Acrivon Predictive Precision Proteomics (AP3)-guided development and prospective clinical registrational-intent phase 2 validation of a response-predictive OncoSignature test for the CHK1/2 inhibitor, ACR-368

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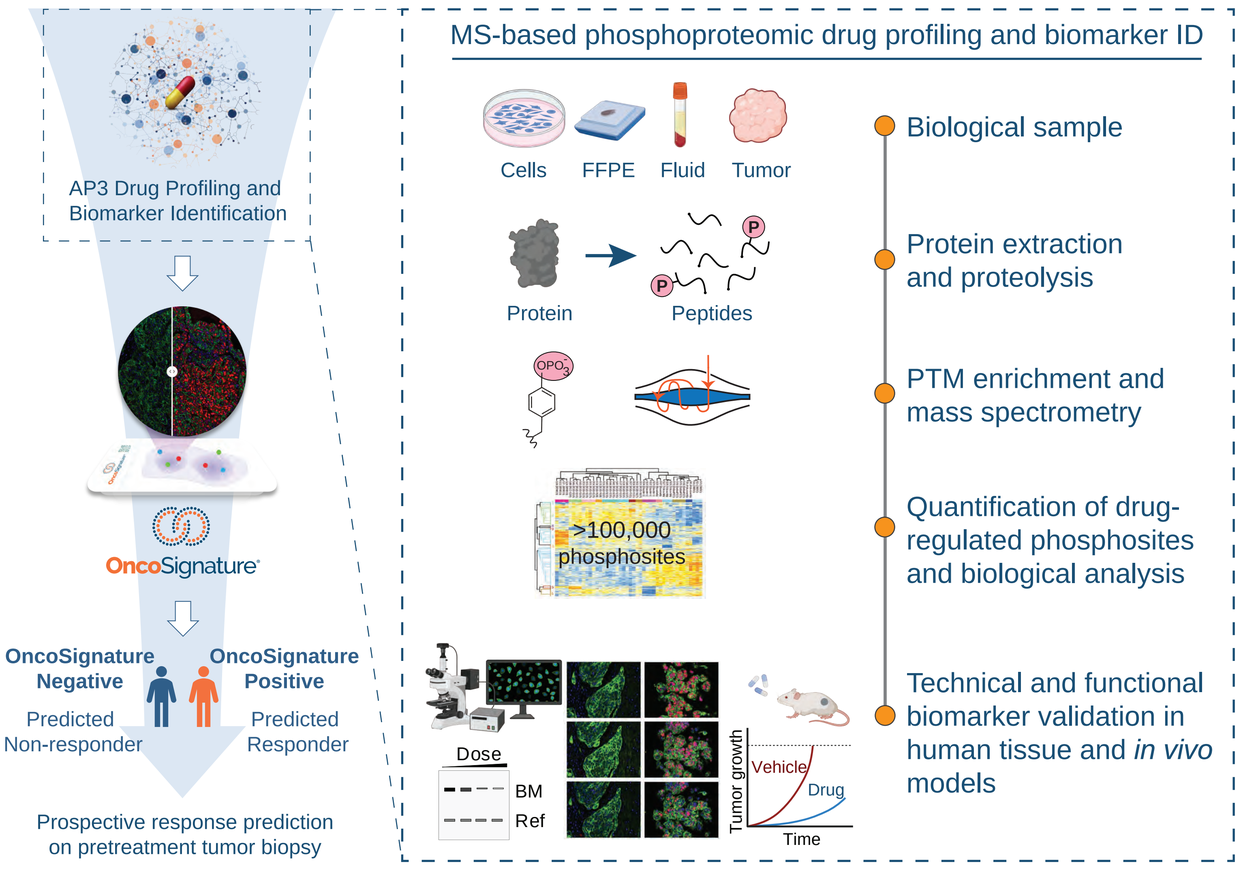
Background

- ACR-368 is a potent and selective clinically advanced CHK1/2 inhibitor which has demonstrated durable, single-agent activity across a proportion of patients with advanced solid tumors.
- As with other DNA Damage Response (DDR) pathway inhibitors, genomic biomarkers have proven unsuccessful in predicting response to ACR-368, hitherto limiting its clinical success.
- Carefully selected protein biomarkers can directly measure the tumor-driving mechanisms and match these with the compound's mechanism of action independent of genomic alterations, making these vulnerabilities ideal for patient response prediction.

Approach and Results

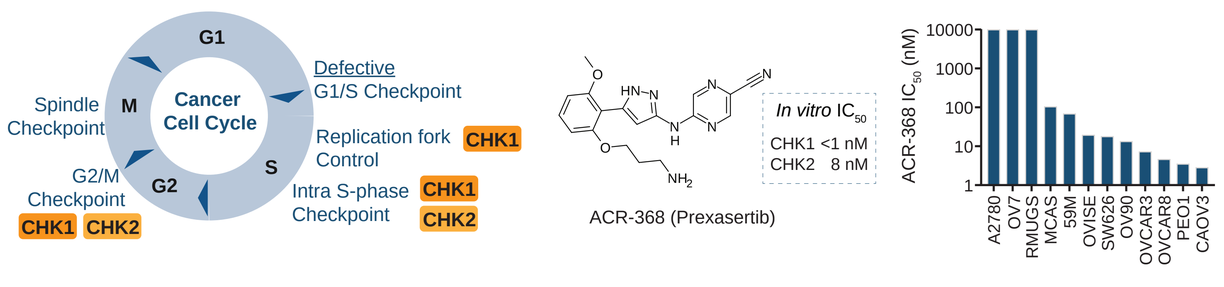
- Leveraging our Acrivon Predictive Precision Proteomics (AP3) approach, mass spectrometry (MS) based quantitative phosphoproteomics profiling was used to identify 3 classes of functionally orthogonal candidate biomarkers predictive of sensitivity to ACR-368.
- A biomarker from each class was selected for the ACR-368 OncoSignature Assay based on pre-specified criteria. The ACR-368-tailored multiplexed immunofluorescence test quantitatively measures each biomarker specifically in tumor cell nuclei.
- The ACR-368 OncoSignature assay was used for preclinical indication finding to identify endometrial cancer as a sensitive indication, and shown to discriminate ACR-368-sensitive and non-sensitive endometrial PDX models ($p < 0.003$ (Wilcoxon)).
- Ongoing registrational-intent Phase 2 trial (NCT05548296) interim data has verified the predicted clinical activity with a confirmed overall response rate (ORR) = 62.5% (95% CI, 30.4-86.5) observed in prospectively-selected ACR-368 OncoSignature-positive patients with endometrial cancer; all confirmed responders had progressed on prior anti-PD-1 inhibitor therapy.

Acrivon Predictive Precision Proteomics (AP3)

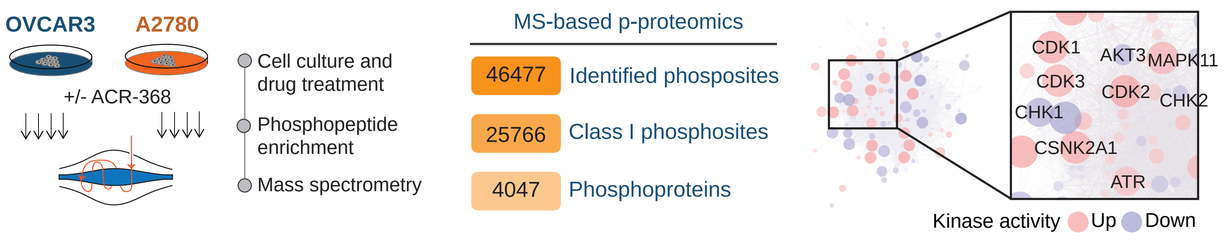


AP3-Driven Characterization of ACR-368 in Cancer Cells

(A) ACR-368, a selective inhibitor of CHK1 and CHK2 kinases, inhibits ovarian cancer cell line growth



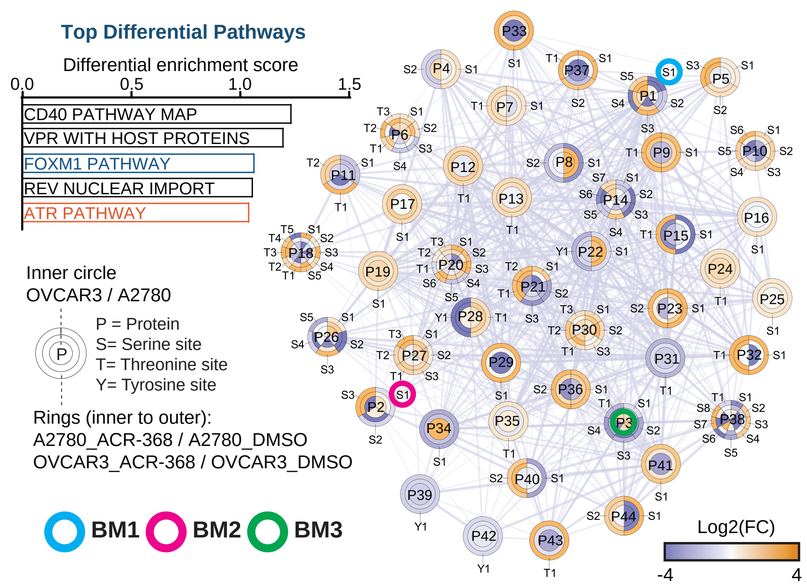
(B) AP3 profiling of ACR-368 identifies >25k confident p-sites and reveals drug-regulated kinases



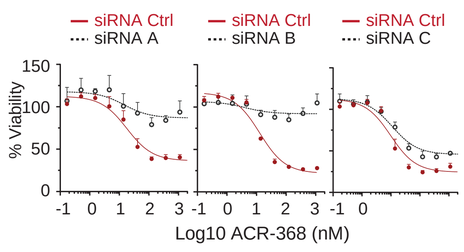
(A) Cancer cell cycle checkpoint regulation by CHK1 and CHK2 (left), chemical structure of the CHK1/CHK2 inhibitor ACR-368 (middle), ACR-368 IC_{50} in ovarian cancer cell lines (right). **(B)** Experimental outline (left), number of identified p-sites in ACR-368 treated OVCAR3 (100 nM, 2h) (middle), and inferred kinase activity in ACR-368 treated OVCAR3 (right).

AP3 Approach Identifies Predictive Functionally Validated Biomarkers for ACR-368

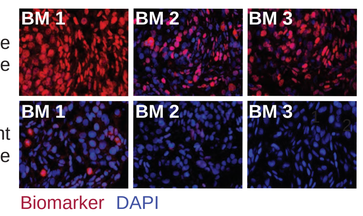
(A) ACR-368 regulated pathways and phospho-protein network



(B) Validating functional biomarker dependency

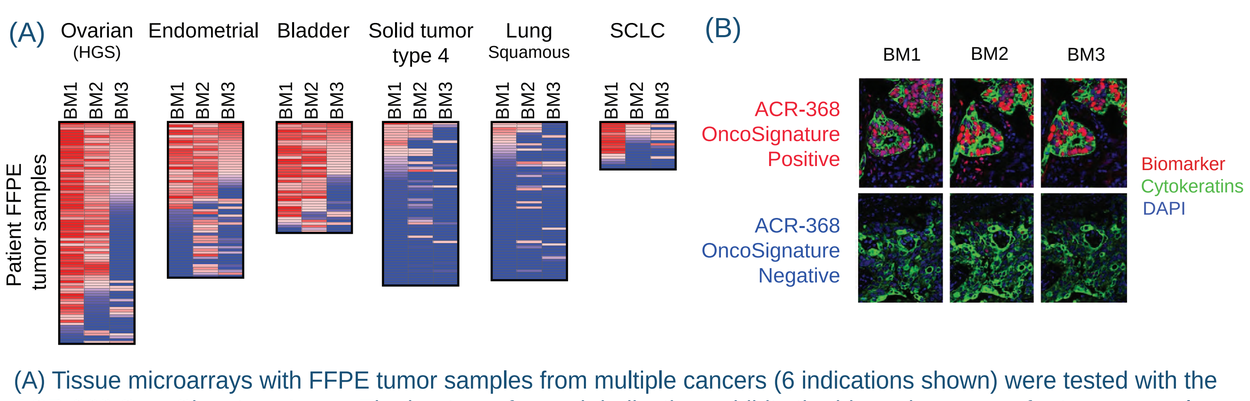


(C) Protein biomarker levels correlate with *in vitro* drug response



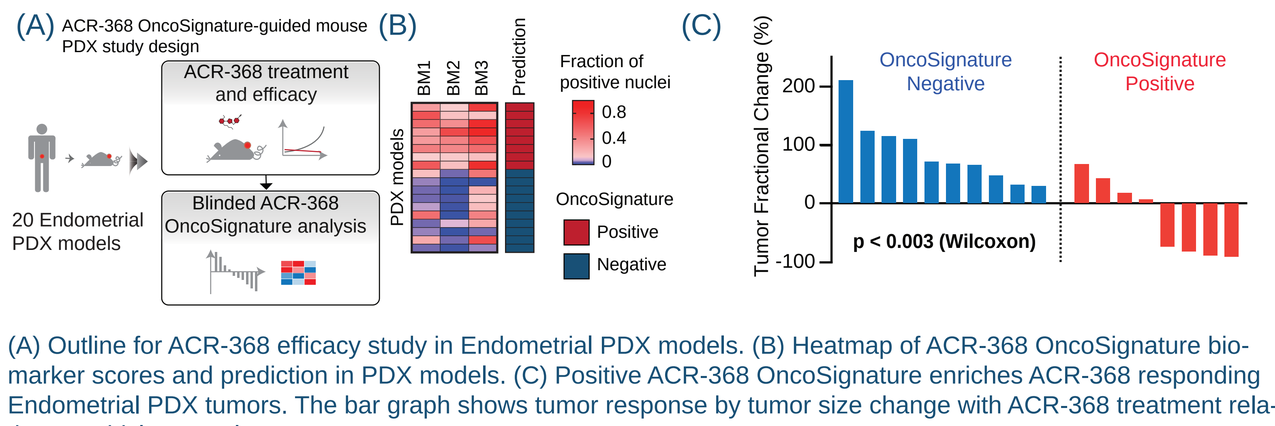
(A) Top differentially ACR-368 regulated pathways were compared in sensitive OVCAR3 and resistant A2780 cells and a quantitative STRING-based network representing the drug-regulated phosphosites was used for candidate biomarker identification. **(B)** siRNA mediated knockdown of candidate predictive biomarkers abrogates sensitivity to ACR-368 in OVCAR3 cells. **(C)** Example of nuclear biomarker levels in sensitive and resistant cell lines.

ACR-368 OncoSignature-Based Indication Finding



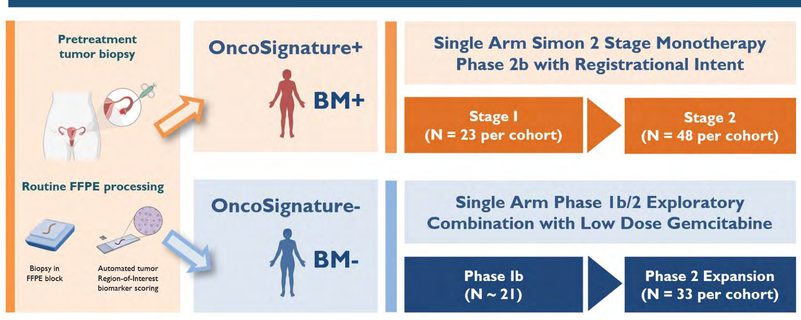
(A) Tissue microarrays with FFPE tumor samples from multiple cancers (6 indications shown) were tested with the ACR-368 OncoSignature Assay. The heatmap for each indication exhibits the biomarker scores for tumor samples. (B) Examples of composite images of ACR-368 OncoSignature positive (top) and negative (bottom) tumor samples.

ACR-368 OncoSignature-Based Response Prediction in Endometrial PDX model



(A) Outline for ACR-368 efficacy study in Endometrial PDX models. (B) Heatmap of ACR-368 OncoSignature biomarker scores and prediction in PDX models. (C) Positive ACR-368 OncoSignature enriches ACR-368 responding Endometrial PDX tumors. The bar graph shows tumor response by tumor size change with ACR-368 treatment relative to vehicle control.

ACR-368 OncoSignature-Based Patient Selection



Prospective clinical trial design
BM+ subjects are treated with single-agent ACR-368 as part of a potentially registrational Phase 2 Simon 2 stage design. BM- subjects are treated with ACR-368 + LDG based on AP3-predicted sensitization to ACR-368 in an exploratory Phase 1b/2 trial

Overall Response	BM+	BM-
	N = 8	N = 15
	N (%)	N (%)
CR	0 (0)	1 (7)
cPR	5 (63)	0 (0)
uPR	0 (0)	1 (7)
SD	1 (13)	6 (40)
PD	2 (25)	7 (47)
cORR (95% CI)	62.5% (30.4, 86.5)	6.7% (0.84, 31.8)
OncoSignature BM+ vs BM- Segregation P = 0.009		

Data current as of 25July2024, includes efficacy-evaluable subjects (at least one on-treatment scan) enrolled after OncoSignature threshold lock (BM+ and BM-) at RP2D for LDG (BM-); cPR = confirmed partial response, uPR = unconfirmed partial response, cORR = confirmed overall response rate; Nonparametric bootstrap method for calculating OncoSignature BM+ vs BM- segregation; Agresti-Coull method for calculating 95% confidence interval (CI).

- Confirmed ORR in BM+ endometrial cancer subjects = **62.5%** [N=8; 95% C.I. (30.4, 86.5)]
- OncoSignature segregation of BM+ vs BM- (N = 23; **P = 0.009**)

Conclusions and Future Directions

- Using our proprietary and actionable AP3 approach, which integrates MS-based phosphoproteomics and quantitative multiplexed IF-staining of drug-tailored biomarkers, we developed a response-predictive OncoSignature test for ACR-368 that enables response prediction to ACR-368 treatment.
- Endometrial carcinoma was identified as a new tumor type predicted to be particularly sensitive to ACR-368 based on preclinical AP3-OncoSignature indication finding.
- The prediction was evaluated in our ongoing registrational intent Phase 2 trial demonstrating a confirmed ORR of 62.5% and segregation of responders from non-responders (N = 23; BM+ vs BM-, **P = 0.009**).