Poster # 239

Potent Synergy Between CHK1/2 Inhibitor ACR-368 and the ADC Payload Topoisomerase I Inhibitor: Rationale for ADC + ACR-368 Combination Therapy

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Introduction

- Antibody-drug conjugates (ADCs) enable targeted delivery of cytotoxic payloads to tumor cells, improving the therapeutic index compared with conventional chemotherapy. Topoisomerase I (Topo I) inhibitors are potent ADC payloads that trap the Topo I-DNA cleavage complex, leading to replication-fork collapse and tumor cell death. However, this also activates the DNA damage response through CHK1/2-dependent cell cycle checkpoints, attenuating cytotoxicity and enhancing resistance to Topo I-containing ADCs.
- CHK1/2 inhibition exploits this therapeutic vulnerability, abrogating these checkpoints, increasing replication stress, and thereby enhancing the efficacy of Topo I inhibitors across multiple tumor models. Consistent with this, treatment with the potent, selective CHK1/2 inhibitor ACR-368 (prexasertib) combined with irinotecan has demonstrated encouraging clinical activity in heavily pretreated patients with sarcomas who had progressed on prior irinotecan therapy.[1] Taken together, these data provide a strong rationale for combining ACR-368 with Topo I inhibitor–based therapies.
- Using the Acrivon Predictive Precision Proteomics (AP3) platform, we previously identified endometrial cancer as a tumor type predicted to be particularly sensitive to ACR-368, which has been shown and is being further evaluated in a Phase 2 registrational trial.
- In a panel of endometrial cancer cell lines, the combination of ACR-368 with exatecan or SN38 demonstrated synergy in a majority of these, with comparable synergy scores between both Topo I inhibitors. Pathway mechanisms underlying Topo I inhibitor sensitivity and resistance and the potent, synergistic activity with ACR-368 were identified using AP3 Generative Phosphoproteomics.

AP3 Identifies Mechanisms of Topoisomerase I Inhibitor Sensitivity

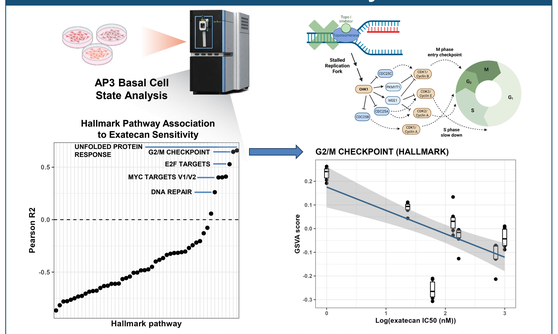


Fig 1. AP3 Identifies G2/M Checkpoint Pathway Association With Increased Sensitivity to Topo I Inhibition and Rationale for CHK1/2 Inhibition with ACR-368. Pearson correlation of baseline phosphoproteomics-derived Hallmark pathway enrichment scores with Topo I inhibitor (exatecan) IC_{50} values across eight human endometrial cancer cell lines. IC_{50} values were determined by 3-day CellTiter-Glo (CTG) assays (see Fig. 2A). Pathway schematic (top right) highlights G2/M checkpoint regulation downstream of CHK1.[2]

ACR-368 Combines Synergistically with Topo I Inhibitors Across Sensitive and Insensitive Endometrial Models

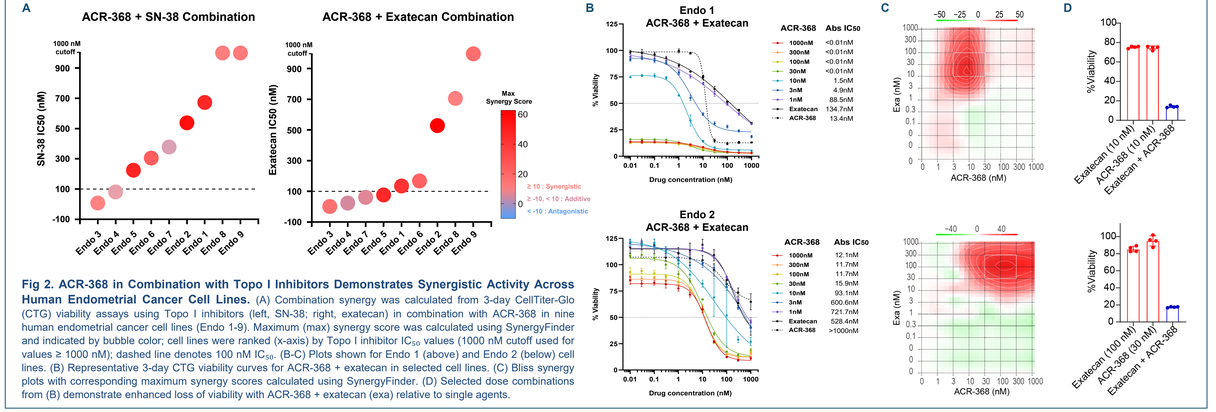


Fig 2. ACR-368 in Combination with Topo I Inhibitors Demonstrates Synergistic Activity Across Human Endometrial Cancer Cell Lines. (A) Combination synergy was calculated from 3-day CellTiter-Glo (CTG) viability assays using Topo I inhibitors (left, SN-38; right, exatecan) in combination with ACR-368 in nine human endometrial cancer cell lines (Endo 1-9). Maximum (max) synergy score was calculated using SynergyFinder and indicated by bubble color; cell lines were ranked (x-axis) by Topo I inhibitor IC_{50} values (1000 nM cutoff used for values ≥ 1000 nM); dashed line denotes 100 nM IC_{50}. (B-C) Plots shown for Endo 1 (above) and Endo 2 (below) cell lines. (B) Representative 3-day CTG viability curves for ACR-368 + exatecan in selected cell lines. (C) Bliss synergy plots with corresponding maximum synergy scores calculated using SynergyFinder. (D) Selected dose combinations from (B) demonstrate enhanced loss of viability with ACR-368 + exatecan (exa) relative to single agents.

AP3 Reveals Exatecan-Mediated Suppression of Cell Cycle Programs, Relieved by ACR-368

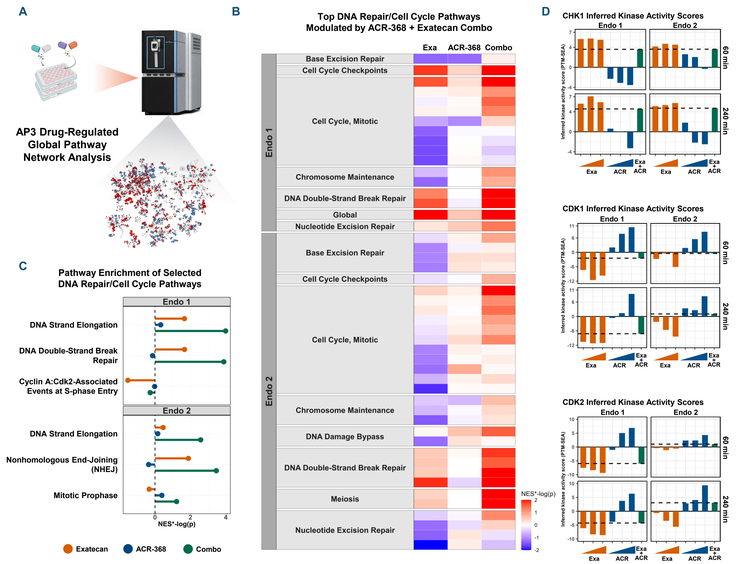


Fig 3. ACR-368 Uncouples Cell Cycle Control from Exatecan-Induced DNA Damage. (A) AP3 pathway analysis reveals global networks of biological pathways in response to drug treatment. (B) Heatmap of normalized enrichment scores (NES) for DNA repair-related pathways from the Reactome database following 60 min drug treatment (10 nM ACR-368, 10 nM exatecan, and their combination) in Endo 1 and Endo 2 cells. Pathways shown are those that exhibited a significant difference in enrichment in the combination treatment compared with single-agent treatment. (C) Pathway enrichment scores of select cell cycle/DNA repair pathways showing differential modulation by treatment as in (B). (D) Inferred kinase activity scores calculated from phosphorylation levels of annotated substrates from the PSP and IKIP databases in Endo 1 and Endo 2 cells after 60 and 240 minutes of single drug treatment (10, 30, 100 nM) or combination (10 nM ACR-368 + 10 nM exatecan). Bars represent the PTM-SEA scores of inferred kinase activities; positive scores indicate increased activity relative to DMSO control and negative values indicate decreased activity. Dashed line indicates the inferred activity level post-combination treatment. (E) Log-normalized phospho-peptide abundance; points represent individual replicate measurements, lines indicate the median across replicates at each dose (1, 10, 30, 100 nM exatecan; 3, 10, 30, 100 nM ACR-368) and in combination (10 nM ACR-368 + 10 nM exatecan). Boxes represent the interquartile range. Dashed horizontal line denotes the DMSO (-) control level for each condition.

ACR-368-Mediated Checkpoint Release Drives Replication Catastrophe with Topo I Inhibitor

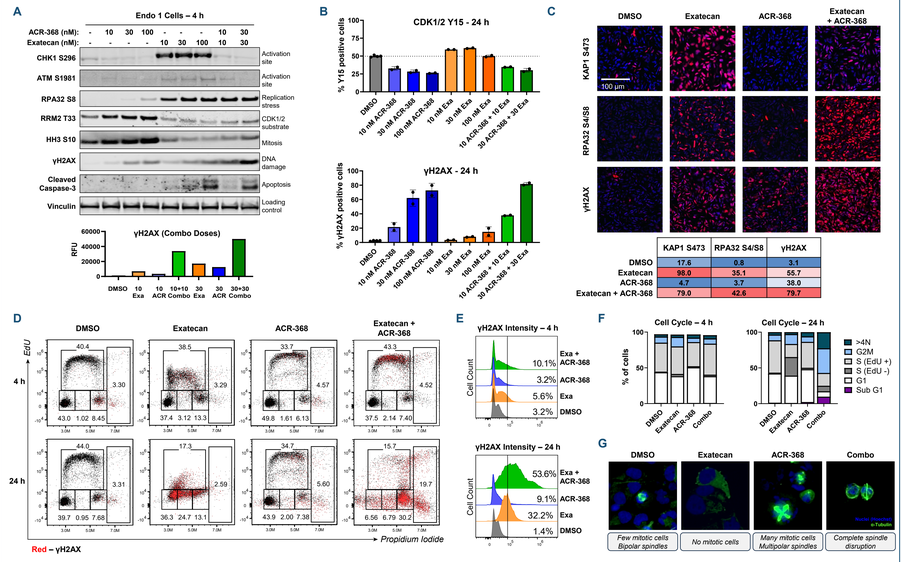
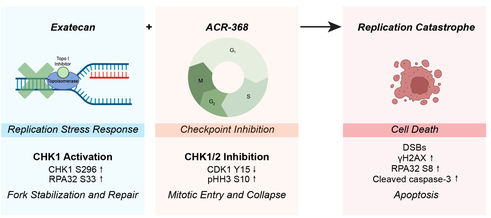


Fig 4. ACR-368 Suppresses the CHK1/2 Checkpoint Response Induced by Exatecan, to Enhance Cell Death by Replication Catastrophe and Mitotic Collapse. (A) Above, Western blot validation of selected cell cycle DNA repair pathway members after 4h treatment with ACR-368 (ACR) and exatecan in Endo 1 cells; below, quantification of γH2AX signal (combination doses shown). (B) CDK1/2 Y15 (above) and γH2AX (below) levels after 24h of treatment with ACR-368, exatecan, or the combination, measured by immunofluorescence and quantified relative to DMSO control. (C) Immunofluorescence staining of cell pellets prepared from Endo 1 cells treated for 4 hours with 30 nM exatecan, 30 nM ACR-368, or the combination. Quantification of staining, shown below, was performed using HALO® software. (D-F) Endo 1 cells treated with 10 nM exatecan, 10 nM ACR-368, or in combination for 4 h (top) or 24 h (bottom). Cell cycle distribution was evaluated by flow cytometry using propidium iodide (PI) and EdU incorporation, with γH2AX high cells (red) overlaid on the cell-cycle profiles (D); histograms of γH2AX intensity (E); and quantification of cell cycle distribution (F). (G) Representative images from Endo 1 cells treated for 24 h with 30 nM exatecan, 30 nM ACR-368, or the combination, captured at 20X on a CX5 high content imager (Blue: nuclei, Hoechst stain; Green: α-tubulin).

Conclusions



- AP3 Generative Phosphoproteomics identified modulation of the G2/M checkpoint pathway as associated with increased sensitivity to Topo I inhibition, providing a mechanistic rationale for combination with ACR-368, a first-in-class CHK1/2 inhibitor currently in an ongoing registrational intent Phase 2b study in endometrial cancer, that enhances G2/M pathway activation through checkpoint inhibition.
- ACR-368 demonstrated synergistic activity with Topo I inhibitors in both Topo I inhibitor–sensitive and –insensitive models, supporting the potential to overcome resistance to Topo I inhibitor–based ADCs.
- Phosphosite and kinase activity signatures identified by AP3 implicated replication stress response and checkpoint inhibition as key mechanisms underlying the synergistic cell death observed with the combination.
- Synergy was observed at low doses of both agents, suggesting the potential for dose reduction while maintaining antitumor activity, which may provide opportunity to move ACR-368 toward frontline settings in combination with Topo I inhibitor-payload ADCs.

References:

[1] Slotkin et al. *JCO Oncol Adv* (2025)

[2] Diagram modified from Gorecki, Andrs & Korabecny. *Cancers* (2021), and di Rorà et al. *J Hematol Oncol* (2020)

Pathway diagrams / figure icons created with use of BioRender.com