Introduction: Adjunctive radiotherapy plays an important role in the treatment of specific pediatric musculoskeletal malignancies. However, crippling limb-length discrepancy can occur when the irradiated field is inclusive of an open physis. Recent work using both in vitro and in vivo models has demonstrated that prophylactic radioprotection and/or supportive radiotherapy strategies may have utility in reducing the severity of this adverse effect, suggesting great translational promise. Prior to promoting efficacious agents to clinical trial, selectivity must also be demonstrated, so as to avoid undesirable interactions with clinically relevant, radiosensitive tumors. To date, there are no established orthotopic xenograft models of soft-tissue sarcoma which allow physiologically relevant evaluation of efficacy and selectivity of various therapies and the adverse skeletal effects of these therapies may produce in skeletally immature animals. Our hypotheses were that 1) an orthotopic inoculation human rhabdomyosarcoma cells into nude mice would establish a ‘primary’ tumor; 2) that radiotherapy would produce dose-dependent improvement in tumor regression and survival; 3) that radiotherapy would result in dose-dependent reduction of limb length. The present work sought to examine the effects of radiotherapy on limb lengthening in an orthotopic murine xenograft model of pediatric rhabdomyosarcoma. Secondarily, we have validated a bioluminescent assay procedure for evaluation of tumor engraftment and growth and metastatic dissemination in this model.

Methods: A human alveolar rhabdomyosarcoma cell line, RC13/SICRH-30 (ATCC, Manassas, VA) was transfected with a luciferase-expressing plasmid vector(pEGFP-Luc, Clontech, Mountain View, CA) using Lipo-fection LTX/Plus(Invitrogen, Carlsbad, CA). Transfected cells were then selected with G418 (10μg/ml) for 14 days. Isolated colonies were further expanded under 5μg/ml G418 selection for 10 doublings, yielding a stably transfected variant, RC13-lucA2. Expression of the Luc gene was verified by RT-PCR; in vitro luciferase activity was demonstrated by incubation with 15μg/ml d-luciferin (Caliper Life Sciences., Hopkinton, MA). All animal procedures were performed in accordance with an IACUC approved protocol. For the in vivo study, a total of n=18, 4-week-old male Ncr nude mice (Taconic Labs, Germantown, NY) were anesthetized with Telazol (15mg/kg, IP), and radiographed. While sedated, the mice were inoculated with 2x10^5 RC13-lucA2 cells suspended in 50μL Matrigel (BD Biosciences, San Jose, CA), injected into the distal right quadriceps. At 24 hours and at 7 days following inoculation, the IVIS-50 (Caliper) bioluminescence imaging (BLI) unit was used to verify tumor engraftment. Prior to imaging studies, the mice received 150mg/kg d-luciferin intraperitoneally, and allowed 10 minutes for circulation and signal leveling. During imaging studies, the mice were anesthetized with isoflurane gas in O₂. Luciferase activity data was recorded over a period of 5 minutes and expressed as photon flux (p/s). Serial BLI scans were performed on a weekly basis thereafter for up to 12-weeks post-inoculation (Fig 1).

Results: Two mice expired from anesthetic complication during inoculation. All remaining mice demonstrated a measurable luciferase signal over the injection site at 1 and 7 days following inoculation, with modest attrition of signal value between scans taken on day 1 and 7 (Fig 1), indicating 100% engraftment. Subsequently, aggressive local tumor growth, was observed in non-irradiated and 2Gy-irradiated mice, necessitating euthanization of all of these animals between 56 and 64 days of inoculation; three mice displayed signal loci suggestive of thoracic or retroperitoneal dissemination. Median survival was 63 days for the 0 Gy and 2 Gy groups (p=0.7390, Mantel-Cox Log Rank test), whereas survival of mice exposed to 10Gy ranged 56 to 84 days (Median 77 days p=0.0529, Table 1). Both 2Gy and 10Gy x-radiation exposure significantly reduced tibial and femoral length relative to the contralateral limb (p<0.5, paired T-test, Table1).

Conclusion: We have successfully developed a tumor bearing animal model that allows quantitative measurement of tumor burden and treatment response, as well as radiation-induced limb-length discrepancy. This model will be used in future work to document the safety, specificity and efficacy of radioprotectant compounds, and may have use in testing novel pharmacologic strategies for the treatment of rhabdomyosarcoma.

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Figure 1: Serial BLI: Luciferase imaging measured as photon flux (p/s). Luciferase activity was persistently and significantly reduced by 10Gy x-rays, whereas 2Gy was not significantly different from un-irradiated control mice. Data shown is average ± SD. Brackets demonstrate significant difference by repeated-measures ANOVA (p<0.05).

Figure 2: Representative histology and immunohistochemistry. A) H&E, 10x, a)H&E, 40x; B MyoD1; C) IHC neg. control; D) Myogenin.