Combination of complete estrogen receptor antagonist, OP-1250, and CDK4/6 inhibitors enhances tumor suppression and inhibition of cell cycle-related gene expression

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Background

- OP-1250 is a complete estrogen receptor antagonist (CERAN) and selective estrogen receptor degrader (SERD) that is currently in Phase 1/2 clinical trials for the treatment of estrogen receptor positive (ER+)/ human epidermal growth factor receptor 2 negative (HER2-) breast cancer
- OP-1250 has shown monotherapy efficacy against estrogen receptor 1 (ESR1) wild-type and mutant preclinical breast cancer models,[1,2] displayed favorable pharmacokinetic properties,[2] and effectively inhibited estrogen-induced proliferative and transcriptional activity[3,4]
- Inhibitors of cyclin-dependent kinases 4 and 6 (CDK4/6) are a standard first-line treatment for ER+ advanced or metastatic breast cancer in combination with endocrine therapy

Methods

- Xenograft studies
  - Athymic female, nude mice were supplemented with estradiol and implanted with either MCF-7 (cell line-derived) or ST941 (patient-derived) tumor cells subcutaneously and were randomized into groups when the tumor volume reached 200-275 mm^3
  - Mice were treated for 28 days with either vehicle, OP-1250, palbociclib, ribociclib, OP-1250 with palbociclib, or OP-1250 with ribociclib. Tumor samples were collected, and mice were euthanized when the volume reached 2,000 mm^3 or at the end of the study. ST941 xenograft studies were extended 28 days post cessation of treatment to monitor possible regrowth of tumors and animal survival
- RNA-seq
  - RNA was extracted from frozen tumor samples, enriched for mRNA using Oligo d(T) beads, and prepared for sequencing following manufacturer instructions of the Illumina NEBNext Ultra II RNA Library Prep Kit
  - Sequencing was conducted on an Illumina HiSeq instrument, with 20-30 million reads generated per sample
  - Gene counts and differential gene expression, carried out using CLC Genomics Workbench, were calculated by first filtering out reads that mapped exclusively to the murine reference genome
  - Pathway analysis was performed using the Ingenuity Pathway Analysis software
- Drug combination analysis
  - To compare the effect of the combination therapy with the 2 individual monotherapies (1 mg/kg OP-1250 and 25 mg/kg palbociclib, given separately), we looked at the log-fold changes for each of these 3 conditions for each gene
  - The effects of the monotherapies were combined according to the Bliss independence model. Using the effect of the combination therapy and the Bliss independence model as an (x, y) coordinate pair, the distance to the y=x diagonal was calculated. A value of 0 indicates that the combination therapy and combined monotherapies had comparable effects. A more negative value indicates that the combination therapy had a stronger repression on gene expression, while a more positive value indicates a stronger enhancement of gene expression

References

1. Hodges-Gallagher et al., Abstract P5-05-02, Abstracts: 2019 San Antonio Breast Cancer Symposium; December 10-14, 2019; San Antonio, Texas. **2.** Hodges-Gallagher et al., Abstract 4376, Proceedings: AACR Annual Meeting 2020; April 27-28, 2020 and June 22-24, 2020; Philadelphia, PA. **3.** Sun et al., Proceedings: 2021 ICA-AACR Precision Cancer Medicine International Conference; Japan, September 10-12, 2021. **4.** Parisian et al., Abstract 5375, Proceedings: AACR Annual Meeting 2022; April 8-13, 2022; New Orleans, LA. **5.** Chan et al., Poster P3-07-15, 2022 San Antonio Breast Cancer Symposium; December 6-10, 2022; San Antonio, Texas.

Acknowledgments

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Results

Treatment groups: OP-1250 and CDK4/6 inhibitor combination xenograft studies

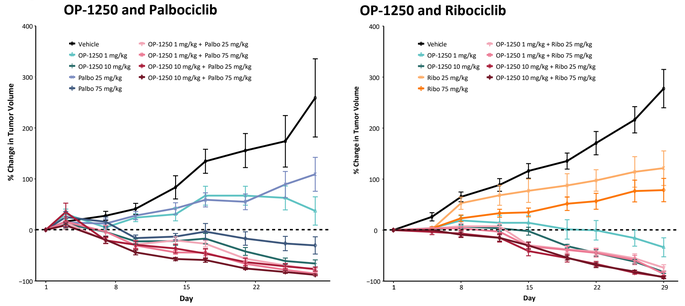
CERAN: OP-1250

CDK4/6 inhibitor: Palbociclib		None	25 mg/kg	75 mg/kg
None	Vehicle	25 mg/kg Palbo	75 mg/kg Palbo
1 mg/kg	1 mg/kg OP-1250	1 mg/kg OP-1250 + 25 mg/kg Palbo	1 mg/kg OP-1250 + 75 mg/kg Palbo
10 mg/kg	10 mg/kg OP-1250	10 mg/kg OP-1250 + 25 mg/kg Palbo	10 mg/kg OP-1250 + 75 mg/kg Palbo

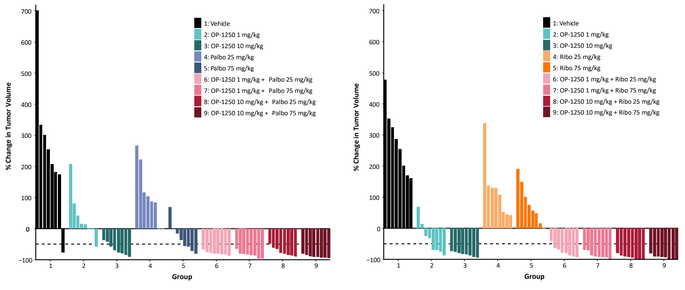
CDK4/6 inhibitor: Ribociclib		None	25 mg/kg	75 mg/kg
None	Vehicle	25 mg/kg Ribo	75 mg/kg Ribo
1 mg/kg	1 mg/kg OP-1250	1 mg/kg OP-1250 + 25 mg/kg Ribo	1 mg/kg OP-1250 + 75 mg/kg Ribo
10 mg/kg	10 mg/kg OP-1250	10 mg/kg OP-1250 + 25 mg/kg Ribo	10 mg/kg OP-1250 + 75 mg/kg Ribo

OP-1250 and CDK4/6 inhibitor combination enhances tumor shrinkage in ER+/HER2- MCF-7 xenograft model

Figure 1. Change in xenograft volume over treatment duration in MCF-7 model



Above is a graph of change in tumor volume over time with listed treatments in the MCF-7 breast cancer model, with horizontal dotted line representing tumor stasis.

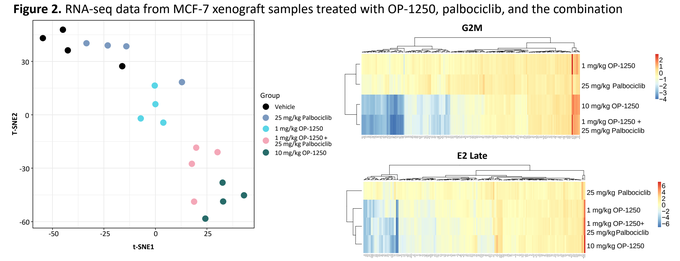


Waterfall plots of change in tumor volume of individual animals within each group.

Combination treatment resulted in the most pronounced tumor regression.

OP-1250 and palbociclib combination reduces transcriptional expression of genes associated with cell cycle progression

Figure 2. RNA-seq data from MCF-7 xenograft samples treated with OP-1250, palbociclib, and the combination



t-SNE plot of MCF-7 xenograft samples from listed treatment groups.

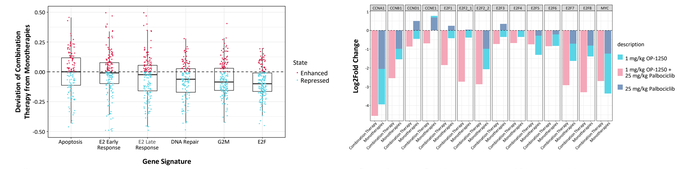
10 mg/kg dose of OP-1250 and 1 mg/kg OP-1250 + 25 mg/kg palbociclib groups showed greatest transcriptional changes from vehicle.

Heatmaps comparing expression of genes associated with the G2M and E2 Late gene signatures across treatment groups. Red corresponds to high expression relative to vehicle and blue corresponds to low expression.

Low dose monotherapies of OP-1250 and palbociclib did not significantly inhibit cell cycle-related gene expression, in contrast to 10 mg/kg dose of OP-1250 and OP-1250 + palbociclib combination. All OP-1250-containing groups inhibited estrogen signaling genes.

OP-1250 and palbociclib combination displays greater than additive effects on cell cycle repression relative to monotherapies

Figure 3. Gene expression of MCF-7 xenograft samples comparing monotherapy and OP-1250 + palbociclib combination



Differential gene expression comparison between combination therapy and monotherapies in hallmark gene sets (gsea). each data point represents a gene in the listed gene signature. the vertical axis depicts a data point's distance to the y = x diagonal, where 0 represents no change from expected result based on monotherapy values.

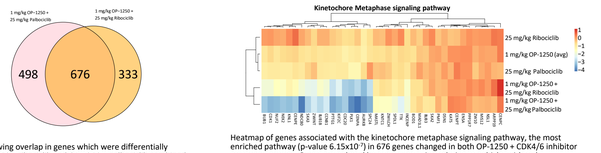
Cell cycle-related G2M and E2F gene signatures are more repressed in the combination therapy than predicted by the effect of the monotherapies

Comparison of log2-fold change values for 14 cell cycle-related genes between combination therapy (pink) and monotherapies (blue and purple for 1 mg/kg OP-1250 and 25 mg/kg palbociclib respectively). Stacked bars represent the combined effect of the monotherapies according to the Bliss independence model.

Greater transcriptional repression is observed for combination treatment in most cell cycle genes analyzed, representing greater than additive effects of combination treatment.

OP-1250 combination with palbociclib or ribociclib results in similar transcriptional changes in cell cycle-related pathway

Figure 4. Gene expression changes in OP-1250 and CDK4/6 inhibitor-treated MCF-7 xenografts



Venn diagram showing overlap in genes which were differentially expressed in OP-1250 + ribociclib and OP-1250 + palbociclib combined treatment xenografts relative to vehicle.

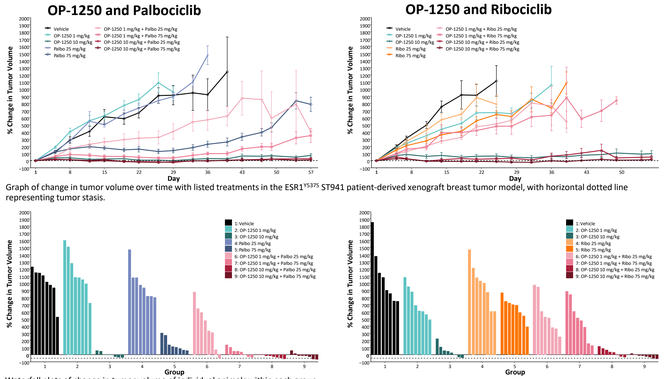
The majority of differentially expressed genes was shared between the 2 combination treatments.

Heatmap of genes associated with the kinetochore metaphase signaling pathway, the most enriched pathway (p-value 6.15x10^{-7}) in 676 genes changed in both OP-1250 + CDK4/6 inhibitor treatment groups. Genes are colored by gene expression relative to vehicle, with red corresponding to high expression and blue corresponding to low expression. OP-1250 1 mg/kg treatment groups from 2 xenograft studies were averaged.

Combination therapy resulted in greater suppression of the kinetochore metaphase pathway relative to monotherapies.

OP-1250 and CDK 4/6 inhibitor combination shrinks tumors in ESR1^{Y537S} tumor model ST941

Figure 5. Change in xenograft volume over treatment duration in ESR1^{Y537S} ST941 model



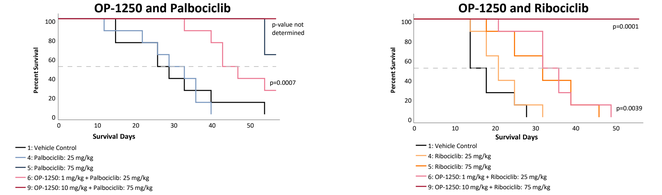
Graph of change in tumor volume over time with listed treatments in the ESR1^{Y537S} ST941 patient-derived xenograft breast tumor model, with horizontal dotted line representing tumor stasis.

Waterfall plots of change in tumor volume of individual animals within each group.

Combination treatment resulted in enhanced tumor growth inhibition or shrinkage.

Addition of OP-1250 to palbociclib or ribociclib enhances tumor inhibition and prolongs survival in an ESR1^{Y537S} PDX model

Figure 6: Kaplan Meier curves of ST941 PDX model treated with CDK4/6 inhibitor or OP-1250 and CDK4/6 inhibitor relative to vehicle



Kaplan Meier graphs of animal survival over time in mice implanted with the ESR1^{Y537S} ST941 PDX model . Left, comparison between vehicle, palbociclib monotherapy and low and high dose groups of palbociclib + OP-1250 . Right, comparison between vehicle, ribociclib monotherapy and low and high dose groups of ribociclib + OP-1250. Shown are p-values comparing CDK4/6 monotherapy and OP-1250 + CDK4/6 combination treatment using the log-rank (Mantel-Cox) test. P-values cannot be determined if total survival for both groups does not drop below 50%. Log-rank (Mantel-Cox) test between CDK4/6 monotherapy and OP-1250 + CDK4/6 combination revealed addition of OP-1250 significantly extended survival where p-values could be determined.

Conclusions

- 10 mg/kg dose of OP-1250 effectively inhibits tumor growth or shrinks tumors in xenograft studies of both ESR1 wild-type and mutant breast cancer models
- Combination of 10 mg/kg OP-1250 and 75 mg/kg CDK4/6 inhibitor shrinks ER+ MCF-7 and ESR1^{Y537S} ST941 xenograft models
- The addition of OP-1250 to palbociclib or ribociclib improves tumor growth inhibition and animal survival in an ESR1^{Y537S} PDX model
- The combination of OP-1250 and CDK4/6 inhibitors results in greater suppression of transcription related to cell cycle progression than the sum of monotherapies
- OP-1250 is in Phase 1/2 clinical development in combination with CDK4/6 inhibitors palbociclib and ribociclib
  - Clinical data on OP-1250 in combination with palbociclib is being presented in SABCS poster P3-07-15

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