Evolving Toward Precision Oncology in Osteosarcoma

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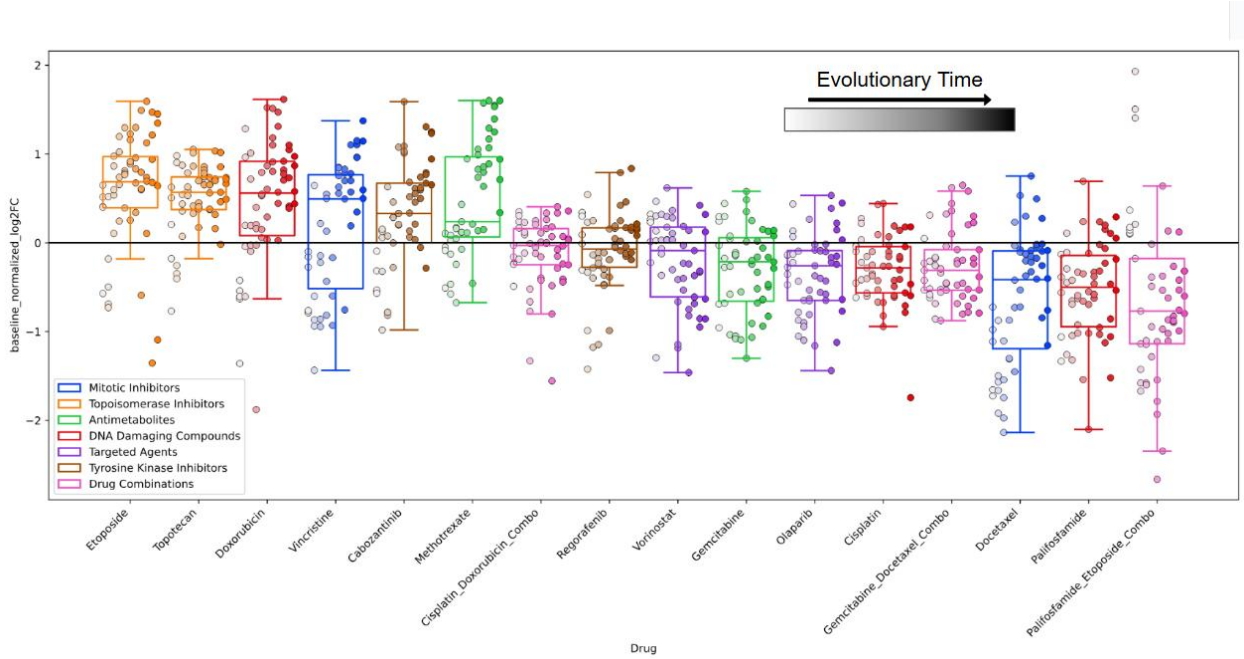
Significance and Hypothesis: We aimed to model the evolution of chemoresistance to methotrexate, doxorubicin, and cisplatin (MAP) in vitro and to characterize collateral drug sensitivity and resistance patterns over time. These data can be used to inform second-line treatment strategies in chemo resistant primary or recurrent disease.

Innovation: Here we present a novel clinically relevant *in vitro* model of evolved resistance to MAP chemotherapy to guide evolutionary approaches to personalized second line chemotherapy.

Approach: Five evolutionary replicates of MG63.3 osteosarcoma cells were treated with six complete cycles of pulsed, clinically relevant doses of cisplatin (IC10/30) and doxorubicin (IC10/30), alternating with methotrexate (IC50). Dose-response curves were obtained after each chemotherapy cycle to assess resistance to MAP, as well as a drug screen for collateral responses to 16 drugs and combinations. RNA sequencing and transcription factor activity inference were performed across treatment timepoints to temporally explore transcriptional correlates of drug response. Latent factor analysis was used to generate a predictive model correlating transcriptome to drug response.

Results: Cells developed resistance to doxorubicin (1.49–2.59 fold) and methotrexate (0.92–2.87 fold) but remained sensitive to cisplatin. Collateral resistance emerged to etoposide, topotecan, and cabozantinib, while sensitivity increased to palifosfamide, vorinostat, and gemcitabine/docetaxel. Collateral responses varied temporally, with some increasing linearly with evolution of MAP resistance, and some transient or stochastic (Fig. 1). Transcriptional profiling revealed increased activity of E2F and ERG and context-dependent roles for interferon signaling. Latent factor modeling identified shared resistance programs enriched for MYC targets, interferon signaling, and apoptosis. Resistance phenotypes persisted after drug withdrawal and cryopreservation.

Discussion and Impact: Our model reveals temporally dynamic, heterogeneous collateral responses during the development of chemoresistance to MAP in osteosarcoma. While cross-resistance to several agents emerged, collateral sensitivity to palifosfamide, vorinostat, and gemcitabine/docetaxel supports a second line use for these agents. Our model can form the basis of transcriptionally guided second line therapy to preempt or exploit resistance evolution. Validation in patient derived cell lines and samples is in process.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Mitotic Inhibitors
Topoisomerase Inhibitors
Antimetabolites
DNA Damaging Compounds
Targeted Agents
Tyrosine Kinase Inhibitors
Drug Combinations
Etoposide
Topotecan
Doxorubicin
Vincristine
Cabozantinib
Methotrexate
Cisplatin_Doxorubicin_Combo
Regorafenib
Vorinostat
Gemcitabine
Olaparib
Cisplatin
Gemcitabine_Docetaxel_Combo
Docetaxel
Palifosfamide
Palifosfamide_Etoposide_Combo
Drug