in vivo *28*: 1-12 (2014)

Improved Survival of Mice After Total Body Irradiation with 10 MV Photon, 2400 MU/min SRS Beam

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Abstract. *Background/Aim: We evaluated the radiobiological effects of stereotactic radiosurgery (SRS) photon beams on survival of C57BL/6NTac mice following total body irradiation. Materials and Methods: Survival of Lewis lung carcinoma (3LL) cells was tested after irradiation using 6 MV: 300 MU/min or 1400 MU/min; or 10 MV: 300 MU/min or 2400 MU/min. Survival of C57BL/6NTac mice after a dose which is lethal to 50% of the mice in 30 days (LD50/30) (9.25 Gy) total body irradiation (TBI) and 21 Gy to orthotopic 3LL tumors was tested. We quantitated levels of organ-specific gene transcripts by Real Time Polymerase Chain Reaction (RT-PCR). Results: While 3LL cell survival and inhibition of orthotopic tumor growth was uniform, 10 MV photons at 2400 MU/min TBI led to significantly greater survival (p=0.0218), with higher levels of intestinal (Sod2), (Gpx1), (Nrf2), and (NFκB) RNA transcripts. Conclusion: Clinical 10 MV-2400 cGy/min SRS beams led to unexpected protection of mice on TBI and increased radioprotective gene transcripts.*

Clinical trials using stereotactic radiosurgery (SRS) have demonstrated effective tumor control with escalating doses (1). Ionizing irradiation therapy using accelerated dose delivery and higher beam energy has improved dosimetry, shortened treatment duration, and has the potential to enhance clinical efficiency, minimize patient discomfort and target motion (1). Stereotactic body radiotherapy (SBRT) using these principles reduces the toxicity of protocols involving concomitant chemotherapy (2-5). Because tumor target volumes in SRS are smaller and doses higher, it has been assumed that the normal tissue radiobiology of currently evaluable (SRS) photon energies and dose rates is uniform and should not require investigation.

Increased irradiation dose rate may saturate the cellular damage response to ionizing radiation, while lower dose rates should better-allow repair of DNA damage (2-5); however, the known radiobiological effects of dose rate (2-5) should not be relevant to the dose rates used in these studies.

We compared clinically-utilized 6 MV with 10 MV photons, and dose rates of 300 MU/min, 1400 MU/min, and 2400 MU/min in SRS with respect to *in vitro* and *in vivo* measurements of tumor control and normal tissue response in a mouse model.

Materials and Methods

Mice and animal care. C57BL/6NTac adult female mice (Taconic Farms, Hudson, NY, USA) were housed five per cage and maintained according to University of Pittsburgh Institutional Animal Care and Use Committee (IACUC)-directed laboratory conditions. Veterinary care was provided by the Division of Laboratory Animal Research of the University of Pittsburgh. All protocols were IACUC-approved (University of Pittsburgh Protocol 1201406).

In vitro clonogenic irradiation survival curves. A Lewis lung carcinoma cell culture line (3LL) was established from a lung tumor from C57BL/6 mice (6). Cells were suspended at 1×10^6 cells/ml and irradiated in suspension to doses ranging from 0 to 800 cGy using dose rates and 6 MV or 10 MV beam energies of the Truebeam linear accelerator (Varian STx Medical Systems, Palo Alto, CA, USA) including 300 MU/min, 1400 MU/min, and 2400 MU/min dose rate for clinical SRS Linear Accelerator parameters. Cells were plated in 4-well Linbro tissue culture plates (MP Biomedicals, LLC, Salon, OH, USA) as previously described (7, 8) and incubated at 37˚C, in 21% oxygen, with 5% CO_2 for 7-14 days, and stained with crystal violet. Colonies of greater than 50 cells were counted using a GelCount colony counter (Oxford Optronix, Oxford, UK). Triplicate *in vitro* clonogenic radiation survival curves were analyzed by both linear-quadratic model and the single-hit multi-target model, and were compared using the final slope representing multiple-event killing (D_0) and the extrapolation number measuring the width of the

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+Presented at the American Society of Therapeutic Radiology and Oncology, 55th Annual Meeting, Atlanta, GA, September 22-25, 2013.

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Key Words: Stereotactic radiosurgery, dose rate, photon energy.

shoulder on the radiation survival curve (ñ) (7). Results for D_0 and ñ are presented as the mean±standard error of the mean (SEM) from at least three measurements. The two-sided two sample *t*-test was used to compare means of different groups (7).

Mouse TBI. A TrueBeam STx linear accelerator (Varian Medical Systems), which is commonly used for SRS in current radiation oncology was configured for TBI mouse studies. C57BL/6NTac female adult mice (N=15 to 20 per group in each of triplicate experiments) were irradiated to the LD 50/30 TBI dose (9.25 Gy) using each of four configurations: dose rate of 300 MU/min (300 cGy/min) with 6 or 10 MV photon beams; dose rate of 1400 MU/min (1400 cGy/min) with a 6 MV flattening filter free (FFF) photon beam; or dose rate of 2400 MU/min (2400 cGy/min) with 10 MV FFF photon beam.

The field size for each beam tested was set to 40 × 40 cm with a source to skin distance (SSD) of 100 cm. Mice were irradiated in groups of five in a plexiglass block of 20 cm × 20 cm × 3 cm with a 12 cm × 9 cm × 2 cm section cutout into which the mice were placed for irradiation. The plexiglass container was placed in the center of the irradiation field on 3 cm of bolus and was surrounded by a 5 cm minium bolus to provide full scatter condition to the plexiglass phantom. The mice were anesthesized using nembutal before irradiation. During irradiation, the mice were covered by 1 cm bolus when irradiated with 6 MV photons, and a 2.0 cm bolus for 10 MV photons to standardize for differences in the dose build-up.

To verify uniformity of dose delivered, several thermoluminescent dosimeter (TLD) measurements were performed with each mouse in each experiment. Sixteen TLDs were chosen with a dose response difference within 3% and were divided into eight groups with two TLD chips for each group. For all measurements, TLD chips were placed as close to the central axis as possible: one group was placed on top of each mouse in the central position, one in the midline, and one at the bottom. A total of six groups of TLD chips were used for each of the two beam energies investigated. Two groups of TLD chips were used as control groups for the two energies and irradiated to the dose of 9.25 Gy under each machine calibration condition (SSD=100 cm at dose maximium point with a 10 cm × 10 cm field). The irradiated doses at top, middle and bottom positions of the central mouse in each group of five were determined by comparison of the average readings at different positions with those from control detectors. All mice were followed for survival after LD 50/30 irradiation to 9.25 Gy by TBI. This dose induces the hematopoietic syndrome in C57BL/6NTac mice (7). Mice were maintained according to IACUC-directed laboratory conditions.

RT-PCR analysis of tissue levels of gene transcripts for irradiation-inducible transcription factors, growth factors, inflammatory cytokines, adhesion molecules, and antioxidant enzymes. Representative mice from each group were sacrificed either one or seven days following 9.25 Gy TBI delivered using each configuration: 6 MV photons at 300 MU/min or 1400 MU/min, or 10 MV photons at 300 MU/min or 2400 MU/min. Brain, heart, liver, intestine and bone marrow were removed and frozen on dry ice. RNA was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions, quantified using a spectrophotometer, and stored at −80˚C. Reverse transcription of 2 μg of total RNA to complementary DNA (cDNA) was accomplished using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol.

In subsequent steps, expression of specific RNA moieties was quantitated and included: *Gapdh* (Gen-Bank: NM_008084.2), *Sod2* (Gen-Bank: NM_013671.3), *TgfB* (Gen-Bank: NM_011577), Gpx1 (Gen-Bank: NM_008160.6), *Nrf2* (Gen-bank: NM_010902.3), *NfκB* (Gen-Bank: NM_008689.2), *Sp1* (Gen-Bank: NM_013672.2), *Fas* (Gen-Bank: NM_007987.2), bone marrow specific B2m (Gen-Bank: NM_009735), and brain-specific *Stx1* (Gen-Bank: NM_016801.3). Each transcript was quantitated by real-time polymerase chain reaction (RT-PCR), as previously described (7). Ninety-six-well plates were prepared with 10 μl of Taqman Gene Expression Master mix, 5 μl of RNase-free water, 1 μl of the corresponding Taqman Gene Expression probe, and 4 μl of cDNA (totaling 2 μg cDNA) using the Eppendorf epMotion 5070 automated pipetting system (Eppendorf, Westbury, NY, USA). The cDNA was amplified with 40 cycles of 95˚C (denaturation) for 15 s and 60˚C (annealing and elongation) for 1 min using the Eppendorf Realplex2 Mastercycler.

Data for each gene transcript were normalized by calculating the differences (ΔCt) from the Ct-GUSB and Ct-Target genes. Subsequently, the relative increase or decrease in expression of each transcript was calculated by comparing the reference gene with the target gene (ΔΔCt) and using the formula for relative expression ($=2^{\Delta\Delta Ct}$). The results are presented as the percentage increase in RNA above baseline levels in control non-irradiated C57BL/6NTac mice.

Orthotopic tumor irradiation. Female C57BL/6NTac mice were injected in the hind limb with 3LL cells (6). Cells were suspended at 1×10^7 cells/ml, and 1×10^6 (100 μl) was injected subcutaneously into the right hind leg. Mice were observed daily and when tumors became palpable (seven days later), gross tumor volume was measured in millimeter by handheld caliper forceps. Mice were anesthesized using nembutal and placed on 3 cm of bolus with the tumor bearing leg placed in an 8 × 8 cm field with the remainder of the mouse shielded. Hind leg targeted radiation treatment volume received 21 Gy delivered with 6 MV photons at 300 MU/min to 10 mice or 10 MV FFF photons at 2400 MU/min to 10 mice. Ten control mice were not irradiated. Triplicate experiments were carried out.

Mice were covered with 1 cm of bolus when irradiated with 6 MV photons or 2 cm bolus when irradiated with 10 MV photons. Mice were irradiated at 100 cm SSD. Tumor volume was measured daily following irradiation and size plotted. When one dimension of any tumor grew to 12 mm or more, the mouse was sacrificed as specified by IACUC regulations. Tumor volume data are given as the mean±standard deviation (SD) for each of the three groups (6 MV 300 MU/min, 10 MV 2400 MU/min and non-irradiated) for each day of measurement.

Statistics. The effect of cesium gamma cell irradiation dose rates on 3LL survival *in vitro* was analyzed with the single-hit multitarget model where D_0 (final slope representing multiple-event killing) and ñ (extrapolation number measuring width of the shoulder on the radiation survival curve) were calculated (8). Data for D_0 and ñ were compared between groups with the two-sided two-sample *t*-test.

In the mouse survival comparison study, C57BL/6NTac mice were irradiated to 9.25 Gy (LD50/30)TBI using different dose rates and photon energies, and followed-up for survival. Mouse survival curves were estimated with the Kaplan–Meier method and compared between groups with the two-sided log-rank test.

The RT-PCR transcript data are summarized as the mean±standard deviation in each group. For each gene and each organ (liver, heart, intestine, brain or bone marrow) at each time

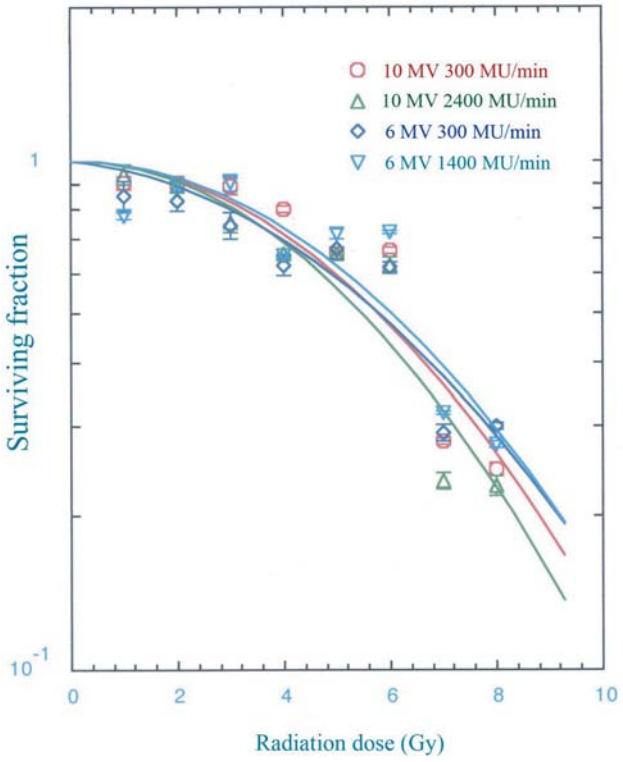

Figure 1. *Changes in dose rate do not affect Lewis lung carcinoma (3LL) in vitro irradiation survival curves. 3LL cells were irradiated to doses ranging from 0 to 8 Gy at 300, 1400 or 2400 cGy per min using 6 MV in 10 MV energies as indicated and plated in 4-well tissue culture plates. Seven days later, the cells were stained with crystal violet and colonies greater than 50 cells were counted and analyzed using linear quadratic and single-hit, multitarget models. There were no significant differences between the curves.*

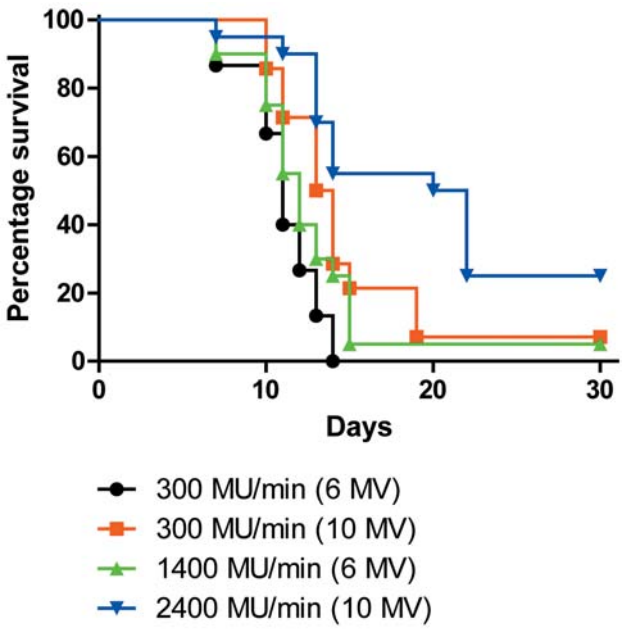

Figure 2. *Mice treated with 10 MV photons have increased total body irradiation (TBI) survival. C57BL/6NTac female mice received TBI to 9.25 Gy using 300 MU/min with 6 MV photons, 300 MU/min using 10 MV photons, 1400 MU/min using 6 MV photons or 2400 MU/min using 10 MV photons. Mice treated with 2400 MU/min using 10 MV photons had a significantly increased survival compared to the other treatment groups (Table III). In addition, mice treated with 300 MU/min using 10 MV photons had significantly increased survival compared to mice treated with 6 MV photons at 300 MU/min.*

Table I. *Effect of true beam dose rate and beam energy on 3LL Lewis Lung carcinoma clonogenic radiation survival curves.*

Dose rate and photon energy	Do (Gy)	ñ
300 MU/min + 6 MV	1.7±0.3	10.0±6.7
300 MU/min + 10 MV	1.7±0.2 (p=0.6605)	6.8±4.5 (p=0.7551)
1400 MU/min + 6 MV	1.9±0.3 (p=0.9243)	13.3±6.1 (p=0.7559)
2400 MU/min + 10 MV	2.0±0.1 (p=0.9217	9.9±2.7 (p=0.9915)

p-Values compare treatments with 300 MU/min at 6 MV. Results are for triplicate experiments.

point (24 h or seven days after irradiation), the four treatments (6 MV 300 MU/min, 6 MV 1400 MU/min, 10 MV 300 MU/min, and 10 MV 2400 MU/min) were compared by one-way ANOVA followed by multiple comparisons. These *post-hoc* multiple comparisons were performed with F-tests by using the CONTRAST statement in SAS Proc GLM (SAS Institute, Inc, Cary, NC, USA). The following comparisons were made: 6 MV 300 MU/min to 10 MV 2400 MU/min; 6 MV 1400 MU/min to 10 MV 2400 MU/min; 10 MV 300 MU/min to 10 MV 2400 MU/min; 10 MV 1400 MU/min to the average of the other three treatment groups.

Mouse tumor volume data are summarized by mean±standard deviation for each of the three groups (*i.e.* 6 MV photons at 300 MU/min, 10 MV photons at 2400 MU/min and the non-irradiated group) at each day of measurement, where data for sacrificed mice were included in the mean and SD calculations at subsequent time points. The volume data were log-transformed, and a linear mixed model was built on the transformed data, where group and day of measurement and their interaction were used as fixed explanatory variables, and the within subject variable, day of measurement, as a repeated measure. The F-test was used to test for the overall significance of the interaction between group and day of measurement. A significant interaction indicates a significant difference in tumor growth rate between groups. Comparison between the three groups was also performed for each day of measurement using the normalized tumor volume. The normalized tumor volume was calculated for each mouse by dividing the volume at each time point by the data at time 0. We compared the normalized tumor volume between pairs of groups for each day of measurement using Wilcoxon rank sum tests.

In all the above tests, a *p*-value of less than 0.05 was regarded as significant. As an exploratory analysis, we did not adjust *p*-values for multiple comparisons.

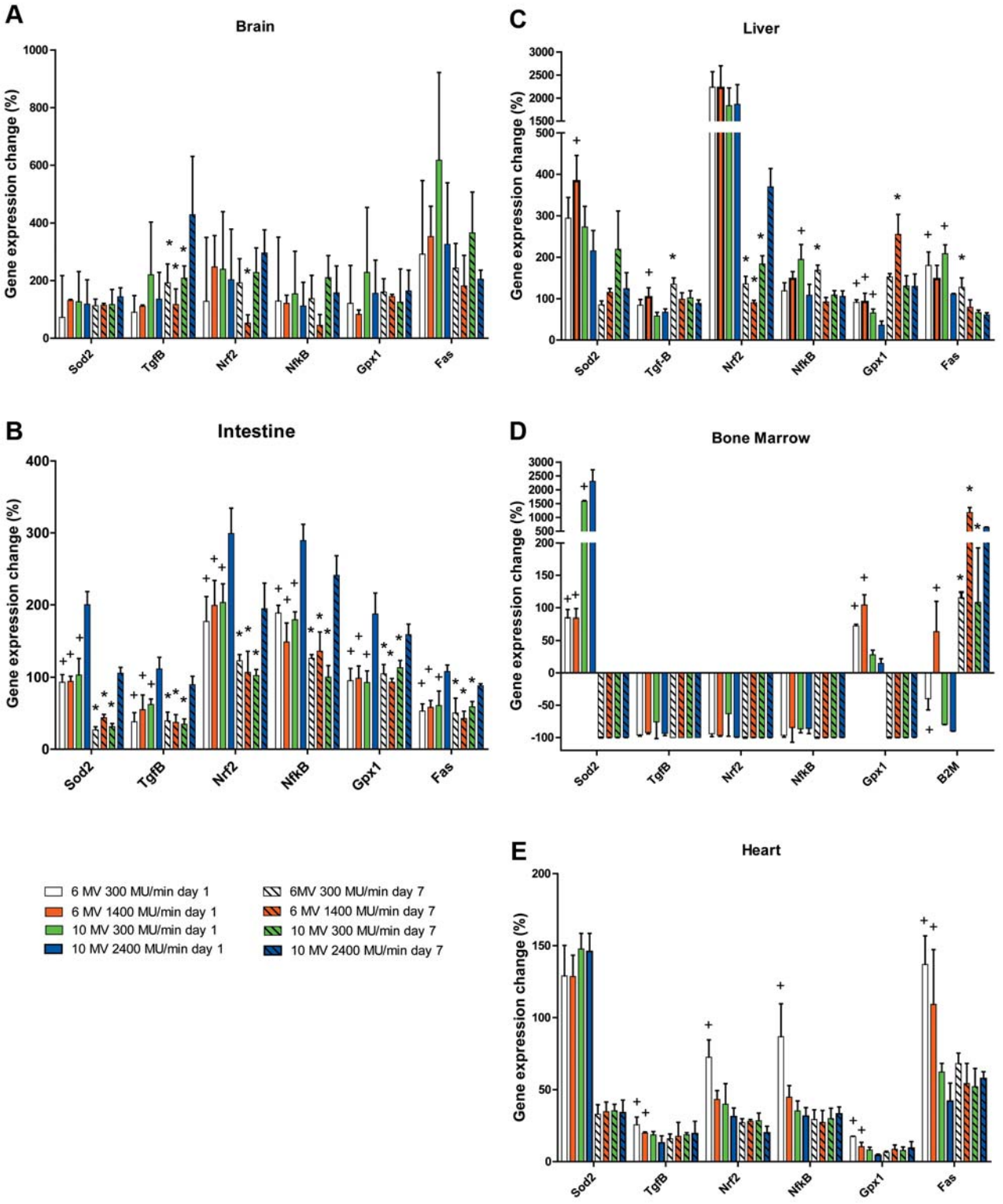

Figure 3. *Level of gene transcripts following total body irradiation (TBI) depends on energy photons. C57BL/6NTac female mice received TBI to 9.25 Gy at 300 MU/min using either 6 or 10 MV photons, 1400 MU/min using 6 MV photons, or 2400 MU/min with 10 MV photons. On day 1 or 7 following irradiation, mice were sacrificed from each group with the brain, heart, liver, intestine, or bone marrow removed, and frozen in liquid nitrogen. RNA was extracted and real time PCR was used to determine gene expression for TgfB, Sod2, Nrf2, NFkB, Sp1, Gpx1, Fas, Stx1 and B2-microglobulin (7).*

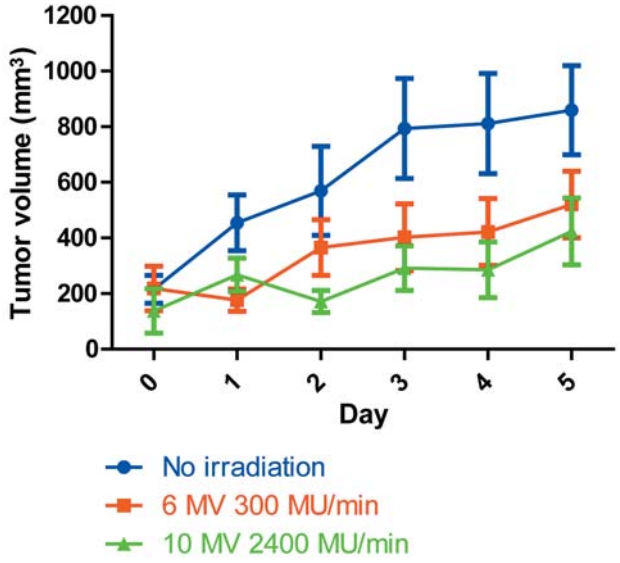

Figure 4. *Reductions in growth of irradiated (3LL) orthotopic tumors is not affected by photon energy. Mice bearing 3LL orthotopic tumors on the right rear leg were irradiated to 21 Gy using 300 MU/min with 6 MV photons or 2400 MU/min using 10 MV photons. The tumors were measured and tumor volumes determined immediately before irradiation and daily following irradiation. Irradiation of the tumors resulted in decreased tumor growth with no significant difference between the two treatment groups.*

Results

No significant effect of SRS dose rate on 3LL cell line clonogenic survival. Triplicate *in vitro* clonogenic irradiation survival curves with 3LL cells were first carried out using the different dose rates and photon energy as described in Materials and Methods. The data showed no significant difference in D_0 or ñ between the three dose rates and two photon energies (Figure 1, Table I). These dose rates were utilized for orthotopic tumor experiments with 3LL tumors on the linear beam accelerator.

TLD measurements confirm uniform radiation dose delivery to mice by 6 MV and 10 MV photons using the SRS linear accelerator platform. Following TLD placement as described in the Materials and Methods section, with placement of detectors above, between, and below mice receiving 9.25 Gy TBI, the measurements confirmed there was no significant difference in average overall TBI dose delivered between 6-MV and 10-MV photons (Table II).

Unexpected effect of dose rate and beam energy on mouse survival after TBI. The effects of the differences in dose rate and photon energy were next compared in mice after TBI in triplicate experiments. There were significant differences in survival following TBI between the groups of mice (Table III, Figure 2 and Figure 5). The combination of 2400 MU/min dose rate and 10 MV photons was clearly less lethal for the same TBI dose.

Table II. *Thermoluminescent dosimeters (TLD) measurement of the radiation dose delivered to mice using 6-MV or 10-MV photons. C57BL/6NTac mice were irradiated to 9.25 Gy using either using 6 MV photons at 300 or 1400 MU/min, or 10 MV photons at 300 or 2400 MU/min. TLDs were placed on top, between, or under the mice. The dose delivered using 6 MV photons was not statistically different from that delivered using 10 MV photons.*

	6 MV Photon	10 MV Photon	*p*-Value for 6 MV *vs*. 10 MV
Bottom	9.3±0.05 Gy	9.26±0.21 Gy	0.8855
Middle	9.51±0.26 Gy	9.68±0.11 Gy	0.6082
AboveTop	9.59±0.33 Gy	9.58±0.21 Gy	0.9909
Mean	9.46±0.21 Gy	9.5±0.18 Gy	0.8844

Beam energy-and dose rate-dependent induction of antioxidant and inflammatory cytokine RNA transcripts in vivo. We sacrificed representative mice and excised tissues at one or seven days after TBI using each set of conditions and analyzed RNA transcript levels in serial tissues. There were clear effects of the beam configuration of 10 MV at 2400 MU/min. Intestine at both 1 and 7 days after 9.25 Gy TBI using 10-MV photons at 2400 MU/min showed significantly increased expression of RNA transcripts for Sod2, Gpx1, TgfB, Nrf2, and NfkB ($p<0.05$; Table IV, Figure 3A and B). Liver tissue removed seven days after irradiation using 10-MV photons at 2400 MU/min showed persistent, significantly increased expression of *Nrf2* ($p<0.0001$) compared to other groups, which were elevated at day 1 (Figure 3C, Table V). Following 10-MV photons at 2400 MU/min, bone marrow at one day post irradiation, showed a significant elevation of *Sod2* ($p<0.0001$), and there was a significant increase in microglobulin B-2 at day 7 (Figure 3D, Table IV). In contrast, heart tissue showed no significant differences in any transcript level between the tested energy and dose rate combination at day 7 (Figure 3E) (Tables IV-XII).

Orthotopic tumor control is unaffected by dose rate or beam energy. On day 0, immediately before irradiation, the average orthotopic tumor size was 190 mm^2. Groups of 10 mice then received hind leg irradiation to 21 Gy, using either 6 MV photons at 300 MU/min or 10 MV photons at 2400 MU/min. Three subgroups of mice included: non-irradiated tumor controls, and mice to 21 Gy with 6 MV 300 MU/min or 10 MV at 2400 MU/min (total 30 mice). Average tumor size was measured daily and compared between groups. There was a significant reduction in tumor size in mice that were irradiated

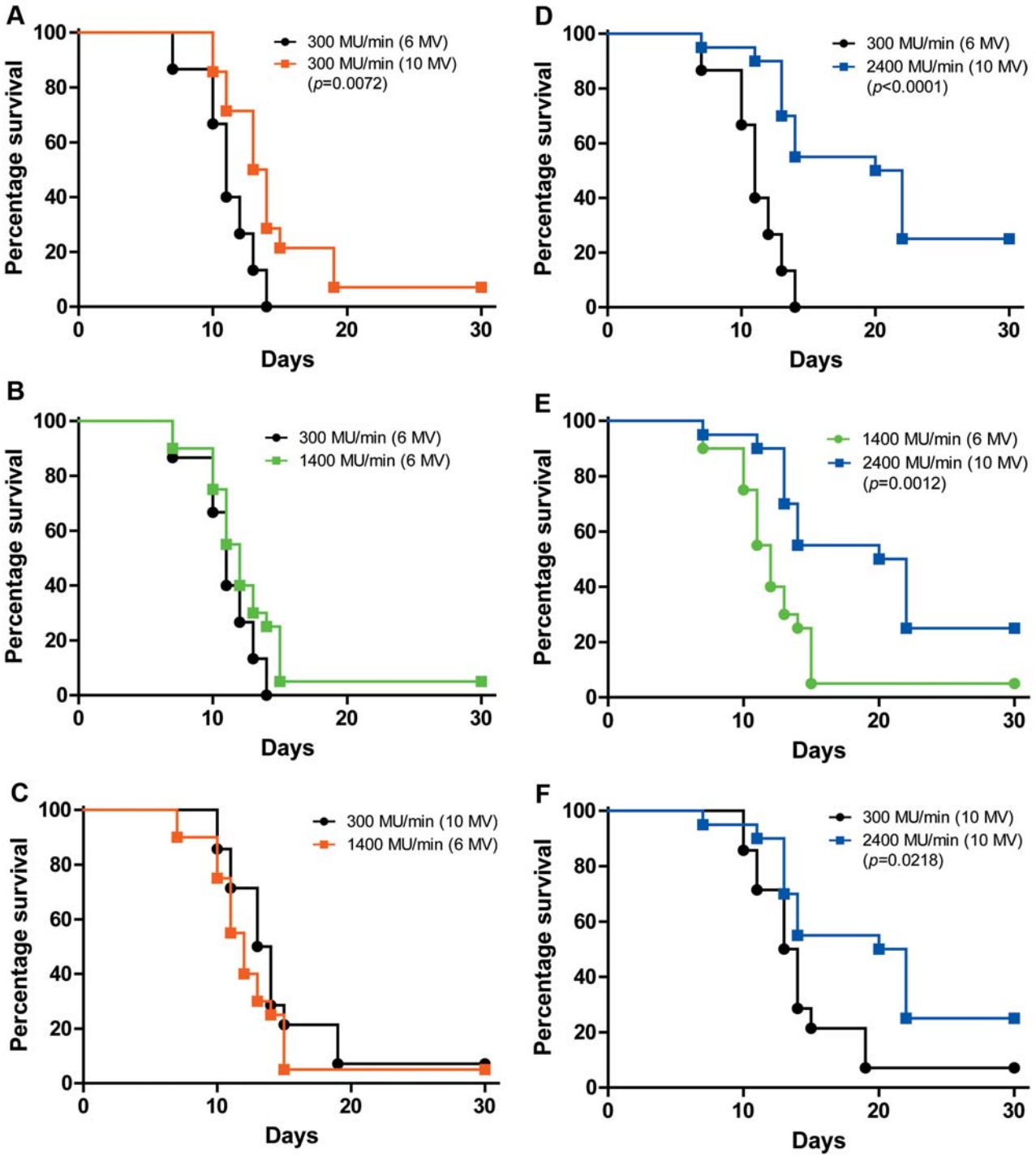

Figure 5. *C57B2/6NTac mice were irradiated to 9.25 Gy total body irradiation using different dose rates and photon energies. Mice were followed for survival. Comparisons of different treatment groups are shown.*

compared to controls (472 mm^3 *vs.* 860 mm^3) ($p<0.05$) (Tables XIII-XIV). Both irradiated groups showed the same reduction in tumor size. There was no significant difference in tumor size reduction between the 6 MV, 300 MU/min and 10 MV, 2400 MU/min irradiation treatment groups (520±111 *vs.* 423±129, respectively) ($p=0.9097$) (Tables XIII-XIV).

Based on the linear mixed model, F-tests for the difference between growth rates for the group treated with 6 MV

Table III. *Mice irradiated using 10 MV photons have increased survival. C57BL/6NTac mice were irradiated to 9.25 Gy with 300, 1400, or 2400 monitor units per min (MU) and either 6 MV or 10 MV photons and followed for the development of the hematopoietic syndrome at which time the mice were sacrificed. The data shown in Figure 2 were analyzed using a log-rank statistical test and the resulting p-values are shown. Mice irradiated at 2400 MU/min and 10 MV photons had a decreased chance of developing hematopoietic syndrome and dying compared to those of other treatment groups.*

Comparison of different treatment groups	*p*-Value
300 MU/min with 6 MV photons *vs*. 2400 MU/min with 10 MV photons	<0.0001
1400 MU/min with 6 MV photons *vs*. 2400 MU/min with 10 MV photons	0.0012
300 MU/min with 10 MV photons *vs*. 2400 MU/min with 10 MV photons	0.0218
300 MU/min with 6MV photons *vs*. 300 MU/min with 10 MV photons	0.0072
300 MU/min with 6 MV photons *vs*. 1400 MU/min with 6 MV photons	0.1085
300 MU/min with 10 MV photons *vs*. 1400 MU/min with 6 MV photons	0.2586

Table IV. *Manganese Superoxide Dismutase (SOD2) RNA transcripts: Expression in tissues from mice irradiated with 9.25 Gy. Data for each group is presented as mean ± standard deviation. The four treatment groups were compared with one-way ANOVA followed by post-hoc comparisons with F-tests using the CONTRAST statement in SAS Proc GLM. The results of the ANOVA F-tests are given in the last column, where a significant p-value indicates that the four groups are not the same. For the post-hoc tests, p1 is the p-value for the comparison with 10 MV 2400 MU/min group, and p2 is the p-value for comparing the 10 MV 2400 MU/min group with the average of the other three groups. p1 and p2 were calculated only when the p-value in the last column is significant, otherwise all groups are regarded as the same which means p1 and p2 are both non-significant.*

Organ and time after radiation		Treatment groups				ANOVA
		6 MV 300 MU/min	6 MV 1400 MU/min	10 MV 300 MU/min	10 MV 2400 MU/min	*p*-value
Liver	24 h	294.3±49.9 (n=3)	383.3±62.2 (n=3)	273.3±49.5 (n=3)	215.5±48.8 (n=2)	
		*p*1=0.1501	*p*1=0.0108	*p*1=0.2745	*p*2=0.0454	0.0498
	7 days	85.5±9.0 (n=3)	115.3±9.1 (n=3)	219.3±92.1 (n=3)	124.3±38.3 (n=3)	0.0525
Heart	24 h	129.0±21.2 (n=2)	128.7±14.7 (n=3)	147.7±10.8 (n=3)	146.0±12.5 (n=3)	0.3071
	7 days	33.0±6.6 (n=3)	34.7±6.7 (n=3)	35.4±4.4 (n=3)	34.0±8.7 (n=3)	0.9753
Intestine	24 h	93.2±10.5 (n=3)	94.4±7.0 (n=3)	103.2±22.8 (n=3)	200.3±18.2 (n=3)	<.0001
		*p*1<0.0001	*p*1<0.0001	*p*1=0.0001	*p*2<0.0001	
	7 days	27.0±4.2 (n=2)	43.9±4.6 (n=3)	31.6±4.1 (n=3)	105.7±8.1 (n=3)	<.0001
		*p*1<0.0001	*p*1<0.0001	*p*1<0.0001	*p*2<0.0001	
Brain	24 h	72.8±144.5 (n=3)	131.6±3.2 (n=3)	126.8±104.4 (n=3)	118.7±83.9 (n=3)	0.8765
	7 days	113.2±22.8 (n=3)	115.2±6.0 (n=3)	117.6±51.5 (n=3)	144.0±31.1 (n=3)	0.6336
Bone Marrow	24 h	84.9±12.3 (n=3)	84.4±14.2 (n=3)	1589.8±23.2 (n=3)	2305.2±419.3 (n=3)	<.0001
		*p*1<0.0001	*p*1<0.0001	*p*1=0.0031	*p*2<0.0001	
	7 days	–100.0±0.1 (n=3)	–99.9±0.3 (n=3)	–99.7±0.5 (n=3)	–99.6±0.5 (n=3)	0.6476

photons at 300 MU/min and the non-irradiation group, and the group treated with 10 MV photons at 2400 MU/min and the non-irradiation group were significant (p=0.0066 and 0.0024, respectively). The difference in the growth rate between 6 MV photons at 300 MU/min and 10 MV photons at 2400 MU/min was non-significant (p=0.1600) (Figure 4).

Discussion

We investigated whether the dose rates and photon energies used in current clinical SRS protocols differed detectably with respect to *in vitro* and *in vivo* parameters of irradiation effects in a mouse model. TBI-irradiated mice, orthotopic tumor-bearing mice, and *in vitro* clonogenic survival curves for the tumor cell line 3LL were used to determine the relative effect of each beam configuration on tumor cell biology compared to normal tissue.

The data show no effect of dose rate or beam energy on clonogenic 3LL cell survival *in vitro*. Furthermore, orthotopic 3LL tumors in the hind limb demonstrated no detectable difference in tumor growth inhibition after 21 Gy irradiation with different beam energies and dose rates. In contrast, we detected a significantly increased survival of mice treated to 9.25 Gy TBI at 10 MV, 2400 MU/min compared to all other

Table V. *Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) RNA Transcripts: Expression in tissues of 9.25 Gy irradiated mice. Data for each group is presented as mean±standard deviation. The four treatment groups were compared with one-way ANOVA followed by post-hoc comparisons with F-tests using the CONTRAST statement in SAS Proc GLM. In the last column shows the results of the ANOVA F-tests, where a significant p-value indicates that the four groups are not the same. For the post-hoc tests, p1 is the p-value for the comparison with 10 MV 2400 MU/min group, and p2 is the p-value for comparing 10 MV 2400 MU/min group with the average of the other three groups. P1 and p2 are calculated only when the p-value in the last column is significant, because if the p-value in the last column is non-significant, then all groups are regarded as the same which means p1 and p2 are both non-significant.*

Organ and time after radiation		Treatment groups				*p*-Value for the ANOVA F test
		6 MV 300 MU/min	6 MV 1400 MU/min	10 MV 300 MU/min	10 MV 2400 MU/min	
Liver	24 h	2236.7±335.0 (n=3)	2220.3±480.4 (n=3)	1837.7±382.5 (n=3)	1865.0±425.7 (n=2)	0.5347
	7days	136.0±17.8 (n=3)	90.5±5.7 (n=3)	184.0±19.3 (n=3)	369.7±44.2 (n=3)	<.0001
		*p*1<0.0001	*p*1<0.0001	*p*1<0.0001	*p*2<0.0001	
Heart	24 h	72.6±12.0 (n=2)	43.3±6.1 (n=3)	39.9±14.3 (n=3)	31.3±6.0 (n=3)	0.0152
		*p*1=0.0028	*p*1=0.1852	*p*1=0.3308	*p*2=0.0193	
	7days	27.1±2.7 (n=3)	28.1±1.2 (n=3)	28.5±5.2 (n=3)	20.0±4.6 (n=3)	0.0740
Intestine	24 h	177.7±33.8 (n=3)	199.3±34.6 (n=3)	203.4±25.7 (n=3)	299.2±35.3 (n=3)	0.0079
		*p*1=0.0018	*p*1=0.0056	*p*1=0.0070	*p*2=0.0012	
	7days	123.0±8.5 (n=2)	106.9±29.1 (n=3)	102.5±8.3 (n=3)	194.7±35.5 (n=3)	0.0094
		*p*1=0.0168	*p*1=0.0037	*p*1=0.0028	*p*2=0.0018	
Brain	24 h	128.7±221.4 (n=3)	247.9±108.4 (n=3)	239.4±200.0 (n=3)	203.4±175.0 (n=3)	0.8457
	7days	192.1±83.7 (n=3)	53.1±28.3 (n=3)	228.4±85.2 (n=3)	295.8±80.5 (n=3)	0.0206
		*p*1=0.1217	*p*1=0.0037	*p*1=0.2930	*p*2=0.0225	
Bone Marrow	24 h	–94.3±4.3 (n=3)	–96.9±1.3 (n=3)	–63.1±35.0 (n=3)	–99.3±0.4 (n=3)	0.1095
	7days	–99.7±0.6 (n=3)	–99.9±0.1 (n=3)	–99.6±0.5 (n=3)	–99.6±0.5 (n=3)	0.7574

Table VI. *Sp1 RNA Transcripts: Expression in tissues of 9.25 Gy irradiated mice. Data for each group is presented as mean±standard deviation. The four treatment groups were compared with one-way ANOVA followed by post-hoc comparisons with F-tests using the CONTRAST statement in SAS Proc GLM. In the last column are the results of the ANOVA F-tests, where a significant p-value indicates that the four groups are not the same. For the post-hoc tests, p1 is the p-value for the comparison with 10 MV 2400 MU/min group, and p2 is the p-value for comparing 10 MV 2400 MU/min group with the average of the other three groups. P1 and p2 are calculated only when the p-value in the last column is significant, because if the p-value in the last column is non-significant, then all groups are regarded as the same which means p1 and p2 are both non-significant.*

Organ and time after radiation		Treatment groups				*p*-Value for the ANOVA F test
		6 MV 300 MU/min	6 MV 1400 MU/min	10 MV 300 MU/min	10 MV 2400 MU/min	
Liver	24 h	163.7±27.6 (n=3)	271.7±73.5 (n=3)	173.3±9.1 (n=3)	127.3±6.1 (n=2)	0.0267
		*p*1=0.3778	*p*1=0.0073	*p*1=0.2722	*p*2=0.0562	
	7days	79.3±11.1 (n=3)	61.0±6.6 (n=3)	133.7±30.8 (n=3)	107.3±20.7 (n=3)	0.0089
		*p*1=0.1193	*p*1=0.0203	*p*1=0.1388	*p*2=0.2574	
Heart	24 h	100.5±2.1 (n=2)	64.3±5.5 (n=3)	58.3±4.2 (n=3)	45.4±4.8 (n=3)	<.0001
		*p*1<0.0001	*p*1=0.0014	*p*1=0.0105	*p*2<0.0001	
	7days	28.0±4.1 (n=3)	36.1±7.8 (n=3)	18.9±2.5 (n=3)	29.6±5.8 (n=3)	0.0276
		*p*1=0.7257	*p*1=0.1762	*p*1=0.0412	*p*2=0.6074	
Intestine	24 h	148.7±7.2 (n=3)	154.3±41.2(n=3)	169.0±17.3 (n=3)	246.3±34.5 (n=3)	0.0100
		*p*1=0.0030	*p*1=0.0042	*p*1=0.0104	*p*2=0.0016	
	7days	31.6±4.1 (n=2)	16.6±2.7 (n=3)	19.1±4.2 (n=3)	99.4±17.0 (n=3)	<.0001
		*p*1=0.0001	*p*1<0.0001	*p*1<0.0001	*p*2<0.0001	
Brain	24 h					
	7days					
Bone Marrow	24 h	–92.7±4.7 (n=3)	–97.3±0.7 (n=3)	–67.5±9.5 (n=3)	–94.2±3.6 (n=3)	0.0006
		*p*1=0.7506	*p*1=0.5241	*p*1=0.0004	*p*2=0.0549	
	7days	–100.0±0.1 (n=3)	–99.2±0.2 (n=3)	–99.0±1.0 (n=3)	–99.6±0.7 (n=3)	0.2939

Table VII. *Nuclear factor Kappa-light-chain-enhancer of activated B cells (NfkB) RNA Transcripts: Expression in tissues of 9.25 Gy irradiated mice. Data for each group is presented as mean±standard deviation. The four treatment groups were compared with one-way ANOVA followed by post-hoc comparisons with F-tests using the CONTRAST statement in SAS Proc GLM. In the last column are the results of the ANOVA F-tests, where a significant p-value indicates that the four groups are not the same. For the post-hoc tests, p1 is the p-value for the comparison with 10 MV 2400 MU/min group, and p2 is the p-value for comparing 10 MV 2400 MU/min group with the average of the other three groups. P1 and p2 are calculated only when the p-value in the last column is significant, because if the p-value in the last column is non-significant, then all groups are regarded as the same which means p1 and p2 are both non-significant.*

Organ and time after radiation		Treatment groups				*p*-Value for the ANOVA F test
		6 MV 300 MU/min	6 MV 1400 MU/min	10 MV 300 MU/min	10 MV 2400 MU/min	
Liver	24 h	119.3±19.0 (n=3)	147.7±17.5 (n=3)	195.0±35.5 (n=3)	108.5±26.2 (n=2)	0.0217
		*p*1=0.6554	*p*1=0.1360	*p*1=0.0075	*p*2=0.0562	
	7days	168.3±12.7 (n=3)	92.3±10.7 (n=3)	109.1±10.5 (n=3)	105.6±13.3 (n=3)	0.0002
		*p*1=0.0002	*p*1=0.2071	*p*1=0.7323	*p*2=0.0567	
Heart	24 h	86.9±22.8 (n=2)	44.8±8.0 (n=3)	35.3±6.9 (n=3)	31.6±6.0 (n=3)	0.0036
		*p*1=0.0008	*p*1=0.1786	*p*1=0.6904	*p*2=0.0138	
	7days	29.3±6.7 (n=3)	27.3±8.3 (n=3)	29.9±7.2 (n=3)	33.2±4.8 (n=3)	0.7692
Intestine	24 h	188.8±10.6 (n=3)	149.1±26.4 (n=3)	180.1±10.1 (n=3)	289.3±22.6 (n=3)	<.0001
		*p*1=0.0002	*p*1<0.0001	*p*1=0.0001	*p*2<0.0001	
	7days	126.4±5.2 (n=2)	136.3±26.7 (n=3)	100.3±15.9 (n=3)	241.0±27.2 (n=3)	0.0005
		*p*1=0.0008	*p*1=0.0007	*p*1=0.0001	*p*2=0.0001	
Brain	24 h	129.6±221.4 (n=3)	121.3±28.2 (n=3)	154.5±147.1 (n=3)	112.2±82.0 (n=3)	0.9837
	7days	137.7±80.6 (n=3)	45.0±37.4 (n=3)	210.5±76.1 (n=3)	157.8±93.0 (n=3)	0.1280
Bone Marrow	24 h	–96.8±2.7 (n=3)	–84.6±22.7 (n=3)	–86.6±6.2 (n=3)	–86.0±8.0 (n=3)	0.6281
	7days	–99.8±0.4 (n=3)	–99.9±0.1 (n=3)	–99.6±0.5 (n=3)	–99.6±0.2 (n=3)	0.7233

Table VIII. *Transforming Growth Factor-β (TgfB) RNA Transcripts: Expression in tissues of 9.25 Gy-irradiated mice. Data for each group are presented as mean±standard deviation.The four treatment groups were compared with one-way ANOVA followed by post-hoc comparisons with F-tests using the CONTRAST statement in SAS Proc GLM. In the last column are the results of the ANOVA F-tests, where a significant p-value indicates that the four groups are not the same. For the post-hoc tests, p1 is the p-value for the comparison with 10 MV 2400 MU/min group, and p2 is the p-value for comparing 10 MV 2400 MU/min group with the average of the other three groups. P1 and p2 are calculated only when the p-value in the last column is significant, because if the p-value in the last column is non-significant, then all groups are regarded as the same which means p1 and p2 are both non-significant.*

Organ and time after radiation		Treatment groups				*p*-Value for the ANOVA F test
		6 MV 300 MU/min	6 MV 1400 MU/min	10 MV 300 MU/min	10 MV 2400 MU/min	
Liver	24 h	84.3±13.6 (n=3)	103.3±23.1 (n=3)	58.2±8.9 (n=3)	67.5±7.8 (n=2)	0.0398
		p1=0.2698	*p1*=0.0381	*p1*=0.5277	*p2*=0.2690	
	7days	135.3±14.7 (n=3)	98.7±15.5 (n=3)	101.0±17.3 (n=3)	88.6±8.6 (n=3)	0.0194
		p1=0.0041	*p1*=0.4190	*p1*=0.3243	*p2*=0.0435	
Heart	24 h	25.7±5.2 (n=2)	19.9±0.8 (n=3)	18.7±2.2 (n=3)	13.0±4.9 (n=3)	0.0307
		p1=0.0054	*p1*=0.0471	*p1*=0.0861	*p2*=0.0096	
	7days	15.9±3.3 (n=3)	17.7±9.6 (n=3)	18.8±1.3 (n=3)	19.5±8.5 (n=3)	0.9188
Intestine	24 h	38.3±12.5 (n=3)	55.0±20.3 (n=3)	62.3±7.4 (n=3)	111.5±16.2 (n=3)	0.0017
		p1=0.0003	*p1*=0.0016	*p1*=0.0037	*p2*=0.0003	
	7days	39.5±12.0 (n=2)	37.3±10.7 (n=3)	34.9±7.1 (n=3)	89.7±11.6 (n=3)	0.0009
		p1=0.0011	*p1*=0.0004	*p1*=0.0003	*p2*=0.0001	
Brain	24 h	90.8±57.7 (n=3)	111.4±4.2 (n=3)	220.4±182.7 (n=3)	136.1±92.5 (n=3)	0.5006
	7days	192.2±65.7 (n=3)	117.5±53.3 (n=3)	208.6±42.4 (n=3)	429.1±202.0 (n=3)	0.0430
		p1=0.0315	*p1*=0.0091	*p1*=0.0417	*p2*=0.0087	
Bone Marrow	24 h	–96.1±1.7 (n=3)	–92.3±2.0 (n=3)	–75.9±25.8 (n=3)	–93.8±3.3 (n=3)	0.2894
	7days	–100.00±0.00 (n=3)	–100.00±0.00 (n=3)	–100.00±0.00 (n=3)	–100.00±0.00 (n=3)	–

Table IX. *Fas ligand RNA Transcripts: Expression in tissues of 9.25 Gy-irradiated mice. Data for each group are presented as mean±standard deviation. The four treatment groups were compared with one-way ANOVA followed by post-hoc comparisons with F-tests using the CONTRAST statement in SAS Proc GLM. In the last column are the results of the ANOVA F-tests, where a significant p-value indicates that the four groups are not the same. For the post-hoc tests, p1 is the p-value for the comparison with 10 MV 2400 MU/min group, and p2 is the p-value for comparing 10 MV 2400 MU/min group with the average of the other three groups. P1 and p2 are calculated only when the p-value in the last column is significant, because if the p-value in the last column is non-significant, then all groups are regarded as the same which means p1 and p2 are both non-significant.*

Organ and time after radiation		Treatment groups				*p*-Value for the ANOVA F test
		6 MV 300 MU/min	6 MV 1400 MU/min	10 MV 300 MU/min	10 MV 2400 MU/min	
Liver	24 h	180.5±32.1 (n=3)	147.3±33.1 (n=3)	208.7±21.2 (n=3)	111.0±1.4 (n=2)	0.0242
		*p*1=0.0263	*p*1=0.1860	*p*1=0.0056	*p*2=0.0151	
	7days	127.5±22.7 (n=3)	79.3±17.7 (n=3)	66.7±5.7 (n=3)	61.0±5.0 (n=3)	0.0022
		*p*1=0.0006	*p*1=0.1692	*p*1=0.6529	*p*2=0.0159	
Heart	24 h	137.0±19.8 (n=2)	109.3±38.0 (n=3)	62.3±6.0 (n=3)	42.0±12.5 (n=3)	0.0083
		*p*1=0.0026	*p*1=0.0087	*p*1=0.3126	*p*2=0.0058	
	7days	68.2±7.2 (n=3)	54.3±14.0 (n=3)	52.1±12.6 (n=3)	57.7±4.7 (n=2)	0.3544
Intestine	24 h	53.2±9.8 (n=3)	58.2±9.6 (n=3)	60.8±20.0 (n=3)	108.0±9.0 (n=3)	0.0027
		*p*1=0.0008	*p*1=0.0015	*p*1=0.0021	*p*2=0.0004	
	7days	50.4±20.6 (n=2)	42.8±10.1 (n=3)	59.0±7.2 (n=3)	88.2±2.8 (n=3)	0.0052
		*p*1=0.0052	*p*1=0.0010	*p*1=0.0107	*p*2=0.0011	
Brain	24 h	292.3±254.5 (n=3)	353.0±104.8 (n=3)	618.0±303.5 (n=3)	325.9±213.3 (n=3)	0.3538
	7days	243.7±85.1 (n=3)	182.0±105.6 (n=3)	365.6±141.3 (n=3)	204.9±31.6 (n=3)	0.1871

Table X. *Glutathione peroxidase 1 (Gpx1) RNA Transcripts: Expression in tissues of 9.25 Gy-irradiated mice. Data for each group is presented as mean±standard deviation. The four treatment groups were compared with one-way ANOVA followed by post-hoc comparisons with F-tests using the CONTRAST statement in SAS Proc GLM. In the last column are the results of the ANOVA F-tests, where a significant p-value indicates that the four groups are not the same. For the post-hoc tests, p1 is the p-value for the comparison with the 10 MV 2400 MU/min group, and p2 is the p-value for comparing 10 MV 2400 MU/min group with the average of the other three groups. P1 and p2 are calculated only when the p-value in the last column is significant, because if the p-value in the last column is non-significant, then all groups are regarded as the same which means p1 and p2 are both non-significant.*

Organ and time after radiation		Treatment groups				*p*-Value for the ANOVA F test
		6 MV 300 MU/min	6 MV 1400 MU/min	10 MV 300 MU/min	10 MV 2400 MU/min	
Liver	24 h	91.3±6.0 (n=3)	91.9±21.3 (n=3)	65.9±9.1 (n=3)	36.0±9.9 (n=2)	0.0079
		*p*1=0.0027	*p*1=0.0025	*p*1=0.0438	*p*2=0.0028	
	7days	152.7±7.8 (n=3)	255.3±48.0 (n=3)	130.7±24.8 (n=3)	130.0±29.5 (n=3)	0.0032
		*p*1=0.3970	*p*1=0.0011	*p*1=0.9796	*p*2=0.0434	
Heart	24 h	17.5±0.2 (n=2)	10.5±2.9 (n=3)	8.1±1.9 (n=3)	4.4±0.8 (n=3)	0.0009
		*p*1=0.0001	*p*1=0.0062	*p*1=0.0532	*p*2=0.0007	
	7days	6.5±0.7 (n=3)	8.7±2.9 (n=3)	7.9±2.3 (n=3)	9.5±4.5 (n=3)	0.6530
Intestine	24 h	95.3±17.0 (n=3)	98.8±17.0 (n=3)	92.9±16.0 (n=3)	187.4±29.1 (n=3)	0.0012
		*p*1=0.0006	*p*1=0.0007	*p*1=0.0005	*p*2=0.0002	
	7days	104.9±12.9 (n=2)	93.1±5.2 (n=3)	113.3±10.1 (n=3)	159.3±14.6 (n=3)	0.0008
		*p*1=0.0010	*p*1=0.0002	*p*1=0.0014	*p*2=0.0001	
Brain	24 h	121.3±131.6 (n=3)	83.3±15.2 (n=3)	228.6±225.1 (n=3)	156.1±114.9 (n=3)	0.6543
	7days	160.7±45.8 (n=3)	144.9±7.3 (n=3)	125.4±115.2 (n=3)	164.1±72.3 (n=3)	0.9057
Bone Marrow	24 h	72.7±2.5 (n=3)	104.4±15.5 (n=3)	28.0±7.1 (n=3)	14.6±7.3 (n=3)	<.0001
		*p*1=0.0001	*p*1<0.0001	*p*1=0.1179	*p*2<0.0001	
	7days	–99.6±0.5 (n=3)	–100.0±0.1 (n=3)	–99.9±0.1 (n=3)	–99.3±0.6 (n=3)	0.2903

Table XI. *Syntaxin (Stx1) RNA Transcripts: Expression in tissues of 9.25 Gy irradiated mice. Data for each group is presented as mean±standard deviation. The four treatment groups were compared with one-way ANOVA followed by post-hoc comparisons with F-tests using the CONTRAST statement in SAS Proc GLM. In the last column are the results of the ANOVA F-tests, where a significant p-value indicates that the four groups are not the same. For the post-hoc tests, p1 is the p-value for the comparison with 10 MV 2400 MU/min group, and p2 is the p-value for comparing 10 MV 2400 MU/min group with the average of the other three groups. P1 and p2 are calculated only when the p-value in the last column is significant, because if the p-value in the last column is non-significant, then all groups are regarded as the same which means p1 and p2 are both non-significant.*

Organ and time after radiation		Treatment groups				*p*-Value for the ANOVA F test
		6 MV 300 MU/min	6 MV 1400 MU/min	10 MV 300 MU/min	10 MV 2400 MU/min	
Brain	24 h	111.0±73.0 (n=3)	283.3±198.1 (n=3)	203.5±135.0 (n=3)	272.1±243.0 (n=3)	0.6224
	7days	551.2±69.7 (n=3)	272.1±68.7 (n=3)	434.1±167.2 (n=3)	430.2±345.9 (n=3)	0.4402

Table XII. *Beta-2 microglobin (B2m) RNA Transcripts: Expression in tissues of 9.25 Gy irradiated mice. Data for each group is presented as mean±standard deviation. The four treatment groups were compared with one-way ANOVA followed by post-hoc comparisons with F-tests using the CONTRAST statement in SAS Proc GLM. In the last column are the results of the ANOVA F-tests, where a significant p-value indicates that the four groups are not the same. For the post-hoc tests, p1 is the p-value for the comparison with 10 MV 2400 MU/min group, and p2 is the p-value for comparing 10 MV 2400 MU/min group with the average of the other three groups. P1 and p2 are calculated only when the p-value in the last column is significant, because if the p-value in the last column is non-significant, then all groups are regarded as the same which means p1 and p2 are both non-significant.*

Organ and time after radiation		Treatment groups				*p*-Value for the ANOVA F test
		6 MV 300 MU/min	6 MV 1400 MU/min	10 MV 300 MU/min	10 MV 2400 MU/min	
Bone Marrow	24 h	–40.1±17.2 (n=3)	63.8±46.0 (n=3)	–79.5±0.8 (n=3)	–90.0±0.3 (n=3)	0.0002
		*p*1=0.0379	*p*1=0.0001	*p*1=0.6159	*p*2=0.0024	
	7days	115.4±8.5 (n=3)	1181.5±177.0 (n=3)	108.2±84.2 (n=3)	643.8±12.2 (n=3)	<.0001
		*p*1=0.0002	*p*1=0.0002	*p*1=0.0002	*p*2=0.0281	

Table XIII. *Tumor volume at each day of measurement. Data are summarized as mean±standard deviation.*

Group	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
No irradiation	215.1±225.7 (n=10)	454.1±354.4 (n=10)	569.4±532.2 (n=10)	793.0±572.6 (n=10)	811.8±551.5 (n=10)	859.9±501.2 (n=10)
6 MV 300 MU/min	218.2±231.7 (n=10)	176.3±158.8 (n=10)	365.0±311.9 (n=10)	402.0±354.8 (n=10)	421.7±366.3 (n=10)	520.9±352.1 (n=10)
10 MV 2400 MU/min	137.9±131.0 (n=10)	267.1±195.3 (n=10)	171.9±134.7 (n=10)	291.0±292.3 (n=10)	285.5±319.1 (n=10)	423.1±414.0 (n=10)

irradiation groups. Furthermore, there was early (days 1 and 7 after TBI) organ-specific increased elevation of RNA transcripts for several irradiation-inducible genes in the 10 MV at 2400 MU/min group. Irradiated brain and intestine showed a significantly greater increase in Sod2, Gpx1, TgfB, Nrf2, NfkB, and Stx1 transcripts seven days following 9.25 Gy TBI using 10 MV photons at 2400 MU/min. Furthermore, one day post-irradiation, bone marrow from mice irradiated using 10 MV beam at 2400 MU/min showed a significantly greater increase in *Sod2* transcripts compared to other combinations. The elevation in *Sod2* observed in the bone marrow at 24 h, and subsequent elevation of *Sod2* and other protective transcripts in brain and intestine at day 7 may have been responsible for the survival advantage in mice treated with 10 MV photons at 2400 MU/min. Further studies will be required to confirm that increased Sod2 or other radioprotective transcripts prolonged survival of mice receiving 10 MV photons at 2400 MU/min.

The present data using a mouse model for the radiobiology of current clinically-utilized SRS beams

Table XIV. *Normalized tumor volume in irradiated mice. Data are summarized as mean±standard deviation. P1 is the p-value for comparison with the non-irradiated group. P2 is the p-value for comparison with the 6 MV 300 group. For these p-values, the two-sided Wilcoxon rank sum tests were used. Significant differences are shown in red.*

	Day					
Group	0	1	2	3	4	5*
No irradiation	1±0 (n=10)	5.0±8.7 (n=10)	5.4±8.5 (n=10)	6.6±8.1 (n=10)	7.2±8.1 (n=10)	9.6±10.8 (n=10)
6 MV 300 Mu/min	1±0 (n=10)	1.4±0.8 (n=10)	2.5±1.4 (n=10)	2.6±1.7 (n=10)	2.7±1.7 (n=10)	4.8±4.7 (n=10)
		*p*1=0.1041	*p*1=0.3447	*p*1=0.0376	*p*1=0.0211	*p*1=0.2123
10 MV 2400 Mu/min	1±0 (n=10)	2.7±2.4 (n=10)	1.7±1.0 (n=10)	2.5±2.0 (n=10)	2.6±2.8 (n=10)	4.3±3.7 (n=10)
		*p*1=0.6776	*p*1=0.0640	*p*1=0.0140	*p*1=0.0211	*p*1=0.1212
		*p*2=1.0000	*p*2=0.1859	*p*2=0.7337	*p*2=0.4727	*p*2=0.9097

*Test at day 5 does not have strong power because about half of the mice were sacrificed before this date due to size of tumor.

revealed that normal tissue responses may be different at different dose rates and beam energies. The configuration of 10 MV photons at 2400 MU/min produced a significant increase in mouse survival after TBI. It is established that the repair kinetics of tumors and normal tissues may differ depending on the irradiation dose rate, but should not be influenced by beam energy. That 10 MV photons increased survival even at 300 MU/min supports the notion that it was the 10 MV beam which produced the effect (6, 9, 12, 13).

The mechanism of improved survival in mice treated at 2400 MU/min with 10 MV photons TBI is not yet known. Further studies are required to determine the mechanism and clinical relevance of this unexpected finding.

Acknowledgements

Supported by NIAID U19-A1068021. This project used the UPCI animal facility that is supported in part by award P30CA047904.

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Received October 7, 2013
Revised November 8, 2013
Accepted November 12, 2013