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Perspective

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Towards clinical translation of FLASH radiotherapy

Marie-Catherine Vozenin[1], Jean Bourhis[1] & Marco Durante[2,3]

Abstract

The ultimate goal of radiation oncology is to eradicate tumours without toxicity to non-malignant tissues. FLASH radiotherapy, or the delivery of ultra-high dose rates of radiation (>40 Gy/s), emerged as a modality of irradiation that enables tumour control to be maintained while reducing toxicity to surrounding non-malignant tissues. In the past few years, preclinical studies have shown that FLASH radiotherapy can be delivered in very short times and substantially can widen the therapeutic window of radiotherapy. This ultra-fast radiation delivery could reduce toxicity and thus enable dose escalation to enhance antitumour efficacy, with the additional benefits of reducing treatment time and organ motion-related issues, eventually increasing the number of patients who can be treated. At present, FLASH is recognized as one of the most promising breakthroughs in radiation oncology, standing at the crossroads between technology, physics, chemistry and biology; however, several hurdles make its clinical translation difficult, including the need for a better understanding of the biological mechanisms, optimization of parameters and technological challenges. In this Perspective, we provide an overview of the principles underlying FLASH radiotherapy and discuss the challenges along the path towards its clinical application.

Sections

[1]Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland. [2]GSI Helmholtzzentrum für Schwerionenforschung, Biophysics Department, Darmstadt, Germany. [3]Technische Universität Darmstadt, Institute of Condensed Matter Physics, Darmstadt, Germany. e-mail: m.durante@gsi.de

Introduction

Shortly after the discovery of X-rays in 1895, several studies showed the feasibility of radiotherapy — that is, eradicating tumours using ionizing radiation[1]. These initial experiences demonstrated that high doses of radiation can kill cancer cells but simultaneously induce toxicity in surrounding non-malignant tissues (commonly referred to as 'normal tissues' in the radiation oncology field). In other words, to be therapeutically exploited, radiation should enable tumour control at a dose lower than that causing severe toxicity (Box 1). The therapeutic window is the dose region between the normal tissue complication probability (NTCP) and the tumour control probability (TCP) curves (Fig. 1); widening this window is the main goal of radiotherapy research. For example, dose fractionation (generally 2 Gy per fraction, five fractions per week up to a curative dose) spares non-malignant tissue with a limited effect on the TCP, thus widening the therapeutic window[2]. In the past few years, a safe reduction of the margins around the tumours has become feasible thanks to improvements in image-guided radiotherapy, additionally sparing non-malignant tissues. In turn, this approach enables the number of fractions to be reduced and the dose per fraction to be increased, especially in patients with early stage tumours or oligometastatic disease (commonly treated with stereotactic body radiotherapy (SBRT))[3]. Conventional fractionation with external beam therapy involves delivering 1.8–2 Gy per fraction in 30 fractions, while hypofractionated regimens, which involve fewer doses of larger intensity (such as 6–8 Gy per fraction in three to five fractions), are increasingly used. Nevertheless, the treatment of radioresistant tumours remains restricted by non-malignant tissue complications and metastatic spread. Therefore, enhancing the differential effect of radiotherapy using selective tumour radiosensitization and enhancing non-malignant tissue protection remain the overarching goals for researchers and clinicians.

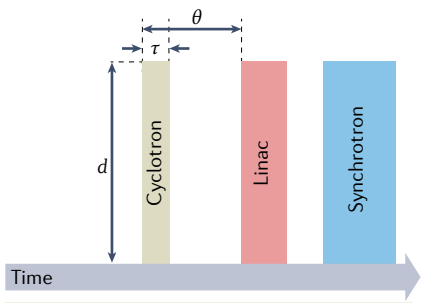
The standard dose rate delivered during a radiotherapy treatment is in the range 0.5–20 Gy/min, depending on the technology used (Box 1). Higher intensities were considered worth testing to spare non-malignant tissues in patients receiving radiotherapy as early as the 1960s, when Dewey and Boag were the first to show that bacteria are more radioresistant to ultra-high dose rates of radiation (3–6 × 10^9 Gy/min) than to conventional dose rates (CONV). Interestingly, the profile of radioresistance to ultra-high dose rates was similar to that observed under hypoxic conditions, in which bacteria have the greatest resistance to radiation[4]. The increased radioresistance at ultra-high dose rates was then confirmed in mammalian cultured cells[5,6] and in vivo in murine intestine and skin[7–9]. Despite these early results, interest waned over the years owing to the common belief that ultra-high dose rates would spare non-malignant tissue and cancer cells in a similar way[9], and to the technical challenges of achieving ultra-high dose rates in clinical settings.

In the 2010s, a paradigm-shifting set of experiments[10] was performed in the frame of a collaboration between Institut Curie, Institut Gustave Roussy (Paris) and Centre Hospitalier Universitaire Vaudois (CHUV, Lausanne). This study suddenly revived the role of ultra-high dose rates in radiotherapy, critically evaluating the effects of FLASH irradiation on both non-malignant and tumour tissue, and demonstrated that ultra-high dose rate irradiation can widen the therapeutic window killing tumours while sparing non-malignant tissues. The study was performed using the Kinetron LINAC, a linear accelerator (linac) delivering 4.5 MeV electrons originally built to investigate pulsed radiolysis and, thus, able to reach extremely high dose intensities. The degree of lung fibrosis and growth control of orthotopic syngeneic lung tumours and human xenografted tumours were similar in mice irradiated to the whole-thorax region with a single dose of 15–17 Gy using 4.5 MeV electrons or conventional γ-rays (1.8 Gy/min); however, no pulmonary fibrosis was found when electrons where delivered in less than 200 ms at an ultra-high dose rate (>40 Gy/s), whilst tumour control remained unchanged. This unexpected differential effect was named the FLASH effect (Fig. 1) and has since been replicated in different preclinical models using radiation of different qualities[11–17]. Interestingly, as the field progressed, the fact that quoting average dose rate is an oversimplification became obvious and, at present, the FLASH effect is known to depend on the combination of multiple beam parameters and biological factors (Box 1). In this Perspective, we discuss the biological basis of the FLASH effect, which is being intensively investigated[18–21], and the clinical translation initiatives already underway[22–24]. We also address several questions that remain to be answered before FLASH radiotherapy can be used in clinical settings at a large scale[25].

Box 1

Key parameters in radiotherapy

- The dose per pulse (*d*) is measured in gray (1 Gy=1 J/kg); *d* is in the range of milligray and >1 Gy with conventional and FLASH radiotherapy, respectively.
- Total dose (*D*): $D=nd$, where *n* is the number of pulses in the treatment. With conventional radiotherapy, *D* is 2–8 Gy per fraction and up to 60–90 Gy in target tissue (although doses in non-malignant tissues are always lower). With FLASH radiotherapy, an effect is generally observed at *D* > 8 Gy in a single fraction.
- Delivery time (*T*): $T=n(\tau+\theta)$; *T* is >1 min and <200 ms with conventional and FLASH radiotherapy, respectively.
- Mean dose rate: D/T. The mean dose rate is ~1 Gy/min and >40 Gy/s with conventional and FLASH radiotherapy, respectively.
- Duty cycle (DT): $DT=\omega\tau(\%)$. DT is 0.1–50% depending on the values of τ and θ, regardless of the radiotherapy modality.



Pulse duration (τ)	~ns–µs	~µs–ms	~ms
Repetition rate (ω=1/θ)	~kHz–MHz	~Hz–kHz	~Hz

FLASH technology

The FLASH effect has been reported to occur with virtually all radiation modalities used in radiotherapy, but has been tested in preclinical settings and small tumour volumes (~1 cm^3), and using high doses in a single

fraction and from a single direction (as discussed in 'FLASH preclinical evidence'). Most FLASH studies performed to date involved dedicated experimental pulsed electron beams (Box 1). The repetition rate and duty cycle depend on the type of accelerator used as source of radiation. The currently available preclinical data suggest that, in terms of physical parameters, the FLASH effect is generally observed at average dose rates of ≥40 Gy/s, with total irradiation times of <500 ms and at total doses of ≥10 Gy[19,21,26]. For clinical applications, however, these conditions need to be scaled up to large volumes (~1 l), applicable using fractionation (usually three or more fractions) and multiple beam directions (generally more than four, delivered using either rotating gantries or different angles) to shape the dose conformally on the tumour[27]. These FLASH conditions are very challenging to achieve with the currently available technology, with which a single fraction lasts generally >1 min.

Radiation sources

Electrons. More than 90% of patients who receive radiotherapy are treated with high-energy (≥6 MV) X-rays, produced by bremsstrahlung ('braking radiation', or the photon emission by charged particles subjected to an acceleration) of electrons accelerated in linacs. Owing to the poor efficiency of the bremsstrahlung process, the first FLASH experiments were all performed with electrons from linacs[20]. These devices are dedicated electron accelerators[10,28,29] or modified medical accelerators, originally designed for treatment with electrons or X-rays[30–32]. Other devices designed for industrial use[33] or intraoperative radiotherapy[34] can achieve ultra-high dose rates, and several companies are currently developing FLASH-dedicated linacs[35–37].

The main clinical limitation of the electron beams currently used is their low penetration depth (2–3 cm at most) (Fig. 2) and short source–target distance (~50 cm) that restrict their clinical application to the treatment of superficial tumours of limited size (1–2 cm). However, the development of very high-energy electrons (VHEEs) (100–200 MeV)[38,39] for FLASH might overcome these limitations. Indeed, VHEEs have a better depth–dose profile than X-rays (Fig. 2) and, owing to their penetration profile, can be used to irradiate most deep-seated tumours in humans.

Currently, only a handful of facilities are available to study VHEEs. However, the possibility of using VHEEs in FLASH has triggered several new initiatives[40], such as the CERN–CHUV project, planning to use accelerating gradients of 100 MeV/m and designed for delivery of radiation in 50 ms with high conformality to patients with large and deep-seated tumours. The most ambitious, albeit immature, projects rely on laser-driven accelerators, in particular, plasma accelerators[41] or dielectric laser accelerators[42], that have the potential to reach gradients of >250 MeV/m, meaning that these tabletop accelerators could produce VHEEs in a much more compact way than the radiofrequency technology currently used for electron acceleration. A major problem of laser technology, however, is that large doses are delivered in ultra-short (femtosecond) pulses with high shot-to-shot fluctuations in beam parameters. This phenomenon impairs reproducibility and accuracy of the dose delivered which is non-acceptable in clinical settings. Moreover, we do not know if the region of dose rates and ultra-short (femtosecond to nanosecond) pulses reached with lasers could still produce the FLASH effect or, instead, generate an 'overflash' that would reduce the benefit observed with the machines used to date.

X-rays. FLASH conditions cannot be achieved with conventional X-ray machines, but soft X-rays (that is, those with energies <1 MeV) can be produced at very high intensity with synchrotron light sources, which are large rings in which electrons are accelerated close to the speed of

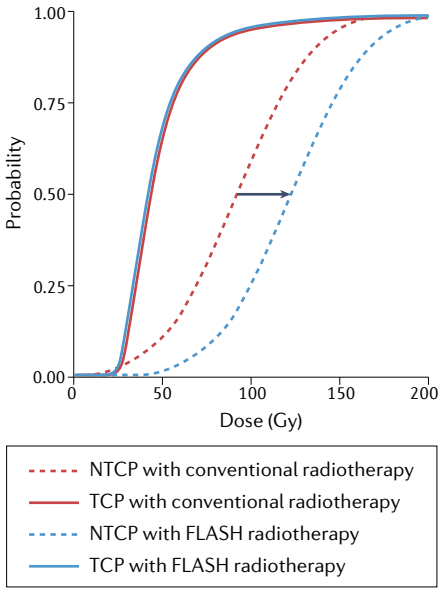


Fig. 1 | FLASH radiotherapy and the therapeutic window. Radiotherapy is only curative in the therapeutic window, that is, the separation between the curves describing tumour control probability (TCP) and normal tissue complication probability (NTCP) — of note, at higher doses the NTCP is shifted. Widening the therapeutic window is a basic goal of radiation oncology research because, if the curves are well separated, the dose to the tumour can be increased, reaching a TCP of ~100%, whereas the NTCP remains at <5%. This chart represents the typical TCP curve for a patient with non-small-cell lung cancer receiving conventional radiotherapy and the corresponding NTCP curve for late lung fibrosis[50]. With FLASH radiotherapy, the TCP curve remains essentially the same but the NTCP curve is shifted to the right (black arrow) by a dose-modifying factor in the range 10–50% (meaning that higher doses are needed for the same probability of complications) and thus widening the therapeutic window.

the light and bremsstrahlung radiation is extracted by deflection. Only a few of these advanced light sources have beamlines dedicated to irradiation of biological targets that can be used in preclinical experiments with FLASH[43]. Of note, the first evidence that ultra-high dose rates reduce brain injury compared with CONV came from an experiment in mice at the European Synchrotron Radiation Facility (ESRF) (Grenoble)[44], discussed in 'FLASH preclinical evidence'. Whilst synchrotron radiation is being used in preclinical studies of FLASH[45,46], it has not so far been applied in the clinic and such application would be extremely difficult owing to the necessity to bring patients to the few facilities available and the very low energy radiation achieved with the synchrotron (~100 keV at the ESRF). An orthovoltage cabinet based on two 150 kVp fluoroscopy systems that can deliver ultra-high dose rates of 40–240 Gy/s has been designed at the Johns Hopkins University (Baltimore), but its use will be restricted to preclinical studies[47].

In a study with results published in 2022, the Chengdu THz Free Electron Laser (CTFEL) group in China produced high-energy X-rays (6–8 MeV) with a superconductive linac reaching dose rates ~1 kGy/s[48]. In comparison with conventional X-rays, FLASH conditions led to a slight decrease in tumour growth rates and enabled sparing of the thorax and abdomen. This study provides the first evidence that FLASH conditions can be achieved with conventional, high-energy X-rays and has paved the way for several other projects currently under way. In the USA, the PHASER platform is an ambitious new high-energy X-ray machine being

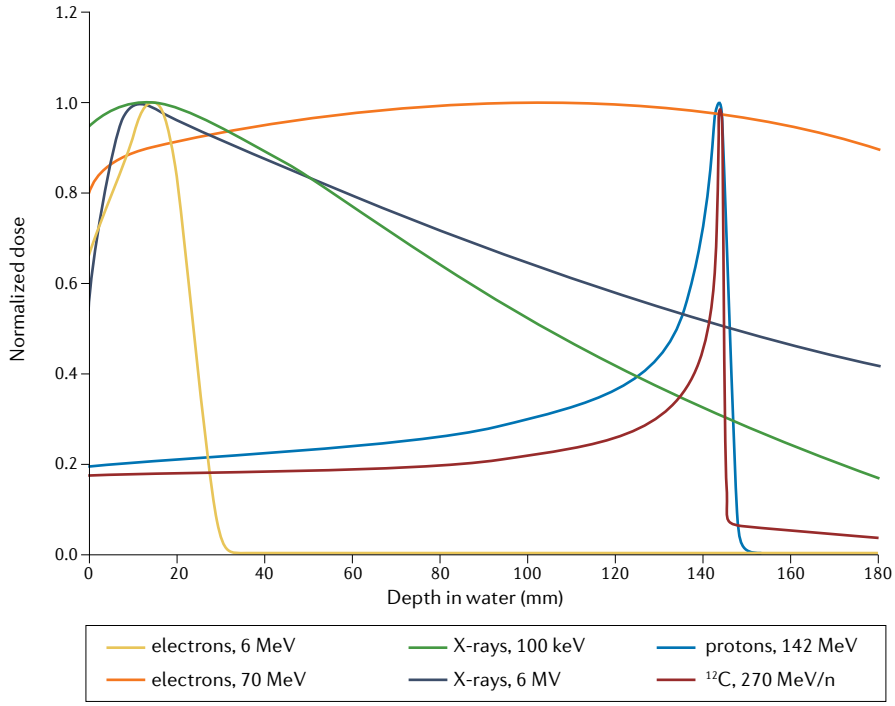


Fig. 2 | Radiation quality achieved with FLASH. The technology currently available enables production of different types of radiation at very high intensities. This chart shows a Monte Carlo simulation of the depth–dose distributions of different types of radiation including electrons, X-rays, protons and heavy ions at different energies. Simulation obtained using GEANT4, courtesy of L. Volz (GSI Helmholtz Centre, Darmstadt). n, nucleon.

developed at the Stanford Linear Accelerator Center (SLAC) (Stanford)[49] that incorporates several technological improvements, such as a new radiofrequency power source, omission of a rotating gantry in favour of 16 stationary beamlines, and imaging guidance.

Protons. Owing to a favourable depth–dose distribution, protons have clear physical advantages over electrons and photons for use in radiotherapy[50] (Fig. 2). The Bragg peak (that represents the maximum energy loss of charged particles immediately before they stop) enables conformal treatments with only a few proton beams, and thus enables more sparing of non-malignant tissue than X-ray therapy. With >100 facilities in operation worldwide, proton therapy is becoming a widespread and accepted radiotherapy modality.

Most centres delivering proton therapy use cyclotron accelerators and pencil beam scanning to accelerate the beam and irradiate the target volume[51]. Pencil beam scanning is a delivery system in which a small beamlet of a few millimetres is scanned over tumour layers ~3 mm thick, and energy variation switches to reach the next tumour slice, producing a spread-out Bragg peak (SOBP) to cover the tumour volume. Clinical isochronous cyclotrons can produce a quasi-continuous beam current at variable energies in the range 80–250 MeV (Box 1). They can easily reach intensities of >60 Gy/s at a fixed energy, considering that they can provide beam currents over an order of magnitude higher than those used in the clinical mode (1–10 nA). The main limitation of using protons in FLASH radiotherapy relates to beam delivery. Indeed, most preclinical studies have been performed in the plateau region of the Bragg curve (Fig. 2), at which a single, monoenergetic beam can be used[52]. In clinical settings, the 3D volumetric scanning used is too slow (each energy change takes ~1 s) to reach FLASH conditions in the whole tumour, which would only be achieved if the speed was increased by at least two orders of magnitude[53].

The most mature approach for clinical applications of proton-based FLASH (pFLASH) involves hybrid active–passive systems using patient-specific 3D-range modulators[54,55], in which the pencil beam is scanned in 2D on a ridge filter that passively produces an SOBP. Irradiation time is therefore only limited by the raster scanning, which is extremely fast (<1 s). These systems are already used in preclinical research facilities using protons[56] and heavy ions[57], and are currently being tested in clinical facilities.

Protons are therefore the most mature technology for clinical translation of FLASH. The definition of dose rate in pencil beam scanning, however, is controversial because achieving ultra-high dose rates is obviously easier in the single beamlet than in the whole volume. New parameters, such as the dose-averaged dose rate[58] or spot peak dose rate[59], have been introduced to define pFLASH in treatment planning. In the future, FLASH treatment planning will require optimization of more parameters, such as beam current and scanning speed, as well as a clear definition of the dependence on fractionation, and of the dose and volume threshold for the FLASH effect[60].

Similar to electron-based therapy, laser-driven accelerators are potentially ideal for proton therapy. To date, the highest proton energy (~100 MeV) has been achieved with a laser of the Rutherford Appleton Laboratory (Chilton, UK)[61]. In preclinical radiobiology studies, a dose of 4 Gy was delivered to a tumour located at a depth of ~40 mm in a mouse ear using ~60 MeV laser-generated protons at Helmholtz-Zentrum Dresden–Rossendorf (Dresden)[62]. The laser has the potential to deliver 20 Gy in a single shot of ~10 ns, thus obviously being in the FLASH-regimen, but these conditions have not yet been applied in preclinical studies and routine use in clinical settings will require large improvements that can only be accomplished in many years[63,64].

Heavy ions. Ions heavier than protons present additional advantages for radiotherapy, especially owing to their high relative biological effectiveness in the Bragg peak region[65]. A dozen centres worldwide are treating patients with high-energy carbon ions and the Heidelberg Ion

Beam Therapy Center (HIT) (Heidelberg) has started treatments with helium ions in the past few years[66]. However, heavy ions are accelerated in synchrotrons, in which FLASH conditions are very difficult to achieve because they generally store a single bunch of particles and the extraction cycle is ~1 s. FLASH conditions can only be achieved using high-current injection. At HIT, dose intensities of >50 Gy/s were reached both with helium[67] and carbon[68] ions on small targets (~10 × 10 mm). Preclinical experiments in cultured cells have been performed at the Gunma University Hospital (Maebashi) with carbon ions at dose rates of up to 195 Gy/s[69]. For larger volumes, the necessary beam currents can be achieved at the synchrotron of the GSI Helmholtz Centre (Darmstadt), thanks to the intensity upgrade required for the new Facility for Antiprotons and Ion Research (FAIR)[70] on the same GSI campus. For comparison, medical synchrotrons for carbon ions are currently limited to ≤10^9 ions per spill, while >10^{11} ions per spill can be reached at GSI–FAIR. These conditions make it feasible to irradiate small mammals at intensities of >100 Gy/s using a single spill with a duration of ≤150 ms[71].

Preclinical experiments delivering FLASH radiation with heavy ions are highly relevant to understanding the mechanism of the FLASH effect. All the current biochemical models of FLASH[71,72] are indeed dependent on the radiation quality, and therefore these studies can be used to benchmark the models. For clinical translation, the same hybrid active–passive approach described for protons could work for heavy ions. The alternative approach of fast beam extraction (in the microsecond range) is complicated by the necessity to measure the radiation flux and to scan the beam over the region of interest.

Dosimetry

Beam dosimetry for FLASH conditions is challenging regardless of the radiation source used. Most preclinical experiments have used radiochromic films, which are dose-rate independent[28] and provide high spatial resolution, but they measure the dose following the exposure. Real-time, online dose monitoring is mandatory to deliver a defined dose in radiotherapy, and is generally performed using ionization chambers, although most commercial ionization chambers have saturation and reduced ion collection efficiency at high dose rates[29]. The ideal dosimetry for FLASH radiotherapy should have high time resolution and a wide dynamic range that should enable monitoring doses and dose rates higher than those routinely measured at present. UHDpulse, a European consortium of metrology institutes[73], and a joint task group from the American Association of Physicists in Medicine, the European Society for Radiotherapy and Oncology, and the European Federation of Organisations for Medical Physics, have been formed to address dosimetry aspects of FLASH radiotherapy[74].

Other groups studying these aspects have proposed several solutions, including the use of advanced ionization chambers with correction factors, solid state detectors, chemical dosimeters and luminescent dosimeters[75]. A newly designed 2D strip-segmented ionization chamber array that fits on to the proton scanning nozzle has excellent spatial, temporal and dosimetric performances for pFLASH[76]. In summary, although FLASH dosimetry is an important problem, it seems solvable when considering clinical applications owing to the wide range of sensors available.

FLASH radiobiology

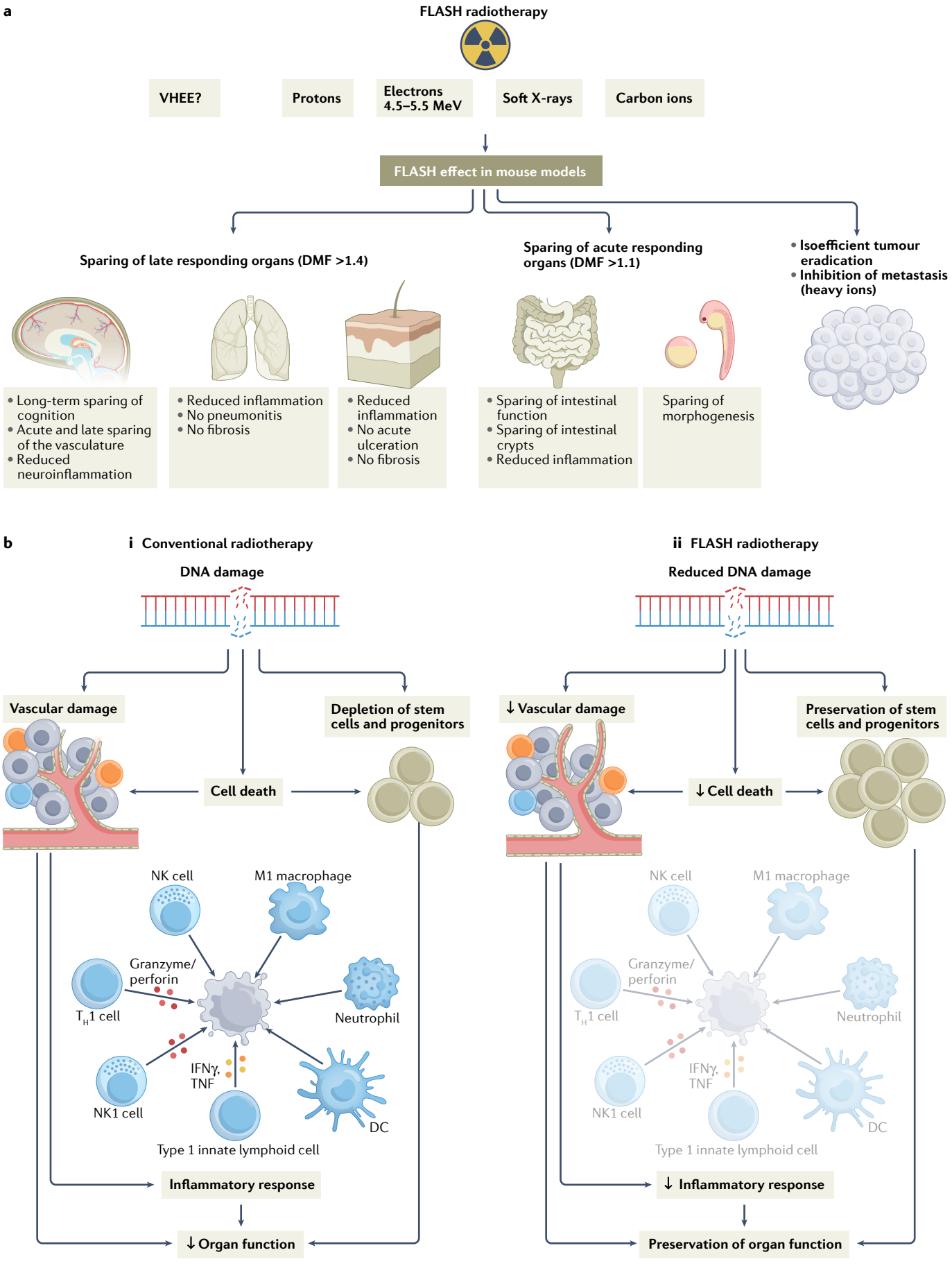
In the past few years, several teams around the world have reproduced the FLASH effect using different beam modalities in various organs and across several animal species (Fig. 3). Although the fundamental physical parameters required to produce the FLASH effect remain to be further investigated and optimized, preclinical radiobiology experiments have been performed with two major aims: demonstrating the feasibility of delivering FLASH radiotherapy with different beams (Fig. 3a), and elucidating the mechanistic basis underlying the FLASH effect (Fig. 3b).

FLASH preclinical evidence

Non-malignant (normal) tissue. Given that sparing non-malignant tissue is the first hallmark of the FLASH effect, traditional preclinical models of radiation-induced toxicity have been used to investigate the FLASH capabilities of various beams compared with irradiation at CONV. At the CHUV, an Oriatron 5.5 MeV electron linac was used for a comprehensive dose rate escalation study that revealed a robust sparing of learning and memory using FLASH at mean dose rates of ≥60 Gy/s (instantaneous dose rate ~10^5 Gy/s)[77]. Similar findings were made in studies using whole-brain irradiation and neurocognitive validation with synchrotron X-rays at the ESRF with a mean dose rate of 37 Gy/s (instantaneous dose rate ~10^4 Gy/s)[44], and with a modified clinical electron linac at Stanford University at mean dose rates of >200 Gy/s[78]. Functional and molecular studies were then performed to elucidate the long-term neurocognitive effects of FLASH radiotherapy[79], with remarkable results. The results of all the cognitive tests performed were statistically indistinguishable between non-irradiated and FLASH-irradiated mice, whereas cognition was permanently altered in mice receiving conventional radiation (0.1 Gy/s). The dose-modifying factor (DMF), or the ratio of doses that cause the same effect, was 1.4 with cognitive sparing obtained with a single dose of 10 Gy delivered in a single pulse (10^7 Gy/s) that was lost at 14 Gy. Cognition sparing was also lost upon carbogen breathing[79], supporting the idea of a combined role for oxygen and reduced reactive oxygen species as contributory to sparing toxicities.

In a study of skin toxicity conducted at CHUV[80], a female Göttingen minipig (43 kg) was irradiated with prescribed electron doses (28–34 Gy to the skin surface) of conventional radiation (~5 Gy/min) on one side of the back and FLASH radiation (ten pulses, 90 ms and 280 Gy/s) on the other side of the back (Supplementary Fig. 1). Compared with CONV, skin toxicities were less severe with FLASH up to 3 years of follow-up[73].

The first negative results of preclinical studies using FLASH radiation were published around the same time. Unlike some of the aforementioned studies, which were performed in late-responding organs (lung and brain), these studies evaluated acute-responding tissues. In both studies performed in mice, severe depletion of lymphatic and crypt cells occurred in blood and intestine, respectively, after exposure to FLASH radiation (35–41 Gy/s) generated using an electron linac[81] and at the Australian Synchrotron[45], and whole abdominal exposure to FLASH was more lethal than conventional radiation. In a study in zebrafish embryos[82], FLASH irradiation was found to be feasible but had a similar effect on morphogenesis to that of CONV. The reasons for these contrasting results remain uncertain but several factors might have been involved, such as differences in dose rates and volumes, and possible technological issues related to the beam parameters. Indeed, the same group that obtained negative results in zebrafish subsequently reported evidence of a protective FLASH effect in the same model using higher mean dose rates and shorter treatment times[83]. Another explanation for the disparate results is that the studies focused on highly proliferating structures (gut and embryo) that might require higher intensities to uncover the FLASH effect. This idea is supported by other studies with electron beams[16,84,85] and proton beams[86,87], in which the measured DMF for intestine was lower (~1.1). In fact, crypt


a
FLASH radiotherapy
VHEE?
Protons
Electrons 4.5–5.5 MeV
Soft X-rays
Carbon ions
FLASH effect in mouse models
Sparing of late responding organs (DMF >1.4)
Sparing of acute responding organs (DMF >1.1)
• Isoefficient tumour eradication
• Inhibition of metastasis (heavy ions)
• Long-term sparing of cognition
• Acute and late sparing of the vasculature
• Reduced neuroinflammation
• Reduced inflammation
• No pneumonitis
• No fibrosis
• Reduced inflammation
• No acute ulceration
• No fibrosis
• Sparing of intestinal function
• Sparing of intestinal crypts
• Reduced inflammation
Sparing of morphogenesis
b
i Conventional radiotherapy
DNA damage
Vascular damage
Cell death
Depletion of stem cells and progenitors
NK cell
M1 macrophage
Granzyme/ perforin
T_H1 cell
Neutrophil
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Preservation of stem cells and progenitors
NK cell
M1 macrophage
Granzyme/ perforin
T_H1 cell
Neutrophil
IFNγ, TNF
NK1 cell
DC
Type 1 innate lymphoid cell
↓ Inflammatory response
Preservation of organ function

Fig. 3 | Response of various tissues to different radiation modalities. a, The FLASH effect has been characterized in mouse models, both in non-malignant tissues and in tumours. **b**, In the tumour microenvironment, the molecular response of cells to radiation exposure at conventional dose rates (i) has been well characterized and is known to involve a complex series of events that leads to the loss of tissue homeostasis. The direct action of radiation on DNA has cytotoxic effects on differentiated, progenitor and stem cells, followed by indirect stimulation of reactive oxygen species and inflammatory mediators. Radiation causes vascular alterations and release of thrombotic factors that are involved in the recruitment and extravasation of immune cells, and in the deep remodelling of the extracellular matrix and stromal compartment. These abnormal processes are associated with genomic alterations, persistent modulation of gene expression and alteration of cellular phenotypes, leading to organ dysfunction. Interestingly, at isodose, tissue responses to FLASH dose rates (ii) do not induce this altered cascade of molecular events, and organ function is preserved. Indeed, studies have shown lower levels of DNA damage[67,85,104,105], apoptosis[11,78] and inflammation[11,78,106,107], as well as preservation of the progenitor and stem cell pools[44,70,104,139], and vessels[140,141]. DC, dendritic cell; DMF, dose-modifying factor; IFNγ, interferon-γ; NK, natural killer; T_H1, T helper 1; TNF, tumour necrosis factor; VHEE, very high-energy electrons.

protection was found to be maximal with a single electron pulse at 3.3×10^6 Gy/s[88] and with protons at >78 Gy/s[86].

Given that proton beams are readily suitable for clinical application, major centres delivering this modality have developed research programmes on pFLASH. Experiments in the plateau region before the Bragg peak (Fig. 2) showed toxicity-sparing effects in the skin, muscle and bone of mice and dogs[89]. Skin toxicity and leg contracture were also significantly reduced in C57Bl6 mice exposed to 250 MeV protons delivered as pFLASH (115 Gy/s) compared with proton-based conventional radiotherapy (1 Gy/s)[90]. A comprehensive dose–response study (20–50 Gy) of skin toxicity in CDF1 mice using pencil beam protons at different doses indicated a DMF in the range 1.44–1.58 for pFLASH versus conventional proton-based therapy[91]. As previously noted, a pFLASH regimen in the SOBP can be reached using a ridge filter. Using this approach, a protective effect was demonstrated with abdominal pFLASH irradiation in mice[92,93].

The first evidence of a sparing effect of FLASH with other accelerated charged particles has been obtained using helium[67] and carbon ions[68] in mammalian cells cultured under hypoxic conditions. At the GSI Helmholtz Centre, dose rates of ~100 Gy/s can be reached with a single pulse (~150 ms) of ^{12}C ions[71], enabling irradiation of mice. In such an experiment, irradiation of a hindlimb osteosarcoma with a dose of 18 Gy under FLASH conditions led to tumour control and substantial sparing of the muscle tissue[94]. In summary, the preclinical evidence generated to date supports the clinical application of FLASH using accelerated charged particles.

Tumour control. The currently available preclinical evidence of a FLASH effect in studies using electrons, protons, heavy ions or X-rays consistently shows efficient tumour control, even if these experiments were generally focused on short-term tumour growth delays. Long-term antitumour effects have been shown in immunocompetent Fisher rats inoculated with glioblastoma cells intracranially or subcutaneously. The animals were completely cured with FLASH or conventional radiotherapy. Moreover, cured animals were able to reject tumour cells upon re-challenge[95].

The iso-efficacy of FLASH and conventional radiotherapy has been demonstrated in simple subcutaneous xenograft and syngeneic models, in more complex orthotopic models implanted into the brain, lung and abdominal cavity, and in genetically engineered mouse models[10,26,84–86,89,90,92,94,95]. These studies support the idea that tumour eradication is independent of dose rate at least after exposure to a single dose. Given that fractionated radiotherapy regimens are the standard of care for the treatment of solid tumours, the evaluation of the effect of fractionation is important. Owing to its sparing effect on non-malignant tissue, the delivery of FLASH radiotherapy using conventional fractionation would enable the addition of more fractions to the total dose, providing a simple and safe approach for its implementation in the clinic. Unfortunately, the results of experiments assessing the FLASH effect using conventional fractionation are not yet available; however, in a mouse model of glioblastoma, the effects of hypofractionated regimens of 2 × 7 Gy and 3 × 10 Gy delivered as FLASH radiotherapy on tumour growth and mouse survival were indistinguishable from those using isodoses of conventional irradiation[26]. These conditions provided equivalent levels of protection in non-malignant tissues.

Nowadays, anticancer treatment generally involves the combination of radiotherapy and systemic therapies (with cytotoxic, targeted and/or immunotherapeutic agents)[96–98]. The reduced inflammatory response to FLASH radiotherapy described in non-malignant tissue and the potential sparing effect on circulating lymphocytes (that remains to be experimentally demonstrated)[81] might contribute to enhancing the efficacy and reducing the toxicity of combinations involving this radiotherapy modality[99]. Indeed, the FLASH effect is expected to reduce damage to circulating lymphocytes compared with the effect of conventional radiotherapy because of the correlation between treatment time and circulating blood dose[100]. Immune cell sparing is very important to elicit a robust immune response when radiotherapy is combined with immunotherapy[101], and clinical data suggest that radiation-induced lymphopenia has a negative effect on concomitant immunotherapy[102,103]. Therefore, FLASH radiotherapy could be associated with great clinical benefit when delivered in combination with immunotherapy.

Mechanistic studies

Exploring the biological mechanisms underlying the FLASH effect is an ongoing effort that has yielded some important findings. In non-malignant tissues, the well-characterized cascade known to be activated after exposure to ionizing radiation is simply not activated when this radiation is delivered under FLASH conditions (Fig. 3b). Indeed, preclinical evidence shows that FLASH delivery reduces the effect of radiation on DNA damage[67,85,104,105], apoptosis[10,78], fibrosis and secretion of inflammatory molecules[10,78,106,107] consistently in various non-malignant tissues, suggesting that they are tolerant of FLASH irradiation. Conversely, tumours seem to be equally sensitive to FLASH and conventional radiotherapy, with similar levels of DNA damage, cytotoxicity and activation of pathways leading to cell death[85]. The basis of this intriguing differential response is one of the most important and challenging questions for the oncology community and several hypotheses are under investigation.

A popular hypothesis is that FLASH induces rapid radiation-induced oxygen consumption and, subsequently, transient local oxygen depletion that would be protective to non-malignant tissue but not tumours[15,19,72,108,109]. The concept of oxygen depletion was described 40 years ago[9,110] and has been considered in several modelling studies of the FLASH effect in the past few years[111–115]. Nevertheless, theoretical and experimental investigations cast doubt on the accuracy of this hypothesis[116–118]. Oxygen depletion requires doses much higher (>100 Gy) than those in the range in which the FLASH effect is observed (around 10 Gy), and the consumption is lower at high dose rates because of radical recombination[116–118]. An alternative explanation is that the reactive oxygen species that function as signalling and damaging moieties in cells interact with molecules involved in redox metabolism, contributing to the FLASH effect[119–121]. High oxygen levels have been shown to abolish the FLASH effect in vivo, suggesting that a low oxygen tension might

contribute to the protection of non-malignant tissue[79]. In vitro experiments have also shown protection at high dose rates only when cells are in intermediate oxygen concentration, between hypoxic and fully oxic conditions[68]. Of note, most non-malignant tissues have oxygen levels in the range 3–7% (physioxia), much lower than normoxia (20%), while most tumours have median oxygen levels of <2%[122]. This different oxygen concentration seems to be important for the sparing effect of FLASH in healthy but not in malignant tissues. Spatial oxygen heterogeneity (for example, in small hypoxic regions within well-oxygenated non-malignant tissues) might also contribute to the sparing effect of FLASH[123]. Moreover, major differences between the redox metabolism of non-malignant and tumour cells might promote survival in the former but exacerbate cell death in the latter following FLASH[119].

Another popular hypothesis to explain the FLASH effect involves the contribution of the tumour microenvironment and the immune response, in particular, the possible FLASH-induced sparing of immune cells[124]. The potential reduction of immunosuppressive signals after FLASH exposure is based on two observations made in various mouse models: that of distal effects in lung metastases after irradiation of a tumour in the hindlimb with 240 MeV per nucleon ^{12}C ions at the GSI synchrotron[94], and the lack of induction of TGFβ1 signalling[10,89,104]. However, whether the reduction of distant metastases[94] is specifically associated with high-energy heavy ions, which can be very effective in combination with immunotherapy also at CONV[125], or also applies to sparsely ionizing radiation is unclear. Another possibility is that the effect of FLASH radiotherapy on metastasis results from the modulation of adhesion and migration properties of tumour cells, which are known to be modified by heavy ions[126]. Further investigation of this issue is certainly of the utmost importance. By contrast, the isoefficacy between FLASH and conventional radiotherapy observed in immunocompetent[79] and immunocompromised animals[26] does not provide evidence of any specific immune response in FLASH-induced tumour control. A study of the immune response in a mouse model of ovarian cancer irradiated with electrons at conventional and FLASH intensities[84] showed that both modalities increase intratumoural T cell infiltration in the absence or presence of anti-PD-1 antibodies, ultimately inducing similar antitumour responses.

A final important point is the possible existence of FLASH-resistant tumours. The majority of preclinical research on FLASH has been performed in mouse hosts engrafted with mouse or human cell lines cultured over extended times in vitro, resulting in an experimental bias that might have underestimated the possibility that a diverse population of patients with various cancer types could have FLASH-resistant tumours. To our knowledge, only a single study of FLASH has been performed to date with primary human tumours generated from acute lymphoblastic leukaemia T cells isolated from three different patients. The results of this study suggest that such resistant tumour subtypes might exist[127], emphasizing the need for further studies, preferably using freshly isolated human samples and/or patient-derived xenografts, but also spontaneously developing tumours in a genetically heterogeneous range of hosts (such as domestic animals or outbred rodent strains).

Studies with curative intent

Studies in domestic mammals

The implementation of FLASH with curative intent has initially been conducted in domestic cats with cancer. The first phase I trial involved six cats with T1–T2 superficial squamous cell carcinomas of the nasal planum that were irradiated using a 5.5 MeV linac. This dose-escalation trial from 26 Gy to 41 Gy suggested that a single dose of 30 Gy delivered in ten pulses and at 300 Gy/s is safe and effective[80]. These results provided the rationale for a phase III trial comparing 30 Gy delivered in three pulses at 1,500 Gy/s and a fractionated standard regimen (90% isodose) in cats and minipigs. This study was the first to provide information on long-term tumour control, which was similar in both arms[128]. However, three of seven cats receiving FLASH radiotherapy and none of those animals receiving standard-of-care radiotherapy developed late toxicities consisting of mandibular osteoradionecrosis at 9–15 months (Supplementary Fig. 2), leading to termination of the trial. We have proposed several explanations for the observed toxicity profile: (1) the use of a single, very high radiation dose (>30 Gy) increased the risk of complications; (2) the administration of the 30 Gy FLASH protocol, but not conventional radiation, was frontal and performed without any bolus; (3) the dose rate selected might have been suboptimal; and (4) we cannot exclude inter-individual variations in radiosensitivity among the three cats with complications. The FLASH protocol, however, resulted in robust local tumour control, supporting the hypothesis that FLASH radiotherapy does not have a protective effect on cancer cells.

Another trial was conducted in dogs with various cancer types[129]. Treatments were performed with a modified clinical linac able to deliver ultra-high dose rates (400–500 Gy/s mean dose rate, 7×10^5 Gy/s instantaneous dose rate) with doses in the range 15–35 Gy. No short-term toxicity was observed but tumour responses were heterogeneous and, given the mixed initial population included in the study, they could not be evaluated. Another major limitation of this study is its short follow-up duration (6 months), that obviated the detection of any potential late toxicities. Short-term investigations (a few days after irradiation) were also performed in dogs with osteosarcoma[89] irradiated with pFLASH. The results confirmed prior results obtained in mice[89] showing that conventional proton-based but not pFLASH radiation induced TGFβ1 at up to 5 days after irradiation.

These studies highlight several relevant points for clinical translation: (1) long-term toxicity follow-up is crucial, especially given that FLASH operates at high doses per fraction, which is a limiting factor for late effects; (2) the implementation of FLASH radiotherapy at higher doses and single fractions might not be feasible in human patients and, therefore, investigations delivering FLASH using standard fractionation regimens should be prioritized as a safe first step; (3) the selection of the beam parameters is also crucial (for example, electron beams with intermediate energy (<10 MeV) provide a heterogenous dose distribution that is difficult to predict entirely).

Early clinical experience

The rationale for the clinical translation of FLASH radiotherapy relies on the robustness and reproducibility of the FLASH effect in most of the preclinical models tested so far, but also on the potential clinically meaningful magnitude of benefit that could be achieved. Understanding the effect of volume, organ and dose on the FLASH-induced DMF is the next challenge, especially for low radiation doses per fraction. High doses per fraction will remain challenging to use in a clinical setting, especially in large radiation fields, owing to the increasing risk of late effects. Additional relevant information on several aspects also remains to be obtained. One of them is the influence of pauses (seconds to a few minutes) on the FLASH effect. Such information is of the utmost importance to understand whether FLASH beams have to converge simultaneously or sequentially. The optimal beam parameters and thresholds also remain to be defined, although concurring evidence suggests that the overall treatment time, generally <200 ms

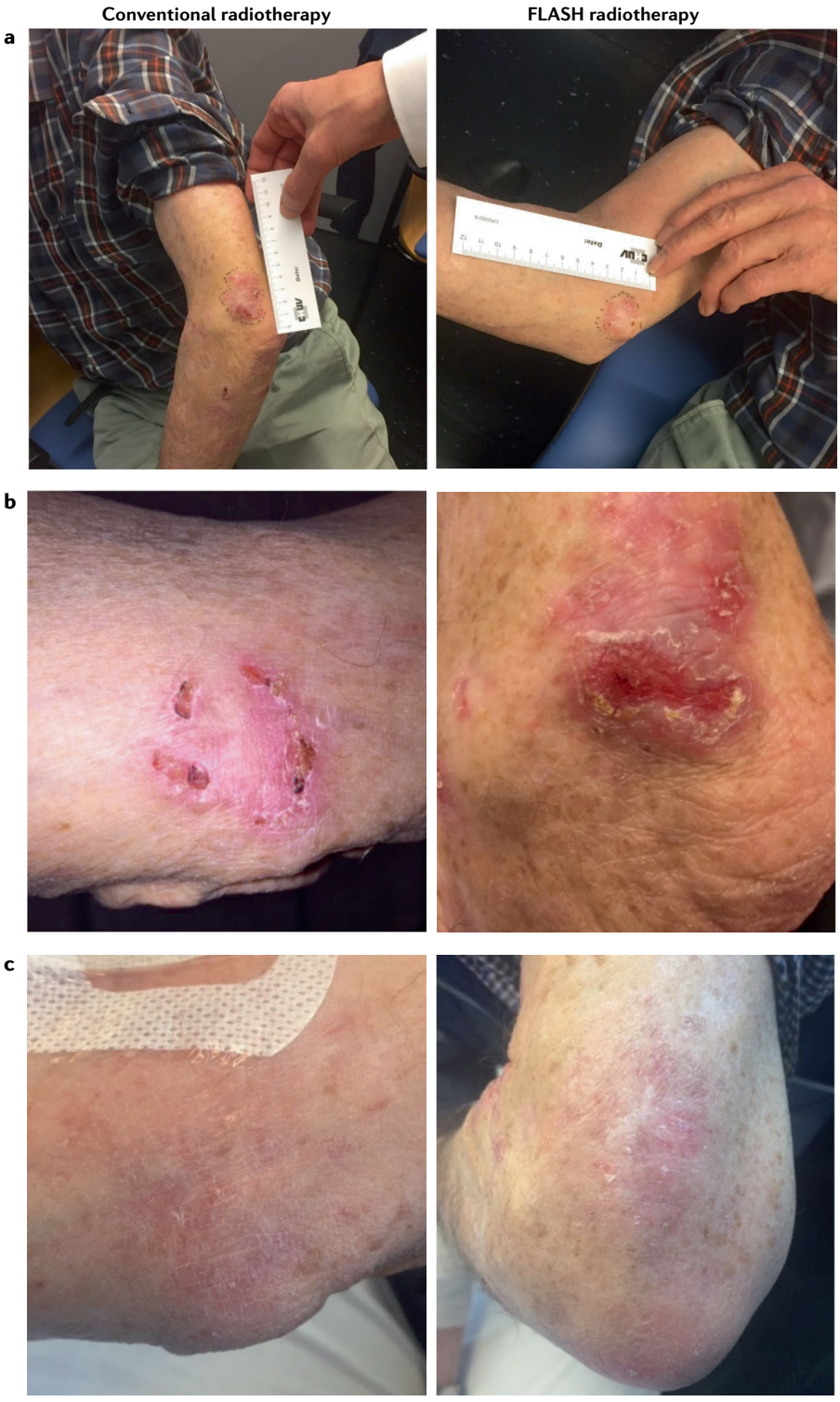

Fig. 4 | Treatment of cutaneous lymphoma with FLASH radiotherapy. a, FLASH and conventional radiotherapy were directly compared in a 75-year-old patient who presented with two cutaneous lymphoma lesions. The same single dose (15 Gy) was delivered on the same day either in 90 ms as FLASH radiotherapy, or in 2.87 min as conventional radiotherapy. **b**, The maximal grade of skin reaction was detected around week 3, with a grade 1 reaction in both treated lesions. **c**, The skin recovered a normal appearance around day 85 after either FLASH or conventional radiotherapy. These data suggest that, in this dose range, the incidence of acute skin reactions is comparable with the two radiotherapy modalities.

for electrons and <400–500 ms for protons, seems to be one of the key parameters to achieve a consistent effect.

With these limitations in mind, clinical translation has started using electron beams with intermediate energy. The first FLASH treatment was delivered at CHUV to a patient with skin lymphoma using 5.4 MeV electrons, and delivering a single fraction of 15 Gy in 90 ms was deemed safe and feasible[23]. The same patient received the same dose in a different lesion as conventional radiotherapy.

Both treatments resulted in similar acute and late (at 2 years) effects[24] (Fig. 4). Therefore, these results suggest that the difference between FLASH and conventional radiotherapy can be difficult to detect in this dose range and when skin is the target organ.

The FAST-01 trial (NCT04592887) is ongoing at the Cincinnati Children's/UC Health Proton Therapy Center (Cincinnati) and has completed accrual with ten patients with bone metastases. The primary end point is the clinical workflow feasibility of delivering an 8 Gy single dose with a broad proton beam at a FLASH minimum dose rate of 40 Gy/s. The beam is used in the plateau region, that is, no Bragg peak is delivered to the patient (Fig. 2). Treatment-related adverse events and workflow feasibility are co-primary end points while pain relief is a secondary end point. A clinical trial aiming to deliver pFLASH therapy to thoracic tumours is in preparation at the same institution.

The IMPULSE trial (NCT04986696) performed at CHUV is a phase I dose-escalation trial of doses from 22 Gy to 34 Gy delivered using 9 MeV electrons to patients with skin metastases from melanoma. Two cohorts are included, one for small tumour fields (<30 cm^3) and a second one for large tumour fields (up to 100 cm^3). Dose escalation is being done by increasing the dose per pulse while keeping the number of pulses and overall treatment time constant (ten pulses in 90 ms). The primary end point of the study is the maximal tolerated dose associated with acute skin reactions (grade <3), and the secondary end points are late adverse events (12 months) and tumour control. The trial is ongoing, with eight patients and no dose-limiting toxicities reported at the two first dose levels (22 Gy and 24 Gy), allowing dose escalation to continue.

In the coming years, several new clinical initiatives will be launched either with pFLASH or FLASH based on low-energy electrons, which are the two most advanced technologies for immediate clinical translation. All these clinical evaluations will focus primarily on the feasibility and safety of using FLASH parameters in patients, but will also perform early assessment of the antitumour efficacy and, thus, the curative potential of FLASH radiotherapy in patients with cancer. The most informative studies will be those that directly compare FLASH and conventional radiotherapy, with all the irradiation parameters being similar except the dose rate. Some of these randomized trials include LANCE, for patients with skin cancers (planned to start in 2023 at CHUV), a second randomized trial from the FLASHKnife Consortium for patients with cutaneous cancers (to start in 2023) and a trial of intraoperative FLASH therapy for abdominal and head and neck cancers (to start at the CHUV in 2023). A midterm aim of these trials should be to understand which technical pathways should be used for delivering highly conformal FLASH therapy to deep-seated and large tumours, two scenarios with a clear unmet clinical need for more efficient and better tolerated radiotherapy. Typically, good candidates for FLASH clinical translation are tumours with extremely low radiocurability rates, such as glioblastomas, inoperable brain metastases or inoperable lung cancers, for which we expect an increased differential effect between tumours and surrounding non-malignant tissues on the basis of the remarkable sparing of non-malignant brain or lung tissues seen in preclinical studies using FLASH. Future relevant clinical settings will also be largely determined by the magnitude of the FLASH effect with fractionated radiotherapy — that is, if a FLASH effect is seen essentially at high dose per fraction ranges, its clinical use in large fields would be limited (for example, as boost). In the long term, if initial studies of FLASH radiotherapy produce promising results, potential additional advantages of

Box 2

Key questions on the implementation of FLASH radiotherapy

What are the optimal parameters?

Preclinical experiments have been performed using a set of highly specific conditions, including physics parameters (including, but not limited to, dose rate, irradiation time, fractionation and total dose), radiation geometry and regimen, and biochemical and intrinsic biological conditions (such as oxygen tension, hypoxia, tumour and tissue type, and individual sensitivity to FLASH). We do not know the optimal parameter range, but have already identified a number of windows in which FLASH could be tested clinically. A safe approach would be to work within a defined set of physical and biological parameters that are already used in clinical treatments and increase only the dose rate. Eventually, whether the FLASH effect can be applied to all patients or tumours or whether it will be restricted to subpopulations will ultimately dictate its application.

Is the technology that enables reaching FLASH conditions ready for clinical implementation?

Although this technology is still not mature, this is not the main hurdle towards clinical application of FLASH radiotherapy. Some solutions are already used in clinical settings that are designed to generate proof-of-principle results, whilst others will be implemented soon (for example, protons with range modulators or intraoperative radiotherapy machines). Even if performing preclinical experiments in FLASH conditions with virtually all radiation types becomes possible in a few years, the necessary quality assurance for full clinical implementation will require longer times and additional developments. The remaining challenges include irradiation of large volumes (≥1 l), irradiation of deep-seated tumours, reliable online dosimetry, use of standard fractionation (such as 2 Gy per fraction for 30 fractions) and implementation of highly conformal FLASH radiotherapy. An alternative strategy to overcome the problem of irradiation of large volumes could be to use FLASH only in a tumour subvolume, such as hypoxic regions or the boundary between the tumour and an organ at risk. The suitability of this strategy could be explored in a reasonable timeline with accelerated charged particles in a clinical setting.

What is the mechanism of the FLASH effect?

A better understanding of the FLASH effect and the possibility of controlling its occurrence will facilitate meaningful clinical implementation.

this modality could be further addressed, such as a reduced number of fractions and very short beam-on time, subsequently improving workflows and reducing waiting lists as well as facilitating tumour motion management.

Conclusions

At present, FLASH radiotherapy has largely sparked the imagination and interest of radiation scientists and oncologists. The advantages of ultra-short treatments at high doses of radiation go beyond the potential widening of the therapeutic window, because short treatment times could also improve the comfort of the patient and the workflow of clinical centres, even if imaging time will remain a limiting factor for accelerating such workflows. Typically, FLASH radiotherapy is delivered in tenths of a second compared with minutes with conventional radiotherapy. This fact brings the added advantage of mitigating problems related to organ or tumour motion, a highly relevant problem in radiotherapy using X-rays[130] and especially particles[131]. For tumours in the thoracic region, FLASH radiotherapy can be delivered with respiratory gating and in combination with real-time, online imaging[132,133]. The use of FLASH would be more complicated for tumours in areas with high motion, such as the pelvic or abdominal region, given that a risk of missing the target is possible depending on the interval between imaging and treatment and/or the capability of tracking. A potential advantage of FLASH radiotherapy is that it could expand the safe use of hypofractionation, and perhaps even the extension of high-dose single-fraction treatments to volumes substantially larger than the those currently used when delivering SBRT[134,135] or carbon ion therapy[136,137]. Single-fraction treatments would also remove the uncertainty regarding the effects of fractionation on FLASH and could be applied in selected tumours and tissues known to be tolerant of high doses of irradiation, such as the skin. Making hypofractionation a safe and effective option for more tumour types will obviously have great advantages for patients and medical centres.

Is FLASH ready to be used in clinical settings? The preclinical evidence generated with electrons, protons, heavy ions and X-rays is consistent, and strongly suggests improvements in sparing of non-malignant tissues without compromising tumour control. This evidence now makes it necessary to test FLASH radiotherapy following the general guidance used for new treatments in oncology, starting with early safety and efficacy trials (phase I/II). A few early phase clinical trials using FLASH-validated beams and known windows of values are ongoing and planned, and their results will be essential for safety and preliminary efficacy assessments before planning larger trials. Nevertheless, additional preclinical studies are certainly needed before full implementation is possible. These studies need to address several questions (Box 2).

Clinical trials are already ongoing, and we expect that their first results will indicate the feasibility and safety of FLASH irradiation in patients rather than provide definite answers on its potential advantages. Nevertheless, initiating phase I trials is necessary to assess whether the current strong investment in research on FLASH radiotherapy is justified. Uncertainties should not stop early safety trials. A somewhat similar situation happened with heavy ion-based therapy, when uncertainty over its relative biological effectiveness was adduced to stop any clinical trial[138]. Instead, phase I/II clinical trials using accelerated carbon ions have shown the advantages and limitations of heavy nuclei in cancer therapy — a similar scenario is possible with FLASH. Thus, full implementation should be made cautiously, although the results from preclinical research that are quickly accumulating need to be exploited. Given that FLASH is typically a tissue effect, in vitro experiments have limited value and animal models remain the best preclinical tool, although human organoids might provide useful hints on the mechanisms. In translational and clinical research, studies on the dose and fraction dependence, tissue specificity, combined treatments and, of course, phase I trials are the highest priority. The future of FLASH radiotherapy will strongly depend on the results of these experiments and the answers to some key questions, including those we have discussed herein.

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Correspondence should be addressed to Marco Durante.

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