



In vitro Effects of Zoledronic Acid and Radiation Therapy on Canine Osteosarcoma



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Background

- Osteosarcoma (OS) accounts for 85% of canine bone cancers. Canine osteosarcoma has similar characteristics to human OS making it a good comparative model.
- A common treatment for canine OS is amputation with adjuvant chemotherapy, and in non-surgical candidates localized radiation therapy (RT) is utilized.
- In recent studies, bisphosphonates have been added to OS treatment protocols to prevent bone resorption and potentially increase the effectiveness of RT.
- Studies on the combined treatment of bisphosphonates and RT in human malignancies have exhibited synergy in primary and metastatic cell lines.

Objective

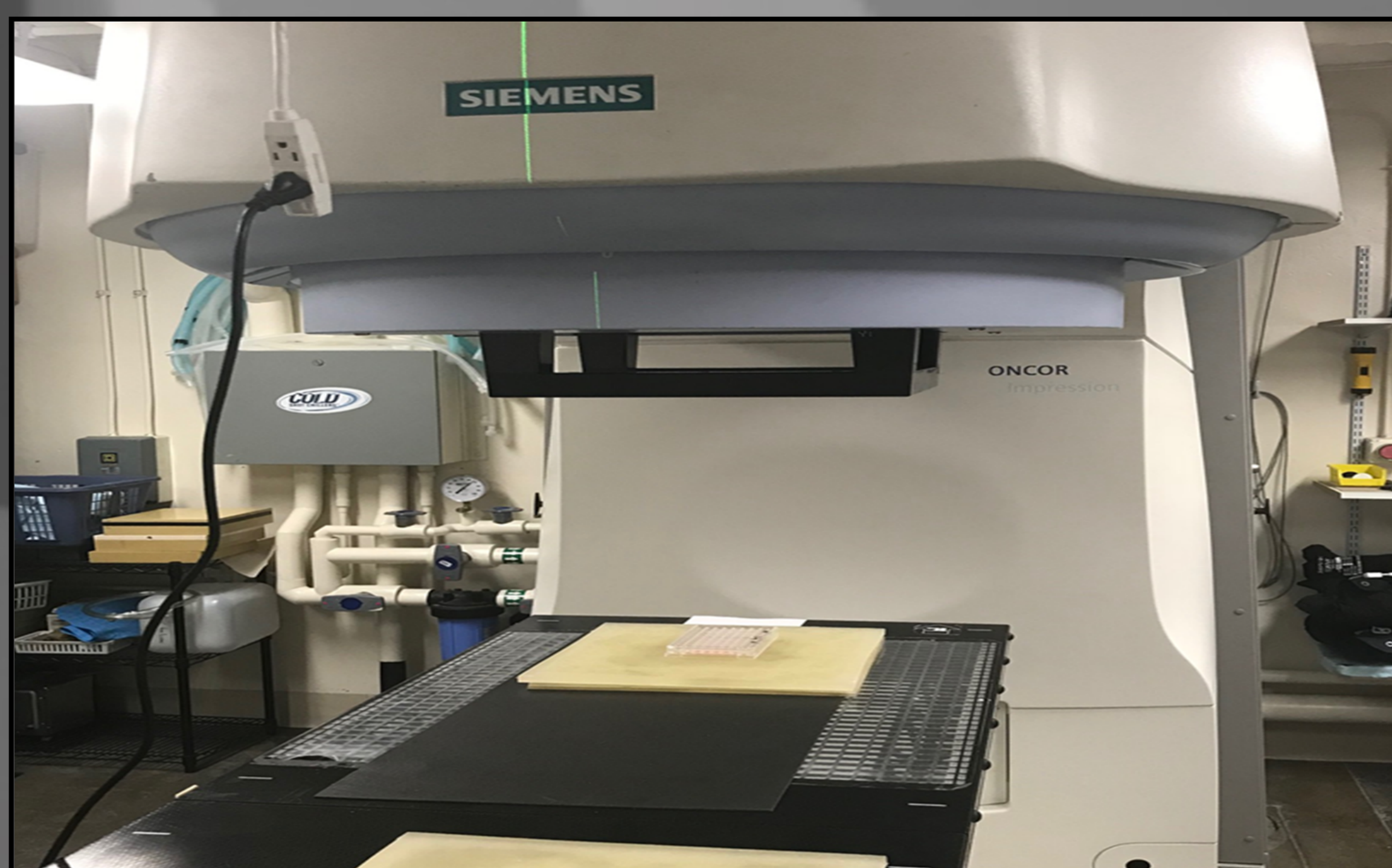
- Evaluate the effect of bisphosphonate zoledronic acid and radiation therapy in canine OS cells.

Hypothesis

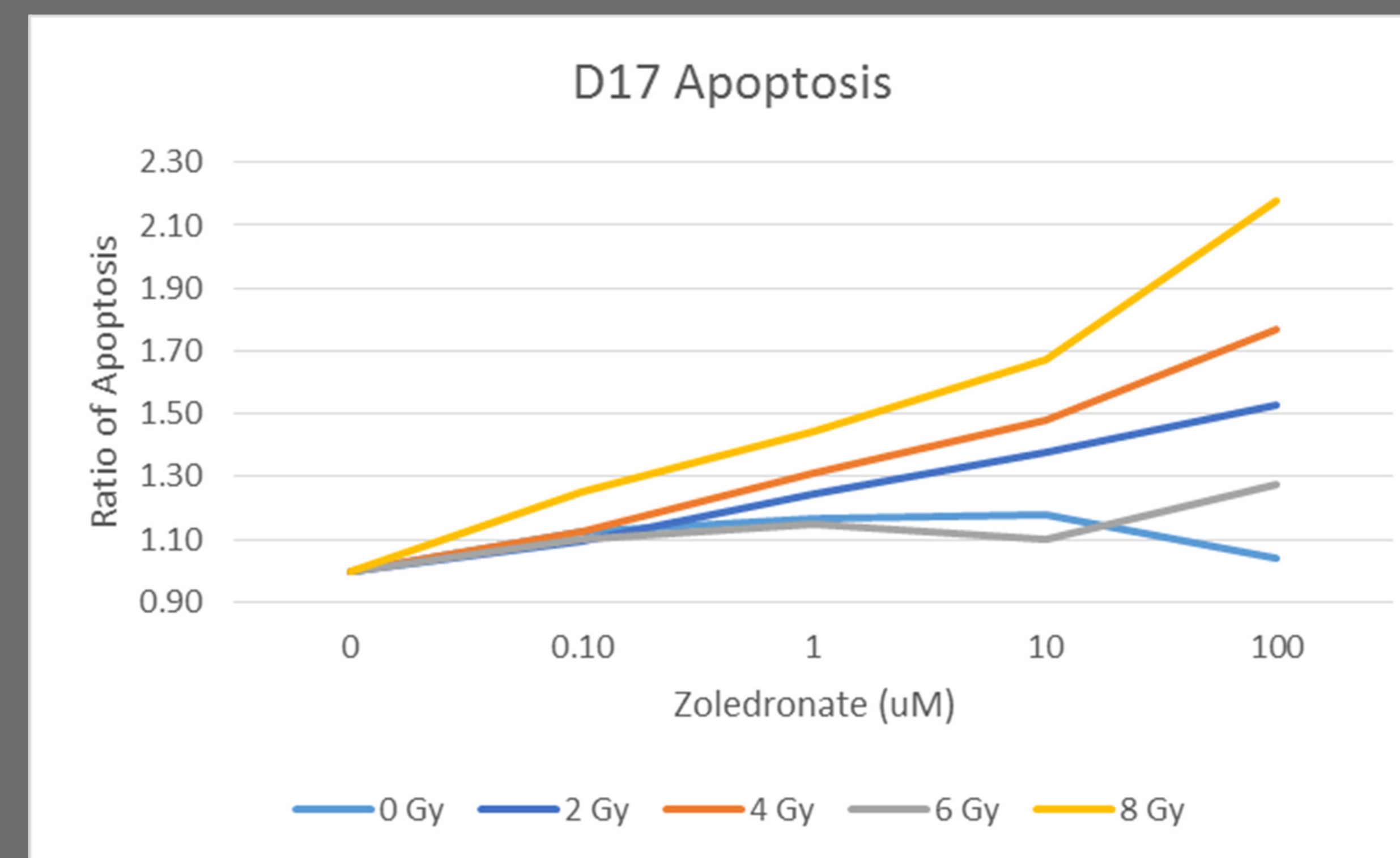
- Combination zoledronic acid and radiation therapy will significantly increase apoptosis in canine OS cells compared to either agent alone.

Materials and Methods

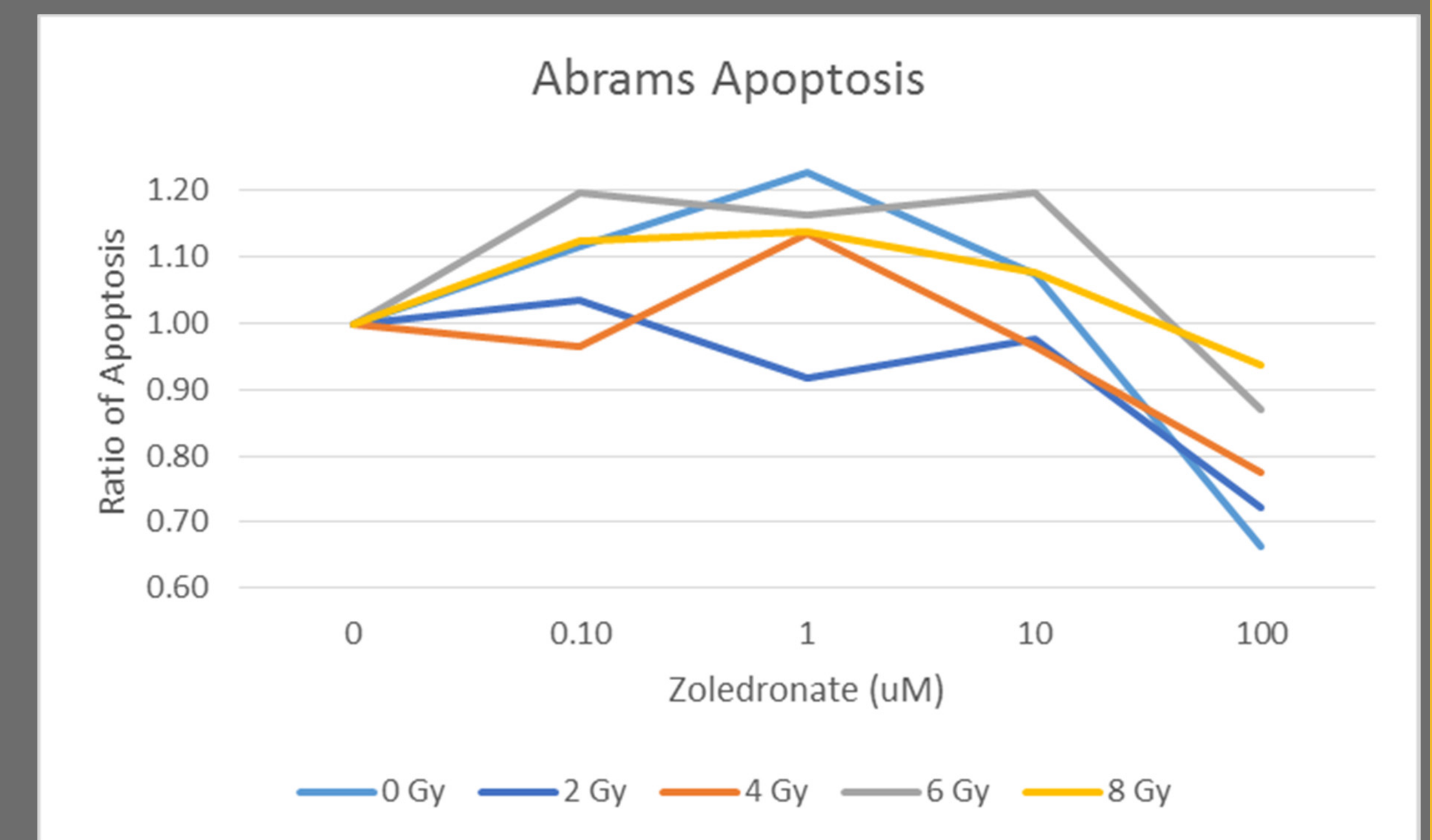
- Abrams and D-17 canine OS cell lines were grown in 96 well plates with 5×10^3 cells per well and incubated for 24 hours at 37°C.
- Cells were treated with 0, 0.10, 1.0, 10, and 100 μM concentrations of zoledronic acid (biologically achievable).
- The cells were radiated at 0, 2, 4, 6, and 8 Gy with a linear accelerator (shown below).
- Cell viability, cytotoxicity, and apoptosis were measured for each group via ApoTox-Glo™ Triplex assay (Promega) at 24 hours after treatment.



Results



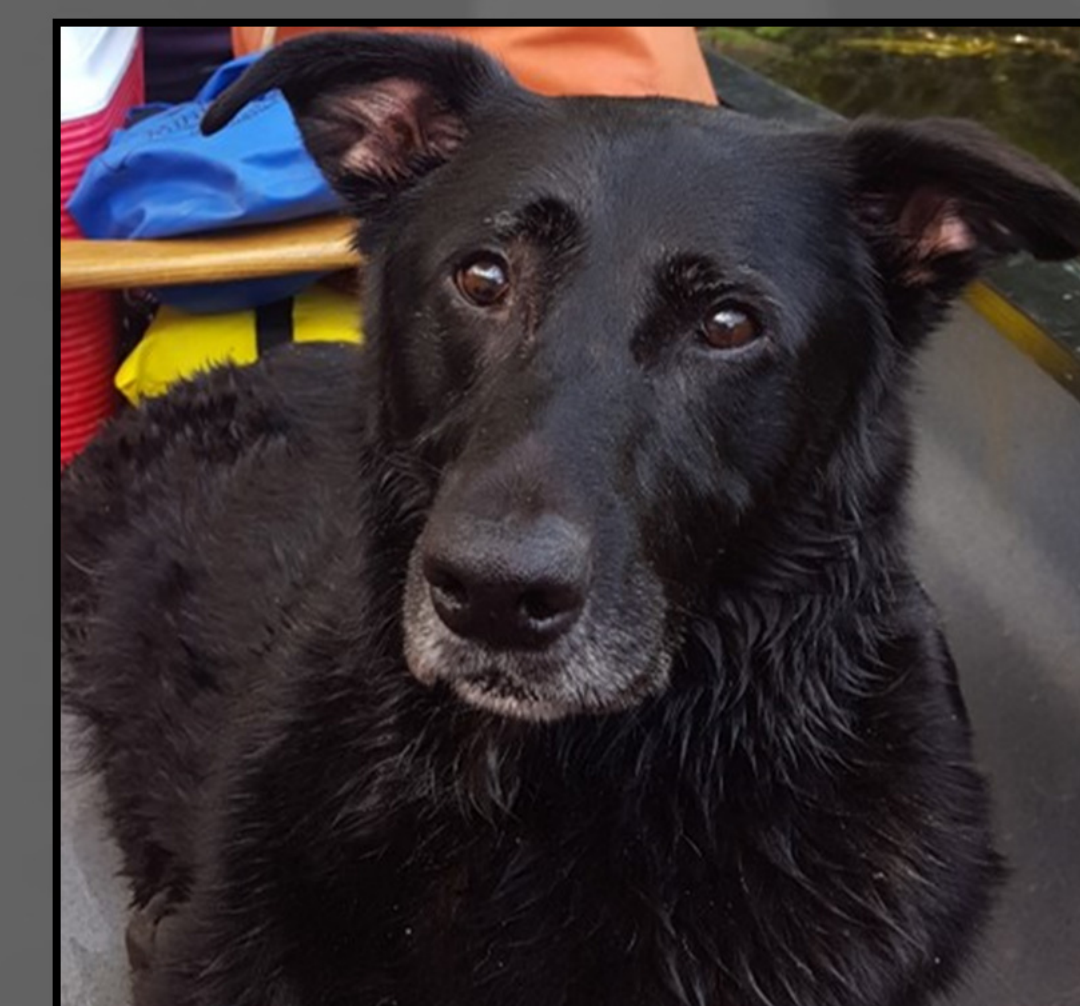
- D17 has cellular characteristics more similar to a primary tumor. Apoptosis increased as zoledronate concentration increased. All irradiated groups except the 6 Gy group had an increase in slope and ratio of apoptosis.



- Abrams has cellular characteristics more similar to a metastatic tumor. Apoptosis increased in each irradiated group. However, increasing concentrations of zoledronate appeared to have a more parabolic effect on apoptosis.

Discussion

- The D17 curves support the notion of synergy between zoledronate and radiation therapy. Several theories are possible for the parabolic Abrams lines:
 - Combination therapy is actually inducing necrosis, therefore fewer cells are available for apoptosis analysis.
 - The Abrams line, which mimics metastatic cells, may be more sensitive to a cell cycle inhibitor. Zoledronate may be having an anti-tumor effect on this population.
 - At higher concentrations, zoledronate may be protective. While unlikely, further analysis of cell viability is necessary in this population of cells.
- Analysis of viability and cytotoxicity is ongoing. We will construct a dose isobologram involving apoptosis.
- We have previously shown a statistically significant increase in apoptosis in cells treated with 4 Gy radiation and 10 μM zoledronate ($p < 0.001$), compared to either treatment alone.
- We will perform comet assays to evaluate DNA strand breaks in cells treated with these agents.
- We expect to find a difference in the number and quality of DNA strand breaks, with relatively more double stranded breaks occurring in the combination group, likely contributing to the increase in apoptosis.
- We have implemented this *in vitro* work into clinical practice. Currently, combination zoledronate and radiation therapy is our first-line option for non-surgical canine patients with osteosarcoma. We have currently treated 15 patients in this fashion. Analysis of those patients' response is ongoing.



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