SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 28: 602, 2024

Emergence of FLASH-radiotherapy across the last 50 years (Review)

MENGHUA LI[1,2*], SEN ZHOU[1*], GUOFU DONG[1,2] and CHANGZHEN WANG[1,2]

[1]Beijing Key Laboratory for Radiobiology, Beijing Institute of Radiation Medicine, Beijing 100850, P.R. China;
[2]Department of Biochemistry, Basic Medical College of Anhui Medical University, Hefei, Anhui 230032, P.R. China

Received May 16, 2024; Accepted September 2, 2024

DOI: 10.3892/ol.2024.14735

Abstract. A novel radiotherapy (RT) approach termed FLASH-RT, which irradiates areas at ultra-high dose rates, is of current interest to medical researchers. FLASH-RT can maintain equivalent antitumor effects while sparing healthy tissue compared with conventional RT (CONV-RT), which uses low dose rates. The sparing effect on healthy tissue after FLASH-RT is known as the FLASH effect. Owing to the FLASH effect, FLASH-RT can raise the maximum tolerable dose to control tumor growth or eradicate the tumor and provide a new strategy for clinical RT. However, definitive irradiation conditions for reproducing the FLASH effect and the biological mechanism of the FLASH effect have not yet been fully elucidated. The efficacy of FLASH-RT is controversial despite its successful application in clinical RT. The present review recapitulates the progression of FLASH-RT and critically comments on the hypothesis of the FLASH effect. In addition, the review expounds on the current issues with regard to the differential phenomena between *in vitro* and *in vivo* studies, and elaborates on the challenges for the application of FLASH-RT that need to be addressed in the future.

Contents

Correspondence to: Professor Guofu Dong or Professor Changzhen Wang, Beijing Key Laboratory for Radiobiology, Beijing Institute of Radiation Medicine, 27 Taiping Road, Haidian, Beijing 100850, P.R. China
E-mail: dongguofu052@126.com
E-mail: wangcz2002@aliyun.com

*Contributed equally

Key words: FLASH effect, FLASH-radiotherapy, ultra-high dose rate, ionizing radiation, tumor

1. Introduction

In recent years, due to the large number of cancer cases and cancer-associated deaths, and the burden of rising treatment costs, there has been an urgent need to improve the therapeutic efficacy of malignant tumor treatment techniques. Radiotherapy (RT) is one of the most widely used cancer treatments, playing an irreplaceable role for curable malignancies. Despite killing tumor cells, RT inevitably damages the healthy tissue surrounding the tumor, significantly affecting the prognosis and quality of life of treated patients. Therefore, a critical principle underlying clinical RT dosimetry is maximizing the dose to the target region while minimizing the dose to the adjacent healthy tissue. In the past 30 years, advanced RT techniques, such as intensity-modulated radiation therapy and image-guided radiation therapy, have been used to precisely adjust the dose distribution and improve the efficacy the tumor treatment (1). The use of particles such as protons and heavy ions, which have unique radiophysical and biological properties, as radiation sources, has also led to improvements in RT techniques (2). Despite the advances made in modern RT techniques, owing to the diversity of tumor types, the radioresistance of tumors and the complexity of RT techniques, the development of new high-precision, high-dose, high-efficacy and low-toxicity RT techniques remains a constant endeavor.

FLASH-RT may be a seminal technique due to its low toxicity to healthy tissue and consequent promising clinical RT applications, and has interested researchers worldwide. The term FLASH was first coined by Favaudon *et al* (3) in 2014. The study demonstrated that FLASH-RT could maintain toxicity to the tumor while sparing healthy tissues surrounding the tumor compared with conventional RT (CONV-RT). The mean dose rates of FLASH-RT (typically >40 Gy/sec) are generally several orders of magnitude higher than CONV-RT (typically ≤0.01 Gy/sec). Currently, FLASH-RT has provided exciting results as a new clinical treatment (4). Although FLASH-RT is expected to be a breakthrough in tumor RT, the exact conditions and underlying biological mechanisms behind the emergence of the FLASH effect remain unclear. The present review discusses the factors that may influence the emergence of the FLASH effect and highlights the proposed hypotheses accounting for the mechanism of the FLASH effect.

At the same time, a comparison of *in vivo* and *in vitro* research shows discrepancies in the biological effect of FLASH irradiation. Therefore, the present review retrospectively assesses the *in vivo* and *in vitro* research, and considers the contributions of oxygen concentration and immune response to this discrepancy. Meanwhile, current issues and the future direction of FLASH-RT are discussed to provide a reference for its clinical application.

2. Possible mechanisms responsible for the FLASH effect

Although multiple studies (5,6) have observed the FLASH effect, the underlying biological mechanism responsible for the FLASH effect remains elusive. Some researchers have proposed hypotheses to explain it, such as oxygen depletion and immune regulation. However, these hypotheses have been challenged with the deepening of the FLASH research.

Oxygen depletion hypothesis mechanism. Dewey and Boag (7) originally proposed the oxygen depletion hypothesis based on the oxygen fixation model and the oxygen effect. It is well known that radiation induces ionization of DNA and H_2O, resulting in direct and indirect damage to DNA, which may

$$H_2O \rightarrow e^-_{aq} + H\bullet + \bullet OH + H_2 + H_2O_2 \qquad \text{(Reaction 1)}$$

lead to cell death. The radiolysis of H_2O created radicals such as hydrated electron (e^-_{aq}), •OH, and H• (8).

Following irradiation, DNA radicals (DNA•) caused by

$$DNA\bullet + RSH \rightarrow RS\bullet + DNA \qquad \text{(Reaction 2)}$$

hydroxyl radicals (•OH) reacting with DNA are innocuous in anoxic conditions owing to the reaction with reducing species

$$DNA\bullet + O_2 \rightarrow DNAOO\bullet \qquad \text{(Reaction 3)}$$

such as thiols.

Nevertheless, in the presence of oxygen, the DNA radicals are fixed by oxygen to form irreversible deleterious species.

Reaction 2 and reaction 3 are competitive reactions. As a result, the radiological sensitivity enhances in the presence of oxygen owing to the fixation of DNA damage. This is a simplified illustration of the oxygen fixation model and oxygen effect. FLASH irradiation delivers the total dose over an extremely short duration with an ultra-high instantaneous dose

$$\bullet H + O_2 \rightarrow HO_2\bullet \qquad \text{(Reaction 4)}$$

$$e^-_{aq} + O_2 \rightarrow O_2^-\bullet \qquad \text{(Reaction 5)}$$

rate causing a high initial concentration of radicals. Among these radicals, e^-_{aq} and H• consume bulk oxygen, as a result, forming superoxide anions (O_2^-•) and hydroperoxyl (HO_2•), respectively, leading to local oxygen depletion.

Therefore, in the absence of oxygen, the enhancement of radioresistance improves the cell survival rate in normal tissues. However, owing to low oxygen levels in tumors (hypoxic), FLASH irradiation induces small change in radiosensitivity of tumor. The physical and chemical events of the oxygen depletion hypothesis, incidentally, occur at millisecond timescale (9-13).

This hypothesis seems convincing, as it is difficult for reoxidation to occur during the duration of flash light exposure, typically a millisecond timescale. Therefore, FLASH irradiation induces less DNA insult in hypoxia resulting in an increased survival rate. However, a critical question is whether FLASH irradiation can deplete oxygen or not. Two studies have been conducted to answer this question via constructed computational model and direct measurement, respectively (14,15). The direct measurements of oxygen level showed a small oxygen change in the range of 1-3 mm Hg at a 20-Gy dose, with a dose rate of 270 Gy/sec, suggesting that FLASH irradiation cannot deplete oxygen *in vivo*. Normal tissues *in vivo* are in a physoxic condition with a partial O_2 pressure ranging from 20-50 mmHg (15,16). Consequently, the oxygen depletion hypothesis may not entirely account for the FLASH effect. Given the potential for achieving local oxygen depletion if cells are at low oxygen levels, the consideration that the FLASH effect occurs in hypoxic cells, such as stem cells, as proposed by Pratx and Kapp (9,17), is plausible. The computational model showed that it is possible to achieve local oxygen depletion at a large dose (e.g., 10 Gy) with ultra-high mean dose rates (e.g., 100 Gy/sec) *in vivo* (14). Furthermore, the FLASH effect was observed at the range of 1.6-4.4% oxygen concentration (12.24-33.66 mmHg) via *in vitro* experiments (18).

Although there exists a discrepancy between the computational model and direct measurement, the underlying consensus is that FLASH irradiation is insufficient to deplete oxygen at normoxic conditions, suggesting that the FLASH effect may not be represented *in vitro* (normoxia, ~139 mmHg) (19). This deduction is consistent with the *in vitro* experimental results that there is no significant difference in cell survival rate between FLASH and CONV irradiation. Consequently, we consider that the oxygen depletion hypothesis may be only partly responsible for the FLASH effect. Other mechanisms must exist to explain the FLASH effect. In fact, some studies have pointed out that the interactions among radiochemical radicals are pivotal for explaining the FLASH effect (20-22).

Mechanism of free radical interaction. As early as 1969, Berry *et al* (20) proposed that FLASH irradiation induced a high local initial free radical concentration resulting in radical-radical interaction. As a result, the number of free radicals reduces and subsequently induces less damage to the biomolecule. In addition, as mentioned by Koch (21), the deposited energy followed by electron track is nonhomogeneous and may cause a local high radical density. In high radical density areas, radical-radical interaction can occur. Based on this assertion, the damage to DNA was simulated in a simulation box full of H_2O and O_2 following FLASH irradiation. The results demonstrated that the levels of innoxious non-ROS are higher at FLASH irradiation than at CONV irradiation. In addition, the population of ROS at the initial time of FLASH irradiation is high and rapidly decreases, and is ultimately lower than that at CONV irradiation with time (22). This simulation is consistent with the aforementioned assertion suggesting that the interaction among high-density radicals reduces the free radicals that may damage biomolecules. Moreover, an *in vivo* study also observed lower levels of ROS at FLASH irradiation than at

CONV irradiation (23). Therefore, the interaction of radicals resulting in the reduction of deleterious radicals may be responsible for the FLASH effect.

Immune regulation mechanism. Some studies have suggested that the FLASH effect may relate to immune regulation. Several experiments have shown that FLASH irradiation activates different inflammatory response pathways and induces less activation of gliocytes compared with CONV radiation in the brain (23-25). However, these studies only showed an association between immune regulation and the FLASH effect, not causation, and the tangible mechanism of FLASH radiation resulting in a reduction of inflammation and ultimately causing the FLASH effect remains elusive. Meanwhile, Jin *et al* (26) proposed that FLASH-RT could control normal tissue toxicity and tumors by reducing the killing of circulating immune cells. This relative protection of the immune system allows the body to mitigate the toxicity of radiation to normal cells and achieve tumor control. Nevertheless, this study is only a theoretical simulation and needs to be experimentally verified.

DNA integrity hypothesis. Shi *et al* (6) suggested that the FLASH effect may be related to the integrity of DNA. The deposition of a CONV-RT dose takes hundreds of seconds, which means that some DNA molecules break due to the energy levels before the dose transfer is completed, resulting in partial DNA damage and damage to the integrity of the DNA. This means that during FLASH radiation, DNA breaks and instability rarely occurs until dose delivery is complete. On the other hand, genomic instability has been considered as a marker of cancer for more than a decade (27). Therefore, in tumor cells, even in FLAS-RT, due to the inherent instability of the genome, a large amount of tumor cell DNA damage will be caused, so as to achieve the same tumor killing effect as CONV-RT.

In conclusion, the proposed hypotheses tried to explain the FLASH effect from different perspectives. However, none can fully explain the mechanism of the FLASH effect. It is possible that the FLASH effect depends on the combination of all mechanisms. For demonstrating these hypotheses, practical validation experiments are indispensable.

3. Differences between *in vitro* and *in vivo* experiments

The previous fundamental studies of FLASH-RT are chiefly in *vitro* studies, in which the observed phenomena are quite different from those in *in vivo* studies (Tables SI and SII). Through examination of these studies, it can be observed that there is no difference in cell survival between FLASH and CONV irradiation, suggesting that the FLASH effect cannot occur at the cell level. However, nearly all *in vivo* studies observed the FLASH effect in a variety of animal models (5,6,18,28). What caused this differential phenomenon and why the FLASH effect emerged in a number of *in vivo* studies is worth considering. Therefore, the *in vitro* and *in vivo* studies were we retrospectively assessed to decipher the potential reason responsible for this.

In vitro research. Several reviews have meticulously depicted the *in vitro* results of FLASH irradiation (29-32). Given that, the present review aims to systematically list *in vitro* studies with experimental conditions, assay endpoint and the occurrence of the FLASH effect to dissect the regular patterns that produce the FLASH effect. Table SI shows that the assay endpoint of irradiation-induced biological effects is frequently characterized by cell colony formation, DNA double-strand breaks (DSBs) and cell arrest. Although there are differences in physical irradiation parameters, the biological effects are generally consistent in these studies.

A cell colony assay is considered a gold standard to validate the radiobiological effect of irradiation in *in vitro* studies. There was no difference in cell colony formation rate between FLASH and CONV irradiation in most studies (33-41). However, a few studies reported that the cell survival rate at FLASH irradiation was higher than at CONV irradiation (7,18,20,28). Notably, one of these studies showed the significance of cell survival rate between FLASH and CONV irradiation only in specific O_2 conditions (1% O_2 in N_2) (7). In addition, physical irradiation parameters and cell lines were similar between the study by Fouillade *et al* (28) and that by Beddok *et al* (38); however, they attained discrepant results, suggesting that the emergence of the FLASH effect was volatile *in vitro*.

Furthermore, the volatility of DSBs in some studies also hinted that the FLASH effect was not ubiquitous *in vitro* (28,37,39,42,43). Notably, with one exception (43), other studies indicated that the number of DSBs was significantly different between FLASH and CONV irradiation in the normal cell line, whereas the number of DBSs produced by the two irradiations was not significantly different in tumour cell lines (Fig. 1).

Consequently, we could speculate that FLASH irradiation, relative to CONV irradiation, induces less molecule damage in normal cells, but has equivalent toxicity in tumor cells. This phenomenon is analogous to what was observed in animal models (Table SII), suggesting that the FLASH effect seems only occur at the molecular level in normal cells rather than in tumor cells *in vitro*. One study showed no difference in DSBs between FLASH and CONV irradiation in a normal cell line (43). It is plausible that different irradiation sources may account for this result. FLASH proton irradiation with high linear energy transfer (LET) and relative biological effectiveness may induce a biological effect equivalent to low LET CONV X-ray irradiation.

As aforementioned, a regular pattern can be observed in which the FLASH effect barely occurs at the cellular level and only occurs in normal cells rather than tumor cells at the molecular level *in vitro*. According to the aforementioned hypotheses, the disappearance of the FLASH effect at cellular level can be attributed to the oxygen concentration being too high to deplete oxygen *in vitro*. However, there are still some uncertainties remaining: i) the reason for the sparing effect at the molecular level in normal cells; ii) the reason that FLASH irradiation cannot magnify the FLASH effect from molecular level to cellular level; and iii) what is responsible for the discrepant results between normal and tumor cells at the molecular level. The present review attempts to answer these questions based on known experimental results and hypotheses. For point i), we consider that the interaction among radicals plays a vital role, since the oxygen depletion

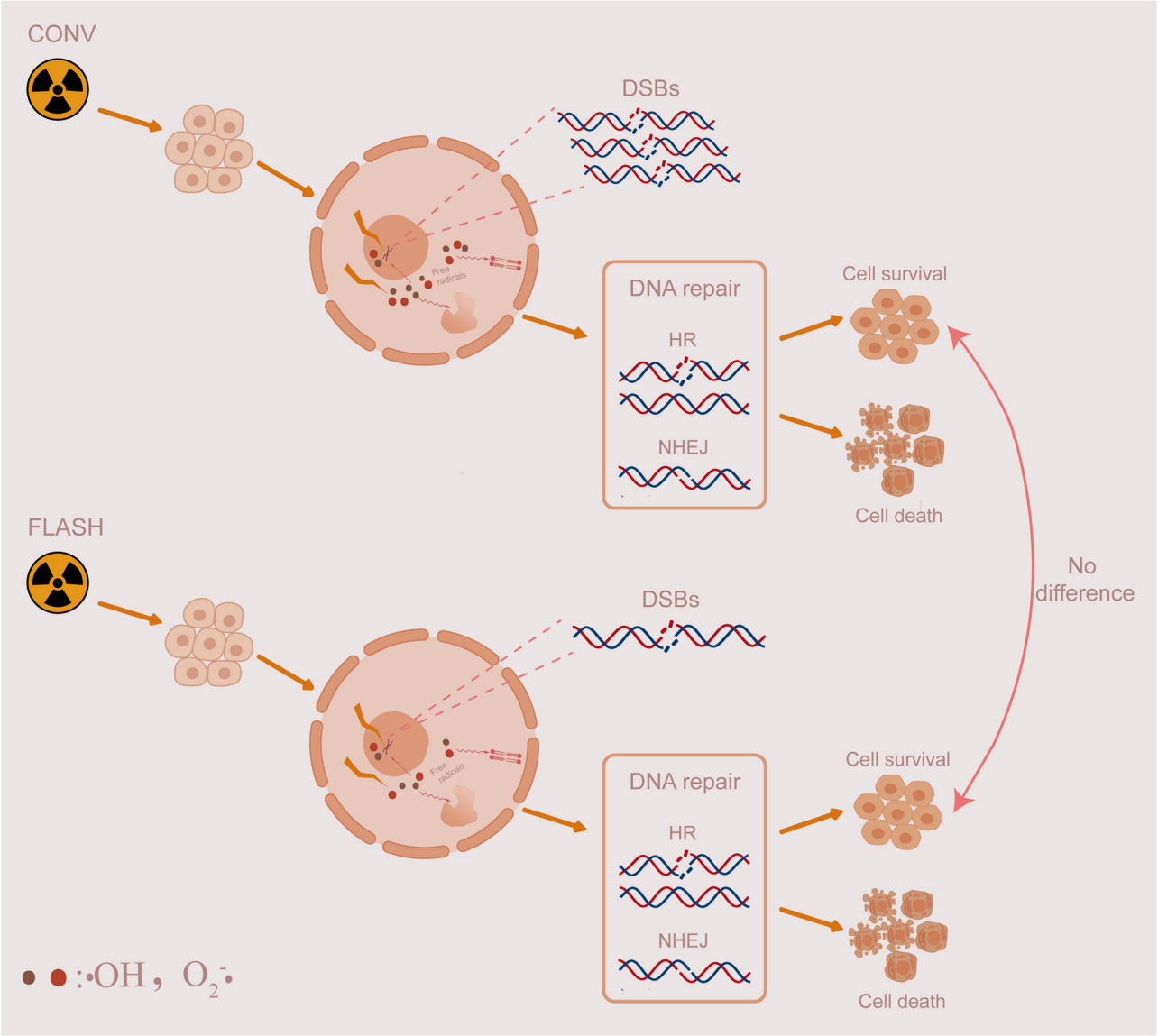

Figure 1. Differences in DNA damage induced by FLASH and CONV irradiation. In normal cell lines, there is a significant difference in DSBs between FLASH and CONV irradiation, with CONV irradiation causing more DSBs. Radiation-induced DNA damage triggers a DNA repair pathway consisting of NHEJ and HR to repair DNA damage. Incorrect and unrepaired DNA damage leads to cell death due to chromosome aberration, and there is no difference in cell survival rate between FLASH and CONV irradiation. CONV, conventional; DSB, double-strand break; NHEJ, non-homologous end joining; HR, homologous recombination.

hypothesis is not eligible *in vitro*. The reduction of deleterious radicals inducing lower DSBs level is persuasive.

For point ii), there are no experimental results or proposed hypotheses for reference. Therefore, an explanation must be proposed via the theoretical mechanism. DNA damage is generally considered the central cause of cell death after irradiation, especially after clinical RT doses. Undeniably, irradiation-induced cell death is comprised of other pathways, such as membrane-dependent signaling pathways and bystander responses, which are associated with oxidative damage to all biomolecules (nucleic acids, proteins and lipids) (44-47). In addition, DNA damage induced by irradiation triggers the DNA repair pathways consisting of non-homologous end joining and homologous recombination to repair DNA lesions. Incorrect and unrepaired DNA lesions cause chromosomal aberrations related to cell death (48). In this respect, a previous study indicated no sparing effect in chromosomal aberrations after FLASH and CONV irradiation (49). Consequently, we consider that the process of DNA repair and oxidative stress responses may diminish the sparing effect from the molecular level to a cellular effect (Fig. 1).

For point iii), given that normal cells and tumor cells are in the same context with regard to culture conditions *in vitro*, the oxygen depletion hypothesis does not seem suitable to explain the discrepant results in DSBs between normal and tumor cells at the molecular level. However, oxidative stress caused by radiation may be associated with the discrepancy. Tumor cells, relative to normal cells, display higher background levels of ROS and have robust Fenton-type reactions, which could magnify the production of ROS (50,51). In addition, according to the aforementioned hypotheses, FLASH irradiation induces

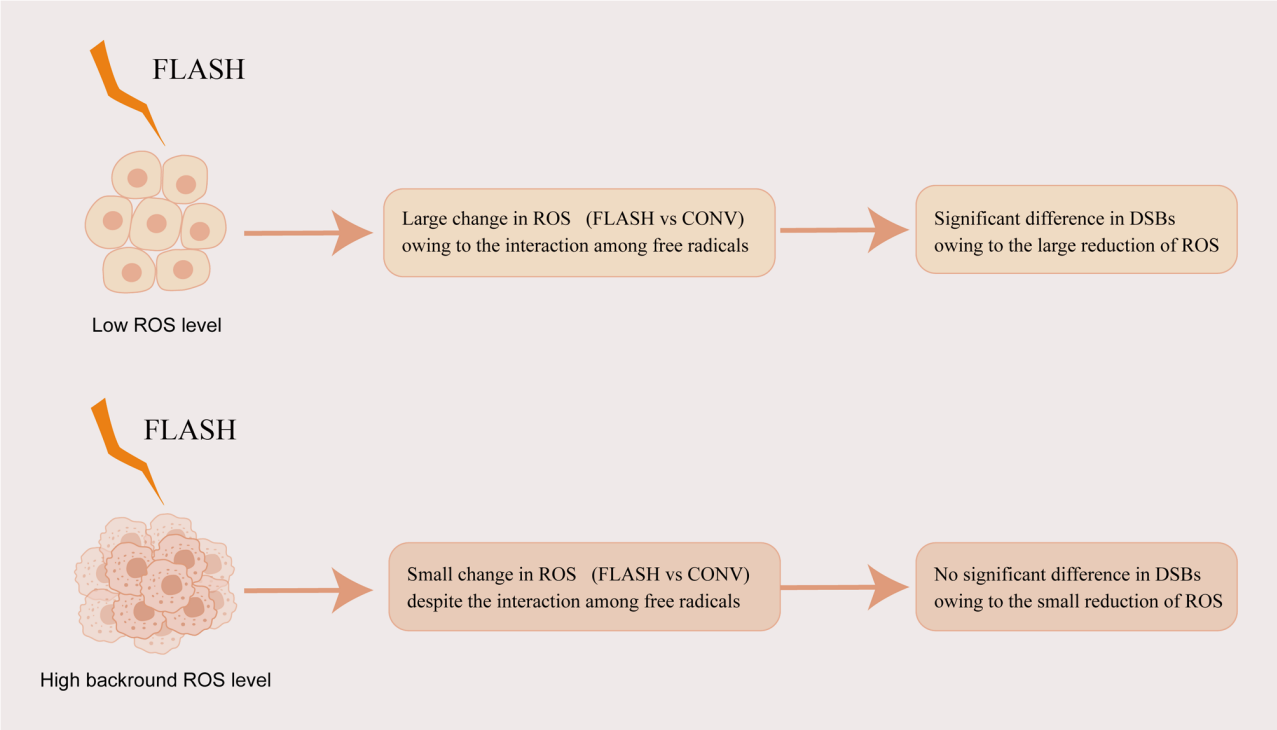

Figure 2. Different background levels of ROS cause the differences in radiation-induced DSBs. Compared with normal cells, tumor cells show higher ROS background levels, and so the decrease of ROS induced by FLASH irradiation is only a small change in relation to the high level of ROS in tumor cells. By contrast, the decrease of ROS induced by FLASH irradiation is a big change in normal cells. CONV, conventional; DSB, double-strand break; ROS, reactive oxygen species.

a lower level of ROS owing to the interaction among free radicals compared with CONV irradiation. Therefore, the reduction of ROS induced by FLASH irradiation may be a small change in tumor cells due to the high background level of ROS. By contrast, the reduction of ROS induced by FLASH irradiation is a large change in normal cells. Consequently, the small change of ROS is insufficient to cause the significant difference in DSBs in tumor cells, which is different from normal cells (Fig. 2).

Although the present review observes and illustrates the regular pattern of FLASH effects produced *in vitro* and proposes mechanistic insights to explain this pattern, it is essential to point out that this pattern is not rigorous owing to the limited experimental results. Further studies are indispensable to validate the accuracy of this pattern.

In vivo research. Unlike the ambiguous results *in vitro*, the FLASH effect ubiquitously occurs in animal models after FLASH irradiation. Currently, in various animal models (e.g., mice, zebrafish, pigs and cats), the FLASH effect has been reported to occur in the central nervous system, lung, intestines and skin (3,6,23,52,53).

The study of FLASH irradiation *in vivo* commenced in the study by Favaudon *et al* (3). The study observed lung fibrosis formation and lung tumor survival after FLASH and CONV electron irradiation. The results indicated that FLASH irradiation induced less lung fibrosis than CONV irradiation. By contrast, FLASH and CONV irradiation possessed comparable tumour killing capacity, suggesting that FLASH irradiation diminishes the insult to normal tissues, and reduces the incidence and severity of post-irradiation complications (3). A further study indicated that FLASH irradiation protected lung progenitor cells from excessive damage, and induced less cell senescence and DNA damage in the lung. However, the FLASH effect disappeared in Terc$^{-/-}$ mice, indicating that *Terc* may be an important gene target for FLASH irradiation to alleviate lung injury (28). Another study also indicated that FLASH irradiation could attenuate the radiation-induced insult to intrathoracic tissues (54). This study demonstrated that use of high energy X-rays as a FLASH irradiation source for sparing normal tissue is technically feasible.

Montay-Gruel *et al* (23-25,55,56) from Lausanne University Hospital, Switzerland, focused on the phenotypic and molecular characteristics of the brain exposed to FLASH irradiation, and united other institutions to validate the FLASH effect on the brain. Severe brain damage, cognitive deficits and neurogenic decompensation at the mean dose rate of ≤30 Gy/sec were observed in normal mouse brains at a 10-Gy dose. However, at higher dose rates, especially those >100 Gy/sec, the cognitive function was spared, suggesting that the sparing effect was more pronounced at higher dose rates. Subsequently, pulsed X-rays were verified as a FLASH irradiation source that could help retain cognitive memory capacity (24). The study indicated that the sparing of cognitive function is associated with the reduction in ROS and that following the relief of oxidative stress, it limits microglial activation and attenuates neuroinflammatory responses (23). Moreover, the difference in astrogliosis and neuroinflammatory responses between FLASH and CONV irradiation may contribute to the sparing effect in the brain. FLASH

irradiation did not trigger TLR4 expression and astrogliosis compared with CONV irradiation, although both irradiations induced the activation of the complement cascade (25). Normal mice were used as the subjects in the aforementioned studies, which cannot indicate that FLASH irradiation possesses the same antitumour effect as CONV irradiation; therefore, Montay-Gruel *et al* (56) established an orthotopic murine glioblastoma model and simulated clinical fractioned RT to investigate the feasibility of FLASH-RT. The results showed that fractioned FLASH-RT could delay tumor growth similar to fractioned CONV-RT, and the FLASH effect was more pronounced after the delivery of hypo-fractionated regimens (56). Other studies indicated that the reduction of ROS, the neuroinflammatory response, the retention of microvascular integrity and dendritic spine density, the reduction of toxicity to the endocrine system and the decrease of apoptosis in neurogenic regions may be related to the sparing of cognition after FLASH irradiation to a substantial degree (57-59).

In addition to the brain and thorax, other anatomical sites of mice, such as the abdomen (54,60-62), skin (63,64) and even the whole body (65), were probed after FLASH irradiation. A series of sparing phenomena, such as the retention of gastrointestinal function, the low mortality of stem cells, the attenuation of the gastrointestinal syndrome, the proliferation of intestinal crypt cells and the high survival rates of mice, were observed after abdominal FLASH irradiation. Moreover, mice with pancreatic flank tumors subjected to abdominal FLASH and CONV irradiation showed iso-efficient tumor delay, suggesting that the FLASH effect can occur in the abdomen (62). Compared with CONV irradiation, electron and proton FLASH irradiation induced lower toxicity to the skin (63,64). Whole-body FLASH irradiation induced less toxicity to hematopoietic stem cells in immunocompromised mice, suggesting that FLASH-RT may be well applied to the treatment of acute lymphoid leukemia (65).

For translation to clinical applications, large mammals such as mini-pig, cat and canine patients subjected to FLASH irradiation were used to evaluate the safety and feasibility of FLASH-RT (52,66). The overall results indicated that FLASH irradiation induced lower toxicity to the normal tissues, with slight side effects, and inhibited tumor growth, suggesting that the FLASH effect can also occur in higher mammals (52).

Although most studies revealed the FLASH effect was ubiquitous *in vivo*, some studies failed to observe the FLASH effect at all (40,53,54,67). For example, in one study, at a dose rate of 35 Gy/sec, the electron FLASH irradiation induced more mucosal damage in the gastrointestinal tract compared with CONV irradiation, suggesting the reproducibility of the FLASH effect may be more stringent for the dose rate range (40). Furthermore, the FLASH effect may be concealed by the high dose of FLASH and CONV irradiation, and the radioresistance of different tissues (54).

The present review summarizes the *in vivo* research data consisting of assay conditions and physical irradiation parameters in Table SII. In contrast to the ambiguous results *in vitro*, the FLASH effect could be produced by different adapted irradiation parameters in the majority of *in vivo* research. It is worth considering what accounts for this discrepant phenomenon between *in vitro* and *in vivo* studies. As aforementioned, the oxygen level is much lower *in vivo* in comparison with that *in vitro*, which may be sufficient to achieve oxygen depletion, resulting in the FLASH effect. In addition, *in vivo* studies, relative to *in vitro* studies, are implemented in the systematic organism encompassing complex interactions of all tissues. Therefore, as researchers have found, immune responses may also be responsible for the discrepancy.

4. Factors influencing the FLASH effect

We attempt to explore the occurrence patterns of the FLASH effect through currently published studies. Physical irradiation parameters, such as the mean dose rate, instantaneous dose rate, pulse width, total exposure time, pulse repetition frequency, total dose and fractioned dose, are noted to be different between FLASH and CONV irradiation, which is presumably responsible for the occurrence of the FLASH effect. In addition, the levels of oxygen may also be a key factor contributing to it.

The mean dose rate is a critical physical parameter to distinguish between CONV and FLASH irradiation. Therefore, the mean dose rate is a key factor influencing the occurrence of the FLASH effect. For example, in a previous study, the proportion of normal human lung fibroblast senescent cells decreased with increasing irradiation dose rates (39). A gradient of mean dose rates of irradiation showed that 30 Gy/sec was the threshold for displaying the sparing effect (55). However, the minimum mean dose rate representing the FLASH effect is uncertain, although most studies define FLASH irradiation with a mean dose rate of ≥40 Gy/sec, as initially stated by Favaudon *et al* (3). We consider that the scope of the mean dose rate could potentially be extended. There are some pieces of evidence to support this consideration. In one study, at a dose rate of 37 Gy/sec, the sparing effect of cognitive function was observed (24). Another study simulated veritable biological responses after FLASH irradiation in animal models and deduced that the minimum dose rate to display the FLASH effect was 57 Gy/sec, which is close to the mean dose rate applied in various preclinical experiments (68). As alluded to here, the occurrence of the FLASH effect demands an adapted mean dose rate. However, a solid minimum mean dose rate is not currently attainable owing to the limited data available.

Researchers are realizing that it is not rigorous to define FLASH irradiation by the mean dose rate alone for further research. Other physical irradiation parameters, such as the total exposure time and the instantaneous dose rate, may be equally important as the mean dose rate. One review has elegantly indicated that the FLASH effect is associated with the combination of relevant parameters, such as the number of pulses, instantaneous dose rate and total exposure time (<200 msec) (69). In addition, the fractionated dose may be an important factor influencing the FLASH effect. In traditional clinical RT regimens, a fractionated dose (<10 Gy) is usually used. However, most *in vivo* studies performed FLASH irradiation at single doses of ≥10 Gy. Given this, a study simulated clinical RT to explore whether the FLASH effect occurs in the fractioned FLASH-RT regimen or not (56). Results showed that the benefits of FLASH-RT were more pronounced in the hypo-fractionated RT regimens (7-10 Gy), while the FLASH effect was not observed when the single dose was too large (14 Gy).

In addition to the physical parameters of FLASH irradiation, the level of oxygen also affects the occurrence of the FLASH effect. As aforementioned, the sparing effect is ubiquitous *in vivo* rather than *in vitro*. The current review has shown that the level of oxygen plays an important role in this discrepancy. Another review also has discussed the importance of oxygen, which may be responsible for this discrepant phenomenon (70). Therefore, we consider the level of O_2 may be directly associated with the FLASH effect.

5. Challenges and prospects

The investigation into FLASH irradiation is a treasure trove that has yet to be mined fully. Despite the fact that the FLASH effect has been demonstrated in a number of studies, it is still a very young research field, and there are many challenges to overcome from the pre-clinical research to clinical practice. Several issues still need to be addressed to bring FLASH radiation to maturity. For example: i) The reproducibility conditions for the FLASH effect are not entirely clear. As aforementioned, certain studies have failed to observe this phenomenon (40,43,53,65). We consider that the key parameters that would reproduce the FLASH effect in these studies are not being used at the required standards. Therefore, the FLASH effect cannot be observed. To best investigate the mechanism of the FLASH effect, the key factors that may influence the occurrence of the FLASH effect must be figured out. ii) There are still technical challenges to achieving FLASH dose rates with clinically modified equipment. The majority of studies use clinical linear electron accelerators retrofitted to emit FLASH electron beams, owing to the lack of difficulty in developing such retrofits. However, the electron beam can only treat superficial tumors due to its limited dose distribution at depth (71). Future treatment of deep tumors will require FLASH X-rays or proton beams; however, there are technical challenges to be resolved to prepare clinical equipment capable of delivering FLASH X-rays. Currently, the apparatus producing FLASH X-rays are only available at large facilities, such as the European Synchrotron Radiation Facility in Europe (24) and the Platform for Advanced RT Research in China (54). When treating tumors with a proton beam, the beam must be scattered or scanned to cover the target volume. However, the scanning may reduce the dose rate and ultimately cannot trigger the FLASH effect (72). Therefore, beam flow systems, scanning speeds and monitoring of ionization chambers available for FLASH proton RT systems also need to be modified (73). iii) FLASH dosimetry and dose monitoring systems need to be improved. Given that FLASH irradiation delivers a dose in an instant period, there is a clinical need for monitoring systems that can achieve real-time monitoring of FLASH irradiation dose. It is vital to develop methods and dose monitoring systems for accurately measuring the delivered dose of FLASH irradiation. Several studies have made progress in this area (74-77). McManus *et al* (74) demonstrated that conventional alanine dosimeters, film dosimeters and pyroelectric dosimeters are all suitable for absolute dose measurements of FLASH irradiation. Jorge *et al* (75) and Petersson *et al* (76) developed an empirical ionization chamber model for FLASH radiation dosimetry. However, the dose monitoring is inaccurate, when the dose rate is too high in each pulse. Therefore, further development of appropriate ionization chambers and empirical models is needed. In addition, Oraiqat *et al* (77) established an image-guided approach for the real-time measurement of deep tissue doses during FLASH-RT. Although these studies have built the foundation for a clinical shift to FLASH-RT, the accuracy of related technologies remains to be verified. iv) The safety of FLASH-RT requires further validation. Although the first FLASH-RT patient was treated well (4), the treatment of superficial tumors alone is not sufficient for clinical RT. One clinical study alone is not representative, and the feasibility and safety of FLASH-RT need to be verified in the future to ensure as much safety as possible. v) The mechanism of the FLASH effect still needs to be experimentally investigated. The role of the oxygen depletion hypothesis and other hypotheses in the FLASH effect, and the relationship between these hypotheses, still require further study.

To conclude, researchers in previous studies tended to primarily devise irradiation parameters based on average dose rate during experimental design, ignoring significant physical parameters, such as instantaneous dose rate, pulse width and radiation dose fractionation. The optimization of radiation parameters is crucial for the future application of FLASH technology. Therefore, researchers must delve deeper into the rationality of physical irradiation parameters and endeavor to devise experiments that encompass a variety of these parameters. This will facilitate a clearer understanding of the conditions under which the FLASH effect manifests. At the same time, researchers must not overlook the long-term consequences of FLASH irradiation in future investigations, as they are intricately linked to the prognosis of patients in potential clinical applications. The FLASH-RT could be a revolutionary advancement in clinical RT in the future. Some radiation-resistant tumors that require larger doses for treatment would be well treated via FLASH-RT, which could provide a high dose threshold to overcome excessive toxicity to healthy tissues surrounding tumors. Furthermore, preclinical studies have shown that all irradiation sources, such as electron beams, X-rays and proton beams, can achieve the FLASH effect, suggesting the universality of FLASH-RT in the future. In terms of laboratory and preclinical research, it is advisable to embark on studies focusing on the integration of FLASH-RT in tumor RT with immunotherapy, with the aim of thoroughly elucidating the underlying mechanisms of FLASH-RT and assessing the potential value of this combined therapeutic approach. In summary, this comprehensive review, embracing pivotal research and pertinent overviews associated with FLASH-RT, serves as a valuable resource for a profound exploration of the mechanisms underlying the diverse applications of this technology.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

Not applicable.

Authors' contributions

SZ and MHL conceived the study, searched the literature and wrote the original manuscript. MHL, GFD, and CZW participated in the writing and subsequent revision of the article. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Yi J, Huang X, Gao L, Luo J, Zhang S, Wang K, Qu Y, Xiao J and Xu G: Intensity-modulated radiotherapy with simultaneous integrated boost for locoregionally advanced nasopharyngeal carcinoma. Radiat Oncol 9: 56, 2014.
2. Luhr A, von Neubeck C, Pawelke J, Seidlitz A, Peitzsch C, Bentzen SM, Bortfeld T, Debus J, Deutsch E, Langendijk JA, *et al*: 'Radiobiology of Proton Therapy': Results of an International expert workshop. Radiother Oncol 128: 56-67, 2018.
3. Favaudon V, Caplier L, Monceau V, Pouzoulet F, Sayarath M, Fouillade C, Poupon MF, Brito I, Hupé P, Bourhis J, *et al*: Ultrahigh dose-rate FLASH irradiation increases the differential response between normal and tumor tissue in mice. Sci Transl Med 6: 245ra93, 2014.
4. Bourhis J, Sozzi WJ, Jorge PG, Gaide O, Bailat C, Duclos F, Patin D, Ozsahin M, Bochud F, Germond JF, *et al*: Treatment of a first patient with FLASH-radiotherapy. Radiother Oncol 139: 18-22, 2019.
5. Dai Y, Liang R, Wang J, Zhang J, Wu D, Zhao R, Liu Z and Chen F: Fractionated FLASH radiation in xenografted lung tumors induced FLASH effect at a split dose of 2 Gy. Int J Radiat Biol 99: 1542-1549, 2023.
6. Shi X, Yang Y, Zhang W, Wang J, Xiao D, Ren H, Wang T, Gao F, Liu Z, Zhou K, *et al*: FLASH X-ray spares intestinal crypts from pyroptosis initiated by cGAS-STING activation upon radioimmunotherapy. Proc Natl Acad Sci USA 119: e2208506119, 2022.
7. Dewey DL and Boag JW: Modification of the oxygen effect when bacteria are given large pulses of radiation. Nature 183: 1450-1451, 1959.
8. Land EJ: Pulse radiolysis and flash photolysis: Some applications in biology and medicine. Biochimie 62: 207-221, 1980.
9. Pratx G and Kapp DS: A computational model of radiolytic oxygen depletion during FLASH irradiation and its effect on the oxygen enhancement ratio. Phys Med Biol 64: 185005, 2019.
10. Becker D and Sevilla MD: 3 - The Chemical Consequences of Radiation Damage to DNA: Advances in Radiation Biology. Elsevier, 1993: 121-80.
11. Alper T and Howard-Flanders P: Role of oxygen in modifying the radiosensitivity of E. coli B. Nature 178: 978-979, 1956.
12. Van den Heuvel F, Vella A, Fiorini F, Brooke M, Hill MA and Maughan T: Incorporating oxygenation levels in analytical DNA-damage models-quantifying the oxygen fixation mechanism. Phys Med Biol 66: 145005, 2021.
13. Wilson JD, Hammond EM, Higgins GS and Petersson K: Ultra-High dose rate (FLASH) radiotherapy: Silver bullet or fool's gold?. Front Oncol 9: 1563, 2020.
14. Petersson K, Adrian G, Butterworth K and McMahon SJ: A quantitative analysis of the role of oxygen tension in FLASH radiation therapy. Int J Radiat Oncol Biol Phys 107: 539-547, 2020.
15. Cao X, Zhang R, Esipova TV, Allu SR, Ashraf R, Rahman M, Gunn JR, Bruza P, Gladstone DJ, Williams BB, *et al*: Quantification of oxygen depletion during FLASH irradiation in vitro and in vivo. Int J Radiat Oncol Biol Phys 111: 240-248, 2021.
16. McKeown SR: Defining normoxia, physoxia and hypoxia in tumours-implications for treatment response. Br J Radiol 87: 20130676, 2014.
17. Pratx G and Kapp DS: Ultra-High-Dose-Rate FLASH irradiation may spare hypoxic stem cell niches in normal tissues. Int J Radiat Oncol Biol Phys 105: 190-192, 2019.
18. Adrian G, Konradsson E, Lempart M, Bäck S, Ceberg C and Petersson K: The FLASH effect depends on oxygen concentration. Br J Radiol 93: 20190702, 2020.
19. Keeley TP and Mann GE: Defining physiological normoxia for improved translation of cell physiology to animal models and humans. Physiol Rev 99: 161-234, 2019.
20. Berry RJ, Hall EJ, Forster DW, Storr TH and Goodman MJ: Survival of mammalian cells exposed to X rays at ultra-high dose-rates. Br J Radiol 42: 102-107, 1969.
21. Koch CJ: Re: Differential impact of FLASH versus conventional dose rate irradiation: Spitz *et al*: Radiother Oncol 139: 62-63, 2019.
22. Abolfath R, Grosshans D and Mohan R: Oxygen depletion in FLASH ultra-high-dose-rate radiotherapy: A molecular dynamics simulation. Med Phys 47: 6551-6561, 2020.
23. Montay-Gruel P, Acharya MM, Petersson K, Alikhani L, Yakkala C, Allen BD, Ollivier J, Petit B, Jorge PG, Syage AR, *et al*: Long-term neurocognitive benefits of FLASH radiotherapy driven by reduced reactive oxygen species. Proc Natl Acad Sci USA 116: 10943-10951, 2019.
24. Montay-Gruel P, Bouchet A, Jaccard M, Patin D, Serduc R, Aim W, Petersson K, Petit B, Bailat C, Bourhis J, *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 129: 582-588, 2018.
25. Montay-Gruel P, Markarian M, Allen BD, Baddour JD, Giedzinski E, Jorge PG, Petit B, Bailat C, Vozenin MC, Limoli C and Acharya MM: Ultra-High-Dose-Rate FLASH irradiation limits reactive gliosis in the brain. Radiat Res 194: 636-645, 2020.
26. Jin JY, Gu A, Wang W, Oleinick NL, Machtay M and Spring Kong FM: Ultra-high dose rate effect on circulating immune cells: A potential mechanism for FLASH effect?. Radiother Oncol 149: 55-62, 2020.
27. Hanahan D: Hallmarks of Cancer: New Dimensions. Cancer Discov 12: 31-46, 2022.
28. Fouillade C, Curras-Alonso S, Giuranno L, Quelennec E, Heinrich S, Bonnet-Boissinot S, Beddok A, Leboucher S, Karakurt HU, Bohec M, *et al*: FLASH irradiation spares lung progenitor cells and limits the incidence of radio-induced senescence. Clin Cancer Res 26: 1497-1506, 2020.
29. Marcu LG, Bezak E, Peukert DD and Wilson P: Translational Research in FLASH radiotherapy-from radiobiological mechanisms to in vivo results. Biomedicines 9: 181, 2021.
30. Esplen N, Mendonca MS and Bazalova-Carter M: Physics and biology of ultrahigh dose-rate (FLASH) radiotherapy: A topical review. Phys Med Biol 65: 23TR03, 2020.
31. Borghini A, Vecoli C, Labate L, Panetta D, Andreassi MG and Gizzi LA: FLASH ultra-high dose rates in radiotherapy: Preclinical and radiobiological evidence. Int J Radiat Biol 98: 127-135, 2022.
32. Omyan G, Musa AE, Shabeeb D, Akbardoost N and Gholami S: Efficacy and toxicity of FLASH radiotherapy: A systematic review. J Cancer Res Ther 16: 1203-1209, 2020.
33. Tillman C, Grafstrom G, Jonsson AC, Jönsson BA, Mercer I, Mattsson S, Strand SE and Svanberg S: Survival of mammalian cells exposed to ultrahigh dose rates from a laser-produced plasma x-ray source. Radiology 213: 860-865, 1999.
34. Shinohara K, Nakano H, Miyazaki N, Tago M and Kodama R: Effects of single-pulse (<=1 ps) X-rays from laser-produced plasmas on mammalian cells. J Radiat Res 45: 509-514, 2004.
35. Auer S, Hable V, Greubel C, Drexler GA, Schmid TE, Belka C, Dollinger G and Friedl AA: Survival of tumor cells after proton irradiation with ultra-high dose rates. Radiat Oncol 6: 139, 2011.

36. Doria D, Kakolee KF, Kar S, Litt SK, Fiorini F, Ahmed H, Green S, Jeynes JCG, Kavanagh J, Kirby D, *et al*: Biological effectiveness on live cells of laser driven protons at dose rates exceeding 109Gy/s. AIP Advances 2: 011209, 2012.
37. Laschinsky L, Baumann M, Beyreuther E, Enghardt W, Kaluza M, Karsch L, Lessmann E, Naumburger D, Nicolai M, Richter C, *et al*: Radiobiological effectiveness of laser accelerated electrons in comparison to electron beams from a conventional linear accelerator. J Radiat Res 53: 395-403, 2012.
38. Beddok A, Fouillade C, Quelennec E nad Favaudon V: OC-0030:: In vitro study of FLASH vs. conventional dose-rate irradiation: Cell viability and DNA damage repair. Radiotherapy and Oncology 123: S9-S10, 2017.
39. Buonanno M, Grilj V and Brenner DJ: Biological effects in normal cells exposed to FLASH dose rate protons. Radiother Oncol 139: 51-55, 2019.
40. Venkatesulu BP, Sharma A, Pollard-Larkin JM, Sadagopan R, Symons J, Neri S, Singh PK, Tailor R, Lin SH and Krishnan S: 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 9: 17180, 2019.
41. Kiefer J and Ebert M: The effect of ultra-high dose-rate beta-ray irradiation in aerobic and hypoxic conditions on the survival of diploid yeast. Biophysik 6: 271-274, 1970.
42. Zlobinskaya O, Dollinger G, Michalski D, Hable V, Greubel C, Du G, Multhoff G, Röper B, Molls M and Schmid TE: Induction and repair of DNA double-strand breaks assessed by gamma-H2AX foci after irradiation with pulsed or continuous proton beams. Radiat Environ Biophys 51: 23-32, 2012.
43. Hanton F, Chaudhary P, Doria D, Gwynne D, Maiorino C, Scullion C, Ahmed H, Marshall T, Naughton K, Romagnani L, *et al*: DNA DSB repair dynamics following irradiation with laser-driven protons at ultra-high dose rates. Sci Rep 9: 4471, 2019.
44. Prise KM, Schettino G, Folkard M and Held KD: New insights on cell death from radiation exposure. Lancet Oncol 6: 520-528, 2005.
45. Desouky O, Ding N and Zhou G: Targeted and non-targeted effects of ionizing radiation. J Radiat Res Appl Sci 8: 247-254, 2015.
46. Kim W, Lee S, Seo D, Kim D, Kim K, Kim E, Kang J, Seong KM, Youn H and Youn B: Cellular stress responses in radiotherapy. Cells 8: 1105, 2019.
47. Jeggo PA and Löbrich M: DNA double-strand breaks: Their cellular and clinical impact?. Oncogene 26: 7717-7719, 2007.
48. Nikjoo H, Emfietzoglou D, Liamsuwan T, Taleei R, Liljequist D and Uehara S: Radiation track, DNA damage and response-a review. Rep Prog Phys 79: 116601, 2016.
49. Purrott RJ and Reeder EJ: Chromosome aberration yields induced in human lymphocytes by 15 MeV electrons given at a conventional dose-rate and in microsecond pulses. Int J Radiat Biol Relat Stud Phys Chem Med 31: 251-256, 1977.
50. Spitz DR, Buettner GR, Petronek MS, St-Aubin JJ, Flynn RT, Waldron TJ and Limoli CL: An integrated physico-chemical approach for explaining the differential impact of FLASH versus conventional dose rate irradiation on cancer and normal tissue responses. Radiother Oncol 139: 23-27, 2019.
51. Benfeitas R, Uhlen M, Nielsen J and Mardinoglu A: New challenges to study heterogeneity in cancer redox metabolism. Front Cell Dev Biol 5: 65, 2017.
52. Vozenin MC, De Fornel P, Petersson K, Favaudon V, Jaccard M, Germond JF, Petit B, Burki M, Ferrand G, Patin D, *et al*: The advantage of FLASH radiotherapy confirmed in mini-pig and cat-cancer patients. Clin Cancer Res 25: 35-42, 2019.
53. Beyreuther E, Brand M, Hans S, Hideghéty K, Karsch L, Leßmann E, Schürer M, Szabó ER and Pawelke J: Feasibility of proton FLASH effect tested by zebrafish embryo irradiation. Radiother Oncol 139: 46-50, 2019.
54. Gao F, Yang Y, Zhu H, Wang J, Xiao D, Zhou Z, Dai T, Zhang Y, Feng G, Li J, *et al*: First demonstration of the FLASH effect with ultrahigh dose rate high-energy X-rays. Radiother Oncol 166: 44-50, 2022.
55. Montay-Gruel P, Petersson K, Jaccard M, Boivin G, Germond JF, Petit B, Doenlen R, Favaudon V, Bochud F, Bailat C, *et al*: Irradiation in a flash: Unique sparing of memory in mice after whole brain irradiation with dose rates above 100Gy/s. Radiother Oncol 124: 365-369, 2017.
56. Montay-Gruel P, Acharya MM, Goncalves Jorge P, Petit B, Petridis IG, Fuchs P, Leavitt R, Petersson K, Gondré M, Ollivier J, *et al*: Hypofractionated FLASH-RT as an effective treatment against glioblastoma that reduces neurocognitive side effects in mice. Clin Cancer Res 27: 775-784, 2021.
57. Simmons DA, Lartey FM, Schuler E, Rafat M, King G, Kim A, Ko R, Semaan S, Gonzalez S, Jenkins M, *et al*: Reduced cognitive deficits after FLASH irradiation of whole mouse brain are associated with less hippocampal dendritic spine loss and neuroinflammation. Radiother Oncol 139: 4-10, 2019.
58. Alaghband Y, Cheeks SN, Allen BD, Montay-Gruel P, Doan NL, Petit B, Jorge PG, Giedzinski E, Acharya MM, Vozenin MC and Limoli CL: Neuroprotection of radiosensitive juvenile mice by ultra-high dose rate FLASH irradiation. Cancers (Basel) 12: 1671, 2020.
59. Allen BD, Acharya MM, Montay-Gruel P, Jorge PG, Bailat C, Petit B, Vozenin MC and Limoli C: 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 194: 625-635, 2020.
60. Loo BW, Schuler E, Lartey FM, Rafat M, King GJ, Trovati S, Koong AC and Maxim PG: Delivery of ultra-rapid flash radiation therapy and demonstration of normal tissue sparing after abdominal irradiation of mice: International Journal of Radiation Oncology*Biology*Physics 98: pE16, 2017.
61. Levy K, Natarajan S, Wang J, Chow S, Eggold JT, Loo PE, Manjappa R, Melemenidis S, Lartey FM, Schüler E, *et al*: Abdominal FLASH irradiation reduces radiation-induced gastrointestinal toxicity for the treatment of ovarian cancer in mice. Sci Rep 10: 21600, 2020.
62. Diffenderfer ES, Verginadis II, Kim MM, Shoniyozov K, Velalopoulou A, Goia D, Putt M, Hagan S, Avery S, Teo K, *et al*: Design, implementation, and in vivo validation of a novel proton FLASH radiation therapy system. Int J Radiat Oncol Biol Phys 106: 440-448, 2020.
63. Soto LA, Casey KM, Wang J, Blaney A, Manjappa R, Breitkreutz D, Skinner L, Dutt S, Ko RB, Bush K, *et al*: FLASH irradiation results in reduced severe skin toxicity compared to conventional-dose-rate irradiation. Radiat Res 194: 618-624, 2020.
64. Cunningham S, McCauley S, Vairamani K, Speth J, Girdhani S, Abel E, Sharma RA, Perentesis JP, Wells SI, Mascia A and Sertorio M: FLASH proton pencil beam scanning irradiation minimizes radiation-induced leg contracture and skin toxicity in mice. Cancers (Basel) 13: 1012, 2021.
65. Chabi S, To THV, Leavitt R, Poglio S, Jorge PG, Jaccard M, Petersson K, Petit B, Roméo PH, Pflumio F, *et al*: Ultra-high-dose-rate FLASH and conventional-dose-rate irradiation differentially affect human acute lymphoblastic leukemia and normal hematopoiesis. Int J Radiat Oncol Biol Phys 109: 819-829, 2021.
66. Konradsson E, Arendt ML, Bastholm Jensen K, Børresen B, Hansen AE, Bäck S, Kristensen AT, Munck Af Rosenschöld P, Ceberg C and Petersson K: Establishment and initial experience of clinical FLASH radiotherapy in canine cancer patients. Front Oncol 11: 658004, 2021.
67. Smyth LML, Donoghue JF, Ventura JA, Livingstone J, Bailey T, Day LRJ, Crosbie JC and Rogers PAW: Comparative toxicity of synchrotron and conventional radiation therapy based on total and partial body irradiation in a murine model. Sci Rep 8: 12044, 2018.
68. Zhou S, Zheng D, Fan Q, Yan Y, Wang S, Lei Y, Besemer A, Zhou C and Enke C: Minimum dose rate estimation for pulsed FLASH radiotherapy: A dimensional analysis. Med Phys 47: 3243-3249, 2020.
69. Bourhis J, Montay-Gruel P, Gonçalves Jorge P, Bailat C, Petit B, Ollivier J, Jeanneret-Sozzi W, Ozsahin M, Bochud F, Moeckli R, *et al*: Clinical translation of FLASH radiotherapy: Why and how?. Radiother Oncol 139: 11-17, 2019.
70. Vozenin MC, Hendry JH and Limoli CL: Biological benefits of ultra-high dose rate FLASH radiotherapy: Sleeping beauty awoken. Clin Oncol (R Coll Radiol) 31: 407-415, 2019.
71. Rahman M, Trigilio A, Frenciosini G, Moeckli R, Zhang R and Böhlen TT: FLASH radiotherapy treatment planning and models for electron beams. Radiother Oncol 175: 210-221, 2022.
72. van Marlen P, Dahele M, Folkerts M, Abel E, Slotman BJ and Verbakel WFAR: Bringing FLASH to the Clinic: Treatment planning considerations for ultrahigh dose-rate proton beams. Int J Radiat Oncol Biol Phys 106: 621-629, 2020.
73. Jolly S, Owen H, Schippers M and Welsch C: Technical challenges for FLASH proton therapy. Phys Med 78: 71-82, 2020.

74. McManus M, Romano F, Lee ND, Farabolini W, Gilardi A, Royle G, Palmans H and Subiel A: The challenge of ionisation chamber dosimetry in ultra-short pulsed high dose-rate Very High Energy Electron beams. Sci Rep 10: 9089, 2020.
75. Jorge PG, Jaccard M, Petersson K, Gondré M, Durán MT, Desorgher L, Germond JF, Liger P, Vozenin MC, Bourhis J, *et al*: Dosimetric and preparation procedures for irradiating biological models with pulsed electron beam at ultra-high dose-rate. Radiother Oncol 139: 34-39, 2019.
76. Petersson K, Jaccard M, Germond JF, Buchillier T, Bochud F, Bourhis J, Vozenin MC and Bailat C: High dose-per-pulse electron beam dosimetry-A model to correct for the ion recombination in the Advanced Markus ionization chamber. Med Phys 44: 1157-1167, 2017.
77. Oraiqat I, Zhang W, Litzenberg D, Lam K, Ba Sunbul N, Moran J, Cuneo K, Carson P, Wang X and El Naqa I: An ionizing radiation acoustic imaging (iRAI) technique for real-time dosimetric measurements for FLASH radiotherapy. Med Phys 47: 5090-5101, 2020.