Combining Palazestrant, a CERAN, and Everolimus, an mTOR Inhibitor, Enhances Tumor Suppression in ER+/HER2- Breast Cancer Models

Abstract # 211

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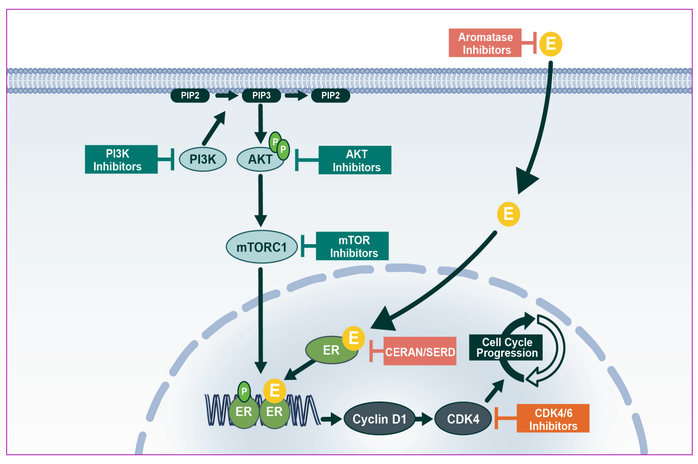
Background

- In patients with advanced estrogen receptor-positive (ER+)/human epidermal growth factor receptor 2-negative (HER2-) breast cancer, the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) axis is implicated in acquired resistance to standard-of-care (SoC) agents[1,2]
- Targeting this axis with an mTOR inhibitor (everolimus), in combination with an endocrine agent (**Figure 1**), has demonstrated clinical benefit[3,4]

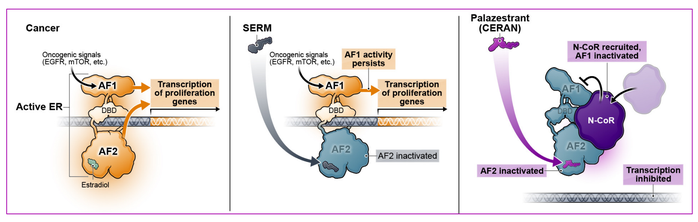
Figure 1: Signaling cascade showing targets of SoC agents for ER+ breast cancer



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; **PIP2**, phosphatidylinositol 4,5-bisphosphate; **PIP3**, phosphatidylinositol 3,4,5-trisphosphate; **SoC**, standard-of-care; **SERD**, selective estrogen receptor degrader.

- Palazestrant (OP-1250), is an orally bioavailable complete estrogen receptor antagonist (CERAN) (**Figure 2**) that exhibits a long half-life and combines well with CDK4/6 inhibitors[5,6]
- Palazestrant has demonstrated favorable tolerability and efficacy in patients with heavily pretreated ER+/HER2- metastatic breast cancer (MBC) [7]
- A Phase 3 study of palazestrant monotherapy vs SoC in second-/third-line (2/3L) ER+/HER2- MBC is ongoing (OPERA-01; NCT06016738)

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



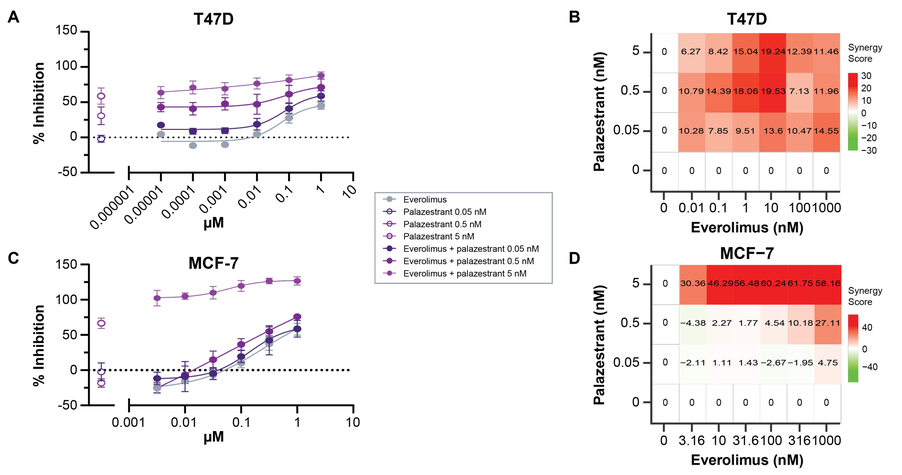
AF, activation factor; **CERAN,** complete estrogen receptor antagonist; **DBD,** DNA binding domain; **EGFR,** epidermal growth factor receptor; **ER,** estrogen receptor; **mTOR,** mammalian target of rapamycin; **N-CoR,** nuclear receptor corepressor; **SERM,** selective estrogen receptor modulator.

Here we show that palazestrant combines effectively with everolimus in preclinical models of ER+ breast cancer.

Palazestrant and everolimus synergize in vitro to inhibit proliferation of *ESR1*^{WT}- and *ESR1*^{Y537S}-mutant cell lines

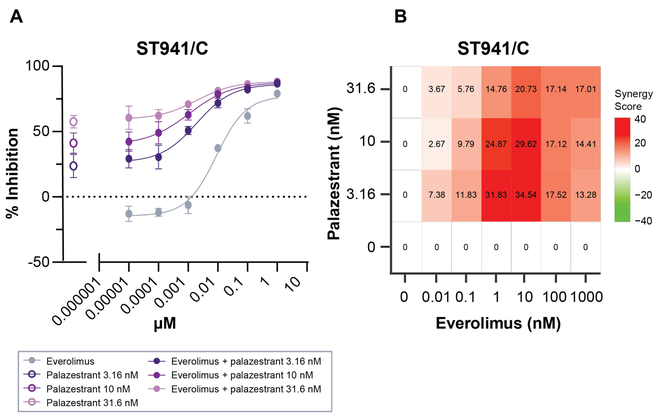
- Everolimus alone weakly inhibited proliferation of ER+/HER2-, *ESR1*^{WT} immortalized breast cancer cell lines; palazestrant potently inhibited growth of these cell lines (**Figure 3A,C**)
- Combining palazestrant with everolimus resulted in greater anti-proliferative activity than either agent alone (**Figure 3A,C**); scores from synergy plots suggested that palazestrant and everolimus act synergistically when combined (**Figure 3B,D**)

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



- The ST941/C model is a cell line derivative of the heterozygous *ESR1*^{Y537S} ST941 ER+/HER2- patient-derived xenograft (PDX) model
- Both everolimus and palazestrant inhibited proliferation of ST941/C (**Figure 4A**)
- When combined, greater antiproliferation was observed, and synergy was indicated by synergy scoring (**Figure 4B**)

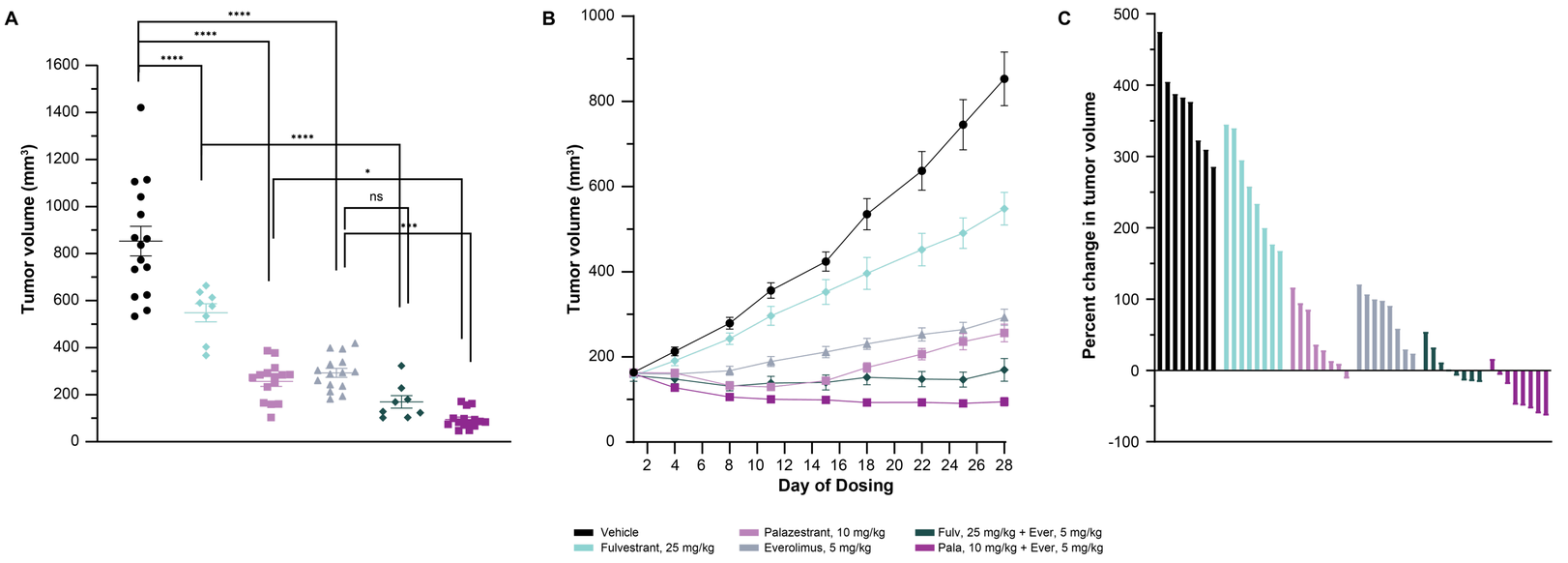
Figure 4: Dose-response curves and synergy plots for ST941/C



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

- Palazestrant and everolimus robustly inhibited T47D tumor growth as monotherapies (**Figure 5A,B**)
- Combination of palazestrant and everolimus significantly enhanced tumor growth inhibition compared to each agent alone (**Figure 5A,B**)
- Fulvestrant monotherapy was less effective than palazestrant monotherapy; combination of fulvestrant with everolimus improved tumor growth inhibition, but was not significantly different compared to everolimus alone (**Figure 5A,B**)
- Tumor regression was observed for all but one animal treated with the palazestrant/everolimus combination (**Figure 5C**)

Figure 5: Scatter plot (**A**), tumor volume over time (**B**), and waterfall plot (**C**) of a 28-day T47D xenograft tumor model

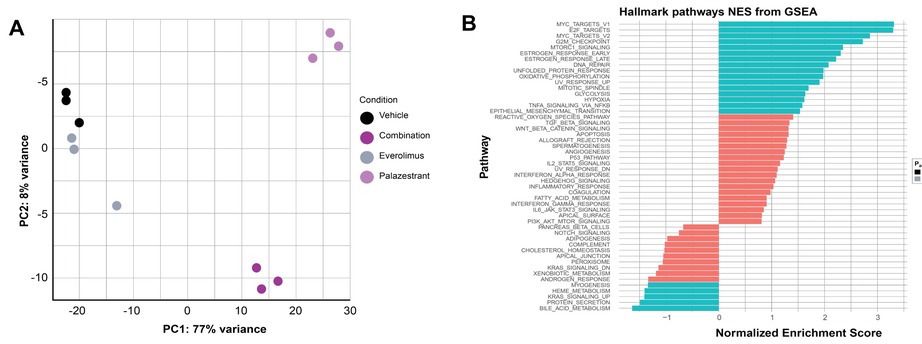


Sidak's multiple comparison test; ****$P < 0.0001$; ***$0.0001 \le P < 0.0001$; **$0.001 \le P < 0.01$; *$0.01 \le P < 0.05$; ns $P > 0.05$
ns, not significant.

Palazestrant and everolimus cause transcriptional changes associated with cell cycle progression and apoptosis

- RNA sequencing of xenografts revealed more gene expression changes with palazestrant monotherapy than everolimus, and unique variability in the combination treatment (**Figure 6A**)
- In addition to E2 and mTOR, the top differentially expressed gene signatures with combination treatment were proliferation and metabolism related (**Figure 6B**)
- Palazestrant and combination treatment had similar effects on expression of E2 early and G2/M genes (**Figure 7A,B**)
- Combination treatment partially reversed the palazestrant-induced changes in the expression of PI3K-AKT-mTOR gene signatures (**Figure 7C**)
- Apoptosis genes were uniquely affected with the combination compared to monotherapy treatments (**Figure 7D**)

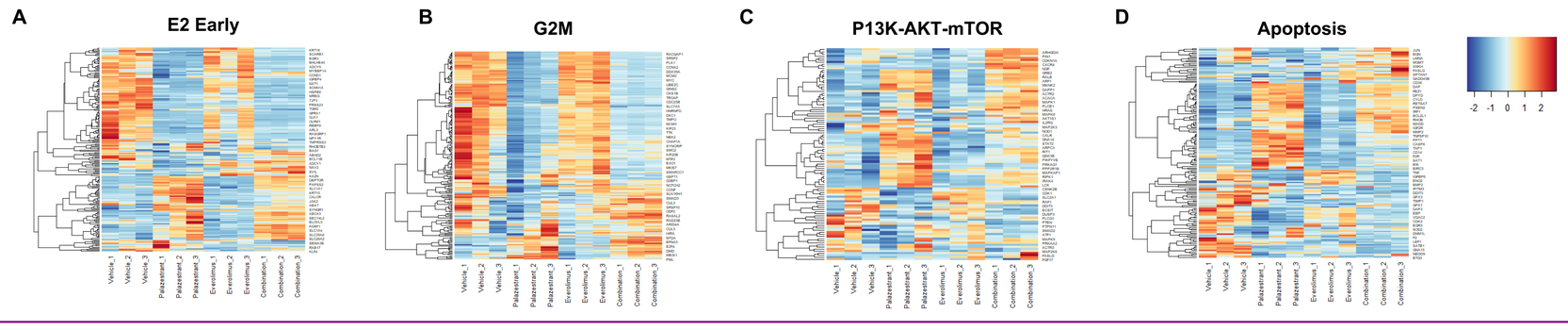
Figure 6: PCA plot analyzing similarity between treatment group clusters (**A**); significant differential regulation of hallmark gene signatures with palazestrant and everolimus combination treatment (**B**)



PCA, principal component analysis

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

Figure 7: Heatmaps showing low (blue) and high (red) expression of hallmark gene signatures associated with E2 early (**A**), G2/M (**B**), PI3K-AKT-mTOR (**C**), and apoptosis (**D**)



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 everolimus, fixed concentrations of palazestrant, and 100 pM estradiol for 7 to 14 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 the mammary fat region. Mice were randomized into groups when the tumor volume reached ~150 mm^3 and were treated for 28 days with vehicle, palazestrant at 10 mg/kg, fulvestrant at 25 mg/kg, everolimus at 5 mg/kg, or combinations thereof.

RNA-Sequencing (RNA-Seq)

- Total RNA was extracted from snap-frozen xenograft tumors using the MagMAX™ mirVana™ Total RNA Isolation Kit on the KingFisher Apex instrument following manufacturer's instructions for tissue samples. For each sample, 500-2000 ng of total RNA was then used for Illumina® Stranded mRNA Prep. Libraries were sequenced on an Illumina NovaSeq X as paired-end 150-nt reads. Sequence reads were analyzed with the STAR alignment – DESeq2 software pipeline.

Conclusions

- **Palazestrant and everolimus demonstrate synergy in vitro and in vivo and robustly inhibit T47D tumor growth.**
- **Combining palazestrant with everolimus downregulates cell cycle and upregulates apoptosis gene signatures.**
- **These data support clinical investigation of the combination of palazestrant and everolimus.**
- **A Phase 1/2 study of palazestrant with everolimus in patients with advanced or metastatic ER+/HER2- breast cancer is ongoing (NCT05508906).**

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For more details about OP-1250-003 or OPERA-01, please visit clinicaltrials.gov