Mechanisms and rational combinations with GP-2250, a novel oxathiazine derivative, in ovarian cancer

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ABSTRACT

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.

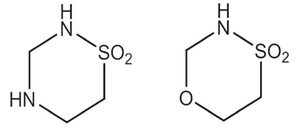


Figure 1. Molecular structure of TRLT and GP-2250. GP-2250, 1,4,5-oxathiazan-dioxide-4,4 is an oxathiazan derivative like TRLT with a molecular weight of 137.25 g/mol.

OBJECTIVES

To determine the biological effects of GP-2250, we investigate the underlying mechanism of action and the therapeutic effect of GP-2250 in combination with the PARP inhibitors, olaparib, niraparib, and rucaparib and with bevacizumab in ovarian cancer models *in vivo* and *in vitro*

MATERIALS & METHODS

- Ovarian cancer cell lines: A2780, Coav3, HeyA8, HeyA8-MDR, Kuramochi, OVCAR3, OVCAR4, OVCAR5, OVACR8, SKOV3
- *In vitro* assays: Cell viability assay, Western blotting, Colony formation assay, Reverse-phase protein array, Ultra-high resolution mass spectrometry analysis
- *In vivo* model of ovarian cancer: Pharmacodynamic study and therapeutic experiment using OVCAR8 ovarian cancer model

RESULTS

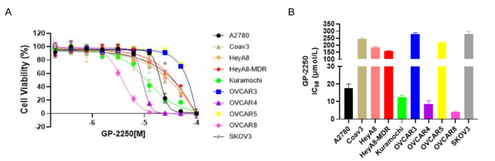


Figure 1. The cytotoxic effect of GP-2250 on ovarian cancer cells. (A) Cell viability assay. (B) IC50 of GP-2250.

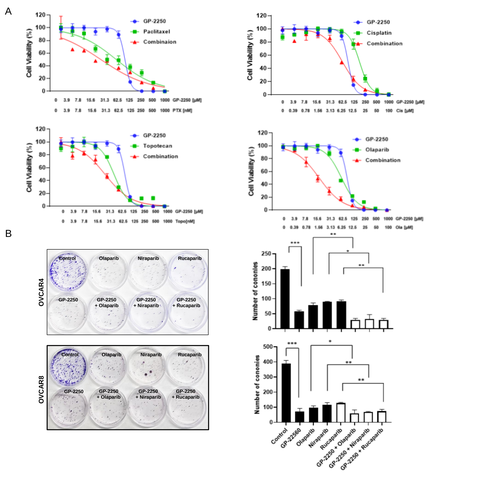


Figure 2. Effect of GP-2250 and standard of care chemotherapy drugs on ovarian cancer cells. (A) Cell viability assay. (B) Colony formation assay. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (Student *t*-test).

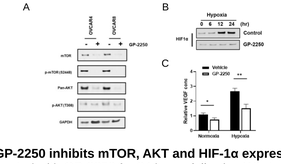
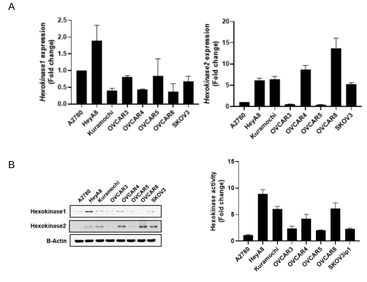


Figure 3. GP-2250 inhibits mTOR, AKT and HIF-1α expression. (A) Ovarian cancer cells were treated with GP-2250 for 24 hours following Western blotting. (B) GP-2250 inhibits HIF-1α expression. (C) ELISA assay of VEGF secretion. *$P < 0.05$; **$P < 0.01$ (vs. control; Student *t*-test).



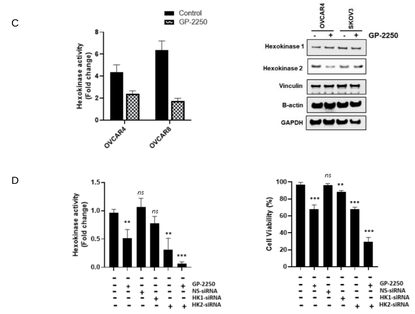


Figure 4. GP-2250 decreases glycolysis via inhibition of hexokinase2 activation and expression. (A) mRNA. (B) protein and activity of hexokinase. (C) GP-2250 inhibits hexokinase activity and protein expression levels. (D) siRNA targeting hexokinase1 and 2. *ns*, not significant, **$P < 0.01$; **$P < 0.001$ (*vs.* control; Student *t*-test).

Figure 5. Pharmacodynamic study. (A) Schematic of *in vivo* PD study of GP-2250. (B) Western blot and AKT kinase assay. (C) Immunohistochemistry analysis of tumor tissue.

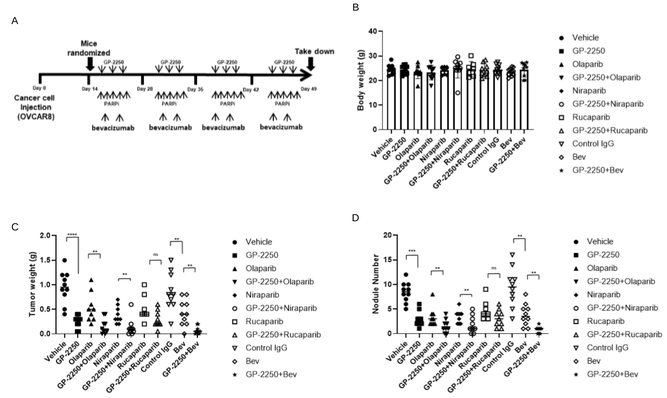


Figure 6. Antitumor effect of GP-2250 combined with olaparib or bevacizumab in an OVCAR8 mouse model. (A) Schematic of in vivo experiment. Body weight (B), tumor weight (C), and nodule number (D). *ns*, not significant, **$P < 0.01$; ***$P < 0.001$; ****, $P < 0.0001$ (Student *t*-test).

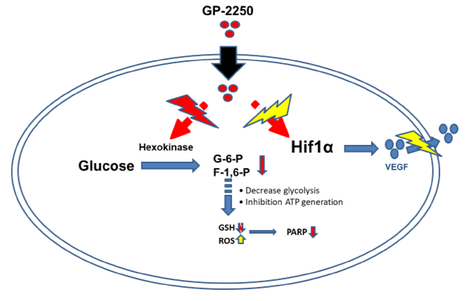


Figure 7. Proposed mechanism of GP-2250

CONCLUSION

- **GP-2250's antineoplastic effects on ovarian cancer cells include inhibition of glycolysis via modulation of HK2 activation and expression and inhibition of HIF-1-induced VEGF secretion.**
- **GP-2250 combination with PARP inhibitors or bevacizumab is well tolerated and shows profound antitumor efficacy.**
- **Our study suggests rational combinations for testing in clinical trials.**

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