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FLASH Radiotherapy: Ultra-High Dose Rates to Spare Healthy Tissue

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FLASH Radiotherapy: Ultra-High Dose Rates to Spare Healthy Tissue

Purpose A recent addition to the treatment options in external beam therapy, so-called FLASH radiotherapy, shows remarkable healthy-tissue-sparing properties in a number of pre-clinical studies without impacting the overall treatment efficacy. Its potential in clinical applications is attracting a great deal of interest in the medical community. The use of ultra-high dose rates at extremely short irradiation times has been shown to significantly enhance the differential effects between normal and tumor tissue. This makes it possible to increase treatment doses without further harming the surrounding healthy tissue. While most studies to date have focused on the use of electron beams, X-ray and proton FLASH radiotherapy have also shown beneficial effects, although for these latter two the results still need to be independently confirmed. Furthermore, the mechanisms underlying the biological effects remain to be elucidated. Very recently, the FLASH effect has been demonstrated in the first human patient, with promising results, supporting further clinical studies. **Conclusion** This review will present an overview of the investigations into FLASH radiotherapy to date.

Keywords: FLASH-RT, ultra-high dose-rate irradiation, normal tissue protection, differential effect

Introduction

A recent series of publications by the Franco-Swiss team from the Institute Curie and Centre Hospitalier Universitaire Vaudois has the potential to change the way external beam therapy is applied to cancer patients all over the world. For decades, researchers have been searching for a way to eradicate tumor tissue while sparing the surrounding healthy tissue. Many of the advances made in this field have focused on beam targeting, including dose fractionation and precision imaging (Favaudon *et al.* 2014). Dose fractionation has been shown to increase the normal tissue tolerance to radiation therapy (Bourhis *et al.* 2006), and improving the precision with which the dose is delivered to the tumor limits the volume of the healthy tissue that is irradiated alongside the tumor,

and hence also limits healthy tissue toxicity. However, a third method to decrease healthy tissue toxicity has recently appeared, which exploits the biological differences between tumor and healthy tissue. FLASH radiotherapy (FLASH-RT) is attracting great interest in the radiation oncology community, as advances in reducing normal-tissue toxicities have been obtained by increasing the dose rate of the incident beams to dose rates exceeding 40 Gy/s. Thus far the effects have been observed in a variety of animal models, including mice, cats and a mini-pig. Following very encouraging preclinical results the first clinical study has just been published only five years after the first animal study, suggesting this treatment method could soon become commonplace in the clinic. This review will give a brief overview of the work that has been done to date on FLASH therapy using electrons, X-ray and protons beams, and conclude with future perspectives.

Electron FLASH radiotherapy

In FLASH-RT, single short treatment pulses (< 500 ms) are delivered at ultrahigh dose rates exceeding 40 Gy/s, far exceeding the commonly used dose rates of around 0.05 Gy/s. After first descriptions appeared in literature some decades ago (Hornsey and Alper 1966, Field and Bewley 1974), FLASH-RT was rediscovered in 2014 by the Vozenin group (Favaudon *et al.* 2014). An electron linac able to generate 4.5 MeV pulses at a high beam current is used by the group for the FLASH-RT, while other groups are known to work with 20 MeV electron beams (Schüler *et al.* 2017), photons (Montay-Gruel *et al.* 2018, Smyth *et al.* 2018) , or proton beams (Beyreuther *et al.* 2019, Buonanno *et al.* 2019).

FLASH in mice

Following in vitro results which suggested that for a fixed total dose the genomic instability for ultra-high dose rate radiation pulses was much lower than at conventional dose rates (Ponette *et al.* 2000, Fernet, V. Ponette, E. Deniaud-Alex 2002), FLASH therapy has to date been studied in a number of animal models: mice, zebrafish, cats and a pig (Bourhis *et al.* 2019). A well-established lung fibrosis model in mice was presented as the first proof-of-principle study (Favaudon *et al.* 2014). Doses up to 30 Gy were delivered in single doses at either FLASH (≥40 Gy/s) or conventional (CONV) (≤0.03 Gy/s) dose rates, and the effect of ultrahigh dose-rate irradiation on both normal and tumor tissue in the lungs was investigated. The FLASH doses were generated by an electron linac and compared against CONV irradiation using either the same LINAC with electron beams at low currents, a ^{137}Cs source irradiator, or a 200 kV x-ray generator. Very significant reductions in normal tissue damage were found with FLASH-RT (Favaudon *et al.* 2014) (Montay-Gruel *et al.* 2017), while the overall treatment efficacy did not appear to differ at similar doses between the two treatment methods. While pulmonary fibrosis was found for 100% of the animals irradiated with the conventional method, there was a complete lack of acute pneumonitis and late lung fibrosis observed with FLASH (Favaudon *et al.* 2015). These studies showed that the treatment delivery time largely influences normal tissue toxicity, while maintaining its anti-tumor effectiveness.

The healthy-tissue sparing effects of FLASH therapy have also been observed by irradiation of the abdomen of mice. Electron beam therapy at CONV dose rates of 0.05 Gy/s, and FLASH dose rates of 70 Gy/s and 210 Gy/s were compared at similar total doses between 10 and 22 Gy. For these studies 20 MeV electrons were used, as opposed to lower energy electrons (6 MeV), since the higher energy electrons have the advantage of a much smaller absorbed dose variation with depth (Schüler *et al.* 2017).

A significant increase in survival was observed for FLASH irradiation, where 90% of the FLASH irradiated mice survived 20 days, whereas only 29% of the CONV irradiated mice survived this same period of time, confirming the results of the Swiss-Franco group (Loo *et al.* 2017).

Further studies were implemented to determine whether the observed effect also holds for object recognition ability in mice following brain irradiations (Montay-Gruel *et al.* 2017), as well as for long-term memory deficits in mice (Montay-Gruel *et al.* 2018). The brain response to FLASH-RT was chosen for investigation as it is a well-defined and robust radiobiology model, where cognitive impairments are the most frequently described functional defects (Montay-Gruel *et al.* 2017). Radiation-induced neurotoxicity is a severe issue in both adult and pediatric brain tumor patients, and the healthy-tissue sparing advantage presented by FLASH therapy could potentially be an efficient way of enabling increased delivered doses while minimizing brain damage. In preclinical mouse models, neurogenesis occurs already within 1 month after a 10 Gy irradiation, and this model, thus, lent itself to the quantification of the effect of FLASH-RT on the functioning of mice brains. The mice received either FLASH-RT or CONV-RT, irradiating their entire brain to assess the effect on the memory recognition capabilities of mice. Mice were irradiated with a 10 Gy dose at dose rates between 0.1 and 500 Gy/s, with thermo-luminescent dosimeters (TLD's) being used for dose monitoring. Increasing the dose rate 1000 times showed a significant increase in the preservation of memory (Montay-Gruel *et al.* 2017). Mice irradiated with 10 Gy at a CONV dose rate had a significant drop in performance of Object Recognition tests 2 months post-irradiation, whereas those with 10 Gy FLASH did not perform any differently from the control group. Differences in memory recognition were observed at 30 Gy/s and lower dose rates. The sparing of spatial cognition and memory upon

FLASH-RT administered to the brains of mice was independently confirmed by Simmons et al (Simmons *et al.* 2019), using a FLASH irradiation system with a clinical accelerator, reaching FLASH dose rates up to 210 Gy/s, compared to CONV dose rates of on average 0.13 Gy/s (Schüler *et al.* 2017). FLASH-RT was associated with reduced cognitive deficits, decreased neuroinflammation and sparing of the hippocampal dendritic spine integrity (Simmons *et al.* 2019). It is suggested that these effects occur at the level of induction of inflammatory responses, although more research is required to fully understand the mechanisms at work (Simmons *et al.* 2019). Long-term radiation-induced learning and memory deficits in non-tumour bearing mice irradiated with FLASH therapy was assessed at 6 months post-exposure (Montay-Gruel *et al.* 2019). At the same delivered dose, CONV-RT (0.07 – 0.1 Gy/s) caused permanent neurocognitive alterations, whereas FLASH-RT did not induce changes in behavior of memory (Montay-Gruel *et al.* 2019), where the threshold for cognitive sparing was found to be around 30 Gy/s. The authors suggest that the lower levels of reactive oxygen species produced with FLASH therapy are in part responsible for the lower healthy-tissue toxicity. Doubling the oxygen concentration in the brain during irradiation was shown to be sufficient to reverse the FLASH neurocognitive benefits (Montay-Gruel *et al.* 2019).

FLASH in higher mammals

Translational studies have been performed looking at the potential benefits of electron-linac FLASH therapy in higher mammals – cats and a mini-pig. A translational study in domestic animals was commenced in cats with advanced squamous-cell carcinoma (Vozenin *et al.* 2019). Six previously untreated cats with nasal squamous cell carcinoma were enrolled for a dose escalation FLASH therapy trial. The doses were escalated from 25 Gy for the first to 41 Gy for the sixth cat, but no dose-limiting toxicity was observed

in any of the cats. At 6 months post irradiation all the cats had achieved complete remission, and three of them were still disease-free after 18 months (Vozenin *et al.* 2019). Because of the enhanced tolerance of healthy tissue with FLASH therapy, larger doses can be delivered giving an enhanced degree of tumor control. Although all cats revealed permanent depilation restricted to the field of irradiation no other permanent toxicities were observed and the cats were not limited in food uptake or smelling capability, making FLASH-RT a promising technique also for the treatment of cats (Vozenin *et al.* 2019). A second study to facilitate transfer of FLASH-RT to the clinic involved the irradiation of a pig skin, which was used to mimic the reactions of a human skin to the different dose rate irradiations (FLASH-RT at 300 Gy/s vs CONV-RT at 0.08 Gy/s). Transient acute toxicity was found 3 weeks after irradiation, but lasted only 4 weeks for doses smaller than 31 Gy. Hair follicles were preserved, whereas in conventional radiotherapy they were found to be permanently destroyed. No other acute toxicity was observed for the FLASH-RT, whereas skin fibronecrosis was observed in the low dose rate irradiated skin patches. A dose protective factor increase of FLASH-RT of at least 20% as compared to CONV-RT was estimated, and together with the results in cats provided rationale for to start clinical studies in humans.

First human patient study

The very first human patient study has recently concluded with promising results (Bourhis *et al.* 2019). A 75-year-old man with CD30+ T-cell cutaneous lymphomas had been treated with a range of chemotherapeutics as well as a total of 110 different irradiations with kV or MV X-rays as well as low energy electrons prior to the FLASH therapy. However, his skin had very poor tolerance for conventional radiation exposures, and would typically get acute skin reactions. A 15 Gy FLASH-RT dose was administered in 90 ms to one of his most resistant and progressive skin lesions with a

diameter of 3.5 cm. Skin reactions did not exceed grade 1, which was minimal compared to earlier CONV exposures for this patient. While no definite conclusions can be drawn based on only one patient, the study has shown that the high FLASH dose could be administered safely, with complete, durable and rapid tumor response in the follow-up period of 6 months, supporting further implementation of FLASH therapy in the clinic (Bourhis *et al.* 2019).

Photon FLASH radiotherapy

Only a few studies have been performed to date using photons to further test the tissue-sparing effect of FLASH therapy. A group in Australia used either MRT (microbeam radiation therapy) or SBBR (synchrotron broad beam radiation therapy) available at the Imaging and Medical Beamline (IMBL) of the Australian Synchrotron for total body irradiation at FLASH dose rates in mice (Smyth *et al.* 2018). The group performed a systematic dose-escalation study comparing CONV therapy (at 0.05 Gy/s) against MRT (around 300 Gy/s) and SBBR (at 37 – 41 Gy/s). With SBBR beams, no difference was found with CONV beams. The authors indicate that this could be due to the relatively low FLASH dose rate used. However, long-term tracking of the animals irradiated with the MRT beams revealed more pronounced sub-normal weight compared to the control irradiation. No evidence of normal tissue sparing effects was found using either MRT or SBBR beams in this study. On the other hand, a study at the European Synchrotron Radiation Facility, using also MRT for the delivery of FLASH-X-ray, did find beneficial effects of FLASH-X-rays on normal tissue (Montay-Gruel *et al.* 2018). They delivered either a FLASH-X-ray or CONV-X-ray dose of 10 Gy to mouse brains, with mean dose-rates of 37 Gy/s and 0.05 Gy/s respectively. Novel object recognition tests were performed at 2 and 6 months p.i. No decrease in recognition ratio was observed compared to the control group, similar to the results obtained in the electron beam

FLASH studies. A clear contrast with CONV-RT was observed, where CONV irradiation induced both irreversible memory alteration as well as a large decrease in hippocampal cell division, whereas hippocampal cell division was preserved under X-ray FLASH therapy.

Proton FLASH radiotherapy

Proton therapy facilities operating with pencil beam scanning (PBS) are able to produce very high instantaneous dose-rates in the range of 200 Gy/s. However, the duration of the scanning process drops the actual mean dose-rate (1–2 Gy/min) which limits investigations of the FLASH effect with protons over large irradiation fields (Montay-Gruel *et al.* 2018). In fact, dose rates of 5 Gy/s are already regarded as ultra-high dose rates in this field. The effect of FLASH-proton therapy on 'normal' human lung fibroblast cells has been investigated by Buonanno et al. at dose rates between 0.05 Gy/s to 1000 Gy/s, considering both acute as well as long term effects in cells (Buonanno *et al.* 2019). FLASH proton therapy did not appear to influence acute effects, as no significant difference in overall cell survival was observed between the low and high dose rate at a dose of 10 Gy. However, the DSB induction at 1000 Gy/s dose rate was significantly lower compared to 0.05 Gy/s and 100 Gy/s at a total administered dose of 20 Gy which might influence delayed harmful effects. This beneficial long-term effect could be due to the high proton dose rate affecting the long-term balance between anti- and pro-inflammatory molecules whereas CONV dose rates might tip the balance toward the latter. (Buonanno *et al.* 2019). At the University Proton Therapy Dresden, zebrafish embryos were irradiated at either a CONV dose rate (5 Gy/min) or at FLASH dose rates (100 Gy/s) (Beyreuther *et al.* 2019). No significant influence of the proton dose rate was observed on either the morphological integrity of the embryo's or overall survival in this study. The different small-scale energy

distribution of protons as compared to electrons could be a reason for the lack of beneficial response to FLASH irradiation. Another reason can be found in the relatively late developmental stage of the zebrafish embryos used in the study, making them less susceptible to radiation. Hence, further studies of this type are justified, as indicated by a recent study irradiating the whole thorax of mice with either proton FLASH (at dose rates of 40 Gy/s) or conventional dose rates (1 Gy/s). This is the only study to date which has shown evidence of tissue-sparing with ultra-high dose rate protons. A reduction in lung fibrosis up to 30% was found together with improved overall survival of the FLASH irradiated mice, suggesting that also with proton-FLASH studies a beneficial tissue sparing effect can be obtained (Girdhani *et al.* 2019).

Conclusions and future outlook

It is generally agreed that the FLASH effect is present for electron radiotherapy, resulting in remarkable healthy tissue-sparing effects while still retaining the anti-tumor efficacy. After the experimental validation in a number of small-animal models, the recent successful completion of the first human patient study supports further clinical evaluation. A few studies have looked at the FLASH effect using X-rays and proton irradiation, and while the FLASH effect appears to be present for X-ray irradiation and has been shown in mice for proton therapy, in vitro studies using protons were not able to show this effect. Further research will be required to determine if the FLASH effect can reproducibly be shown in X-ray and proton therapy. To date there are no known studies of FLASH therapy in ion beam therapy.

The mechanism behind the differential effects seen in tumour and normal tissues is still not very well understood. Hypotheses include the induction of transient hypoxia or the differential activation of DNA damage pathways (Smyth *et al.* 2018), as well as the formation of reactive organic hydroperoxide upon the depletion of local tissue

oxygen by FLASH doses (Spitz *et al.* 2019), but these will need to be validated experimentally to fully understand the biological effects induced by FLASH therapy. Cell studies have shown that the primary response to irradiation can occur as fast as within one second in some cell lines (Ponette *et al.* 2000), indicating that Poly(ADP-ribose) polymerase (PARP-1) could be involved (Fernet, V. Ponette, E. Deniaud-Alex 2002).

While current data seems to support large-scale clinical implementation of FLASH therapy, a number of issues still need to be resolved to make this possible. Next to the understanding of the biological effects, it is important to understand the effect of irradiation at FLASH dose rates on radiation-induced anti-tumour immunity (Harrington 2019), as well as optimize the dose rates and doses used in FLASH therapy for further clinical translation. Parameters such as dose per pulse administered, dose-rate within the pulse, and number of pulses delivered have to be carefully controlled (Bourhis *et al.* 2019). Furthermore, there are only very few systems available which are capable of delivering low-energy electron beams at the dose-rates required for FLASH therapy at the required field sizes. The further development of X-ray, proton, and possibly heavy ion FLASH will present technical challenges for the delivery of the dose rates requiring a very high power accelerator (Bourhis *et al.* 2019). Therefore, while FLASH therapy has been shown to significantly improve the differential effect between tumors and normal tissue, a better understanding of the biological effects is required, and a number of technical challenges need to be solved before it can be implemented in the clinic on a large scale.

Declaration of Interest

The author declares no conflicts of interest

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