Review article

FLASH RADIOTHERAPY AS NEW PERSPECTIVE IN RADIOTHERAPY TECHNOLOGY

Flash radioterapija kot nova možnost v radioterapevtski tehnologiji

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ABSTRACT

Purpose: The purpose of this article is to present FLASH radiotherapy as a new radiation therapy method, to explain its mechanisms of action, to present possible sources and devices of radiation, and to identify its advantages and disadvantages compared to conventional radiotherapy.

Methods: Articles were reviewed for this study in online scientific research over the last 10 years (2012–2022). The Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram was used to document and report on all decisions made during the study selection process for this review paper.

Results and Discussion: Most studies have found that FLASH-RT reduces toxicity to healthy tissue adjacent to a tumour. At present, there is a lack of suitable radiation devices for the use of FLASH-RT, and it will be necessary to adapt existing devices.

Conclusion: FLASH-RT could be used in highly radioresistant tumours where CONV-RT would cause too much damage to healthy tissue with an increase in radiation dose. It could also be useful in tumours where CONV-RT is successful but too toxic for healthy tissue adjacent to a tumour. A great deal of research is required before the clinical implementation of FLASH-RT to determine the optimal dose rate, doses for different types of cancer with most the favourable effect/toxicity ratio and technical solution (i.e. radiation source).

Keywords: FLASH radiotherapy, radiotherapy, neoplasms, radiotherapy dosage

IZVLEČEK

Namen: Namen članka je predstaviti FLASH radioterapijo (FLASH-RT) kot novo obsevalno metodo, pojasniti do sedaj znane mehanizme delovanja, predstaviti možne vire in naprave sevanja ter ugotoviti kakšne so njene prednosti in pomanjkljivosti v primerjavi s konvencionalno radioterapijo (CONV-RT).

Metode in materiali: Za raziskavo so bili pregledani članki, objavljeni v zadnjih desetih letih (2012-2022) v spletni bazi podatkov. Za sistematični pregled literature in metaanalizo je bil uporabljen diagram za lažji izbor člankov, ki opisujejo značilnosti FLASH-RT.

Rezultati in razprava: Pri večini študij je bilo ugotovljeno, da FLASH-RT zmanjša toksičnost na zdrava tkiva ob tumorju. Trenutno je premalo primernih obsevalnih naprav za uporabo FLASH-RT in bo zato potrebno prilagoditi obstoječe naprave.

Zaključek: FLASH-RT bi lahko uporabili pri zelo radiorezistentnih tumorjih, kjer bi pri CONV-RT z višjo obsevalno dozo preveč poškodovali zdravo tkivo. Uporabna bi bila tudi pri tumorjih, kjer je CONV-RT uspešna, a ima preveč stranskih učinkov na zdrava tkiva ob tumorju. Pred klinično uporabo bo potrebno napraviti še veliko raziskav in ugotoviti: hitrost doze, dozni odmerek za različne vrste raka in najugodnejše razmerje med učinkom in toksičnostjo ter tehnično rešitev (tj. vir sevanja).

Ključne besede: FLASH radioterapija, radioterapija, neoplazme, dozni odmerki v radioterapiji

INTRODUCTION

Radiotherapy is one of the main types of treatment in oncology. In recent decades, a new radiation therapy method called FLASH radiotherapy (FLASH-RT) has been developed, and has been found to have fewer early and late radiation side effects, and the same antitumour efficacy. This is referred to as the FLASH effect. This could make FLASH-RT the main radiotherapy method in the future (1, 2). FLASH-RT is defined as irradiation with a single ultra-high dose rate (≥ 40 Gy/s) radiotherapy. FLASH irradiation is approximately 400 times faster than conventional irradiation (~5 Gy/min) (1).

The FLASH effect was first reported by Dewey and Boag in 1959. At that time, they irradiated Serratia marcescens bacteria with 1.5 MV X-rays at ultra-high dose rates. This study showed that bacteria in a nitrogen-oxygen mixture containing 1% oxygen were more radiosensitive than in a 100% nitrogen environment after irradiation at normal dose rates (1000 rad/min). However, lower radiosensitivity was observed when ultra-high dose rates (10-20 kilorad/2μs) were applied in the same nitrogen-oxygen mixture. Their study thus highlighted the fact that irradiation at ultra-high dose rates can protect bacteria better than conventional radiotherapy (CONV-RT) at normal dose rates (1).

FLASH-RT was first used in humans in 2018 at the University Hospital of Lausanne in Switzerland. The patient was a 75-year-old man who was diagnosed with CD30+ T-cell cutaneous lymphoma in 1999. From 2008 to 2018, the patient received CONV-RT, which successfully treated the lymphoma, but experienced severe side effects on the skin adjacent to the tumour. In 2018, he was treated with FLASH-RT using a total dose of 15 Gy delivered in 10 x 1 μs pulses (≥ 106 Gy/s, 1.5 Gy per pulse) with a total treatment time of 90 ms. The tumour was initially 3.5 cm in size and started to shrink after 10 days. Complete tumour response was achieved after 36 days and lasted five months. From the beginning, when the irradiated lesion started to shrink, there were only mild redness and minor oedema around the irradiation site, which was different from the patient's problems after conventional irradiation, where the surrounding tissue was more severely damaged and took three to four months to heal (2).

Flash-RT mechanism hypotheses

There are several different hypotheses regarding the mechanisms of FLASH-RT. However, the exact mechanism of action of FLASH-RT and its effects on cells are not yet known. The most commonly used hypotheses to explain the effects of FLASH-RT are the oxygen deprivation hypothesis, the role of reactive oxygen species (ROS) and redox reactions, the immune hypothesis and the differential response of normal and tumour tissue hypothesis (3).

Oxygen deficiency hypothesis

Oxygen is a critical molecule in the biological effect of FLASH-RT. It is known that hypoxic tissues are more radioresistant than oxygen-rich tissues. Radiochemical oxygen depletion occurs in FLASH-RT (4). There is an instantaneous consumption of oxygen, which is significantly faster than reoxygenation. Transient radioresistance occurs in healthy tissue due to transient hypoxia. There is thus less toxicity to such tissue (2, 5). This phenomenon is not as pronounced in CONV-RT because the dose rates are lower and repeated several times, so oxygen is replaced in between and the oxygen concentration in the irradiated tissue changes less (4).

ROS role hypothesis and redox biology

After irradiation with photons and electrons, water is radiolysed and ROS are formed, which cause 60–70% of indirect DNA damage, while 30–40% of the DNA damage is caused by direct interaction between the radiation and the DNA. If there is a lot of oxygen in the tissue, more ROS are produced and more DNA is damaged. This also explains why hypoxic tumours are more radioresistant than well-oxygenated tumours (2).

It is also hypothesised that ROS and other free radicals alter biochemical reactions in normal and tumour tissue, and thus contribute to the FLASH effect. This was also shown in a study where zebrafish embryos were irradiated with FLASH-RT and CONV-RT, and it was determined that there were fewer side effects after FLASH-RT. However, when the zebrafish were placed in an environment with ROS scavengers one hour before irradiation, no differences were identified. They concluded that FLASH-RT increases radioresistance in normal tissue due to a decrease in ROS (1). A study in which zebrafish embryos were irradiated with both radiotherapies confirmed the hypothesis that ROS and other free radicals alter biochemical reactions in tissue (2).

Normal and tumour tissue are distinguished both by the generation of free radicals and by the course of redox reactions. The same dose of FLASH-RT as CONV-RT triggers different redox pathways and a lower burden of pro-oxidants because they scavenge free radicals faster than tumour cells. In tumour tissue, peroxidation chain reactions take longer to occur, causing the accumulation of free radicals, resulting in cell damage and destruction (5).

Immune hypothesis

The FLASH effect is thought to be mediated by inflammatory and immune responses. TGF-beta is important as a pro-inflammatory cytokine and is thought to be involved in the different effect of FLASH-RT compared to CONV-RT. In an in vitro study, the level of TGF-beta in human lung fibroblasts was monitored and found to be less after FLASH-RT with proton beams than with conventional irradiation. The production was only 1.8 times higher in FLASH-RT than in non-irradiated tissue, and 6.5 times higher in CONV-RT, suggesting that FLASH-RT significantly reduced chronic inflammation relative to CONV-RT (2).

Similarly, another study in mice confirmed that CONV-RT increased the levels of five of the ten cytokines observed, whereas FLASH-RT increased only three. The exact effect of TGF-beta is not yet known, but it is thought to be involved in the anti-tumour immune response. It is thought to suppress the immune system and promote cancer progression, increasing the need for inhibitors of the TGF-beta pathway (2).

Hypothesis of differential response of normal and tumour tissue

It was hypothesised that different types of DNA damage after the two irradiations trigger different responses in healthy

and tumour tissue. Solid tumours are mostly hypoxic, so they will not be protected from the transient hypoxia induced by FLASH-RT, whereas healthy tissues will be, resulting in a differential effect. Cancer and normal cells have different abilities to scavenge hydrogen peroxide products (1). It has been found that it is precisely due to different redox metabolism, different levels of ROS and redox metals, such as labile iron, that normal cells scavenge the free radicals generated during irradiation more efficiently. The authors also found out that cancer cells have higher levels of labile iron and transferrin receptors, which results in an increase in catalytic processes (Fenton reaction) that convert hydrogen peroxide into hydroxyl free radicals, causing more oxidative damage in cancer cells. Healthy cells have less labile iron, and scavenge hydroperoxides formed more rapidly after FLASH-RT (3, 4).

Impact on radiotherapy

FLASH-RT has the potential to change the theory of radiobiology (1). The first change could be in the five Rs of radiobiology: DNA repair, reoxygenation, repopulation, redistribution and intrinsic radiosensitivity. The duration of FLASH-RT is too short for reoxygenation, repopulation and redistribution to occur, but the effect of FLASH-RT may be related to two Rs: DNA repair and intrinsic radiosensitivity (1). Another modification may be the threshold dose to healthy tissue, as pre-clinical studies have confirmed that a higher dose of FLASH-RT is required to induce the same level of toxicity as CONV-RT. This was confirmed in a study where CONV-RT irradiation with a dose of 15 Gy induced pulmonary fibrosis, whereas FLASH-RT irradiation with a dose of 20 Gy did not induce the same effect, even after 36 weeks. A similar finding was made in another study where CONV-RT irradiation at 17 Gy induced severe skin lesions, while FLASH-RT irradiation at 15 and 20 Gy did not. (1). A third option is a comprehensive change in treatment strategy. FLASH-RT can only be performed once for a very short period of time, so concomitant chemoradiotherapy cannot be performed. Only neoadjuvant and adjuvant chemotherapy can be performed (1). The fourth option is a change in the number of fractions in radiotherapy. FLASH-RT is only performed once and could therefore displace CONV-RT (1).

Devices and radiation sources

In addition to the dose rate and the duration of FLASH-RT, the radiation source is also important. Electrons, photons and protons can be used (1). Most research has used linear accelerator electron beams. These beams are limited to the treatment of superficial cancers and intraoperative radiotherapy due to their low penetration and limited energy (4 to 20 MeV) (2). Higher energy electron beams could also be used, i.e. high-energy electron beams with energies of 100 to 250 MeV. Such beams have good depth penetration and are less sensitive to tissue heterogeneity than X-rays (4). Photon beams from linear accelerators are not sufficiently intense to achieve the required high doses with current technology. However, X-rays from synchrotrons have been successfully used (3). Synchrotron sources have similar beam energies to X-ray tubes, but also have the potential to use spatially fractionated, ultra-high-dose microbeam radiation therapy (MRT). The disadvantage is that synchrotrons are large, expensive and few in number (4). In proton beam radiotherapy, the penetration of the beams is deeper and facilitates the irradiation of deeper tumours. Another advantage is that most of the beam energy is deposited in a narrow area at Bragg's peak, facilitating the precise targeting of the tumour volume while protecting surrounding healthy tissue and organs at risk (2).

The aim of this review article is to present FLASH-RT as a new irradiation method, to explain the currently known mechanisms of action, to present possible sources and devices of radiation, and to identify its advantages and disadvantages compared to CONV-RT.

METHODS

The studies used in this paper were found in online scientific research databases and were published in the last 10 years (including 2012 to 2022). To simplify the literature review, we selected some exclusion criteria, such as studies published in the period before 2012, studies that are not in English, papers without full text and papers not related to the theme of our study. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram was used to document and report on all decisions made during the study selection process for this review paper (Diagram 1).

RESULTS AND DISCUSSION

The results present a systematic review of irradiation results for studies investigating toxicity to healthy tissue. The essential characteristics expected from FLASH-RT are equal or even higher antitumour efficacy and lower toxicity to healthy tissue adjacent to a tumour. The effects of FLASH-RT have been studied in various animal models of mice, rats, zebrafish, pigs and cats, and in organs such as lungs, skin, intestines and brain. The results of in vitro and in vivo studies were also compared. Researchers were also interested in the effects of FLASH-RT from different radiation sources. Most reported that there were fewer adverse effects on healthy tissue after FLASH-RT compared to CONV-RT (Table 1).

In 2014, Favaudon reported that the use of FLASH-RT to treat lung tumours can lead to a complete response, and reduce early and late toxicity affecting normal lung tissue. To investigate toxicity, he used healthy mice in which the lungs were irradiated, and the occurrence of pneumonitis and fibrosis was assessed. One group was irradiated with a high single dose of FLASH-RT (≥ 40 Gy/s) and the other group was conventionally irradiated at a dose rate of 0.003 Gy/s. After CONV-RT at 17 Gy, severe pneumonitis and fibrosis occurred in all mice, whereas FLASH-RT at the same dose resulted in neither pneumonitis nor fibrosis, but only at 30 Gy. At 17 Gy, FLASH-RT also prevented TGF-beta activation (6).

Similar conclusions were reached by Vozenin et al. (2019), who irradiated the skin of mini-pigs and cats in their study. For FLASH-RT, they used two prototype linear accelerators, the Kinetron (4.5 MeV) and the Oriatron (6 MeV) for the electron source, and a wider range of dose rates. They irradiated 10 equally sized circular patches of skin in each pig. Five different doses ranging from 22 to 34 Gy were used. A dose rate of 5 Gy/min was used for CONV-RT and 300 Gy/s for FLASH-RT. After 36 weeks, skin biopsies were taken. FLASH-

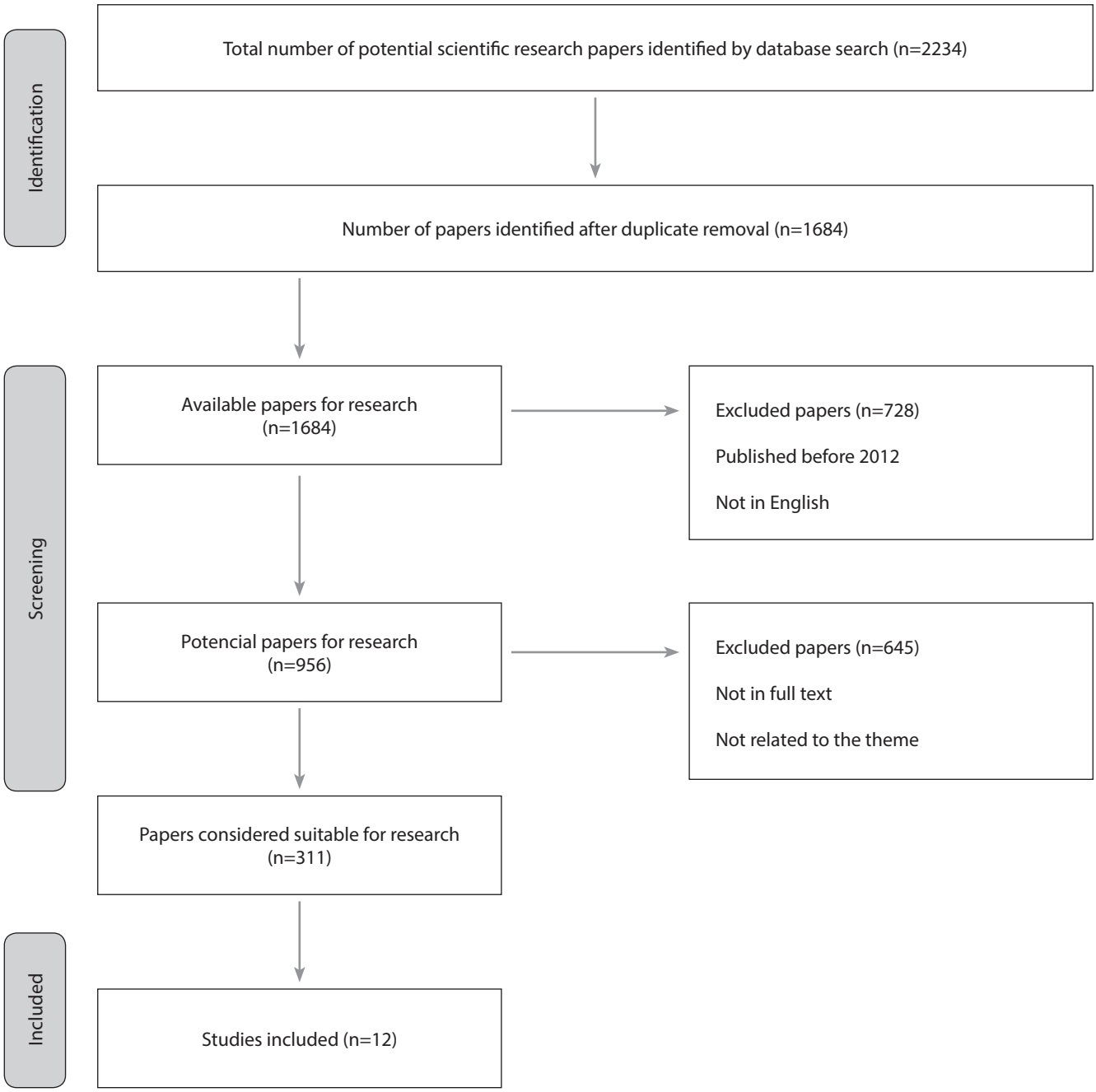

Diagram 1: Selection of documents for systematic review

RT had fewer side effects: only transient depilation occurred, but hair follicles were preserved. CONV-RT resulted in permanent hair follicle damage, skin fibronecrosis, epithelial ulceration and hyperkeratosis. In another study, he used cats irradiated for locally advanced squamous cell carcinoma of the nasal planum. A worse antitumour effect was observed with CONV-RT. FLASH-RT used a single dose, while different dose rates (from 25 to 41 Gy) were used to find the maximum acceptable dose. They were followed up for 18 months. There was permanent depilation at the irradiation site, but no disturbance of olfaction and nutritional functions. Tumour response was complete after six months and three of the six cats were still disease-free after 18 months. The results of this study are promising because larger mammals were studied and this would be more easily transferable to human research (7).

Table 1: Irradiation results for studies investigating toxicity to healthy tissues

Author	Model	Observed variable	Total dose (Gy)	Dose rate (Gy/s)		Modality of radiation	Which RT has the advantage?
				CONV-RT	FLASH-RT		
Favaudon et al. (2014)	Mice – Thoracic irradiation	Onset of pneumonitis and pulmonary fibrosis	17	≤ 0.03	≥ 40	electron	**FLASH-RT**
Vozenin et al. (2019)	Mini pigs – Skin irradiation	Skin toxicity	22-34	0.08	300	electron	**FLASH-RT**
Vozenin et al. (2019)	Cats – Skin irradiation	Skin toxicity	25-41	0.08	300	electron	**FLASH-RT**
Montay-Gruel et al. (2017)	Mice – Whole brain irradiation	Cognitive skills	10	0.1	30–5.6x106	electron	**FLASH-RT**
Montay-Gruel et al. (2018)	Mice – Whole brain irradiation	Cognitive skills	10	0.05	37	X-ray	**FLASH-RT**
Alaghband et al. (2020)	Mice (juvenile) – Brain irradiation	Cognitive skills	8	7.7×103	4.4×106	electron	**FLASH-RT**
Diffenderfer et al. (2020)	Mice – Abdomen irradiation	Acute cell loss and late fibrosis	12-18	0.5-1	60-100	proton	**FLASH-RT**
Venkatesulu et al. (2019)	Mice – Heart and spleen irradiation	Level of lymphocytes in the circulation	0-8	0.1	35	electron	**CONV-RT**
Venkatesulu et al. (2019)	Mice – Abdomen irradiation	Toxicity	16	0.1	35	electron	**CONV-RT**

Montay-Gruel et al. (2017) assessed cognitive skills after whole brain irradiation with FLASH-RT and CONV-RT in two separate studies. They used electrons from a linear accelerator for FLASH-RT in the first study, and synchrotron-generated X-ray radiation in the second. They found that FLASH-RT better preserved memory and neurogenesis in the hippocampus, with more than 37% of preserved neurogenesis clusters found in mice after FLASH-RT, but only 14% with CONV-RT. CONV-RT reduced cognitive abilities and significantly reduced cell divisions in the hippocampus (8, 9). Moreover, a study by Alahband (2020) showed that FLASH-RT after the irradiation of mouse brains better preserves the memory, learning and socialisation abilities of these mice for four months after FLASH-RT, whereas CONV-RT impairs these functions. This in turn suggests that FLASH-RT also gives encouraging results in the long term, which would be very good if FLASH-RT were used in the treatment of paediatric patients (10).

Diffenderfer (2020) also compared the two proton radiotherapies. He irradiated the abdomen of healthy mice, whole or only part. After FLASH-RT, he found greater cell preservation in intestinal crypts and better crypt regeneration. Analysis of the muscle layer in the intestine also showed less fibrosis after FLASH-RT, or changes comparable to those in non-irradiated mice. The effect of proton FLASH-RT on the tumour was then studied. Pancreatic cancer cells were inoculated and this area was irradiated. Both radiotherapies had the same effect on the tumour (11).

However, a few studies have found that there were more side effects after FLASH-RT. Venkatesulu et al. (2019) also observed that both radiotherapies caused lymphopenia, but this was more severe with FLASH-RT. There was even more severe gastrointestinal toxicity after whole abdomen irradiation and the worse survival of mice with FLASH-RT (12).

It is difficult to compare all studies published to date because the authors do not use the same conditions for both irradiation techniques. Some use electrons as the radiation source for FLASH-RT and photons for CONV-RT. The shape of the irradiation field is also important, as it is different if the irradiation field is circular or square, even if the same area has been irradiated. Vozenin et al. (2019) point out that often in in vitro studies, oxygen concentrations were significantly higher than in vivo. Due to such non-physiological oxygen concentrations (21%), the FLASH effect may not occur in these studies, but is observed when concentrations are physiological (3 to 7%) (5).

CONCLUSION

FLASH-RT is a new irradiation method that was first mentioned in 1959, but has only started to be studied again more intensively in the last two decades. The major benefits expected from this method are reduced toxicity to healthy tissue adjacent to a tumour, and an equal or, in some tumour types or conditions, even better antitumour effect than in CONV-RT. The mechanism of action of FLASH-RT is not yet fully understood, but there are some hypotheses that try to explain it. Various studies comparing FLASH-RT with CONV-RT are ongoing, but so far only in animals. There is only one known

example of FLASH-RT in humans, which is not sufficient to translate this method into clinical use. Extensive research is needed before this can be done to optimize the dose rate for different types of cancer, and to determine the dose with the most favourable effect/toxicity ratio. It will also be necessary to determine which radiation source is most appropriate for this type of radiation, which will require intensive technological developments in the field of irradiation devices.

FLASH-RT could be used for highly radioresistant tumours, where CONV-RT would damage healthy tissue if an increase in radiation dose would be used to overcome radioresistancy. It would also be useful for tumours where CONV-RT is successful in order to further reduce side effects on healthy tissue adjacent to a tumour.

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