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PROTON THERAPY SPECIAL FEATURE: REVIEW ARTICLE

FLASH and minibeams in radiation therapy: the effect of microstructures on time and space and their potential application to protontherapy

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ABSTRACT

After years of lethargy, studies on two non-conventional microstructures in time and space of the beams used in radiation therapy are enjoying a huge revival. The first effect called "FLASH" is based on very high dose-rate irradiation (pulse amplitude ≥10^6 Gy/s), short beam-on times (≤100 ms) and large single doses (≥10 Gy) as experimental parameters established so far to give biological and potential clinical effects. The second effect relies on the use of arrays of minibeams (*e.g.,* 0.5–1 mm, spaced 1–3.5 mm). Both approaches have been shown to protect healthy tissues as an endpoint that must be clearly specified and could be combined with each other (*e.g.,* minibeams under FLASH conditions). FLASH depends on the presence of oxygen and could proceed from the chemistry of peroxyradicals and a reduced incidence on DNA and membrane damage. Minibeams action could be based on abscopal effects, cell signalling and/or migration of cells between "valleys and hills" present in the non-uniform irradiation field as well as faster repair of vascular damage. Both effects are expected to maintain intact the tumour control probability and might even preserve antitumoural immunological reactions. FLASH *in vivo* experiments involving Zebrafish, mice, pig and cats have been done with electron beams, while minibeams are an intermediate approach between X-GRID and synchrotron X-ray microbeams radiation. Both have an excellent rationale to converge and be applied with proton beams, combining focusing properties and high dose rates in the beam path of pencil beams, and the inherent advantage of a controlled limited range. A first treatment with electron FLASH (cutaneous lymphoma) has recently been achieved, but clinical trials have neither been presented for FLASH with protons, nor under the minibeam conditions. Better understanding of physical, chemical and biological mechanisms of both effects is essential to optimize the technical developments and devise clinical trials.

INTRODUCTION

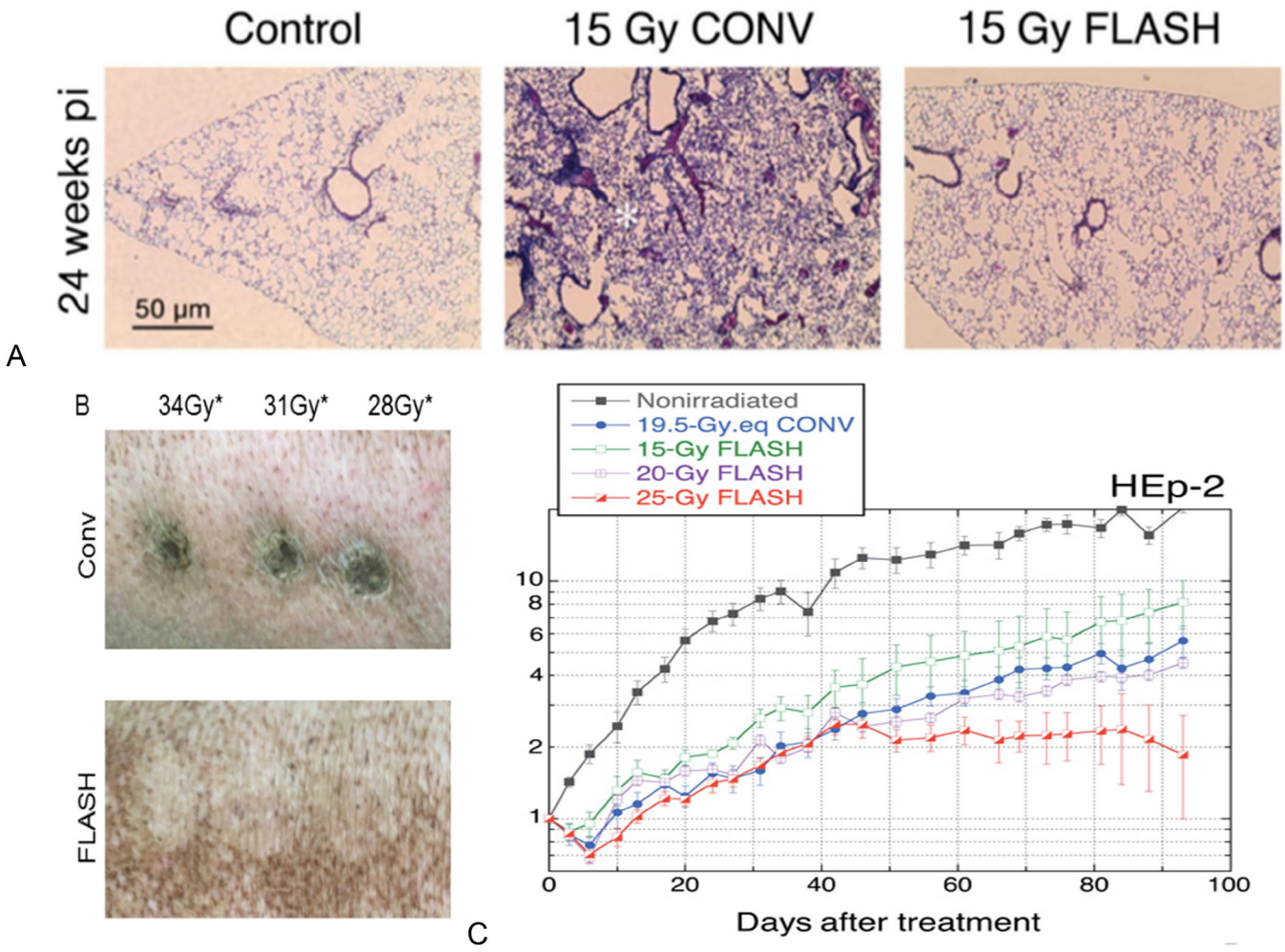
In this review, we evaluate two approaches in the domains of time and space devoted to healthy tissue preservation in radiation therapy (RT): (a) single dose -and potentially hypofractionated- irradiation at very high dose-rate known as "FLASH", and (b) irradiations with an array of minibeams. While FLASH experiments started with electron beams, minibeams have a rationale on microbeams produced by synchrotron X radiation. Studies in related fields (oxygen effect, high dose-rates, irradiation through grids etc) have gone through a recent redefinition, paving the way for clinical applications. Both effects, independently and even combined, have great potential to be successfully achieved in clinics using proton beams, combining their inherent preservation of doses after the distal range with their high dose rate and focusing properties of pencil beams.

The FLASH effect

Investigations on animal models and evidence of a FLASH effect : towards a clinical trial

Recently Favaudon et al,[1,2] investigated ***lung fibrogenesis*** in C57BL/6J mice receiving 15–17 Gy in bilateral thorax irradiation with 4.5 MeV pulsed electron beams. Animals were exposed in single doses to short pulses (typically 1–10 Gy

Figure 1. (A) Differential induction of pulmonary fibrosis by FLASH vs CONV irradiation in C57BL/6J mice, 24 weeks after single dose irradiation with 4.5 MeV electrons[1] . (B) Skin of pig 36-week post-RT with fibronecrotic lesions in CONV (5 Gy/min) irradiated spots and the normal appearance of the skin in FLASH (300 Gy/s) irradiated spots[3] . (C) Indistinguishable evolution of HEp-2 tumour xenografts after FLASH and CONV irradiations.[1]



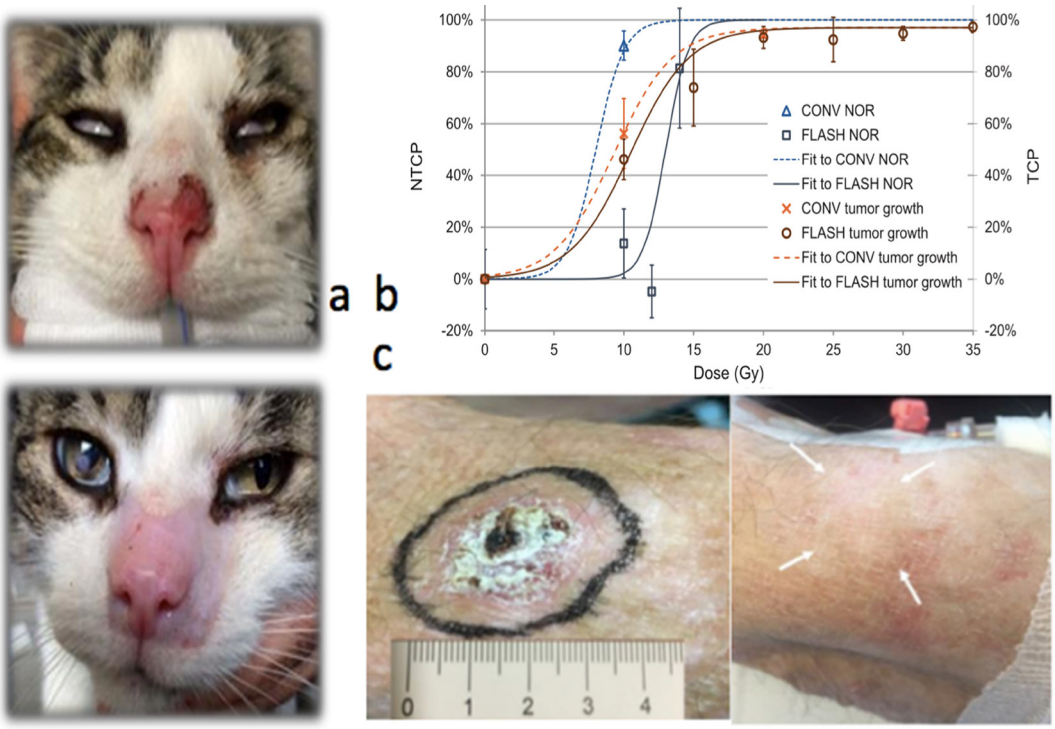
in 1 µs) given in sequence at 5–10 ms interval in such a way that the total beam-on time was ≤100 ms in most instances (FLASH). In a control arm (CONV), mice were exposed to "conventional" dose-rate irradiation (≤0.03 Gy/s). CONV treatment triggered lung fibrosis associated with activation of the TGF-β (trans-forming growth factor–β) cascade in 100% of animals, whereas no complications developed after doses of FLASH below 23 Gy at 36 weeks after irradiation (Figure 1a). In contrast, FLASH was as efficient as CONV in the repression of tumour growth of human HBCx-12A and HEp-2 tumour xenografts in nude mice and syngeneic TC-1 Luc$^+$ orthotopic lung tumours in C57BL/6J mice (Figure 1c).

Vozenin et al,[3] found that FLASH irradiation spares pig skin at doses that inevitably induce necrosis when irradiated in the CONV mode (Figure 1b). The dose-equivalent difference between the two modalities was ≥20% in terms of dose. The authors presented excellent results in progression-free survival of cat-patients irradiated under FLASH conditions treated for a carcinoma of the nasal planum,(Figure 2a) with no long term toxicity.

FLASH therapy was also proven to be advantageous for normal brain sparing in rodents.[6–8] The long-term neurocognitive benefit of FLASH relative to CONV-RT was attributed to a reduction in the level of neuroinflammation and reactive oxygen species (ROS), thus raising the question of the role of oxygen in the FLASH effect. Tumor Control Probability and Normal Tissue Complication Probability with FLASH were evaluated for glio-blastoma in mouse brain (Figure 2b) by Bouhris et al. [4]

Normal tissue sparing after abdominal irradiation of mice under FLASH irradiation was demonstrated by Loo et al[9] using a modified clinical linac with a 20 MeV electron beam. The lethal dose LD50 moved from 14.7 Gy at 0.05 Gy/s (beam-on time 294 s) to 18.3 Gy at 210 Gy/s (beam-on time 0.087 s). FLASH-irradiated Zebrafish embryos[7] showed significantly fewer alterations in body length development compared to CONV irradiation. However, the protective effect of proton

Figure 2. (a) FLASH irradiation of cat-patients with locally advanced T2/T3N0M0 squamous cell carcinoma of the nasal planum as part of a Phase I single dose escalation trial (25–41 Gy). The results showed 84% progression-free survival (PFS) and complete local control at 16 months. No long-term toxicity was observed except from permanent depilation in the irradiated area. Images from personal communication of M.C. Vozenin, and see[3] for details. (b) Tumour control probability (central curves) in implanted glioblastoma and normal tissue complication probability in mouse brain (through Novel Object recognition) for FLASH and conventional irradiations[4]. (c) Response at 5 months of a cutaneous lymphoma in the first human patient treated under FLASH conditions.[5] *Figs. 2b,c reprinted from[4,5], copyright (2019), with permission from Elsevier.*



FLASH could not be reproduced with Zebrafish embryos by Beyreuther et al,[10] due probably to different beam and biological conditions adopted in the study (lower max dose rate in a pulse, longer delay post-fertilization of the Zebrafish). Conversely, Diffenderfer et al[11] using an innovative cyclotron facility delivering a 230 MeV proton beam operated at a mean dose-rate of 78 ± 9 Gy/s, provided the first demonstration of small intestine sparing from loss of stem cells and radio-induced fibrosis by proton-FLASH. Consistent with the initial observations[12] this sparing effect was specific of normal cells and did not extend to tumour xenografts, suggesting a high potential of the FLASH methodology in the treatment of solid gastrointestinal malignancies.

In vitro studies published so far hardly gave evidence of a FLASH effect in terms of cell survival. Early studies using immortalized (tumoural) cell lines, reviewed by Zackrisson et al[13] did not give evidence of a differential response to nano-microsecond pulses of radiation compared to continuous, conventional dose-rate irradiation in terms of clonogenic survival. In a recent review, Colangeno and Azzam[14] reported nine studies with no effect of ultra-high dose rates with protons with acute toxicity as an endpoint, all of them performed under ambient atmospheric conditions (21% O2) what is considered as one of the potential reasons for these results.

This situation is changing. Yet a parallel with the FLASH effect is not straightforward, in some cell lines short pulses of radiation were found to elicit rapid, transient changes of radiosensitivity depending on DNA damage recognition by poly(ADP-ribose) polymerase I.[15,16] Second, Buonanno et al,[17] using low-energy proton beams (4.5 Mev) to irradiate normal human lung fibroblasts at very high dose-rate (10^3 Gy/s) reported recently a mitigation of long-term radio-induced senescence and expression of TGF-β1. And third, Fouillade et al just showed that, relative to CONV, FLASH spares normal lung fibroblasts grown *in vitro* from a specific subset of DNA double-strand breaks and limits the incidence of radio-induced senescence in lung stem cells both *in vitro* and *in vivo*.[18]

Current hypotheses on the mechanisms underlying the FLASH effect

In a recent overview, Vozenin et al[19] proposed that the differential response to FLASH irradiation between normal tissues and tumours stems from a lower pro-oxidant burden in the former. Data from the same team[3] also suggest that FLASH has minimal impact on skin stem cells consistently with what was already reported for neural[6] and intestinal stem cells.[9,11] That will potentially be one of the underlying mechanisms to reduce the toxicity on the normal tissues that will be able from replication and differentiation of stem cells. Along the same lines, Buonnano

et al[17] proposed that normal tissue sparing by FLASH proceeds from a combination of related effects such as a reduction in the complexity of damage to DNA, cell senescence and radiation-induced chronic inflammatory processes.

Recently Montay-Gruel et al[7] showed that the FLASH effect depends on the partial pressure of oxygen. The effect of oxygen on the response of cells or tissues exposed to large doses of radiation at very high dose-rates has been known for decades,[12,20,21] in particular by evaluating the mouse tail resistance to epithelial radionecrosis when short pulses of electrons induce oxygen depletion, including preirradiation and daily fractionation.[22] Even then, the analysis of the dose needed to induce oxygen depletion and the risk of protecting also tumour cells were factors that halted further developments in the field.[23] *In vitro* studies highlighted the role of oxygen as a radiosensitizer yet Berry and Hall[24] alternatively proposed that radical-radical interactions might play a major role in the response to ultrahigh dose-rate irradiation.

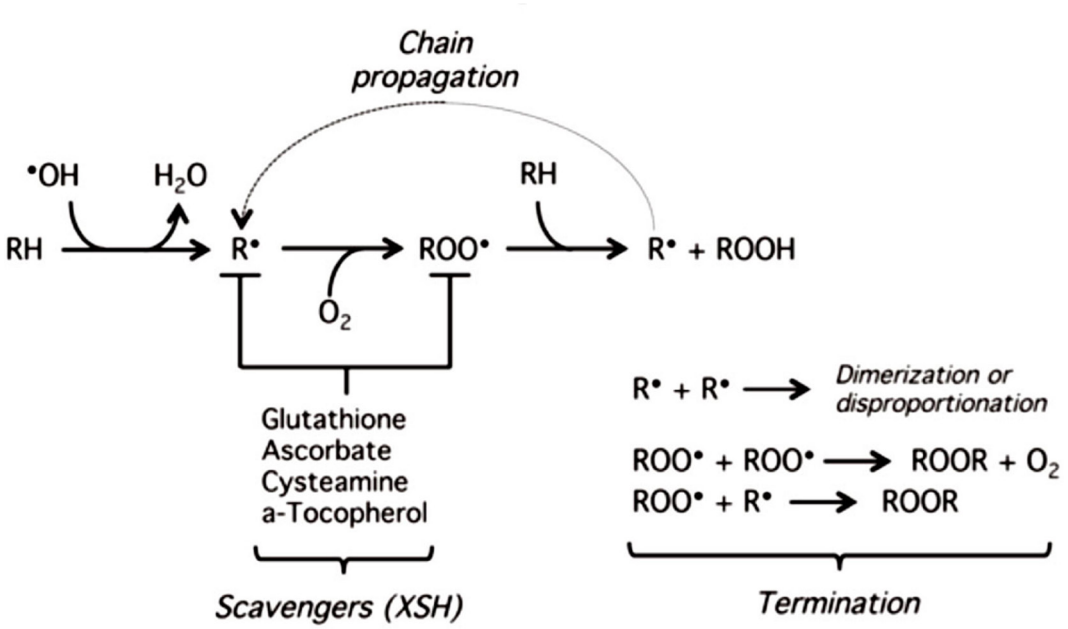
Rothwell[25] modelled the processes of oxygen diffusion and reaction in cells, suggesting that ultra-high dose-rates cause temporary oxygen depletion as the mechanism behind the FLASH effect. Durante et al[26] also stressed oxygen depletion as a possible mechanism for reduction of the damage after exposure to ultra-high dose-rate irradiation, yet for them the mechanism underlying the effect observed in the FLASH radiotherapy remained to be elucidated. Pratx and Kapp[27,28] developed a model including the rate of oxygen diffusion through the tissue, its consumption by metabolically active cells and its radiolytic depletion to estimate the relative decrease in cell radiosensitivity. They suggested that the FLASH effect should be effective to protect normal tissue by sparing already hypoxic stem cell niches. It must still be elucidated if the stem cell preservation is not a cause but a consequence of the absence of initial damage and how much this can explain effects such as preservation of brain functions.

Spitz et al,[18] based on the fact that FLASH delivers four order of magnitude higher instantaneous dose rate over conventional photon and electron beams, proposed a mechanism where differences between the decay rates of ROO and ROOH produced in normal tissue *vs* tumours may explain the beneficial therapeutic ratio of FLASH, along with the differences in the labile iron pool: normal tissues can more effectively regulate endogenous levels of labile Fe, so Fenton-type reactions will be limited in normal *vs* cancer tissues.

Another hypothesis has been formulated by Favaudon et al. (second international symposium on ultrahigh dose-rate FLASH radiation therapy. Lausanne, September 12–13, 2018, Switzerland) related to competition between radical recombination, thiol-induced scavenging and oxygen uptake by the primary carbon-centred radicals at the origin of peroxyradicals ROO$^{\bullet}$ (Figure 3). This model is consistent with the chemistry of peroxyradicals[29] and in the line of that proposed earlier by Berry and Hall.[24]

Recent data from Fouillade et al[30] shows that FLASH minimizes the induction of pro-inflammatory genes and persistent DNA damage and facilitates radiation recovery by sparing murine lung progenitor cells and limiting the incidence of radio-induced senescence. These studies also show that FLASH elicits differential DNA damage in normal (stem) cells maintained in culture in equilibrium with air, a property that is not shared by tumoural cells, thus suggesting that the oxygen is not the sole mediator of the FLASH effect and opening the route to studies of DNA repair in relation to cell cycle progression and free radical chemistry.

Figure 3. Chemical model eliciting competition between second-order recombination, thiol-induced scavenging and oxygen uptake by carbon-centred radicals in lipids (Favaudon, personal communication).



Technical platforms, dosimetry and virtual calculations of FLASH

Technical platforms

Most of the pioneering and collaborative work done by the Institute Curie in France[1] and the Lausanne[3] teams to study the FLASH effect has been done with ***4–6 MeV electrons*** not readily suitable to clinical work, except for intraoperative radiation therapy (IORT) or treatment of superficial tumours. Irradiations were performed using a Kinetron LINAC (4.5 MeV electrons, CGR-MeV, Buc, France)[31] and an Oriatron LINAC (eRT6; 6 MeV electrons, PMB-Alcen, Peynier, France).[32] The wide range of dose rates (Gy/min to kGy/s and up to 50 Gy in a single 2 μs pulse) was obtained by varying the LINAC gun-grid tension, the pulse repetition frequency, the pulse width, and the source-to-surface distance (SSD).

Schüler et al[33] achieved 220 Gy/s at 1 cm depth for $a > 4$ cm field size with 90% homogeneity throughout a 2 cm thick volume for small animal experiments with ***electron beams of 9 and 20 MeV*** in clinical mode into the head of a Varian Clinac medical accelerator. Proposals for using very *high-energy electrons (VHEE),* able to also deliver very high intensity beams, are under development.[34]

Montay-Gruel et al[35] used the ID17 Biomedical Beamline of the ESRF synchrotron to deliver whole-brain mice irradiations with ***225 kV X-rays*** at peak dose-rates of 12 kGy/s and mean dose-rates of 37 Gy/s, in such a way that the beam-on time was 0.27 s. Smyth et al,[36] using also synchrotron radiation (93 to 124 KeV), did not find evidence of normal tissue sparing when irradiating mice with high *vs* conventional dose rates, but their maximal dose rate was of 41 Gy/s and the authors stated that it could be "too low to elicit" a protective FLASH effect.

The production of megavoltage ***photon*** beams required for FLASH conditions is impaired by target cooling technical limitations in LINACs..[37] The first clinical device that will provide FLASH conditions has been conceived by the Stanford Team originally for fast irradiations reducing the effect of organ movements. Their Pluridirectional High-energy Agile Scanning Electronic Radiotherapy (PHASER) includes original solutions to integrate 16 linacs with an electron beam scanned on stationary targets, producing very rapid electronically scanned highly intensity-modulated photon beams of 10 MV.[38]

An alternative of high interest is to move to ***proton*** beams. Patriarca et al[39] implemented a system with a single scattering foil, a ridge filter and a high current monitoring system upstream from the isocentre. The purpose was to use passive proton beams, avoiding in a first approach the time structure of a proton pencil beam scanning system. Dose rates exceeding 40 Gy/s at energies between 138 and 198 MeV were obtained from a 106 MHz cyclotron (IBA, Belgium) for a field size of 12 × 12 mm.

Technical set ups for scanning beam of protons with FLASH dose rates are being produced at several facilities[40] and the use of high-frequency proton LINACs has been proposed for FLASH by Kolano et al..[41]

Physical dosimetry

The translation of experiments to clinical setups needs the implementation of accurate physical dosimetry of high intensity pulsed beams with detectors showing reproducibility and linearity for monitoring, calibrating and performing quality controls of clinical beams (*e.g.,*based on ionization chambers traceable to calibration laboratories) and also to perform any kind of *in-vivo* dosimetry in these single or hypofractionated treatments.

A combination of detectors has been used to reduce uncertainties in dosimetry. Hendry et al[22] mention the use of a Faraday cup, Ferrous sulphate dosimetry and FLi *in vivo* dosimetry when using electron beams of 10 MeV with 1–50 pulses per second of 0.5–5 μs length each, varying the dose per pulse from 0.0017 to 3 Gy. Favaudon et al[1] used submicrosecond, time-resolved determination of the electron fluence and chemical dosimetry based on methyl viologen to measure the dose absorbed in water.

Buonanno et al[17] and Petersson et al[42,43] tested different monitors and dosimeters (Gafchromic™ EBT3, TLD, Alanine pellets, Markus and a custom made parallel plate ion chambers, and a methyl viologen dosemeter), concluding on their independence of the dose-rate and a level of uncertainty in the order of 5%. These approaches have been used for *in vivo* dosimetry.[3,5,32]

For protons, Busold and Heese[44] presented a high cross-linearity in measurements with a Faraday cup, a transmission monitor chamber, a parallel plate ion chamber and Gafchromic films in a scanned 250 MeV pencil beam, up to the maximal clinically available beam currents of 350 nA as used for FLASH experiments.

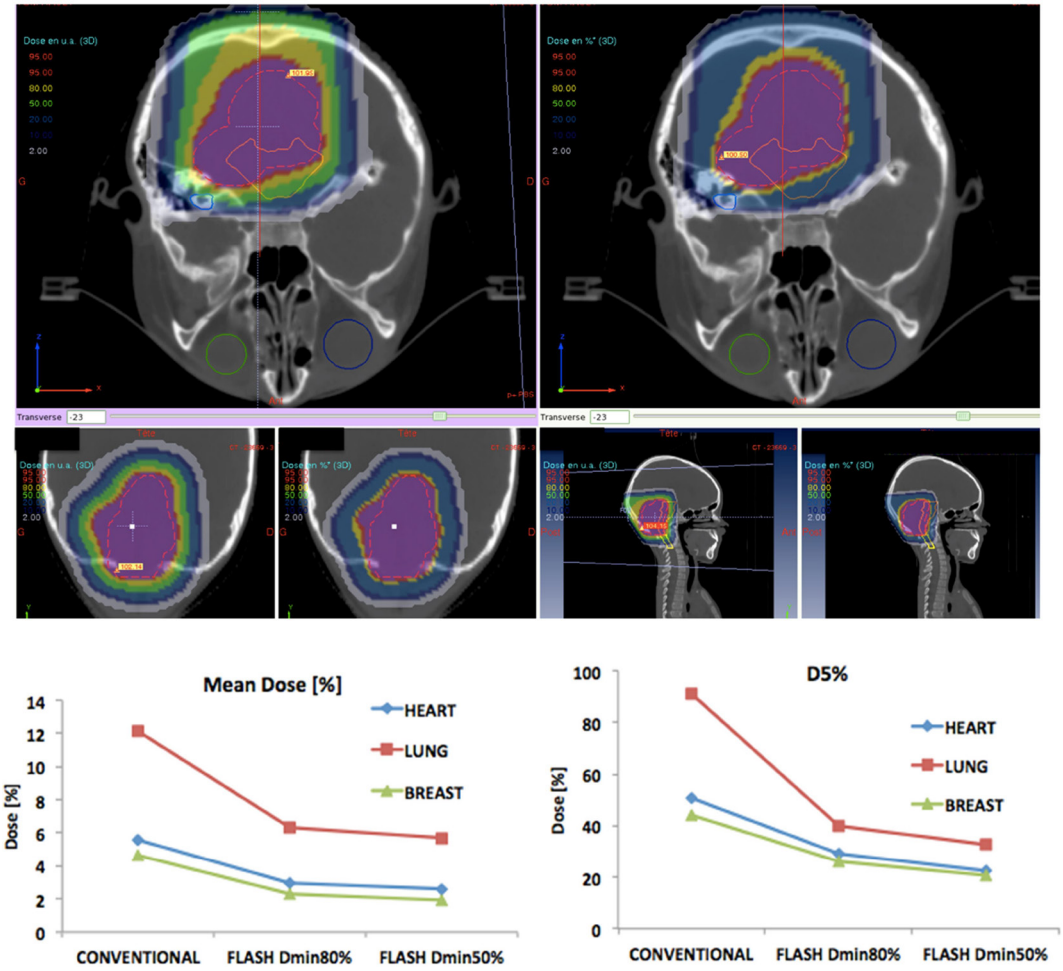
Simulations and virtual dosimetry

In the studies of skin tolerance in pigs and veterinary treatments of cancer in cats,[3] a reconstruction of the dose distribution was performed using a CT-scan of the pig and cats and a commercial treatment planning system (TPS). No modelling of the time effect nor a FLASH effect estimation were included.

For protons, Mazal et al[45] presented a prospective model to take into account individual parameters such as the minimal and maximal dose required to achieve a FLASH effect assuming the dose rate and the maximal time to irradiate are fulfilled (Figure 4). Using the concept of a relative biological effectiveness (RBE) for FLASH in the healthy tissues, they suggest that the main benefit of the FLASH effect could be seen in a layer of high to mid doses around the target, depending on the required minimal dose to achieve the FLASH effect.

Van de Water et al[46] recently evaluated the spatially varying instantaneous dose rates for different intensity-modulated proton therapy (IMPT) planning strategies and delivery scenarios. They proposed the "dose-averaged dose-rate" (DADR) metric, defined for each voxel as the dose-weighted mean of the instantaneous dose-rates of all spots (*i.e.,* pencil beams). Calculations for head and neck cases using the Varian ProBeam system (Varian, Palo Alto, USA) showed that increased beam intensities, spot-reduced planning and hypofractionation were required to achieve FLASH compatible dose rates.

Figure 4. Virtual simulation of the modification of dose distribution in the treatment of (a) a posterior fossa (medulloblastoma), for a single conventional beam of protons (left) and the case where a FLASH effect is achieved in healthy tissue for doses higher than a Dmin (right). (b) A mediastinal tumour with a single proton beam: mean and D5% (dose delivered to the 5% of the volume of a given structure) for critical structures when the FLASH effect is activated at 80 or at 50% of the target dose (Mazal et al, personal communication).



Clinical trials of FLASH radiotherapy

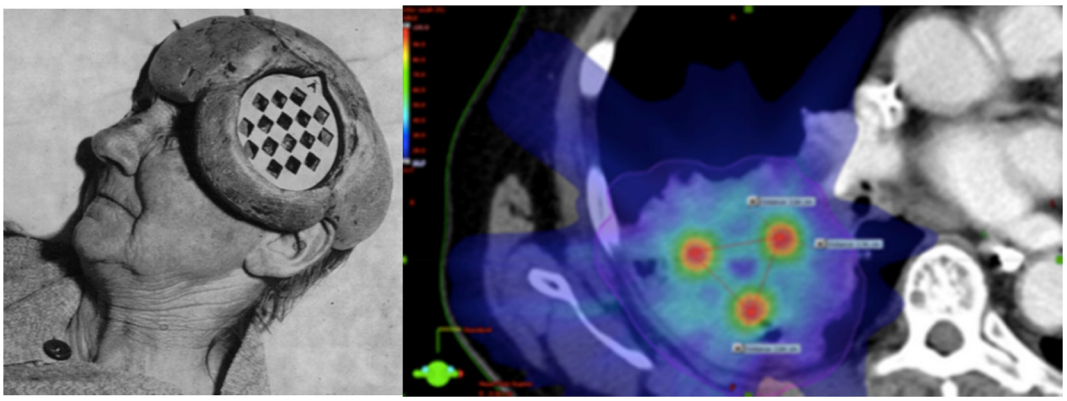
The main data supporting the clinical translation of FLASH were reviewed by Bourhis et al [4] who explored its feasibility, the key irradiation parameters (dose, dose-rate within the pulse and overall time of irradiation) and the potential technologies needed (low and very high energy electrons, protons and X-rays) for successful clinical trials. Symonds and Jones, in an Editorial in July 2019,[47] concluded that "FLASH radiotherapy clinical trials using photons or even protons may start in the next few years." The team of Bourhis et al[5] effectively announced recently the treatment of the first patient with the explicit conditions necessary to evaluate the FLASH effect in clinics. A 75-year-old patient with a multiresistant CD30 +T cell cutaneous lymphoma disseminated throughout the whole skin surface had been previously treated with localized skin radiation therapy for various ulcerative and/or painful cutaneous lesions, progressing despite systemic treatments and with poor general tolerance. A 3.5 cm diameter skin tumour was treated with 15 Gy in 90 ms using 10 pulses of 1μs at 10 ms interval of a 5.6-MeV electron beam from the eRT6 LINAC designed for FLASH. The tumour response was complete with a short follow-up of 5 months (Figure 2c). A Grade 1 epithelitis and a transient Grade 1 oedema in soft tissues surrounding the tumour were observed. Optical coherence tomography observations showed that FLASH irradiation preserved the thickness of the epidermis and the basal membrane, with limited damage to the vascularization. These observations are promising and demonstrate the feasibility of this approach, first with electron beams (*e.g.,* for superficial tumours and for IORT) but also with proton beams that can achieve these conditions for small size targets.

Spatial fractionation of the dose in radiation therapy: the minibeams and microbeams in radiation therapy

Historical GRID and present use of LATTICE radiation therapy (LRT)

The concept of spatially modulating the dose in RT was first proposed in the early 20th century by A. Kohler.[48,49] He proposed the use of a grid collimator to spare skin toxicity when

Figure 5. (a) *studies of the connective tissue reactions to radiation using a sieve-chess technique, leading later to GRID palliative radiation of bulk tumours reducing complications in healthy tissue*[51]. (b) *Lattice radiotherapy (LRT) in voluminous non-small cell lung cancer.*[52] *Fig.5a reprinted from*[51] *by permission from Springer Nature on behalf of Cancer Research UK, copyright (1949).*



deep-seated tumours were to be treated with the orthovoltage machines existing at that time ("GRID therapy"). In practice, he pushed the X-ray tube's lead-shielded housing against a stiff grid of 1 mm^2 iron wires woven 3.0–3.5 mm on centre, taped tightly to the skin over a thin chamois. GRID therapy was disparaged or ignored until the 1930s[50] and was used since then and "rediscovered" using Co-60 units and megavoltage beams provided by medical linear accelerators, for example to shrink bulky malignancies for palliative cases(Figure 5a). [49–51,53–63]

By adjusting the old 2D grid technique into a 3D lattice using multiple high-dose areas (called "vertices"), high-dose radiation is delivered with high-energy photons (6–18 MeV) within the tumour and not in the peripheral areas adjacent to normal tissues.(Figure 5b)[52,64] ,

Amendola et al[52] hypothesized that with this Lattice Radio-Therapy (LRT) bystander effects may be induced in peripheral neoplastic cells while avoiding toxicity to adjacent normal structures. It may allow for possible immune modulation of T-cells within the irradiated tissues, which is currently a trend in medical oncology.

While LRT is oriented towards improving the effects in the tumour volume, GRID was developed to preserve healthy tissue. Exploring further this second concept, it was noted that the reduced output of LINACs and the important lateral scattering of MV make it that large beam sizes (>1 cm^2) need to be used. This is not favourable for tissue sparing, as important scattering results in low differences between doses at the irradiated and not irradiated sectors into the beam areas, and pushed the research in other directions.

The concept and trials of microbeams and minibeams : platforms based on X-rays and proton beams

The observation of a highly non-linear inverse relationship between normal tissue radiosensitivity and tissue volume was exposed by Zeman and co-workers[65] in the 1950s, as part of investigation of biological effects of cosmic rays on brain tissue. Using a 22.5 MeV deuteron beams, they found that the dose required to produce a radiogenic lesion in mouse brain increased from 300 Gy to 10kGy when the diameter of the beam was reduced from 1000 to 25 μm.

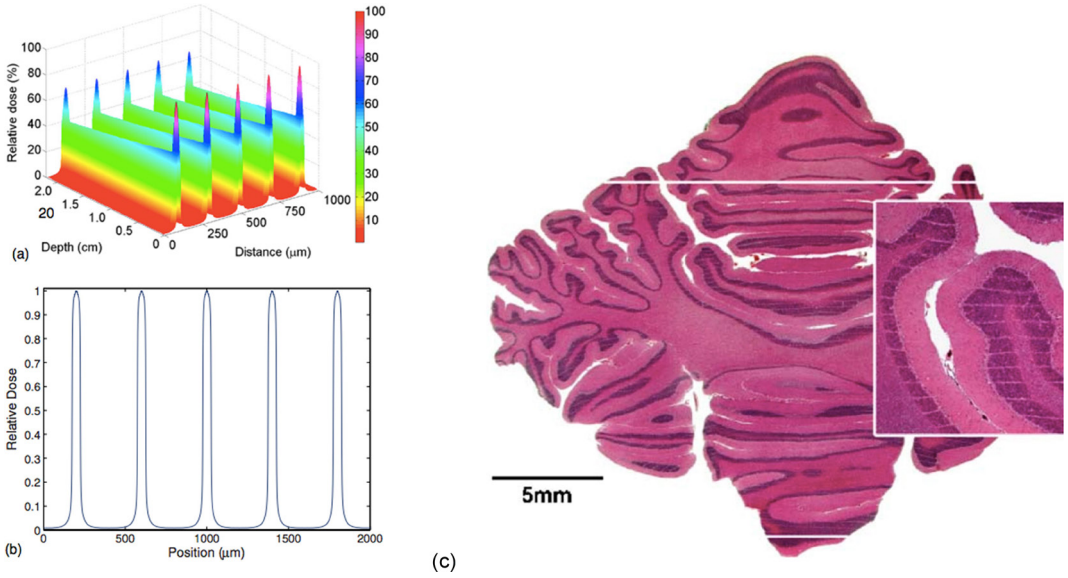
This effect was exploited in the 1990s thanks to the advent of third-generation ***synchrotron sources*** providing ***kilovoltage X-ray beams*** with negligible beam divergence and high brilliance, such as the Brookhaven National Lab or the European Synchrotron Radiation Facility.[66] In 1992, Slatkin and colleagues proposed the concept of microbeam radiation therapy (MRT),[67,68] using 25–100 μm wide beams spaced by 200–400 μm. The most common metrics to characterize an array of microbeams is the peak-to-valley dose ratio PVDR = Dpeak/Dvalley(Figure 6a and b), while several other dose-volume metrics have been proposed.[69]

Numerous experiments, mainly concentrated on the central nervous system, have shown an extraordinary normal tissue sparing.[71–78] . Figure 6cshows the results of one of the pioneers and more emblematic experiments in MRT: the cerebellum of five 47-day-old Weanling Piglets (surrogate for the radiosensitive infant human cerebellum) were irradiated with 20 μm wide beams and peak-entrance doses reaching 600 Gy in one fraction. The piglets were followed for more than one year, and no signs of developmental, behavioural, or radiological damage was observed.

MRT has also been shown to delay tumour growth and in some cases induce tumour ablation in different kinds of tumours in rodents.[78–85]

The need for complex requirements to achieve MRT conditions, such as (a) extremely high dose rates (*e.g.*, 100–10000 Gy/s) to prevent blurring by cardiosynchronous pulsations of the peak and valleys patterns,[86] (b) low-kilovoltage energies (<200 keV) to avoid scattering,[87] and (c) technical solutions related to positioning and dosimetry, triggered the exploration of minibeam radiation therapy (MBRT) instead of Microbeams with slightly larger but still submillimetric beams as presented by Prezado et al.[88] MBRT is less vulnerable to beam smearing than MRT,[89]

Figure 6. (a) Dose as a function of depth in an animal head phantom for a microbeam width of 25 μm and spacing of 200 μm ; the prominent dose near the entrance and exit walls of the phantom is due to the elevated dose to bone and (b) lateral dose profile for a 50 μm wide microbeam separated by 400 μm with a PVDR of 123[69] (c) Horizontal section of the cerebellum of a piglet of 15 months after irradiation with a skin entrance dose of 300 Gy, beam width 27 μm, spacing 210 μm. Some cells and their nuclei directly in the path of microbeams were destroyed. There was no tissue destruction present, nor were there signs of haemorrhage.[70] *Fig.6a reprinted from*[69]*, copyright Institute of Physics and Engineering in Medicine. Reproduced by permission of IOP Publishing. All rights reserved.*



which allows for implementation outside synchrotrons with low-cost equipment, such as conventional research platforms for small animal irradiations with ***conventional X-rays in the range of 160–220 kV***.[90,91] MBRT has also been shown to significantly increase the normal tissue resistance in animal experiments with respect to uniform irradiation while delaying tumour growth.[91–94]

The effect has been observed also with conventional dose rates[90,91] which confirms that the spatial fractionation has an effect *per se*, independent of the high dose rates (similar to the ones used to obtain a FLASH effect) available at synchrotrons.

X-rays MRT or MBRt require the use of low energy photons that do not penetrate much and thus a high dose is deposited in the entrance to achieve a required dose in the tumour.

The increasing availability of ***proton*** therapy centres has triggered the exploration of synergies between spatial dose fractionation and the use of protons. The "proton minibeam radiation therapy" (pMBRT)[95] offers the possibility to (a) maintain the spatial fractionation of the dose at the entrance of the beam and in the beam path; (b) produce a more uniform dose than synchrotron radiation in a target at depth (where the multiple scattering of protons makes a wider minibeam); (c) achieve a higher dose at any depth than in the path; and (d) preserve tissues after the target by the inherent property of a determined range of proton beams[95–98]

Minibeams of protons have been produced using slit collimators[40,99,100] and efforts are underway to use magnetically focused beams as an alternate approach to increase the beam efficiency and to reduce the presence of neutrons (Figure 7a and b). The use of single quadrupole Halbach cylinders has been suggested by preliminary Monte Carlo simulation work.[101]

A recent complete review of spatially fractionated proton minibeams approaches has been done by Meyer et al.[102]

The biological evaluations performed to date confirm a remarkable reduction in normal tissue toxicity[103,104] even with supramillimetric beams (Figure 8a). In addition, an equivalent or superior tumour control than with conventional proton irradiations has been observed in tumour bearing rats (Figure 8b).[105,106]

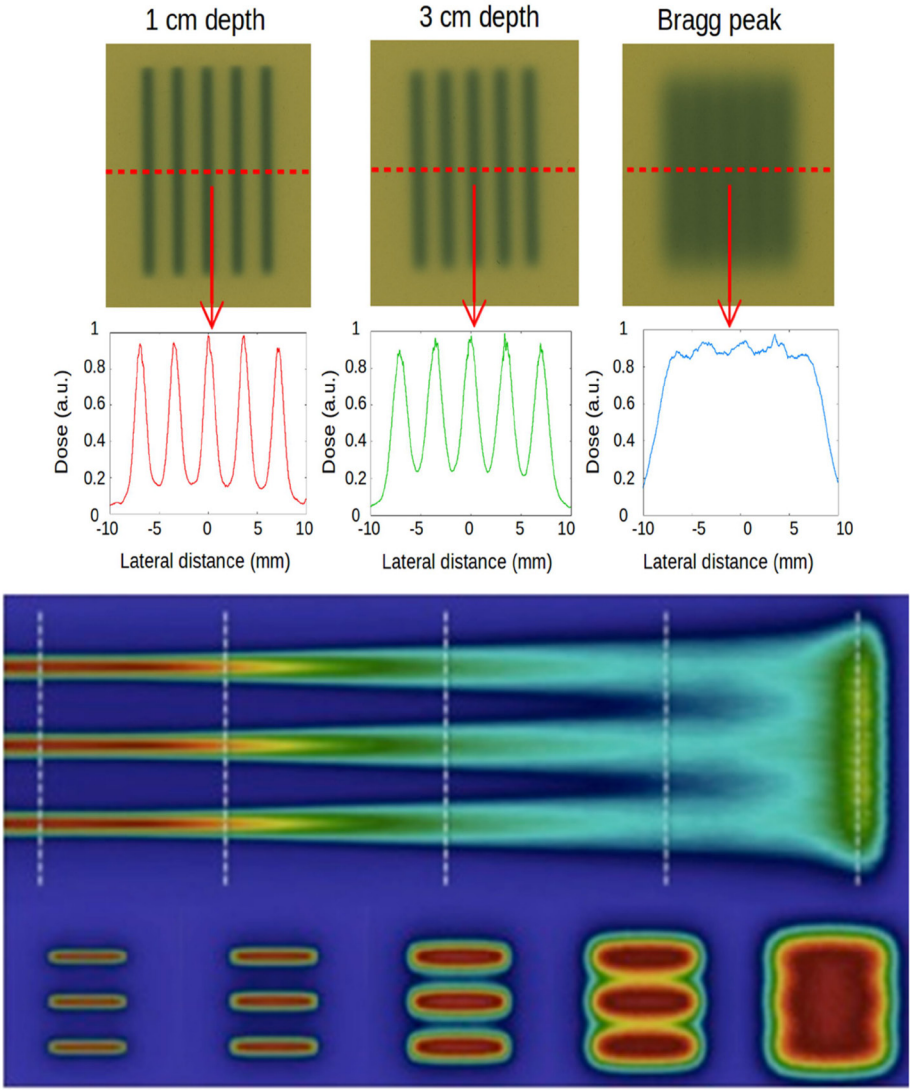
The technique of pMBRT has been implemented at research facilities[96,100] and at a clinical beamline,[99] including with a PBS system.[40] While both have been used for pre-clinical studies, the latter implementation allows for dose rates of 6 Gy/min in multislit conditions and is ready for use in treating patients.

Physical and virtual dosimetry in proton MBRT

Physical dosimetry in microbeams from synchrotron radiation has been extensively evaluated, including detectors such as scintillography and Gafchromic films.[78] In pMBRT, this is still a challenging task due to the very small beam sizes used, even if they are larger than microbeams. The volume averaging effect, or the lack of secondary electron and/or proton equilibrium, plays a non-negligible role.

Ionization chambers do not have enough spatial resolution to resolve the peak and valley regions. Film dosimetry has been widely used.[99,107] Some other options are microdiamond

Figure 7. (a) Array of 5 minibeams 700 mm wide, 3500 mm separation generated by a 5 cm thick brass collimator on a 100 MeV passive proton beam, measured by radiochromic films in water. PVDR varies from eight at the entrance to one at the target level[99]; (b) Monte Carlo simulation of proton minibeams separated by 8.5 mm created using a quadrupole Halbach cylinder with gradient 250 T/m and 10 cm range protons, with PVDR from 19 to 1.[101] *Fig.7a reprinted from*[99]*, Copyright (2016), with permission from John Wiley and sons.*



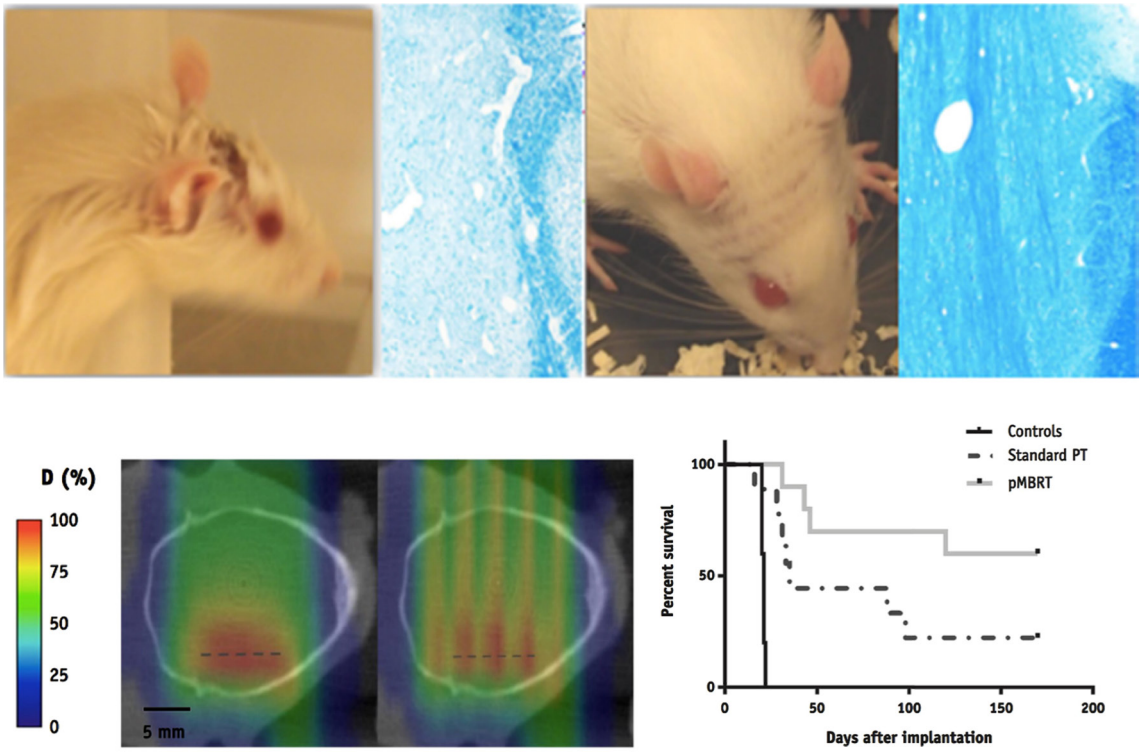
detector (Guardiola et al personal communication, submitted to BJR), the nanoRAzor diode[40] or scintillation detectors (*e.g.,* for *in vivo* measurements), corrected by Gafchromic films.[103]

Examples of calculations in a TPS have been presented using analytical and Monte Carlo methods,[40,108,109] or Monte Carlo generated pencil beams.[103] The codes Peneasy-Penelope and Gate have been used to simulate minibeams of synchrotron kilo-voltage X-rays (xGRT), high-energy electrons (eHGRT), and proton beams (pGRT).[110]

Hypothesis on the effects of spatially fractionated dose and the lack of clinical trials with minibeams in radiation therapy

Recent research in radiobiology has provided new biological insights on the old GRID technique (and applied in the new LATTICE proposals). Bystander factors, such as TNF-a, Tumor Necrosis Factor-related Apoptosis Induced Ligand (TRAIL), and Ceramide[111,112] are induced in cells that are under the open field of the high-dose GRID areas. They are hypothesized to be responsible for initiating the cell death cascade, both in the epithelial and endothelial compartments of the tumour micro-environment including the shielded low-dose regions. Peter et al[113] reported that there is also a robust abscopal effect in distant tumours or metastatic lesions that are not irradiated or treated. Kanagavelu et al[79] indicate that high-dose partial volume LRT irradiation of Lewis lung carcinoma (LLC1) cells, implanted in both hind legs of C57BL/6 mice can cause an improved distant effect than the total tumour volume irradiation through activation of the host immune system.

Figure 8. Response to minibeam irradiations : (a) normal tissues: while there is a moist desquamation and a permanent epilation with conventional irradiation, there is no skin damage and a reversible epilation with minibeams (Prezado, personal comm.), In blue: destruction of the myelin after conventional irradiation and normal myelin organization after minibeams irradiation[104] . (b) evaluation of tumour control: coronal 2D dose distributions in the computed tomography images of a rat's head corresponding to a conventional (seamless) irradiation (left) and a proton minibeam radiation therapy (pMRBT) irradiation (right). (c) Survival curves for the controls, standard PT and pMBRT irradiated tumour-bearing rats.[105] *Fig. 8c reprinted from*[106]*, copyright (2019), with permission from Elsevier. Other reprinted figures with citation in references and/or agreement from authors and/or same coauthors of this paper. Special acknowledgment to Mrs Rocio Dias Sanchez for her work on the copyrights permissions.*



These data strongly suggest that GRID therapy would induce a rapid and higher rate of tumour cell apoptosis in bulky and hypoxic tumours than conventional radiotherapy.

Teams working with microbeams and minibeams state that the biological mechanisms involved in spatially fractionated RT are indeed not well understood yet. The participation of the so-called non-targeted effects has been evoked. They include cell signalling effects such as cohort effects[112] and abscopal effects.[114] Another possible player was hypothesized to be hyperplasia and migration of endothelium and glial cells in the valleys, therefore, minimally irradiated.[115] The so-called microscopic prompt tissue-repair effect,[81] leading to a fast repair of vascular damage, has also been proposed. At the tumour level Bouchet et al[72] reported that MRT from synchrotron radiation (407.6 Gy peak; 6.2 Gy valley-dose) induced significantly longer tumour regrowth delay than uniform broad beam irradiation (6.2 Gy). This was related to a significant 24% reduction in the blood vessel perfusion, a lower cell proliferation index and a greater induction of senescence in B16-F10 murine melanomas implanted into mice ears. Bio-Plex analyses revealed enhanced concentration of monocyte-attracting chemokines associated with leukocytic infiltration attributed mainly to CD8 T cells, NK cells, and macrophages.

Note that in some of these studies, the uniform dose given by broad beams is the one corresponding to the *minimal* valley dose of microbeams or minibeams, while in others the comparative results are done with equal *mean* doses and other metrics.[69]

Smyth et al[36] compared the toxicity of microbeams from synchrotron radiation and broad beams with FLASH dose-rate conditions compared to conventional parameters in different regions of mice. The valley dose was found the best predictor of acute normal tissue toxicity, while acute neurological toxicity was most likely due to the peak doses.

They established dose equivalents between modalities using the median toxic dose TD50.

Kundapur (2019) presented a randomized Phase III study of treating canine denovo brain tumours with 6 MV photon, between standard stereotactic treatment (9 Gy x three fractions) (SRS) *vs* single fraction MBRT (26 Gy to mean dose, 1000 microns size). Between 2013 and 2017, 16 dogs were accrued (eight on SRS and eight on MBRT arm). In SRS-treated dogs, vascular changes were more pronounced and were also seen outside 50% isodoses, while treatment changes were confined to within 50% isodose lines among dogs treated with MBRT.

The SRS treated dogs images and where available post-mortem report showed residual tumour in all of them except one who had a good response. In contrast, the minibeam-treated dogs have almost complete response as noted on the follow-up MRI.

Schultke et al evaluated the potential clinical applications of microbeams and minibeams for both malignant and non-malignant diseases.[116] While different studies are ongoing for clinical applications of synchrotron radiation, to our knowledge no human clinical trial has been started with minibeams, neither with photons nor with protons.

The use of proton minibeams will potentially reproduce or improve the effect of the LRT in target volumes and of GRID in healthy tissue, keeping potential advantages of microbeams while facilitating their implementation, in synergy with the intrinsic features of proton beams to reduce the integral dose to tissues.

Further research is necessary to understand (a) the underlying mechanisms, (b) how these effects are translated when the spatial distribution is modified (no systematic evaluation of the influence of the beam width and spacing on tissue response to spatially fractionated RT has ever been performed) and (c) if the best results for tumour control is to treat homogeneously the tumour or to treat inhomogeneously with higher dose per fraction some subvolumes of the target.

DISCUSSION

The potential evolution in the use of the FLASH effect and minibeams in radiation therapy, specifically in protontherapy

FLASH and minibeams trials are oriented towards protection of critical organs and healthy tissues in general, justifying from the clinical point of view the interest in these new irradiation concepts.

Care must be taken with terminology in this field:

(1) Looking for a single breath hold irradiation, Matsuura et al[117] performed *in vitro* studies of cell survival irradiated at the Bragg peak and at the plateau. They concluded that no dose rate effect exists between conventional and "ultra-high dose rate" (UDR) experiments. However, the maximum value of dose rate in these experiments was around 5 Gy/s, which is <20 fold what is evaluated at present as FLASH effect.
(2) Future plasma laser-based accelerators are able to deliver UDR in ultra-short pulses of electrons and proton beams, in even much shorter times (*e.g.* <<1 ns, dose rate 10^9 Gy/s) than those studied at present for the FLASH effect (≤10^7 Gy/s during the pulse). The biology and clinical feasibility of laser-based beams is already under study after a few years.[118,119] However, the dose per pulse (not the dose rate) in these systems is low, while the FLASH effect requires a large dose delivered in the millisecond time range.

FLASH conditions, as defined in the present works, are in dose rate values between these two last examples.

(3) Biological response to microbeam and minibeam has been, respectively, observed with synchrotron and proton beams, among others. While similarities and differences in between the two approaches are presented in this work, it must be always specified the dose rate achieved in every spatially fractionated setup (*e.g.,* synchrotron radiation Microbeams at higher than 100 Gy/s, protons at conventional or also at very high doses rates) in order to always discriminate if there is an associated effect of spatial fractionation and FLASH effect.

Typical values to achieve FLASH and proton minibeams effects are presented in Table 1 from authors and literature[4,19,63].

It must be stated that for any "definition" of the FLASH concept it is not enough to specify technical parameters such as mean dose rate, peak dose rate, length of irradiation (pulses or total) and the delivered dose as presented in Table 1. As for any radiobiological effect, and taken as an example the RBE, it must also be stated which is the biological, functional, and/or clinical endpoint evaluated as "protection of healthy tissues" (fibrosis, necrosis, neurocognitive effects, etc).

It is necessary to obtain a deeper knowledge on the mechanism and the required parameters to achieve and quantify a FLASH effect and the minibeam optimal parameters, in order to build a coherent model, which will surely include the time and spatial structure of the dose delivery. This will be of interest particularly in proton beams with delivery system based on the scanning of a small pencil beam of high intensity.

As usual in the field, there are at least three issues limiting the development of these new approaches: (a) the understanding of the mechanisms involved, (b) technical limitations, and (c) the safe implementation of clinical protocols with significant follow-up:

(1) Mechanisms: the presence of oxygen and free radical chemistry is crucial to obtain the FLASH effect, while for minibeams the effects in tumour and in healthy tissue seem to include bystander, abscopal, cell migration, fast vascular repair, and senescence. Even if proton beams can be considered as close to photons and electrons in terms of RBE, the conditions to achieve the FLASH effect with proton beams must be specifically studied with both passive and pencil beam systems. The question if the FLASH effect can be produced with heavy ions is also a subject of discussions: high LET could "mask" it or, as suggested by Colangelo and Azzam[14] a synergistic effect could exist if, for example, the normal tissue, being in the plateau region, would elicit a FLASH effect while the tumour cells would not, due to the molecular oxygen generated at the Bragg peak by heavy ions.
(2) Technical: a high interest is manifested by industry around getting FLASH conditions with proton beams. Ion beam applications (Louvain, Belgium) and Varian (Palo Alto, USA) are actively promoting studies among their protontherapy users and patenting technical solutions and procedures. Cyclotrons and synchrocyclotrons seem to be more adapted than synchrotrons to produce very high dose rates. High dose rates have been shown in specific experimental approaches for small targets. Experiments have already been performed with passive and active beams. But the effect

Table 1. *Typical values to achieve FLASH (data from electron beams) and proton minibeam effects (from[4,19,63] and authors opinions).*

FLASH						
Dose	Mean dose rate	Peak dose rate	Irr length	Fractions	Pulse length	Frequency pulses
[Gy]	[Gy/s]	[Gy/s]	[ms]	#	[μs]	[Hz]
5–50	40–2000	1E6 - 3E7	<100	1 - > 1	1–2	100–200
Particle	Oxygen tension	Volume	RBE Tumour	RBE healthy	Target	Tissue/End Point
type		ml			Histol	Functional
e,X,p	10% (130 μM) [Lung] 4% (50 μM) [Brain] 0.3% (4 μM) [Tumour]	2–100	1	≈ 0.6	GBM, lung, nasal squamus cell ca, lymphoma	Pneumonitis, fibrosis, skin necrosis, neurocognition...
MICRO-MINI-GRID BEAMS						
Particle	FWHM	Spacing	Energy	PVDR	Expected gain factor in healthy tissue	Applications
	[μm]	[μm]	[MeV]			
Synchrotron Radiation Microbeams						
X	25–100	100–400	0.05–0.6	50–150	5–50	Tumours, Epilepsy,…
Proton Minibeams						
Protons	500–1000	1000–3500	60–230	10–−20	>4	Tumours, Epilepsy,…
GRID therapy (examples)						
Low E X-rays	1.00*E* + 06	1.50*E* + 06	0.15–0.3	5–6	<2	Reduce skin effect, paliative, reduce mass, pain,…// Bladder, lung, brain,…
High E X-rays	1.00*E* + 06	2.00*E* + 06	1–25	3–5		
Protons	1.00*E* + 06	2.00*E* + 06	60–230	3–5		

of scanning beams (where the superposition of successive spots and energy layers determines a complex pattern of dose deposition in time for a single fraction) remains to be determined.[120]

For the minibeams, it is of the utmost interest to develop the focusing approaches to increase the PVDRs without collimators and using beams other than synchrotron radiation, so opening a promising path towards proton beams. While perfect interlacing of parallel minibeams adds complexity to the practical implementation, crossed interlacing (*e.g.,* orthogonal or non-coplanar beams) could be explored.

(3) Clinical: FLASH will surely be applied into controlled trials on several clinical cases, where high doses are required and tolerances of critical organs are the limiting factor. The FLASH effect has been observed and applied using electron beams from low energy LINACs that dramatically limits the clinical application yet they are well suited to IORT. Protons will have the same approach and rationale as electrons, with the possibility to irradiate deep targets with no loss of their ballistic properties. While large targets could have a maximum benefit of techniques reducing complications, they are also the most difficult to cover achieving FLASH conditions and/or with minibeams. The translation to clinical applications will be facilitated if the chemistry and biology of both effects are kept when moving from a single fraction to a few fractions per treatment and using as delivery system a scanned proton beam

Targeting and the management of organ movements is more and more essential, what are also basic conditions for minibeams and can benefit of the short irradiation time of FLASH if properly delivered.

While FLASH and minibeams effects are still under study, they can be applied independently and are highly complex. A high proportion of the studies with minibeams have intrinsically included high dose rates (what also helps to reduce movements during the irradiation) and FLASH can also be easier to achieve if the irradiated volume is reduced and—maybe—separated in space, as is the case with minibeams. Their complexity and related uncertainties must be understood in their individual or combined implementation.

CONCLUSIONS

FLASH and minibeams are examples of the interest and need to revisit physics, radiation chemistry and radiation biology to have a better understanding of their underlying mechanisms. With

this approach, the internal structures of time and spatial dose distributions could be optimized to set new clinical approaches in radiation therapy, minimizing complications in healthy tissues.

As stated by Harrington,[121] it is also important to evaluate how these effects affect the 5 Rs of radiobiology (repair, reoxygenation, redistribution, repopulation, radiosensitivity, some of them becoming irrelevant), as well as the tumour microenvironment. Care must be taken with unknown effects of these approaches in the short, mid and long term.

Even if the primitive goal of FLASH and minibeams is to reduce complications, it is necessary to make sure that the tumour control efficiency will not be affected, or that can even be improved in some cases, and to evaluate how much these approaches can contribute to immunological response of cancer patients.

Proton beams have specific benefits for each of these two effects individually. And the combination of FLASH and minibeams using proton beams, in spite of their complexity, may help to optimize the benefits of several or all the reviewed aspects, through the following concepts:

(1) the intrinsic advantages of protons to reduce the integral mid and low doses, will be volumetrically combined in synergy with the FLASH and minibeam effects as a whole;
(2) to reduce mid and high equivalent doses in critical organs around the tumour volume using the FLASH effect with high dose rates achievable with proton beams, both with passive or pencil beam approaches;
(3) to reduce healthy tissue complications by the minibeams space modulation in every beam path, where protons can be focalized with a steep penumbra and hence a high peak to valley ratio;
(4) to deliver an homogeneous dose to the target at any depth using the multiple scattering of proton minibeams in depth, and/or with multiple fields, or even setting a controlled inhomogeneous "vertex" doses escalation approach, optimizing intensity modulated proton therapy with robust solutions;
(5) to modify present approaches of immunological responses by the combination of concentration of lattice doses in very short time with a slight increase in LET, and the microstructure in time and space of both effects and
(6) to deliver single or hypofractionated treatments in very short time per fraction, facilitating the treatment of moving organs, specially when using pencil beam approaches and the associated risk of interplay effects, as well as the optimal use of minibeams with minimal risk of movement during the fraction.

Proton beams have in consequence one of the highest potentials to optimize the use of FLASH and Minibeams effects in radiation therapy, individually or in a synergistic combination.

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