Targeting NF1/RAS pathway mutations in high-grade serous ovarian carcinoma (HGSOC) with a novel dual-mechanism ERK 1/2 inhibitor to improve chemotherapy sensitivity

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Topic: Ovarian

Objectives
Mutations in the NF1 tumor suppressor gene result in unregulated activation of the RAS/ERK pathway and increased cellular proliferation. Somatic NF1 mutations have been identified in up to one-third of HGSOC, and are associated with chemotherapy resistance. A novel dual-mechanism ERK 1/2 inhibitor, ASTX029, has been shown to have activity in non-ovarian solid tumor cell lines with BRAF, KRAS, and NRAS mutations. ASTX029 is unique in that it inhibits the kinase activity of ERK and blocks ERK from being phosphorylated itself by MEK. In this study we investigate the role of ASTX029 to improve platinum sensitivity in NF1/RAS pathway mutated HGSOC.

Methods
We selected 3 human cancer cell lines consistent with HGSOC histology: OVSAHO, OVCAR3, and OVCAR7. OVSAHO was selected for its NF1 mutation, OVCAR3 for its RAS mutation, and OVCAR7 as a control. We treated all 3 cell lines with varying concentrations of carboplatin and ASTX029, both alone and in combination. The cytotoxic effect of carboplatin and ASTX029 were determined using a CellTiter Glo Luminenscent Cell Viability Assay. Combenefit software was used to evaluate for drug synergy and determine IC50 of both drugs. Phosphorylation of ERK (p-ERK) and RSK (p-RSK), an ERK substrate, were used as markers for effectiveness of inhibition of the NF1/RAS pathway and measured using Western Blot performed 6 and 24 hours after treatment.

Results
All 3 cell lines demonstrated decreased platinum sensitivity based on IC50, with OVSAHO having the lowest IC50. ASTX029 demonstrated exquisite cytotoxicity at very low concentrations in the OVSAHO cell line, both alone and in combination with carboplatin. OVCAR3 demonstrated moderate sensitivity to ASTX029, but IC50 was more than 10x greater than for OVSAHO. OVCAR7 demonstrated minimal sensitivity with even high doses of ASTX029, and IC50 was not reached. Synergy between carboplatin and ASTX029 was noted at various doses of both drugs for the OVSAHO cell line, using concentrations far below the IC50 of either drug. Synergistic effect was less pronounced for OVCAR3, and no synergy was noted for OVCAR7. Western blot demonstrated a decrease in p-ERK and p-RSK in a dose and time dependent fashion for OVSAHO and OVCAR3, consistent with effective inhibition of the RAS/ERK pathway.

Conclusions
ASTX029, a novel dual-mechanism ERK 1/2 inhibitor, effectively blocks the RAS/ERK pathway in NF1/RAS pathway mutated HGSOC. ASTX029 demonstrates efficacy both as a single-agent and synergy when combined with carboplatin, and may indicate a potential new targeted therapy for chemotherapy resistant