Poster# A097

Global Pharmacodynamic Effects Uncovered With AP3 Phosphoproteomic Profiling of Novel WEE1/PKMYT1 Inhibitor ACR-2316 Reveals The Critical Importance of PLK1 for ACR-2316's Superior Preclinical Activity and Differentiated Mechanism of Action

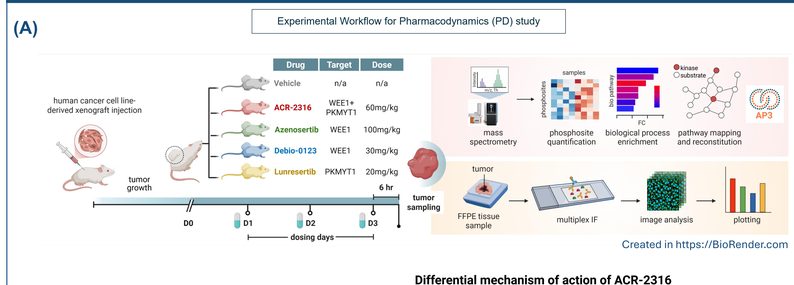
Subodh Kumar[1], Joelle Baddour-Sousounis[1], YingChun Liu[1], Kate Rappard[1], Jessica Hopkins[1], Maria Rodriguez-Zabala[1], Calvin Yang[1], Sam Saliba[1], Francisco Santana[1], Eunice Kwon[1], Georgia Mitsa[2], Martina Pasetto[2], Mohamed Eldeeb[2], Taronish Dubash[1], Portia Lombardo[1], William Dahlberg[1], Amira Elbakry[1], Zachary Best[1], Luka Romero[1], Nina Lipjankic[2], Valentina Siino[2], Ahmed Youssef[1], Bryan Thornlow[1], Corey Xu[1], Yan He[1], Reina Improgo[1], Anil Prasad[1], Sibgat Choudhury[1], Magnus E. Jakobsson[2], Helén Nilsson[2], Caroline Wigerup[2], Lei Shi[1], Ayesha Murshid[1], Joon Jung[1], David Proia[1], Michail Shipitsin[1], Kristina Masson[1] and Peter Blume-Jensen[1]

[1]Acrivon Therapeutics Inc., Watertown, MA, USA, and [2]Acrivon AB, Medicon Village, Lund, Sweden

Introduction

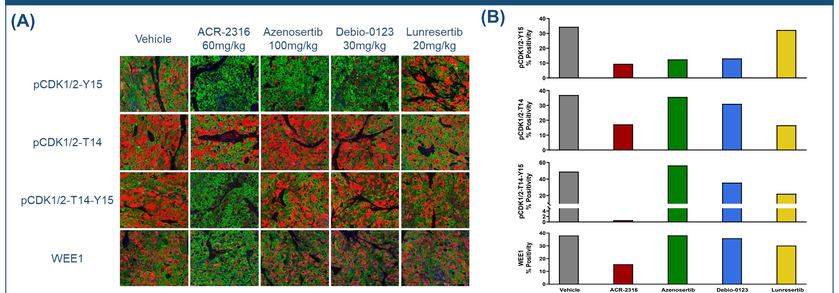
- Proper cell cycle progression and mitotic execution requires timely coordinated activation of the cell cycle kinases, including CDK1, CDK2, and PLK1.
- WEE1 and PKMYT1 kinases act as negative regulators of CDK1/2 through inhibitory phosphorylation of CDK1/2-Y15 and CDK1-T14.
- ACR-2316 is an internally discovered, clinical stage, potentially first- and best-in-class WEE1/PKMYT1 inhibitor uniquely enabled using Acrivon's Predictive Precision Proteomics (AP3) platform.
- ACR-2316 was rationally designed using AP3 to overcome the limitations of previous WEE1 and PKMYT1 inhibitors through robust activation of CDK1, CDK2, and PLK1, to induce potent tumor cell death.
- Here, we present, head-to-head preclinical global pharmacodynamic (PD) and *in vivo* efficacy analyses of ACR-2316 against clinical benchmarks.

Acrivon's Predictive Precision Proteomics (AP3) Platform Identifies ACR-2316 Efficacy-Associated Substrates of CDK1, CDK2 and PLK1 Kinases



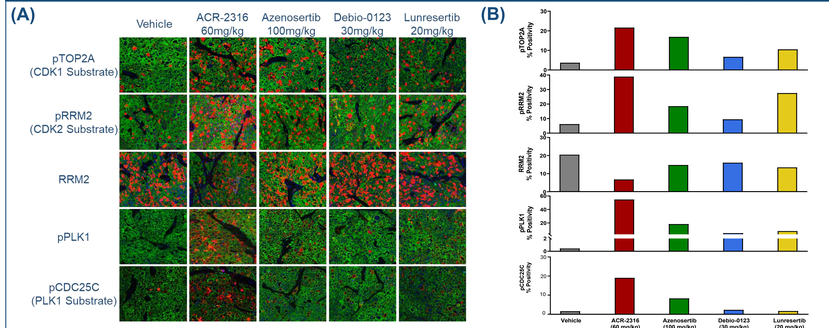
Acrivon's Predictive Precision Proteomics (AP3) Platform confirms that ACR-2316 leads to superior induction of CDK1, CDK2, and PLK1 kinase activities. (A) Experimental workflow of *in vivo* PD studies. Phosphoproteomics and immunofluorescence studies were conducted in ovarian xenograft tumors collected from mice at 6h post-3-day treatment with ACR-2316 at 60 mg/kg, azenosertib at 100 mg/kg, Debio-0123 at 30 mg/kg, and lunresertib at 20 mg/kg. **(B)** PTM-SEA scores heatmap (upper panel heatmap) was generated from unsupervised hierarchical clustering of AP3 phosphoproteomics detected abundance of annotated CDK1, CDK2, and PLK1 substrate phosphopeptides (lower panel heatmap) in ovarian cancer xenograft that were treated as described in (A). **(C)** Mechanistic pathway construction using BioRender for select AP3-identified CDK1, CDK2, and PLK1 substrates based on publicly available data and literature.

ACR-2316 Shows Superior Target Engagement In Vivo Compared to Clinical Benchmarks



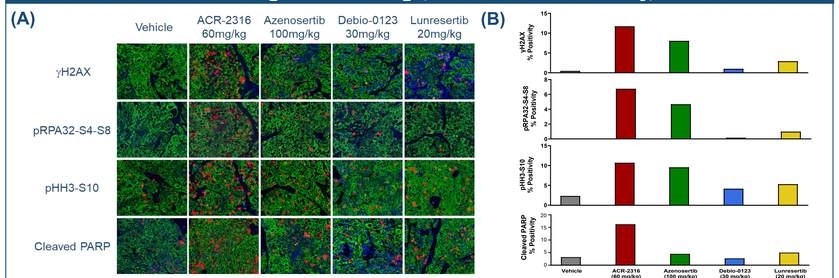
Immunofluorescence staining of ovarian cancer cell line-derived xenograft tumors shows superior target engagement with ACR-2316 compared to clinical benchmarks. (A) Immunofluorescence staining and **(B)** quantitative image analysis scoring for pCDK1/2-Y15, pCDK1/2-T14, pCDK1/2-T14-Y15, and WEE1 in xenograft tumors collected at 6h post-3-day treatment from mice treated with ACR-2316 at 60 mg/kg, azenosertib at 100 mg/kg, Debio-0123 at 30 mg/kg, and lunresertib at 20 mg/kg.

ACR-2316 Leads to Stronger Induction of CDK1, CDK2, and PLK1 Activity



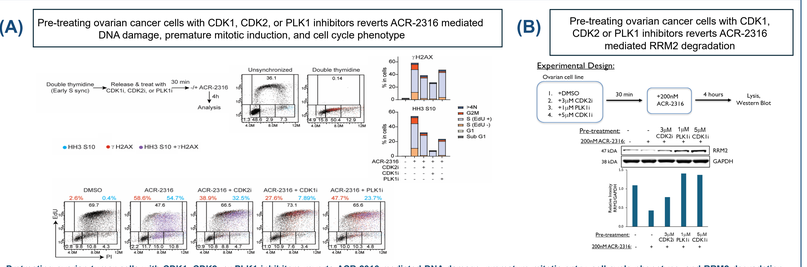
Immunofluorescence staining of ovarian cancer cell line-derived xenograft tumors treated with ACR-2316 shows superior induction of CDK1,CDK2, and PLK1 activity. (A) Immunofluorescence staining and **(B)** quantitative image analysis scoring for pTOP2A, pRRM2, RRM2, pPLK1, and pCDC25C in xenograft tumors collected from mice at 6h post-3-day treatment with ACR-2316 at 60 mg/kg, azenosertib at 100 mg/kg, Debio-0123 at 30 mg/kg, and lunresertib at 20 mg/kg.

ACR-2316 Induces Stronger DNA Damage, Premature Mitotic Entry, And Cell Death



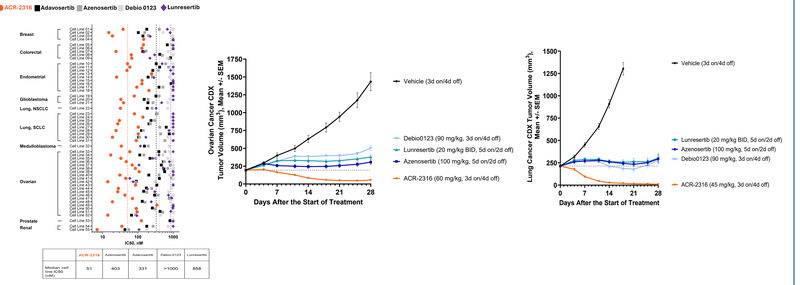
Immunofluorescence staining of ovarian cancer cell line-derived xenograft tumors treated with ACR-2316 shows strong induction of DNA damage, premature mitotic entry, and cell death. (A) Immunofluorescence staining and **(B)** quantitative image analysis scoring for γH2AX, pRPA32-S4-S8, pHH3-S10, and cleaved PARP in xenograft tumors collected from mice at 6h post-3-day treatment with ACR-2316 at 60 mg/kg, azenosertib at 100 mg/kg, Debio-0123 at 30 mg/kg, and lunresertib at 20 mg/kg.

Coordinated Induction of CDK1, CDK2, and PLK1 Activity is Essential for Superior Preclinical Activity of ACR-2316



Pretreating ovarian tumor cells with CDK1, CDK2, or PLK1 inhibitors reverts ACR-2316 mediated DNA damage, premature mitotic entry, cell cycle phenotype, and RRM2 degradation. (A) Ovarian cancer cell lines were synchronized at S phase, then released and pretreated with CDK1i (5μM), CDK2i (5μM), or PLK1i (1μM) for 30 minutes, followed by ACR-2316 (200nM) treatment for 4h with compounds, followed by assessment of EdU (cell cycle), γH2AX (DNA damage), and HH3 S10 (mitotic entry) by flow cytometry. **(B)** Ovarian cancer cells treated as indicated, followed by Western blot for RRM2.

ACR-2316 Demonstrates Superior Single Agent Efficacy in Cell Lines and Xenograft Tumors



	ACR-2316	Azenosertib	Azenosertib	Debio-0123	Lunresertib
Median cell line IC50 (nM)	51	403	331	>1000	858

Superior efficacy of ACR-2316 versus clinical WEE1 and PKMYT1 inhibitors. (A) ACR-2316 displays superior activity (6-day CellTiter Glo cell viability assay) compared to clinical WEE and PKMYT1 inhibitors across all tested human tumor cell lines (n=55 human tumor cell lines, not selected based on underlying genetic mutations). **(B)** & **(C)** ACR-2316 demonstrates superior efficacy compared to clinical WEE1 inhibitors (azenosertib, Debio0123), and PKMYT1 inhibitor (lunresertib) in ovarian cancer (B) and lung cancer (C) xenograft tumor models. Mice were treated as indicated for 28 days at maximum tolerated or allowed by formulation doses. Tumor volume represents Mean ± SEM.

Conclusions

- ACR-2316 is a potential first- and best-in-class dual inhibitor of WEE1 and PKMYT1, rationally designed using Acrivon's proprietary AP3 platform to deliver superior single-agent efficacy.
- ACR-2316-treated xenograft tumors demonstrated stronger target engagement, increased phosphorylation of CDK1, CDK2, and PLK1 substrates, decreased WEE1 protein expression, and superior activation of DNA damage response, mitotic, and apoptotic markers compared to single-target WEE1 or PKMYT1 inhibitors.
- In ovarian and lung cancer xenograft models, treatment with ACR-2316 uniquely resulted in complete tumor regression, whereas treatment with WEE1 inhibitors azenosertib and Debio0123 or the PKMYT1 inhibitor lunresertib, resulted in only stable disease at maximum tolerated/formulable doses.
- Potent activation of PLK1, together with CDK1/2, is essential for the superior activity of ACR-2316.
- 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.