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REVIEW

FLASH radiotherapy: Research process from basic experimentation to clinical application

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Abstract

FLASH radiotherapy (FLASH-RT) has gained attention as an ultra-high dose rate RT in recent years. This treatment significantly shortens the time of RT and reduces the influence of tumor movement caused by breathing or other factors. In addition, it spares the surrounding normal tissues and organs while ensuring the anti-tumor effect. With the efforts of scientific researchers and clinical staff, the FLASH effect has been successfully induced in electron, photon, and proton irradiation. Preliminary research has been carried out to explore its related mechanism. However, this has not yet been fully determined, although oxygen depletion was the proposed primary mechanism discovered. Due to the development of immunotherapy, studies on the involvement of the immune system in the FLASH effect have begun to attract attention. This study reviewed published experimental results to analyze and summarize the feasibility of FLASH-RT widely used in clinical practice, and whether it could be combined with immune checkpoint inhibitors to guide therapy.

KEYWORDS
FLASH, immunotherapy, oxygen depletion, radiotherapy, ultra-high dose rate

1 | INTRODUCTION

Although the morbidity and mortality of cancer has gradually decreased in recent years, it remains the main obstacle to life expectancy in various countries in the 21st century.[1] Surgery, radiotherapy (RT), chemotherapy, targeted therapy, and immunotherapy are important methods for the treatment of malignant tumors. More than half of the patients with malignant tumors require RT.[2] However, the maximum tolerated dose to normal tissues limits the improvement of the tumor irradiation dose and the efficacy of RT. Although conventional fractionated RT (CONV-RT) can provide treatment while giving the normal tissues sufficient recovery time to reduce the adverse reaction induced by radiation, it also provides time for tumor tissue recovery. Therefore, there is an urgent need to develop a new RT strategy to spare normal tissues while increasing the tumor-killing effects of RT. To address this, a study carried out in 2014 presented the differential effect of a rapid, ultra-high dose rate and high precision RT on mice tumor tissues and normal tissues. The study demonstrated FLASH irradiation on tumor tissues for the first time, and it was officially named "FLASH effect".[3] The dose per pulse and the instant dose rate of the pulses in this therapy is 10^3–10^4-fold higher than that of CONV-RT.[4] It reduces radiation complications, but increases the tolerance of normal tissues to ensure anti-tumor function, so as to optimize the biological effects of RT. Furthermore, the rapid pulse of FLASH-RT can also treat tumors that require exercise management, such as lung cancer, where the tumor moves with breathing during irradiation.[5]

2 | ADVANTAGES OF FLASH-RT

2.1 | Normal tissue-sparing effect of FLASH-RT

Normal tissue sparing is one of the advantages of FLASH-RT. In 1971, an experiment on the sensitivity of intestinal tissues in normal mice found that FLASH irradiation can induce hypoxia in irradiated tissues to reduce radiation sensitivity and protect normal tissues when the dose rates were >60 Gy/min.[6] A few years later, experiments on rat skin showed that when the radiation dose rate was as high as 5000 Gy/min, the adverse reactions could be reduced with FLASH irradiation.[7] Both experiments found that the radiation sensitivity of the irradiated tissues did not change in a hypoxic environment. In a 2014 study of normal mice thoracic irradiation by Favaudon et al., CONV-RT (≤0.03 Gy/s) or FLASH-RT (≥40 Gy/s) was used for contrast irradiation.[3] After 8 weeks of 17-Gy CONV-RT in mice, substantial fibrosis was observed at the site of irradiation after 24 weeks. However, no fibrosis was seen in mice irradiated with 17-Gy FLASH-RT. When the FLASH-RT doses were increased to a range of 16–30 Gy, there was only mild pigmentation observed in the irradiated area. Research on the lungs of mice by Fouillade et al. also found that tissues exposed to FLASH recovered more easily and lung fibrosis was reduced.[8] Furthermore, total body irradiation for acute lymphoblastic leukemia showed that the damage to hematopoietic stem cells was greatly reduced with FLASH-RT compared with CONV-RT.[9] All these experiments observed that FLASH-RT spares normal tissues better than CONV-RT.

In addition, FLASH-RT has a protective effect on neurocognition. Montay-Gruel et al. were the first to use linear electron accelerators to carry out CONV-RT (0.1 Gy/s) or single-pulse (1.8 μs) FLASH-RT on normal brain tissues of mice with a standard prescription dose of 10 Gy and test cognition after 2 months.[10] Researchers found that cognitive impairment occurred in the CONV-RT group, whereas it was not observed in the FLASH-RT group. In fact, on dose escalation, the normal tissue was protected when the dose rates reached 30 Gy/s, and the memory of mice was completely preserved when it reached >100 Gy/s with FLASH-RT. These results laid the foundation for the development of FLASH-RT in the field of brain RT.

Encouraged by the success of the aforementioned study, the experimental protocol was changed by using X-rays (photons) instead of electrons to irradiate the whole brain of mice. A consistent result was reported with the electron beam irradiation, and the cognitive ability of mice in the CONV-RT group did not recover after 6 months.[11] In fact, the team continued to observe the protective effect of FLASH-RT on neurocognition by experimenting with proton irradiation using a glioblastoma mouse model.[12] Similarly, Yasaman et al. used low radiosensitivity on a juvenile medulloblastoma mouse model to study the neuroprotective effects of FLASH-RT and CONV-RT, and found that a single fraction dose of 8 Gy of FLASH-RT caused no neurocognitive impairment within 2–4 months.[13] These basic studies provide a theoretical basis for FLASH-RT in clinical pediatric medulloblastoma, glioma, and other brain-related tumors.

The protective effect of FLASH-RT can also be reflected in research on higher mammals. Vozenin et al. carried out a comparative study of FLASH-RT at a dose rate of 300 Gy/s versus CONV-RT at 0.083 Gy/s using one mini-pig and six cats with nasal squamous cell carcinoma.[14] After different skin sites of the mini-pig were irradiated, they observed that the site of FLASH-RT showed no severe toxicity, but mild depilation that occurred 3 weeks post-irradiation. In contrast, the site of CONV-RT showed non-regeneration of the irradiated skin and follicles, and advanced fibrosis. The experiment on cats showed similar results as those on the mini-pig, where low toxicity after FLASH-RT was observed. Not only that, FLASH-RT was found to prolong the progression-free survival of the sick cats. The success of this higher mammalian research has reinforced the clinical application of FLASH-RT.

Despite the positive effects of FLASH-RT, controversial results have also been shown. Venkatesulu et al. carried out an experiment of total abdominal irradiation in mice, and found that FLASH-RT (35 Gy/s) induced a higher gastrointestinal toxicity and more lymphocyte apoptosis compared with CONV-RT (0.1 Gy/s).[15] This result suggests that the normal tissue protective effect of FLASH-RT might be limited by the heterogeneity of tissues or some unknown biological factors. Therefore, further research on the limitations of FLASH-RT must be carried out (Table 1).

2.2 | Anti-tumor function of the FLASH effect

Although the protective effect on normal tissues is notable, the main advantage of FLASH-RT is the anti-tumor response. Favaudon et al. used C57BL/6J mice to observe the differential effect between tumor tissues and normal tissues at an ultra-high dose rate in 2014.[3] Equal doses of FLASH-RT or CONV-RT were administered to human breast cancer cells HBCx-12A, and head and neck tumor cells Hep-2 xenograft models, respectively. The results suggested that both models were consistent in controlling tumor growth. In the syngeneic orthotopic tumor model, FLASH-RT or CONV-RT with a total dose of 15 Gy also achieved the same anti-tumor effect; however, when the dose was increased to 28 Gy, the overall survival time of these mice irradiated by FLASH was prolonged compared with CONV-RT. This conclusion that the survival rate improves more significantly with FLASH-RT than CONV-RT is consistent with the observation of their threshold for toxicity. Loo et al. showed that FLASH-RT caused toxicity in normal tissues with dose rates of 70 Gy/s or 210 Gy/s, which is significantly higher than the 0.05 Gy/s dose rate of CONV-RT that causes toxicity.[16]

Recently, Montay-Gruel et al. used proton FLASH to treat glioma *in situ* in mice, and found that the anti-tumor effects of FLASH-RT and CONV-RT were similar when the mice were irradiated with doses of 10 Gy and 14 Gy, respectively.[12] However, the 14-Gy dose of FLASH-RT was limited to anti-tumor activity. For this reason, they studied the effect of 7 Gy × 2 and 3.5 Gy × 4 dose fractionation irradiation further. Although they failed to obtain a significantly improved overall survival time, tumor growth could be delayed when the dose of segmentation was increased. The results of that study suggest a difference in the

TABLE 1 Summary of outcomes for *in vivo* and *in vitro* studies on the FLASH effect

Researchers	Model	Radiation source	Energy (MeV)	Dose (Gy)	Dose rate (Gy/s)	Reference
Dewey et al.	Bacterial	X-ray	1.5	100–200	$5–10 \times 10^7$	17
Town et al.	HeLa S-3	Electron	15	45	3.5×10^7	18
Berry et al.	HeLa, CHL	X-ray	10	24	7×10^8	19
Hornsey et al.	Mice (intestinal)	Electron	7	11.9	17–83	6
Field et al.	Mice (feet)	Electron	7	24	56–83	7
Favaudon et al.	Mice (thorax)	Electron	4, 6	15, 17	40	3
Loo et al.	Mice (intestinal)	Electron	20	14.7	70–210	16
Montay-Gruel et al.	Mice (brain)	Electron	4.5, 6	10	10–106	10
Montay-Gruel et al.	Mice (brain)	Photon	0.1	10	37	11
Patriarca et al.	Mice (thorax)	Proton	230	12	40	47
Beyreuther et al.	Zebrafish embryo	Proton	224	23	100	48
Bourhis et al.	Human (skin)	Electron	5.6	15	167	45
Buonanno et al..	Human diploid lung fibroblasts	Proton	4.5	20	1000	37
Fouillade et al.	Mice (thorax)	Electron	4.5	17	NA	8
Abel et al.	Mice (thorax)	Proton	NA	15, 17.5, 20	40	51
Girdhani et al.	Mice (thorax)	Proton	NA	15, 17.5, 20	40	34
Montay-Gruel et al.	Mice (brain)	Electron	6	8–80	>100	23
Rama	Mice (thorax)	Proton	NA	18	40	35
Simmons	Mice (brain)	Electron	16, 20	30	300, 200	27
Venkatesulu et al.	Mice (abdomen)	Electron	4.5	16	35	15
Vozenin et al.	Mini-pig, cat	Electron	4.5, 6	22–34	300	14
Adrian et al.	prostate cancer cells	Electron	10	5–18	600	21
Yasaman et al.	Juvenile medulloblastoma mice	Electron	NA	8	NA	13
Diffenderfer et al.	Mice (abdomen, local intestinal)	Proton	230	15, 18	78	49
Chab et al.	Mice	Electron	6	4	200	9
Cunningham et al.	Mice (leg, skin)	Proton	250	35, 15	57, 115	36
Kim et al.	Mice (thorax)	X-ray	0.32	15	352.1	41

CHL, Chinese hamster cells; NA, not available.

FLASH effect caused by the heterogeneity of tissues. This explanation might provide a reference for the doses of segmentation at different sites in subsequent clinical research (Table 1).

3 | MECHANISM OF FLASH-RT

3.1 | Oxygen depletion hypothesis

The mechanism of the FLASH effect has been studied repeatedly, and the hypothesis of oxygen depletion of cells or tissues is the most extensive explanation. In 1959, Dewey et al. found that the radiation sensitivity of bacterial suspensions decreased under oxygen-rich conditions.[17] This premise began the research on ultra-high dose rate irradiation.

A study of radiation treatment of HeLa S-3 cells in an air environment by Town et al. found that when the dose rate reached a high point, the radiation sensitivity decreased with an increase in the dose rate.[18] Afterwards, they carried out a second set of experiments using nitrogen and failed to show the same decrease in sensitivity, which suggested that radiation resistance was related to oxygen consumption. Berry et al. came to the same conclusion by shortening the pulse delivery time, and proposed that oxygen depletion reduces the production of radiation-induced free oxygen radicals, thus avoiding normal tissue damage.[19] This result is consistent with the conclusion presented by Smyth et al., who used a comprehensive physical and

chemical method[20]; the free radicals generated by the differences in redox metabolism between different tissues is a valid indicator for defining the FLASH effect. The disadvantage is that these *in vitro* experiments used an artificial set of oxygen concentrations that does not reflect the physiological environment of the human body.

To verify the oxygen depletion of the FLASH effect in the human body, Gabriel et al. carried out an *in vitro* irradiation experiment on prostate cancer cells under a physiological oxygen concentration, and observed that the protective effect of FLASH-RT on normal tissue depends on oxygen concentration.[21] Computer model analysis also showed that FLASH-RT can protect normal tissues and cells under complete hypoxia.[22] This reinforces the oxygen depletion hypothesis.

In addition to cell experiments, animal experiments have also explored the hypothesis of oxygen depletion. Montay-Gruel et al. found that increasing the oxygen concentration through hydrocarbon respiration could reverse the protective effect of FLASH-RT on normal nervous tissues.[23] Furthermore, this reversal effect was also confirmed in zebrafish embryos. They hypothesized that the ultra-high dose rate of FLASH-RT could consume the local oxygen concentration rapidly to reduce the production of toxic reactive oxygen species, thereby disrupting the pathogenic pathways they mediate. Several experiments have confirmed that the reduction of reactive oxygen species can decrease neuroinflammation, as it activates astrocytes that damage neurocognitive function.[24–28]

These experiments showed that under the ultra-high dose rate irradiation, intracellular oxygen was consumed rapidly. However, the time of the pulse is too short for extracellular oxygen to diffuse into the cell. Therefore, the "hypoxic window" is formed to resist radiation and achieve normal tissue protection. As for the anti-tumor efficacy, we can assume that there is no radiation resistance caused by oxygen consumption, because the tumor tissues themselves are hypoxic. Furthermore, radiotherapy sensitivity remains unchanged.[6,29] In addition, the unstable iron content in tissues might also be involved in the differential effect between normal tissues and tumor tissues after FLASH-RT.[30]

3.2 | Immune regulation hypothesis

Immune regulation has always been an important mechanism in radiotherapy for malignant tumors, but its role in FLASH-RT has not yet been clarified.[31] For this purpose, Jin et al. calculated the number of remaining circulating immune cells after FLASH-RT (200 Gy/min) and CONV-RT (5 Gy/min) separately by simulating the immune system.[32] They found that just 10% of immune cells were killed by FLASH-RT, whereas the killing rate by CONV-RT was as high as 95%. Therefore, FLASH irradiation has a strong protective effect on the immune system, and this result might be beneficial in reducing the radiation-induced toxicity in normal tissues.[33]

In 2014, the differential effect experiment of normal tissues and tumor tissues by Favaudon et al. pointed out that CONV-RT could trigger the tumor growth factor-beta (TGF-β)/SMAD cascade activation pathway and cause pulmonary fibrosis, whereas FLASH-RT could not.[3] Therefore, FLASH-RT can reduce the complications caused by RT and protect normal tissues from apoptosis induction. Several years later, Girdhani et al. used a proton accelerator to assess the molecular mechanism behind FLASH-RT in mice using genome-wide microarray analysis.[34] They found that the cytokines inducing apoptosis of T lymphocytes were increased after CONV-RT, but decreased after FLASH-RT.

A study of pulmonary irradiation in mice by Rama et al. was carried out using immunofluorescence staining on harvested tumor sections.[35] They found that T lymphocytes were recruited into the tumor microenvironment for treatment with FLASH-RT compared with CONV-RT to achieve a better anti-tumor response. This result showed that proton beam FLASH-RT can reshape the tumor immune microenvironment.

Using a clinical pencil beam scanning proton system, Cunningham et al. observed that FLASH-RT decreased the radiation-induced toxicity in normal tissues by reducing the level of TGF-β1.[36] Previous experiments on lung and whole brain irradiation also observed a decrease in the pro-inflammatory factor TGF-β.[11,27,37] A recent proton FLASH study also found that CONV-RT induced more TGF-β1 in murine and canine skin than FLASH-RT.[38] Simultaneously, the study also showed the protective effect of FLASH-RT on the mesenchymal tissues of muscles and bones, and the anti-tumor effects of CONV-RT were shown in mouse sarcoma models. These findings stimulated the research of FLASH-RT in sarcoma and additional cancers, where mesenchymal tissues are at risk. However, there are conflicting data on the role of this pro-inflammatory factor in FLASH-RT. Some studies believe that TGF-β is a key factor that leads to RT resistance directly,[39] and another study showed that FLASH-RT inhibits TGF-β-related signaling pathways, so that the immune system is no longer affected, allowing normal tumor growth.[40]

A recent study by Kim et al. used Lewis lung carcinoma models, and found that FLASH-RT altered the tumor microenvironment by activating myosin light chains for anti-tumor effects, which was not observed in CONV-RT.[41] Therefore, they hypothesized that combined immune checkpoint inhibitors might enhance the efficacy of FLASH-RT. However, their results showed that FLASH-RT produced more reactive oxygen species, which was inconsistent with the results of Montay-Gruel et al.[23]

A study carried out by Yang et al. explored the apoptosis of cancer stem cells and normal cancer cells MCF-7 after FLASH irradiation, and their results showed that cancer stem cells could increase lysosomal-mediated autophagy and reduce cellular apoptosis, as compared with MCF-7, to escalate radiation resistance.[42] That study explored the role of cancer stem cells in FLASH-RT to optimize radiation resistance programs after FLASH-RT in the future. However, whether the immune response triggers the FLASH effect or vice versa has not yet been determined. Immune regulation plays an important role in the mechanism of RT. Therefore, failure to resolve this controversy might have a severe impact on the combination of FLASH-RT and immune-related drugs. Recently, Demaria et al. carried out a virtual discussion on the differences of RT fractionation leading to different immunomodulatory effects, and they explored radiation as an immunomodulatory "drug".[43] These concepts might lay the groundwork in developing the new combination of FLASH-RT and immunotherapy.

4 | CLINICAL APPLICATIONS OF FLASH-RT

Whether the emerging radiotherapy technology could be safely applied to the clinic has always been the focus of concern. In 2019, the Oncology Center of the University of Amsterdam in the Netherlands formulated a lung stereotactic scanning beam proton FLASH plan for seven patients, and compared it with the volumetric modulated arc therapy treatment plan. Their results showed that the protective effect of the FLASH treatment plan on the lung, chest wall, and heart is equivalent to or even better than volumetric modulated arc therapy.[44] The same year, Bourhis et al. reported the first application of FLASH-RT to humans.[45] The patient was a 75-year-old man with a 3.5-cm ulcero-infiltrative tumor on his left forearm. The lesion was observed within 10–44 days after 15-Gy/90 ms FLASH-RT. Grade 1 acute epithelial inflammatory reaction and edema were present at the site of radiation, and there were few corresponding clinical symptoms. Furthermore, the lesion began to shrink 10 days after RT and achieved complete remission after 36 days. There was no recurrence in the 5-month follow-up period. The drawback is that there is only one case in this experiment; therefore, it is impossible to conclude that FLASH-RT can be applied to other human tissues and organs. However, it still proves the effectiveness and safety of FLASH-RT – a single- and high dose-rate RT.

5 | PROGRESS OF FLASH-RT

Decades' worth of *in vivo* and *in vitro* experiments have made FLASH-RT recognized by experts and scholars in various countries because of its low toxicity and high efficiency in the treatment of superficial tumors. However, safety is still the primary issue because of the deep tumor irradiation of high-energy electrons or X-rays, combined with the short pulse and high dose rate of FLASH-RT. Currently, proton radiotherapy can reduce the radiation dose in endangered organs and normal tissues due to its Bragg peak effect. This provides an alternative scheme for the treatment of deep tumors with FLASH-RT.

Previous studies on proton CONV-RT compared the volume control of proton and photon RT, and found that the effect of proton irradiation is better than that of photons. This laid a foundation for exploring the application of proton FLASH-RT.[46] Subsequently, Fouillade et al. explored a proton FLASH-RT device.[47] The researchers used a proton cyclotron at 230 MeV to achieve uniform irradiation with a dose rate of >40 Gy/s at a site of $12 \times 12\ mm^2$, and successfully induced the FLASH effect in the lungs of mice. The feasibility of proton FLASH-RT in animals was shown. The proton FLASH-RT study of pulmonary fibroblasts was carried out by Buonanno et al.[37] In that study, normal human pulmonary fibroblasts were irradiated with dose rates of 0.05–1000 Gy/s, and the FLASH effect was found to have an impact on adverse reactions from radiation only when the dose rates were >20 Gy/s. However, proton dose rates have little effect on the acute radiation damage of cells and are only beneficial for long-term effects. The difference is that in the zebrafish model experiment by Beyreuther et al., the comparison of proton FLASH-RT (100 Gy/s) and CONV-RT (0.083 Gy/s) only confirmed the feasibility of proton FLASH-RT in the treatment of tumors, but failed to conclude whether it is beneficial to embryos.[48] Although this result might be related to the developmental stage of the zebrafish embryo affecting the pulse dose rate of radiation, it still obscured the study of proton FLASH-RT.

To further verify the effect of proton FLASH-RT, a study by Rama et al. involved injecting Lewis lung carcinoma cells into the left lung of C57BL/6J mice.[35] They treated the lungs of the mice with a single fraction dose of 18-Gy proton FLASH-RT at a dose rate of 78 Gy/s or CONV-RT, then measured the tumor volume of mice and observed that FLASH-RT showed better tumor control. Similarly, proton beam irradiation in mouse models of gastrointestinal tumors in another study showed equivalent anti-tumor efficacy of FLASH-RT and CONV-RT.[49] Similarly, Cunningham et al. used proton pencil beam scanning, and found that after irradiating the plasma, skin, and hind limbs of normal mice, less skin toxicity and hind limb contractures were induced with FLASH-RT, whereas mice with head and neck tumor irradiated with FLASH achieved the same anti-tumor effect as with CONV-RT.[36] These studies provided evidence on the tumor control ability of proton FLASH-RT.

The application research of proton FLASH in clinical devices is also ongoing. In March 2019, the first proton FLASH irradiation was carried out favorably at the University Medical Center of Groningen in the Netherlands using the Ion Beam Applications Rotating Gantry Treatment Room with a dose rate of 200 Gy/s.[50] In April 2019, at the annual meeting of the American Association for Cancer Research, the School of Radiation Oncology at the University of Maryland School of Medicine, the Maryland Proton Therapy Center, and Varian, announced results of the first pre-clinical study of FLASH treatment using a clinical device. FLASH treatment can reduce 25%–30% of lung tissue damage, thereby reducing the occurrence of pulmonary fibrosis and decreasing the incidence of radiation dermatitis by an average of 35%.[51] FLASH treatment can protect normal tissues and organs, which is a major breakthrough in the treatment of malignant tumors. This constructive preclinical animal study supports the application of the proton FLASH effect in humans. In 2020, Breiktreutz et al. used a 40-MeV electron beam with a dose rate of 115 Gy/s on a clinical linear accelerator to simulate pediatric whole-brain FLASH-RT using Monte Carlo, providing a driving force for the realization of clinical FLASH-RT.[52] In the same year, the Proton Therapy Center of Cincinnati Children's Hospital Medical Center and University of Cincinnati Medical Center and Varian announced that the first patient in the FAST-01 clinical trial, a feasibility study of FLASH-RT for the treatment of bone metastases, received FLASH irradiation. The trial plans to recruit 10 patients with bone metastases treated with proton FLASH irradiation, the enrollment of patients has been completed, and the feasibility of FLASH treatment clinical workflow and treatment-related side-effects are being evaluated (NCT04592887). The results of this experiment have not yet been announced.

6 | CONCLUSION

In summary, FLASH-RT is a new RT that has attracted increasing attention. There has been an increasing number of clinical and scientific studies focusing on the characteristics of the therapy; ultra-high dose rate, ultra-fast radiation, low toxicity, and anti-tumor effects. Although there have only been a few clinical trials applying FLASH-RT to the treatment of human malignant tumors, the cell experiments and pre-clinical animal studies carried out have laid the foundation for human clinical applications. In particular, the studies on proton FLASH make up for the lack of photon and electron irradiation in deep tumors.[37,47,48]

FLASH-RT has shown many benefits, but there are disadvantages that need to be addressed. First, an unknown mechanism.[53] Even though the current research suggests that the differential effect of normal tissues and tumor tissues could ensure the anti-tumor effect of FLASH-RT while protecting the surrounding normal tissues and organs, there is no convincing evidence for its specific trigger mechanism and appropriate response dose rate for different tissues. Although relevant studies have shown the feasibility of FLASH-RT combined with immunotherapy, its definite regulatory mechanism needs to be further researched.

Second, a short follow-up period and small sample size. The sparing of normal tissues using FLASH-RT has not been verified by long-term follow up in the pre-clinical studies carried out so far, and the problem of small samples is worthy of attention regardless of the study on high mammals (for example, mini-pig and cats) or the first patient treated by FLASH-RT.

Third, technical and financial problems. FLASH-RT requires an ultra-high dose rate, but traditional linear accelerators cannot meet this demand. Although Schuler et al. modified an accelerator that could generate an electron beam with a dose rate of 200 Gy/s, it is only suitable for small animals and superficial tumors.[54] Proton beams irradiate deep tumors, but this accelerator is bulky and expensive. Therefore, some researchers, such as Gao Feng et al., carried out a detailed analysis of dose rates calculated using tumors with different depths.[55] The researchers used a superconducting electron accelerator on the large hertz free electron laser device to establish a photon radiotherapy research platform with a dose rate of up to 2000 Gy/s, such that a coverage depth of dose rate >100 Gy/s could reach 20 cm. In 2021, they used this device to achieve ultra-high dose rate X-rays FLASH irradiation, which provides a basis for future scientific research and clinical applications of FLASH-RT.[56] Further related research needs to be carried out.

Fourth, precise image guidance. Current technologies that achieve FLASH irradiation have a limited penetrative ability that can only reach a depth of a few centimeters beneath the tissue, and an extremely high amount of energy is required to improve it. Considering the characteristics of the short pulse and high dose rate of FLASH-RT, it will cause bodily harm when the positioning is not accurate. In 2019, the PHASER project carried out by Maxim et al. hoped to solve this by providing image-guided highly conformable FLASH-RT.[57]

Fifth, dose segmentation and dose rate. Bourhis et al. carried out low-dose fractionated irradiation on mouse glioma *in situ* and found that FLASH-RT has the same effect as CONV-RT, but further research is still required.[58] Furthermore, Jin et al. showed that the immune cell sparing of FLASH-RT reached its peak only when the single doses were 30–50 Gy, and disappeared at a low dose (2 Gy).[32] In terms of dose rate, although the protective effect of ultra-high dose rate (100 Gy/s) on the nervous system was confirmed,[10] some experiments showed tissue damage at 35 Gy/s.[15]

Sixth, range of target area. The range of the area targeted by the FLASH-RT is small (diameter <4 mm); hence, it is not enough to support extensive clinical application.

In conclusion, FLASH-RT is a promising treatment for malignant tumors, but further research and technical improvements still require the efforts of scientific researchers and clinical staff. The combination of immune checkpoint inhibitors might be a topic for further research.

CONFLICT OF INTEREST

The authors declare that they have read the article and there are no competing interests.

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