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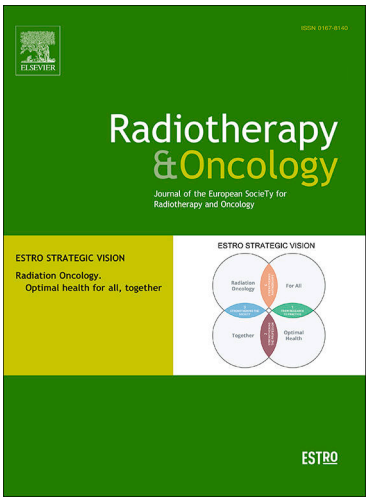

Review Article

Combining FLASH and spatially fractionated radiation therapy: the best of both worlds

Tim Schneider, Cristian Fernandez-Palomo, Annaïg Bertho, Jennifer Fazzari, Lorea Iturri, Olga A. Martin, Verdiana Trappetti, Valentin Djonov, Yolanda Prezado

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Combining FLASH and spatially fractionated radiation therapy: the best of both worlds

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Highlights

- Advantages and current limitations of FLASH and spatially fractionated radiotherapy (SFRT).
- Similarities and differences between FLASH and SFRT.
- Devices for simultaneous application of FLASH and SFRT: current status and perspectives.

Abstract

FLASH radiotherapy (FLASH-RT) and spatially fractionated radiation therapy (SFRT) are two new therapeutical strategies that use non-standard dose delivery methods to reduce normal tissue toxicity and increase the therapeutic index. Although likely based on different mechanisms, both FLASH-RT and SFRT have shown to elicit radiobiological effects that significantly differ from those induced by conventional radiotherapy. With the therapeutic potential having been established separately for each technique, the combination of FLASH-RT and SFRT could therefore represent a winning alliance. In this review, we discuss the state of the art, advantages and current limitations, potential synergies, and where a combination of these two techniques could be implemented today or in the near future.

1. Introduction

Radiation therapy (RT) represents one of the mainstays of cancer treatment with projections estimating that 50% of patients in the EU will require external RT by 2025 (1). Despite the technological advances and remarkable improvements in dose conformation achieved in recent decades (2), normal tissue toxicity remains the primary factor limiting the efficient treatment of radioresistant tumors, lesions near radiosensitive structures (e.g., spinal cord) as well as some pediatric cancers (3). Therefore, the exploration of new therapeutic modalities allowing a further reduction of toxicities is of paramount importance. Two such novel strategies are *spatially fractionated radiation therapy* (SFRT) (4-7) and *FLASH radiotherapy* (FLASH-RT) (8).

In SFRT, the dose is spatially modulated to create alternating regions of high dose, called *peaks*, and low dose, called *valleys*, fundamentally contrasting the flat dose profiles used in conventional radiotherapy (see Figure 1a) (4, 9, 10). Spatial fractionation of the dose is typically achieved by segmenting the irradiation field into several narrow beamlets which are separated by small gaps (usually 1-4 times the beamlet width). The beamlets can be pencil-like (i.e., narrow along all transversal dimensions) or planar (i.e., narrow along only one transversal dimension).

There are four main types of SFRT which mainly differ in the size of the beamlets and the arrangement of the peak and valley regions: While GRID-RT (5) and lattice RT (LRT) (11) use centimeter-scale beamlets, minibeam RT (MBRT) (7) and microbeam RT (MRT) (6) work with beamlets in the range of hundreds and tens of micrometers, respectively (see Figure 1b). Moreover, a two-dimensional dose modulation is used for GRID-RT, MBRT and MRT, giving rise to longitudinally continuous peaks and valleys, whereas LRT is based on a three-dimensional dose modulation characterized by isolated dose hot spots. Further details regarding each technique can be found elsewhere (4).

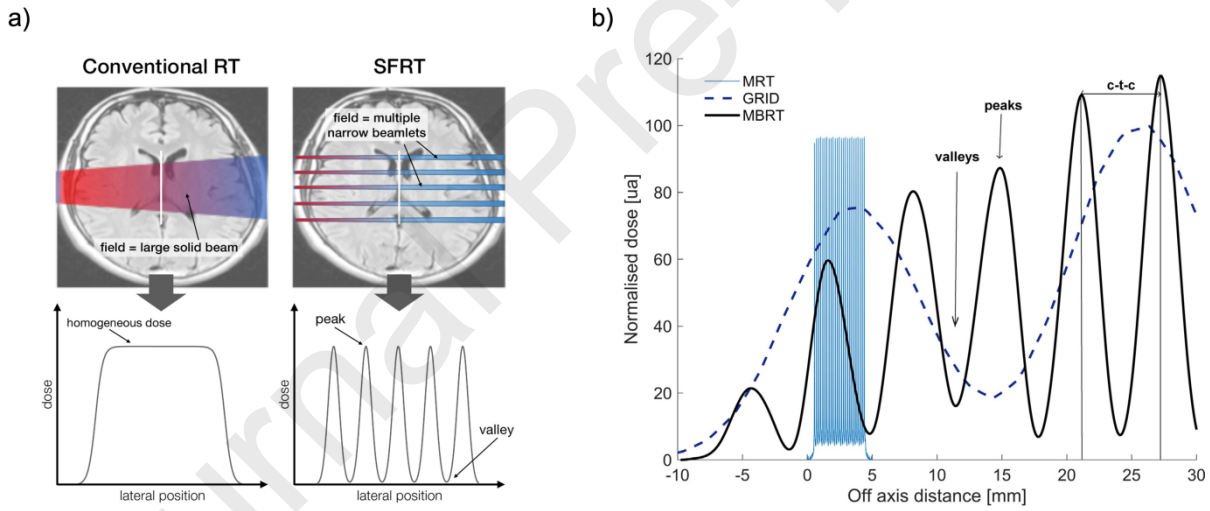

Figure 1. a) The conceptual difference between conventional radiotherapy (RT) and spatially fractionated radiotherapy (SFRT). b) Lateral dose profiles in SFRT and comparison of several forms of SFRT. The minibeam profile (MBRT) is scaled up by a factor of 2 for better visibility. Taken with permission from (10).

Compared to conventional RT, SFRT can provide a remarkable reduction of normal tissue toxicities while simultaneously offering high tumor control rates (4, 9). While GRID-RT and LRT are already seeing clinical use for the treatment of bulky tumors, mainly with palliative intent (4, 9, 12, 13), MBRT and

MRT are being extensively explored in preclinical studies. In this context, MRT was shown to delay or completely ablate tumors in rodent models (14-16) and MBRT experiments with glioma-bearing rats could demonstrate tumor control rates similar to those of conventional RT (17-20) as well as a complete response in some cases (20-22).

Where SFRT focuses on the spatial modulations of the dose, FLASH-RT reconsiders temporal aspects of the dose delivery. The idea is to exploit the so-called *FLASH effect*, a reduction of radiation-induced normal tissue toxicities observed when irradiating with ultra-high dose rates (UHDR) ≥ 40 Gy/s and very short delivery times < 200 ms (8, 23-25). Similarly to SFRT, FLASH-RT has already shown remarkable sparing of normal tissue in various animal models and different organs (8, 26-29) and several studies found comparable tumor control rates between FLASH and conventional RT (8, 30, 31). Particularly promising are the results of the first FLASH-RT patient who, after several unsuccessful treatments with conventional RT, saw a complete recovery of their cutaneous T-cell lymphoma while experiencing only minor adverse skin reactions (32). Moreover, a first clinical trial considering FLASH-RT with protons for the palliative treatment of painful bone metastases (https://clinicaltrials.gov/ct2/show/NCT04592887) has recently been launched and will be completed in December 2022.

These examples illustrate how FLASH-RT and SFRT can modulate biological responses and improve the outcome for patients through the use of non-conventional irradiation parameters. Consequently, experts are showing great interest in these two novel strategies as well as their combination. In this review, we will critically discuss both approaches, address their differences and common features, compare their individual advantages and disadvantages and consider the potential synergies of a combined use. Thereby, we hope to provide a starting point for further reflections and a catalyst for future research into FLASH-SFRT modalities.

2. Irradiation requirements for SFRT and FLASH-RT

The irradiation parameters required to observe the FLASH effect have not yet been fully elucidated (25, 33). While it is generally accepted that FLASH-RT requires the use of UHDR above 40 Gy/s (26, 34), some experiments satisfying this condition yielded negative results. For example, zebrafish embryos exposed to protons at 100 Gy/s and 0.08 Gy/s showed no difference in morphology or survival (35). Such negative results were also found at the limit of 40 Gy/s. No FLASH effect was observed when comparing mice exposed to synchrotron X-rays at 37-41 Gy/s and mice irradiated with conventional X-rays at 0.06 Gy/s (36). Moreover, mice exposed to electrons at 35 Gy/s showed more severe gastrointestinal toxicity and lower survival than a group exposed to 0.1 Gy/s (37).

Indeed, the FLASH effect is not exclusively correlated to the average dose-rate but also to the beam structure (pulse width, frequency, etc.), deposited dose, beam-on time and even the beam energy (23, 33). While FLASH effects have been described after the administration of photons (38), protons (39), and electrons (31), it is not clear how the sparing effect depends on the nature of the particles or their linear energy transfer. In the case of electrons, the number of pulses and the instantaneous *intra-pulse* dose rate (≥ 104 Gy/s) have been shown to be important factors (23, 33). The oxygen content of the tissue also appears to play a non-negligible role (23), however the most important requirement seems to be the total irradiation time, which should be less than 200 ms (24).

In contrast to FLASH-RT, SFRT does not appear to depend on the radiation beam structure, beam-on time, tissue oxygen concentration or dose rate (MRT being the only exception as described below). Instead, the most important conditions for tissue sparing in SFRT are (i) a significant spatial modulation of the dose (meaning a high peak-to-valley dose ratio and low valley doses (4, 40)) and (ii) the beam size (the smaller the beam size, the higher the doses tolerated by the tissue (41)). Additionally, the micrometer-scale beam sizes used in MRT (20-100 µm) require UHDR to prevent the distortion of microbeam patterns due to cardiosynchronous motion (42, 43). Similar to FLASH-RT, the sparing effects of SFRT have been demonstrated with different types of particles, namely photons, protons and even neon ions (4).

3. Biological effects of SFRT and FLASH-RT

This section reviews the biological effects observed in the context of SFRT and FLASH-RT, considering immunity, vascular, physicochemical, and cell signaling effects. A summary is also presented in Figure 2.

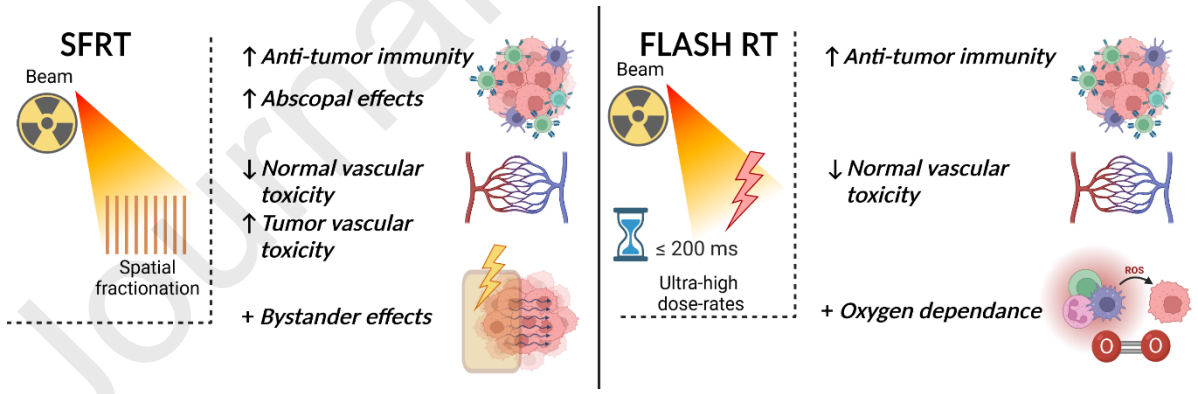

Figure 2. Biological effects in SFRT and FLASH-RT. Created with biorender.com.

3.1 Effects on immunity

One of the presumed mechanisms involved in the efficacy of SFRT is its ability to elicit an anti-tumor immune response (4, 9, 44). Several groups saw tumor infiltration by different subsets of T lymphocytes following both MRT (15, 45, 46) and MBRT (47). Furthermore, an increase of immune cytokine expression in tumors has been observed after MRT (15, 45) and B and natural killer (NK) lymphocyte infiltration was seen after MBRT (47). Another study looking at mice with tumors implanted in both limbs found evidence of abscopal immune responses after SFRT. The contralateral limb showed increased infiltration of both antigen-presenting cells and activated T cells, preceded by increased systemic IFN-γ production, resulting in a delay in tumor growth (48). Further details on immunomodulation after SFRT can be found in recent reviews (4, 45). Finally, a single high dose of radiation to only a partial volume of the tumor was able to cause T-cell infiltration into the tissue and improve the effect in distant tumor sites compared to the conventional beam radiation (49).

On the other hand, FLASH-RT has also been shown to enhance T-cell infiltration in tumors (50, 51) and to increase the concentration of pro-inflammatory cytokines in the serum of mice (34, 52). Moreover, a recent computational study considering the irradiated blood volume predicts a sparing of circulating blood cells at dose rates over 40 Gy/s (53). Irradiation of mice using synchrotron-accelerated carbon ions also showed a decrease of lung metastasis at UHDR compared to conventional dose rates, suggesting a more efficient abscopal effect under high dose rates as well as an immunogenic effect of FLASH-RT (54). However, the molecular mechanisms by which FLASH-RT or SFRT elicit immune responses are still under investigation, and it is plausible that the two modalities trigger distinct cellular mechanisms that could be combined.

3.2 Vascular effects

Regarding vascular effects in SFRT, the differential effects of MRT on normal and tumor tissue have been described in detail (55). MRT has been shown to spare the normal cerebral vasculature, while decreasing the tumoral blood volume fraction and vascular diameter in rat gliosarcomas (56). The effects of MRT on tumor growth delay due to tumor vascular impairment have also been demonstrated in murine melanomas (15). Structural or functional parameters such as blood volume fraction, vascular density, and perfusion have been reported to be intact in normal tissue after MRT irradiations (57, 58). Moreover, MRT exhibits a preferential damaging effect on immature vessels, whereas mature microvasculature is preserved (59). Griffin *et al.* (60) compared the vascular effects of MBRT (500 µm width at 2000 µm spacing) to those of MRT (50 µm width at 200 µm spacing) alone or in combination with an anti-angiogenic drug (alectin-1 targeted anti-angiogenic peptide) in mouse mammary tumors. The same peak doses were used in the two treatment configurations (150 and 75 Gy). The researchers observed a decrease in vascular density in all treated groups, which was enhanced in the groups receiving a concomitant anti-angiogenic drug. Interestingly, they also observed an increase in pericyte density (60).

For GRID-RT and LRT, there is only indirect evidence of their effects on the vasculature. Notably, increased sphingomyelinase activity and ceramide levels have been measured in patients with a complete or partial response to GRID-RT (61). Ceramide has been associated with a sensitization of endothelial cells to radiation-induced apoptosis (62). Similar results were obtained in a preclinical study with LRT (49).

Compared to conventional RT, FLASH-RT has been shown to spare blood vessels in the lungs (8) and to not induce vasodilation of microvessels in the brain (63). Importantly, according to the first human patient treated with FLASH-RT in 2018, the irradiated skin around the tumor showed a limited increase in vascularization, one of the components contributing to the promising evidence of sparing normal tissue (24). At the same time, a complete tumor response was observed in the following 5 months (24).

In summary, the current research supports the idea that both SFRT and FLASH-RT spare the normal vessels, although the underlying molecular mechanisms leading to this phenomenon are likely different. Importantly, all of the above studies on vascular effects after MRT benefited from irradiation at UHDR.

3.3 Physicochemical processes

Early chemical reactions may play a role in later biological responses in FLASH-RT and SFRT. The dependence of the FLASH effect on target oxygenation has led to the assumption that oxygen depletion could be one of its main drivers, although recent experimental data challenges this hypothesis (64). This idea is based on the premise that initial radiation exposure leads to oxygen depletion and, due to the UHDR, re-oxygenation cannot occur between pulses. Recent *in vitro* results describe increased clonogenic survival with FLASH-RT (600 Gy/s) compared to conventional RT (14 Gy/min), with a dependency on oxygen concentration only when doses exceeded 10 Gy (65). Under normoxic conditions with doses below 10 Gy, there was no difference in survival fraction between the two modalities whereas under hypoxic conditions a significant FLASH effect was seen at 18 Gy. In contrast, recent *in vivo* experiments have shown that oxygen depletion does not reach radiobiologically relevant levels of hypoxia after UHDR (66). However, tissues or cellular compartments that are already hypoxic (or close to hypoxic) at the time of irradiation could result in FLASH-associated radioprotection and normal tissue sparing. It has been hypothesized that stem-cell compartments of normal tissues may be one such example of already hypoxic niches spared by FLASH-RT (67). The full picture of the impact of oxygen on the FLASH effect is therefore still missing.

As for SFRT, no oxygen dependence has been described. The free radicals generated after irradiation were also hypothesized to contribute to tissue responses in SFRT. Dal Bello *et al.* (68) recently proposed that the distribution of hydrogen peroxide (H_2O_2) could play a key role in the anti-tumor efficacy of MRT and MBRT. H_2O_2 has a strong oxidizing capacity leading to damage of proteins, lipids, and DNA with a longer half-life than other ROS. Their calculations revealed that H_2O_2 produced in the peak regions diffuses to the valley regions during exposure, leading to a homogeneous H_2O_2 distribution over the target. Experimental evidence is nevertheless needed to confirm the validity of this model.

3.4 Effects on cell signaling

Cellular radiobiological responses are associated with both the direct damage to DNA by energy deposition as well as the indirect damage induced by the formation of ROS and free radicals following radiation-induced hydrolysis of water (69). Unirradiated or partially irradiated cells in the valleys exhibit oxidative DNA damage due to communication with directly targeted cells in the peak regions and due to scattered radiation (70, 71).

This is particularly relevant for SFRT where heterogeneous dose depositions are employed. Indeed, several studies found indications for the presence of cell-cell communications when observing that cancer cell death in the valley regions of SFRT irradiations was greater than the cell death following homogeneous irradiation with the same doses as in the valleys (71-73). Such a bystander effect can also be observed in valley-residing cells shortly after irradiation where it manifests itself in an increased expression of genes involved in apoptosis, DNA repair and cell cycle arrest. In contrast, such an increase was not observed in directly irradiated cells. (73). High-dose bystander effects involved in SFRT have been reviewed elsewhere (74). Abscopal effects (i.e., effects in out-of-field organs and tissues) have also been observed in several SFRT experiments (49, 75, 76). At the systemic level, genotoxic abscopal effects in out-of-field normal tissues have been reported after synchrotron MRT and broad beam irradiation delivered in a FLASH mode with a dose-rate 43 Gy/s (76, 77). As mentioned in section 3.1, carbon ion FLASH-RT showed a decrease in lung metastasis compared to conventional dose rate, which

could be explained either by abscopal effect or by limiting cancer stem cell migration to the tissues (54).

4. Advantages and disadvantages of SFRT and FLASH-RT

SFRT and FLASH-RT have specific advantages and disadvantages associated with each technique, which are summarized in Table 1. The main argument in favor of both techniques is the normal tissue sparing that can be achieved compared to conventional RT. Moreover, both SFRT and FLASH-RT provide tumor control that can be equivalent (and in the case of SFRT sometimes superior) to that of conventional RT. As a result, both techniques allow to widen the therapeutic window, in particular for radioresistant tumors.

Currently, no dependency on oxygen levels has been reported for SFRT. However, this is different for FLASH-RT where a control of partial oxygen saturation is required to maintain healthy tissue preservation which in practice also increases treatment complexity. Another aspect is that SFRT allows to reduce the overall volume of the irradiated tissue. On the other hand, the fast delivery times of FLASH-RT may facilitate the treatment of moving organs and reduce the requirements for organ motion management. This is not the case with SFRT where organ motion (cardiac and respiratory motion) during the treatment might cause blurring of the beam paths when using very small and tightly spaced beamlets such as in MRT. A combination of the two techniques would be beneficial for SFRT because UHDR could reduce artifacts when treating moving organs and the independence from oxygenation levels in SFRT could compensate a potential lack of the FLASH effect.

SFRT and FLASH-RT share the common challenge of an unconventional and demanding dosimetry (78-80). However, while the form of prescribing the dose in FLASH-RT is very similar to conventional RT, many more parameters (peak dose, valley dose, peak-to-valley dose ratio, etc.) would need to be considered in SFRT. This is because the optimal SFRT parameters yielding the best radiobiological response are still unknown.

The optimal beam structure and parameters for achieving the best healthy tissue preservation is also still unclear in FLASH-RT, as various beam structures and irradiation setups have been used in the published experiments. In the same way, the optimal dose rate to benefit from the FLASH effect is still unknown. In view of this important lack of data, the acquisition of comprehensive radiobiological evidence is crucial to further advance with these new techniques.

Concerning patient safety, FLASH-RT requires special ultra-fast monitoring systems on the irradiator, capable to shut off the beam within a few nanoseconds in order to avoid overdosing the patient (79). Moreover, the UHDR necessary for FLASH-RT impose important challenges on the accelerator and beam delivery technology (see section 6).

	SFRT	FLASH-RT
Advantages	- Reduction of toxicities - No dependency on oxygen concentration observed - Equivalent or superior tumor control to that of conventional RT - Reduced irradiated volume	- Reduction of toxicities - No organ motion management needed - Very short treatment times - Tumor control equivalent to that of conventional RT - Similar way of prescribing the dose as conventional RT
Disadvantages	- Challenging dosimetry - The optimal dosimetry and geometry parameters (peak dose, valley dose, peak-to-valley dose ratio, beam widths) not completely known - Different way to prescribe the dose with respect to conventional RT - Organ motion management required	- Challenging dosimetry - Optimal dosimetry and beam parameters not known (dose rate *vs* beam on time) - Clinical treatment of deep-seated tumors only currently feasible with proton beams - Oxygen dependence - Safety issues linked to the very short irradiation times - Limited working dose range

Table 1. Main advantages and disadvantages of SFRT and FLASH-RT applications

5. Potential synergies between SFRT and FLASH-RT

FLASH-RT and SFRT have clear advantages and disadvantages, as discussed above. Their sparing effect has been observed separately and their combined use could be expected to have at least an additive effect.

As explained above, most of the SFRT techniques use conventional dose rates, and lead to a remarkable normal tissue sparing. Synchrotron MRT is the only technique which has been mainly employed with UHDR. However, in a study conducted *in vitro*, the authors found a differential response between tumor cells and normal cells after spatial fractionation in the absence of UHDR, with tumor cells showing greater sensitivity to spatial fractionation than healthy cells (81). This supports the notion that MRT could also be performed at conventional dose rates.

A combined use of UHDR and SFRT has been carried out in numerous experiments at large synchrotron sources using MRT and MBRT modalities (15, 58, 82, 83). In a recent study, the lungs of Fischer rats were irradiated with 50 Gy at UHDR as a broad beam field, microbeam array (50 µm wide with 400 µm spacing), and minibeam array (500 µm wide with 4 mm spacing) (84). The results showed that irradiation with microbeams and minibeams resulted in significantly less lung fibrosis compared to irradiation with broad beams. This demonstrates the importance of administering heterogeneous doses to achieve higher tolerances to radiation.

In the same study, higher doses of 100, 300, and 600 Gy were also administered, but only to compare the effects of microbeams and minibeams (such doses would cause severe radiation toxicity as broad beam). After 100 Gy, there were no significant differences in the induction of pulmonary fibrosis between microbeams and minibeams, making this dose a potential starting point for the development of treatment plans. Only after 300 and 600 Gy did the microbeams, with their smaller width, induce less pulmonary fibrosis than the minibeams. However, these higher doses are too high to be clinically

relevant since there are currently no devices outside of a synchrotron that can deliver photons at the dose rate required to maintain the shape of the microbeams.

In fact, there are no studies demonstrating the biological effects of smeared microbeams on normal tissues or tumors. Although the synergy between UHDR and SFRT has not been demonstrated beyond an additive effect, it is clear that UHDR are necessary to maintain the shape of the microbeam. This suggests that minibeams may be a more viable modality for clinical trials when UHDR are not an option.

In addition to these synchrotron-based studies, proton MBRT at UHDR has been explored at the research facility SNAKE (Superconducting Nanoprobe for Applied nuclear (Kern-) physics Experiment) using low energy proton beams (20 MeV) (85). However, as of today, there is no clear evidence on the potential additive or synergistic effect of that combination.

Finally, there have been MRT studies attempting to decouple the FLASH effect from aspects related to spatial fractionation, but the results are inconclusive. In an *in vivo* study, total body irradiation was given to mice with synchrotron MRT at 291 Gy/s, UHDR broad beam RT at 39 Gy/s, and conventional RT at 0.05 Gy/s (36). Although the delivery times should have allowed the authors to see a FLASH effect (152-305 ms for MRT and 92-230 ms for UHDR broad beam RT versus a few minutes min for conventional RT), they could not detect it even without spatial fractionation. This demonstrates the importance of using an animal model that has been previously validated and shows a FLASH effect before attempting to perform spatial fractionation with UHDR. Biological experiments in well-controlled conditions are therefore warranted to assess the potential advantages of a combination of SFRT and FLASH-RT.

6. Where to combine SFRT and UHDR?

SFRT and FLASH-RT are non-standard approaches in radiation oncology, and the implementation of both techniques presents unique challenges. The primary requirement for SFRT is to produce narrow beams, which can be achieved with mechanical collimators or, in the case of charged particle beams, through magnetic focusing. For FLASH-RT, the primary challenge is to produce dose rates ≥ 40 Gy/s. However, in practice, other factors such as beam pulse structure, irradiation pattern, dosimetry, and monitoring or safety interlocks are also important. A detailed discussion of these aspects is beyond the scope of this review and can be found elsewhere (79). Instead, we decided to focus on how SFRT can be delivered at UHDR. To this end, recent developments, and prospects for the different types of radiation are presented individually.

6.1 Photons

Photons are the most commonly used radiation type in modern RT. While clinical megavoltage linacs can be readily equipped to deliver GRID-RT (5) or LRT (11), the typical dose rates of ~ 0.1 Gy/s (86) are far too low for FLASH-RT. An interesting new approach in this context is the PHASER (Pluridirectional High-energy Agile Scanning Electronic Radiotherapy) project (87), which aims to deliver intensity-modulated megavoltage X-rays at UHDR using high-energy electron beams that are magnetically scanned over a bremsstrahlung target. The resulting X-rays are then routed through a tungsten collimator which could be adapted to deliver SFRT.

While megavoltage photons are a good candidate for GRID-RT and LRT, energies in the range of ~ 50-400 keV are preferred for MRT and MBRT (86, 88) to maintain a satisfactory spatial modulation. As mentioned in section 2, because of their small dimensions, microbeams must be delivered at UHDR to prevent smearing of peak-and-valley patterns caused by cardiosynchronous pulsations (42). Therefore, almost all MRT experiments to date have been performed at synchrotron X-ray sources (86), which can

deliver extremely high dose rates of up to 16,000 Gy/s (14, 40). Large synchrotron facilities are one of the few places where FLASH-SFRT can already be performed today.

However, a disadvantage of large synchrotron facilities is that they are expensive, relatively rare, and not the most suitable for daily clinical use. An alternative could be the Line-Focus X-ray Tube (LFXT) concept recently proposed by Bartzsch and Oelfke (89) which is supposed to deliver microbeams with an average photon energy of 100 keV at peak dose-rates exceeding 100 Gy/s (90). With its small footprint, the LFXT could also be suitable for implementation in a clinical context.

6.2 Protons and ions

Proton therapy currently offers the greatest potential for clinical application of FLASH-RT: Commercial pencil-beam scanning (PBS) systems can achieve dose rates of 40-160 Gy/s at beam energies that allow treatment of deep-seated tumors (39, 91, 92) and a first clinical trial with proton FLASH-RT has recently been initiated (https://clinicaltrials.gov/ct2/show/NCT04592887). In addition, PBS systems have already been used for proton GRID (93) and for proton MBRT experiments (4, 94, 95).

It should be noted, however, that current clinical PBS systems cannot deliver beams smaller than ~ 2-4 mm full width half at maximum (FWHM) (96), so collimators are still required to produce proton minibeams. This implies a reduction in dose rate of at least one order of magnitude (97), which makes achieving UHDR a major challenge. A good example in this context is a recent study at the Paul Scherrer Institute in Switzerland, where experimenters succeeded in modifying a former clinical beamline to deliver 170-250 MeV proton beams at staggering dose rates of 700 to 9000 Gy/s (98). This could pave the way towards collimator-based proton FLASH-MBRT in a clinical setting.

A better alternative would be to avoid collimators altogether and use magnetic focusing to generate minibeams. This concept was first demonstrated at the aforementioned ion microprobe SNAKE in Munich, where beam sizes down to 10 µm (99) and dose rates up to 75 Gy/s (for larger 180 × 180 µm^2 beams) were achieved (100). While the maximum beam energies at SNAKE (currently 20 MeV, 70 MeV with a proposed update (101)) are sufficient for preclinical applications, magnetically focused minibeams with clinical energies may be achieved with a recently proposed nozzle concept design (96). A design study evaluating this nozzle in combination with the proton linac LIGHT (102) found that proton minibeams with widths between 0.6 and 0.9 mm FWHM and energies up to 200 MeV could be delivered at Bragg peak dose rates of ~ 50-1500 Gy/s (97). This could enable the study of proton FLASH-MBRT but also FLASH-GRID-RT in both experimental and, more importantly, clinical contexts.

Beyond protons, studies at Heidelberg Ion Therapy center (HIT) , Germany, have investigated the feasibility of FLASH-RT with carbon (54, 103) and helium ions (104), reporting dose rates of up to 70 and 193 Gy/s, respectively. With a beam size of 5 mm FWHM, the setup of HIT could be considered suitable for ion FLASH-GRID-RT. In addition, Prezado *et al.* have recently demonstrated the generation of neon ion minibeams using collimators (105), but these are unlikely to achieve UHDR.

6.3 Electrons

While electron FLASH-RT is already well established, both with experimental high-current linacs (106, 107) and with modified clinical linacs (38, 108), electron SFRT has been investigated only in some GRID dosimetry studies (109, 110) and theoretical studies (111, 112). One reason is that the use of clinical electron beams (≤ 20 MeV) is restricted to shallow targets and very large beamlet sizes (≥ 2.5 cm) because of their short range and significant lateral scattering (109, 111). Treatment of small or deep-seated lesions requires the use of so-called very high-energy electrons with beam energies ≥ 50 - 300

MeV (113, 114). Consequently, an implementation of electron FLASH-SFRT should provide both high energies and high beam currents.

Currently, such conditions can be achieved at dedicated accelerators such as the NLCTA at SLAC in Stanford, USA which can provide a maximum beam energy of 120 MeV at instantaneous dose rates of up to 10^{7} Gy/s (115). In addition, laser-based acceleration could deliver UHDR and beam energies up to 250 MeV (116, 117). However, such sources typically suffer from both large energy spreads and large beam divergences as well as issues concerning reproducibility. Another candidate for the future may be the aforementioned proposal for the PHASER project (87), which includes plans for a 100-MeV electron linac.

In the meantime, UHDR irradiation of superficial lesions could also be performed with modified linacs for intraoperative RT (IORT), as demonstrated in recent studies reporting average dose rates > 500 Gy/s at energies of 6-9 MeV (118, 119) The modification of IORT linacs could be comparatively easy and quick to implement and Felici *et al.* could obtain beam sizes of about 0.5 mm FWHM in one configuration which would be suitable (118).

7. Discussion

We are witnessing a paradigm shift in RT, driven by the increasing recognition that the physical irradiation parameters (i.e., dose delivery method, beam structure, etc.) play an important role for the biological response. In this context, techniques such as SFRT and FLASH-RT are some examples of how the use of non-conventional dose delivery methods may increase the therapeutic index (12, 29, 120).

Compared to conventional RT, both SFRT and FLASH-RT have already proven that they can offer a remarkable reduction of normal tissue toxicities while providing similar or even superior tumor control (12, 29, 120). Although certain biological aspects may be common to both techniques (such as sparing of normal tissue vasculature and strong induction of T-cell recruitment to the tumor), the underlying processes and pathways might differ on a microscopic level. Consequently, a combination of SFRT and FLASH-RT could have the potential to further increase the therapeutic index which could benefit for instance the treatment of radioresistant tumors. However, a deeper understanding of the involved mechanisms is needed to determine their best complementary use.

Moreover, it is also conceivable that the tissue sparing provided by spatial fractionation could compensate the potential absence of a FLASH effect in parts of the irradiated tissues with inadequate oxygen levels (27) or in situations where very large volumes have to be irradiated (121). On the other hand, the UHDR needed for FLASH-RT could help overcoming challenges in SFRT that arise from organ motion and long exposure times.

Concerning a practical implementation, the combination of SFRT and FLASH-RT can already be realized using low-energy X-rays at large synchrotron facilities as well as with certain innovative devices (line-focus X-ray tube) and at dedicated research installations (SNAKE). New developments, such as new nozzle designs, may enable irradiation with FLASH proton MBRT in clinical centers in the coming years (97).

Finally, FLASH-RT and SFRT come with important challenges for a clinical translation (4, 122). These include safety requirements, the standardization of unconventional dosimetry (protocols and detectors) and the need to integrate additional parameters in the treatment planning (such as the temporal beam structure for FLASH-RT and the peak/valley doses for SFRT). Moreover, the notion of dose as the main mediator for tissue response and the only parameter for treatment prescription will have to be updated and extended. The fact that more and more literature on treatment planning and

dose prescription in SFRT and FLASH-RT is becoming available highlights the drive for their clinical implementation (123-125).

It appears that the exploration of these novel techniques and the associated unconventional irradiation parameters have led us to a radiobiological iceberg of which we can perceive but the tip

As the authors of this review, we believe that we are currently experiencing a very exciting period for RT and a veritable blooming moment, creating new insights and treatment approaches that will fundamentally reshape RT. The best assets of these new strategies: FLASH-RT and SFRT.

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Conflict of interest statement

Tim Schneider and Yolanda Prezado have filed a patent application on proton minibeam generation

“Apparatus and method for proton minibeam radiation therapy, international extension filed, IOBR19INCPR2, 2020. “

Yolanda Prezado has filed a second patent for proton minibeam generation

Scanning dynamic device for minibeams production and method thereof, *EP21306092, 2021*