (Schüler et al. 2017).

Furthermore, Montay-Gruel et al. (2017) showed that FLASH-RT supported memory sparing in healthy mice receiving a whole-brain irradiation (Montay-Gruel et al. 2017). A preservation of neurogenesis and memory in the hippocampus was observed two months after a 10 Gy FLASH irradiation using 4–6 MeV electron beams with a dose rate under 100 Gy/s (Montay-Gruel et al. 2017).

Even though the above-cited experiments have been obtained by megavoltage electron beams from LINACs, it was also demonstrated that X-ray photons at ultra-high dose rates can trigger the FLASH effect as well. Experiments conducted at the ID17 beamline of the European Synchrotron Radiation Facility (ESFR, Grenoble, France) showed that the memory sparing effect of FLASH-RT was observed when irradiating whole-brain mice with synchrotron X-rays delivered at 37 Gy/s, with a resulting preservation of memory, maintenance of hippocampal cell division and reduction of reactive astrogliosis when compared with conventional dose rates from a 225 kV X-ray photon beam (Montay-Gruel et al. 2018).

Long term studies (6 months following exposure) were then performed to find the durability of the neuroprotective FLASH effect. The findings showed that RT at conventional dose rates implied deficits in neurocognitive endpoints, whereas FLASH did not cause long-term radiation-induced alterations of memory and learning as well as anxiety and depression in mice (Montay-Gruel et al. 2019).

Further mechanistic studies also showed that the FLASH benefits were caused by a mechanism involving a lower production of reactive oxygen species (ROS) levels. In fact, the FLASH effect seems to disrupt and bypass ROS-mediated cascades that commonly lead to neurocognitive complications typically found after conventional radiotherapy (Montay-Gruel et al. 2019).

In 2020, Allen and colleagues provided the first evidence that FLASH irradiation also preserves microvasculature integrity (Allen et al. 2020). Both early and late timepoints post-irradiation were analyzed using C57Bl/6J mice exposed to whole-brain irradiation delivered at single doses of 10 or 25 Gy by using conventional (0.09 Gy/s) or FLASH ($>10^6$ Gy/s) irradiation. Although the changes found one day post-irradiation were marginal, FLASH reduced apoptosis in neurogenic regions (Allen et al. 2020).

At one week and one month post-irradiation, conventional irradiation was found to induce vascular dilation. Such results were positively correlated with the alteration of the expression of eNOS, indicative of the potential dysregulation in the blood flow at these times. Moreover, the expression of tight junction proteins that reduced after conventional dose rates, remained unvaried after FLASH irradiation (Allen et al. 2020).

According to very recent preliminary results (Montay-Gruel et al. 2021) the hypofractionation has been found to equally spare normal brain tissue, compared to single fraction irradiation, from toxicity without the impairment of tumor cure. Significantly, animals that received FLASH-RT did not exhibit cognitive alterations either after single dose or hypofractionated regimens (Montay-Gruel et al. 2021). The possibility of delivering a FLASH compliant treatment with hypofractionation is extremely relevant for practical exploitation of FLASH-RT as it may lead to less demanding irradiation beam specifications compared to the single fraction approach.

Moreover, a recent study investigated the impact of ultra-high dose rate FLASH irradiation versus conventional-dose-rate total body irradiation (CONV-TBI) on a humanized murine model of T cell acute lymphoblastic leukemia (T-ALL) and normal human hematopoiesis (Chabi et al. 2020). Immunocompromised mice were conditioned with three T-ALL patient derived xenografts (PDXs) and human blood stem/progenitor cells and 1 month later subjected to FLASH- or CONV-TBI. FLASH therapy resulted in superior killing of leukemia cells and extended mice survival times compared to conventional therapy for two of the three PDXs examined. For the third PDX, conventional therapy resulted in greater leukemia cell kill than FLASH therapy suggesting that inter-patient cancer variability may play a role in the effectiveness of FLASH therapy. Additionally, it was observed that FLASH therapy partially preserved hematopoietic stem/progenitor cell function, which was completely destroyed by conventional radiotherapy. This finding supports the notion that the impact of FLASH-TBI on the hematopoietic system differs from what has been observed in other hypoxic organs and that other mechanisms beyond the hypothesized oxygen depletion are involved in the observed FLASH effect (Chabi et al. 2020).

However, the FLASH effect has not been limited to the mouse model, but it has been confirmed in higher mammals. Indeed, using the radiation-induced depilation and skin fibrosis as acute and late endpoint respectively, a protective effect of FLASH-RT has been pointed out in mini-pigs and cats (Vozenin, De Fornel et al. 2019). FLASH-RT (4.5 MeV electrons at 300 Gy/s) minimized healthy tissue damages of mini-pig skin even when large single doses (24–34 Gy) were applied as compared with conventional dose rates (0.083 Gy/s). The presence of late effects such as fibronecrosis, collagen deposition and skin contracture were greater in animals irradiated with conventional dose-rates. (Vozenin et al. 2019).

In parallel, the impact of FLASH-RT has been investigated in six domestic cats with squamous cell carcinoma of the nose receiving single fractions of ultrahigh dose-rates (25–41 Gy/2) by using electron fields (Vozenin et al. 2019). No dose-limiting toxicity and relatively mild long-term toxicity were observed. In total, the FLASH-RT treatment yielded a favorable outcome with complete response at 3 months for all cat patients and a free-survival rate of 84% at 16 months (Vozenin et al. 2019).

Recent experiments have provided additional evidence of an increased biological tolerance of FLASH-RT in developing zebrafish embryos irradiated with 5–12 Gy. FLASH radiotherapy showed a significant reduction of

morphological alterations following irradiation at doses above 10 Gy (Vozenin et al. 2019).

In this context, the treatment with FLASH-RT of the first human patient is of particular interest, a 75-year-old patient with a cutaneous CD30+ T cell lymphoma (Bourhis et al. 2019). Radiotherapy treatment was given to a 3.5-cm diameter skin tumor with a 5.6-MeV linac suitably planned for FLASH-RT (Oriatron, PMB Alcen, France). FLASH-RT treatment (15 Gy in 10 pulses of 1 μs,) resulted safe and practicable with a favorable outcome (Bourhis et al. 2019) both on tumor and healthy tissue. However, as the authors acknowledged, it is not possible to draw any definite conclusion given the historical and indirect nature of the comparison with conventional RT, as well as the presence of potential confounding factors such as the exposure to concomitant treatments (Bourhis et al. 2019). Additionally, the study was performed in a single patient that does not allow any clinically meaningful evidence for FLASH-RT into clinical context. Adequately powered randomized clinical trials are needed to define whether FLASH-RT is associated with potential clinical benefits.

Despite these important pre-clinical results, the biological mechanisms underlying the sparing effects of healthy tissues with FLASH-RT remain elusive and require extensive pre-clinical investigation.

Redox biology and radiobiological mechanisms of FLASH-radiotherapy

Biological response to radiotherapy is based on the concept of delivering a physical dose of ionizing radiation to cause target cell killing within the tumor tissue.

The ionizing radiation-biological material interaction induces ionization and disruption of chemical bonds that depend on the energetic particle crossing a cell (Hall and Giaccia 2012).

The linear energy transfer (LET) for a particular radiation influences its effectiveness in evoking a biological response (i.e. relative biological effectiveness, RBE). DNA represents the most critical target for radiation-induced lethal damage, but other cellular sites such as membranes and organelles may be crucial (Hall and Giaccia 2012). Low LET radiation (X-rays, gamma rays and beta particles) induces lower concentrations of ionization events and deposits a relatively small amount of energy in a highly dispersed manner (Hall and Giaccia 2012; Phillips and Griffin 1999). Hadrons

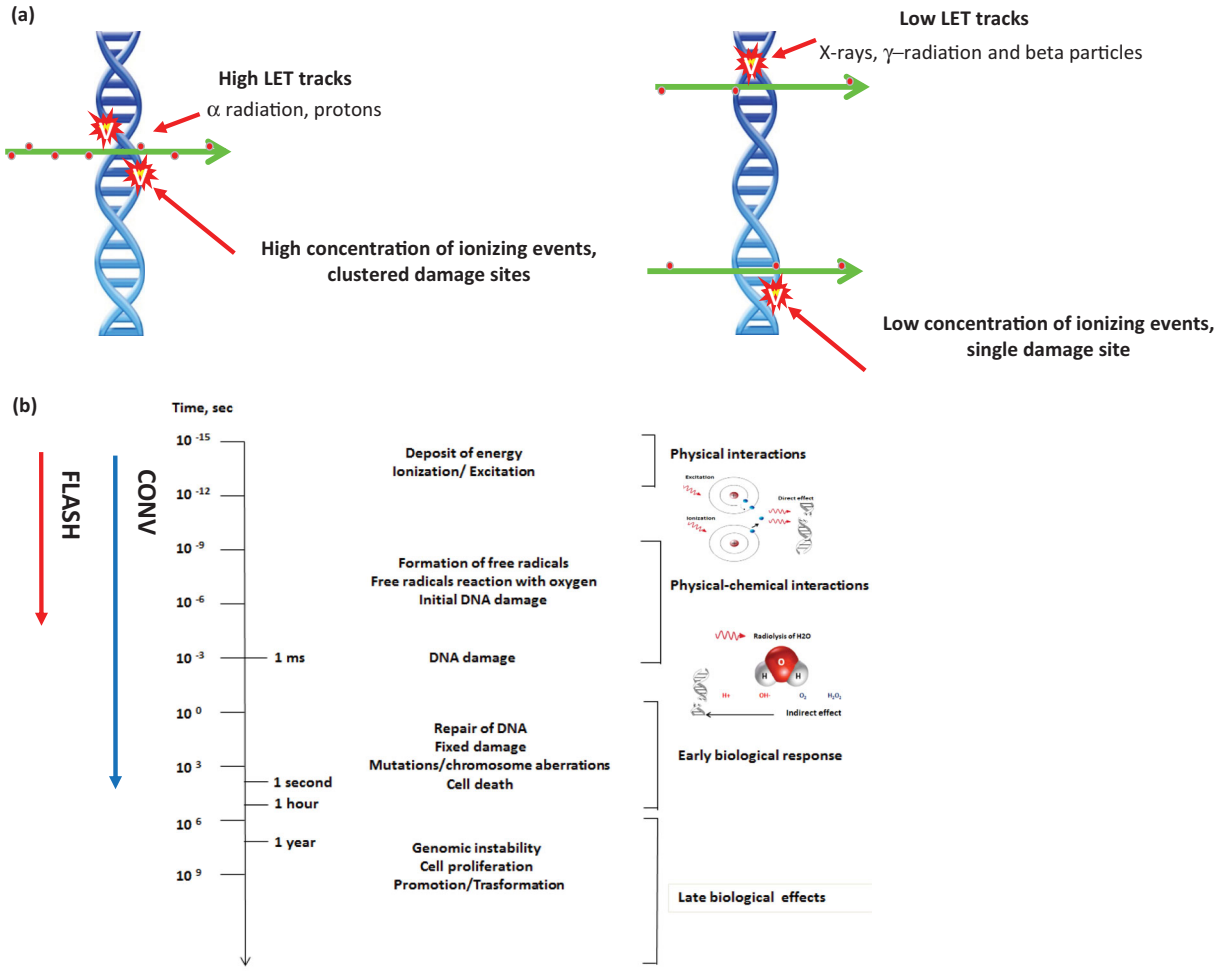

Figure 1. (a) Schematic representation of the types of DNA damage induced by Low-and High-LET radiation, ranging from single and clustered damage sites; (b) time scale of radiation induced events in FLASH irradiation and CONV irradiation.

(protons, alpha rays, and other heavier ions) have an increased ionization density and deposit more energy on the biological target, inducing more effects than the low LET radiations (Figure 1). Radiation-induced ionizations may operate directly on the DNA molecule or indirectly on water, causing the production of reactive species, including the aqueous electron (e^-_{aq}), the highly reactive hydroxyl radical (OH•) and the radical H• (Ward 1988).

The important concept is that physical steps leading to energy deposition and radical formations occur in a very short time (10^{-9}–10^{-3} seconds), although the biological manifestation of physical damage can persist over time (UNSCEAR 2001) (Figure 1).

The ability of cells to repair different types of DNA lesions determines the cell fate and influences the efficacy of radiation in determining cell lethality.

The accuracy of DNA repair depends on the number and spatial proximity of the lesions (clustered DNA damage), which are dependent on LET (Lomax et al. 2013), and on energy deposition or dose-rate (Hall and Brenner 1991).

From a radiation chemistry perspective, it is therefore plausible to hypothesize that the redox reactions following ultra-high instantaneous dose-rates may lead to different biochemical and biophysical effects with a kinetically different path (Spitz et al. 2019), as in the case of laser-plasma accelerated beams (Andreassi et al. 2016). Indeed, it may result in collective effects through spatio-temporal overlap of independent tracks (Fourkal et al. 2011), resulting in an increased complexity of DNA damage, with a resultant decrease in their repair (Babayan et al. 2017).

Additionally, the production of free radicals following the 'instantaneous' FLASH pulse may rapidly decay in a series of biochemical and biophysical events able to modify the entire biological cascade (Figure 1), leading to a reduction of pro-oxidant burdens in healthy tissues with a relative protection compared with conventional radiotherapy (Vozenin et al. 2019).

As discussed many years ago (Town 1967), this sparing effect of normal tissues is attributed to a potential alteration of indirect DNA damage associated with free radicals (oxygen depletion effect) after high dose rate exposure. Oxygen is quickly depleted and, therefore, unavailable for the increased production of free radicals and fixation of DNA damage (Town 1967).

Therefore, the hypothesis of oxygen depletion suggests that local oxygen is depleted faster than reoxygenation can occur, having a negligible impact on already hypoxic tumor tissue and leading to a transient state of radiation-induced hypoxia and, therefore, radioresistance and protection of the normal tissues.

However, there is no conclusive evidence on the oxygen depletion hypothesis, and the inherent biological mechanisms are not fully understood (Durante et al. 2018). Vozenin et al. 2019).

Indeed, it is not easy to explain why oxygen depletion mightspare the healthy tissue but not the tumor, and there is also a possible risk that FLASH-RT might render tumor more resistant to radiotherapy (Wilson et al. 2012; Adrian et al. 2019; Pratx and Kapp 2019).

Other potential mechanisms underlying FLASH-RT

Intrinsic differences between normal tissues and tumor tissues in response to ROS and free radicals have been also hypothesized to contribute to the FLASH effect (Spitz et al. 2019; Zhou 2020). It has been observed that normal cells have lower pro-oxidant burdens during normal redox metabolism and can more effectively reduce the levels of free radicals and hydroperoxides generated from peroxidation chain reaction and Fenton type chain reactions effect (Spitz et al. 2019; Zhou 2020). These differences in redox chemistry and free radical production may explain the differential effect after FLASH-RT (Zhou 2020).

In addition, normal and cancer cells differ in their DNA repair mechanisms in response to radiation, particularly for DNA double strand breaks, and DNA repair is often dysregulated and less efficient in tumor cells (O'Connor 2015). Therefore, the difference in DNA repair kinetics between normal and cancer cells may be consistent with well documented sparing effect of hyperfractionated radiotherapy with reduced single doses.

From a theoretical point of view, other mechanisms are also conceivable, such as an altered inflammatory cellular signaling or the sparing of circulating immune cells (Durante et al. 2018; Buonanno et al. 2019; Pratx and Kapp 2019; Fouillade et al. 2020).

Surprisingly, only few *in vitro* studies have been performed to investigate the hypothesized biological mechanisms driving the radiotherapy response under FLASH conditions.

Using a variety of *in vivo* and *in vitro* analytical approaches, an elegant study investigated the molecular changes induced specifically after FLASH irradiation employing a 4.5 MeV linear electron accelerator, identifying early and late indicators of the differential response of mouse lung to FLASH vs. conventional dose-rate irradiation. In particular, FLASH effect reduces the activation of pro-inflammatory genes and DNA damage response (H2AX and 53BP1 foci) in normal cells, and induces radiation recovery by preserving lung progenitor cells (Fouillade et al. 2020).

Moreover, a recent study investigated the biological effects in normal cells exposed to therapeutic doses of 4.5 MeV proton radiation using ultra-high dose rates, up to 1000 Gy/s. The proton dose rate employed for a FLASH effect showed no influence on the acute biological endpoints, but significantly reduces DSBs represented by the number of γH2AX foci by 20 Gy of proton FLASH-RT when compared with conventional proton irradiation at the same dose. The expression of long-term biological responses were significantly impacted by ultra-high dose rates, as shown by the reduced induction of senescence and expression of pro-inflammatory markers (Buonanno et al. 2019).

Another *in vitro* experiment of FLASH radiotherapy employing irradiation delivered with a LINAC (4.5 MeV electrons) was reported by Beddok et al. (2017) in a study undertaken on normal and cancer lung cell lines. Cells were exposed to 5 Gy with either FLASH (>40 Gy/s) or conventional (0.03 Gy/s) irradiation techniques. DNA damage response (H2AX foci and 53BP1) and cell viability showed

no statistically significant differences between the two modalities for normal and tumor cells (Beddok et al. 2017).

Overall, the radiobiological mechanisms of sparing effect on living cells and animals needs to be further elucidated in order to enhance FLASH-RT application in medicine with a solid biological basis. Carefully designed experiments are needed based upon accelerators systems capable of delivering controlled dose at dose-rates varying from conventional to ultra-high values, to investigate different radiochemical processes occurring in radiobiological systems at different time-scales, down to the sub-ps level. In order to do so, a range of accelerator techniques will have to be employed, from the conventional RF accelerators to the latest plasma accelerators based on optical ultra-short, high intensity pulses that have now been established as powerful laboratory sources and are now entering the stage of industrial development.

Devices for FLASH-RT and development of novel sources of ultra-fast irradiation delivery

FLASH-RT relies on a combination of dose, dose-rate, and time of irradiation that are out of the range of operation of existing conventional clinical linear accelerators. In fact, clinical accelerators for radiotherapy normally deliver dose via beams of X-ray photons with broad energy spectrum produced by bremsstrahlung of primary electrons with typical energy of 6–20 MeV. The conversion of electrons into photons is highly inefficient and severely limits the maximum dose-rate that can be reached at the cross-hair for treatment. In order to have the dose rate required for FLASH-RT, a factor of a thousand or more in power increase of the existing clinical linacs would be required. Attempts are being made to modify existing machines (Lempart et al. 2019) to provide high dose rates using directly the primary 6 MeV electron beam. However, due to the small penetration distance (range) of such electron beams, these modifications will possibly only enable treatment of skin tumors on one side and *in vitro* studies and proof of concept *in vivo* studies on small animals. For this reason, conventional RT relies on three-dimensional dose conformation (or 'dose-painting') through spatially and intensity modulated irradiation from several directions (physical optimization), and on treatment fractionation (biological optimization). Brahme et al. tried to merge physical parameters and radiobiological models in more general cost function that maximizes treatment outcome, by keeping the adverse effect to normal tissues at minimum (Brahme et al. 2001).

In contrast, for the clinical applicability of future FLASH-RT to a wide range of tumors, including deep seated, radiation resistant tumors, high dose-rate beams of highly penetrating radiation will be needed which none of the existing systems can deliver (Bourhis et al. 2019). It is therefore clear that a step change in clinical accelerator technology is needed to provide the therapeutic beam specifications required by the FLASH-RT. Presently, clinical trials with FLASH-RT on deep-seated tumors may be performed with scanned proton beams (van Marlen et al. 2020) covering the total target volume, provided the FLASH effect is not affected by the increase of LET in the Bragg peak or by the scanning of the beam (Wilson et al. 2020).

Indeed, an effective solution for efficient FLASH-RT with low LET beams may be provided by the direct use of accelerated electron beams. In fact, electron beams are not affected by the loss of efficiency of the Bremmstrahlung electron/photon conversion and, therefore, power requirements for the accelerator would be up to three orders of magnitude lower. Also, provided their energy is sufficiently high, electrons can deposit energy at any given depth with little lateral diffusion. The so-called very high-energy electron beams radiation therapy (VHEE-RT) operates at energy between 150 MeV and 250 MeV and presents attractive features as percentage depth dose (PDD) and lateral beam profiles, (DesRosiers et al. 2000), leading to superior dose distribution as compared to external megavoltage photon beams in specific Monte Carlo simulated treatment plans, such as those related to prostate, lung or pediatric cancers (Bazalova-Carter et al. 2015).

In view of the above, a combination of VHEE and FLASH-RT, may provide a complete solution to a clinical translation of this new approach. However, generation of 150–250 MeV electron beams using conventional technology is anyway hindered by the low acceleration gradient of RF cavities that, with the most advanced technologies currently available would require very large accelerator lengths, with prohibitive size and cost of clinical equipment that would inevitably limit access to future FLASH-RT. This limitation has already hindered the development of phase-contrast X-ray imaging based on Synchrotron radiation that, in spite of showing significant performance in early cancer detection (Taba et al. 2018), never passed from the stage of laboratory demonstration to clinical application. It is therefore crucial to invest in the development of accelerator technologies that, in principle, can overcome these limitations and provide compact and affordable accelerators suitable to be placed inside hospitals.

In recent years, new high gradient accelerator schemes have been proposed as alternative solutions. These schemes rely on increasingly higher frequency of the driver electromagnetic field, from the S, C and X microwave/RF band cavities eventually reaching, with a step-change of technology, the optical range, with laser-driven plasma accelerators (LPA). RF accelerators are developing fast and promise to deliver efficient accelerators at increasingly higher energy. Developments of RF technology to deliver high current electron beam, potentially capable of a 300-fold increase of the beam current compared with existing clinical technology are in progress (Maxim et al. 2019). The proposed RF design is aiming at delivering either X-ray beam or high energy electron to meet the requirements of FLASH radiotherapy. However, the energy required for VHEE-RT, between 100 and 250 MeV, may still be too high for a compact RF accelerator. On the other hand, LPA accelerators can easily provide VHEE beams (Labate et al. 2020) with ultra-high instantaneous dose rate with a compact size that can already deliver significant dose per shot, with an instantaneous

dose-rate that is typically greater than expected FLASH dose-rates. Such beams have a sub-mm scale diameter (pencil-beam) that could be scanned to cover larger target volumes, in a similar fashion as currently done to deliver FLASH dose-rate with proton beams. Developments are still needed to reach the specifications of FLASH-RT in terms of dose per fraction over a larger area for clinical treatment. It is envisaged that this could be achieved with the increase of the repetition rate of the driving laser power source from current 10 W-10 Hz to the kW-kHz that is currently being developed for this class of accelerators. A major breakthrough has already been achieved with the recent demonstration at the Extreme Light Infrastructure opening the way to a viable industrial solution (Sistrunk et al. 2019). Novel laser materials are also being applied which may enable even higher efficiency and more compact accelerator footprint.

It is clear however that both RF and laser-driven acceleration will require significant investments to deliver accelerators capable of delivering VHEE beams with high dose rate for clinical use. On the other hand, pre-clinical research is necessary to confirm the effectiveness of the FLASH effect and to understand the underlying fundamental mechanisms. This will lead to the identification of the proper beam specifications for future FLASH-RT.

Conclusion

FLASH ultra-high dose rate in radiotherapy has received great recent attention and is considered an intriguing radiobiological phenomenon that attains comparable tumor control relative to conventional radiotherapy while sparing healthy tissues from severe side effects.

In this context, preclinical studies report that the FLASH effect reduces toxicity maintaining an equal tumor response across different animal models. In addition, the care of the first human patient with FLASH-RT also indicates promising results and supports further clinical evaluations. Nevertheless, the molecular radiobiology underlying ultra-high dose rates is not fully understood, and further experiments are fundamental to understand the biological effects in order to better develop the clinical potential of FLASH-RT.

In particular, it will be important to evaluate several main biological aspects (e.g. DNA damage response, oxidative stress/inflammatory cell signaling, and bystander effects) as likely key effectors in modulating biological differences between normal tissues and tumor tissues.

Additionally, at the preclinical level animal models are essential for further investigating *in vivo* the biological responses at cellular, tissue and whole organism levels.

Future research will also include the design of a FLASH delivery system able to produce beams for treatment of tumors with ultra-high dose rates and for clinical setting. Significant investments are needed to develop RF and laser-driven accelerators capable to deliver VHEE beams with high dose rate for clinical use. A positive outcome of these research activities will be facilitated by a multidisciplinary collaboration across biology, physics and clinical oncology, increasing the potential of a rapid clinical translation of FLASH-RT.

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