Precision Run-on Sequencing (PRO-Seq) Analysis of a Treatment Time Course in ER+ Breast Cancer Cell Lines Profiles the Transcriptional Changes Underlying Response to Complete Estrogen Receptor Antagonist (CERAN) OP-1250

5375

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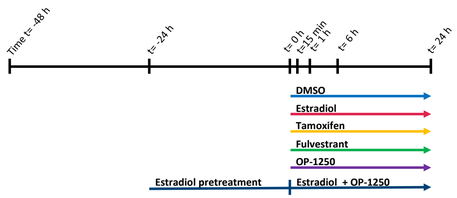
Background

- OP-1250 is an orally bioavailable, small-molecule Complete Estrogen Receptor ANtagonist (CERAN) that potently and completely inactivates the estrogen receptor (ER)
- OP-1250 has been shown to shrink wild-type and ESR1 mutant estrogen receptor-positive (ER+) tumors in multiple preclinical models
- CERANs, including OP-1250 and fulvestrant, lack agonistic estrogen-like effects and completely block the effects of estrogen, whereas selective estrogen receptor modulators (SERMs), such as tamoxifen, have been shown to have some agonistic activity
- Precision run-on sequencing (PRO-Seq) detects nascent RNA and thus can detect transcriptional changes at shorter time scales than those of traditional RNA-Seq methods
- PRO-Seq was applied to antiestrogen treatment in ER+ breast cancer cell lines to profile the direct transcriptional effects of OP-1250, fulvestrant, and tamoxifen

Methods

- PRO-Seq was conducted 15 minutes, 1 hour, 6 hours, and 24 hours after administration of OP-1250, estradiol, fulvestrant, and tamoxifen in 2 ER+ cell lines, MCF7 and CAMA-1 (**Figure 1**)
  - Cells were switched to an estrogen-depleted medium 48 hours prior to compound treatment
  - Treatment with OP-1250 was conducted in the absence of estradiol and after 24 hours of estradiol pretreatment to generate a detailed profile of the time course of OP-1250 action
- Cell culture medium: Richter's minimum essential medium (MEM) modification (Thermo Fisher A1048801) + 5% charcoal dextran-stripped serum (SH30068) + 1× nonessential amino acids + 1× GlutaMAX (Thermo Fisher)
  - Treatments: 316 nM OP-1250, 100 pM estradiol, 316 nM fulvestrant, and 316 nM OH-tamoxifen
- Run-on and subsequent PRO-Seq library preparation experiments were performed on permeabilized cell pellets in a freeze buffer as described in the Mahat et al protocol,[1] but with modifications
  - The RNA libraries were prepared and sequenced using the Illumina NextSeq500 (single read, 1×75 base pairs)
- Data were analyzed and visualized using Arpeggio Insights portal
- Cellular proliferation assay: 1000 cells/well were treated for 7 days with dimethylsulfoxide vehicle, 100 pM estradiol, 316 nM OH-tamoxifen, 316 nM fulvestrant, or 316 nM OP-1250. Cell number was measured at 1, 3, 5, and 7 days after dosing using CyQUANT fluorescent reagent

Figure 1. PRO-Seq Experimental Design



Study experimental design. MCF7 and CAMA-1 cells were treated with the compounds listed and harvested 15 minutes, 1 hour, 6 hours, or 24 hours following treatment. In the estradiol-pretreatment condition, OP-1250 was applied following 24 hours of estradiol treatment.
DMSO, dimethylsulfoxide.

Reference

1. Mahat D, et al. *Nat Protoc.* 2016;11(8):1455-76.

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Disclosures

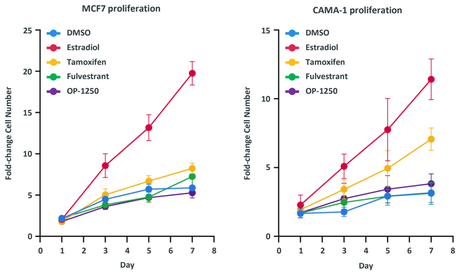
Alison D. Parisian: Olema Oncology: Salary, Ownership interest. **Caitlin Miller:** None. **Fabian E. Ortega:** Olema Oncology: Salary, Ownership interest. **Leslie Hodges-Gallagher:** Olema Oncology: Salary, Ownership interest. **Susanna Barratt:** Olema Oncology: Salary, Ownership interest. **Joey Azofeifa:** None. **Peter J. Kushner:** Olema Oncology: Salary, Ownership interest. **David Kulp:** Olema Oncology: Salary, Ownership interest. **Cyrus L. Harmon:** Olema Oncology: Salary, Ownership interest, IP rights; IDbyDNA: Ownership interest; Primary Diagnostics: Ownership interest

For an e-Print and supplemental slides, scan the above QR code.

Results

- Cellular proliferation assay illustrates stimulation of proliferation by tamoxifen in CAMA-1 cells, but not in MCF7 cells (**Figure 2**)

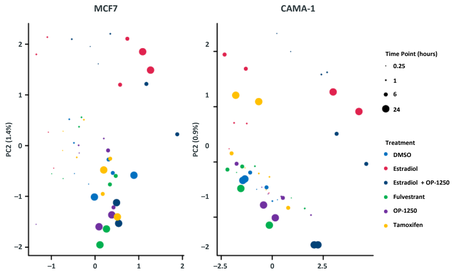
Figure 2. Cell Proliferation in Response to Compound Treatment



Cellular proliferation assay of MCF7 and CAMA-1 cells treated for 7 days with DMSO vehicle, 100 pM estradiol, 316 nM OH-tamoxifen, 316 nM fulvestrant, or 316 nM OP-1250.
DMSO, dimethylsulfoxide.

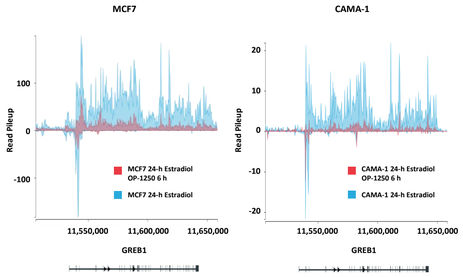
- Principal component analysis demonstrates reversal of estradiol-induced transcriptional changes with OP-1250 treatment (**Figure 3**)
- 6 hours of OP-1250 treatment induces transcriptional changes in canonical estrogen target genes (**Figure 4**)
- Heatmap visualization shows changes in expression of estrogen response early genes following OP-1250 treatment (**Figure 5**)
- Heatmap visualization shows changes in expression of G2/M checkpoint genes following OP-1250 treatment (**Figure 6**)

Figure 3. OP-1250 Treatment Effectively Reverses the Estradiol-Induced Transcriptional Changes



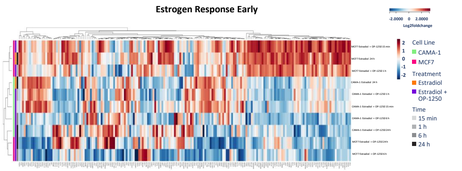
Principal component analysis of all MCF7 and CAMA-1 treatments and time points. Each dot represents a sample, with dot size corresponding to time point and color to treatment condition. The x- and y-axes refer to different principal components based on the sample's full set of TPM values. Dots that are close to each other in the PCA sample are more similar in terms of TPM expression across the gene reference set.
DMSO, dimethylsulfoxide; PCA, principal component analysis; TPM, transcript per million.

Figure 4. OP-1250 Treatment Downregulates Peaks in Canonical Estrogen Target Genes



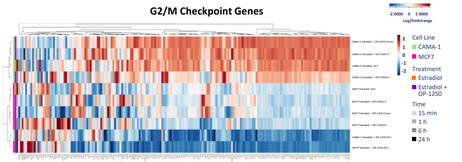
Read distribution at estrogen target gene GREB1 after 6 hours of OP-1250 treatment following estradiol pretreatment compared to 24-hour estradiol treatment. The x-axis refers to the position along the genome, and the y-axis refers to the number of reads aligning to that position normalized for the depth of each sample. Replicates were averaged. Bars that are less than zero are reverse-strand reads; bars above zero are forward-strand reads.

Figure 5. OP-1250 Treatment Reverses Induction of Estrogen Response Early Genes by Estradiol



Heatmap of annotated estrogen response early genes in CAMA-1 and MCF7 cells after 24 hours of estradiol treatment and 15 minutes, 1 hour, 6 hours, and 24 hours of OP-1250 treatment following estradiol pretreatment. Cells are colored based on gene expression, with red indicating high expression and blue low expression. Samples and genes with similar expression patterns are grouped together using hierarchical clustering, with sample relationships depicted by dendrograms at top and left of graph. Colored bars on the left of the graph indicate cell line, treatment condition, and time point of sample.

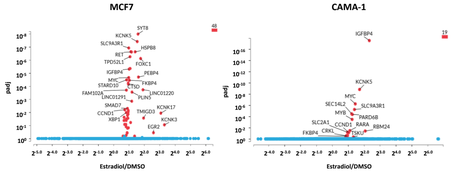
Figure 6. OP-1250 Treatment Reverses Induction of Cell Cycle–Related Genes by Estradiol



Heatmap of annotated G2/M checkpoint genes in CAMA-1 and *MCF7* cells after 24 hours of estradiol treatment and 15 minutes, 1 hour, 6 hours, and 24 hours of OP-1250 treatment following estradiol pretreatment. Cells are colored based on gene expression, with red indicating high expression and blue low expression. Samples and genes with similar expression patterns are grouped together using hierarchical clustering, with sample relationships depicted by dendrograms at top and left of graph. Colored bars on the left of the graph indicate cell line, treatment condition, and time point of sample.

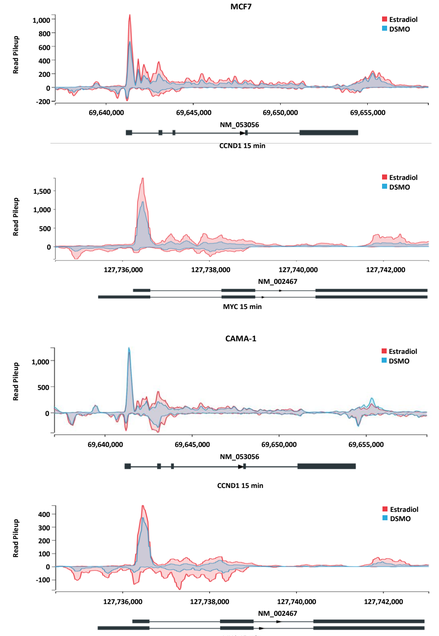
- Within 15 minutes of estradiol treatment, significant transcriptional changes occur in estrogen target genes (**Figure 7; Figure 8**)

Figure 7. Estrogen Induces Transcriptional Changes Within 15 Minutes of Treatment



Volcano plot of genes differentially expressed by 15-minute estradiol treatment relative to vehicle. Each dot represents a gene in the annotation set. Fold change is calculated by taking the depth-normalized expression value of that gene in treatment vs control samples. The *P*-value and adjusted *P*-value are calculated by the DeSeq2 algorithm. The number on the top right-hand corner represents the number of genes changed, and red dots indicate genes with observed change in expression.
DMSO, dimethylsulfoxide.

Figure 8. Transcriptional Changes in Estrogen-Target Genes Begin Within 15 Minutes of Estradiol Treatment

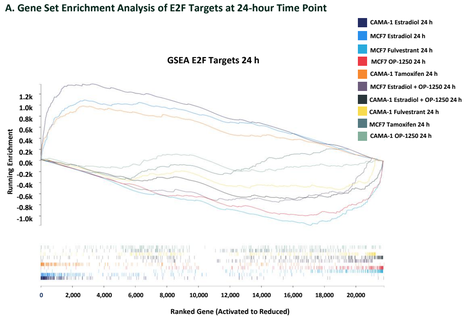


Read distribution at estrogen target genes CCND1 and MYC after 15 minutes of estradiol treatment compared to vehicle control. The x-axis refers to the position along the genome, and the y-axis refers to the number of reads aligning to that position normalized for the depth of each sample. Replicates were averaged. Bars that are less than zero are reverse-strand reads; bars above zero are forward-strand reads.
DMSO, dimethylsulfoxide.

- The 24-hour time point gene set enrichment analysis shows enrichment in transcription factors (**Figure 9A**)
- Heatmap visualization shows changes in expression of cell cycle–related genes after 24 hours of compound treatments (**Figure 9B**)
  - OP-1250 blocks estrogen-stimulated cell cycle induction, whereas tamoxifen demonstrates estrogen-like stimulation in CAMA-1 cells
  - Tamoxifen also looks like an anti-estrogen in MCF7 cells

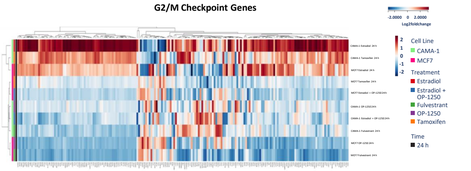
Figure 9. OP-1250 Blocks Estrogen-Stimulated Cell Cycle Induction, Whereas Tamoxifen Demonstrates Estrogen-Like Stimulation in CAMA-1 Cells After 24 Hours of Treatment

A. Gene Set Enrichment Analysis of E2F Targets at 24-hour Time Point



Gene set enrichment analysis (GSEA) of MCF7 and CAMA-1 samples following 24 hours of compound treatment. GSEA calculates how significant a pathway or gene set is for a given differential expression set. In brief, GSEA starts by ranking genes by their log2 fold change. If a gene is within a particular set, a running sum is increased; otherwise, it is decreased. If the peak of this running sum is above what we expect by chance, we can say that the pathway is enriched for a given differential expression set.

B. Heatmap Visualization of Cell Cycle–Related Genes After 24 Hours of Treatment



Heatmap of annotated G2/M checkpoint genes in CAMA-1 and MCF7 cells after 24 hours of compound treatment. Cells are colored based on gene expression, with red indicating high expression and blue low expression. Samples and genes with similar expression patterns are grouped together using hierarchical clustering, with sample relationships depicted by dendrograms at top and left of graph. Colored bars on the left of the graph indicate cell line, treatment condition, and time point of sample.

- Please see supplemental materials for additional data on transcriptional changes and responses after compound treatments

Conclusions

- **Genes that displayed early transcriptional changes in response to the compound treatments tested were identified and are primarily known estrogen response genes**
- **OP-1250 treatment is able to effectively reverse the estradiol-induced transcriptional changes associated with the activated estrogen receptor**
- **Tamoxifen demonstrates estrogen-like transcriptional effects, most pronounced in the CAMA-1 cell line, that are not observed with OP-1250 or fulvestrant treatment**
- **OP-1250 is being evaluated in an ongoing Phase 1/2 clinical trial in patients with locally advanced and/or metastatic ER+, human epidermal growth factor receptor 2-negative (ER+/HER2-) breast cancer (NCT04505826)**

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