Validation of the OncoSignature Assay, an ACR-368-tailored response-predictive quantitative multiplexed immunofluorescent assay for prediction of sensitivity to the CHK1/2 inhibitor ACR-368 in individual patients with cancer

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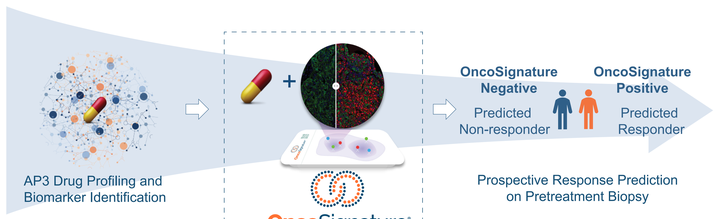
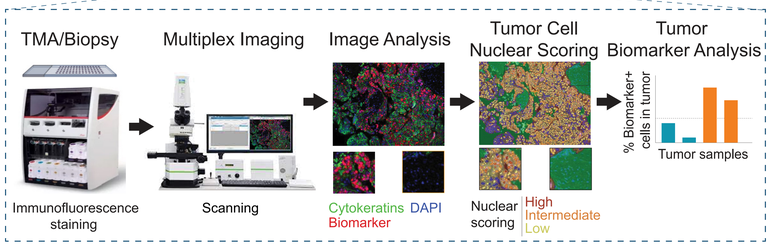
Introduction

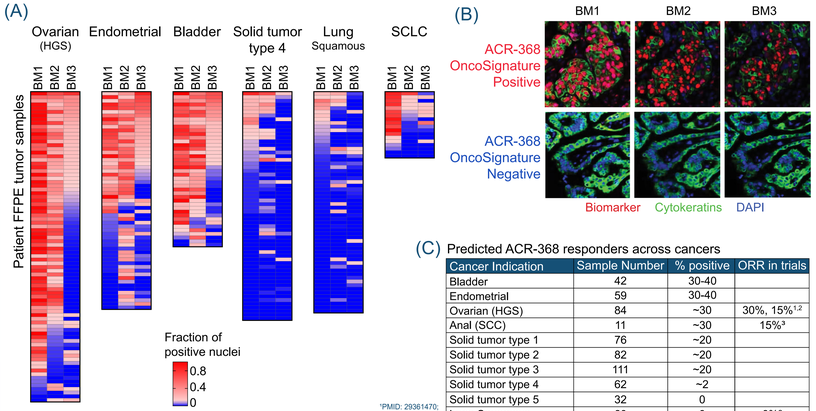
- The clinical stage CHK1/2 kinase inhibitor ACR-368 (Prexasertib) has demonstrated durable single-agent activity in a proportion of patients with high grade serous ovarian, anal, and head & neck cancers. Using Acrivon Predictive Precision Proteomics (AP3), which integrates quantitative phosphoproteomics with our protein multiplex biomarker platform, we identified three functionally orthogonal biomarkers tailored to predict tumor response to ACR-368 treatment.
- We developed an ACR-368 OncoSignature Assay for routine-processed FFPE tumor biopsies based on immunofluorescent staining of the three biomarkers. Our assay involves image analysis and quantitative biomarker measurements in tumor cell nuclei. A sample is ACR-368 OncoSignature positive if all three biomarker scores are above their pre-specified thresholds.
- We validated our predictive ACR-368 OncoSignature Assay in cancer cell lines and ovarian cancer PDX models. We further evaluated the ACR-368 OncoSignature Assay for prediction of sensitivity to ACR-368 in known and new cancer types (indication finding), and in blinded, prospectively-designed studies on pretreatment tumor biopsies from past clinical trials with ACR-368.
- By screening across routine-processed human cancer tissues, we found excellent concordance between ACR-368 OncoSignature predicted prevalence of ACR-368 responders and the ORR observed in previous clinical trials. Moreover, we identified endometrial and bladder cancers as new tumor types predicted sensitive to ACR-368 in approximately 30-40% of cases.
- We confirmed the predicted efficacy in genetically non-modified endometrial cancer PDX models, and further demonstrated that we could predict sensitive from insensitive models with an AUC of 0.88 in blinded studies using the ACR-368 OncoSignature Assay.
- Finally, we evaluated the ACR-368 OncoSignature Assay in blinded, prospectively-designed studies of pretreatment tumor biopsies from past Phase 2 ACR-368 trials in patients with ovarian cancer and demonstrated a statistically significant segregation of responders from non-responders (ORR 47% and 58%, respectively) and increased median progression-free survival (mPFS).

Acrivon OncoSignature Assay Development





ACR-368 OncoSignature Indication Screening



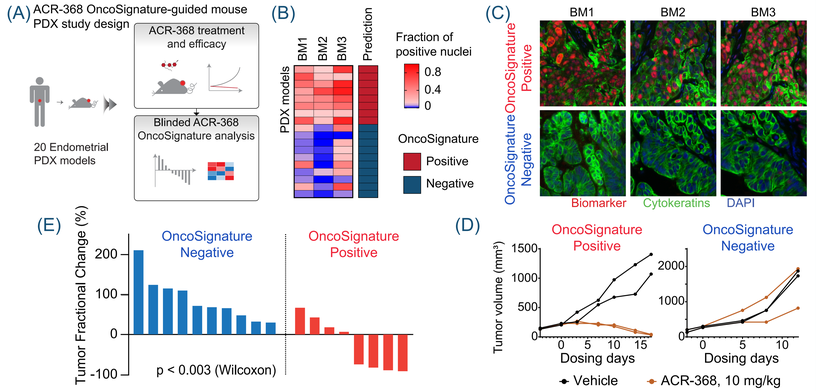
(C) Predicted ACR-368 responders across cancers

Cancer Indication	Sample Number	% positive	ORR in trials
Bladder	42	30-40	
Endometrial	59	30-40	
Ovarian (HGS)	84	~30	30%, 15%[1,2]
Anal (SCC)	11	~30	15%[3]
Solid tumor type 1	76	~20	
Solid tumor type 2	82	~20	
Solid tumor type 3	111	~20	
Solid tumor type 4	62	~2	
Solid tumor type 5	32	0	
Lung Squamous	60	0	0%[3]
SCLC	18	0	0%[4]

[1]PMID: 29361470; [2]PMID: 36192237; [3]PMID: 29643063; [4]PMID: 34034991

(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. (C) Summary table for prevalence of ACR-368 OncoSignature predicted responders across cancers and clinically observed response rates to ACR-368 treatment.

ACR-368 Efficacy Study in Endometrial Cancer 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) Examples of composite images of ACR-368 OncoSignature predicted Endometrial PDX responder and non-responder. (D) Tumor growth curves of the corresponding PDX models treated with vehicle or ACR-368. (E) 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.

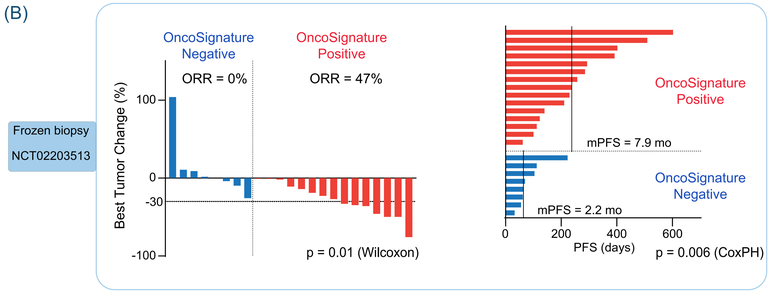
ACR-368 OncoSignature Validation in ACR-368 Clinical Trial Samples

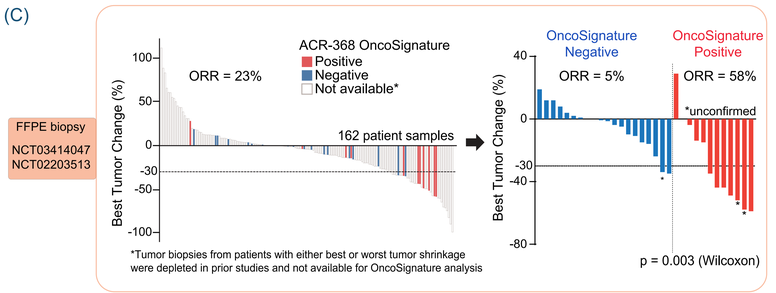
(A)

Clinical Trial ID	NCT02203513	NCT03414047 + NCT02203513
Total number	23	32 (24+8)
Sample type	Frozen biopsy	FFPE biopsy
Best response to ACR-368 treatment		
PR	7	8
SD	11	20
PD	5	4

NCT02203513 Single center (NCI), single arm Ph2 trial of ACR-368 in platinum-resistant or refractory recurrent ovarian cancer.

NCT03414047 Multicenter, single arm Ph2 trial of ACR-368 in platinum-resistant or refractory recurrent ovarian cancer.





(A) Summary of frozen and FFPE biopsy samples cohorts. (B) Blinded frozen pretreatment ovarian cancer biopsies from a single center ACR-368 monotherapy Phase 2 trial (NCT02203513) were analyzed by ACR-368 OncoSignature Assay. ACR-368 OncoSignature prediction grouped patient responses by best change in tumor size (left) and by progression free survival (right). (C) Blinded FFPE pretreatment ovarian cancer biopsies from the multi-center ACR-368 monotherapy Phase 2 trials NCT03414047 and NCT02203513 were analyzed by ACR-368 OncoSignature Assay (left). ACR-368 OncoSignature prediction grouped patient responses by best change in tumor size (right).

Conclusions

- Employing our AP3 platform, which combines MS-based phosphoproteomics and quantitative multiplexed-IF staining of drug-tailored biomarkers, we developed a response-predictive test for ACR-368 that enables identification of responders to ACR-368 treatment.
- A clinical trial (NCT05548296) evaluating the efficacy of ACR-368 based on the ACR-368 OncoSignature test status is currently recruiting in patients with platinum-resistant ovarian, endometrial, and urothelial cancer.