Evolution-Informed Extinction Therapy: A Novel Treatment Paradigm for Osteosarcoma

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Significance & Hypothesis/Techniques: Osteosarcoma (OS) is an aggressive pediatric bone cancer with high mortality and limited therapeutic advances over the past several decades. Standard treatment uses six repeated cycles of MAP therapy (methotrexate, doxorubicin, cisplatin), which can select for chemo-resistant cell populations, underscoring the need for novel therapeutic strategies. Evolution-informed cancer studies leverage eco-evolutionary principles such as competition and cellular fitness to develop innovative therapies. Extinction therapy (ET) is an emerging curative strategy that aims to drive a tumor to extinction using a multi-strike approach: first reducing tumor size and heterogeneity to a population bottleneck, then exploiting vulnerabilities of surviving cells. We hypothesized that an ET regimen for OS would be both more efficacious and less morbid than standard repeated dosing approaches.

Innovation: This study introduces ET as a novel framework for OS treatment, representing a conceptual shift from conventional repeated dosing toward an evolution-informed, adaptive strategy. Clinically, it leverages high-throughput drug screening to identify collateral sensitivities at the population bottleneck, enabling rational, data-driven selection of second-strike agents tailored to post-bottleneck OS cell populations.

Approach: OS cell line population dynamics were characterized after cisplatin and doxorubicin treatment using dose-response curves and lineage tracing. Second-line agents for the ET regimen were identified via two methods: querying the National Comprehensive Cancer Network (NCCN) for approved second-line agents, and conducting a high-throughput drug screen (HTDS) of 147 FDA-approved oncology agents to identify collateral sensitivities between chemo-naive OS cells and cisplatin and doxorubicin-treated OS cells. ET regimens were then tested across multiple *in vitro* models, including 2D models (143B and 17-3X cell lines) and 3D organoid models (151-2 and 162-4). Efficacy was assessed by growth metrics and one-way ANOVA.

Results: Dose-response curves and lineage tracing revealed that OS cell lines variably respond to an NCCN-guided ET approach based on the size of the achievable bottleneck effect after cisplatin and doxorubicin (**Figure 1**). HTDS identified multiple agents with collateral sensitivities in bottlenecked OS cells (**Figure 2**). A HTDS-guided ET regimen significantly outperforms repetitive single-agent dosing in 143B cells and both organoid lines (**Figure 3**).

Discussion and Impact: ET aims to drive a heterogeneous tumor population to extinction after an initial bottleneck event. These *in vitro* findings demonstrate that ET is a more efficacious, and potentially less morbid, approach than traditional repeated dosing for OS. Future work will validate ET approaches in *in vivo* models and employ computer simulation to optimize treatment sequencing and timing.

References:

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3. Somarelli, J. A. (2021). The hallmarks of Cancer as Ecologically Driven Phenotypes. Frontiers in Ecology and Evolution, 9. https://doi.org/10.3389/fevo.2021.661583
4. Reed, D. R., Metts, J., et al. (2020). An evolutionary framework for treating pediatric sarcomas. Cancer, 126(11), 2577–2587. https://doi.org/10.1002/cncr.32777

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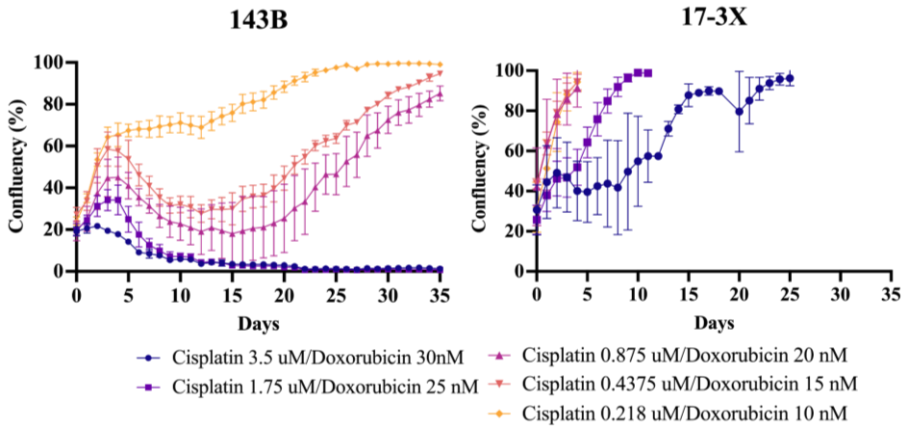

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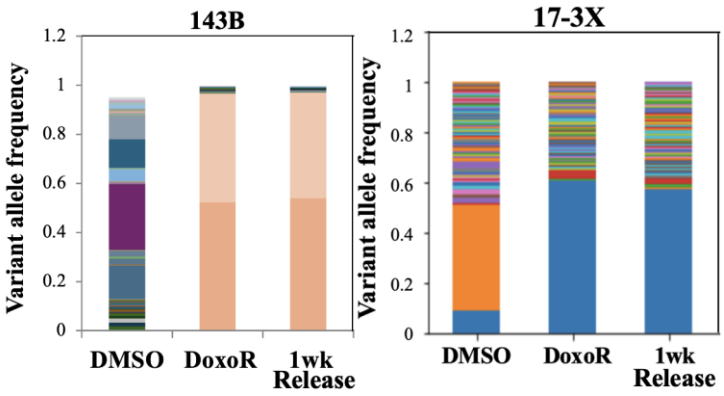

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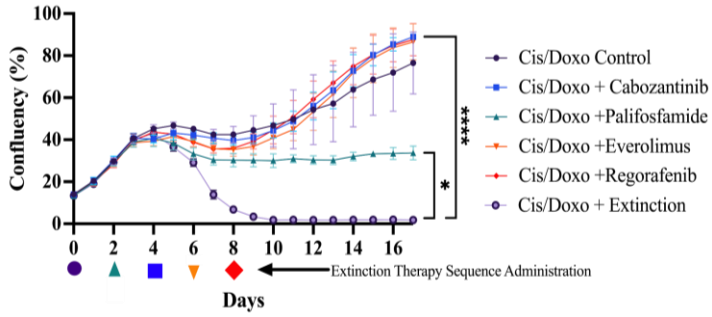

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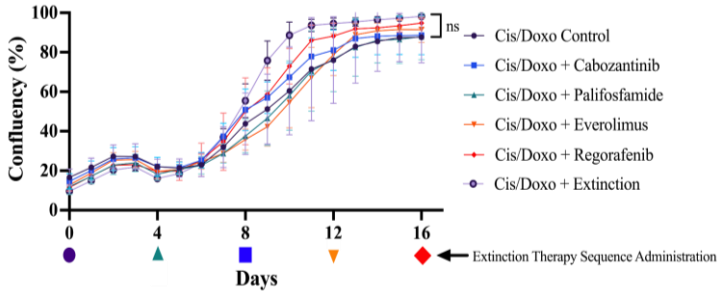

Figure 1. Differences in bottleneck effect between 143B and 17-3X OS cell lines affect ET potential. (A) Cisplatin and doxorubicin dose response curves. 143B and 17-3X OS cell lines dosed with variable dosages of cisplatin and doxorubicin for a 24-hour pulse. **(B) Variant allele frequency by cell line**. Barcoding and lineage tracing system to monitor population dynamics in 143B and 17-3X OS cell lines. Treatment groups were control (DMSO), doxorubicin-resistant (DoxoR), and 1 week release after doxorubicin treatment. **(C-D) Confluency of 143B cells (C) and 17-3X OS (D) cells treated with NCCN-guided ET regimen.** All lines were dosed with cisplatin (143B: 0.875 µM/17-3X: 2.5 µM) and doxorubicin (143B: 20nM/17-3X: 30 nM) at t=0 days. Second line agents were dosed once at t=2 days for a 24-hour pulse. Extinction therapy agents were dosed for a 24-hour pulse with a 24-hour break between agents, as shown by the legend below the x-axis. Doses used were palifosfamide (143B: 4 µM/17-3X: 4 µM), cabozantinib (143B: 200nM/17-3X: 300 nM), everolimus (143B: 100nM/17-3X: 250 nM), and regorafenib (143B: 1.5 µM/17-3X: 2.5 µM). Outcome was cell confluency using IncuCyte. One-way ANOVA was run with * representing $p < 0.05$, **** representing $p < 0.0001$, and "ns" representing non-significant.

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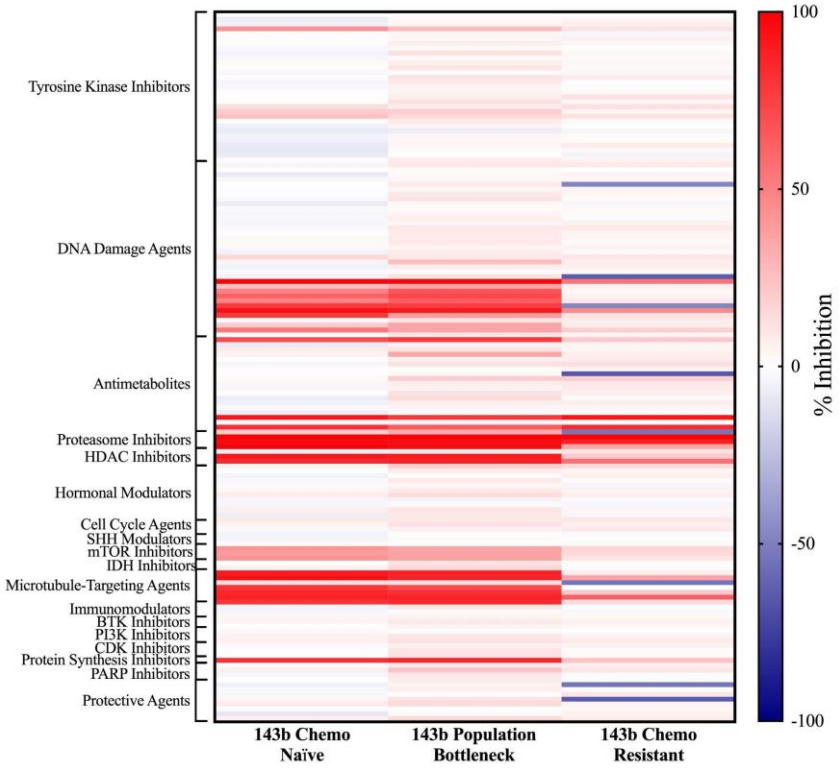

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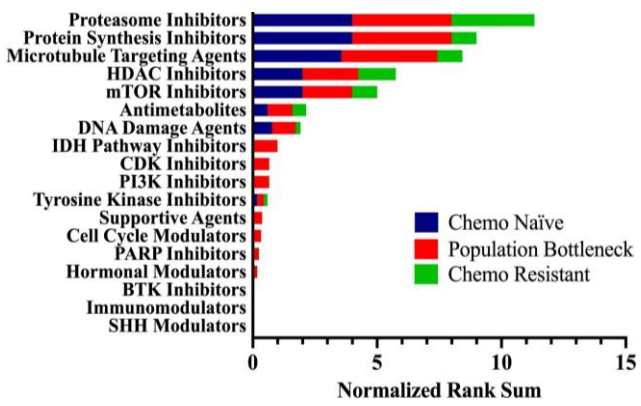

Figure 2. High-throughput drug screen identifies collateral sensitivities at the population bottleneck. A HTDS was performed comparing responses between 143B cells treated with a single pulse of cisplatin 0.875 µM/doxorubicin 20nM, chemo-naïve cells, and cells resistant to cisplatin and doxorubicin. All drugs dosed at 100nM for 72 hours. Outcome was CellTiter-Glo. **(A) Overall heat map of % inhibition by drug**. Drug screen results grouped by drug class and cell line. Higher values (red) represent higher drug inhibition and lower values (blue) represent lower drug inhibition. **(B) Sum rank scoring by drug class.** Drugs were individually 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 based on number of drugs per group.

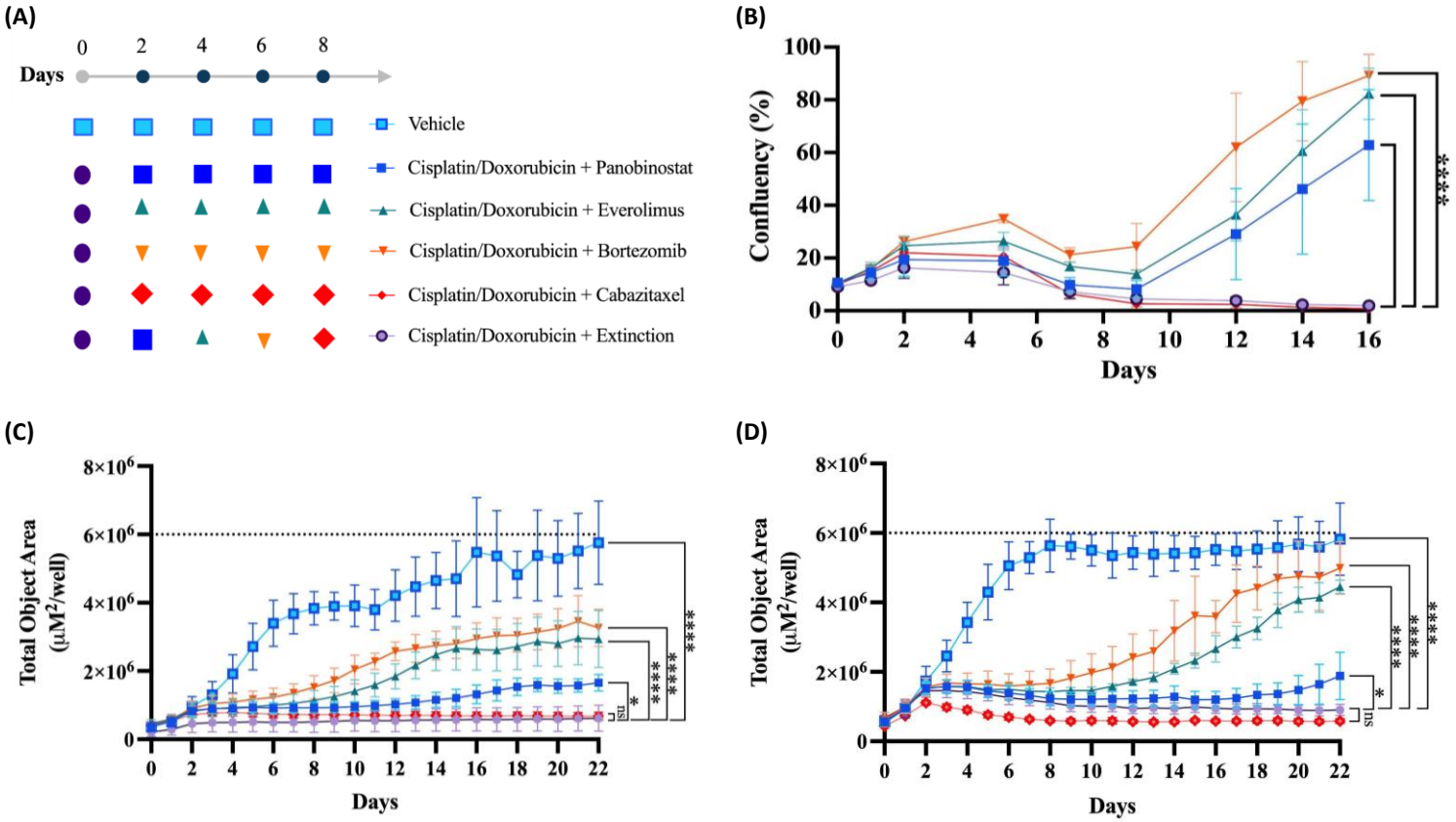

Figure 3. HTDS-guided ET regimen significantly outperforms repetitive dosing of nearly all single agents in cell and organoid models. (A) Dosing schedule for *in vitro* experimentation. Shapes represent the drug given on each day for each of the experimental arms. **(B) Confluency of 143B OS cells treated with HTDS-guided ET regimen.** All lines were dosed with 24 hours of cisplatin (0.875 µM) and doxorubicin (20 nM) at t=0 days. Second line agents were dosed for 24 hours per pulse starting on t=2 days according to Figure 3A. Doses used were panobinostat (2.5 nM), everolimus (100 nM), bortezomib (0.25 nM), and cabazitaxel (5 nM). Outcome was cell confluency using IncuCyte. One-way ANOVA run with **** representing $p < 0.0001$. **(C-D) Confluency of 151-2 (C) and 162-4 (D) organoid treated with HTDS-guided ET regimen.** All lines were dosed with 24 hours of cisplatin (4.375 µM) and doxorubicin (100 nM) at t=0 days. Second line agents were dosed for 24 hours per pulse starting on t=2 days according to Figure 3A. Doses used were panobinostat (50 nM), everolimus (500 nM), bortezomib (2 nM), and cabazitaxel (12.5 nM). Outcome was cell confluency using IncuCyte. One-way ANOVA was run with * representing $p < 0.05$, **** representing $p < 0.0001$, and "ns" representing non-significant.