

# The FLASH effect—An evaluation of preclinical studies of ultra-high dose rate radiotherapy: Supplementary Materials

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## 1 Additional Graphs (not in main body)

Figure 1: Pearson's Correlation Coefficients in heat map form to show the correlations between each dosimetric parameter and the corresponding endpoint. The values range between -1 and 1, where the extremities (closest to -1 and 1) have the deepest colour and the weakest correlations (closer to 0) have a weak colour. Statistically significant correlations are identifiable by an asterisk at the top right of the corresponding correlation coefficient. Key: TIS- Therapeutic Index Score, TCS- Tumour Control Score, NTSS- Normal-tissue Sparing Score, ILS- Increased Lifespan,  $S_1$ - Survival % at 1 month,  $S_2$ - Survival % at 2 month,  $S_3$ - Survival % at 3 month.

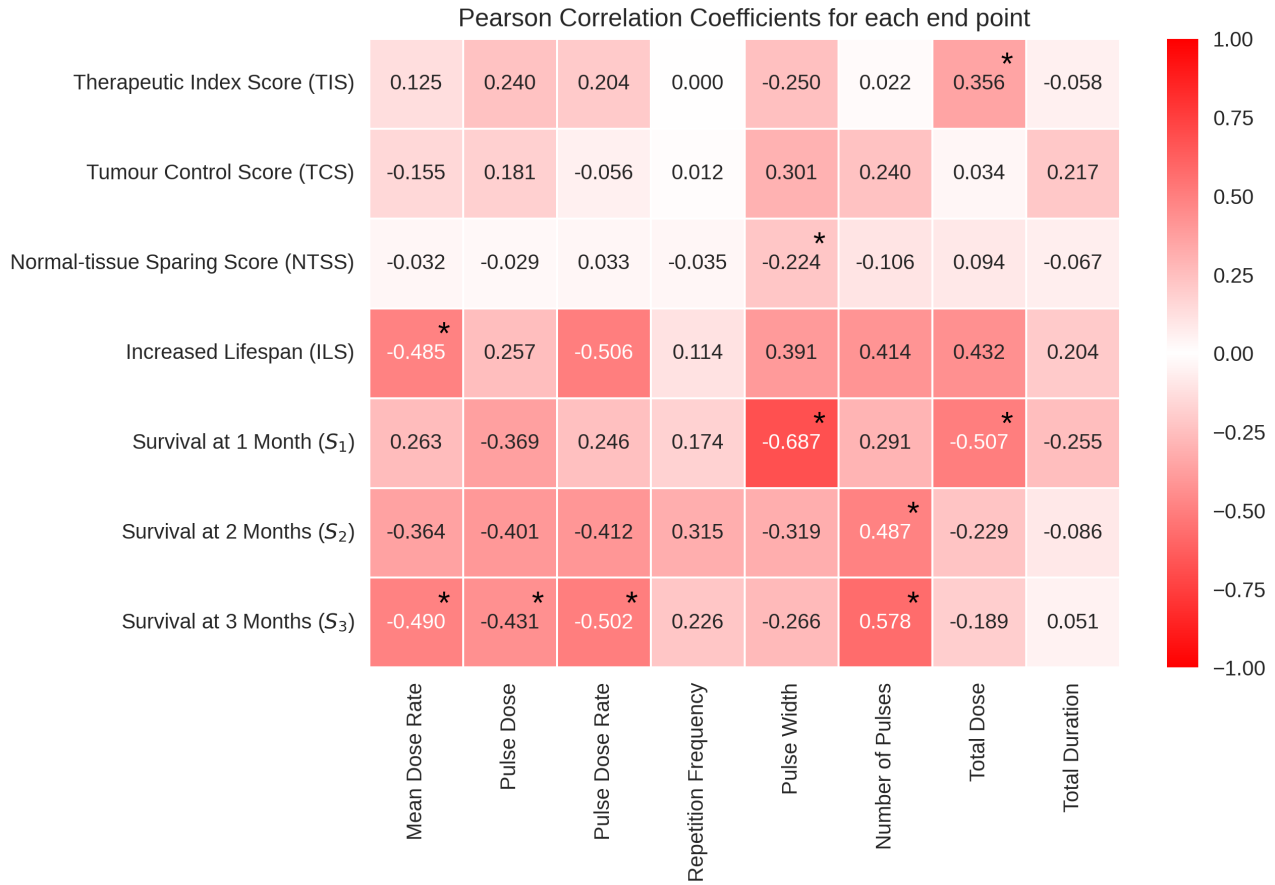


Figure 2: Pearson's Correlation Coefficients in heat map form to show the correlations between the log of each dosimetric parameter and the corresponding endpoint **for all data with mean and pulse dose rates above 30Gy/s**. The values range between -1 and 1, where the extremities (closest to -1 and 1) have the deepest colour and the weakest correlations (closer to 0) have a weak colour. Statistically significant correlations are identifiable by an asterisk at the top right of the corresponding correlation coefficient. Key: TIS- Therapeutic Index Score, TCS- Tumour Control Score, NTSS- Normal-tissue Sparing Score, ILS- Increased Lifespan,  $S_1$ - Survival % at 1 month,  $S_2$ - Survival % at 2 month,  $S_3$ - Survival % at 3 month.

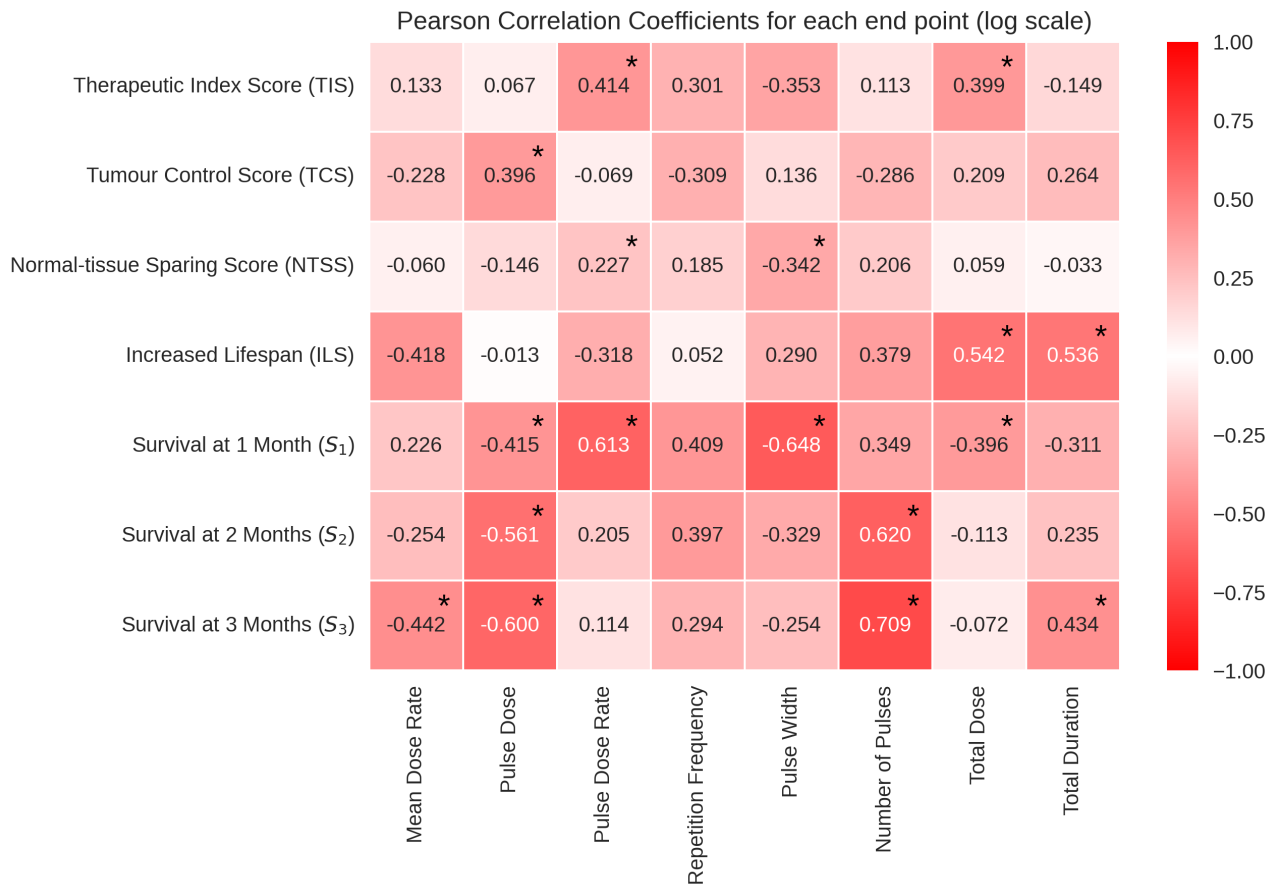
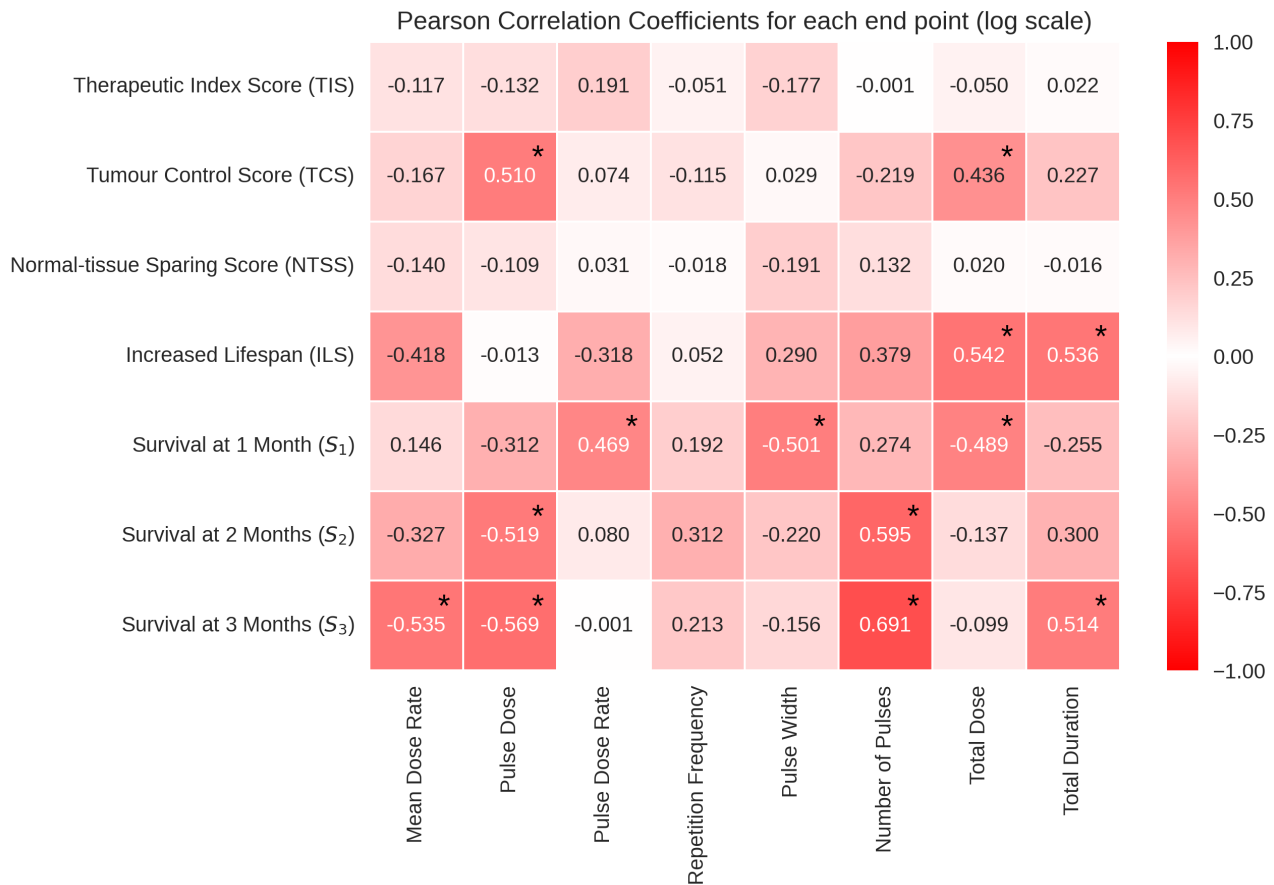
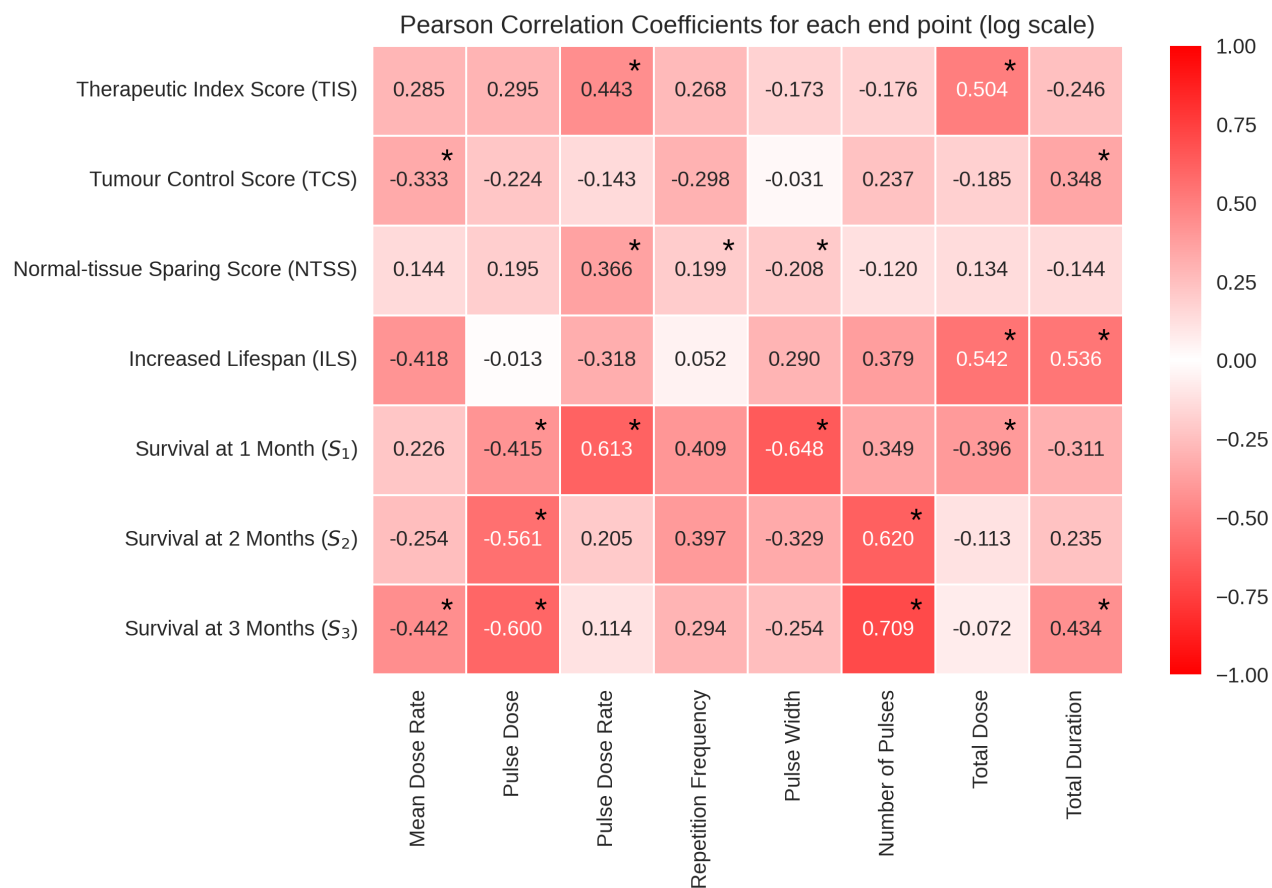


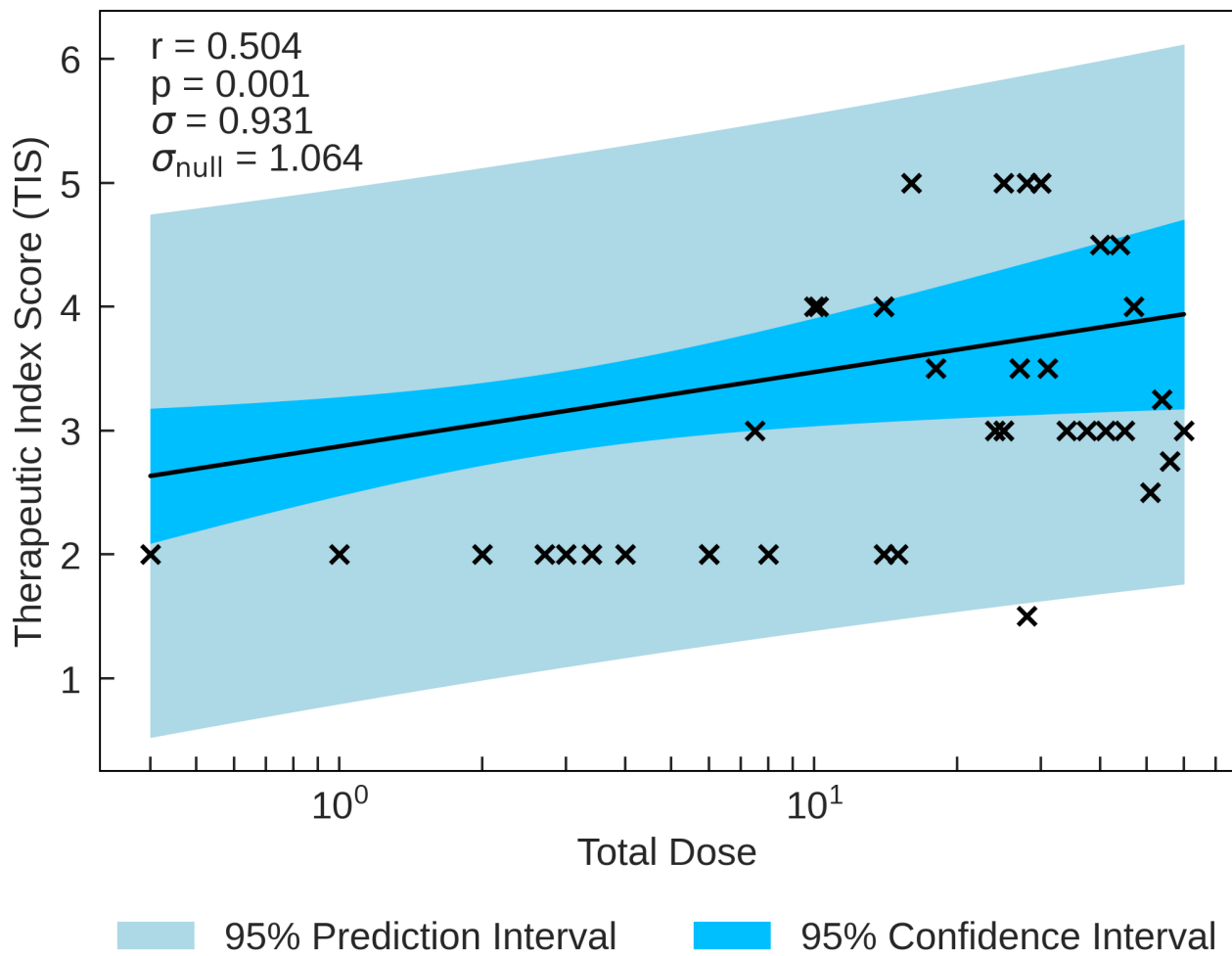
Figure 3: Pearson's Correlation Coefficients in heat map form to show the correlations between the log of each dosimetric parameter and the corresponding endpoint **for all data with mean and pulse dose rates above 40Gy/s**. The values range between -1 and 1, where the extremities (closest to -1 and 1) have the deepest colour and the weakest correlations (closer to 0) have a weak colour. Statistically significant correlations are identifiable by an asterisk at the top right of the corresponding correlation coefficient. Key: TIS- Therapeutic Index Score, TCS- Tumour Control Score, NTSS- Normal-tissue Sparing Score, ILS- Increased Lifespan,  $S_1$ - Survival % at 1 month,  $S_2$ - Survival % at 2 month,  $S_3$ - Survival % at 3 month.



## 2 Full Versions of Graphs (in main body)

Figure 4: Pearson's Correlation Coefficients in heat map form to show the correlations between the log of each dosimetric parameter and the corresponding endpoint. The values range between -1 and 1, where the extremities (closest to -1 and 1) have the deepest colour and the weakest correlations (closer to 0) have a weak colour. Statistically significant correlations are identifiable by an asterisk at the top right of the corresponding correlation coefficient. Key: TIS- Therapeutic Index Score, TCS- Tumour Control Score, NTSS- Normal-tissue Sparing Score, ILS- Increased Lifespan,  $S_1$ - Survival % at 1 month,  $S_2$ - Survival % at 2 month,  $S_3$ - Survival % at 3 month.





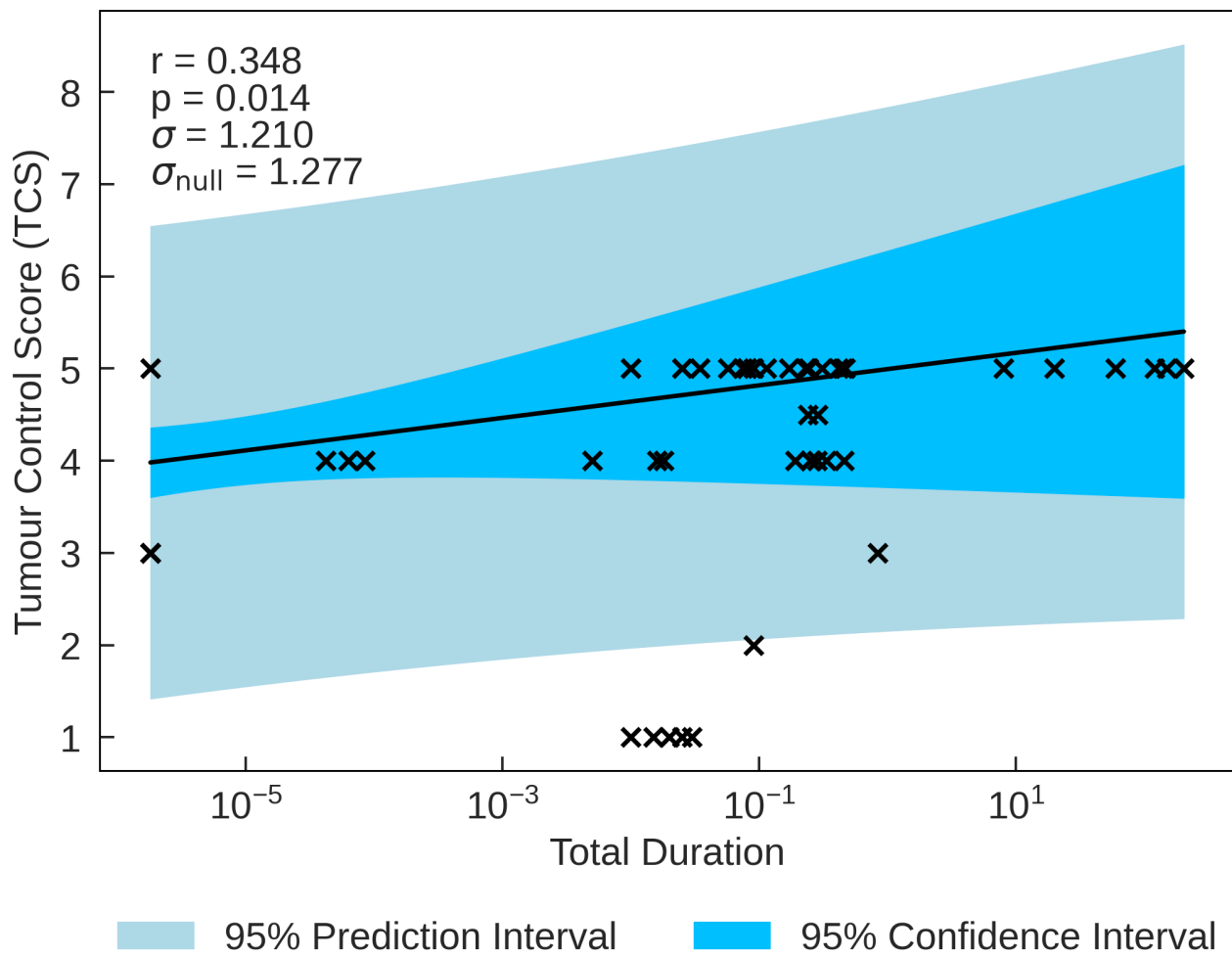


Figure 6: TCS plotted against the strongest dosimetric parameter, Total Time. There is a moderate positive correlation between the parameters, suggesting that an increase in irradiation duration may increase tumour control.

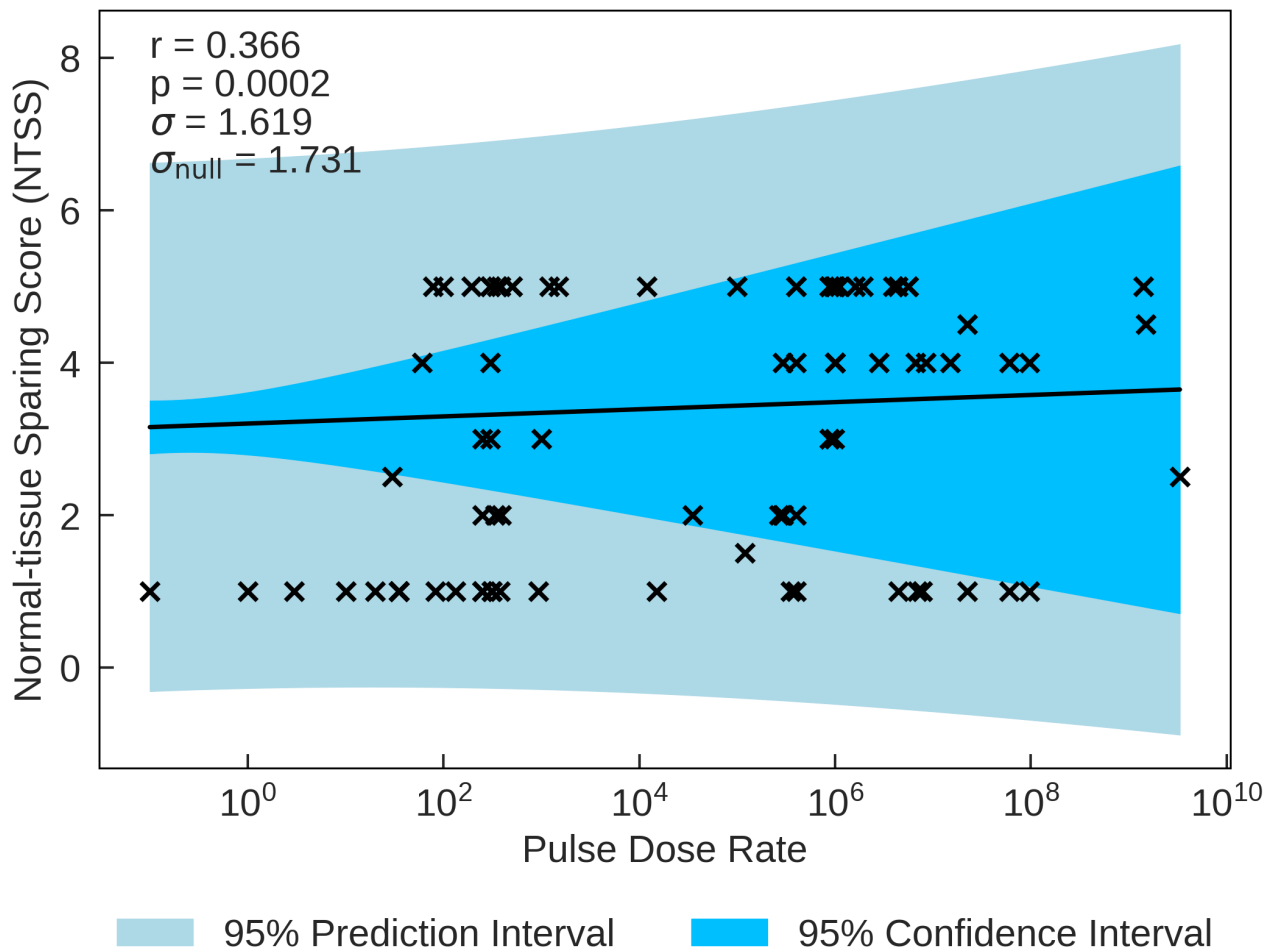


Figure 7: NTSS plotted against the strongest dosimetric parameter, Pulse Dose Rate. There is a moderate positive correlation between the parameters, suggesting that an increase in the dose rate of each pulse may increase the chance of observing a sparing effect in normal tissue.



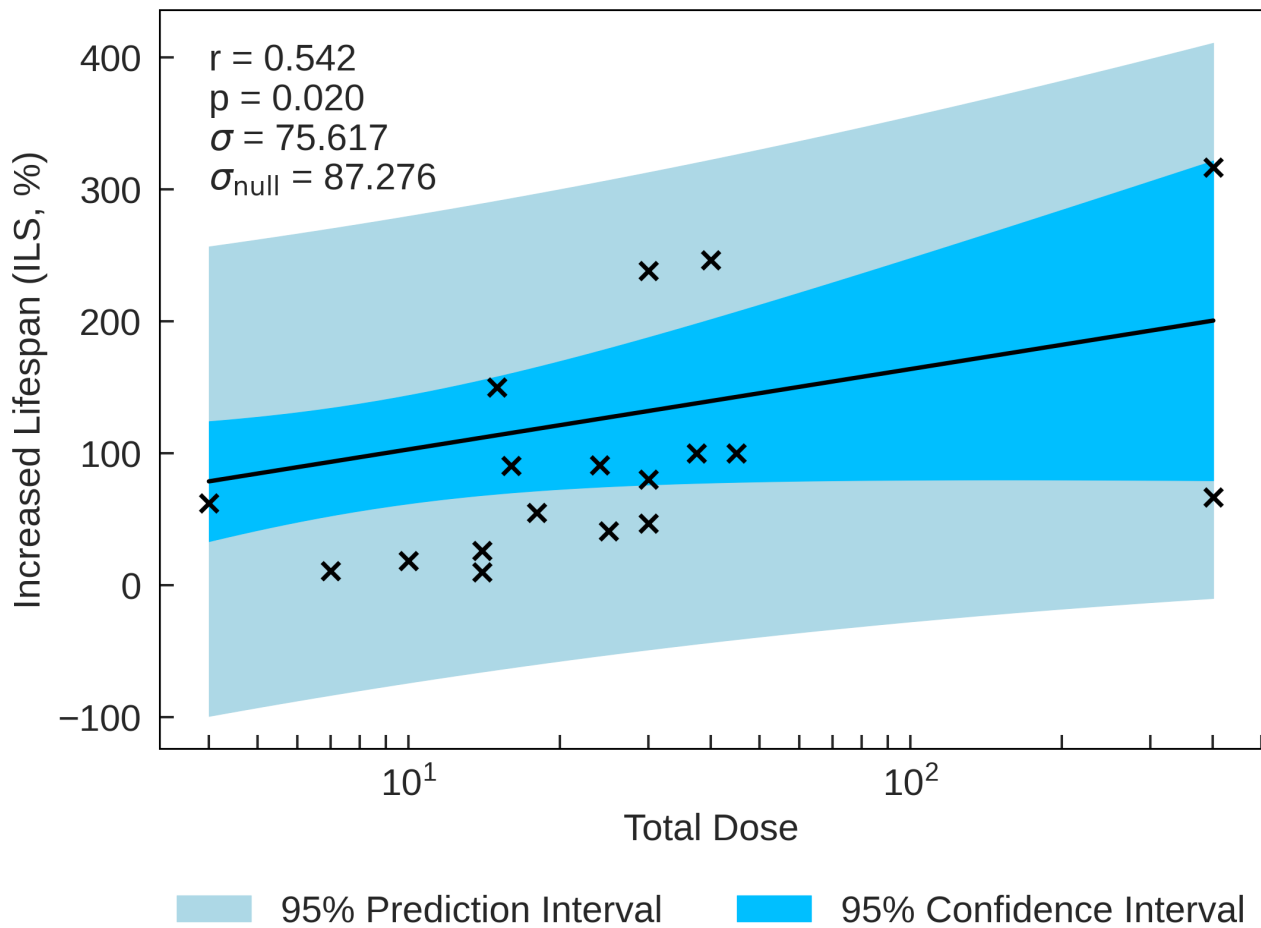


Figure 8: ILS plotted against the strongest dosimetric parameter, Total Dose. There is a strong positive correlation between the parameters, illustrating that an increase in dose can increase the lifespan of small animals.

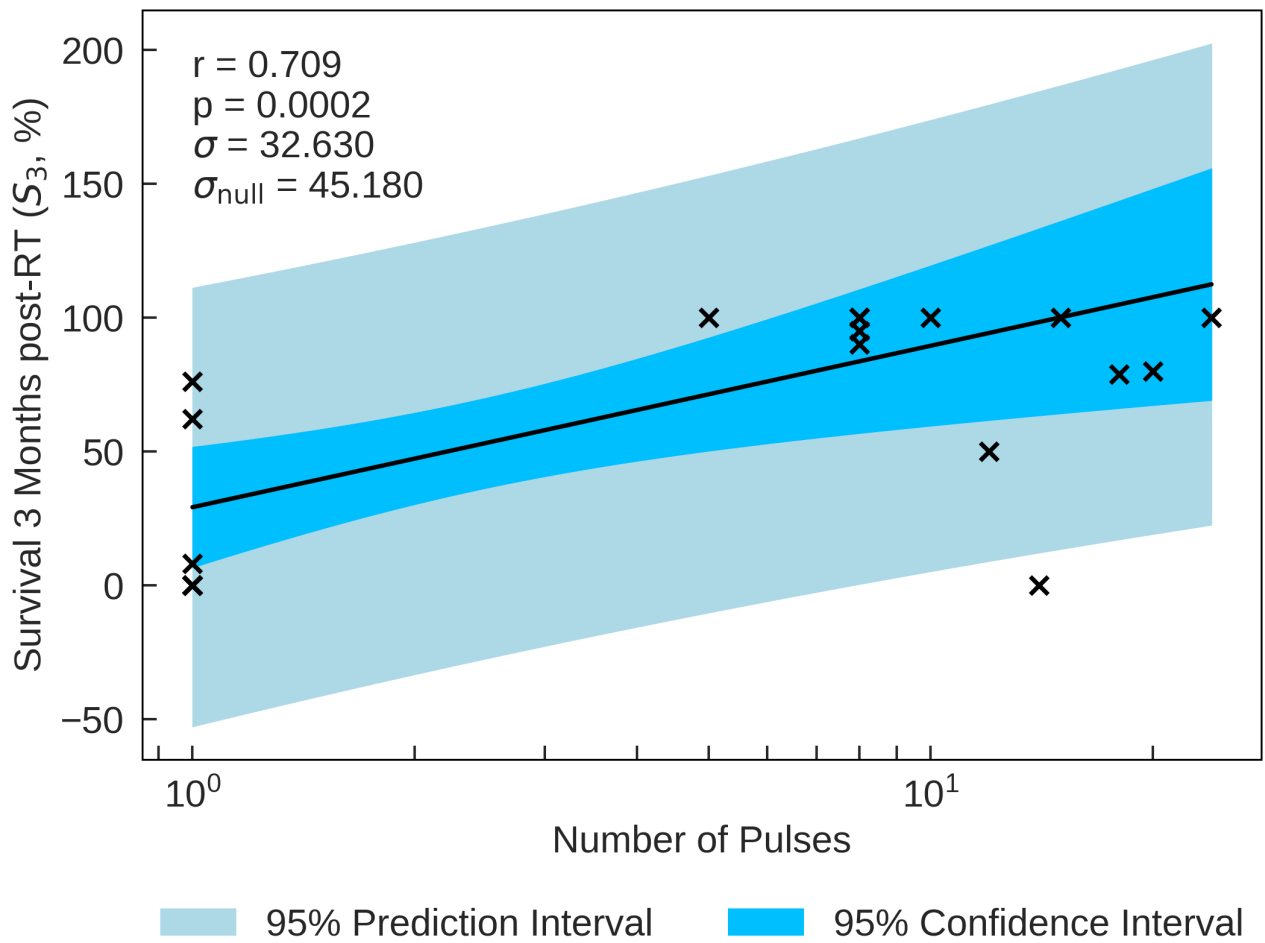


Figure 9: SS percentage plotted against the strongest dosimetric parameter, Number of Pulses. There is a strong positive correlation between the parameters, illustrating that an increase in the Number of Pulses can increase the survival time of small animals.

### 3 Data Tables

Table 1: Full table of beam parameters including the DOI of each study with the corresponding Increased Lifespan (ILS).

DOI	Mean Dose Rate (Gy/s)	Pulse Dose Rate (Gy/s)	Pulse Width (μs)	Pulse Dose (Gy)	Repetition Frequency (Hz)	Number of Pulses	Total Dose (Gy)	Total Duration (s)	Energy (MeV)	ILS
10.3390/cancers12092656	1.29E+04						4.01E+02	3.11E-02	1.04E-01	6.67E+01
10.3390/cancers12092656	1.17E+04						4.01E+02	3.43E-02	1.04E-01	3.17E+02
10.1158/1078-0432.ccr-20-0894	5.60E+06	5.56E+06	1.80E+00	1.00E+01	1.00E+02	1.00E+00	1.00E+01	1.80E-06	6.00E+00	1.85E+01
10.1158/1078-0432.ccr-20-0894	7.80E+06	7.78E+06	1.80E+00	1.40E+01	1.00E+02	1.00E+00	1.40E+01	1.80E-06	6.00E+00	2.58E+01
10.1158/1078-0432.ccr-20-0894	1.90E+06	1.94E+06	1.80E+00	3.50E+00	1.00E+02	1.00E+00	4 x 3.50E+00	1.80E-06	6.00E+00	1.06E+01
10.1158/1078-0432.ccr-20-0894	3.90E+06	3.89E+06	1.80E+00	7.00E+00	1.00E+02	1.00E+00	2 x 7.00E+00	1.80E-06	6.00E+00	9.77E+00
10.1158/1078-0432.ccr-20-0894	5.60E+06	5.56E+06	1.80E+00	1.00E+01	1.00E+02	1.00E+00	3 x 1.00E+01	1.80E-06	6.00E+00	4.66E+01
10.1016/j.radonc.2021.11.004	1.00E+03	1.00E+03	1.80E+04	1.80E+01	5.56E+01	1.00E+00	1.80E+01	1.80E-02	8.00E+00	5.49E+01
10.1016/j.radonc.2021.11.005	7.00E+02	7.00E+02	4.29E+04	3.00E+01	2.33E+01	1.00E+00	3.00E+01	4.29E-02	8.00E+00	2.38E+02
10.1016/j.ijrobp.2020.10.012	2.00E+02	1.11E+06	1.80E+00	2.00E+00	1.00E+02	2.00E+00	4.00E+00	2.00E-02	6.00E+00	6.20E+01
10.1016/j.adro.2022.101011	5.71E+05	2.86E+05	3.50E+00	2.00E+00	2.86E+05	4 x 3.00E+00	8 x 3.00E+00	4.20E-05	1.00E+01	9.10E+01
10.1016/j.adro.2022.101011	5.95E+05	2.98E+05	3.50E+00	2.08E+00	2.86E+05	6 x 3.00E+00	12.5 x 3.00E+00	6.30E-05	1.00E+01	1.00E+02
10.1016/j.adro.2022.101011	5.36E+05	2.68E+05	3.50E+00	1.88E+00	2.86E+05	8 x 3.00E+00	15 x 3.00E+00	8.40E-05	1.00E+01	1.00E+02
10.1038/s41598-022-16612-6	6.60E+01	1.33E+02	3.00E+04	4.00E+00	3.33E+01	2 x 4.00E+00	2 x 8.00E+00	2 x 1.20E-01		9.05E+01
10.1038/s41598-022-16612-6	7.40E+01	7.35E+01	2.43E+04	1.79E+00	3.33E+01	2 x 7.00E+00	2 x 1.25E+01	2 x 1.70E-01		4.09E+01
10.1667/RADE-20-00090	1.80E+02	4.00E+05	5.00E+00	2.00E+00	9.00E+01	1.50E+01	3.00E+01	1.67E-01	1.60E+01	8.00E+01
10.1667/RADE-20-00090	1.80E+02	4.00E+05	5.00E+00	2.00E+00	9.00E+01	2.00E+01	4.00E+01	2.22E-01	1.60E+01	2.46E+02
10.1126/scitranslmed.3008973	6.00E+01						1.50E+01	2.50E-01	4.50E+00	1.50E+02

Table 2: Full table of beam parameters including the DOI of each study with the corresponding % survivals ( $S_M$ ).

DOI	Mean Dose Rate (Gy/s)	Pulse Dose Rate (Gy/s)	Pulse Width (μs)	Pulse Dose (Gy)	Repetition Frequency (Hz)	Number of Pulses	Total Dose (Gy)	Total Duration (s)	Energy (MeV)	One month survival %	Two month survival %	Three month survival %	Six month survival %
10.3390/cancers12092656	1.29E+04						4.01E+02	3.11E-02	1.04E-01	0.00E+00	0.00E+00	0.00E+00	0.00E+00
10.3390/cancers12092656	1.17E+04						4.01E+02	3.43E-02	1.04E-01	3.50E+01	3.50E+01	3.50E+01	3.50E+01
10.1101/2019.12.12.873414	2.16E+02	1.00E+06	2.00E+00	2.00E+00	1.08E+02	8.00E+00	1.60E+01	7.41E-02	1.60E+01	9.50E+01	9.50E+01	9.50E+01	
10.1158/1078-0432.ccr-20-0894	5.60E+06	5.56E+06	1.80E+00	1.00E+01	1.00E+02	1.00E+00	1.00E+01	1.80E-06	6.00E+00	1.00E+02	0.00E+00	0.00E+00	0.00E+00
10.1158/1078-0432.ccr-20-0894	7.80E+06	7.78E+06	1.80E+00	1.40E+01	1.00E+02	1.00E+00	1.40E+01	1.80E-06	6.00E+00	1.00E+02	0.00E+00	0.00E+00	0.00E+00
10.1158/1078-0432.ccr-20-0894	1.90E+06	1.94E+06	1.80E+00	3.50E+00	1.00E+02	1.00E+00 4 x 3.50E+00		1.80E-06	6.00E+00	8.30E+01	8.00E+00	0.00E+00	0.00E+00
10.1158/1078-0432.ccr-20-0894	3.90E+06	3.89E+06	1.80E+00	7.00E+00	1.00E+02	1.00E+00 2 x 7.00E+00		1.80E-06	6.00E+00	1.00E+02	0.00E+00	0.00E+00	0.00E+00
10.1158/1078-0432.ccr-20-0894	5.60E+06	5.56E+06	1.80E+00	1.00E+01	1.00E+02	1.00E+00 3 x 1.00E+01		1.80E-06	6.00E+00	1.00E+02	8.00E+01	8.00E+00	0.00E+00
10.1016/j.radonc.2021.11.004	1.00E+03	1.00E+03	1.80E+04	1.80E+01	5.56E+01	1.00E+00	1.80E+01	1.80E-02	8.00E+00	1.00E+02	2.00E+01	0.00E+00	0.00E+00
10.1016/j.radonc.2021.11.004	1.20E+03	1.20E+03	2.50E+04	3.00E+01	4.00E+01	1.00E+00	3.00E+01	2.50E-02	8.00E+00	9.00E+01	9.00E+01	7.60E+01	0.00E+00
10.1016/j.radonc.2021.11.004	9.37E+02	9.37E+02	1.60E+04	1.50E+01	6.25E+01	1.00E+00	1.50E+01	1.60E-02	8.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
10.1016/j.radonc.2021.11.004				1.20E+01		1.00E+00	1.20E+01		8.00E+00	6.20E+01	6.20E+01	6.20E+01	
10.1016/j.jrobp.2020.10.012	2.00E+02	1.11E+06	1.80E+00	2.00E+00	1.00E+02	2.00E+00	4.00E+00	2.00E-02	6.00E+00	1.00E+02	1.00E+02		
10.1038/s41598-019-53562-y	3.50E+01	3.50E+01	4.57E+05	1.60E+01	4.57E-01	1.00E+00	1.60E+01	4.57E-01	2.00E+01	0.00E+00	0.00E+00	0.00E+00	0.00E+00
10.1016/j.adro.2022.101011	5.71E+05	2.86E+05	3.50E+00	2.00E+00	2.86E+05	4 x 3.00E+00	8 x 3.00E+00	4.20E-05	1.00E+01	1.00E+02	1.00E+02	5.00E+01	
10.1016/j.adro.2022.101011	5.95E+05	2.98E+05	3.50E+00	2.08E+00	2.86E+05	6 x 3.00E+00	12.5 x 3.00E+00	6.30E-05	1.00E+01	1.00E+02	9.00E+01	7.88E+01	
10.1016/j.adro.2022.101011	5.36E+05	2.68E+05	3.50E+00	1.88E+00	2.86E+05	8 x 3.00E+00	15 x 3.00E+00	8.40E-05	1.00E+01	1.00E+02	1.00E+02	1.00E+02	
10.1038/s41598-022-16612-6	6.60E+01	1.33E+02	3.00E+04	4.00E+00	3.33E+01	2 x 4.00E+00	2 x 8.00E+00	2 x 1.20E-01		1.00E+02	1.00E+02	1.00E+02	
10.1038/s41598-022-16612-6	7.40E+01	7.35E+01	2.43E+04	1.79E+00	3.33E+01	2 x 7.00E+00	2 x 1.25E+01	2 x 1.70E-01		6.40E+01	0.00E+00	0.00E+00	0.00E+00
10.1667/RADE-20-00090	1.80E+02	4.00E+05	5.00E+00	2.00E+00	9.00E+01	5.00E+00	1.00E+01	5.56E-02	1.60E+01	1.00E+02	1.00E+02	1.00E+02	1.00E+02
10.1667/RADE-20-00090	1.80E+02	4.00E+05	5.00E+00	2.00E+00	9.00E+01	8.00E+00	1.60E+01	8.89E-02	1.60E+01	1.00E+02	1.00E+02	1.00E+02	1.00E+02
10.1667/RADE-20-00090	1.80E+02	4.00E+05	5.00E+00	2.00E+00	9.00E+01	1.00E+01	2.00E+01	1.11E-01	1.60E+01	1.00E+02	1.00E+02	1.00E+02	1.00E+02
10.1667/RADE-20-00090	1.80E+02	4.00E+05	5.00E+00	2.00E+00	9.00E+01	1.50E+01	3.00E+01	1.67E-01	1.60E+01	1.00E+02	1.00E+02	1.00E+02	1.00E+02
10.1667/RADE-20-00090	1.80E+02	4.00E+05	5.00E+00	2.00E+00	9.00E+01	2.00E+01	4.00E+01	2.22E-01	1.60E+01	1.00E+02	8.00E+01	8.00E+01	5.30E+01
10.1126/scitranslmed.3008973	6.00E+01						1.50E+01	2.50E-01	4.50E+00	4.00E+01	2.00E+01		
10.1126/scitranslmed.3008973	6.00E+01						2.30E+01	3.83E-01	4.50E+00	8.00E+01	6.00E+01		
10.1126/scitranslmed.3008973	6.00E+01						2.80E+01	4.67E-01	4.50E+00	8.90E+01	6.00E+01		
10.1038/s41598-020-78017-7	2.16E+02	4.00E+05	5.00E+00	2.00E+00	1.08E+02	8.00E+00	1.60E+01	7.41E-02	1.60E+01	9.00E+01	9.00E+01	9.00E+01	

Table 3: Full table of beam parameters including the DOI of each study with the corresponding TIS, TCS and NTSS.

[illegible]

Table 4: % survivors of Glioma-bearing rats at 3 months post FLASH (ultra-high dose rates, dose rate of FLASH-RT recorded in columns 2 and 3) vs CONV (conventional radiotherapy, conventional dose rates). Average and standard error recorded for both modalities.

Journal Citation	Mean Dose Rate (Gy/s)	Pulse Dose Rate (Gy/s)	% survivors post FLASH	% survivors post CONV
[1]	5600000.00	5555555.56	0.00	0.00
[2]	571428.57	285714.29	50.00	48.00
[2]	595238.10	297619.05	78.80	100.00
[2]	535714.29	267857.14	100.00	93.00
[3]	66.00	133.33	100.00	85.00
[3]	74.00	73.53	0.00	0.00
		Average	54.80	54.33
		Error	7.71	7.63

## References

- [1] P. Montay-Gruel, M. M. Acharya, P. Gonçalves Jorge, B. Petit, I. G. Petridis, P. Fuchs, R. Leavitt, K. Petersson, M. Gondré, J. Ollivier, R. Moeckli, F. Bochud, C. Bailat, J. Bourhis, J.-F. Germond, C. L. Limoli, and M.-C. Vozenin, “Hypofractionated FLASH-RT as an effective treatment against glioblastoma that reduces neurocognitive side effects in mice,” *Clin. Cancer Res.* **27** (Feb., 2021) 775–784.
- [2] E. Konradsson, E. Liljedahl, E. Gustafsson, G. Adrian, S. Beyer, S. E. Ilaahi, K. Petersson, C. Ceberg, and H. Nittby Redebrandt, “Comparable long-term tumor control for hypofractionated FLASH versus conventional radiation therapy in an immunocompetent rat glioma model,” *Adv. Radiat. Oncol.* **7** (Nov., 2022) 101011.
- [3] E. Liljedahl, E. Konradsson, E. Gustafsson, K. F. Jonsson, J. K. Olofsson, C. Ceberg, and H. N. Redebrandt, “Long-term anti-tumor effects following both conventional radiotherapy and FLASH in fully immunocompetent animals with glioblastoma,” *Sci. Rep.* **12** (July, 2022) 12285.