Radiobiology Behind Dose Fractionation in Ewing Sarcoma

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Background

Ewing sarcoma (ES) is an aggressive bone and soft tissue sarcoma that most frequently occurs in adolescent patients. The multimodal treatment for ES consists of chemotherapy, radiation, and surgery. Unfortunately, >10% of patients have recurrent disease, indicating room for improvement in radiation efficacy. Radiation creates reactive oxygen species that cause double strand breaks (DSB) to tumor cell DNA in the presence of oxygen. Thus, increasing oxygenation of tumors may enhance radiation efficacy. Studies have shown that exercise improves tumor vascular function which enhances oxygenation. **We hypothesize that exercise will enhance RT efficacy in an A673 ES murine model. To test this, we first determined the radiosensitivity of ES cells *in vitro*.**

Materials and Methods

We performed clonogenic assays, the gold standard in measuring radiosensitivity, on A673 Ewing tumor cells in increasing seeding densities (100, 200, 300, 500, 1000, 1500, 2000, 3000) in 6 well plates. Plates were irradiated at 2, 4, 5, and 7Gy with a clinical linear accelerator (6MV photon beam), the same machine used to treat patients. Backscatter and buildup material were used to place the cells at a water-equivalent depth of 10 cm during irradiation mimicking the location of a tumor in the human body. After 8-14 days, colonies (50 cells) were stained using Crystal Violet 0.5% and counted using ImageJ. The plating efficiency (PE) and survival fractions (SF) were calculated, and the dose-survival curves were generated by plotting the SF as a function of the dose on a semi-logarithmic scale (Prism Software) and fitted using linear quadratic model.

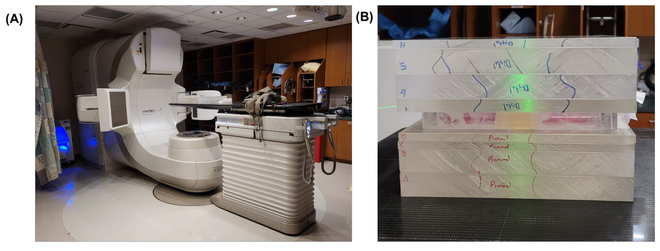

Fig. 1 (A). LINAC was used for external beam radiation treatments of the plates (B). Acrylic set-up was used for accurate calibration of the radiotherapy beams, to avoid dosimetry error and to mimic the body

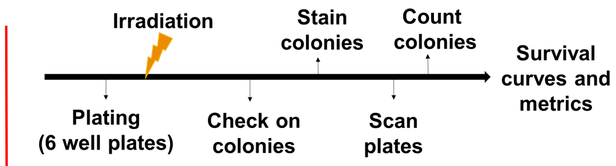

Fig. 2. Timeline of the clonogenic assay. Plates are seeded with different cell densities and irradiated after 24h with the LINAC. When ready, the colonies are stained, scanned, and enumerated using Fiji software.

Results

We first determined the appropriate seeding density at 0Gy (100, 400, 800). After 12 days of incubation at 400 and 800, we determined the plating efficiency using the formula: PE=Number of colonies/Number of cells plated. **Therefore, for the irradiation experiment, we proceeded with a lower seeding density of 100 and 200 at 0Gy. The aim of the clonogenic assay is to reach at least 10% survival with one of the 4 doses plus 0Gy. With increasing radiation dose, more cells must be plated to achieve this survival goal**

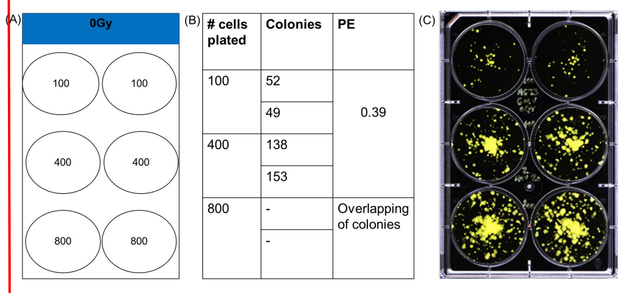

Fig. 3. Plating efficiency of A673 at 0Gy. (A) Set up. In this experiment seeding densities of 100, 400 and 800 were tested. (B) Summary of colonies counted and PE determination. (C) Colonies stained using a crystal violet solution (0.5%) were scanned using Black & White negative, Epson scanner.

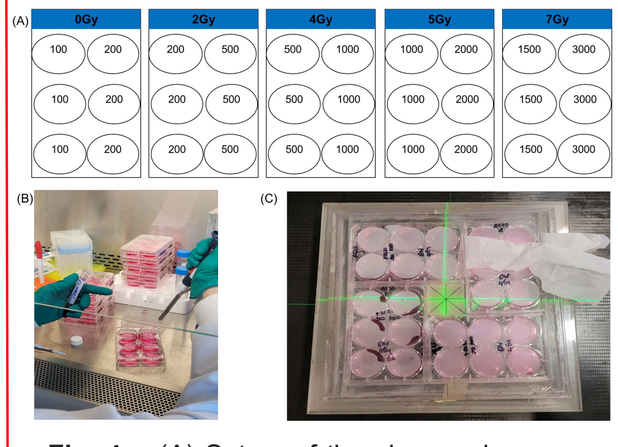

Fig. 4. (A) Set-up of the clonogenic assays in 6-well plates. We plated four batches of A673 cells for more experimental accuracy. (B) Wells were loaded according to the experimental set-up. (C) Plate replicates assigned the same dose were arranged in acrylic build-up material 24 hours after plating.

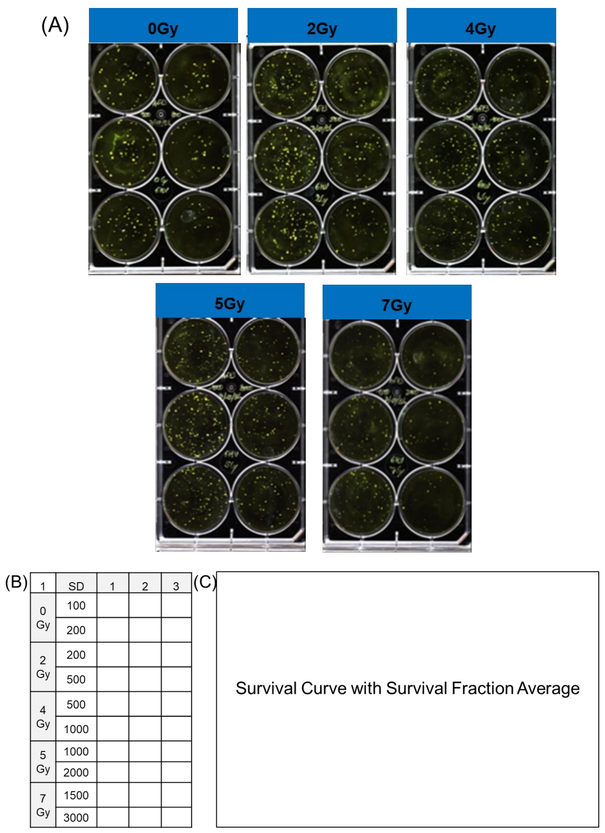

Fig. 5. (A) Representative clonogenic assay of A673 cell line. (B) Enumeration of the colonies (C) Radiation cell survival curve for A673 cells.

We performed 3 more replicates of the A673 clonogenic assays. Figure 5 shows a dose dependent decrease of the survival fraction shows that the 10% survival is likely to obtained with a dose of ___Gy for all batches.

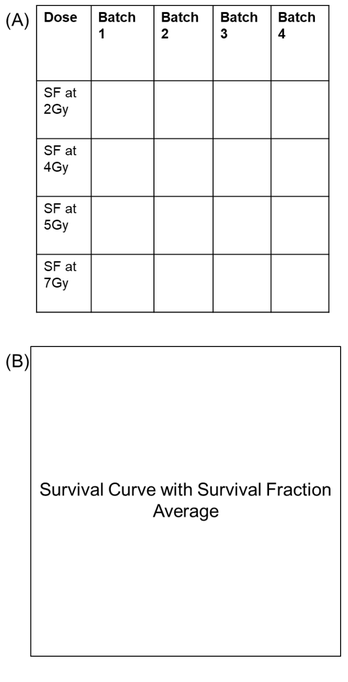

Fig. 6. Summary of the clonogenic data. (A) The SF of the replicates are similar for each dose of radiation. (B) Survival curve of all data.

We expect to calculate the metrics which include the α/β ratio, where the α components corresponds to non-repairable lethal damage and β to repairable DSB (linear quadratic model). These values are statistically determined with Graphpad when we build the Survival curve. In radiobiology, we used the α/β as a reliable estimate of radiation response. Late responding tissue like sarcomas are characterized by a low ratio (3 or lower). We expect this to be reflected with our experiment. Knowing the α/β, the BED can be determined. This reflects the sensitivity to dose fractionation.

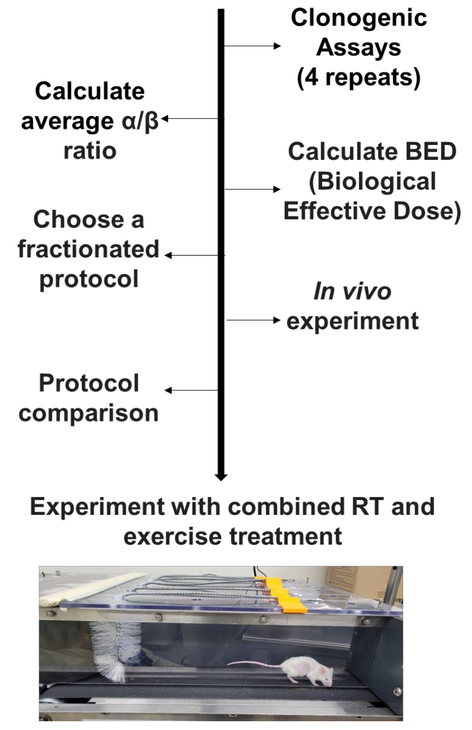

Fig. 7 Extension of *in vitro* experiment to murine model timeline

Conclusions

The α/β ratio and BED will be used to design a fractionation protocol to be used in combination with treadmill exercise in mice studies.

Dose per Fraction	6Gy
Total Dose	
α / β	
BED	~150

https://www.mdcalc.com/radiation-biologically-effective-dose-bed-calculator

Table. 1 Possible fractionation protocol for murine model. For patients, the BED should be >100 to achieve 85% of local tumor control. To have the same effect, murine models should have a BED>150. Conventional radiation therapy using 2Gy/fraction over a period of 6 weeks. For the mice mice experiment, we could use an hypofractionated protocol.

References

1) Patel et al. ASCO Journals 2021.
2) Garcia et al. J Cancer Biol 2020.