# Combined Effects of Zoledronic Acid and Radiation Therapy on Canine Osteosarcoma Cells

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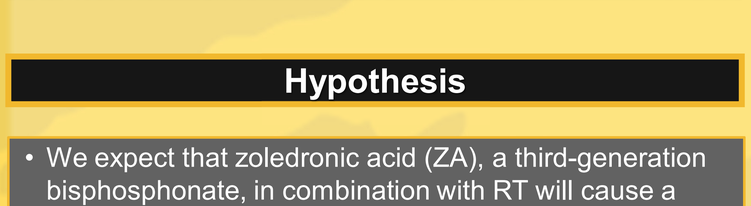
## Background

- Osteosarcoma (OS) accounts for the majority of bone cancers in dogs as well as human children and young adults, making it a suitable comparative oncology model.
- A common treatment for canine OS is amputation with adjuvant chemotherapy, and in non-surgical candidates localized radiation therapy (RT) is utilized.
- Recently, bisphosphonates have been added to OS treatment protocols to increase the effectiveness of RT.
- Studies on the combined treatment of bisphosphonates and RT in human and murine cancer cells have exhibited synergy in primary and metastatic models.
- Synergy has yet to be studied in canine cancer cells.



## Hypothesis

- We expect that zoledronic acid (ZA), a third-generation bisphosphonate, in combination with RT will cause a significant increase in apoptosis of canine OS cells compared to either ZA or RT treatment alone.

## Materials and Methods

- Abrams and D-17 canine OS cell lines were seeded in 96 well plates (2,000 cells/well) and incubated for 24 hours at 37ºC.
- Cells were evaluated under four conditions: control with no treatment (Group 1), treatment with10 µM of ZA (Group 2), treatment with 4 Gy of RT (Group 3), and treatment with both ZA and RT (Group 4).
- Group 2 cells were treated with 10 µM ZA for 24 hours, Group 3 cells were irradiated (4Gy) using a linear accelerator (Siemen's Oncor, CA) at 48 hours, and Group 4 cells were treated with 10 µM ZA at 24 hours then irradiated at 48 hours.
- Cell viability, cytotoxicity, and apoptosis were measured for each group via Triplex ApoTox assay (Promega, WI) at 24 and 48 hours after treatment.
- Statistical significance of detected differences were calculated for each treatment group using a two-tailed Student T-test.

## Results

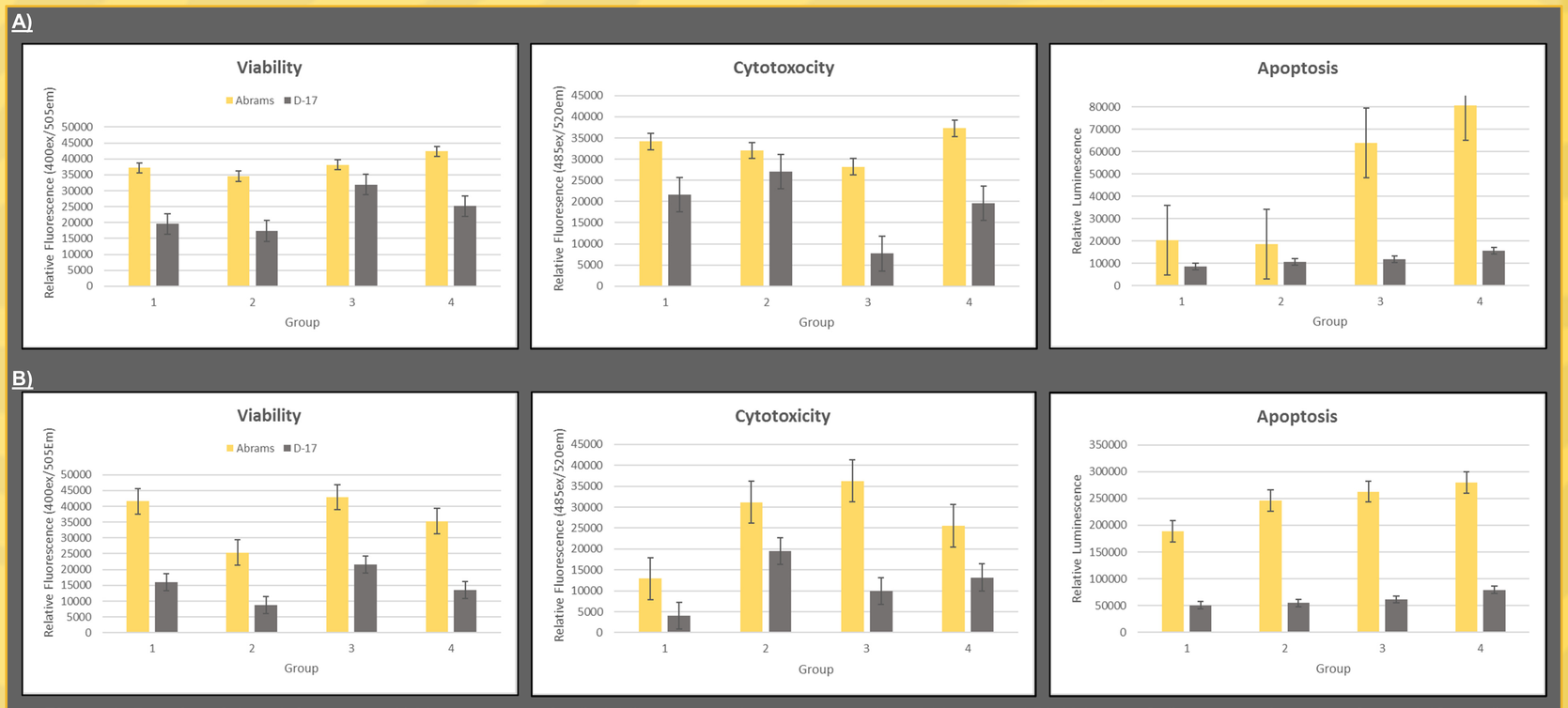



**Figure 1: Results of the triplex assay.** A) Measurement of cell viability, cytotoxicity, and apoptosis at 24 hours and B) 48 hours. Cells were evaluated as Group 1 (Control), Group 2 (10 µM ZA only), Group 3 (RT only) and Group 4 (10 µM ZA and RT). In both cell lines, the differences in Group 4 cell viability and cytotoxicity were insignificant compared to control at either time point. However, both cell lines exhibited a significant increase in apoptosis with the Group 4 treatment at both time points.

## Conclusion

- At 24 hours, the Abrams cell line showed no significant changes in viability ($p > .05$) or cytotoxicity ($p > .05$) with the combined treatment of ZA and RT, but significant increases in apoptosis ($p < .0001$) were seen compared to controls.
- At 48 hours, the same held true for the Abrams cells with no significant difference in viability ($p > .05$) or cytotoxicity ($p > .05$), but a significant increase in apoptosis ($p < .0001$) compared to the control.
- At 24 hours, the D-17 cell line showed no significant difference in viability ($p > .05$) or cytotoxicity ($p > .05$) , but showed a significant increase in apoptosis ($p < .0001$)) when compared to the control.
- At 48 hours, D-17 cells showed no significant changes in viability or cytotoxicity with the combined treatment, but significant increases in apoptosis ($p = .001$) were seen compared to controls.
- These results demonstrate that the combination therapy of ZA and RT causes significant effects on canine OS cells by causing increased apoptosis at both 24 and 48 hours, especially in the highly metastatic Abrams line.
- We plan to continue investigating varying concentrations and doses of ZA and RT to find optimal synergy with the two therapies in canine OS cells.

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