Abstract #11243 – Three-Dimensional Drug-Screening Platform for Progression of Osteosarcoma Micrometastases Demonstrates Heterogeneous Response to MAP Chemotherapy

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Background: Osteosarcoma accounts for the majority of deaths from bone tumors, which are the 3rd most common cause of cancer-related death in adolescents and young adults. Lethal metastasis occurs primarily in the lungs, where ~80% of patients harbor subclinical micrometastases at diagnosis. Treatment includes conventional MAP chemotherapy (methotrexate, doxorubicin, and cisplatin); however, this regimen and thus overall survival has remained largely unchanged for thirty years. It is therefore necessary to identify novel therapeutics targeting the metastatic progression of osteosarcoma to improve patient outcomes.

Questions/purposes: This study employed three-dimensional osteosarcoma spheroids (sarcospheres) for the following purposes: (1) to develop and validate a drug-screening platform targeting the progression of osteosarcoma micrometastases and (2) to determine the impact of MAP chemotherapy across multiple cell lines using this model.

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. Volume and resazurin reduction were measured. Fluorescence images were obtained after treatment with MAP using probes for live, dead, and apoptotic cells (caspases 3/7). Results are presented as the mean ± SD of n=3-4 independent experiments.

Results: Therapeutically relevant sarcosphere diameters of 300-500µm were achieved for 143B, MG63.3, and LM7 cells lines at 2,500, 1,000, and 8,000 cells seeded, respectively at Day 0 (Figure 1A). Growth rates from Day 0-2 were also cell line-dependent and greater for small sarcospheres (Figure 1B). Incubation with resazurin for six hours provided the highest signal-to-noise ratio within the linear range of the assay. In a prior study, several epithelial-based spheroids required EDTA-mediated permeabilization to accurately measure resazurin reduction. However, EDTA did not show a significant effect in our mesenchymal model. Resazurin reduction and volume were related to the number of cells seeded and correlated with each other (Spearman correlation p-values < 0.0001 for all cell lines, Figure 1C, D). Uniformity analysis of untreated sarcospheres showed little variation (Figure 1E, F, G) and mean Z'-factors for both volume and resazurin reduction were > 0.5 for all cell lines, a cut-off widely considered to indicate an "excellent" assay.

Response to MAP therapy was cell line-dependent (Figure 2A-I). MG63.3 and LM7 sarcospheres exhibited greater than 2,000-fold resistance to methotrexate (GIC_{50} = 88µM ± 36 and 174µM ± 16, respectively), compared to the 143B cell line (GIC_{50} = 0.04µM ± .01). GIC_{50} represents the drug concentration at which 50% of growth from Day 0-2 is inhibited. Volume and resazurin reduction produced similar results at low drug concentrations. However, at high drug concentrations, volume was limited by the persistence of non-viable cells and did not significantly decrease below the Day 0 threshold. Resazurin reduction continued to decline at high drug concentrations. Finally, fluorescent imaging of live, dead, and apoptotic cell populations within 143B and LM7 sarcospheres showed a cell line-dependent response. Doxorubicin induced the greatest apoptotic and dead cell staining in 143B sarcospheres, while LM7 sarcospheres demonstrated more apoptotic and dead cell staining after cisplatin therapy. Methotrexate induced the least apoptotic and dead cell staining in both cell lines.

Conclusions: This study developed and validated a novel drug-screening platform for progression of osteosarcoma micrometastases. It also highlights heterogeneity within osteosarcoma cell lines by demonstrating differences in growth rates, susceptibility to MAP therapy, and mechanisms of cell death. These findings reflect the known heterogeneity in osteosarcoma patients and underscore the importance of evaluating multiple tumors in osteosarcoma research. Although an *in vitro* model, the described approach is an ideal starting point for drug screening in osteosarcoma because it is tailored to evaluate micrometastatic disease. We recently applied this approach to an FDA-approved anticancer drug library. After further *in vitro* evaluation, promising therapeutics will be explored *in vivo* and if effective, could be repurposed for clinical trials without the associated costs of traditional drug discovery.

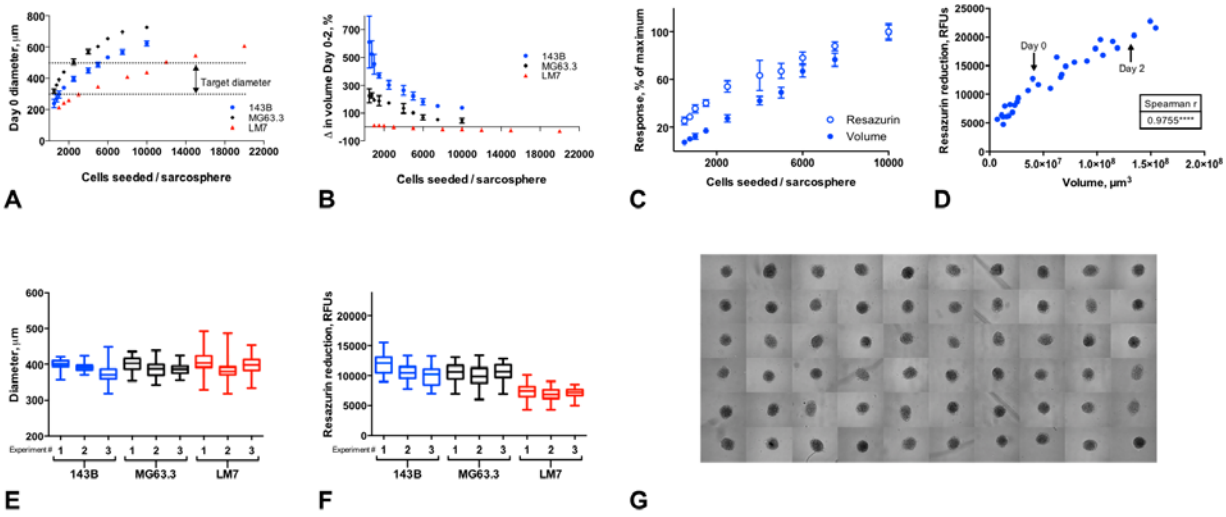

Fig. 1A-G The relationship between cell number per sarcosphere and both Day 0 diameter (**A**) and volumetric growth Day 0-2 (**B**) is dependent on cell line (dashed lines indicate the target diameter for future studies, mean ± SD). Volume and resazurin reduction by 143B cells on Day 0 correlate with cell number per sarcosphere (**C**) and with each other (**D**; arrows represent typical values on Day 0 and Day 2 for all subsequent experiments, mean ± SD, **** = $p < 0.0001$). Box-and-whisker plots depict uniformity of sarcosphere diameter (**E**) and resazurin reduction (**F**) on Day 0 across multiple experiments for each cell line (n = 60 sarcospheres/experiment). Representative light microscopy of a Day 0 MG63.3 plate is shown (**G**).

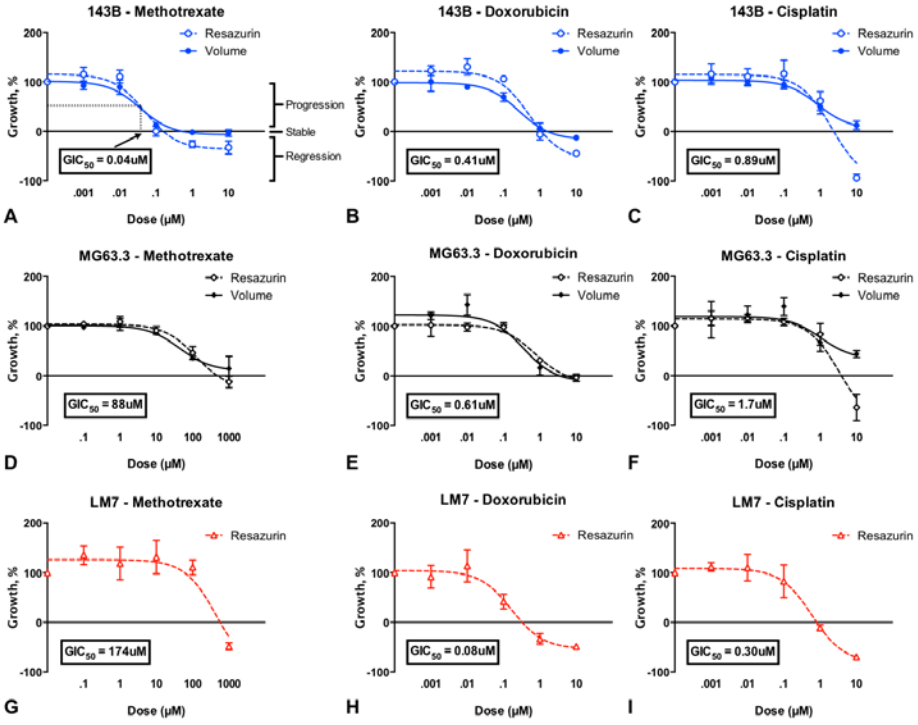

Fig. 2A-I Concentration-response curves demonstrate variable effects on volume and resazurin reduction across cell lines (143B, **A-C**; MG63.3, **D-F**; LM7, **G-I**) after treatment with individual MAP drugs (mean ± SD). Annotated brackets in (**A**) correlate clinical disease status with growth values, defined as the change in assay response since Day 0 relative to untreated controls. Dashed lines and arrow (**A**) depict the location of the GIC_{50} for resazurin reduction, which is provided for each cell type and treatment. LM7 volume (not shown) did not change in control or treatment groups.