FLASH radiotherapy: bridging revolutionary mechanisms and clinical frontiers in cancer treatment – a narrative review

Running title: FLASH radiotherapy in cancer treatment

Jae Sik Kim[1], Hak Jae Kim[2,3]

[1]Department of Radiation Oncology, Soonchunhyang University Seoul Hospital, Soonchunhyang University College of Medicine, Seoul, Korea.

[2]Department of Radiation Oncology, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea.

[3]Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea.

Correspondence: Hak Jae Kim

Department of Radiation Oncology, Seoul National University Hospital,

101 Daehak-ro, Jongno-gu, Seoul, 03080, Korea.

Tel: +82-2-2072-2520; Fax: +82-2-765-3317; E-mail: khjae@snu.ac.kr

Abstract

FLASH radiotherapy (FLASH-RT) is an innovative approach that delivers ultra-high dose rates (UHDR) exceeding 40 Gy in less than a second, aiming to widen the therapeutic window by minimizing damage to normal tissue while maintaining tumor control. This review explores the advancements, mechanisms, and clinical applications of FLASH-RT across various radiation sources. Electrons have been predominantly used due to technical feasibility, but their limited penetration depth restricts clinical application. Protons, offering deeper tissue penetration, are considered promising for treating deep-seated tumors despite challenges in beam delivery. Preclinical studies demonstrate that FLASH-RT reduces normal tissue toxicity in the lung, brain, skin, intestine, and heart without compromising antitumor efficacy. The mechanisms underlying the FLASH effect may involve oxygen depletion leading to transient hypoxia, reduced DNA damage in normal tissues, and modulation of immune and inflammatory responses. However, these mechanisms are incompletely understood,

and inconsistent results across studies highlight the need for further research. Initial clinical studies, including treatment of cutaneous lymphoma and bone metastases, indicate the feasibility and potential benefits of FLASH-RT in patients. Challenges for clinical implementation include technical issues in dosimetry accuracy at UHDR, adaptations in treatment planning systems, beam delivery methods, and economic considerations due to specialized equipment requirements. Future directions will involve comprehensive preclinical studies to optimize irradiation parameters, large-scale clinical trials to establish standardized protocols, and technological advancements to overcome limitations. FLASH-RT holds the potential to revolutionize radiotherapy by reducing normal tissue toxicity and improving therapeutic outcomes, but significant research is required for real-world clinical applications.

Keywords: DNA damage; Electrons; Hypoxia; Neoplasms; Protons

Introduction

Background

Radiotherapy (RT) is a crucial component of antitumor therapies, and approximately 30% of cancer patients in Korea undergo RT [1]. RT must strike a balance between efficiently killing tumor cells and minimizing damage to normal tissue [2]. This constraint complicates the administration of an adequate tumoricidal dose, presenting a significant challenge, especially in the context of repeated RT [3,4]. Although numerous biological studies have been conducted to prevent or mitigate RT-induced acute and late toxicities, there have also been significant advancements in radiation technology over the past few decades. These include developments in intensity-modulated RT, stereotactic body RT, and adaptive RT [5–10]. Moreover, technologies aided by artificial intelligence have been integrated into these advancements [11]. Despite these improvements, the radiation oncology community continues to explore new methods to expand the therapeutic window, as there remain unmet medical needs.

One of these innovative approaches is FLASH radiotherapy (FLASH-RT), which delivers irradiation at an ultra-high dose rate (UHDR) exceeding 40 Gy in less than a second [12]. This method can significantly shorten treatment times compared to conventional RT. In 1959, Dewey and Boag first observed that the radiosensitivity of *Serratia marcescens* decreased when exposed to 1.5 MV X-rays at a UHDR of 10-20 kilorads/2 μs, thereby protecting the bacteria compared to exposure at conventional dose rates—a phenomenon now known as the FLASH effect [13]. The term FLASH-RT was first introduced in a 2014 study by Favaudon et al. [14]. Subsequent preclinical experiments with mammalian cells and animal models have shown that UHDR irradiation, compared to conventional RT, provides a similar antitumor effect while also protecting normal tissue [12]. In 2019, a case report detailed the treatment of a patient with T-cell cutaneous lymphoma using FLASH-RT, noting that the approach was feasible and resulted in favorable outcomes for both the lymphoma and normal skin [15].

When cells and tissues are irradiated, a series of physical, chemical, and biological reactions occur [16]. However, after FLASH-RT, these reactions do not advance to the biochemical phase [16]. Several radiobiological hypotheses have been proposed to explain the differential effects of FLASH-RT on normal and tumor tissues, including oxygen depletion, DNA damage, and the immune/inflammatory response [17]. Nevertheless, the exact mechanism of action of FLASH-RT is still not well understood.

Objectives

The purpose of this review is to explore the revolutionary advancements and underlying mechanisms of FLASH-RT in cancer treatment. By integrating findings from both preclinical and clinical studies, this review aims to highlight the therapeutic potential and challenges of FLASH-RT, ultimately bridging the gap between cutting-edge radiobiological research and clinical application.

Ethics statement

As this study is a literature review, it did not require institutional review board approval or individual consent.

FLASH-RT beam delivery devices

FLASH-RT is theoretically feasible with all contemporary radiotherapy equipment; however, most research has primarily utilized pulsed electron beams [18]. In this session, we will introduce the characteristics of FLASH-RT according to different radiation sources. Nonetheless, implementing FLASH-RT in clinical settings using current RT modalities presents technical challenges, including the need for multiple beam directions to ensure tumor conformity [19].

Electrons

The initial studies on FLASH-RT were performed using low-energy electrons (~25 MeV) from either experimental or medical linear accelerators [20]. Conventional C-arm and intraoperative radiotherapy devices have also been successful in achieving UHDR [21]. These devices are readily available for UHDR, and several vendors are developing electron beam FLASH-dedicated linear accelerators [18]. However, the clinical application of this technology is restricted by the inherent properties of electrons, which include a low penetration depth of only a few centimeters, a short source-target distance (~50 cm), and a significant lateral penumbra [18,21]. Consequently, only skin cancers or tumors located within 2-3 cm of the body surface are currently suitable for treatment with FLASH-RT [22].

The use of very high energy electrons (VHEE, 50-250 MeV) has been suggested as an effective method for delivering therapeutic doses to tumors deep within the body using external electron beam FLASH-RT [22]. VHEE beams demonstrate relative insensitivity to body inhomogeneities compared to protons [23]. Although VHEE beams are associated with high entrance and exit doses, employing multi-directional beams can offer a benefit by sparing the skin [23,24]. However, due to the technical challenges associated with

electron acceleration, VHEE research is limited to a few facilities, including the Photo Injector Test facility at Deutsches Elektronen-Synchrotron in Zeuthen and the European Organization for Nuclear Research Linear Electron Accelerator for Research facility [21].

Photons

Currently used linear accelerators in RT are unsuitable for photon beam-based FLASH-RT [18]. The primary reason is the high inefficiency in converting the electron beam to a photon beam, largely due to electron heat deposition [21,22]. This inefficiency necessitates the generation of a factor of 1000 more electrons than current equipment can handle, presenting a significant challenge that must be overcome [22]. Furthermore, technology that can accelerate this vast quantity of electrons and convert them into photons is also required [22].

Unlike high-energy beams, X-rays with energies <1 MeV can achieve FLASH conditions through a synchrotron [18]. The European Synchrotron Radiation Facility was the first to demonstrate that a UHDR synchrotron light source could reduce brain injury in mice following whole-brain irradiation [25]. Johns Hopkins University successfully achieved UHDR (40-240 Gy/s) using two 150 kVp fluoroscopy systems [26]. Development projects for new accelerators specifically designed for conventional, high-energy photon UHDR beams are currently in progress. Notable examples include the superconductive linac (6-8 MeV) from the Chengdu THz Free Electron Laser group and the Pluridirectional High-energy Agile Scanning Electronic Radiotherapy platform from the Stanford Linear Accelerator Center [18,21].

Protons

Protons, unlike electrons and photons, possess a unique physical property known as the Bragg peak, where they deposit most of their energy at a specific depth just before stopping. This characteristic enables proton therapy to concentrate the dose on the tumor site while reducing exposure beyond the Bragg peak, thereby minimizing the risk of side effects [27]. Clinical isochronous cyclotrons utilized in proton therapy can deliver intensities exceeding 60 Gy/s at a fixed energy. With proton energies surpassing 200 MeV, these cyclotrons are capable of treating deep-seated tumors [18,21]. As a result, protons are considered the most advanced technology for the clinical application of FLASH therapy [18].

Proton therapy employs pencil beam scanning to accurately target tumor volumes. However, a

significant limitation of proton-based FLASH therapy stems from this method of beam delivery, as existing technology cannot accommodate the rapid energy modulation needed [18]. In clinical settings, it is difficult to achieve FLASH conditions across the entire tumor due to the current speed of 3D volumetric scanning. Each energy change in the scanning process takes about one second, which is too slow to satisfy FLASH criteria [18].

To achieve FLASH conditions throughout the entire tumor, the scanning speed must be increased by at least two orders of magnitude [18].

Researchers are actively working to overcome this challenge and bring this technology into routine clinical use. Hybrid active-passive systems featuring patient-specific 3D range modulators are currently being tested in clinical facilities and offer extremely rapid irradiation times (<1 s) [18]. Additionally, laser-driven accelerators are under development at Helmholtz-Zentrum Dresden-Rossendorf [18].

Heavy ions

The depth-dose distribution of heavy ions exhibits a Bragg peak similar to that observed with protons; however, unlike protons, heavy ions also display a tail region [21]. Heavy ions offer additional advantages in RT, including a sharper lateral penumbra and higher relative biological effectiveness [18,21]. Despite these advantages, the global number of heavy ion centers remains limited. Consequently, research on FLASH-RT using heavy ions has been relatively sparse, partly due to the technical challenges associated with the synchrotron accelerators needed to achieve UHDR [21]. Currently, the Heidelberg Ion Beam Therapy Center has achieved dose rates exceeding 50 Gy/s using both helium and carbon ions. Meanwhile, Gunma University Hospital has reached dose rates up to 195 Gy/s with carbon ions, and the GSI Helmholtz Center for Heavy Ion Research has exceeded 100 Gy/s [18].

In vivo **studies on FLASH-RT**

The burgeoning interest in UHDR FLASH-RT is driven by recent preclinical studies that have shown its potential to protect various normal tissues (Table 1). These promising results have sparked a surge of in vivo research focused on understanding the underlying mechanisms and improving clinical applications. RT often results in significant toxicity, which complicates the administration of high doses to tumors [28–30].

Given FLASH-RT's ability to preserve normal tissues, it could facilitate dose escalation, thus improving tumor control while maintaining similar levels of normal tissue toxicity. However, it is crucial to acknowledge that not all studies have demonstrated the FLASH effect, with some yielding negative outcomes. This underscores the urgent need for additional research to better comprehend the factors that affect the efficacy of FLASH-RT.

Lung

The first groundbreaking proof of concept study in 2014, using a lung fibrosis mouse model, demonstrated that 17 Gy FLASH-RT prevented lung fibrosis and radiation-induced acute apoptosis in blood vessels and bronchi. At 30 Gy, FLASH-RT was less fibrogenic than conventional RT, although it still caused some pulmonary fibrosis [14]. The morphology of lung tissue exposed to 20 Gy of either single or 10-pulse (fractionated) FLASH resembled that of non-irradiated tissue, with only minimal neutrophil infiltration. This finding was consistent with observations from 30 Gy FLASH whole-thorax irradiation [31,32]. In contrast, the conventional RT group showed additional changes, including thickening of the alveolar septum and interstitial hemorrhage [31].

The study conducted by Fouillade et al. provides compelling evidence that FLASH irradiation offers protective effects on lung tissue compared to conventional RT [33]. The researchers observed that FLASH irradiation significantly reduced DNA damage in irradiated lung tissues and decreased the proliferation of lung progenitor cells following injury. Through single-cell RNA sequencing and histological analyses, it was revealed that FLASH reduced the activation of pro-inflammatory genes, diminished progenitor cell proliferation, and curtailed stem cell senescence, all factors contributing to lung fibrosis. Notably, lungs treated with FLASH showed a greater potential for tissue regeneration, exhibiting fewer signs of persistent DNA damage and senescence compared to those treated with conventional RT. However, these protective effects were absent in Terc−/− mice, which have notably shortened telomeres and deficient telomerase activity, underscoring the critical role of telomere integrity and progenitor cell populations in harnessing the full benefits of FLASH.

In a study comparing thorax-irradiated mice, the FLASH group exhibited an 81% lower risk of mortality compared to the conventional group. By the end of the follow-up period, survival rates were 100% in the control group, 90% in the FLASH group, and 50% in the conventional group [32].

Brain

Montay-Gruel et al. were the first to show that after 10 Gy whole-brain irradiation in mice, spatial memory was impaired at conventional dose rates, but remained unchanged at dose rates of ≥100 Gy/s [34]. This preservation of memory was attributed to the reduced impact of FLASH-RT on neurogenesis in the subventricular zone of the hippocampus compared to conventional dose rates. Montay-Gruel and colleagues also demonstrated this neuroprotective effect using synchrotron-generated X-rays for FLASH-RT [25]. Additionally, radiation-induced reactive astrogliosis was less severe following FLASH treatment than with conventional RT [25,35]. In later experiments, FLASH-RT was associated with long-term neurocognitive benefits over a 6-month period, reduced neuroinflammation, and preserved neuronal structure [36]. Not only was astrogliosis less pronounced in the FLASH group compared to conventional RT, but there was also no significant increase in the number of activated microglia in the hippocampus or brain cortex [36,37]. The dendritic area, branches, and length, which were significantly reduced after conventional RT compared to the control, were maintained in the FLASH-irradiated brain [36]. Similar results were observed in the number, density, and volume of dendritic spines.

Although the juvenile mouse brain is known to be radiosensitive, the FLASH effect was still observed [38,39]. Further research has shown that FLASH-RT not only spares the neurogenic niche but also preserves pituitary function, as evidenced by stable growth hormone levels [38]. Even with hypofractionated regimens, it prevented radiation-induced neurocognitive complications in the normal brain [39,40]. Other researchers have also validated the relationship between FLASH-RT and reduced cognitive deficits in both spatial and non-spatial object recognition, as well as associated neurodegeneration, at the electrophysiological, molecular, and structural levels [39,41,42].

In experiments involving proton-based FLASH, no significant differences were observed in locomotion, exploratory behavior, spontaneous activity, or anxiety levels among the control, FLASH, and conventional RT groups [43]. However, in the object recognition task used to assess memory, both the control and FLASH groups demonstrated good recall of the familiar object, in contrast to the rats that received conventional RT. Analysis of the immune cell populations in the brain parenchyma revealed that infiltration of peripheral immune cells ($CD45^{high}$) occurred irrespective of the dose rate. Additionally, there was a 4-fold reduction in microglia compared to non-irradiated tissue, with no variations noted across different dose rates.

These beneficial effects of FLASH might result from reduced production of reactive oxygen species

or less pronounced increases in pro-inflammatory cytokines following FLASH-RT [36,41]. Additionally, they may be associated with the preservation of cerebrovascular integrity through the protection of tight junctions or aquaporin-4 levels [37,39,44].

Skin

FLASH-RT has the potential to mitigate radiation-induced skin reactions, which could significantly reduce both acute and late skin reactions in the treatment of head and neck cancers and extremity soft tissue sarcoma when integrated into future clinical practice [45–48]. When murine skin was subjected to UHDR proton therapy, the skin reaction score was lower compared to that observed with conventional proton therapy [45–47]. Additionally, there were fewer instances of epidermal necrosis, skin stem cell depletion, hair follicle atrophy, inflammation, epidermal hyperplasia, myofiber atrophy, and bone remodeling [46,47,49,50]. The potential benefits of FLASH-RT have also been demonstrated in mini-pigs; however, severe late skin necrosis, which was volume-dependent, developed but eventually resolved [51,52]. Regarding lymphedema, no differences were noted in the incidence or progression between the dose rates; however, the severity was greater in mice that received conventional RT [46]. This reduction in side effects translated into a survival benefit for mice that received ≤40 Gy, although no survival difference was observed between the two dose rates at the 45 Gy dose [45,46].

Zhang et al. reported the oxygen dependence of the FLASH effect [48]. FLASH irradiation improved skin contraction (25-30 Gy), epidermal thickness (25 Gy), and collagen deposition (25 Gy). However, when irradiation occurred in a 100% oxygen environment or under hypoxic conditions induced by restricting blood flow through leg constriction, the tissue-sparing effect of FLASH was lost.

In transcriptome analysis, pathways such as apoptotic signaling, keratinocyte differentiation, and cornification were upregulated in the group that received conventional proton therapy. Conversely, these changes were not observed in the UHDR group [46]. Transforming growth factor (TGF)-β1 expression was also observed at lower levels following FLASH-RT [46,47]. The levels of chemokine ligand-1 and granulocyte-colony stimulating factor (G-CSF) increased, while those of granulocyte-macrophage colony-stimulating factor (GM-CSF) decreased following conventional RT [47]. The GM-CSF/G-CSF ratio, which inversely correlates with tissue toxicity, was reduced in the conventional group, suggesting increased tissue toxicity [53]. In the FLASH group, interleukin (IL)-6 levels rose, although no significant differences in

cytokine levels were noted between the 57 Gy/s and 115 Gy/s dose rates [47]. However, when proton irradiation was delivered at 930 Gy/s, no differences were observed in the levels of TGF-β1, IL-1α, IL-1β, and tumor necrosis factor-α in the blood [50].

Intestines

FLASH experiments using electrons demonstrated more favorable crypt survival at doses between 7.5-12.5 Gy after whole-abdominal irradiation than after conventional dose rate irradiation [54]. However, this sparing effect decreased as the number of FLASH pulses increased or as the interval between pulses extended, leading to a longer delivery time. When 15 Gy of proton irradiation was administered to the whole abdomen, there was a smaller reduction in proliferating cells within the jejunum crypts in the FLASH group than in the conventional RT group [55,56]. After intestinal irradiation at 15 or 18 Gy, the extent of intestinal fibrosis was similar to that observed in non-irradiated tissue [32,55].

Some studies have shown that abdominal FLASH irradiation reduces mortality in mice suffering from radiation-induced gastrointestinal syndrome compared to conventional irradiation [32,57]. This protective effect is believed to stem from FLASH irradiation's ability to decrease chromosomal damage and apoptosis in the crypt base columnar cells of the jejunum, thereby helping to preserve intestinal function and epithelial integrity [57]. Additionally, the beneficial impact of FLASH X-rays may be associated with differing inflammatory responses, including reduced activation of the cyclic guanosine monophosphate–adenosine monophosphate synthase-stimulator of interferon genes (cGAS-STING) pathway and changes in the redox status within the intestinal crypts [58,59].

Fecal samples were utilized for gut microbiome analysis, revealing that overall α-diversity and evenness declined across all irradiated groups, although richness decreased solely in the conventional group [54]. In the β-diversity analysis, the cluster of the FLASH-treated group was closer to that of the control group, suggesting fewer alterations in the microbiome.

Heart

The impact of FLASH-RT on the heart remains largely unexplored. Until recently, research in this area continued to be scarce, with the heart being an uncharted area in FLASH-RT studies. It was not until 2024 that the first study addressing the effects of FLASH-RT on cardiac tissue was published, marking a significant

advancement in our understanding of how this innovative RT might influence cardiac function.

A recent study investigated the impact of proton FLASH-RT aimed specifically at the cardiac apex, delivering a precise 40 Gy dose [60]. The research utilized γH2AX staining to evaluate DNA damage, which was found to be limited to the lower third of the heart, with no impact on adjacent tissues. Bulk RNA sequencing of cardiac tissue revealed distinct pathway regulations based on the treatment approach. In the FLASH-RT group, pathways related to cytoplasmic translation, mitochondrion organization, and adenosine triphosphate synthesis were upregulated. In contrast, pathways involved in tissue morphogenesis and the regulation of developmental growth were downregulated. A key finding was that FLASH-RT reduced cardiac inflammation and profibrotic responses, leading to decreased myocardial fibrosis. Unlike conventional RT, FLASH-RT maintained heart functionality at levels similar to those of non-irradiated controls.

Tumors

Tumor cell killing was not altered, as reported by Favaudon et al., who found that both xenograft human tumor and syngeneic orthotopic lung tumor models exhibited equivalent tumor growth inhibition when comparing FLASH-RT (4.5 MeV electrons, 60 Gy/s) with conventional RT [14]. Similarly, other studies employing various tumor models and FLASH sources have demonstrated comparable levels of histological tumor cell damage, regardless of the dose rate and fractionation [31,40,43,46,47,49,55,56,61–68]. In some instances, tumor growth was even more delayed with FLASH-RT than with conventional RT [32]. In a separate study using 250 MeV proton beams, no difference in lung tumor diameter was observed between 18 Gy FLASH (60 Gy/s) and conventional irradiation; however, there was a significant reduction in proliferating tumor cells following FLASH, indicating a meaningful decrease in lung tumor burden [69]. The survival of tumor-bearing mice was found to be equivalent to or better with FLASH-RT compared to conventional RT [14,32,40,56,67].

Although numerous studies have explored the interaction between the tumor immune microenvironment (TIME) and FLASH-RT, the results have so far been varied and inconsistent [43,62,69]. Some researchers have observed that FLASH retains its antitumor efficacy even in severely immunodeficient mice, suggesting the existence of an antitumor mechanism that may function independently of the immune response [66]. FLASH-RT has been shown to enhance cytotoxic T-cell infiltration into tumors and reverse the immunosuppressive phenotype [69]. There was an increase in $CD8^+$ T-cell recruitment to the tumor,

accompanied by a decrease in the infiltration of immunosuppressive regulatory T-cells (Treg). Additionally, macrophage polarization shifted towards an M1-like phenotype, which facilitated increased lymphocyte infiltration in lung tumors. Furthermore, FLASH-RT suppressed the expression of programmed death-1 (PD-1) and its ligand (PD-L1)

In an orthotopic glioma rat model, tumor-infiltrating lymphocytes, including $CD4^+$ and $CD8^+$ T-cells, increased at both conventional dose rates and UHDR (226 MeV proton, 257 Gy/s) [43]. Interestingly, Treg levels also increased in both groups. Additionally, there were observed increases in natural killer cells and B cells, suggesting that cranial irradiation activates adaptive immunity. However, in the FLASH group, no increase in tumor myeloid cells was noted.

A very recent study using an orthotopic syngeneic mouse model of brainstem diffuse midline glioma explored high-resolution profiling of the TIME following FLASH (9 MeV electron, 90 Gy/s) and conventional dose-rate RT [62]. The methods employed included single-cell RNA sequencing and flow cytometry. Analysis of $CD45^+$ cells revealed that both the FLASH and conventional groups displayed similar proportions of immune subsets, with microglia as the predominant population. As an acute effect of RT, both FLASH and conventional irradiation triggered a type 1 interferon (IFN1) response in microglia. However, by day 10 post-RT, the FLASH group exhibited a dose-rate-dependent reduction in the IFN1 response in microglia, indicating a distinct temporal pattern and suggesting that microglial activation by FLASH was transient during the early stages. Regarding non-resident myeloid cells, such as macrophages and dendritic cells, which represented a minor fraction of the TIME, an early IFN1 response was observed in the conventional group, but in the FLASH group, this response was not clearly defined until day 10 post-RT. Despite these temporal immune changes, no significant differences in tumor control were noted between the dose rates, highlighting an area for future research.

Several trials have explored FLASH-RT in animals with cancer, using electrons in the 4.5-12 MeV range [70–74]. In one study, seven cats with T1/2N0M0 squamous cell carcinoma of the nasal planum received 30 Gy of radiation. All remained tumor-free for one year, with only one case showing progression thereafter [73]. Another trial involved six cats with locally advanced T2/T3N0M0 tumors treated with 25-41 Gy, achieving an 84% progression-free survival rate at 16 months [74]. Additionally, a collaborative effort between researchers from Denmark and Sweden applied FLASH-RT to canine cancer patients with superficial malignant tumors. The treatment was effective, although it was associated with a potential risk of

osteoradionecrosis [75–77].

Clinical studies with FLASH-RT

The clinical application of FLASH-RT was first demonstrated in 2018 when a 75-year-old patient with multi-resistant $CD30^+$ T-cell cutaneous lymphoma received treatment at Lausanne University Hospital in Switzerland (Table 2) [15]. A skin tumor measuring 3.5 cm was exposed to 15 Gy of radiation in just 90 milliseconds using a 5.6-MeV linac. The tumor began to shrink 10 days after treatment with FLASH-RT, achieving a complete response by day 36, which was sustained for five months. Regarding adverse effects, the patient experienced asymptomatic grade 1 epithelitis and grade 1 edema in the surrounding skin, which had previously undergone extensive RT. Optical coherence tomography showed no reduction in epidermal thickness or disruption of the basal membrane, except for a slight increase in vascularization. Subsequently, the patient underwent two additional treatments of 15 Gy each at different sites on the same day (dose rates, 166 Gy/s and 0.08 Gy/s, respectively) [78]. Over the next 2 years, both treatment sites exhibited similar levels of acute and late skin toxicity, with no differences in tumor response noted (Table 2).

The FAST-01 trial, a pioneering first-in-human study of FLASH-RT, involved 10 patients with symptomatic bone metastasis [79]. This trial, presented at the 2022 Annual Meeting of the American Society for Radiation Oncology, suggested that FLASH-RT could be a promising treatment for particularly resistant tumors. It targeted one to three painful bone metastases in the extremities, administering an 8 Gy single fraction to 12 metastatic sites using a FLASH-enabled proton therapy system at a dose rate of $\geq$40 Gy/s. The primary outcomes, which included workflow feasibility and radiation-related toxicities, demonstrated favorable results comparable to those of conventional RT (as detailed in Table 2). Among the 12 treated metastatic lesions, pain was completely alleviated at six sites, and symptoms partially improved at two sites.

Several clinical trials involving FLASH-RT have recently been initiated and are currently recruiting patients: NCT04986696 (phase I, metastatic melanoma), NCT05524064 (phase I, bone metastases, FAST-02), and NCT05724875 (phase II, skin cancers) (Table 3). These trials mark a significant step forward in investigating the safety and efficacy of FLASH-RT, potentially providing cancer patients with faster and less toxic treatment options.

Biological mechanisms behind FLASH-RT

The biological mechanisms by which FLASH irradiation reduces damage to non-malignant tissues while maintaining effective tumor control, as compared to conventional irradiation, remain under active investigation and are not yet fully understood. Several hypotheses have been proposed to explain these differential effects, each with its own limitations. In this section, we introduce three key biological mechanisms: oxygen depletion, DNA damage, and immune/inflammatory response. Additionally, other emerging hypotheses, such as minimal mitochondrial damage or the preservation of normal flora induced by FLASH, are also under consideration [80]. A deeper understanding of these mechanisms is essential for optimizing FLASH-RT and successfully translating its benefits into clinical practice.

Oxygen depletion hypothesis

The oxygen depletion hypothesis is currently the most widely accepted theory. It is based on the principle that oxygen acts as a critical radiosensitizer in RT; thus, tissues with a high oxygen supply are more radiosensitive [81]. FLASH irradiation rapidly depletes oxygen, leaving insufficient time for oxygen to be replenished from the surrounding circulating blood [80]. This results in acute hypoxic conditions that lead to transient radioresistance, thereby sparing normal tissue [82]. Conversely, tumors, with their inherently abnormal blood vessels, are already adapted to hypoxic conditions. This adaptation explains why the dose rate does not significantly impact the tumor cells' susceptibility to radiation [17].

A limitation of this hypothesis is that while FLASH-RT resulted in greater oxygen consumption compared to conventional RT, it did not completely deplete all the oxygen [83]. Furthermore, the oxygen levels associated with higher cell survival rates following FLASH-RT vary significantly across experiments, ranging from severely hypoxic conditions (<0.5%) to oxygen-rich environments like those found in the lungs [80]. This variability suggests that the oxygen depletion hypothesis may not fully explain the FLASH effect.

An alternative explanation has been proposed, suggesting that reactive oxygen species, which serve dual roles as signaling and damaging agents within cells, may interact with molecules involved in redox metabolism. This interaction could potentially play a pivotal role in the FLASH effect [18].

DNA damage hypothesis

Cell fate after irradiation is primarily determined by DNA damage, specifically unrepaired DNA double-strand breaks [84]. Several studies have shown that DNA damage is less severe after FLASH irradiation [33,37,61]. This reduction in DNA damage helps preserve stem and progenitor cells across various tissues, consequently decreasing toxicity [33,34,38,46,55,56]. However, while this effect accounts for the sparing of normal tissue, it does not completely explain the sustained antitumor activity. Although the precise mechanisms are still not fully understood, it is possible that differences in the activation of downstream pathways after DNA damage—such as DNA repair pathways, the cGAS-STING pathway, or the immune system—between normal and tumor cells could contribute [17].

Immune and inflammatory hypothesis

FLASH significantly reduces the duration of radiation exposure, which is anticipated to decrease the volume of irradiated blood and aid in preserving circulating immune cells from depletion [50,85]. However, several *in vivo* studies have yielded negative results, showing no significant difference in the circulating immune cell populations between FLASH and conventional dose rates [43,59,86]. Instead, while further detailed research is necessary, it is generally observed that there is an increase in cytotoxic T-cell infiltration into tumors [43,69]. Conversely, the persistence of the antitumor effect in immunocompromised animals suggests that this effect cannot be solely attributed to the immune response [66].

FLASH also reduces TGF-β and pro-inflammatory gene expression, as well as the release of pro-inflammatory cytokines, thereby mitigating stress response and inflammation [33,41,46,47,58–60,87]. This contributes to the preservation of normal tissue, exemplified by the reduction of neuroinflammation in the normal brain, which in turn supports the maintenance of neurological function [25,35–37]. Given that the TGF-β pathway is a pharmacological target in cancer therapy, the reduction of TGF-β expression induced by FLASH could enhance antitumor activity, similar to the effects of TGF-β antagonists [88].

Current cancer treatment is witnessing a revival of interest in immunotherapy, particularly in its integration with RT [89]. In this context, the immune response elicited by FLASH provides compelling insights that may herald a new phase in radioimmunotherapy. First, FLASH reduces the expression of PD-1 and PD-L1, thereby inhibiting the tumor's ability to evade the immune system [69,90]. In a study using an ovarian cancer mouse model, abdominopelvic irradiation followed by PD-1 therapy led to enhanced tumor control in

both conventional and UHDR settings, without an increase in toxicity compared to using FLASH alone [91]. This treatment also resulted in a lower Treg-to-T-effector ratio and a higher level of $CD8^+$ T-cell infiltration within the tumor. While immunotherapy alone often yields only modest response rates, these findings are noteworthy as they indicate that FLASH-RT can significantly improve the effectiveness of PD-1/PD-L1 inhibitors.

Challenges in the clinical application of FLASH-RT: current issues and future directions

Recent preclinical studies and ongoing clinical trials have advanced the clinical application of FLASH-RT significantly. However, numerous challenges must be overcome before it can be routinely implemented in clinical settings. Key considerations for preclinical studies include: (i) research has been limited to a small number of normal tissues, which may lead to unexpected side effects when FLASH-RT is used clinically; (ii) the extent of the protective effect varies based on tissue type and physical parameters; (iii) there are inconsistent results among different studies; and (iv) most studies have utilized high single doses, necessitating further research to determine if the FLASH effect is achievable with lower doses and fractionated regimens [17,92]. Addressing these issues is essential for the successful integration of FLASH-RT into standard clinical protocols.

In addition to these challenges, it is important to note that some studies have not observed the beneficial effects of FLASH [86,93–95]. One study compared high dose-rate synchrotron broad-beam radiotherapy (37-41 Gy/s) with a mean photon energy of 124 keV to conventional RT (0.05-0.06 Gy/s) with 93 keV. It found that synchrotron broad-beam radiotherapy did not demonstrate the FLASH effect of sparing normal tissue compared to conventional RT [93]. The irradiated mice exhibited weights below normal compared to control mice and experienced disruption of normal crypt-villus units following abdominal irradiation. Additionally, cranial irradiation led to neurological deficits, while thoracic partial irradiation caused inflammatory responses and long-term lung damage.

In a mouse model investigating radiation-induced lymphopenia, both cardiac and splenic irradiation were administered using 20 MeV electron FLASH-RT (35 Gy/s) and conventional RT (0.1 Gy/s) [86]. For both cardiac and splenic irradiation, researchers employed a multi-fraction regimen of 2 Gy (or 1 Gy) per day over 5 days, as well as a single fraction of 10 Gy (or 5 Gy). The findings indicated a decrease in $CD3^+$, $CD4^+$, $CD8^+$, and $CD19^+$ lymphocytes, regardless of the dose rate or fractionation regimen used. Notably, the FLASH-RT group showed a more significant reduction in lymphocyte counts following splenic irradiation compared to the

conventional RT group. In a model of gastrointestinal mucosal injury following whole-abdominal irradiation, acute gastrointestinal toxicity was more severe in the FLASH-RT group after a 16 Gy single fraction. All mice in the FLASH-RT group died within 7 days, whereas those in the conventional RT group survived until day 15.

A recent study demonstrated the absence of tissue-protective effects with FLASH-RT [94]. After partial abdominal FLASH proton irradiation at a rate of 120 Gy/s, survival rates were notably lower in the FLASH group compared to those in the conventional RT group at doses ranging from 15.1 to 18 Gy. Additionally, measurements of proliferating crypt cells and the thickness of the muscularis externa revealed no significant differences. Similarly, there were no variations in circulating lymphocyte counts. These findings indicate that the effectiveness of FLASH irradiation may be subject to multiple influencing factors and that FLASH irradiation could potentially result in adverse outcomes if not properly managed.

When using zebrafish embryos and proton-based FLASH irradiation, no significant protective effect was observed [95]. A comparison of FLASH irradiation (100 Gy/s) using 224 MeV protons with conventional RT (5 Gy/min) revealed no differences in embryonic survival attributable to the varying proton dose rates. Apart from a decreased incidence of pericardial edema following FLASH irradiation, there were no differences in the rate of embryo malformations, specifically spinal curvature, between the two irradiation methods.

The negative results observed in these studies underscore the importance of thoroughly examining the underlying factors. It is possible that the low dose rates and the specific experimental setup played a role in these outcomes [96]. Determining the optimal dose rate to preserve the integrity of normal tissue remains an unresolved issue. Future research should focus on identifying the most effective dose, dose rate, pulse, and fraction size to reduce complications in normal tissues for specific organs [86]. These experiments should be carefully designed to mirror clinical treatment scenarios, ensuring that the results are relevant to real-world applications. Given these challenges, ongoing research and sustained attention are crucial to effectively address these issues and advance the field.

One technical issue pertains to dosimetry. Current dosimetry protocols and equipment, designed for much lower dose rates than those used in FLASH, struggle with accurate measurements at UHDR [97]. The ion chambers typically employed in clinical settings are significantly affected by ion recombination at UHDR, resulting in substantial uncertainties [21,98]. Another challenge involves the development of treatment plans that can accurately deliver the desired dose at UHDR to the specific target location [21]. To address this,

modifications are necessary in the treatment planning system to not only calculate and display the dose distribution in patients but also evaluate the 3D dose rate distribution [21]. Additionally, the beam delivery system needs further development. For optimal conformity to the RT target, beams are usually delivered from multiple angles, which requires the use of rotating gantry systems instead of fixed gantry setups in FLASH-RT [19].

From an economic perspective, FLASH-RT is currently available at only a few institutions, and the equipment required for proton therapy, suitable for treating deep tumors, is extremely expensive. Electron therapy, on the other hand, is only effective for superficial tumors. Photon equipment, which is more widely used globally and less costly than proton therapy, can treat deep-seated tumors. Therefore, it is essential to develop photon-based FLASH-RT equipment to make this treatment more accessible and economically viable [12].

Conclusions

FLASH-RT represents an exciting avenue for improving therapeutic outcomes in oncology, characterized by its ability to deliver UHDR radiation while minimizing damage to normal tissues. This approach has shown promise in both preclinical and initial clinical studies, offering efficacy in tumor control and reduced toxicity. Despite these positive findings, numerous biological and technical challenges remain. The precise mechanisms underlying the FLASH effect are complex and not yet fully understood, necessitating further investigation into the oxygen depletion hypothesis and other potential explanations. Additionally, implementing FLASH-RT in clinical settings requires improvements in dosimetry, treatment planning, and beam delivery systems to meet the specific requirements of UHDR. Future research and clinical trials are essential to address these challenges and validate the long-term safety and effectiveness of FLASH-RT across a broader range of cancers. As this technology evolves, it holds the potential to revolutionize radiation therapy, offering more effective and less toxic treatment options for patients.

ORCID

Jae Sik Kim: https://orcid.org/0000-0002-0039-8667

Hak Jae Kim: https://orcid.org/0000-0003-3602-2263

Authors' contribution

Project administration: Kim JS, Kim HJ

Conceptualization: Kim JS, Kim HJ

Funding acquisition:: Kim HJ

Writing - original draft: Kim JS

Writing - review & editing: Kim JS, Kim HJ

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Funding

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (2023R1A2C1002539) to Hak Jae Kim.

Data availability

Not applicable

Acknowledgments

Not applicable.

Supplementary materials

Not applicable.

References

1. Kim E, Jang W Il, Yang K, Kim M-S, Yoo HJ, Paik EK, et al. Clinical utilization of radiation therapy in Korea between 2017 and 2019. Radiat Oncol J 2022;40:251–9. https://doi.org/10.3857/roj.2022.00500.

[2] Carlos-Reyes A, Muñiz-Lino MA, Romero-Garcia S, López-Camarillo C, Hernández-de la Cruz ON. Biological Adaptations of Tumor Cells to Radiation Therapy. Front Oncol 2021;11:718636. https://doi.org/10.3389/fonc.2021.718636.

[3] Embring A, Onjukka E, Mercke C, Lax I, Berglund A, Bornedal S, et al. Re-Irradiation for Head and Neck Cancer: Cumulative Dose to Organs at Risk and Late Side Effects. Cancers (Basel) 2021;13:3173. https://doi.org/10.3390/cancers13133173.

[4] Florez MA, De B, Chapman B V., Prayongrat A, Thomas JG, Beckham TH, et al. Safety and efficacy of salvage conventional re-irradiation following stereotactic radiosurgery for spine metastases. Radiat Oncol J 2023;41:12–22. https://doi.org/10.3857/roj.2022.00353.

[5] Ren H, Wu Q, Sun Z, Fang M, Liu J, Luo J. Research progress and treatment of radiation enteritis and gut microbiota. Radiat Oncol J 2023;41:61–8. https://doi.org/10.3857/roj.2023.00346.

[6] Chen HHW, Kuo MT. Improving radiotherapy in cancer treatment: Promises and challenges. Oncotarget 2017;8:62742–58. https://doi.org/10.18632/oncotarget.18409.

[7] Song JY, Chie EK, Kang S-H, Jeon Y-J, Ko Y-A, Kim D-Y, et al. Dosimetric evaluation of magnetic resonance imaging-guided adaptive radiation therapy in pancreatic cancer by extent of re-contouring of organs-at-risk. Radiat Oncol J 2022;40:242–50. https://doi.org/10.3857/roj.2022.00332.

[8] Şenkesen Ö, Tezcanlı E, Abacıoğlu MU, Özen Z, Çöne D, Küçücük H, et al. Limited field adaptive radiotherapy for glioblastoma: changes in target volume and organ at risk doses. Radiat Oncol J 2022;40:9–19. https://doi.org/10.3857/roj.2021.00542.

[9] Jia-Mahasap B, Madla C, Sripan P, Chitapanarux I, Tharavichitkul E, Chakrabandhu S, et al. Stereotactic radiosurgery for limited brain metastasis using three different techniques: helical tomotherapy, volumetric modulated arc therapy, and cone-based LINAC radiosurgery. Radiat Oncol J 2022;40:232–41. https://doi.org/10.3857/roj.2022.00136.

[10] Lee J, Kim HJ, Kim WC. CyberKnife-based stereotactic radiosurgery or fractionated stereotactic radiotherapy in older patients with brain metastases from non-small cell lung cancer. Radiat Oncol J

2023;41:258–66. https://doi.org/10.3857/roj.2023.00563.

[11] Lee Y, Choi HJ, Kim H, Kim S, Kim MS, Cha H, et al. Feasibility of artificial intelligence-driven interfractional monitoring of organ changes by mega-voltage computed tomography in intensity-modulated radiotherapy of prostate cancer. Radiat Oncol J 2023;41:186–98. https://doi.org/10.3857/roj.2023.00444.

[12] Lin B, Gao F, Yang Y, Wu D, Zhang Y, Feng G, et al. FLASH Radiotherapy: History and Future. Front Oncol 2021;11:644400. https://doi.org/10.3389/fonc.2021.644400.

[13] Dewey DL, Boag JW. Modification of the Oxygen Effect when Bacteria are given Large Pulses of Radiation. Nature 1959;183:1450–1. https://doi.org/10.1038/1831450a0.

[14] Favaudon V, Caplier L, Monceau V, Pouzoulet F, Sayarath M, Fouillade C, et al. Ultrahigh dose-rate FLASH irradiation increases the differential response between normal and tumor tissue in mice. Sci Transl Med 2014;6:245ra93. https://doi.org/10.1126/scitranslmed.3008973.

[15] Bourhis J, Sozzi WJ, Jorge PG, Gaide O, Bailat C, Duclos F, et al. Treatment of a first patient with FLASH-radiotherapy. Radiother Oncol 2019;139:18–22. https://doi.org/10.1016/j.radonc.2019.06.019.

[16] Vozenin M-C, Hendry JH, Limoli CL. Biological Benefits of Ultra-high Dose Rate FLASH Radiotherapy: Sleeping Beauty Awoken. Clin Oncol 2019;31:407–15. https://doi.org/10.1016/j.clon.2019.04.001.

[17] Hageman E, Che P-P, Dahele M, Slotman BJ, Sminia P. Radiobiological Aspects of FLASH Radiotherapy. Biomolecules 2022;12:1376. https://doi.org/10.3390/biom12101376.

[18] Vozenin M-C, Bourhis J, Durante M. Towards clinical translation of FLASH radiotherapy. Nat Rev Clin Oncol 2022;19:791–803. https://doi.org/10.1038/s41571-022-00697-z.

[19] MacKay R, Burnet N, Lowe M, Rothwell B, Kirkby N, Kirkby K, et al. FLASH radiotherapy: Considerations for multibeam and hypofractionation dose delivery. Radiother Oncol 2021;164:122–7. https://doi.org/10.1016/j.radonc.2021.09.011.

[20] Schüler E, Acharya M, Montay-Gruel P, Loo BW, Vozenin M, Maxim PG. Ultra-high dose rate electron beams and the FLASH effect: From preclinical evidence to a new radiotherapy paradigm. Med Phys 2022;49:2082–95. https://doi.org/10.1002/mp.15442.

[21] Kim MM, Zou W. Ultra-high dose rate FLASH radiation therapy for cancer. Med Phys 2023;50:58–

61. https://doi.org/10.1002/mp.16271.

[22] Matuszak N, Suchorska WM, Milecki P, Kruszyna-Mochalska M, Misiarz A, Pracz J, et al. FLASH radiotherapy: an emerging approach in radiation therapy. Reports Pract Oncol Radiother 2022;27:343–51. https://doi.org/10.5603/RPOR.a2022.0038.

[23] Lagzda A, Angal-Kalinin D, Jones J, Aitkenhead A, Kirkby KJ, MacKay R, et al. Influence of heterogeneous media on Very High Energy Electron (VHEE) dose penetration and a Monte Carlo-based comparison with existing radiotherapy modalities. Nucl Instruments Methods Phys Res Sect B Beam Interact with Mater Atoms 2020;482:70–81. https://doi.org/10.1016/j.nimb.2020.09.008.

[24] Whitmore L, Mackay RI, van Herk M, Jones JK, Jones RM. Focused VHEE (very high energy electron) beams and dose delivery for radiotherapy applications. Sci Rep 2021;11:14013. https://doi.org/10.1038/s41598-021-93276-8.

[25] Montay-Gruel P, Bouchet A, Jaccard M, Patin D, Serduc R, Aim W, et al. X-rays can trigger the FLASH effect: Ultra-high dose-rate synchrotron light source prevents normal brain injury after whole brain irradiation in mice. Radiother Oncol 2018;129:582–8. https://doi.org/10.1016/j.radonc.2018.08.016.

[26] Rezaee M, Iordachita I, Wong JW. Ultrahigh dose-rate (FLASH) x-ray irradiator for pre-clinical laboratory research. Phys Med Biol 2021;66:095006. https://doi.org/10.1088/1361-6560/abf2fa.

[27] Nangia S, Burela N, Noufal MP, Patro K, Wakde MG, Sharma DS. Proton therapy for reducing heart and cardiac substructure doses in Indian breast cancer patients. Radiat Oncol J 2023;41:69–80. https://doi.org/10.3857/roj.2023.00073.

[28] Mandava A, Koppula V, Kandati M, Raju KVVN. Synchronous radiation-induced enterovesical and enterocervical fistulas in carcinoma of the uterine cervix. Radiat Oncol J 2023;41:297–300. https://doi.org/10.3857/roj.2023.00500.

[29] Seo SH, Pyo H, Ahn YC, Oh D, Yang K, Kim N, et al. Pulmonary function and toxicities of proton versus photon for limited-stage small cell lung cancer. Radiat Oncol J 2023;41:274–82. https://doi.org/10.3857/roj.2023.00773.

[30] Ellahham S, Khalouf A, Elkhazendar M, Dababo N, Manla Y. An overview of radiation-induced heart disease. Radiat Oncol J 2022;40:89–102. https://doi.org/10.3857/roj.2021.00766.

[31] Dai Y, Liang R, Wang J, Zhang J, Wu D, Zhao R, et al. Fractionated FLASH radiation in

xenografted lung tumors induced FLASH effect at a split dose of 2 Gy. Int J Radiat Biol 2023;99:1542–9. https://doi.org/10.1080/09553002.2023.2194403.

[32] Gao F, Yang Y, Zhu H, Wang J, Xiao D, Zhou Z, et al. First demonstration of the FLASH effect with ultrahigh dose rate high-energy X-rays. Radiother Oncol 2022;166:44–50. https://doi.org/10.1016/j.radonc.2021.11.004.

[33] Fouillade C, Curras-Alonso S, Giuranno L, Quelennec E, Heinrich S, Bonnet-Boissinot S, et al. FLASH Irradiation Spares Lung Progenitor Cells and Limits the Incidence of Radio-induced Senescence. Clin Cancer Res 2020;26:1497–506. https://doi.org/10.1158/1078-0432.CCR-19-1440.

[34] Montay-Gruel P, Petersson K, Jaccard M, Boivin G, Germond J-F, Petit B, et al. Irradiation in a flash: Unique sparing of memory in mice after whole brain irradiation with dose rates above 100 Gy/s. Radiother Oncol 2017;124:365–9. https://doi.org/10.1016/j.radonc.2017.05.003.

[35] Montay-Gruel P, Markarian M, Allen BD, Baddour JD, Giedzinski E, Jorge PG, et al. Ultra-High-Dose-Rate FLASH Irradiation Limits Reactive Gliosis in the Brain. Radiat Res 2020;194:636–45. https://doi.org/10.1667/RADE-20-00067.1.

[36] Montay-Gruel P, Acharya MM, Petersson K, Alikhani L, Yakkala C, Allen BD, et al. Long-term neurocognitive benefits of FLASH radiotherapy driven by reduced reactive oxygen species. Proc Natl Acad Sci 2019;116:10943–51. https://doi.org/10.1073/pnas.1901777116.

[37] Dokic I, Meister S, Bojcevski J, Tessonnier T, Walsh D, Knoll M, et al. Neuroprotective Effects of Ultra-High Dose Rate FLASH Bragg Peak Proton Irradiation. Int J Radiat Oncol 2022;113:614–23. https://doi.org/10.1016/j.ijrobp.2022.02.020.

[38] Alaghband Y, Cheeks SN, Allen BD, Montay-Gruel P, Doan N-L, Petit B, et al. Neuroprotection of Radiosensitive Juvenile Mice by Ultra-High Dose Rate FLASH Irradiation. Cancers (Basel) 2020;12:1671. https://doi.org/10.3390/cancers12061671.

[39] Allen BD, Alaghband Y, Kramár EA, Ru N, Petit B, Grilj V, et al. Elucidating the neurological mechanism of the FLASH effect in juvenile mice exposed to hypofractionated radiotherapy. Neuro Oncol 2023;25:927–39. https://doi.org/10.1093/neuonc/noac248.

[40] Montay-Gruel P, Acharya MM, Gonçalves Jorge P, Petit B, Petridis IG, Fuchs P, et al. Hypofractionated FLASH-RT as an Effective Treatment against Glioblastoma that Reduces Neurocognitive Side Effects in Mice. Clin Cancer Res 2021;27:775–84.

https://doi.org/10.1158/1078-0432.CCR-20-0894.

[41] Simmons DA, Lartey FM, Schüler E, Rafat M, King G, Kim A, et al. Reduced cognitive deficits after FLASH irradiation of whole mouse brain are associated with less hippocampal dendritic spine loss and neuroinflammation. Radiother Oncol 2019;139:4–10. https://doi.org/10.1016/j.radonc.2019.06.006.

[42] Limoli CL, Kramár EA, Almeida A, Petit B, Grilj V, Baulch JE, et al. The sparing effect of FLASH-RT on synaptic plasticity is maintained in mice with standard fractionation. Radiother Oncol 2023;186:109767. https://doi.org/10.1016/j.radonc.2023.109767.

[43] Iturri L, Bertho A, Lamirault C, Juchaux M, Gilbert C, Espenon J, et al. Proton FLASH Radiation Therapy and Immune Infiltration: Evaluation in an Orthotopic Glioma Rat Model. Int J Radiat Oncol 2023;116:655–65. https://doi.org/10.1016/j.ijrobp.2022.12.018.

[44] Allen BD, Acharya MM, Montay-Gruel P, Jorge PG, Bailat C, Petit B, et al. Maintenance of Tight Junction Integrity in the Absence of Vascular Dilation in the Brain of Mice Exposed to Ultra-High-Dose-Rate FLASH Irradiation. Radiat Res 2020;194:625–35. https://doi.org/10.1667/RADE-20-00060.1.

[45] Soto LA, Casey KM, Wang J, Blaney A, Manjappa R, Breitkreutz D, et al. FLASH Irradiation Results in Reduced Severe Skin Toxicity Compared to Conventional-Dose-Rate Irradiation. Radiat Res 2020;194:618–24. https://doi.org/10.1667/RADE-20-00090.

[46] Velalopoulou A, Karagounis I V., Cramer GM, Kim MM, Skoufos G, Goia D, et al. FLASH Proton Radiotherapy Spares Normal Epithelial and Mesenchymal Tissues While Preserving Sarcoma Response. Cancer Res 2021;81:4808–21. https://doi.org/10.1158/0008-5472.CAN-21-1500.

[47] Cunningham S, McCauley S, Vairamani K, Speth J, Girdhani S, Abel E, et al. FLASH Proton Pencil Beam Scanning Irradiation Minimizes Radiation-Induced Leg Contracture and Skin Toxicity in Mice. Cancers (Basel) 2021;13:1012. https://doi.org/10.3390/cancers13051012.

[48] Zhang Q, Gerweck LE, Cascio E, Yang Q, Huang P, Niemierko A, et al. Proton FLASH effects on mouse skin at different oxygen tensions. Phys Med Biol 2023;68:10.1088/1361-6560/acb888. https://doi.org/10.1088/1361-6560/acb888.

[49] Sørensen BS, Sitarz MK, Ankjærgaard C, Johansen JG, Andersen CE, Kanouta E, et al. Pencil beam scanning proton FLASH maintains tumor control while normal tissue damage is reduced in a mouse

model. Radiother Oncol 2022;175:178–84. https://doi.org/10.1016/j.radonc.2022.05.014.

[50] Rudigkeit S, Schmid TE, Dombrowsky AC, Stolz J, Bartzsch S, Chen C-B, et al. Proton-FLASH: effects of ultra-high dose rate irradiation on an in-vivo mouse ear model. Sci Rep 2024;14:1418. https://doi.org/10.1038/s41598-024-51951-6.

[51] Vozenin M-C, De Fornel P, Petersson K, Favaudon V, Jaccard M, Germond J-F, et al. The Advantage of FLASH Radiotherapy Confirmed in Mini-pig and Cat-cancer Patients. Clin Cancer Res 2019;25:35–42. https://doi.org/10.1158/1078-0432.CCR-17-3375.

[52] Rohrer Bley C, Wolf F, Gonçalves Jorge P, Grilj V, Petridis I, Petit B, et al. Dose- and Volume-Limiting Late Toxicity of FLASH Radiotherapy in Cats with Squamous Cell Carcinoma of the Nasal Planum and in Mini Pigs. Clin Cancer Res 2022;28:3814–23. https://doi.org/10.1158/1078-0432.CCR-22-0262.

[53] MOSER C, JENSEN PØ, PRESSLER T, FREDERIKSEN B, LANNG S, KHARAZMI A, et al. Serum concentrations of GM-CSF and G-CSF correlate with the Th1/Th2 cytokine response in cystic fibrosis patients with chronic Pseudomonas aeruginosa lung infection. APMIS 2005;113:400–9. https://doi.org/10.1111/j.1600-0463.2005.apm_142.x.

[54] Ruan J-L, Lee C, Wouters S, Tullis IDC, Verslegers M, Mysara M, et al. Irradiation at Ultra-High (FLASH) Dose Rates Reduces Acute Normal Tissue Toxicity in the Mouse Gastrointestinal System. Int J Radiat Oncol 2021;111:1250–61. https://doi.org/10.1016/j.ijrobp.2021.08.004.

[55] Diffenderfer ES, Verginadis II, Kim MM, Shoniyozov K, Velalopoulou A, Goia D, et al. Design, Implementation, and in Vivo Validation of a Novel Proton FLASH Radiation Therapy System. Int J Radiat Oncol 2020;106:440–8. https://doi.org/10.1016/j.ijrobp.2019.10.049.

[56] Kim MM, Verginadis II, Goia D, Haertter A, Shoniyozov K, Zou W, et al. Comparison of FLASH Proton Entrance and the Spread-Out Bragg Peak Dose Regions in the Sparing of Mouse Intestinal Crypts and in a Pancreatic Tumor Model. Cancers (Basel) 2021;13:4244. https://doi.org/10.3390/cancers13164244.

[57] Levy K, Natarajan S, Wang J, Chow S, Eggold JT, Loo PE, et al. Abdominal FLASH irradiation reduces radiation-induced gastrointestinal toxicity for the treatment of ovarian cancer in mice. Sci Rep 2020;10:21600. https://doi.org/10.1038/s41598-020-78017-7.

[58] Shi X, Yang Y, Zhang W, Wang J, Xiao D, Ren H, et al. FLASH X-ray spares intestinal crypts from

pyroptosis initiated by cGAS-STING activation upon radioimmunotherapy. Proc Natl Acad Sci 2022;119:e2208506119. https://doi.org/10.1073/pnas.2208506119.

[59] Zhu H, Xie D, Yang Y, Huang S, Gao X, Peng Y, et al. Radioprotective effect of X-ray abdominal FLASH irradiation: Adaptation to oxidative damage and inflammatory response may be benefiting factors. Med Phys 2022;49:4812–22. https://doi.org/10.1002/mp.15680.

[60] Kim K, Kim MM, Skoufos G, Diffenderfer ES, Motlagh SAO, Kokkorakis M, et al. FLASH Proton Radiation Therapy Mitigates Inflammatory and Fibrotic Pathways and Preserves Cardiac Function in a Preclinical Mouse Model of Radiation-Induced Heart Disease. Int J Radiat Oncol 2024;119:1234–47. https://doi.org/10.1016/j.ijrobp.2024.01.224.

[61] Levy K, Natarajan S, Wang J, Chow S, Eggold JT, Loo PE, et al. Abdominal FLASH irradiation reduces radiation-induced gastrointestinal toxicity for the treatment of ovarian cancer in mice. Sci Rep 2020;10:21600. https://doi.org/10.1038/s41598-020-78017-7.

[62] Padilla O, Minns HE, Wei H-J, Fan W, Webster-Carrion A, Tazhibi M, et al. Immune Response following FLASH and Conventional Radiation in Diffuse Midline Glioma. Int J Radiat Oncol 2024;119:1248–60. https://doi.org/10.1016/j.ijrobp.2024.01.219.

[63] Kim Y-E, Gwak S-H, Hong B-J, Oh J-M, Choi H-S, Kim MS, et al. Effects of Ultra-high doserate FLASH Irradiation on the Tumor Microenvironment in Lewis Lung Carcinoma: Role of Myosin Light Chain. Int J Radiat Oncol 2021;109:1440–53. https://doi.org/10.1016/j.ijrobp.2020.11.012.

[64] Konradsson E, Liljedahl E, Gustafsson E, Adrian G, Beyer S, Ilaahi SE, et al. Comparable Long-Term Tumor Control for Hypofractionated FLASH Versus Conventional Radiation Therapy in an Immunocompetent Rat Glioma Model. Adv Radiat Oncol 2022;7:101011. https://doi.org/10.1016/j.adro.2022.101011.

[65] Cao N, Erickson DPJ, Ford EC, Emery RC, Kranz M, Goff P, et al. Preclinical Ultra-High Dose Rate (FLASH) Proton Radiation Therapy System for Small Animal Studies. Adv Radiat Oncol 2024;9:101425. https://doi.org/10.1016/j.adro.2023.101425.

[66] Almeida A, Godfroid C, Leavitt RJ, Montay-Gruel P, Petit B, Romero J, et al. Antitumor Effect by Either FLASH or Conventional Dose Rate Irradiation Involves Equivalent Immune Responses. Int J Radiat Oncol 2024;118:1110–22. https://doi.org/10.1016/j.ijrobp.2023.10.031.

[67] Liljedahl E, Konradsson E, Gustafsson E, Jonsson KF, Olofsson JK, Ceberg C, et al. Long-term anti-

tumor effects following both conventional radiotherapy and FLASH in fully immunocompetent animals with glioblastoma. Sci Rep 2022;12:12285. https://doi.org/10.1038/s41598-022-16612-6.

[68] Liljedahl E, Konradsson E, Linderfalk K, Gustafsson E, Petersson K, Ceberg C, et al. Comparable survival in rats with intracranial glioblastoma irradiated with single-fraction conventional radiotherapy or FLASH radiotherapy. Front Oncol 2024;13:1309174. https://doi.org/10.3389/fonc.2023.1309174.

[69] Shukla S, Saha T, Rama N, Acharya A, Le T, Bian F, et al. Ultra-high dose-rate proton FLASH improves tumor control. Radiother Oncol 2023;186:109741. https://doi.org/10.1016/j.radonc.2023.109741.

[70] Konradsson E, Arendt ML, Bastholm Jensen K, Børresen B, Hansen AE, Bäck S, et al. Establishment and Initial Experience of Clinical FLASH Radiotherapy in Canine Cancer Patients. Front Oncol 2021;11:658004. https://doi.org/10.3389/fonc.2021.658004.

[71] Børresen B, Arendt ML, Konradsson E, Bastholm Jensen K, Bäck SÅ, Munck af Rosenschöld P, et al. Evaluation of single-fraction high dose FLASH radiotherapy in a cohort of canine oral cancer patients. Front Oncol 2023;13:1256760. https://doi.org/10.3389/fonc.2023.1256760.

[72] Gjaldbæk BW, Arendt ML, Konradsson E, Bastholm Jensen K, Bäck SÅJ, Munck af Rosenschöld P, et al. Long-term toxicity and efficacy of FLASH radiotherapy in dogs with superficial malignant tumors. Front Oncol 2024;14:1425240. https://doi.org/10.3389/fonc.2024.1425240.

[73] Rohrer Bley C, Wolf F, Gonçalves Jorge P, Grilj V, Petridis I, Petit B, et al. Dose- and Volume-Limiting Late Toxicity of FLASH Radiotherapy in Cats with Squamous Cell Carcinoma of the Nasal Planum and in Mini Pigs. Clin Cancer Res 2022;28:3814–23. https://doi.org/10.1158/1078-0432.CCR-22-0262.

[74] Vozenin M-C, De Fornel P, Petersson K, Favaudon V, Jaccard M, Germond J-F, et al. The Advantage of FLASH Radiotherapy Confirmed in Mini-pig and Cat-cancer Patients. Clin Cancer Res 2019;25:35–42. https://doi.org/10.1158/1078-0432.CCR-17-3375.

[75] Konradsson E, Arendt ML, Bastholm Jensen K, Børresen B, Hansen AE, Bäck S, et al. Establishment and Initial Experience of Clinical FLASH Radiotherapy in Canine Cancer Patients. Front Oncol 2021;11:658004. https://doi.org/10.3389/fonc.2021.658004.

[76] Børresen B, Arendt ML, Konradsson E, Bastholm Jensen K, Bäck SÅ, Munck af Rosenschöld P, et

al. Evaluation of single-fraction high dose FLASH radiotherapy in a cohort of canine oral cancer patients. Front Oncol 2023;13:1256760. https://doi.org/10.3389/fonc.2023.1256760.

[77] Gjaldbæk BW, Arendt ML, Konradsson E, Bastholm Jensen K, Bäck SÅJ, Munck af Rosenschöld P, et al. Long-term toxicity and efficacy of FLASH radiotherapy in dogs with superficial malignant tumors. Front Oncol 2024;14:1425240. https://doi.org/10.3389/fonc.2024.1425240.

[78] Gaide O, Herrera F, Jeanneret Sozzi W, Gonçalves Jorge P, Kinj R, Bailat C, et al. Comparison of ultra-high versus conventional dose rate radiotherapy in a patient with cutaneous lymphoma. Radiother Oncol 2022;174:87–91. https://doi.org/10.1016/j.radonc.2021.12.045.

[79] Mascia AE, Daugherty EC, Zhang Y, Lee E, Xiao Z, Sertorio M, et al. Proton FLASH Radiotherapy for the Treatment of Symptomatic Bone Metastases. JAMA Oncol 2023;9:62–9. https://doi.org/10.1001/jamaoncol.2022.5843.

[80] Lin B, Huang D, Gao F, Yang Y, Wu D, Zhang Y, et al. Mechanisms of FLASH effect. Front Oncol 2022;12:995612. https://doi.org/10.3389/fonc.2022.995612.

[81] Spiro IJ, Ling CC, Stickler R, Gaskill J. Oxygen Radiosensitisation at Low Dose Rate. Br J Radiol 1985;58:357–63. https://doi.org/10.1259/0007-1285-58-688-357.

[82] Lv Y, Lv Y, Wang Z, Lan T, Feng X, Chen H, et al. FLASH radiotherapy: A promising new method for radiotherapy (Review). Oncol Lett 2022;24:419. https://doi.org/10.3892/ol.2022.13539.

[83] Jansen J, Knoll J, Beyreuther E, Pawelke J, Skuza R, Hanley R, et al. Does FLASH deplete oxygen? Experimental evaluation for photons, protons, and carbon ions. Med Phys 2021;48:3982–90. https://doi.org/10.1002/mp.14917.

[84] Forster JC, Douglass MJJ, Phillips WM, Bezak E. Stochastic multicellular modeling of x-ray irradiation, DNA damage induction, DNA free-end misrejoining and cell death. Sci Rep 2019;9:18888. https://doi.org/10.1038/s41598-019-54941-1.

[85] Jin J-Y, Gu A, Wang W, Oleinick NL, Machtay M, (Spring) Kong F-M. Ultra-high dose rate effect on circulating immune cells: A potential mechanism for FLASH effect? Radiother Oncol 2020;149:55–62. https://doi.org/10.1016/j.radonc.2020.04.054.

[86] Venkatesulu BP, Sharma A, Pollard-Larkin JM, Sadagopan R, Symons J, Neri S, et al. Ultra high dose rate (35 Gy/sec) radiation does not spare the normal tissue in cardiac and splenic models of lymphopenia and gastrointestinal syndrome. Sci Rep 2019;9:17180. https://doi.org/10.1038/s41598-

019-53562-y.

[87] Tang R, Yin J, Liu Y, Xue J. FLASH radiotherapy: A new milestone in the field of cancer radiotherapy. Cancer Lett 2024;587:216651. https://doi.org/10.1016/j.canlet.2024.216651.

[88] Kim B-G, Malek E, Choi SH, Ignatz-Hoover JJ, Driscoll JJ. Novel therapies emerging in oncology to target the TGF-β pathway. J Hematol Oncol 2021;14:55. https://doi.org/10.1186/s13045-021-01053-x.

[89] Zheng Y, Liu X, Li N, Zhao A, Sun Z, Wang M, et al. Radiotherapy combined with immunotherapy could improve the immune infiltration of melanoma in mice and enhance the abscopal effect. Radiat Oncol J 2023;41:129–39. https://doi.org/10.3857/roj.2023.00185.

[90] Jiang X, Wang J, Deng X, Xiong F, Ge J, Xiang B, et al. Role of the tumor microenvironment in PD-L1/PD-1-mediated tumor immune escape. Mol Cancer 2019;18:10. https://doi.org/10.1186/s12943-018-0928-4.

[91] Eggold JT, Chow S, Melemenidis S, Wang J, Natarajan S, Loo PE, et al. Abdominopelvic FLASH Irradiation Improves PD-1 Immune Checkpoint Inhibition in Preclinical Models of Ovarian Cancer. Mol Cancer Ther 2022;21:371–81. https://doi.org/10.1158/1535-7163.MCT-21-0358.

[92] Tang R, Yin J, Liu Y, Xue J. FLASH radiotherapy: A new milestone in the field of cancer radiotherapy. Cancer Lett 2024;587:216651. https://doi.org/10.1016/j.canlet.2024.216651.

[93] Smyth LML, Donoghue JF, Ventura JA, Livingstone J, Bailey T, Day LRJ, et al. Comparative toxicity of synchrotron and conventional radiation therapy based on total and partial body irradiation in a murine model. Sci Rep 2018;8:12044. https://doi.org/10.1038/s41598-018-30543-1.

[94] Zhang Q, Gerweck LE, Cascio E, Gu L, Yang Q, Dong X, et al. Absence of Tissue-Sparing Effects in Partial Proton FLASH Irradiation in Murine Intestine. Cancers (Basel) 2023;15:2269. https://doi.org/10.3390/cancers15082269.

[95] Beyreuther E, Brand M, Hans S, Hideghéty K, Karsch L, Leßmann E, et al. Feasibility of proton FLASH effect tested by zebrafish embryo irradiation. Radiother Oncol 2019;139:46–50. https://doi.org/10.1016/j.radonc.2019.06.024.

[96] Kacem H, Almeida A, Cherbuin N, Vozenin M-C. Understanding the FLASH effect to unravel the potential of ultra-high dose rate irradiation. Int J Radiat Biol 2022;98:506–16. https://doi.org/10.1080/09553002.2021.2004328.

[97] Marcu LG, Bezak E, Peukert DD, Wilson P. Translational Research in FLASH Radiotherapy—From Radiobiological Mechanisms to In Vivo Results. Biomedicines 2021;9:181. https://doi.org/10.3390/biomedicines9020181.

[98] Marcu LG, Bezak E, Peukert DD, Wilson P. Translational Research in FLASH Radiotherapy—From Radiobiological Mechanisms to In Vivo Results. Biomedicines 2021;9:181. https://doi.org/10.3390/biomedicines9020181.

[99] Liljedahl E, Konradsson E, Linderfalk K, Gustafsson E, Petersson K, Ceberg C, et al. Comparable survival in rats with intracranial glioblastoma irradiated with single-fraction conventional radiotherapy or FLASH radiotherapy. Front Oncol 2024;13:1309174. https://doi.org/10.3389/fonc.2023.1309174.

[100] Kim K, Kim MM, Skoufos G, Diffenderfer ES, Motlagh SAO, Kokkorakis M, et al. FLASH Proton Radiation Therapy Mitigates Inflammatory and Fibrotic Pathways and Preserves Cardiac Function in a Preclinical Mouse Model of Radiation-Induced Heart Disease. Int J Radiat Oncol 2024;119:1234–47. https://doi.org/10.1016/j.ijrobp.2024.01.224.

Table 1. Summary of *in vivo* studies demonstrating the effect of FLASH on normal tissues

Irradiated site	Model	FLASH source (energy)	FLASH dose rate (Gy/s)	FLASH dose* (Gy)	Main assessment	Reference
Lung	Mouse	Electrons (4.5 MeV)	60	17 or 30	Pulmonary fibrosis Apoptosis	Favaudon et al. (2014) [14]
Lung	Mouse	Electrons (4.5 MeV)	60	17	Pulmonary fibrosis Cell proliferation RNA sequencing DNA damage Radiation-induced senescence	Fouillade et al. (2020) [33]
Lung	Mouse	Photons (8 MeV)	1200	30	Survival Pathological analysis	Gao et al. (2022) [32]
Lung	Mouse	Photons (1.25 MeV)	200	20 (1 or 10 pulses)	Pathological analysis	Dai et al. (2023) [31]
Brain	Mouse	Electrons (4.5 or 6 MeV)	5.6M†	10	Cognitive function Neurogenesis	Montay-Gruel et al. (2017) [34]
Brain	Mouse	Electrons (6 MeV)	>100	10	Cognitive function Neuroinflammation Neuronal morphology	Montay-Gruel et al. (2019) [36]
Brain	Mouse	Electrons (16 or 20 MeV)	200 or 300	30	Cognitive function Neuroinflammation Neuronal morphology Cytokine assay	Simmons et al. (2019) [41]
Brain	Mouse	Electrons (6 MeV)	2.5K	10 or 25	Cerebrovascular structure Apoptosis within neurogenic regions	Allen et al. (2020) [44]
Brain	Mouse	Electrons (6 MeV)	5.6M	10	Astrogliosis Astrocytic/microglial expression	Montay-Gruel et al. (2020) [35]
Brain	Mouse	Electrons (6 MeV)	2.5K - 7.8M	10, 14, 25‡, 14/4 Fx, 14/2 Fx, or 30/3 Fx,	Cognitive function	Montay-Gruel et al. (2021) [40]
Brain	Mouse	Electrons (6 MeV)	1.6M	30/10 Fx	Long-term potentiation	Limoli et al. (2023) [42]
Brain	Mouse	Photons (102 keV)	37	10	Cognitive function Neurogenesis	Montay-Gruel et al. (2018) [25]

					Neuroinflammation	
Brain	Mouse	146.6 MeV	120	10	Neuroinflammation DNA damage Cerebrovascular structure HMGB1 expression	Dokic et al. (2022) [37]
Brain	Mouse, juvenile	Electrons (6 MeV)	4.4M	8	Cognitive function Neurogenesis Neuroinflammation Pituitary function	Alaghband et al. (2020) [38]
Brain	Mouse, juvenile	Electrons (6 MeV)	5.6M	20/2 Fx	Cognitive function Neuroinflammation Neuronal morphology Long-term potentiation Cerebrovascular structure	Allen et al. (2023) [39]
Brain	Rat	Electrons (10 MeV)	≥429	20-30	Hair loss	Liljedahl et al. (2024) [99]
Brain	Rat	Protons (226 MeV)	257	25	Cognitive function Neuroinflammation Circulating immune cell	Iturri et al. (2023) [43]
Skin	Mouse	Electrons (16 MeV)	180	10-40	Survival Skin damage	Soto et al. (2020) [45]
Skin	Mouse	Protons (250 MeV)	57 or 115	35	Skin damage Leg contracture TGF-β1 expression Cytokine assay	Cunningham et al. (2021) [47]
Skin	Mouse	Protons (230 MeV)	69-124	30	Survival Skin/muscle/bone damage Skin inflammation Lymphedema RNA sequencing TGF-β1 expression	Velalopoulou et al. (2021) [46]
Skin	Mouse	Protons (250 MeV)	71-89	40-60	Skin damage Fibrosis	Sørensen et al. (2022) [49]
Skin	Mouse	Protons (230 MeV)	~130	25-45	Skin damage	Zhang et al. (2023) [48]

Skin	Mouse	Protons (20 MeV)	930	23 or 33	Skin damage Ear swelling/inflammation Cytokine assay	Rudigkeit et al. (2024) [50]
Skin	Mini-pig	Electrons (4.5 or 6 MeV)	~300	22-34	Skin damage	Vozenin et al. (2019) [74]
Skin	Mini-pig	Electrons (6 MeV)	150	31	Skin damage	Rohrer Bley et al. (2022) [73]
Abdomen	Mouse	Electrons (16 MeV)	216	14 or 16	Survival Body weight Intestinal damage Intestinal crypts Stool pellets Apoptosis DNA damage	Levy et al. (2020) [61]
Abdomen	Mouse	Electrons (6 MeV)	2M-6M	5-19.9	Intestinal crypts Fecal microbiome analysis	Ruan et al. (2021) [54]
Abdomen	Mouse	Photons (6 MV)	110-120	13 or 25/5 Fx	Intestinal crypts Fibrosis Immune cell infiltration cGAS-STING activation	Shi et al. (2022) [58]
Abdomen	Mouse	Photons (6 MV)	>150	10 or 15	Survival Body weight Intestinal damage Intestinal crypts Immune cell infiltration Complete blood count Cytokine assay ROS, antioxidant enzyme, and lipid peroxidation response	Zhu et al. (2022) [59]
Abdomen	Mouse	Photons (8 MeV)	700 or 937	12 or 15	Survival Pathological analysis	Gao et al. (2022) [32]
Abdomen	Mouse	Protons (230 MeV)	94 (whole-abdomen) or 63 (focal abdomen)	15 (whole-abdomen) or 18 (focal abdomen)	Intestinal crypts Fibrosis	Diffenderfer et al. (2020) [55]
Abdomen	Mouse	Protons	106.2-108.2	15	Intestinal crypts	Kim et al. (2021) [56]

		(230 MeV)	(SOBP) or 107.1-118.5(entrance region)			
Heart	Mouse	Protons (230 MeV)	122.65	40	DNA damage RNA sequencing TGF-β1/TNF-α/αSMA expression Myocardial fibrosis Echocardiography	Kim et al. (2024) [100]

*Used to assess the effects on normal tissue.

†To investigate the differences in FLASH-induced neuropreservation at various dose rates, intermediate dose rates of 1, 3, 10, 30, 100, and 500 Gy/s were also used.

‡Delivered only to the right hemisphere.

cGAS, cyclic guanosine monophosphate–adenosine monophosphate synthase; HMGB1, high mobility group box protein 1; K, 10^3; M, 10^6; PD-1, programmed death-1; PD-L1, programmed death ligand 1; RNA, ribonucleic acid; ROS, reactive oxygen species; RT, radiotherapy; SMA, smooth muscle actin; SOBP, spread-out Bragg peak; STING, stimulator of interferon genes; TGF, transforming growth factor; TNF, tumor necrosis factor.

Table 2. Clinical experiences with FLASH radiotherapy

Authors	Cancer	Design	Population	No. of patients	Radiation source (energy, dose rate)	Treatment	Main outcomes
Bourhis et al. (2019) [15]	Lymphoma	Case report	Multi-resistant CD30+ T-cell cutaneous lymphoma	1	Electrons (5.6 MeV, 166 Gy/s)	15 Gy in a single fraction	• Follow-up of 5 months • Rapid, complete, and durable tumor response • Grade 1 epithelitis and edema • Intact epidermis and basal membrane • Limited increased vascularization
Gaide et al. (2022) [78]	Lymphoma	Case report	Multi-resistant CD30+ T-cell cutaneous lymphoma	1 (2 sites)	Electrons (5.6 MeV, 166 Gy/s)	15 Gy in a single fraction - Right elbow: 0.08 Gy/s - Left distal arm: 166 Gy/s	• Follow-up of 2 years • Rapid, complete, and durable tumor response • Grade 1 acute epithelitis at both treated sites • Mild late radiodermatitis at both treated sites
Mascia et al. (2023) [79]	Bone metastasis	Prospective Single arm Feasibility study	10 patients with 1-3 symptomatic bone metastases in the extremities (except for the feet, hands, or wrists)	10 (12 sites)	Protons (250 MeV, 51-61 Gy/s)	8 Gy in a single fraction	• Median follow-up of 4.8 months (range, 2.3-13.0) • Average patient time on the treatment couch 18.9 minutes (range, 11-33) • No device-related treatment delays • Transient pain flares (2-9 days post-FLASH) in 4 of the 12 sites (33%) • Pain relief in 8 of the 12 sites (67%) • No pain in 6 of the 12 sites (50%) • No grade ≥3 FLASH-related toxicity

Table 3. Overview of ongoing clinical trials involving FLASH radiotherapy

NCT identifier	Cancer	Design	Population	Estimated enrollment	Radiation source	Treatment	Primary endpoint	Status* (study start date)
NCT04986696	Malignant melanoma	Phase I Non-randomized Dose escalation	Multiple skin metastases PD after systemic treatment	46	Electrons	7 dose levels (22, 24, 26, 28, 30, 32, and 34 Gy in a single fraction)	MTD or RP2D	Recruiting (July 1, 2021)
NCT05524064	Bone metastasis	Phase I Single arm	1-3 symptomatic bone metastasis in the thorax	10	Protons	8 Gy in a single fraction	Toxicity Patient-reported pain relief Pain medication use	Recruiting (March 8, 2023)
NCT05724875	Skin cancer	Phase II Randomized	T1-2N0M0 cutaneous squamous cell carcinoma or basal cell carcinoma	60	Electrons	FLASH-RT vs. Conventional RT (T1, 22 Gy in a single fraction; T2, 30 Gy in 5 fractions)	Skin toxicity (≥ grade 3) Local control rate	Recruiting (June 22, 2023)

*From https://clinicaltrials.gov/ (accessed on July 26, 2024)

MTD, maximum tolerated dose; NCT, National Clinical Trial; PD, progressive disease; RP2D, recommended phase II dose; RT, radiotherapy.