

ACR-2316: a novel potent, selective WEE1/PKMYT1 inhibitor rationally designed for superior single agent activity using Acrivon Predictive Precision Proteomics (AP3) for biological SAR



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INTRODUCTION

Normal cells, as well as cancer cells, rely on the cell cycle checkpoints (G1/S-, intra-S-, G2/M-checkpoints) to maintain genomic integrity. Cancer cells often have defective G1/S checkpoint and are hypothesized to be particularly sensitive to inhibition of intra-S and G2/M-checkpoints that are regulated by WEE1 and PKMYT1. Although clinical activity with WEE1 inhibitors has been observed in certain tumor types, understanding of key resistance mechanism enables the rational design of more selective and potent compounds, aiming for improved therapeutic index, suitable for clinical monotherapy development that can target these resistance mechanisms to achieve better clinical activity and tolerability.

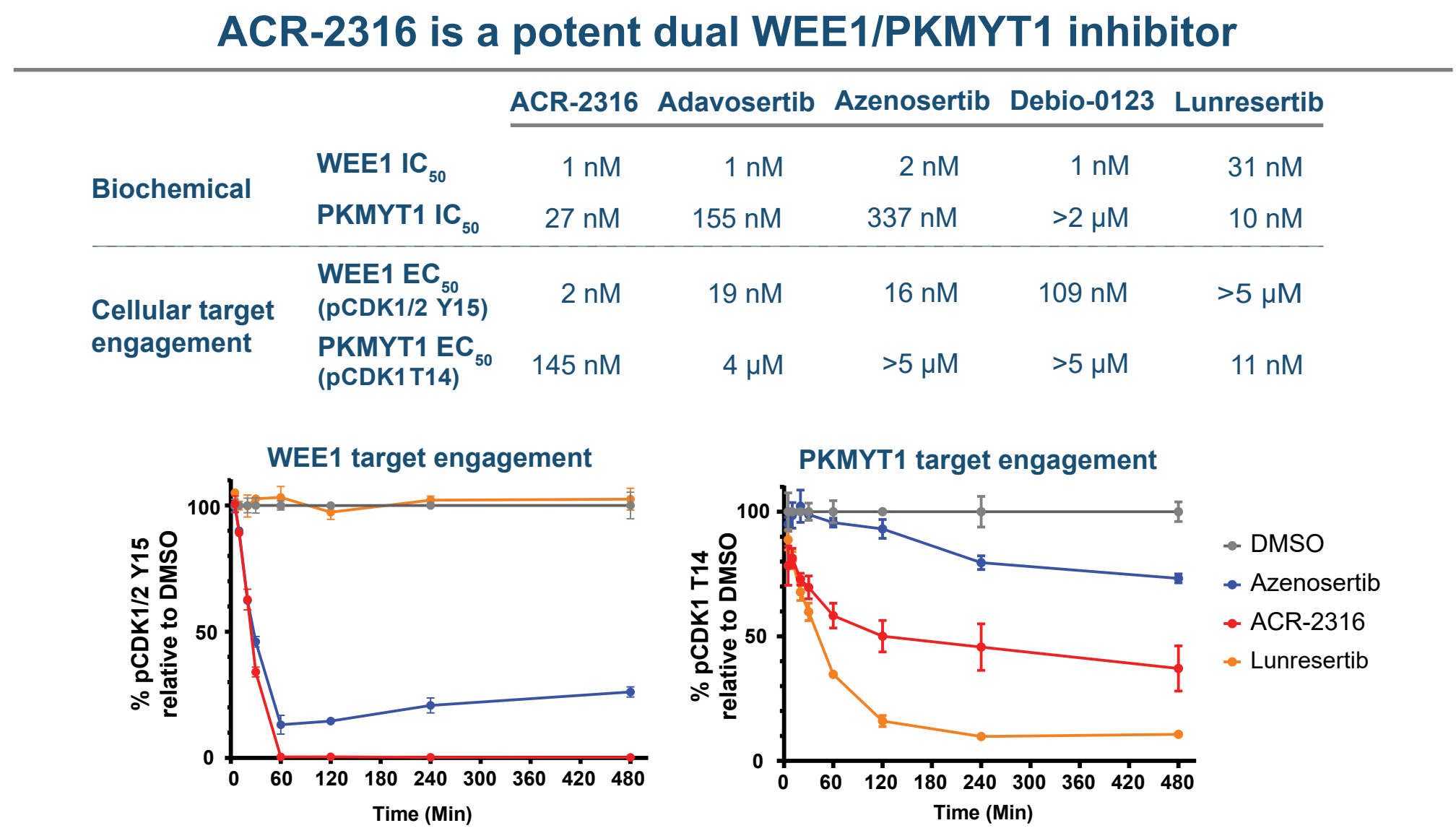
Acrivon's proprietary proteomics-based patient responder identification platform, Acrivon Predictive Precision Proteomics, or AP3, is engineered to measure compound-specific effects on the entire tumor cell protein signaling network and drug-induced resistance mechanisms in an unbiased manner. These distinctive and actionable capabilities enable AP3's direct application for biological SAR and drug design optimization for potent single agent activity by ensuring an optimal target selectivity profile to overcome drug-induced resistance mechanisms, with the goal of clinical monotherapy development and potential for an accelerated development and regulatory pathway.

Here we describe Acrivon's discovery of ACR-2316, a potent, selective WEE1/PKMYT1 inhibitor designed for superior single-agent activity as demonstrated in preclinical studies against benchmark inhibitors. This compound was specifically designed using AP3 to overcome limitations of WEE1-specific inhibition through balanced inhibition of PKMYT1, which our AP3 platform uncovered as a dominant WEE1 inhibitor-induced drug resistance mechanism. Using structure-guided drug design, we achieved exquisite selectivity for WEE1 and PKMYT1 ensuring primarily mechanism-based reversible adverse events. In head-to-head preclinical studies against benchmark clinical molecules, ACR-2316 demonstrates superior potency and activity compared to current clinical WEE1 or PKMYT1 inhibitors. Patient dosing has begun in the ongoing Phase 1 monotherapy clinical trial for ACR-2316, designed to assess the safety and tolerability of ACR-2316.

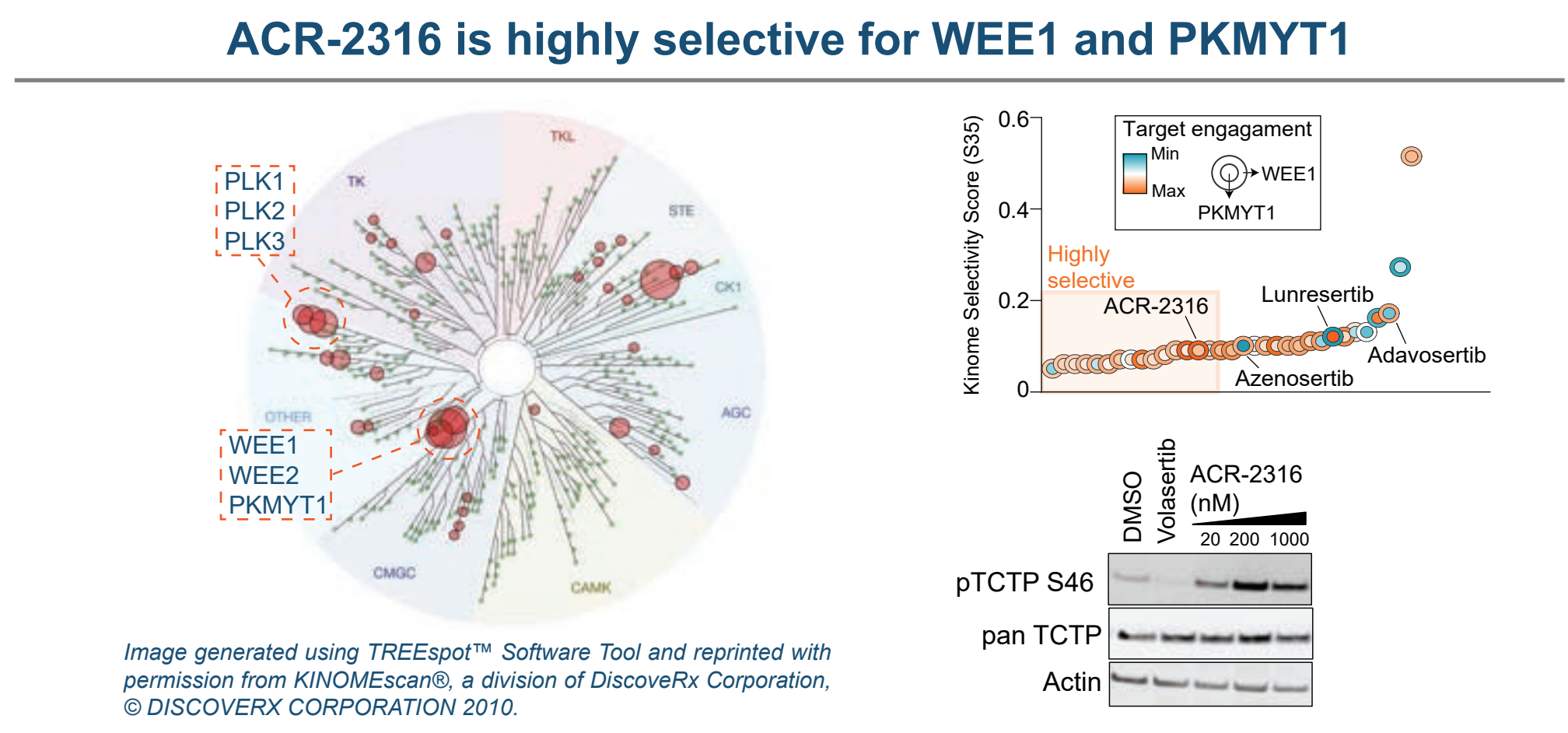
CONCLUSION

- ACR-2316 is a novel, selective dual WEE1/PKMYT1 inhibitor, uniquely designed using AP3 for superior single agent activity to overcome limitations of current WEE1 and PKMYT1 inhibitors, aiming for clinical monotherapy development.
- ACR-2316 demonstrates superior single agent anti-cancer activity and pro-apoptotic cell death in a broad spectrum of in vitro cancer line models including those insensitive to WEE1 or PKMYT1 inhibitors.
- ACR-2316 induces tumor regression in CDX and PDX tumor mouse models with superior activity compared to clinical benchmark inhibitors in head-to-head studies.
- Preclinical data across different dosing regimens show a high degree of flexibility based on anti-tumor efficacy, a result of the AP3-driven selectivity profile, suggesting a favorable clinical therapeutic index
- Preclinical IND-enabling GLP safety-toxicity studies demonstrate that adverse events are mechanism-based but transient and fully reversible.
- The ongoing Phase 1 monotherapy clinical trial for ACR-2316 is designed to assess the safety and tolerability of ACR-2316.

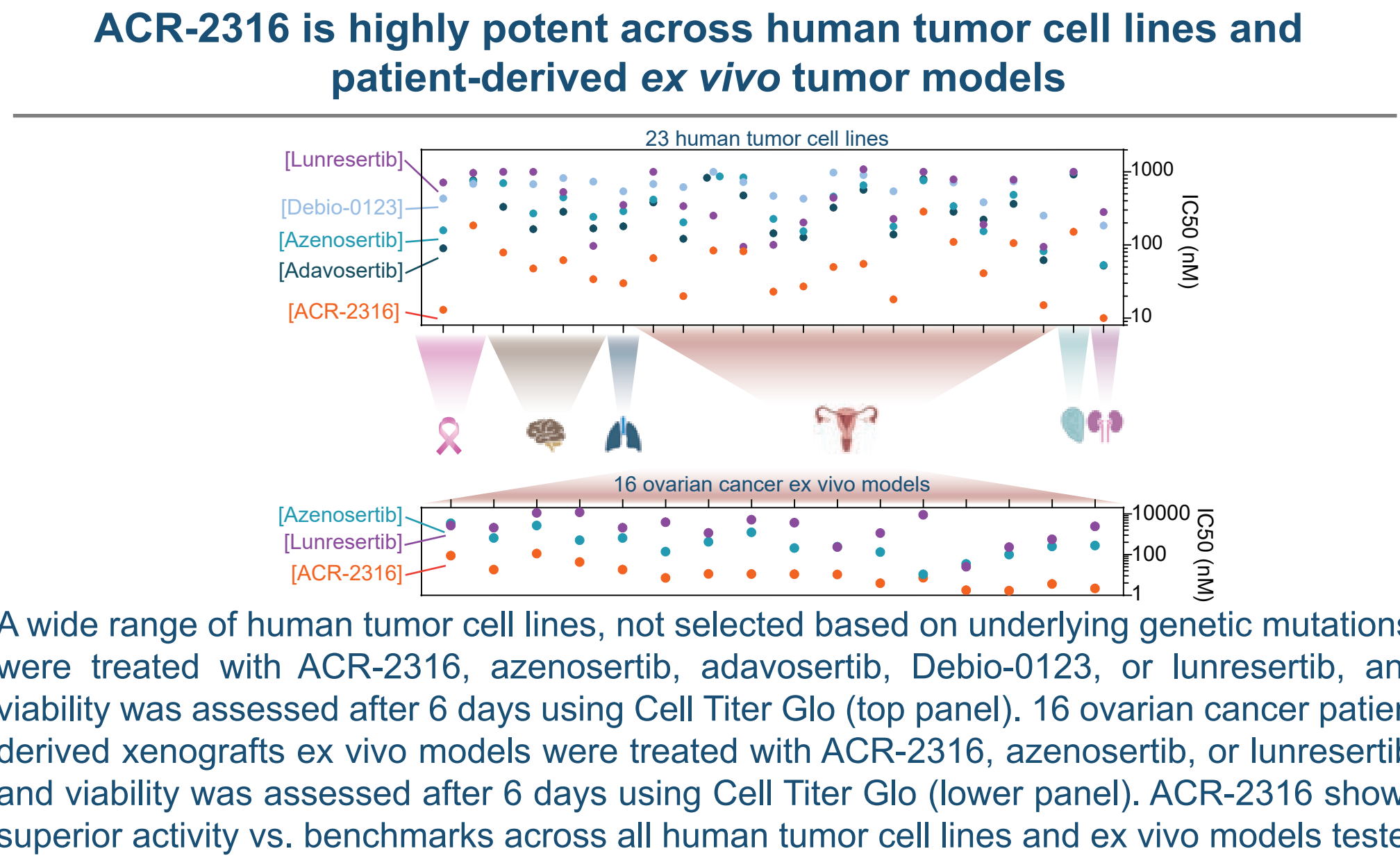
RESULTS



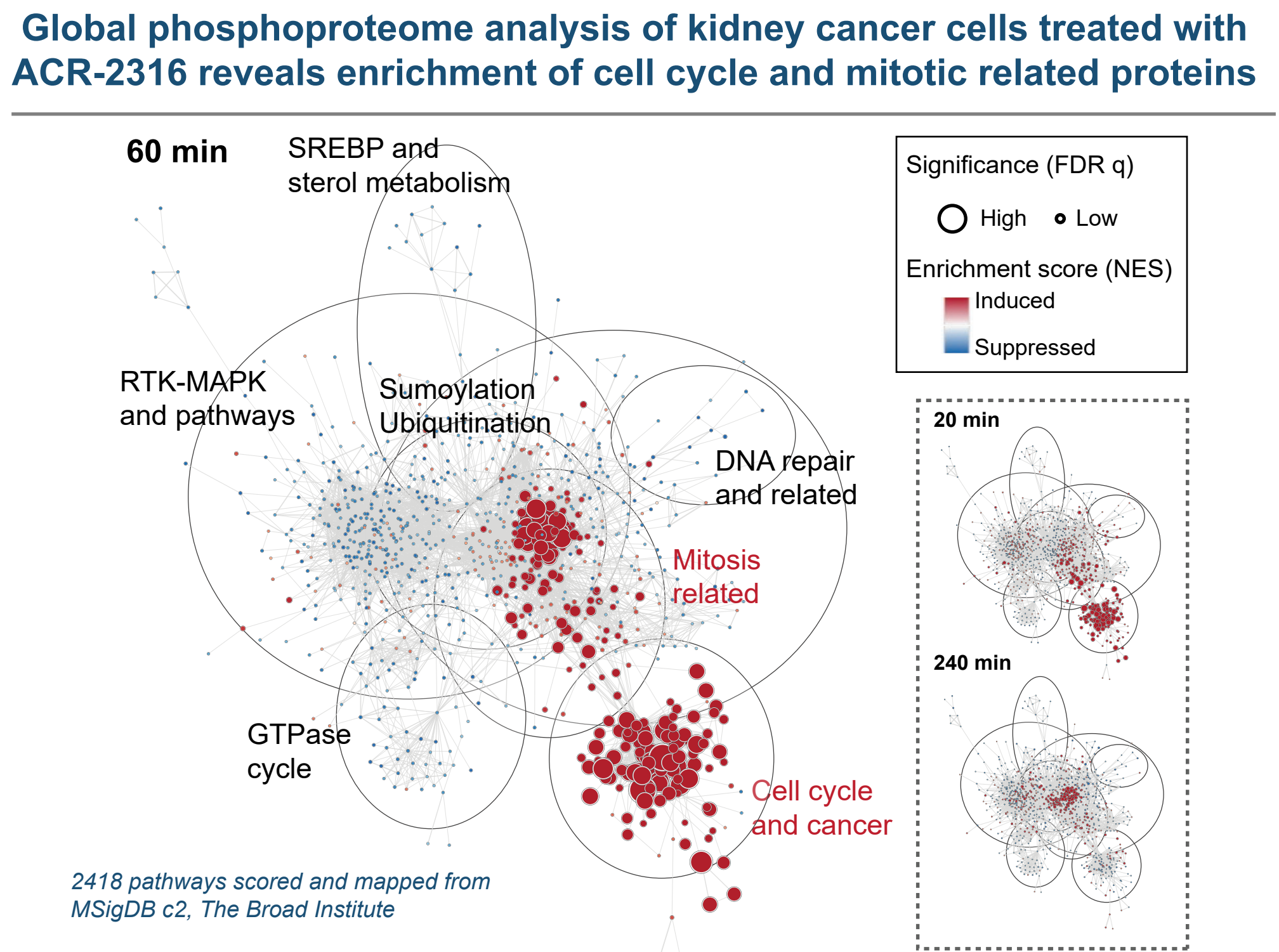
A LanthaScreen Europium (Eu) Kinase Binding Assay was used to determine binding affinities (IC₅₀) to WEE1 and PKMYT1 for ACR-2316, adavosertib, azenosertib, Debio-0123, and lunresertib. WEE1 target engagement (EC₅₀) was assessed using quantitative high content immunofluorescence imaging of nuclear pCDK1/2 Y15 in a human kidney cancer cell line, and PKMYT1 target engagement (EC₅₀) was assessed by the pCDK1 T14 AlphaLISA sandwich immunoassay in a human medulloblastoma cell line (top table). Kinetics of pCDK1/2 Y15 and pCDK1 T14 inhibition in a human kidney cancer cell line treated for 5 - 480 minutes with 20 nM (left) or 200 nM (right) ACR-2316, azenosertib, or lunresertib (bottom graphs).



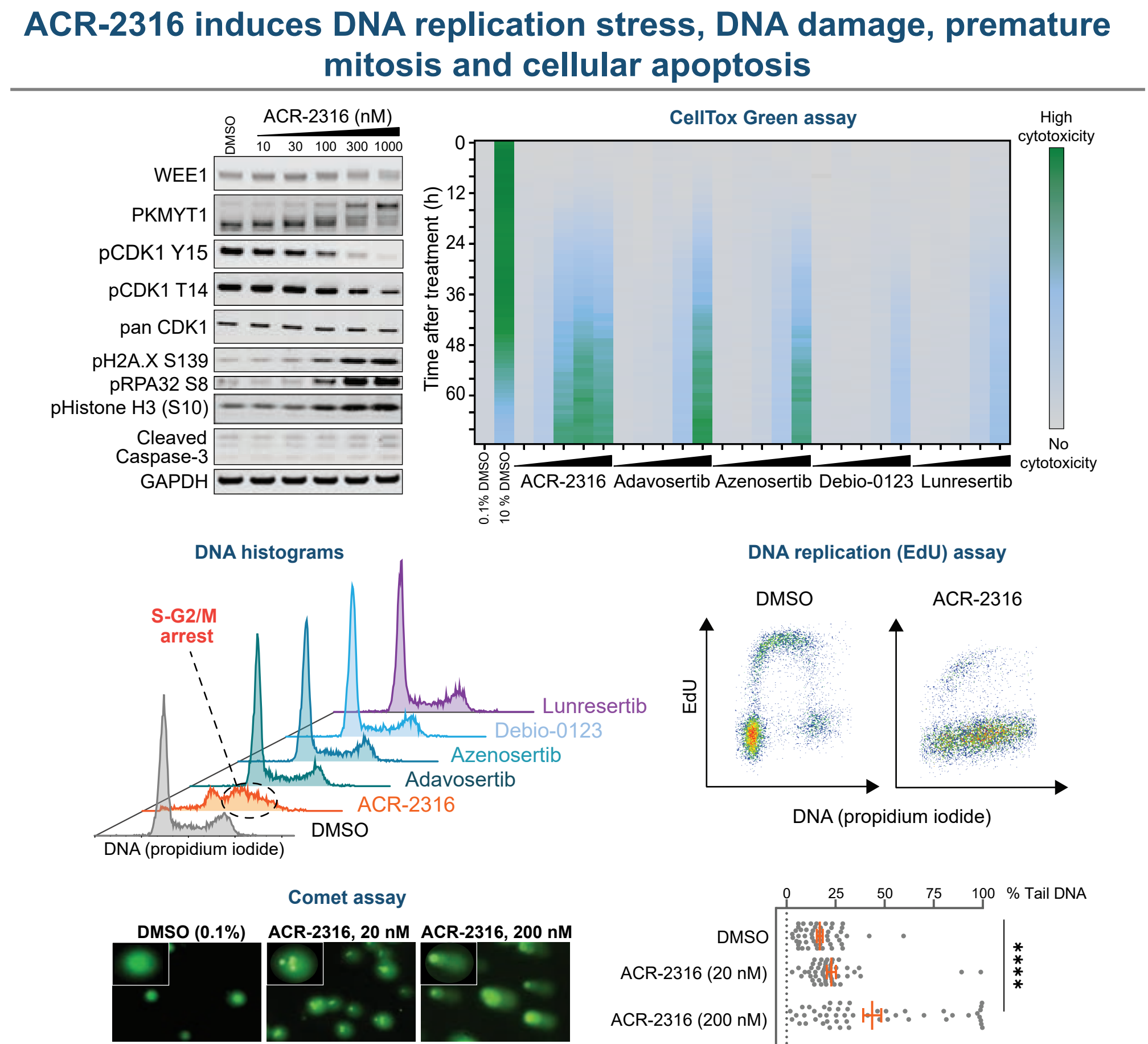
ACR-2316 selectivity was assessed by measuring the binding of ACR-2316 to a comprehensive panel of human kinases included in the scanMAX Kinase Assay Panel at 1 µM drug concentration (left). A compound set of n=33, including ACR-2316 and analogs, azenosertib, adavosertib, and lunresertib were plotted for kinase selectivity score (S35) (y-axis) and target engagement depicted for each compound (upper right). Ovarian cancer cells were treated with PLK1 inhibitor volasertib (1000 nM) and increasing doses of ACR-2316 and blotted for pTCTP S46. ACR-2316 leads to increased pTCTP S46, indicating that ACR-2316 does not inhibit PLK1 activity in cells (lower right).



A wide range of human tumor cell lines, not selected based on underlying genetic mutations, were treated with ACR-2316, azenosertib, adavosertib, Debio-0123, or lunresertib, and viability was assessed after 6 days using Cell Titer Glo (top panel). 16 ovarian cancer patient derived xenografts ex vivo models were treated with ACR-2316, azenosertib, or lunresertib, and viability was assessed after 6 days using Cell Titer Glo (lower panel). ACR-2316 shows superior activity vs. benchmarks across all human tumor cell lines and ex vivo models tested.

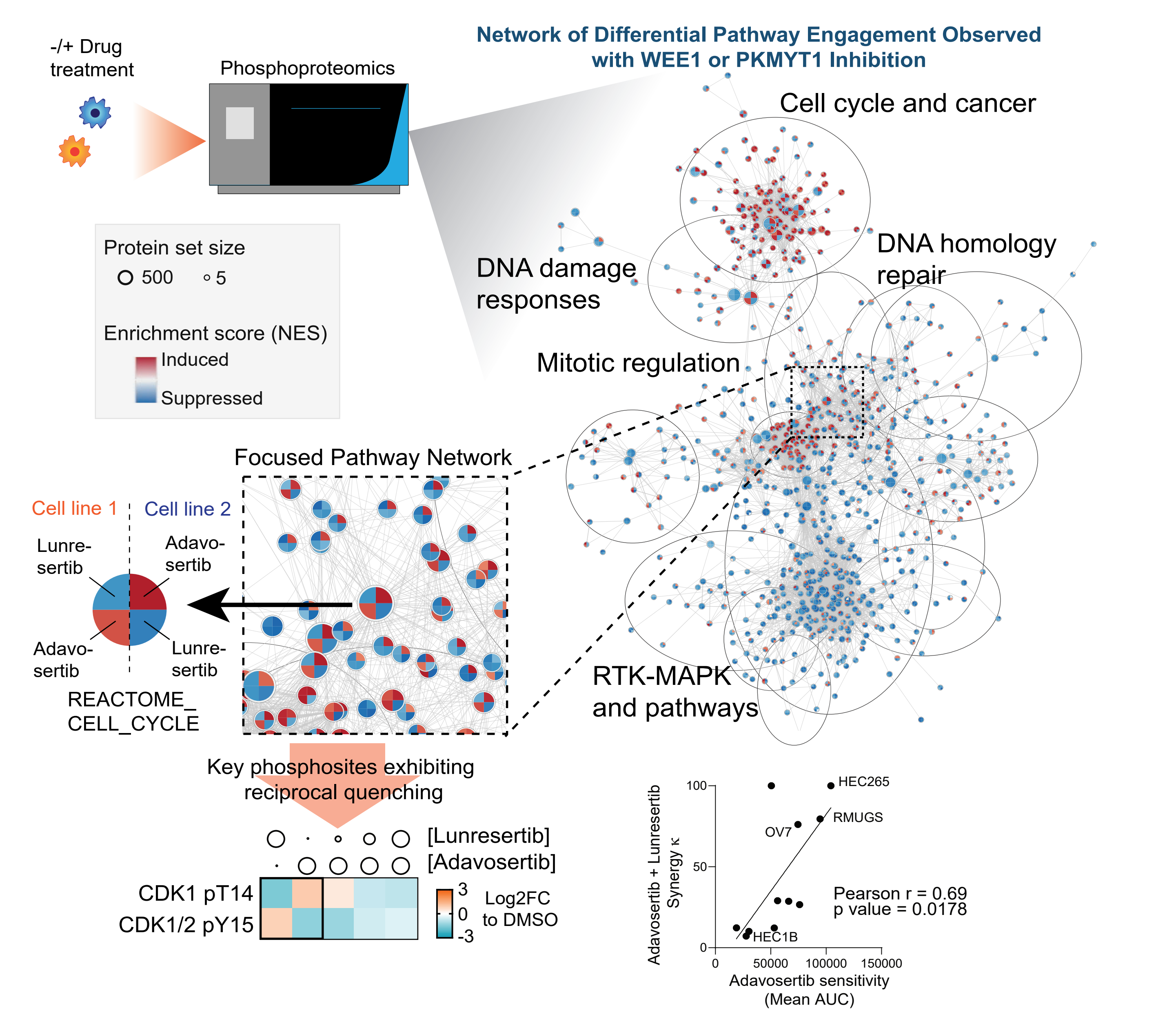


ACR-2316 induced phosphorylation pathway activity relating to cell cycle and mitotic pathways. The phosphoproteomes of ACR-2316 vs. DMSO treated human kidney cancer cells were analyzed via dose dependency (20-200-1000 nM), pathway enrichment, and network construction. Pathway activity was assessed after 20 min, 1 hour, and 4 hours, respectively.



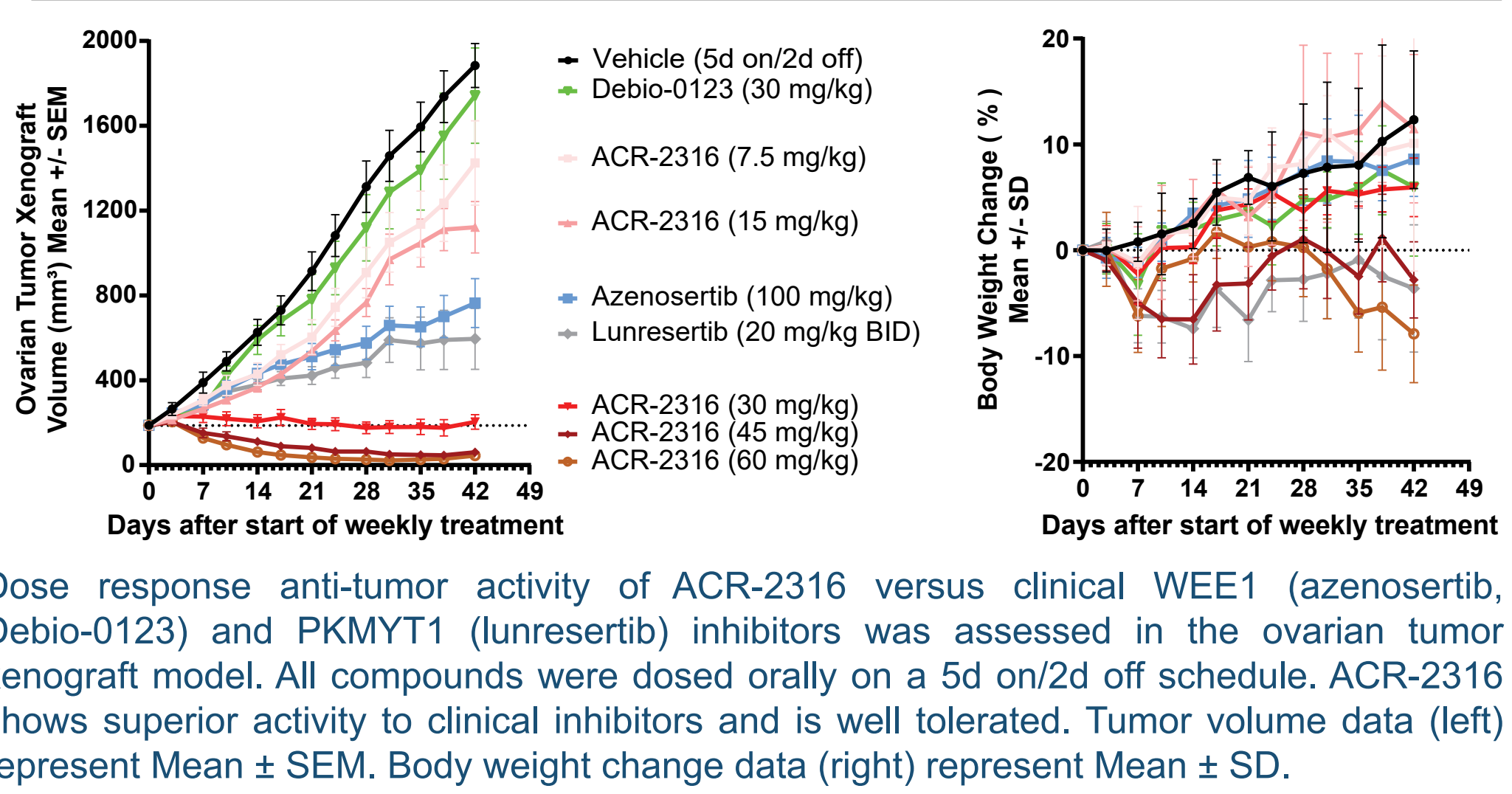
An ovarian cancer cell line was treated with increasing doses of ACR-2316 for 4h and blotted for drug targets (WEE1, PKMYT1), target engagement (pCDK1 Y15/T14), DNA damage (γH2A.X, pRPA32 S8), mitosis (pHistone H3 S10), and apoptosis (cleaved caspase 3) (western blot, upper left). Drug cytotoxicity was measured in ovarian cancer cells using CellTox Green assay for up to 72h (upper right). Disruption of cell cycle was analysed in ovarian cancer cells treated with 100 nM of ACR-2316, adavosertib, azenosertib, Debio-0123, or lunresertib for 24h (DNA histograms, middle panel, left). Disruption of active DNA replication was measured using EdU assay in ovarian cancer cells treated with 100 nM ACR-2316 for 24h (middle panel, right). Induction of DNA breaks was measured using comet assay in ovarian cancer cells treated with 20 nM or 200 nM ACR-2316 for 24h (lower panel left), and the % tail DNA was quantified (lower panel right).

Acrivon's Predictive Precision Proteomics Identifies Signaling Pathways Associated with WEE1 or PKMYT1 Inhibitor Resistance

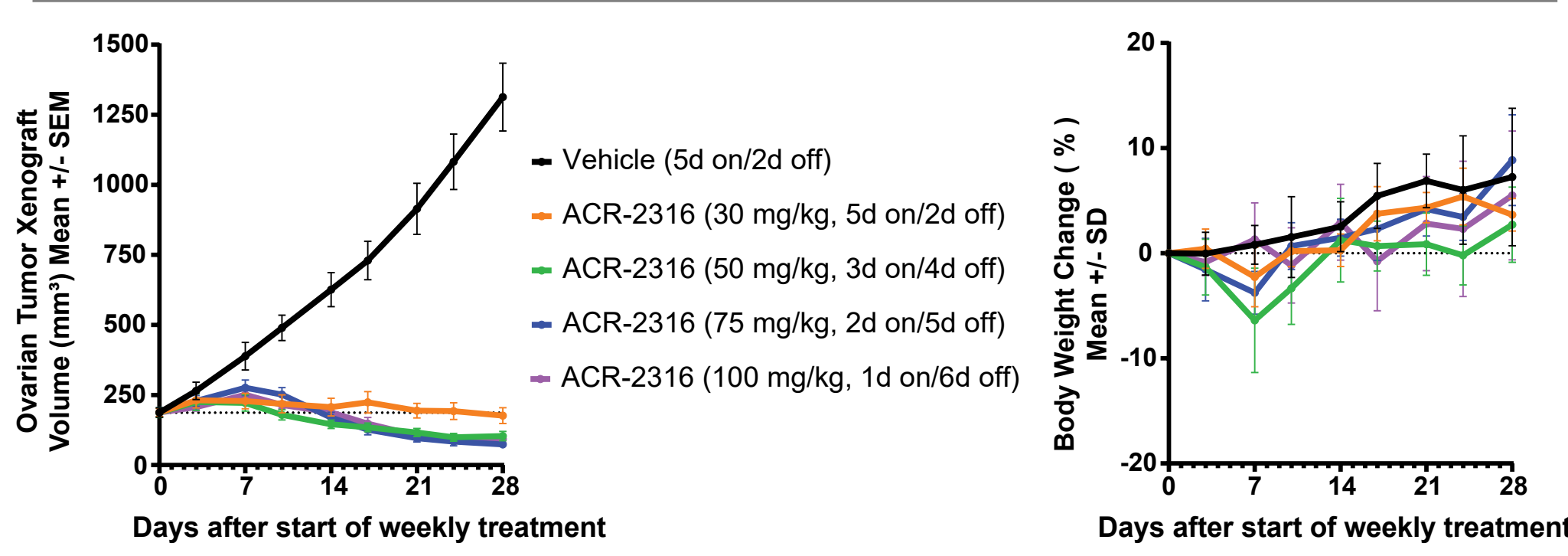


Human tumor cell lines were treated with Adavosertib (200 nM) or Lunresertib (20 nM) for 60 min. Cell lysates were processed for phosphoproteomics mass spectrometry. The drug regulated phosphoproteome was analysed and mapped to cellular pathways to identify resistance mechanisms to WEE1 inhibition. Pathways enriched by WEE1 inhibition included those related to cell cycle, DNA damage response, and mitotic regulation. A subset of these pathways were quenched by PKMYT1 inhibition, with a key phosphosite, CDK1 T14, exhibiting such reciprocal quenching. These data suggest that a combination of WEE1 and PKMYT1 inhibition would be synergistic in terms of fully activating CDK1 and overcoming resistance to WEE1 inhibition. This is supported by correlation between Adavosertib + Lunresertib synergy and WEE1 resistance across human cell line models.

ACR-2316 demonstrates dose-dependent efficacy and near complete regressions in a human ovarian tumor xenograft model

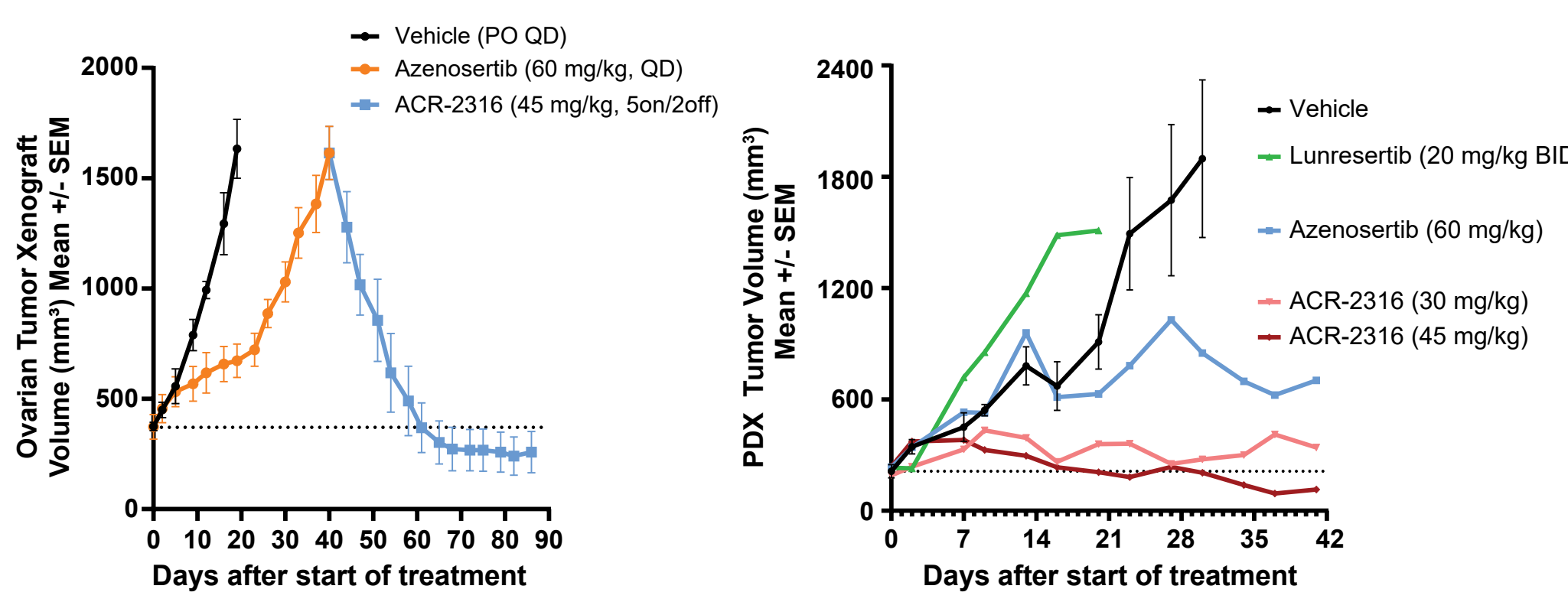


ACR-2316 demonstrates comparable strong efficacy across a broad range of intermittent dosing schedules



Anti-tumor activity of ACR-2316 with different dosing schedules was tested in the ovarian tumor xenograft model. ACR-2316 was administered orally at 100 mg/kg (1d on/6d off), 75 mg/kg (2d on/5d off), 50 mg/kg (3d on/4d off) or 30 mg/kg (5d on/2d off) and demonstrated comparable efficacy at all doses tested. Tumor volume data (left) represent mean ± SEM. Body weight change data (right) represent Mean ± SD.

ACR-2316 promotes rapid regression of ovarian tumor xenografts progressing on azenosertib, and superior anti-tumor activity in an ovarian PDX model compared to clinical WEE1 or PKMYT1 inhibitors



Mice bearing ovarian tumor xenograft tumors were treated with azenosertib at 60 mg/kg daily until progression to TV~1,500 mm³, then switched to ACR-2316 at 45 mg/kg (5d on/2d off). ACR-2316 demonstrates rapid and deep regression of tumors progressing on azenosertib. Tumor volume data (left) represent mean ± SEM. Mice bearing ovarian cancer patient derived xenograft (PDX) tumors were treated on a 5d on/2d off dosing schedule with ACR-2316 at 30 or 45 mg/kg, azenosertib at 60 mg/kg or lunresertib at 20 mg/kg BID. ACR-2316 shows superior activity to clinical WEE1 and PKMYT1 inhibitors. Tumor volume data (right) represent mean ± SEM.