Background
Ewing sarcoma (ES) is an aggressive bone and soft tissue sarcoma that most frequently occurs in adolescents. 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.

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.

Conclusions
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.

References