Combining Palazestrant, a CERAN, and Capivasertib, a pan-AKT Inhibitor, Enhances Tumor Suppression in ER+/HER2- Breast Cancer Models

Abstract # 212

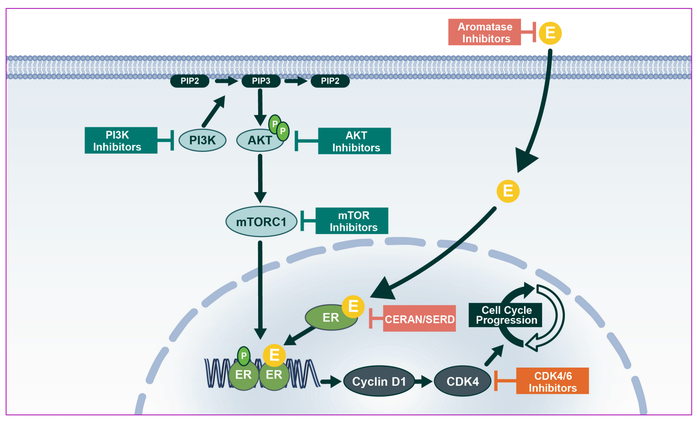
Susanna A. Barratt, Gopinath S. Palanisamy, Brandon Robello, Guadalupe Peña, Raymond A. Ng, David C. Myles

Olema Oncology, San Francisco, CA

Background

- Resistance to endocrine therapies is associated with the acquisition of hyperactive oncogenic mutations in the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) axis[1,2]
- Capivasertib, a pan-AKT inhibitor, was recently approved in combination with fulvestrant for patients with hormone receptor positive (HR+) locally advanced or metastatic breast cancer whose tumors harbor at least one phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA)/serine threonine kinase 1 (AKT1)/ phosphatase and tensin homolog (PTEN) mutation[3,4]
- The activity observed with this combination suggests that combining capivasertib with an endocrine therapy that demonstrates superior estrogen receptor (ER) antagonism may offer greater therapeutic benefit for patients (**Figure 1**)

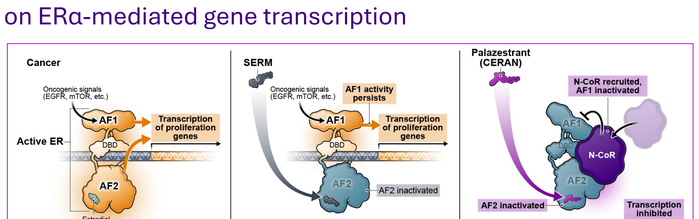
Figure 1: Signaling cascade showing targets of SoC agents for ER+ breast cancer



AKT, protein kinase B; **CDK4/6**, cyclin-dependent kinase 4/6; **CERAN**, complete estrogen receptor antagonist; **E**, estrogen; **ER**, estrogen receptor; **mTOR**, mechanistic target of rapamycin; **mTORC1**, mechanistic target of rapamycin complex 1; **P**, phosphorylation; **PI3K**, phosphatidylinositol-3-kinase; **PIP2**, phosphatidylinositol 4,5-bisphosphate; **PIP3**, phosphatidylinositol 3,4,5-trisphosphate; **SERD**, selective estrogen receptor degrader; **SOC**, standard of care.

- Palazestrant (OP-1250), is a potent, orally bioavailable, and brain-penetrant complete estrogen receptor antagonist (CERAN) (**Figure 2**) that has demonstrated favorable tolerability and efficacy in patients with heavily pretreated ER+/HER2- metastatic breast cancer[5]
- Palazestrant is currently undergoing a Phase 3 clinical trial (OPERA-01) for the treatment of ER+/HER2- breast cancer[6]

Figure 2: Differential effects of estradiol, SERMs, and CERANs on ERα-mediated gene transcription



AF, activation factor; **CERAN**, complete estrogen receptor antagonist; **DBD**, DNA binding domain; **EGFR**, epidermal growth factor receptor; **ER**, estrogen receptor; **mTOR**, mammalian target of rapamycin; **N-CoR**, nuclear receptor corepressor; **SERM**, selective estrogen receptor modulator.

Here we demonstrate the effectiveness of combining palazestrant and capivasertib in ER+ breast cancer models.

Methods

Cell Proliferation Assays

- Cells were plated in 96-well plates at optimized densities in appropriate complete medium and incubated overnight. Cells were treated with serial dilutions of capivasertib, fixed concentrations of palazestrant, and 100 pM estradiol for 8 days.
- Cell number was assessed using CellTiter-Glo or CyQuant and normalized to T=0.

Drug Combination Analysis

- Synergy was evaluated using SynergyFinder 3.0 tools, specifically the Zero Interaction Potency (ZIP) model, which assumes that the combined effect of two molecules is the sum of their individual responses, without any interaction.
- Combinations were normalized to the monotherapy response; deviations >10 from this predicted combined effect indicate synergy (greater effect), between -10 and +10 indicate additive (equal) effect, and <10 indicate antagonism (lesser effect).

Xenograft Studies

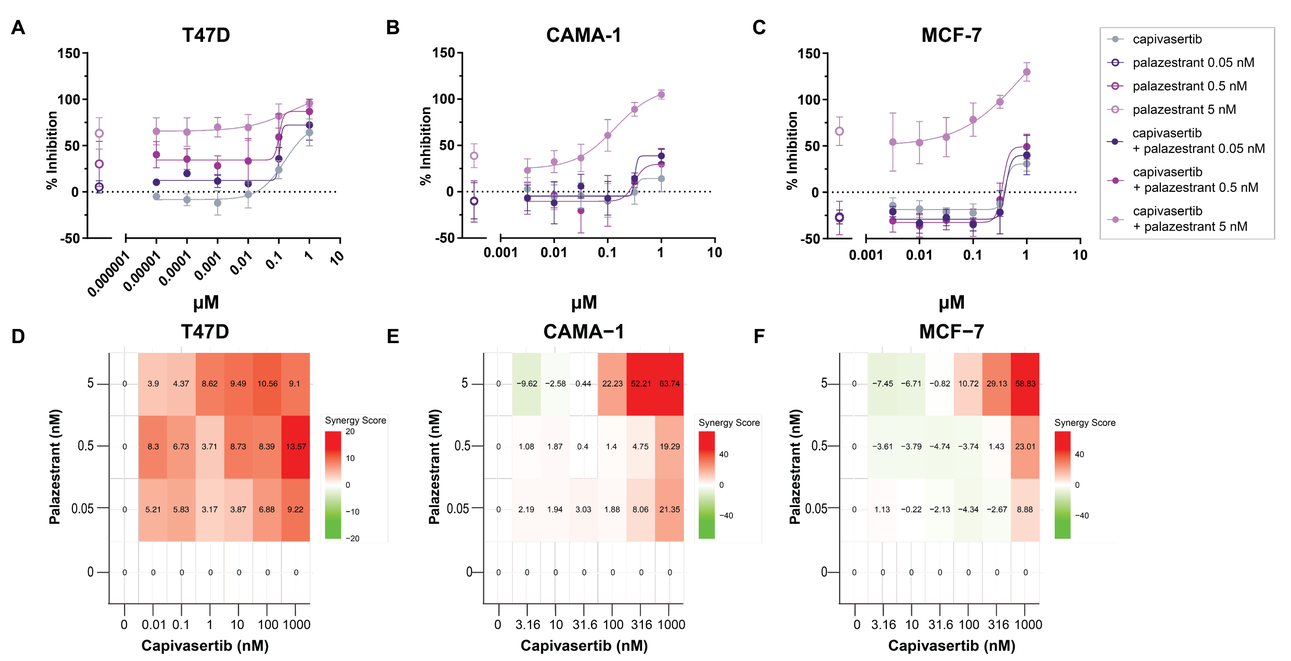
- Female, athymic nude (immune-deficient) mice were supplemented with estradiol and implanted subcutaneously with an ER+ breast cancer cell line, T47D, in mammary fat region. Mice were randomized into groups when the tumor volume reached ~150 mm^3 and were treated for 28 days with either vehicle, palazestrant at 10 mg/kg, fulvestrant at 25 mg/kg, capivasertib at 100 mg/kg or combinations thereof.

RNA-Sequencing (RNA-Seq)

- Total RNA was extracted from snap-frozen xenograft tumors using the Qiagen RNeasy Plus Universal Mini Kit following the manufacturer's instructions. RNA sequencing libraries were prepared using the NEBNext Ultra II RNA Library Prep kit and sequenced on an Illumina NovaSeq X as paired-end 150-nt reads. Sequence reads were trimmed using Trimmomatic v.0.36 and referenced to the GRCh38 human reference genome using the STAR aligner v2.5.2b. Differential gene expression analysis was performed using DESeq2 v1.16.1.

Palazestrant and capivasertib synergize to inhibit proliferation of T47D, CAMA-1, and MCF7 breast cancer cell lines

Figure 3: Dose-response curves (**A-C**) and synergy plots (**D-F**) for T47D (**A,D**), CAMA-1 (**B,E**) and MCF7 cells (**C,F**)

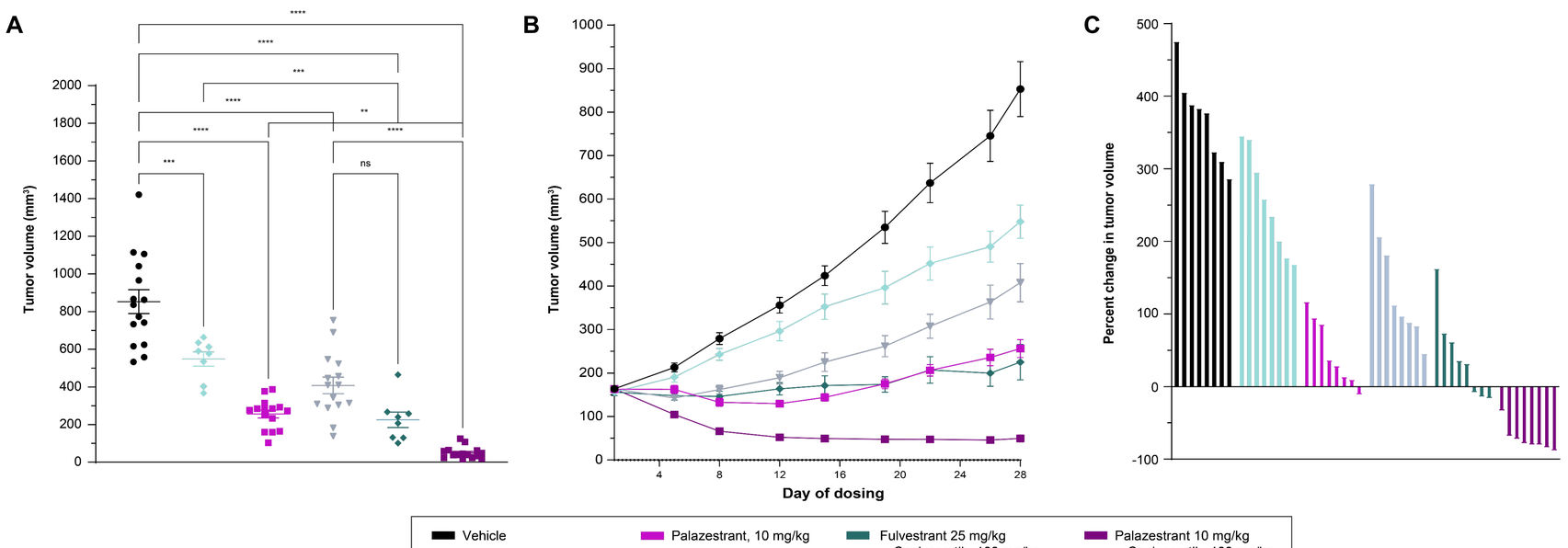


- Capivasertib alone weakly inhibited proliferation of T47D, CAMA-1, and MCF-7 ER+/HER2- immortalized breast cancer cell lines (**Figure 3A-C**)
- Palazestrant alone inhibited proliferation of these cell lines with low nanomolar potencies (**Figure 3A-C**)
- Scores from synergy plots suggested that combining palazestrant and capivasertib inhibited cell proliferation more than either agent alone (**Figure 3D-F**)

Palazestrant in combination with capivasertib significantly inhibits and represses tumor growth in vivo

- As monotherapies, palazestrant was more effective than fulvestrant or capivasertib in inhibiting tumor growth (**Figure 4 A,B**)
- The combination of palazestrant and capivasertib significantly enhanced tumor growth inhibition compared to each agent alone (**Figure 4 A-C**)
- The fulvestrant/capivasertib combination resulted in greater tumor growth inhibition compared to monotherapy treatments; however, the effect was only significant compared to fulvestrant alone (**Figure 4A**)
- Combination of fulvestrant and capivasertib primarily resulted in tumor growth inhibition, while the palazestrant/capivasertib combination yielded complete tumor regression in all animals (**Figure 4C**)

Figure 4: Scatter plot (**A**), tumor volume over time (**B**), and waterfall plot (**C**) of a 28-day T47D xenograft tumor model

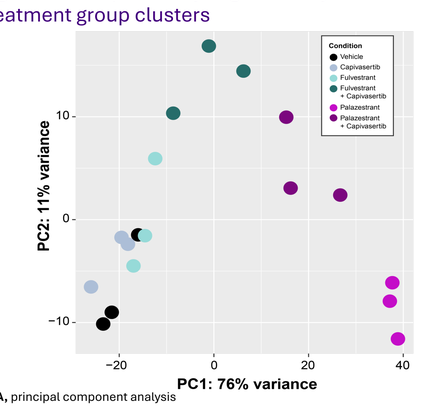


ns, not significant, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

Palazestrant and capivasertib downregulate genes associated with cell cycle progression

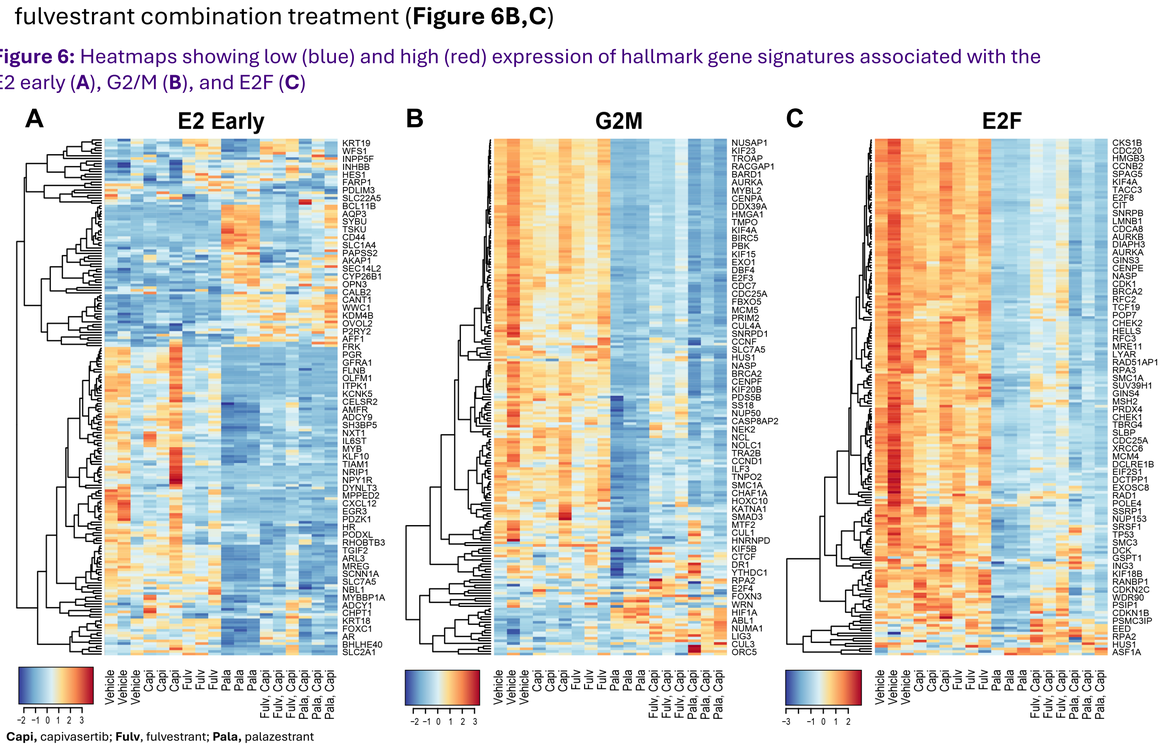
- Sequenced RNA isolated from xenograft tumors indicated that palazestrant treatment altered gene expression to a greater degree than treatment with capivasertib or fulvestrant (**Figure 5**), particularly when specific hallmark gene signatures were analyzed (**Figure 6A-C**)
- Gene sets identified to be enriched following combination treatment with either fulvestrant or palazestrant were broadly mirrored (**Figure 7**; fulvestrant data not shown); however, greater downregulation of G2M and E2F gene signatures was observed with palazestrant compared to fulvestrant combination treatment (**Figure 6B,C**)

Figure 5: PCA plot analyzing similarity between treatment group clusters



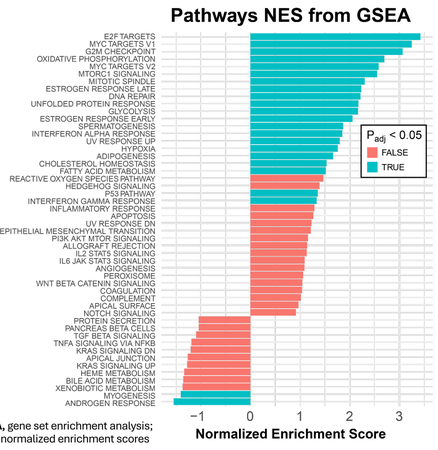
PCA, principal component analysis

Figure 6: Heatmaps showing low (blue) and high (red) expression of hallmark gene signatures associated with the E2 early (**A**), G2/M (**B**), and E2F (**C**)



Capi, capivasertib; **Fulv**, fulvestrant; **Pala**, palazestrant

Figure 7: Significant differential regulation of hallmark gene signatures with palazestrant combination treatment



GSEA, gene set enrichment analysis; **NES**, normalized enrichment scores

Conclusions

- **Palazestrant and capivasertib demonstrate synergistic activity in ER+ breast cancer models, both in vitro and in vivo.**
- **Combining palazestrant and capivasertib increases downregulation of genes associated with cell cycle progression.**
- **Palazestrant demonstrates superior anti-tumor efficacy over fulvestrant when in combination with capivasertib.**
- **These data support clinical investigation of the combination of palazestrant and capivasertib.**

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