Extinction Therapy Approach for Osteosarcoma Using Evolutionary Principles and Collateral Sensitivities

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INTRODUCTION: Patients with high-grade osteosarcoma (OS) face high recurrence rates and 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 approach (MTD). In contrast, "extinction therapy" (ET) leverages evolutionary principles to drive heterogeneous tumors to extinction through multi-strike delivery of sub-MTD therapies. This study aims to develop an ET regimen informed by collateral sensitivities to improve OS outcomes.

METHODS: ET begins with standard doxorubicin and cisplatin to reduce tumor size, followed by sequential second-line agents from established guidelines. Efficacy of ET versus standard therapy is assessed using cell growth via IncuCyte and post-treatment CellTiter-Glo (CTG) in both 143B and 17-3X OS cell lines. A high throughput screen of 147 FDA-approved oncology agents (72 hours, 100nM) is completed on three 143B cell lines: naïve, single-dosed cisplatin (0.875µM) and doxorubicin (20nM), and chronic cisplatin and doxorubicin. Primary outcome is CTG. Statistical analysis uses one-way ANOVA.

RESULTS: Sequential doxorubicin pulses lead to decreased confluency with each subsequent pulse and increased cell viability with each pulse. Compared to standard therapy, ET significantly inhibits OS cell growth and cell viability in 143B cells, however, allows for significantly increased OS cell growth compared to standard therapy in 17-3X cells. The high-throughput screen shows proteasome inhibitors, protein synthesis inhibitors, microtubule-targeting agents, and HDAC inhibitors have the strongest efficacy amongst the drug classes. Comparison of OS cell inhibition between single-pulse and chronic treatment reveals collateral sensitivities, with more drugs showing enhanced killing in both settings compared to naïve cells.

DISCUSSION: 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 and validate the ET regimen by incorporating novel agents from high throughput screening that encourage collateral sensitivities and limit cross-resistance.

SIGNIFICANCE/CLINICAL RELEVANCE: Multiagent chemotherapy is a cornerstone of OS treatment but carries substantial toxicity, with a 59% 3-year event-free survival of high-grade OS. Exploring extinction therapy is paramount to advancing efficacy of OS treatment while reducing morbidity.

IMAGES AND TABLES:

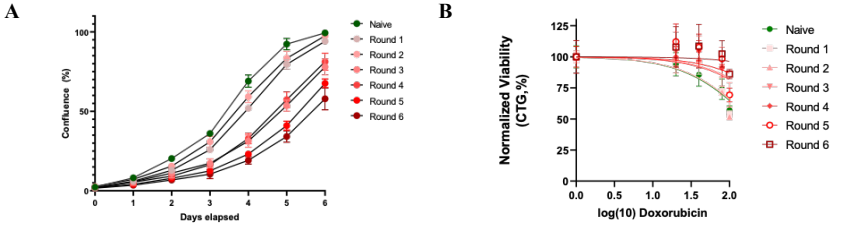

Figure 1. (A) Doxorubicin Resistance Assay, Cell Confluency. Naïve 143B OS cells were treated with six 24-hour pulses of 100nM doxorubicin. **(B) Doxorubicin Resistance Assay, Cell Viability**. CTG was measured after each round to determine cell viability.

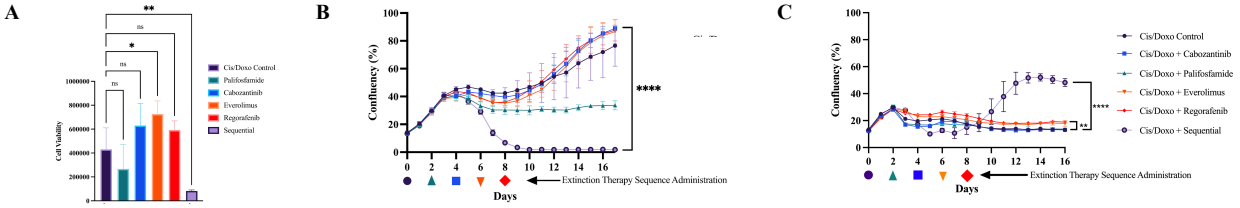

Figure 2. (A) CTG plot of cell viability for 143B naïve extinction therapy approach. "**" represents p value < 0.0039. **(B) Cell confluency of extinction therapy in 143B OS cell line**. All lines dosed with cisplatin (0.875µM) and doxorubicin (20nM) at t=0 days. Second line agents (palifosfamide 4µM, cabozantinib 200nM, everolimus 100nM, and regorafenib 1.5µM) were dosed at t=2 days for 24 hours. "****" represents p < 0.0001. **(C) Cell confluency of extinction therapy in 17-3X OS cell line**. All lines dosed with cisplatin (2.5µM) and doxorubicin (30nM) at t=0 days. Second line agents (palifosfamide 4µM, cabozantinib 300nM, everolimus 250nM, and regorafenib 2.5µM) were dosed at t=2 days for 24 hours.

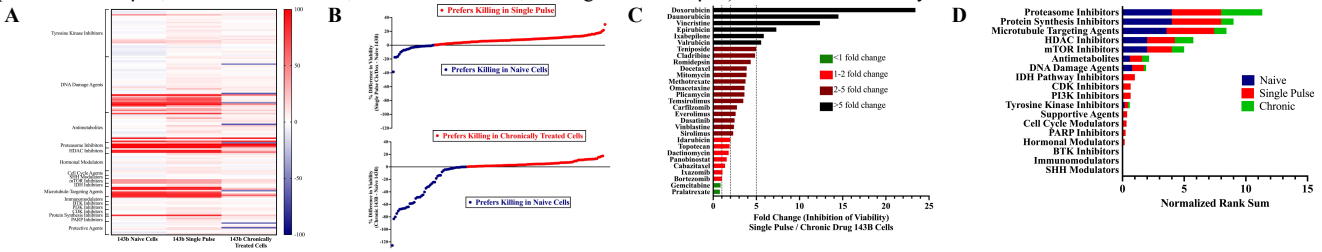

Figure 3. High-throughput screen (A) Overall Heat Map. All chemical screen results shown across 3 cell lines with drugs grouped by drug class. Higher values (red) represent lower cell viability and lower values (blue) represent higher cell viability. **(B.1) % Difference in Viability Between Naive and Single Pulsed (B.2) % Difference in Viability Between Naive and Chronic (C) Fold Change of Killing Between Single Pulse and Chronic.** Agents with % killing >25% in chronic cell line were included. Fold change was calculated between chronic and single pulse. **(D) Sum Rank Graph Grouped by Drug Class.** Individual drugs scored based on % Inhibition of Viability (<10% = 0, 10-25% = 1, 25-50% = 2, 50-75% = 3, >75% = 4). Scores were summed per drug class and normalized by group size.