Using Drug Therapy to Target Androgen Receptor and Aldehyde Dehydrogenase 1A1 in Metastatic Osteosarcoma In Vitro

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Background

Previous studies using the ALDH-inhibitor disulfiram as a single agent to treat metastatic osteosarcoma (OS) in *in vivo* and *in vitro* murine experiments have been promising. However, combination therapy is the clinical standard to prevent treatment resistance and reduce effective doses. Previous pathway analyses predicted androgen receptor (AR) to directly activate ALDH1A1 in OS lung metastases. In the present study, we evaluated the combination of disulfiram and enzalutamide, an AR-inhibitor, in primary, SaOS-2, and metastatic, LM2, human OS cell lines.

Methods

Metastatic OS cell lines SaOS-2 and LM2 were treated with disulfiram and enzalutamide alone and in combination for 72 hours. Single treatment dose curves were created and SynergyFinder 3.0 was used to evaluate the combination matrix data. Promising combination treatment regimens were evaluated using growth curve analysis, scratch migration and clonogenic survival asssays.

Results

Less than 50% LM2 cell viability was observed at 30 µM disulfiram (**Figure 1a**) and 300 µM enzalutamide (**Figure1b**) when treated separately. SaOS-2 cell viability was reduced below 50% at the same doses, however, SaOS-2 was slightly more resistant to both single-agent treatments compared to LM2.

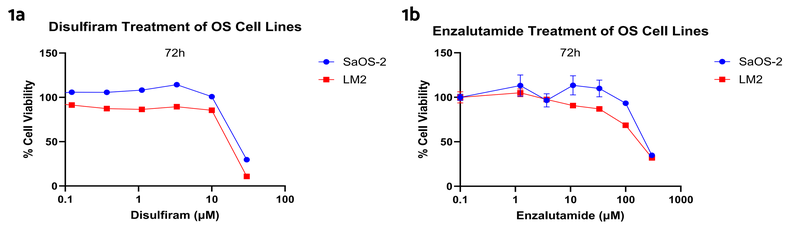

Figure 1. In vitro single-agent disulfiram and enzalutamide treatment reduces cell viability of human primary, SaOS-2, and metastatic, LM2, OS cells.

Disulfiram and enzalutamide had an average Bliss synergy score of -1.39 ± 4.2 (95% CI) for SaOS-2 (**Figure 2a**) and 1.19 ± 2.98 (95% CI) for LM2 **(Figure 2b).**

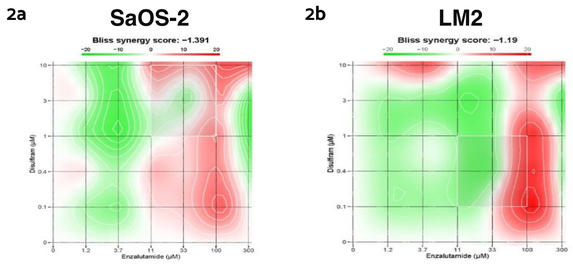

Figure 2. Synergy distribution of multi-dose Enzalutamide and Disulfiram response data for SaOS-2 and LM2.

The highest levels of synergy were observed at the combination of 10 µM disulfiram and 100 µM enzalutamide for SaOS-2 (3.96 Bliss score). The highest synergy (2.42 Bliss score) for LM2 was calculated at the combination dose of 0.1 µM disulfiram and 100 µM enzalutamide. Overall, both drugs appear to be additive without any areas displaying antagonistic scores.

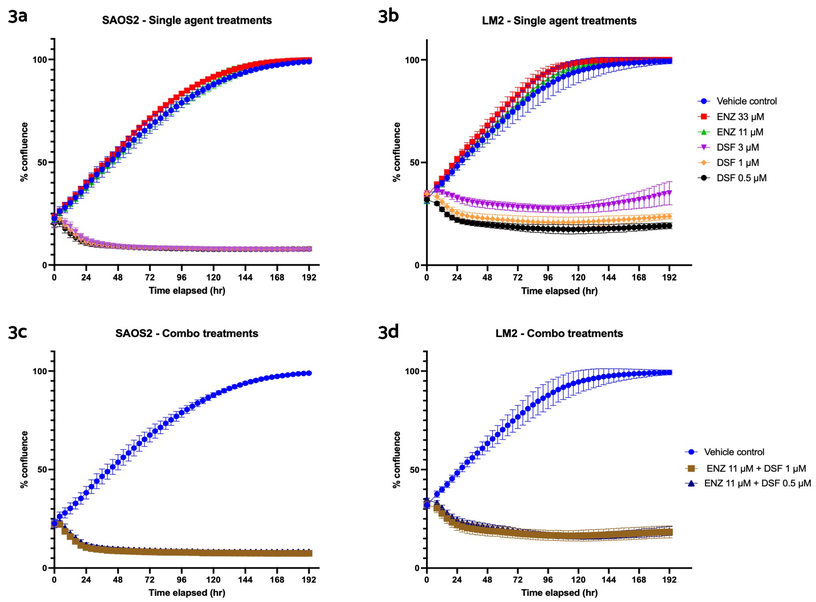

Figure 3. Growth analysis data, collected with the Incucyte SX1, for SaOS-2 and LM2 treated with single-agent and combination disulfiram and enzalutamide over a total duration of 166 hours.

When the OS cells were plated at lower densities prior to treatment, lower concentrations of disulfiram (3 µM) inhibited their growth, and the treatment effect was persistent for 166 hours. Both single enzalutamide doses (11 and 33 µM) were ineffective at inhibiting growth of SaOS-2 and LM2 (**Figure 3a & 3b**). Combining the lowest tested doses of enzalutamide (11 µM) and disulfiram (3 µM) inhibited growth of the OS cell lines, similar to the effects of single-agent disulfiram (**Figure 3c & 3d).**

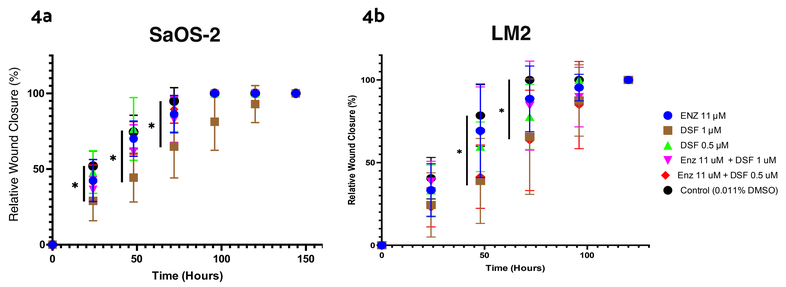

Figure 4. Relative wound closure of SaOS-2 and LM2 cell lines after treatment with enzalutamide and disulfiram separately and in combination.

Disulfiram 1 µM significantly reduced the relative wound closure of SaOS-2 at 24 (p=0.0182), 48 (p=0.0011), and 72 (p=0.0011) hours.

At 48 hours, there was also a significant difference in the relative wound closure of LM2 treated with disulfiram 1 µM (p=0.0184) as well as the combination treatment of enzalutamide 11 µM and disulfiram 0.5 µM (p=0.0309). Additionally, this same combination treatment showed significant differences in relative wound closure of LM2 at 72 hours (**Figure 4**).

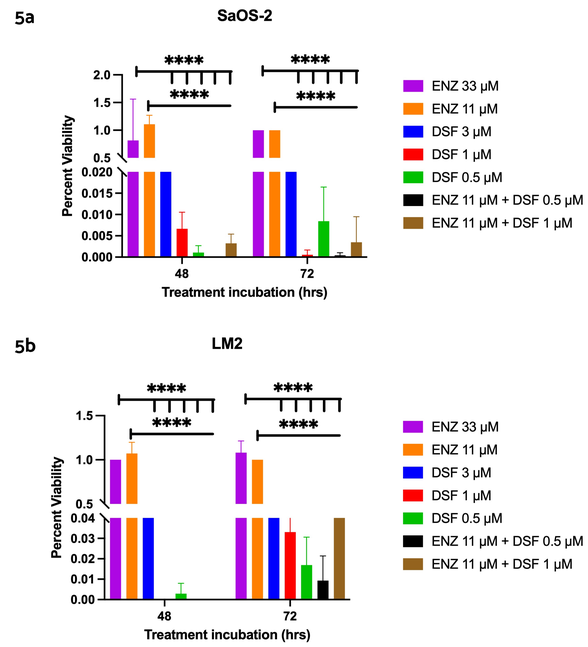

Figure 5. Survival fraction, normalized to untreated (vehicle) controls, of SaOS-2 and LM2 after treatment with single agent and combination doses of disulfiram and enzalutamide after 48 and 72 hours of treatment.

Enzalutamide alone did not significantly lower survival fractions of the OS cell lines. All disulfiram and combination treatments significantly decreased survival of both OS cell lines when compared to enzalutamide monotherapy (and untreated controls). However, combination treatments were not significantly different from each other or in comparison to disulfiram monotherapy (**Figure 5**).

Discussion

Disulfiram and enzalutamide display additive effects when used in combination to treat metastatic OS in vitro. However, the effective dose of enzalutamide remains at least one order of magnitude greater than reported IC_{50} in cancers responsive to AR inhibition. Nevertheless, disulfiram continues to display promising results as a treatment for OS. The development of combination schedules may further reduce effective doses and enhance additive effects.

Acknowledgements

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