Entinostat restores responsiveness to Olaparib in ID8 TP53 null and ID8 TP53 null/BRCA2 null mouse ovarian cancer cell lines

Tyler J. Woodard, MD, Washington University in St. Louis

Topic: Ovarian

Objectives
Poly (adenosine diphosphate [ADP]-ribose) polymerase inhibitors (PARPi) reduce disease progression in patients diagnosed with ovarian cancer. The anticancer effects of PARPi are most effective in tumors with BRCA mutations and homologous recombination (HR) deficiency (HRD). One mechanism of PARPi resistance is through restoration of HR repair. Our group has shown that entinostat (an HDACi) potentiates the effects of olaparib (a PARPi). The objective of this study was to investigate mechanisms of intrinsic PARPi resistance by studying the combination of entinostat and olaparib in HR proficient and HR deficient mouse ovarian cancer cell lines.

Methods
We used the ID8 TP53 null (HR proficient) and ID8 TP53 null/BRCA2 null (HR deficient) mouse ovarian cancer cell lines that harbor molecular features consistent with human high-grade serous ovarian cancer. To recapitulate an ongoing clinical trial using this drug combination, cells were pre-treated with 0.25 µM Entinostat or control for 24 hours, followed by 24 or 72 hours of drug treatment [0.5 µM Entinostat, 10 µM of Olaparib, the combination of both, or control]. Clonogenicity assays were used to assess colony formation and MTS assays were used to measure cell viability and proliferation. We then performed immunofluorescence (IF) staining for RAD51 (a marker for HR repair), γH2AX (a marker of DNA damage) and Ki67 (a marker of proliferation).

Results
As expected in the clonogenicity assay, there was no statistically significant difference in cell survival between the olaparib treated ID8 TP53 null (HR proficient) cells and vehicle-treated controls (p= 0.0993; paired t test). Alternatively, the ID8 TP53 null/BRCA2 null (HR deficient) cell line treated with olaparib alone reduced cell viability compared to control (p=0.0245; paired t test). Clonogenicity assays also demonstrated that the combination of entinostat and olaparib reduced colony formation compared to control in the ID8 TP53 null cell line (p< 0.0001; one-way ANOVA). MTS assays corroborated this treatment effect (p< 0.0001; two way ANOVA). Preliminary data suggests that compared to controls and each drug alone, IF staining for RAD51 and Ki67 expression was reduced in cells treated with combination therapy. Also preliminarily, there was increased number of γH2AX foci in combination treatment compared to controls and each drug alone.

Conclusions
Entinostat restores responsiveness to olaparib in ID8 TP53 null HR proficient mouse ovarian cancer cells that molecularly resemble high-grade serous ovarian carcinoma. These results suggest this drug combination can overcome intrinsic resistance to PARPi and provide additional preclinical support for clinical investigation of this combination for the treatment of HR proficient ovarian cancer.