Kim MS, MS, Glassman D, Ahumada AL, et al. Mechanisms and rational combinations with GP-2250, novel oxathiazine derivative in ovarian cancer. Cancer Research. 2023; 87(7_supplement): 528.

Background: GP-2250 (**Fig. 1**), a novel analog of taurultam (TRLT), has emerged as a potent anti-neoplastic drug; however, the mechanisms underlying its effects are not well understood. Here, we investigated the mechanism of action and the biological effects of GP-2250 using *in vitro* and *in vivo* models.

Methods: We carried out a series of *in vitro* experiments including MTT assay, Annexin V/PI assay, colony formation assay, reverse-phase protein array (RPPA), and HRLC/IC analysis to determine the biological activity of GP-2250 and investigate the mechanism of action. *In vivo* experiments were carried out to determine the therapeutic efficacy of GP-2250 alone and in combination with standard-of-care drugs (e.g., paclitaxel, cisplatin topotecan, and poly ADP-ribose polymerases (PARP) inhibitors.

Results: We investigated the cytotoxic effect of GP-2250 in 10 ovarian cancer cell lines and found that HRD ovarian cancer cells (e.g., Kuramochi, OVCAR4, and OVCAR8) were more vulnerable to GP-2250 than HRP ovarian cancer cells (e.g., A2780 and OVCAR5). In addition, the GP-2250 combination with a PARP inhibitor showed the most synergistic effects. There was no difference among the PARP inhibitors (e.g., olaparib, niraparib, and rucaparib) with regard to the combinatorial effect with GP-2250. RPPA analyses revealed that GP-2250 inhibited hypoxia-inducible factor-1α, AKT, and mTOR activation and expression level. Ultra-high resolution mass spectrometry (HRMS) analysis also revealed that hexokinase2 activity and expression were significantly reduced by GP-2250 treatment. Furthermore, GP-2250 also reduced glycolysis and ATP synthesis in cancer cells. *In vivo* pharmacodynamic experiment using the OVCAR8 mouse model demonstrated that a dose of 500 mg/kg GP-2250 was the most effective in downregulating AKT and mTOR activation and expression. In the *in vivo* therapy experiment using an orthotopic mouse model, a combination of GP-2250 and PARP inhibitors (olaparib, niraparib, or rucaparib) or bevacizumab showed a significant reduction of tumor weights (0.16 ± 0.05 g, 0.13 ± 0.06 g, 0.29 ± 0.05 g, and 0.07 ± 0.03 g, respectively) and nodules (1.56 ± 0.44, 1.89 ± 0.59, 3.11 ± 0.59, and 0.78 ± 0.2, respectively) compared to those treated with a vehicle (tumor weight, 0.95 ± 0.1 g and nodules, 8.4 ± 0.65), control IgG groups (tumor weight, 0.86 ± 0.38 and nodules, 9.4 ± 3.92) or the monotherapy groups; GP-2250 (tumor weight, 2.9 ± 0.48 g, and nodules, 2.9 ± 0.48), olaparib (tumor weight, 0.53 ± 0.09 g, and nodules, 3.3 ± 0.64), niraparib (tumor weight, 0.38 ± 0.05 g, and nodules, 3.4 ± 0.44), rucaparib, (tumor weight, 0.52 ± 0.1 g, and nodules, 4.85 ± 0.79), and bevacizumab (tumor weight, 0.43 ± 0.08 g, and nodules, 3.8 ± 0.71), respectively.

Conclusions: Taken together, our data indicate that GP-2250 exerts profound effects on tumor metabolism and combination with PARP inhibitors or bevacizumab showed promising anti-tumor efficacy. These findings could have implications for the clinical development of GP-2250.