POSTER 85

Combination Therapy Targeting ALDH1a1 and AR May Reduce Metastatic Potential in Osteosarcoma Cells

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Background: For decades, patients diagnosed with metastatic osteosarcoma (OS) have had 5-year survival rates of approximately 10-30%. Sadly, as many as 25% of patients have metastases at the time of their primary OS diagnosis. Almost all (~90%) of these cases of metastasis spread to the lungs. Previously, aldehyde dehydrogenase (ALDH) activity and expression were found to be higher in tumor samples from patients who had or developed metastases. Androgen Receptor (AR) is a predicted upstream regulator of ALDH. Several studies have investigated the role sex steroids may play in the development of OS, but little is known about their role in OS metastases. Both ALDH and AR may be inhibited with current FDA-approved drugs Disulfiram and Enzalutamide, respectively.

Questions/Purposes: This study investigated the ability of Disulfiram and Enzalutamide to reduce human OS cell viability and migration. Human OS cell lines with varying metastatic potentials were treated with each inhibitor alone and in combination.

Methods: SaOS-2 (low metastatic potential) and LM2 (high metastatic potential) human OS cell lines were cultured for all *in vitro* experiments. First, to assess viability, cell lines were grown in 10cm/6-well plates and treated with either Disulfiram, Enzalutamide, or both (Dis+Enz) for 24 hours. After treatment, cells were counted. Next, to assess the cells' ability to migrate, SaOS-2, LM-2, and HT-1080 (positive control) cells were seeded onto transwell membrane inserts in 24-well plates. Cells were treated as described in the viability assay. After the 24-hour treatment incubation, migrated cells were stained with crystal violet and the amount of bound crystal violet was quantified using an acetic acid-dependent elution assay. Migration data was normalized, and all data was analyzed using two-way ANOVA with Tukey's multiple comparisons test.

Results: Both SaOS-2 and LM2 viability was reduced with treatment of Disulfiram and Enzalutamide, respectively. However, combining these drugs allowed for similar cell viability at lower concentrations of one or both drugs (Figure 1). SaOS-2 showed the most significant ($p<0.0001$) decrease in viability when treated with the combination of IC50 Enzalutamide and 2.5 µM Disulfiram (10% IC50 dose). Surprisingly, LM2 was more sensitive to combination therapy and yielded the most significant decrease in viability when treated with 6.25% IC50 Enzalutamide and 1.0 µM Disulfiram (8% IC50 dose). The migration of SaOS-2 was not significantly reduced with either single agent or combination treatment. However, LM2 displayed reduced migration with IC50 Enzalutamide treatment ($p = 0.0004$) and with its respective, diluted combination therapy ($p = 0.0007$) as described (Figure 2).

Conclusions: While patients with primary OS have a 5-year survival rate of approximately 65-70%, most patient deaths are due to metastatic disease. Treatment options for patients with metastatic OS are limited and have not improved for several decades. The role of ALDH and AR in OS development and metastases has not been fully explored even though it has been and area of interest for several years. Here, we described how Disulfiram and Enzalutamide can be used in combination at relatively low concentrations to significantly decrease the viability and migration of OS human cell lines with high metastatic potential (LM2). This study highlights the difference in behavior between primary and

metastatic OS cell lines. While our novel combination therapy showed significant promise for the treatment of OS metastases, the same treatments did not significantly lower viability or migration in SaOS-2. This study is limited to *in vitro* data and should be validated in more complex *ex vivo* or *in vivo* experiments. However, this study demonstrates that treatment with Enzalutamide and Disulfiram may provide better prognoses to patients with metastatic OS.

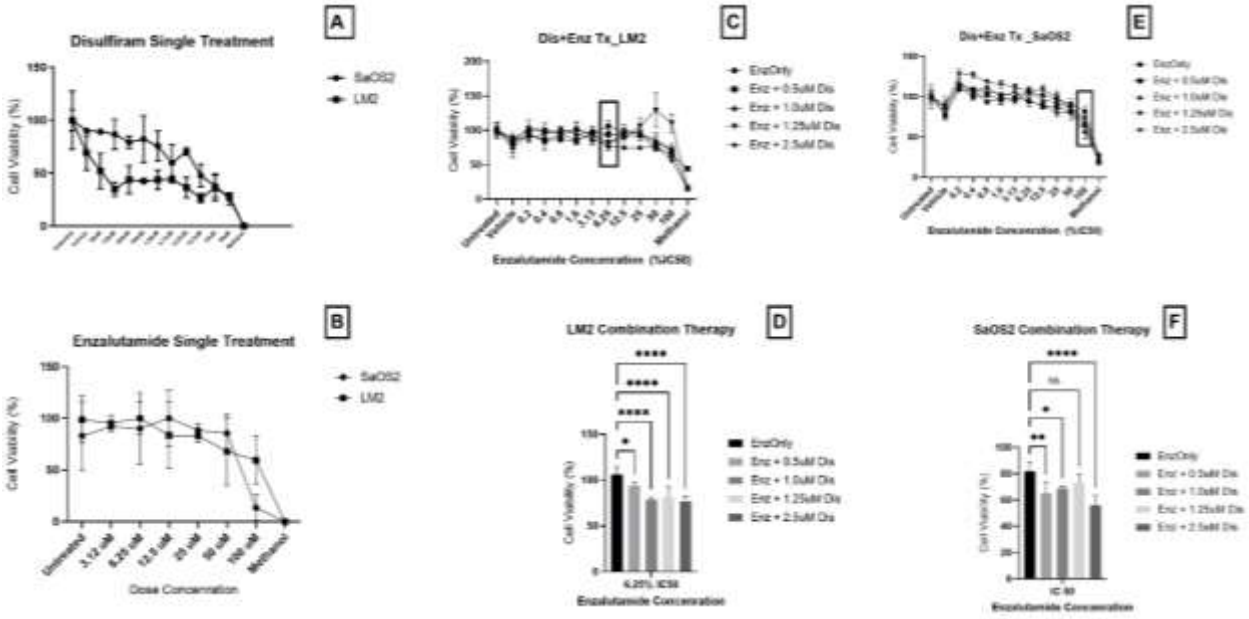

Figure 1: Evaluation of osteosarcoma cell viability due to single agent and combination treatment of Disulfiram and Enzalutamide. Cell viability of SaoS-2 and LM2 over a range of *in vitro* treatment concentrations of Disulfiram only (**A**), Enzalutamide only (**B**) and Enzalutamide with diluted Disulfiram (**C-F**). When compared to Enzalutamide only treatment, 6.25% IC50 Enzalutamide and 1.0 µM Disulfiram yielded the most significant decrease in LM2 cell viability (**C-D**) while IC50 Enzalutamide and 2.5 µM Disulfiram yielded the most significant decrease in SaOS-2 cell viability (**E & F**). Data was analyzed using a one-way ANOVA and Dunnett's multiple comparisons test with significance defined as p*<0.05, p**<0.01, p***<0.001, p****<0.0001 . Error bars are reported as SE.

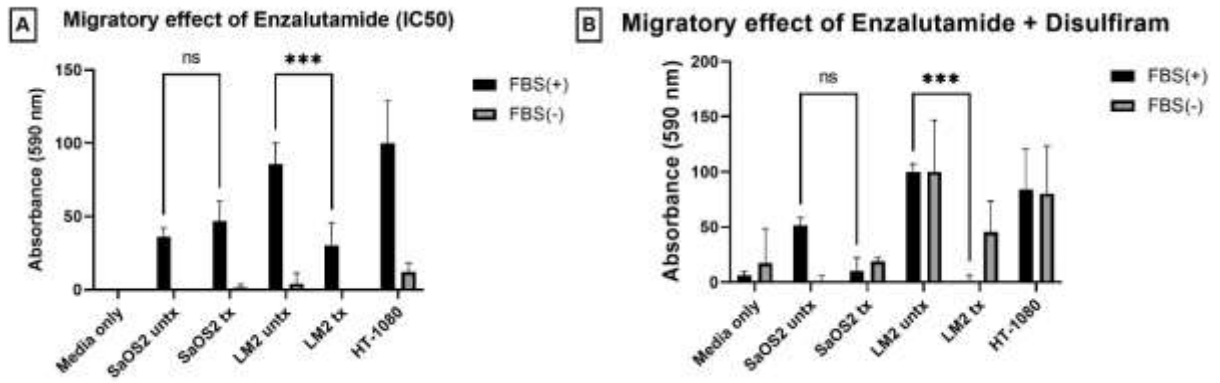

Figure 2: Effect of single agent and combination treatment of Disulfiram and Enzalutamide on osteosarcoma cell migration. SaOS-2, LM2 and HT-1080 (positive control) were plated onto transwell membranes and cultured for 24-hours with their respective treatment. Treating with Enzalutamide only decreased LM2 migration (p***=0.0004) but did not significantly affect SaOS-2 cell migration (**A**). Treating LM2 with a reduced concentration of both drugs yielded a similarly significant decrease in LM2 migration (p***=0.0007) but did not significantly affect SaOS-2 cell migration. Data was analyzed using a two-way ANOVA and Sidak's multiple comparisons test with significance defined as p*<0.05, p**<0.01, p***<0.001, p****<0.0001 . Error bars are reported as SE.