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Scientific Article

Comparable Long-Term Tumor Control for Hypofractionated FLASH Versus Conventional Radiation Therapy in an Immunocompetent Rat Glioma Model

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Abstract

Purpose: To ensure a clinical translation of FLASH radiation therapy (FLASH-RT) for a specific tumor type, studies on tumor control and toxicity within the same biological system are needed. In this study, our objective was to evaluate tumor control and toxicity for hypofractionated FLASH-RT and conventional radiation therapy (CONV-RT) in an immunocompetent rat glioma model.

Methods and Materials: Fisher 344 rats (N = 68) were inoculated subcutaneously with NS1 glioma cells and randomized into groups (n = 9-10 per group). CONV-RT (∼8 Gy/min) or FLASH-RT (70-90 Gy/s) was administered in 3 fractions of either 8 Gy, 12.5 Gy, or 15 Gy using a 10-MeV electron beam. The maximum tumor diameter was measured weekly, and overall survival was determined until day 100. Long-term tumor control was defined as no evident tumor on day 100. Animals were evaluated for acute dermal side effects at 2 to 5 weeks after completed RT and for late dermal side effects at 3 months after initiation of treatment.

Results: Survival was significantly increased in all irradiated groups compared with control animals ($P < .001$). In general, irradiated tumors started to shrink at 1 week post−completed RT. In 40% (23 of 58) of the irradiated animals, long-term tumor control was achieved. Radiation-induced skin toxic effects were mild and consisted of hair loss, erythema, and dry desquamation. No severe toxic effect was observed. There was no significant difference between FLASH-RT and CONV-RT in overall survival, acute side effects, or late side effects for any of the dose levels.

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Conclusions: This study shows that hypofractionated FLASH-RT results in long-term tumor control rates similar to those of CONV-RT for the treatment of large subcutaneous glioblastomas in immunocompetent rats. Neither treatment technique induced severe skin toxic effects. Consequently, no significant difference in toxicity could be resolved, suggesting that higher doses may be required to detect a FLASH sparing of skin.

Introduction

Glioblastoma is a highly aggressive primary brain tumor associated with a short median survival. The development of effective treatment protocols against glioblastoma is a challenge. The standard protocol introduced by Stupp et al includes maximal safe tumor resection followed by radiation therapy (RT) administered as 60 Gy in 30 fractions, 5 days per week, with concomitant and adjuvant temozolomide. With this protocol, median survival in study patients increased from 12.1 months with RT alone to 14.6 months with the addition of temozolomide.[1] Despite aggressive treatment, glioblastoma tumors are highly resistant.[2] Compared with patients who qualify for study inclusion, patients in population-based series have been shown to have worse overall survival, in many cases less than 1 year.[3]

Radiation therapy is one of the few treatments that has provided glioblastoma patients with a survival benefit. However, a limiting factor is the radiation-induced side effects, including neurocognitive decline. With efforts to increase the absorbed dose to a therapeutic level, severe toxic effects arise in sensitive areas of the brain. Therefore, any approach that could improve the therapeutic index by increasing normal tissue tolerance would improve the benefits of RT, allowing increased dose to the tumor to improve tumor control. Contemporary RT techniques, such as hypofractionated RT and stereotactic radiosurgery, are strategies that may offer shorter treatment courses to maximize quality of life and allow for dose intensification for improved tumor control.[4] Still, major problems regarding RT against glioblastoma are both inherent resistance and further development of adaptive radioresistance.[5]

In 2014, a novel approach to broaden the therapeutic window was proposed. By reducing the beam-on time from several minutes to a fraction of a second, Favaudon et al observed significantly less radiation-induced fibrosis in mice lung.[6] The technique of using ultrahigh-dose-rate irradiation was coined FLASH radiation therapy (FLASH-RT). In recent years, a lower toxicity on normal tissue has been confirmed in various animal models and organs[7] as well as in the skin of higher mammals, as seen in a minipig and in cat and canine cancer patients.[8,9] The protective effect appears to be triggered at average dose rates greater than 30 Gy/s.[10] Furthermore, FLASH-RT has been shown to be equally effective as conventional RT (CONV-RT) delivered at average dose rates of a few Gy per minute, in preventing tumor growth.[6,11-16] Considering the previously mentioned evidence, there may be a therapeutic gain with FLASH-RT that can increase the probability of uncomplicated tumor control compared with CONV-RT.

In brain, the use of ultrahigh dose rates has been demonstrated to result in less inflammation compared with conventional dose rates,[17] as well as a higher degree of protection of blood vessels[18] and neurocognitive functions in mice.[10,11,19] A few studies have shown that tumor growth delay is similar for FLASH-RT and CONV-RT in xenograft glioma models.[11,12] However, data on how FLASH-RT compares to CONV-RT with respect to long-term tumor control, which is the goal of curative RT, have not yet been published.

In this study, we used a synergetic subcutaneous glioblastoma model with an infiltrative growth pattern[20] in fully immunocompetent animals. The aim was to evaluate and compare tumor control and treatment toxicity for various doses of hypofractionated FLASH-RT versus CONV-RT by assessing overall survival, tumor growth, and long-term tumor control, as well as the frequency of acute and late local dermal toxic effects.

Methods and Materials

Ethics statement

This study was approved by the animal ethics committee at Lund University with permit ID 5-8-18-02383/2020 and amendment 2021. All efforts were made to minimize animal suffering.

Rat glioma NS1 cells

The NS1 rat glioma cell line is a new green fluorescent protein (GFP)−positive tumor cell line created in our laboratory.[20] The cell line was initiated by treating pregnant homozygous GFP-positive Fischer 344 rats with ENU, where the offspring subsequently developed GFP-positive central nervous system tumors. The NS1 tumor cell line was established from an intraparenchymal tumor growing in the offspring. Rats inoculated with NS1 cells develop cell-rich tumors with an invasive growth pattern. Tumors are positive for glial fibrillary acidic protein, GFP, and express wild-type isocitrate dehydrogenase 1.[21] Sandwich

enzyme-linked immunosorbent assay was used to rule out mycoplasma infection in cells and supernatant and was used according to the manufacturer's instructions (MycoProbe R&D Systems). To verify the GFP signal, cells were cultured for 1 to 2 days in 2-chamber culture slides (Thermo Fisher Scientific) at 37°C in a humidified incubator with 5% CO_2. The medium was removed, and the cells were fixed in 4% paraformaldehyde. Cells were mounted with Eukitt Quick-hardening mounting medium (Sigma-Aldrich), stained with Hoechst (Thermo Scientific) and photographed with a fluorescent microscope fitted with the appropriate wavelength filters (Fig. 1A). In preparation for inoculations, cells were cultured using Iscove's Modified Dulbecco's Medium with addition of 1% mL Na-pyruvate, 1% mL HEPES (4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid), 0.1% mL gentamycin, and 10% inactivated fetal calf serum (heated to 56°C for 30 minutes). After culturing in T25 flasks, the cells were prepared for inoculation by removal of the medium and washed gently with phosphate-buffered saline. Trypsin (Invitrogen) was added, and cells were incubated at 37°C for 1 to 2 minutes to detach the adherent cells from the flask. Thereafter, medium was added, and viable cells were counted. The cells were centrifuged, the supernatant was removed, and the cell pellet was resuspended to achieve the concentration used for inoculation, 1000 cells/μL.

In vivo experiments

Seven- to 10-week-old female Fischer 344 rats (Fisher Scientific, Schwerte, Germany) were purchased and housed in pairs in cages (Taconic type 3 cages, 2 animals/cage) with access to water and fed ad libitum with rat chow. Rats were allowed to acclimatize for 1 week, after which tumor cells (50,000 NS1 cells) were subcutaneously inoculated in the hindleg at experimental day 0 with the

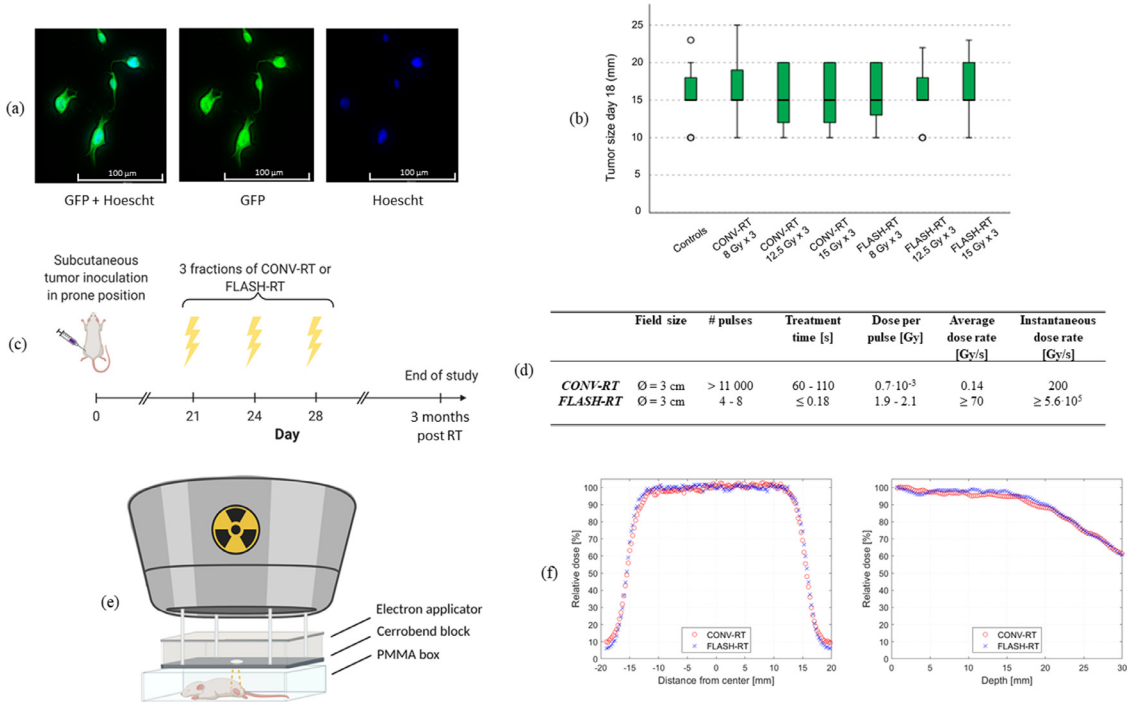


Fig. 1 Study design to compare hypofractionated FLASH radiation therapy (FLASH-RT) and conventional RT (CONV-RT) in a subcutaneous rat glioma model. A, The rat glioma cell line NS1 is a green fluorescent protein (GFP)−positive tumor cell line (left panel). Tumor cells are marked with Hoescht nuclear staining (right panel), and the GFP autofluorescence can be detected (middle panel). B, Animals were randomly assigned between groups (n = 9-10) according to tumor size on day 18 after inoculation, and a Kruskal-Wallis analysis did not show any statistical difference between the groups. The box-and-whiskers plot presents the resulting distribution of tumor size across groups. C, Timeline of the study design. Animals were inoculated in prone position on day 0 and irradiated with either CONV-RT or FLASH-RT on day 21, 24, and 28. D, Treatment parameters for CONV-RT and FLASH-RT. E, For irradiation, an electron applicator with source-to-applicator-end distance of 65 cm was attached to the gantry head of a clinical linear accelerator and fitted with a Cerrobend plate for beam collimation. Rats were placed in PMMA boxes and positioned 1-by-1 in close connection to the Cerrobend plate. F, Dose profiles at 4-mm depth and percentage depth dose curves for CONV-RT (red) and FLASH-RT (blue) measured in a polystyrene phantom placed inside a PMMA box to mimic the rat irradiation setup. This figure was created with BioRender.com.

animals in prone position. The estimated mean weight of the animals at the time of inoculation was 120 to 150 g, depending on age. The inoculation was performed during general anesthesia with isoflurane inhalation. At day 18, all tumors had reached a size ≥ 10 mm, but no tumor was >30 mm, and animals were randomized into different treatment groups. Kruskal-Wallis analysis did not show any difference between the groups (P = 1.0) with respect to tumor size at randomization (Fig. 1B). Sixty-eight animals were included in the study, with 9 or 10 animals per treatment group (n = 10 controls; n = 9 FLASH-RT 8 Gy × 3; n = 10 FLASH-RT 12.5 Gy × 3; n = 10 FLASH-RT 15 Gy × 3; n = 10 CONV-RT 8 Gy × 3; n = 9 CONV-RT 12.5 Gy × 3; n = 10 CONV-RT 15 Gy × 3). Animals in the control group were inoculated with tumor cells without any further treatment. Animals were monitored daily, and those showing signs of paresis, tumor diameter >30 mm, or declined general condition were euthanized. The criteria for euthanasia are defined in the animal ethics permission and in accordance with the acceptance from the ethics board.

Rat irradiation

Radiation therapy was administered in 3 fractions (day 21, 25, and 28) of either 8 Gy, 12.5 Gy, or 15 Gy, using CONV-RT or FLASH-RT (Fig. 1C). The irradiation source was a 10 MeV electron beam from a clinical Elekta Precise linear accelerator (Elekta AB, Stockholm, Sweden). For FLASH-RT delivery, the treatment machine was temporarily modified to deliver ultrahigh-dose-rate electrons in 3.5 μs pulses, as described elsewhere.[22] CONV-RT treatments were delivered during 60 to 110 seconds with an average dose rate of 8 Gy/min and an instantaneous dose rate of 200 Gy/s (Fig. 1D). The FLASH-RT treatments were delivered with 4, 6, or 8 pulses, resulting in total treatment times of ≤180 ms, average dose rates of ≥70 Gy/s, and instantaneous dose rates of $\geq 5.6 \cdot 10^5$ Gy/s. The dose per pulse was adjusted by varying the source-to-surface distance between 65 and 67 cm. For both CONV-RT and FLASH-RT irradiation, an electron applicator was fitted with a Cerrobend plate creating a circular radiation field of 3 cm in diameter. Before treatment, the animals were anesthetized by intraperitoneal injection of Ketalar/Rompun and positioned in custom-made PMMA boxes. For irradiation, the boxes were positioned at the corresponding source-to-surface distance with the tumor in the center of the field (Fig. 1E). The absorbed dose was prescribed at 4-mm depth in the animal. Dosimetry was performed using GafChromic film (XD film, Ashland Advanced Materials, Bridgewater NJ) in a polystyrene phantom placed in one of the boxes to mimic the rat, measuring percentage depth dose curves and dose profiles at 4-mm depth for both the CONV-RT and FLASH-RT beam (Fig. 1F). XD film measurements at 4-mm depth in the phantom were also performed before each treatment session. During treatment, a Farmer-type ionization chamber (NE 2505/3-3A) positioned in a custom-made holder was used for relative output measurements to ensure output stability in FLASH-RT mode.

Tumor growth, overall survival, and treatment response

The maximum tumor diameter (d) was measured for all animals once every week using a caliper. Measurements were carried out by the same personnel throughout the study, blinded to the assigned treatment group. Overall survival was determined from the day of inoculation (day 0) until the criteria for euthanasia were reached. At the end of the study, animals were further divided by treatment response based on the maximum tumor diameter at day 100 (d_{100}), as long-term tumor control ($d_{100} = 0$), stable disease ($0 > d_{100} \leq d_{18}$), tumor progression ($d_{100} > d_{18}$), or animals euthanized during the observation period due to large tumor.

Acute and late radiation-induced skin reactions

Animals were evaluated for acute radiation-induced skin reactions according to a phenotypic grading scale of 1 to 6 (1: normal, 2: hair loss, 3: erythema, 4: dry desquamation, 5: <30% moist desquamation, and 6: >30% moist desquamation) established by de Andrade et al.[23] Observations and toxicity evaluations were performed weekly, 2 to 5 weeks after completed RT. Animals that were euthanized due to large tumors (diameter >30 mm) during the study period were excluded from the analysis to avoid mistaking a subtherapeutically treated fast-growing tumor for local side effects to the skin. At 3 months after initiation of RT, the surviving animals were evaluated for late skin toxic effects by determining the ratio of animals with toxic effects greater than grade 1.

Statistical analysis

SPSS (IBM, Armonk, NY) was used for statistical evaluations. The Kruskal-Wallis test, Mann-Whitney U test, and Fisher exact test were performed for nonparametric analyses. Survival curves were assessed using log rank test. All significance tests were performed with a significance level of 5%.

Results

Dose delivery

The measured beam characteristics demonstrated a full width at half maximum of 3.1 cm for both the FLASH-RT and CONV-RT beams and a therapeutic range of 2.4 cm (Fig. 1F), indicating that the animals were irradiated with good dose coverage across the tumor. Based on the film measurements conducted before each treatment session, all fractions were estimated to be within 5% of the prescribed dose, and 81% (141 of 174) of the fractions were within 3%. The average agreement between the prescribed dose and the estimated dose to the tumor was 0.1% (range, −0.9% to +1.3%) for CONV-RT delivery and 2.5% (range, −0.4% to +5.0%) for FLASH-RT delivery.

Tumor growth and overall survival

In general, irradiated tumors started to shrink at 1 week post−completed RT (Fig. 2). Mean tumor size for each group (solid lines in Fig. 2) was determined at each measuring point until the first animal in the respective group was euthanized. All animals in the control group were euthanized before day 100 owing to tumors exceeding 30 mm in diameter.

Survival was significantly increased in all groups compared with control animals (log rank test, $P < .001$) (Fig. 3A-D). There was no statistically significant difference between animals treated with FLASH-RT and CONV-RT at any of the dose levels. For CONV-RT, there was a statistically significant difference comparing 8 Gy × 3 versus 12.5 Gy × 3 ($P = .007$) or 8 Gy × 3 versus 15 Gy × 3 ($P = .026$), but no difference comparing 12.5 Gy × 3 versus 15 Gy x 3. For FLASH-RT, there was a statistically significant difference comparing 8 Gy × 3 versus 15 Gy × 3 ($P = .012$) but no difference comparing 8 Gy × 3 versus 12.5 Gy × 3 or 12.5 Gy × 3 versus 15 Gy × 3. With the present sample size, power estimation was 0.999 to detect differences in survival between groups (significance level 5%).

Treatment response

In total, 78% (45 of 58) of the irradiated animals were alive on day 100, and in 40% (23 of 58) of the tumors, a long-term tumor control was achieved by RT. For animals irradiated with 8 Gy × 3, a similar tumor control was observed for CONV-RT and FLASH-RT, with 44% (4 of 9) and 40% (4 of 10) of animals either with long-term tumor control or stable disease on day 100, respectively (Fig. 3E). For animals irradiated with 12.5 Gy × 3, 2 animals in the FLASH-RT group were euthanized before the end of the study due to large tumors, whereas long-term tumor control or stable disease was evident for all animals in the CONV-RT group. All animals irradiated with 15 Gy × 3 achieved long-term tumor control or had stable disease, except for 1 animal belonging to the CONV-RT group, which was euthanized 1 week before the end of the observation period due to a large tumor.

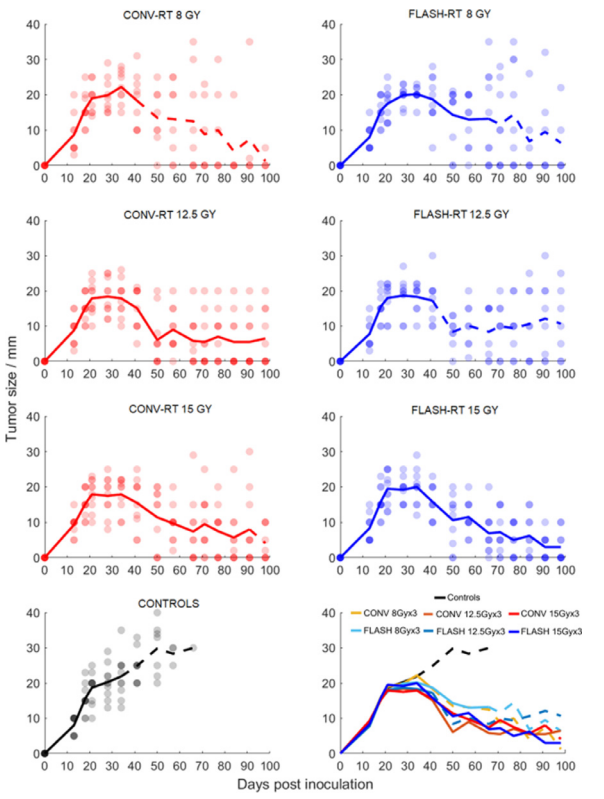
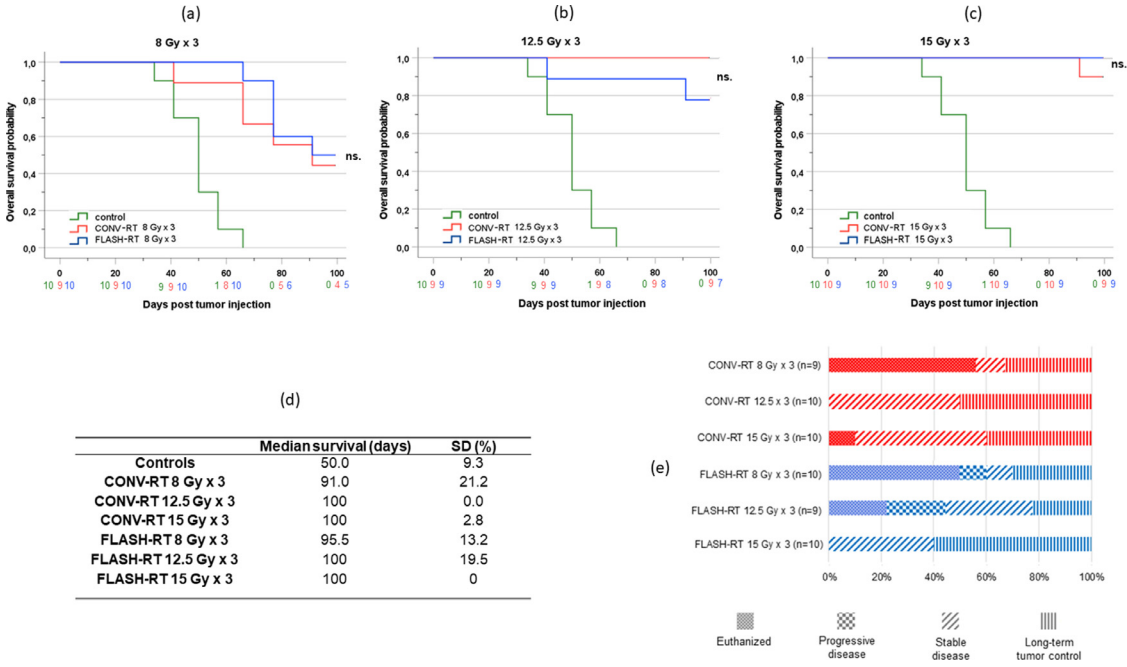


Fig. 2 Tumor growth for subcutaneous glioblastoma inoculated at the flank of Fisher 344 rats and irradiated with hypofractionated conventional radiation therapy (CONV-RT) or FLASH-RT at day 18, 21, and 24, as well as for nonirradiated controls. Dots represent tumor size for each individual animal measured once a week. Calculated mean tumor size for each group is presented as solid lines. Dotted lines indicate the mean tumor size calculated when ≥1 animals had been euthanized during the study period, thus slightly underestimating the actual mean tumor size.

Radiation-induced skin reactions

Radiation-induced skin reactions were generally mild and consisting of hair loss, erythema, and dry desquamation. Acute skin effects at 2 to 5 weeks post−completed RT were dose- and time-dependent (Fig. 4A). There was no significant difference in acute side effects between FLASH-RT and CONV-RT for any of the investigated dose levels at any of the investigated time points (Mann-



	Median survival (days)	SD (%)
Controls	50.0	9.3
CONV-RT 8 Gy x 3	91.0	21.2
CONV-RT 12.5 Gy x 3	100	0.0
CONV-RT 15 Gy x 3	100	2.8
FLASH-RT 8 Gy x 3	95.5	13.2
FLASH-RT 12.5 Gy x 3	100	19.5
FLASH-RT 15 Gy x 3	100	0

Fig. 3 Overall survival and treatment response for glioblastoma-bearing rats irradiated with hypofractionated conventional radiation therapy (CONV-RT) or FLASH-RT, as well as nonirradiated controls. A-C, Kaplan-Meier survival curves for all study groups, demonstrating no significant difference in survival between CONV-RT and FLASH-RT at any of the investigated dose levels (n = 9-10, log rank test). No deaths occurred after CONV-RT 12.5 Gy × 3 and FLASH-RT 15 Gy × 3. Colored numbers below the x-axis represent the number of animals still alive at each time point. D, Median survival and standard deviations (SDs) for each study group. E, Animals irradiated with hypofractionated CONV-RT or FLASH-RT categorized on day 100 as euthanized (dotted pattern), progressive disease (check), stable disease (diagonal stripes), or long-term tumor control (vertical stripes). In total, 78% (45 of 58) of the irradiated animals were alive on day 100.

Whitney *U* test). Most acute side effects healed spontaneously. The ratio of survivors with late side effects greater than grade 1 at 3 months after initiation of RT increased with increasing fraction dose (Fig. 4B). There was no significant difference in late side effects between CONV-RT and FLASH-RT for any of the dose levels investigated (2-sided Fisher exact test).

Discussion

In the present study, we compare hypofractionated FLASH-RT versus hypofractionated CONV-RT for treatment of large subcutaneous glioblastomas in immunocompetent rats. All animals had verified tumors upon initiation of treatments. Treatment doses were chosen to achieve tumor growth delay as well as high probability of long-term tumor control for the highest doses. To obtain toxicity data on the same material, we examined the animals for normal tissue complications at early time points (weekly, 2-5 weeks after RT) and at a late time point (3 months after RT).

Despite recent in vitro findings of a sparing of tumor cells using FLASH-RT,[24] the current study showed no difference in survival between CONV-RT and FLASH-RT for any of the delivered doses. Similar to previous studies on glioblastoma-bearing mice,[11,12] irradiated animals displayed a delayed tumor growth compared with control animals. At doses of 8 Gy × 3, neither FLASH-RT nor CONV-RT was sufficient to achieve adequate tumor control in all animals. However, at higher dose levels most of the animals achieved long-term tumor control or had stable disease on day 100. On the contrary, Montay-Gruel et al showed that no animals with glioblastoma in the brain, treated with 10 Gy × 3 at 3 days after inoculation in immunodeficient animals, lived for 100 days.[11] Local early side effects were time-dependent, indicating that the time elapsed from irradiation to evaluation appears important and that frequent follow-up is needed. There was no severe toxic effect associated with any of the treatments. No difference in acute or late side effects between FLASH-RT and CONV-RT could be resolved for any of the investigated dose levels. Combining these results with previous studies comparing skin toxic effects between FLASH-RT and CONV-RT,[8,13,25-27] it seems that high fraction doses are required to detect a FLASH-sparing effect in the skin. In the first report on a skin-sparing effect of FLASH-RT compared with CONV-RT, single

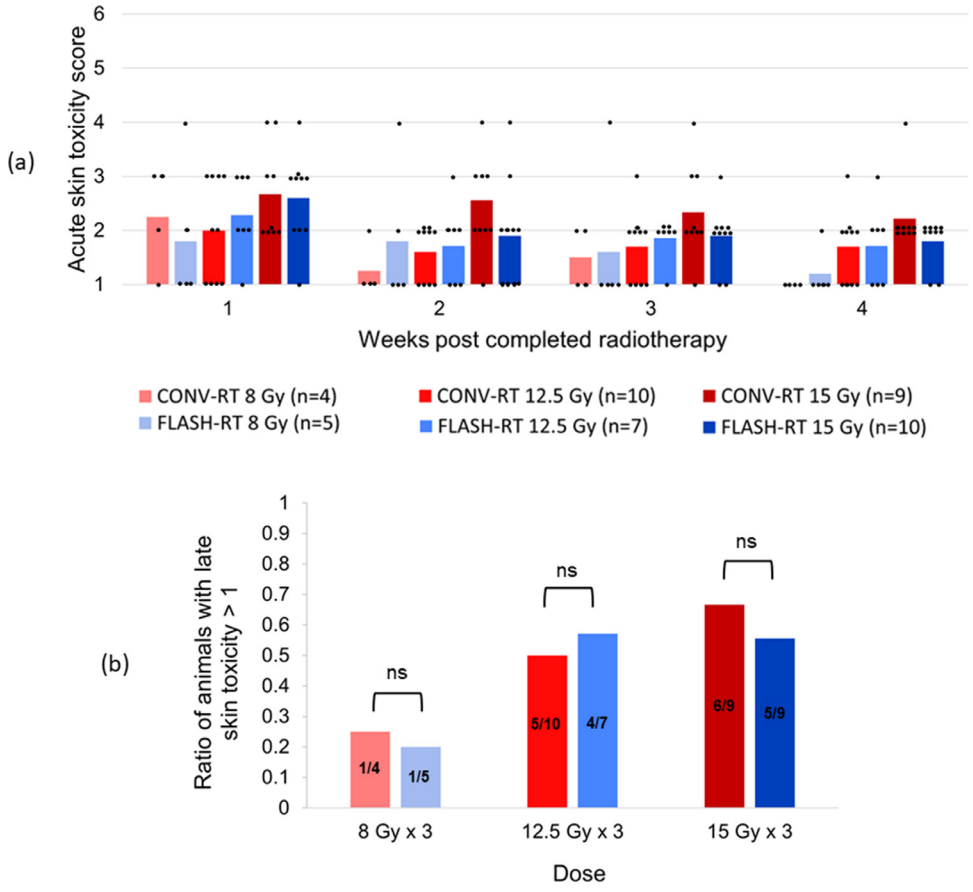


Fig. 4 Severity of skin toxic effects in animals irradiated with conventional radiation therapy (CONV-RT) or FLASH-RT in 3 fractions of either 8 Gy, 12.5 Gy, or 15 Gy. Local dermal side effects were graded on a scale of 1 to 6 (1: normal, 2: hair loss, 3: erythema, 4: dry desquamation, 5: <30% moist desquamation, and 6: >30% moist desquamation). No severe toxic effect (grade >4) was observed. There was no significant difference in acute or late skin toxic effects between CONV-RT and FLASH-RT for any of the dose levels at any time point (Mann-Whitney *U* test and 2-sided Fisher exact test). A, Acute skin toxic effects at 2 to 5 weeks post−completed radiation therapy. Bars represent the average score for each treatment group, and dots represent scores of individual animals. B, The ratio of survivors with late side effects >1 at 3 months after initiated radiation therapy.

fractions in the range 28 to 34 Gy were administered to the skin of a pig.[8] Soto et al found a lower incidence and severity of skin ulcerations for FLASH-RT compared with CONV-RT at doses of 30 and 40 Gy, but no severe toxicity ≤20 Gy [25]. In a direct comparison of 15 Gy FLASH-RT and 15 Gy CONV-RT in a human patient with multiply relapsed cutaneous T-cell lymphoma, Gaide et al did not observe any difference in acute or late effects.[26] Furthermore, our preliminary results on skin toxic effects in flank melanoma-bearing mice show a substantial difference between FLASH-RT and CONV-RT in a single fraction of 25 Gy, compared with no or a small difference for doses in the range 10 to 20 Gy.[28] It should be noted that a large subtherapeutically treated tumor on the flank may influence the scoring of local dermal side effects. To avoid this in the present study, animals that were euthanized due to large tumors during the study period was excluded from the toxicity analysis. At the timepoint for evaluation of late side effect, the animals had either no tumors or small tumors, implying that the evaluation was not compromised by tumor size. However, separate studies of toxic effects in tumor-free animals are needed to completely avoid the tumor as a confounding factor.

In contrast to skin tissue, Montay-Gruel et al demonstrated improved neurocognitive function in nude mice following brain irradiation with hypofractionated 10 Gy × 3 FLASH-RT compared with CONV-RT.[11] Therefore, it is likely that the threshold dose for inducing a FLASH-sparing effect varies between normal tissue types and the environment. For example, it has been shown in vitro that the FLASH effect depends on oxygen concentration.[29] Also, the use of immunocompromised animals may result in a different response than immunocompetent hosts. It is known that RT has immunologic effects that can reshape the tumor microenvironment,[30] and it has been proposed that the FLASH effect can be caused

by a modification of the immune response, as the ratio of irradiated circulating T-lymphocytes is likely to be reduced compared with the longer treatment time used for CONV-RT.[31] Accordingly, we believe that it is important to investigate the FLASH effect in immunocompetent animal models.

The measured beam characteristics indicate that the animals were irradiated with adequate dose coverage across the tumor, and the absorbed dose measurements performed before each treatment session confirmed that the prescribed doses were delivered accurately. For FLASH-RT, the temporal structure of the electron beam has been shown to be important.[7,11,12] In this study, the treatment parameters previously recognized as critical for the FLASH effect (ie, average dose rate, instantaneous dose rate, beam-on time, and fraction dose) were expected to be sufficient to observe a potential FLASH effect.[7,11,12] Using similar temporal parameters, we observed a sparing FLASH effect in our laboratory, both in vitro[24,29] and in vivo.[28] It could be that the fraction doses investigated in this study are too low to observe a sparing effect on skin. However, long-term tumor control was still achieved. Further studies on tumor cure and normal tissue toxicity are required to investigate the therapeutic window at higher doses. FLASH-RT has not yet been explored for standard fractionated treatments, such as the 60 Gy/30 fractions used in the Stupp protocol currently employed for glioblastoma patients. However, as previously discussed, there are indications that higher fraction doses are required to observe a FLASH effect. Ultimately, to ensure a clinical translation of FLASH-RT for a specific tumor type and site, the therapeutic window should be studied in clinically interesting scenarios in models where tumor cure can be achieved.

Although the subcutaneous glioma model cannot be used to draw conclusions about the interactions between the microenvironment in the brain and glioma cells, it is used here as a first step to investigate tumor control and normal tissue complications in multiple treatment groups. In the intracranial setting, additional challenges are encountered, including the blood-brain barrier, which facilitates immune evasion, as well as spread of tumor cells within the brain parenchyma and interactions with the complex microenvironment.[32] In the next step, we will use the glioma model intracranially to further explore the effect of these issues in immunocompetent animals treated with FLASH-RT and CONV-RT.

Conclusion

In the present study, we show that long-term tumor control can be achieved in large subcutaneous glioblastomas without inducing severe skin toxic effects, using both hypofractionated FLASH-RT and CONV-RT. No difference in tumor response between FLASH-RT and CONV-RT could be resolved, and no significant difference in treatment toxicity was found, suggesting that higher doses may be required to detect a FLASH sparing of the skin. This study is the first to show that there is no difference in long-term tumor control rates between FLASH-RT and CONV-RT in immunocompetent glioblastoma-bearing animals.

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