Poster # C112

ACR-2316 is a novel, differentiated, clinical-stage WEE1/PKMYT1 inhibitor designed by Acrivon's Generative Phosphoproteomics AP3 Platform for optimal pro-apoptotic pathway effects in tumor cells resulting in superior preclinical activity

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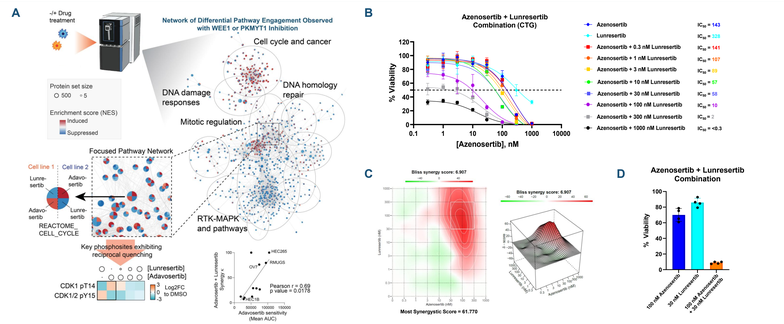
Introduction

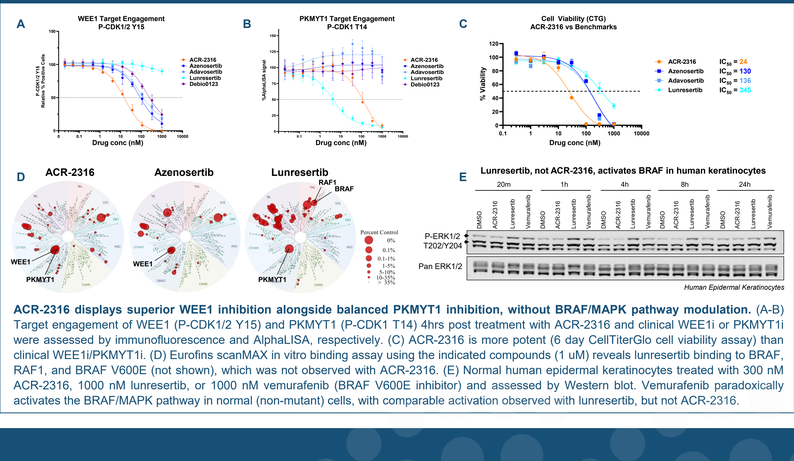
- ACR-2316 is an internally discovered, clinical stage, potentially first- and best-in-class WEE1/PKMYT1 inhibitor specifically designed to overcome the limitations of previous WEE1 and PKMYT1 inhibitors using Acrivon's Predictive Precision Proteomics (AP3) platform.
- Through AP3 profiling, we previously uncovered WEE1 inhibitor-induced resistance mechanisms that were quenched by PKMYT1 inhibition.
- ACR-2316 was rationally designed using AP3 to suppress this resistance mechanism alongside potent WEE1 inhibition and robust activation of CDK1, CDK2, and PLK1, to induce potent tumor cell death.

AP3 "Reciprocal Quenching" reveals actionable resistance mechanisms



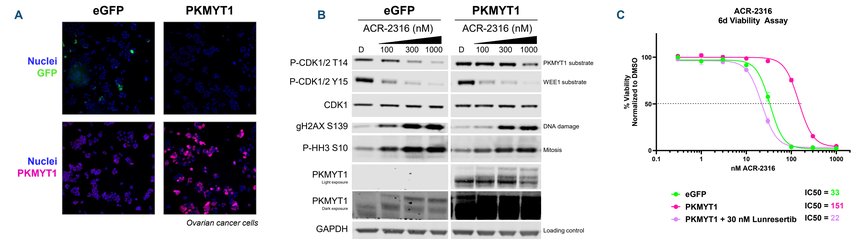
Combination of WEE1i and PKMYT1i is synergistic, supporting rationale for dual targeting (A) The drug regulated phosphoproteome of human tumor cell lines treated 60 min with WEE1i (adavosertib, 200 nM) or PKMYT1i (lunresertib, 20 nM) was assessed by AP3 mass spectrometry profiling and mapped to cellular pathways, which revealed those enriched by WEE1i (including cell cycle, DNA damage response, and mitotic regulation), a subset of which were reciprocally quenched by PKMYT1i, suggesting PKMYT1 inhibition as a mechanism of overcoming WEE1i resistance. (B-D) Combination of azenosertib and lunresertib (6 day cell viability CellTiter Glo assay, shown in an ovarian cancer cell line) displayed strong synergy. All IC_{50}s represented in (nM). Bliss scores calculated using SynergyFinder.

ACR-2316 exhibits class-leading WEE1 potency and balanced PKMYT1 inhibition with high selectivity delivering superior anti-cancer cell activity



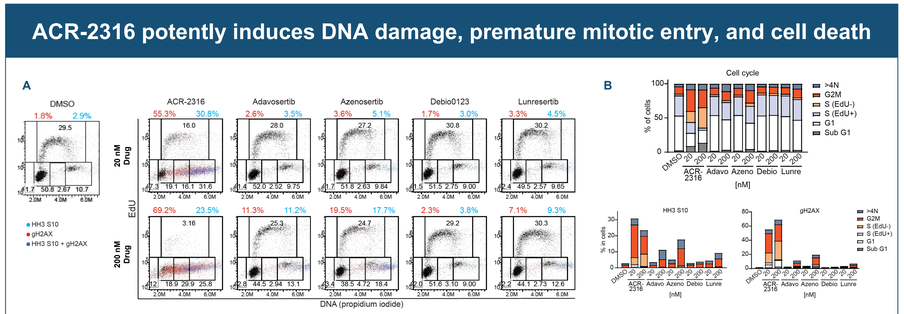
ACR-2316 displays superior WEE1 inhibition alongside balanced PKMYT1 inhibition, without BRAF/MAPK pathway modulation. (A-B) Target engagement of WEE1 (P-CDK1/2 Y15) and PKMYT1 (P-CDK1 T14) 4hrs post treatment with ACR-2316 and clinical WEE1i or PKMYT1i were assessed by immunofluorescence and AlphaLISA, respectively. (C) ACR-2316 is more potent (6 day CellTiterGlo cell viability assay) than clinical WEE1i/PKMYT1i. (D) Eurofins scanMAX in vitro binding assay using the indicated compounds (1 uM) reveals lunresertib binding to BRAF, RAF1, and BRAF V600E (not shown), which was not observed with ACR-2316. (E) Normal human epidermal keratinocytes treated with 300 nM ACR-2316, 1000 nM lunresertib, or 1000 nM vemurafenib (BRAF V600E inhibitor) and assessed by Western blot. Vemurafenib paradoxically activates the BRAF/MAPK pathway in normal (non-mutant) cells, with comparable activation observed with lunresertib, but not ACR-2316.

PKMYT1 targeting contributes to the anti-cancer cell activity of ACR-2316



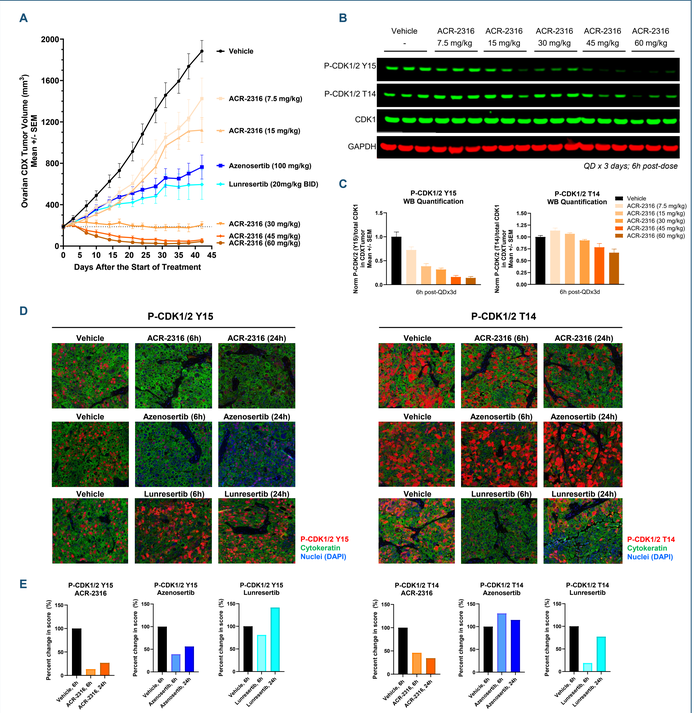
PKMYT1 overexpression reduces ACR-2316 sensitivity, which is reversed by PKMYT1 inhibition. (A) Ovarian cancer cell lines stably expressing eGFP or PKMYT1. GFP (green), PKMYT1 (pink), nuclei (Hoechst stain; blue); images captured at 10X on a CX5 high content imager. (B) Stably expressing cell lines were treated 4h with ACR-2316 and assessed by Western blot. PKMYT1 overexpressing cells display comparable WEE1 engagement (P-CDK1/2 Y15) to eGFP control cells, but maintain P-CDK1/2 T14 (PKMYT1 engagement) levels and display reduced induction of P-Histone H3 (HH3) and gH2AX. (C) 6 day cell viability assay (CellTiter Glo) reveals a >3X loss of sensitivity induced by PKMYT1 overexpression, which was restored by co-treatment with PKMYT1i lunresertib.

ACR-2316 potently induces DNA damage, premature mitotic entry, and cell death



ACR-2316 more potently induces DNA damage, premature mitotic entry, and cell death compared to clinical WEE1/PKMYT1i. (A-B) Ovarian cancer cell lines treated 24h with compounds (20, 200 nM), followed by flow cytometry assessed by EdU (cell cycle), gH2AX (DNA damage), and P-HH3 S10 (mitotic entry). (C) Ovarian cancer cells treated for the indicated times, followed by Western blot. (D) ACR-2316 leads to cell death initiation within 12h, with near-complete loss of viability by 48h observed using live cell imaging in a CellTox Green cytotoxicity assay. (E) ACR-2316 displays superior activity compared to clinical WEE/PKMYT1i across all human tumor cell lines and human patient derived xenograft (PDX) models tested (n=55 human tumor cell lines, not selected based on underlying genetic mutations, tested in 6 day CellTiter Glo (CTG) cell viability assay; n=16 ovarian cancer PDX ex vivo models tested in 6 day 3D-CTG assay). Dashed lines indicate median IC_{50} values, also shown in table at right.

ACR-2316 displays superior preclinical efficacy in vivo at doses that suppress both WEE1 and PKMYT1



Dual inhibition of WEE1 and PKMYT1 is associated with superior preclinical efficacy of ACR-2316. (A) In a human ovarian tumor CDX model (5d on/2d off dosing or as indicated), azenosertib and lunresertib resulted in modest growth inhibition at maximum tolerated/formulable doses, while ACR-2316 demonstrated near complete tumor regression. (B-C) Human ovarian CDX mouse model treated QD x 3d at the indicated doses and assessed 6h post last dose. (B) Doses of ACR-2316 associated with efficacy (45, 60 mg/kg) displayed inhibition of both WEE1 and PKMYT1 (P-CDK1/2 Y15 and T14, respectively) assessed by Western blot of CDX tumor lysates, quantified in (C). (D-E) Human ovarian CDX tumors from mice treated QD x 5d with ACR-2316 (45 mg/kg), azenosertib (100 mg/kg) or lunresertib (20 mg/kg BID), collected 6h or 24h post last dose as indicated. (D) Immunofluorescence staining for CDK1/2 Y15 (left) and T14 (right), quantified in (E), displayed potent inhibition of WEE1 as well as balanced and durable PKMYT1 inhibition induced by ACR-2316.

Conclusions

- WEE1 inhibitor-induced PKMYT1 activation constitutes a resistance mechanism that may limit the clinical efficacy of WEE1 inhibition.
- ACR-2316 is a potential first- and best-in-class WEE1/PKMYT1 inhibitor with superior preclinical efficacy optimized by AP3 pathway-based structure-activity relationships in the intact cell for strong CDK1, CDK2, and PLK1 activation to induce potent tumor cell death.
- Acrivon's ongoing Phase 1 ACR-2316 monotherapy trial has already demonstrated initial clinical activity during dose escalation across several AP3-predicted solid tumors, including confirmed partial response.