<ol data-stringify-type="ordered-list" data-list-tree="true" p-rich_text_list__ordered="" p-rich_text_list--nested"="" data-indent="0" data-border="0"><li data-stringify-indent="0" data-stringify-border="0">Evolution-Informed Extinction Therapy: A Novel Treatment Paradigm for Osteosarcoma

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INTRODUCTION: Patients with high-grade osteosarcoma (OS) face high recurrence rates and significant treatment-related toxicity, despite multiagent chemotherapy (MAP: methotrexate, adriamycin (doxorubicin), cisplatin). Tumor recurrence is in part driven by chemoresistance, which can develop after a maximally tolerated dose (MTD) approach. In contrast, “extinction therapy” (ET) is an emerging curative strategy that leverages eco-evolutionary principles to drive heterogeneous tumors to extinction through multi-strike delivery of sub-MTD therapies targeting unique subpopulations. This study aims to develop an ET regimen that offers a rational path to improved outcomes in OS with reduced toxicity.

METHODS: The first strike of ET uses standard adriamycin and cisplatin to reduce overall tumor size and heterogeneity. Subsequent strikes use sequential second-line agents selected from established guidelines. Efficacy of ET versus standard therapy is assessed using cell growth and post-treatment cell viability. *In vitro* modelling uses a 143B OS cell line; *ex vivo* uses a pulmonary metastasis assay (PuMA). Chemoresistance was tested using repeat sequential dosing of adriamycin at a 99% inhibitory concentration (IC99) in 143B OS cells. Statistical analysis uses one-way ANOVA with Tukey’s post-hoc analysis for multiple test comparisons.

RESULTS: Repeated dosing of doxorubicin shows greater chemoresistance via decreased cell confluency (Figure 1A) and increased cell viability (Figure 1B) with each subsequent round. Compared to standard therapy, ET significantly inhibits OS cell growth (Figure 2A) and significantly reduces OS cell viability (Figure 2B) in *in vitro* testing. ET stabilizes OS metastatic tumor burden within the PuMA *ex vivo* model (Figure 3). In regards to overall toxicity, multiple *in vitro* drug concentrations in the ET regimen were significantly lower than reported in published pharmacokinetic studies.

DISCUSSION AND CONCLUSION: Current agents used in OS demonstrate chemoresistance with repeat use, leading to a need to explore new therapies. ET demonstrates efficacy in preclinical models and represents a promising approach to minimize toxicity and chemoresistance in OS. Future work aims to optimize the current ET regimen by incorporating novel agents and validate ET across multiple *in vitro, ex vivo, and in vivo* OS models.

Figure 1A.

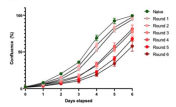

Figure 1B.

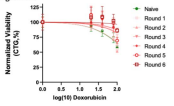

Figure 1. (A) Doxorubicin Resistance Assay, Cell Confluency. Naïve 143B OS cells were treated with six 24-hour pulses of 100 nM doxorubicin. Re-growth was allowed between pulses. Imaging was via IncuCyte. (B) **Doxorubicin Resistance Assay, Cell Viability**. CellTiter Glo (CTG) was measured after each round to determine cell viability by quantifying the number of metabolically active cells via ATP production.

Figure 2A.

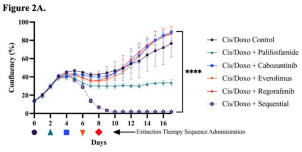

Figure 2B.

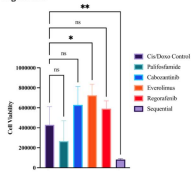

Figure 2. (A) Confluency line graph of extinction therapy approach in 143B naïve OS cell line. All lines were dosed with cisplatin (0.875 µM) and doxorubicin (20 nM) at t=0 days. Second line agents were dosed at t=2 days for a 24-hour pulse. Doses used were palifosfamide (4 µM), cabozantinib (200 nM), everolimus (100 nM), and regorafenib (1.5 µM). Imaging done via IncuCyte. One-way ANOVA completed with $p < 0.0001$. **(B) CTG plot of cell viability for 143B naïve extinction therapy approach**. One-way ANOVA completed between sequential and Cis/Doxo control with p value < 0.0039.

Figure 3.

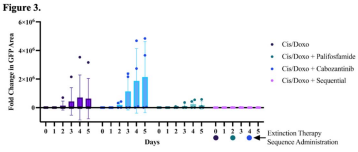

Figure 3. Confluency bar graph of PuMA. All lines were dosed with cisplatin (8.75 µM) and doxorubicin (200 nM) at t=0 days. Second line agents were dosed at t=2 days. Doses chosen were palifosfamide (40 µM) and cabozantinib (2 µM). Imaging done via IncuCyte.