Poster # 4749

Acrivon Predictive Precision Proteomics (AP3) uncovers mechanism of resistance to ACR-368, a clinical-stage CHK1/2 inhibitor, and identifies rational combination treatment

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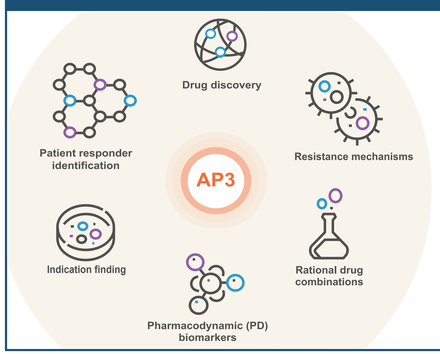
AP3 - Acrivon Predictive Precision Proteomics



Acrivon predictive precision proteomics (AP3) platform enables biological SAR based drug discovery and identification of drug-specific pharmacodynamic and response predictive protein biomarkers for drug indication screening and patient responder identification

In this study, the AP3 platform was used to uncover a key druggable resistance mechanism to ACR-368 and to identify a rational combination treatment to over-come resistance

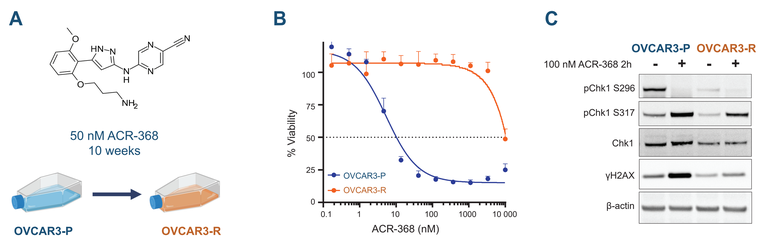
BACKGROUND

- ACR-368 is a potent and selective inhibitor of CHK1/2, key nodes in the DNA Damage Re-sponse (DDR) pathways, with demonstrated durable, single-agent activity in a subset of patients with advanced solid tumors
- As is the case for most DDR modulators, genomic biomarkers have also proven unsuccessful in predicting response to ACR-368, limiting the clinical success rates in patients
- Using the AP3 platform, we developed a drug tailored patient selection test - ACR-368 Onco-Signature - for individualized patient drug response prediction
- A Phase 2 clinical trial is ongoing where patients are treated with ACR-368 monotherapy based on OncoSignature-predicted sensitivity (NCT05548296)

PRESENT STUDY

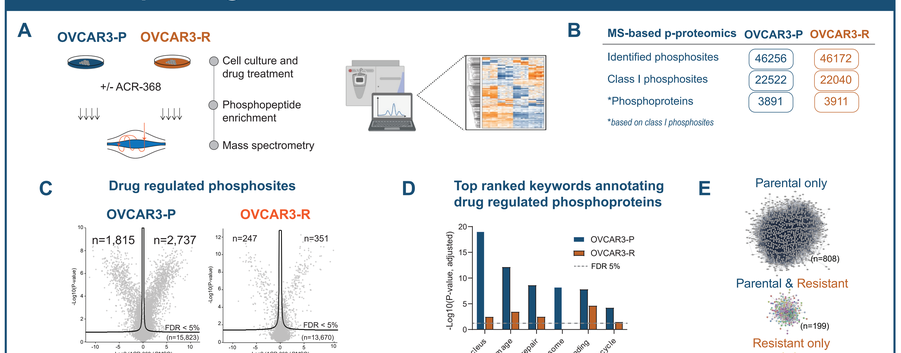
- NIH-OVCAR3 (OVCAR3) ovarian cancer cells were generated durably resistant to ACR-368
- Differential response to ACR-368 treatment was profiled in OVCAR3 Parental (OVCAR3-P) and Resistant (OVCAR3-R) cells using AP3 mass spectrometry
- Comprehensive pathway reconstitution and kinase activity analysis was performed to identify drug resistance mechanisms and actionable vulnerabilities

Generation of ACR-368 resistant ovarian cancer cells



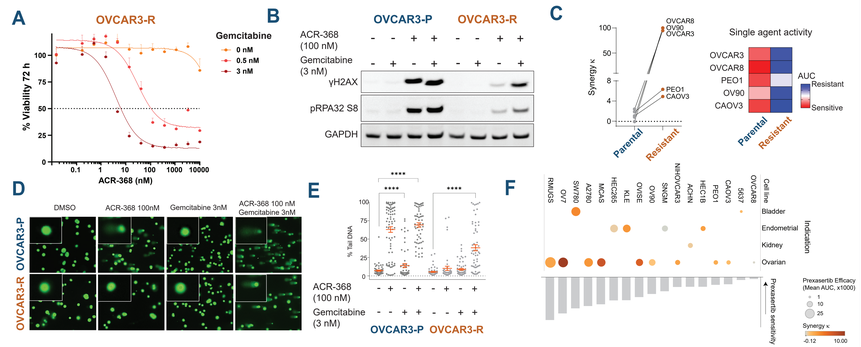
A. OVCAR3 ovarian cancer cells were rendered durably resistant to ACR-368 by culture in continuous presence of 50 nM drug. B. CellTiter Glo assay show drug-induced resistance to ACR-368 in OVCAR3-R cells following 72 hours treatment, compared to OVCAR3 parental cells. C. ACR-368 target engagement was confirmed in both parental and resistant OVCAR3 cells, while induction of DNA damage marker γH2AX was only observed in OVCAR3 parental cells.

AP3 profiling uncovers features linked to ACR-368 resistance



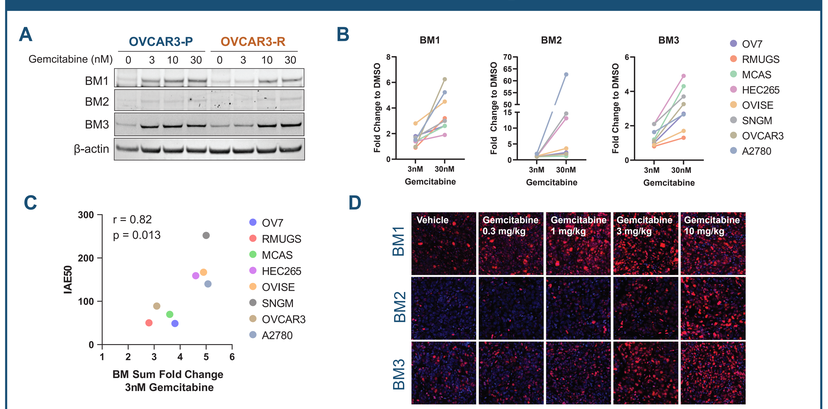
Phosphoproteomic profiling reveals reduced activity around CHK1/2 DDR axis in OVCAR3-R cells. A. Phosphoproteomics workflow. Phosphoproteomes from OVCAR3-P and OVCAR3-R cells treated with ACR-368 were recorded by data independent acquisition mass spectrometry. B. Overview of quanti-fied phosphorylation sites. C. Volcano plots showing ACR-368 regulated phosphosites in relation to DMSO control. D. Top enriched Uniprot keywords associated with regulated phosphoproteins in panel C using Fisher's exact test. E. STRING network visualizations of phosphoproteins underlying terms in panel D. F. Inferred kinase activity networks in ACR-368 treated cells compared to DMSO controls using the Post Translational Modification Set En-richment Analysis (PTM-SEA) approach. G. Heatmap showing unsupervised hierarchical clustering of proteins with differential abundance in OVCAR3-P and OVCAR3-R cells based on Fisher's exact test - DNA damage repair pathway enriched in OVCAR3-R downregulated cluster.

Low dose Gemcitabine sensitizes ovarian cancer cells to ACR-368



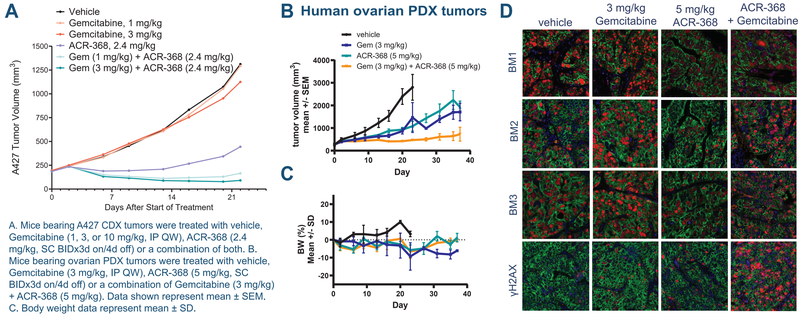
Gemcitabine treatment restores dependency on CHK1/2 activity. A. Gemcitabine sensitize OVCAR3-R cells to ACR-368 in a 72 hours CTG assay. B. Gemcitabine potentiates the effect of ACR-368 and induces markers of DNA damage in OVCAR3-R cells. C. Sensitization to ACR-368 by gemcitabine confirmed in a panel of 5 drug induced ACR-368 resistant cell lines. D. Alkaline comet assay show DNA damage in OVCAR3-R cells treated for 24h with ACR-368 and gemcitabine. E. Quantification of % tail DNA from panel D. F. Sensitization to ACR-368 by gemcitabine validated across a panel of cancer cell lines.

Gemcitabine induces ACR-368 OncoSignature biomarkers



ACR-368 OncoSignature biomarkers predict dependency on CHK1/2 axis activity. Biomarker levels increase as cells become sensi-tized to ACR-368 by low dose gemcitabine. A-B. Induction of ACR-368 OncoSignature biomarkers evaluated by western blot following 24 hours treatment with gemcitabine in OVCAR3-P and OVCAR3-R cells and across a panel of cancer cell lines. C. Biomarker induc-tion by gemcitabine correlates to combination treatment synergy score in cell line panel. D. Dose-dependent induction of ACR-368 OncoSignature biomarkers in A427 CDX model following 24 hours gemcitabine treatment.

Low dose Gemcitabine sensitizes to ACR-368 in CDX and PDX models



A. Mice bearing A427 CDX tumors were treated with vehicle, Gemcitabine (1, 3, or 10 mg/kg, IP QW), ACR-368 (2.4 mg/kg, SC BIDx3d on/4d off) or a combination of both. B. Mice bearing ovarian PDX tumors were treated with vehicle, Gemcitabine (3 mg/kg, IP QW), ACR-368 (5 mg/kg, SC BIDx3d on/4d off) or a combination of Gemcitabine (3 mg/kg) + ACR-368 (5 mg/kg). Data shown represent mean ± SEM. C. Body weight data represent mean ± SD.

A-C. In vivo efficacy and tolerability of ACR-368 and low dose gemcitabine combination therapy in human non-small cell lung cancer CDX and ovarian PDX models. D. Immunofluorescent staining of ovarian PDX tumors show increased levels of OncoSignature bi-omarkers following low dose gemcitabine treatment. DNA damage marker γH2AX is strongly induced in tumors following combi-nation treatment.

CONCLUSIONS

- Using AP3 mass spectrometry based pathway mapping of ACR-368 resistance mechanisms, gemcitabine was identified as a rational combination treatment through restoration of stress around the CHK1/2 signal-ing axis
- Low dose gemcitabine was confirmed to sensitize cancer cells to ACR-368 across a cell line panel and in CDX and PDX models *in vivo*
- A concomitant induction of ACR-368 OncoSignature drug response predictive biomarkers was observed following sensitization by gemcitabine treatment *in vitro* and *in vivo*
- Efficacy and safety of ultra low dose gemcitabine (10 mg/m²) combined with ACR-368 is currently being evaluated in an exploratory Phase1b/2 clinical study in ACR-368 OncoSignature negative patients
- This study demonstrates the potential of AP3 for unbiased elucidation of actionable drug resistance mechanisms and rapid clinical implementation