Abstract of proposed student project (1 page limit. This should mirror the aims page of a grant and CLEARLY indicate the student's role.)

Squamous cell carcinoma (SCC) is the most common type of oral cancer in cats (60% of cases). SCC is an aggressive tumor, often invading into the underlying bone structures and spreading to regional lymphoid organs. Regardless of treatment, it is a uniformly fatal disease with less than 10% of affected cats surviving for more than one year from diagnosis, mostly succumbing from the local disease. Adequate local control with surgical resection is challenging due to location of the tumor, and is associated with a high rate of post-operatory complications. A previous study utilizing feline SCC cell lines has identified a cyclin dependent kinase inhibitor (CDKi), dinaciclib, as a highly effective drug at nanomolar concentrations [1]. Studies have shown that CDKi combined with radiation therapy are associated with an increased cell killing due to synergistic effects. The goal of this study is to evaluate the synergistic effects of dinaciclib in inhibiting the growth of feline SCC when given concomitantly with radiation therapy. We hypothesize that feline SCC cells in vitro treated with dinaciclib and radiation therapy have a smaller surviving fraction than those treated with either modality alone.

Three fully established and commercially available feline SCC cell lines (SCCF1, SCCF2 and SCCF3) will be utilized to test our hypothesis. Cells will be cultured in DMEM media supplemented with 10% fetal bovine serum, 1% antibiotic-antimycotic, 0.1% gentamicin, epidermal growth factor at 10ng/mL and cholera toxin at 0.1nM. Radiosensitivity will be first measured by clonogenic assays in irradiated cells treated with dinaciclib, and untreated cells. SCC cells will be irradiated with single fractions of 0, 2, 4, 6, 8, and 10 Gy, with and without treatment with dinaciclib, using a 6MV linear accelerator located at the UF Small Animal Hospital. After incubating for 7-10 days to allow colony formation, culture dishes are fixed and stained with 0.1% crystal violet for colony counting. Surviving fraction (SF) will be calculated as SF = colonies counted/cell plated, with normalized SF = treated SF/control SF (platting efficiency). The surviving fraction of cells at each dose of radiation will be calculated, and compared to the surviving fraction when radiation is combined with treatment with dinaciclib. Three independent experiments will be carried out. Survival curves will be drawn in log scale using linear-quadratic regression equations, and will be compared using ANOVA and Wilcoxon's test with Prism 9 software (GraphPad). Results from this study can be used as a proof of concept to further investigation of this novel dual modality of treatment in the clinical setting. The student will have an active role in all experimental steps described above, under guidance and supervision of the faculty and biological scientists from the Clinical Sciences Research Laboratory. The student will be first introduced to basic cell culture techniques (culture media preparation, cells trypsinization, cells passage, cell counting). Then, they can be introduced to the clonogenic assay with untreated cells. The next step will be to perform assays with treated cells, and with time working with multiple cell lines at the same time.

References

1. Piegols, H.J., et al., Investigation of novel chemotherapeutics for feline oral squamous cell carcinoma. Oncotarget, 2018. 9(69): p. 33098-33109.