Abstract #11417 - Opportunities for Drug Repurposing in Osteosarcoma: A Screen of FDA-Approved Oncology Drugs in a Micrometastatic Model of Disease

Authors: Christopher Collier, MD; Patrick Getty, MD; Edward Greenfield, PhD

Institution: Case Western Reserve University/University Hospitals Case Medical Center, Cleveland, OH

Background: Osteosarcoma, the most common bone sarcoma, affects approximately 420 children and adolescents annually in the United States. The five-year overall survival rate is 60-70%, but has remained largely unchanged for over thirty years. Distant relapse is thought to be associated with subclinical pulmonary micrometastases despite wide local excision. Novel therapeutics are therefore needed to target the progression of micrometastases and improve patient outcomes. However, as a relatively rare cancer, osteosarcoma challenges traditional drug-discovery because of prohibitive drug-to-market costs and scarcity of clinical trials. Drug repurposing, or the evaluation of established drugs for new indications, is attractive in this setting since safety profiles are known, drugs are already in production, and thus they can be rapidly translated into clinical trials with reduced costs and time. Remarkably, over 40% of FDA-approved anticancer drugs have not been evaluated in osteosarcoma.

Questions/purposes: This study employed three-dimensional osteosarcoma spheroids (sarcospheres) for the following purposes: (1) to identify novel therapeutics for drug repurposing in osteosarcoma by screening an FDA-approved anticancer drug library in a micrometastatic model and (2) to confirm and further characterize promising drugs identified by the screen prior to *in vivo* testing.

Methods: Sarcospheres were generated from highly metastatic human cell lines (143B, MG63.3, and LM7) by a centrifugation-based method, matured for 24 hours, and then incubated with or without drug from Day 0-2. Resazurin reduction was measured on Day 0 and Day 2 to estimate growth. The FDA-approved anticancer drug library, Approved Oncology Drug Set V, was obtained from the NCI Developmental Therapeutics Program. These 114 drugs were screened in all cell lines at 10μM with and without conventional MAP therapy (methotrexate, doxorubicin, and cisplatin). Promising drugs were further characterized if they met the following criteria: large effect on multiple cell lines, additive effect with MAP therapy, unique mechanism of action, and/or limited existing data in osteosarcoma. The 13 drugs selected were exposed to five 10-fold serial dilutions (0.001-10μM) to evaluate potency in each cell line.

Results: Response to treatment was cell line-dependent and varied considerably across the 114 drugs screened (Figure 1). 20% (23/114) of the drugs resulted in negative growth averaged for all cell lines and conditions, which is consistent with regression of disease in this model. HDAC inhibitors (2/2), protease inhibitors (2/2), and topoisomerase inhibitors (8/10) were highly represented in the group resulting in negative growth. MAP therapy to screen for synergy produced large improvements in treatment response for three tyrosine kinase inhibitors: ponatinib, vandetinib, and afatinib. Finally, unbiased hierarchical clustering identified similarities among the three cell lines studied. 143B and MG63.3 cell lines were found to be more similar to each other than the LM7 cell line based on their drug response profiles.

Promising drugs selected for further characterization demonstrated a wide range in potency (Figure 2). Regression modeling was used to estimate the GIC_{50} for each drug and cell line (Figure 2A, B, C; where the GIC_{50} is defined as the concentration at which 50% of growth from Day 0-2 is inhibited). The 13 drugs had over a 1,000-fold range in GIC_{50}. Bortezomib and carfilizomib, both protease inhibitors, were two of the most potent drugs in 143B and MG63.3 cell lines, reaching clinically desired nanomolar potency, but showed more modest potency in the LM7 cell line. Romidepsin (HDAC inhibitor), plicamycin (RNA synthesis inhibitor), and omacetaxine (protein synthesis inhibitor) had GIC_{50} values that were consistently in the nanomolar range for all cell lines.

Conclusions: This study identified several promising agents to target the progression of micrometastatic disease in osteosarcoma. Based on a recent review of the literature, 6/13 identified drugs have not been studied in osteosarcoma (plicamycin, omacetaxine, tenoposide, ponatinib, afatinib, and vandetinib), while 3/13 drugs (romidepsin, carfilizomib, and mitoxantrone) have only limited *in vitro* data supporting efficacy in osteosarcoma. Ongoing studies will further characterize the effects of these 13 drugs on normal cells and sarcospheres with and without the addition of MAP therapy. The most promising drugs will then be characterized *in vivo* and if effective, could be repurposed for clinical trials without the associated costs of traditional drug discovery.

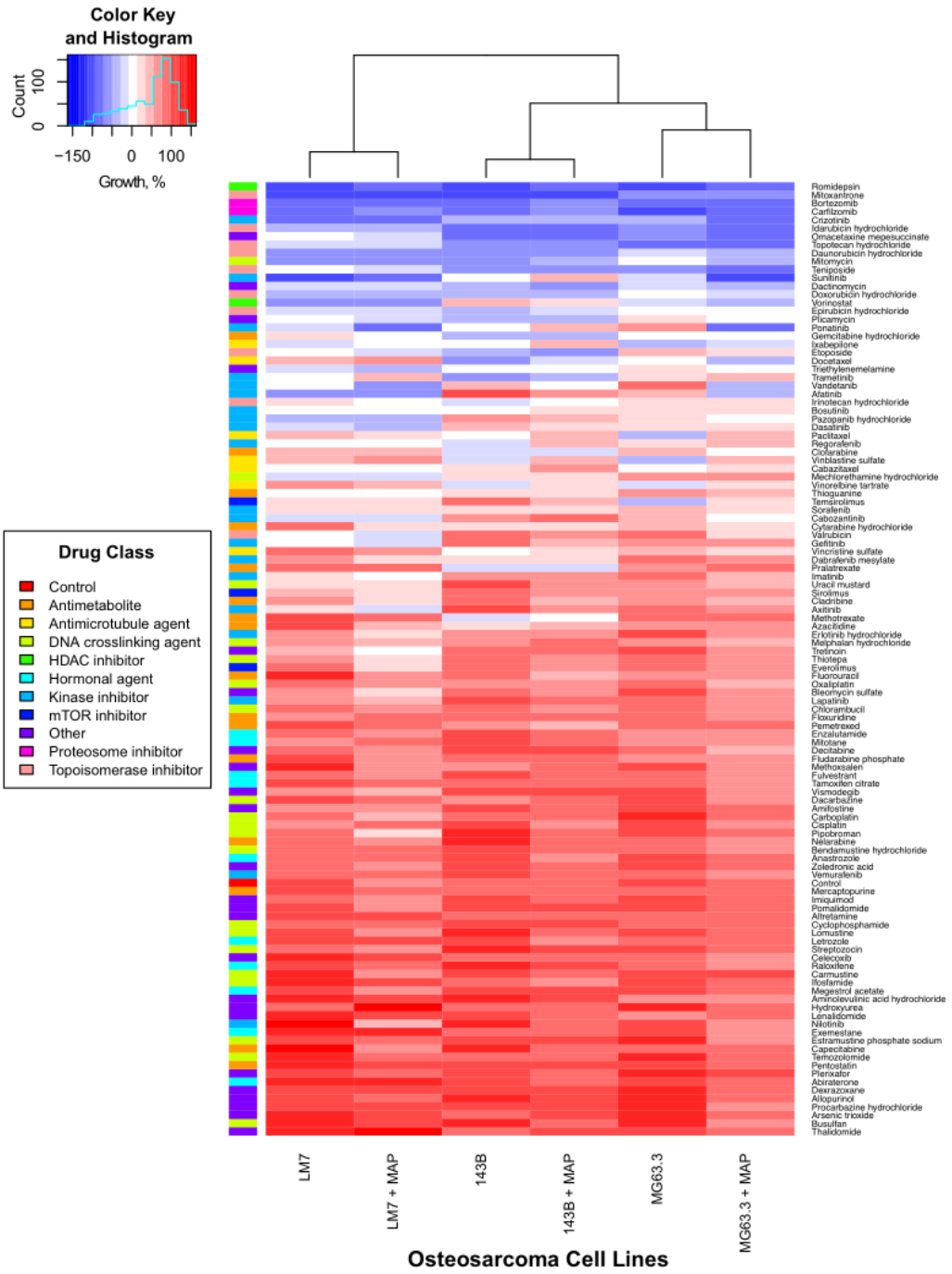

Fig. 1 Heatmap and dendrogram are shown depicting drug response for indicated cell lines and conditions. Color key and histogram demonstrate numerical values and frequencies for growth, calculated as change in sarcosphere growth from Day 0-2 relative to controls. Negative values (blue) represent the most effective drugs, while positive values (red) represent least effective drugs. Drugs were sorted based on mean response across all cell lines and conditions (+/- MAP). Dendrogram at the top of the heatmap illustrates the arrangement of the cell lines based on comparison of responses via unsupervised hierarchical clustering. The height of the tree branch connecting end nodes (cell lines) in this plot is proportional to the value of the intergroup dissimilarity. The color bar on the left of the heatmap shows the classification for each drug.

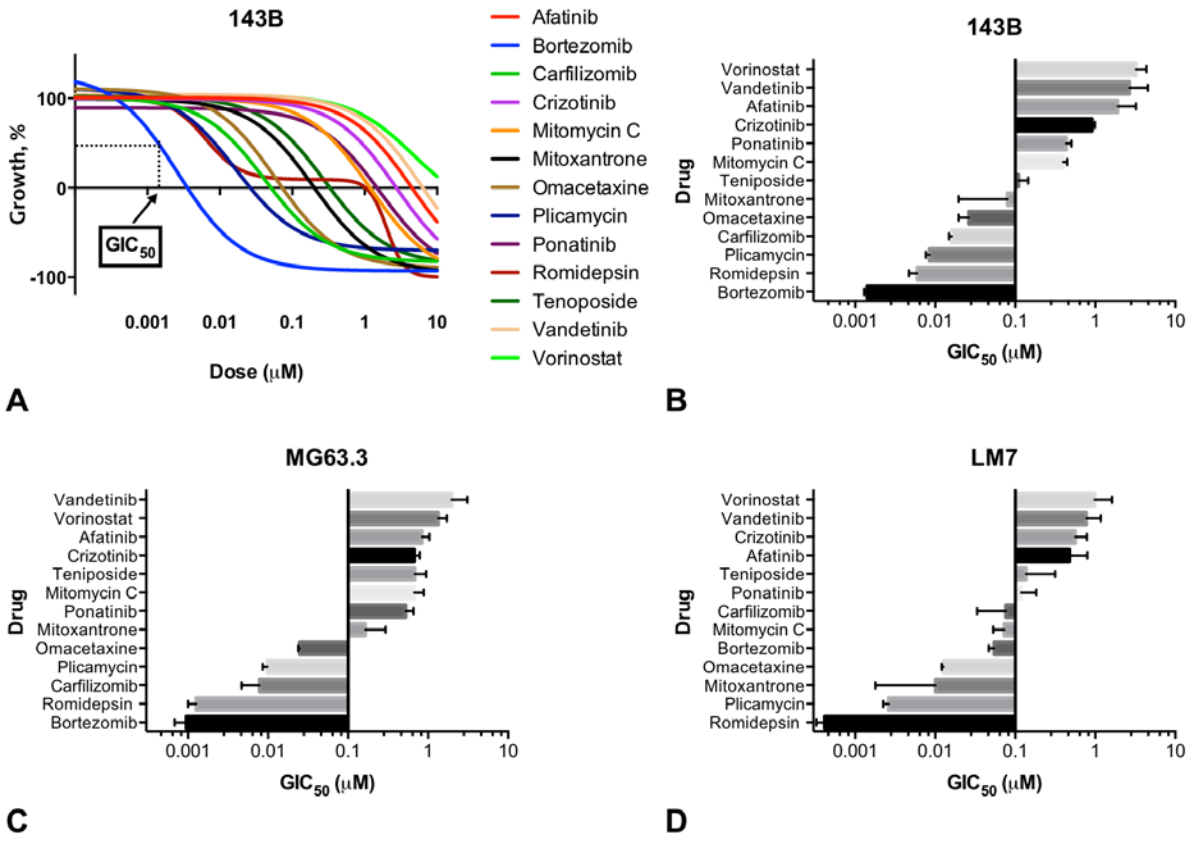

Fig. 2A-D Concentration response curves for the indicated drugs are shown in (**A**) for 143B cells (three parameter logistic regression analysis performed for all drugs, except romidepsin where a biphasic regression provided the best fit, dashed lines and arrow indicate the GIC_{50}). Interpolated GIC_{50} values are presented to illustrate relative potency in 143B (**B**), MG63.3 (**C**), and LM7 (**D**) cell lines (mean ± SD of n=3 independent experiments).