OP-1250 Prevents Tumor Spread in a Model of Metastatic Mutant ERα+ Breast Cancer

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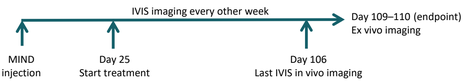
Background

- Estrogen receptor-positive (ER+) breast cancers represent about 70–75% of all breast cancers[1,2]
- ER+ breast cancer can be effectively treated with adjuvant endocrine therapies[3]
  - However, many patients develop resistance and eventually progress to metastatic disease
  - In patients with breast cancer, metastasis can occur in the liver, lungs, brain, and bone
- Acquired mutations in *ESR1*, which encodes estrogen receptor alpha (ERα), contribute to 20–40% of endocrine therapy–resistant metastatic breast cancer (MBC)[4-7]
- OP-1250 is a small molecule Complete Estrogen Receptor ANtagonist (CERAN) that potently and completely inactivates the ER
- **Objective:** To better understand the effects of OP-1250 treatment, both alone and in combination with palbociclib, a CDK4/6 inhibitor, on tumor growth and metastasis in a murine model of aggressive mutant ER+ MBC

Methods

- Michigan Cancer Foundation (MCF)-7 cells engineered to express one of the most common and aggressive ERα mutations, Y537S, were labeled with luciferase and injected via the nipple mammary intraductal (MIND) model into NSG mice
  - Cells were heterozygous for Y537S expression
  - ~250,000 MCF-7 ERα Y537S cells labeled with luciferase were injected in glands 4 and 9
  - 6 NSG mice per treatment group
- At day 25, these luciferase-tagged MCF-7 ERα Y537S tumors were treated with OP-1250, Faslodex® (fulvestrant), palbociclib, or combination treatment (**Figure 1**)
- Tumor growth was continuously monitored every 2 weeks using an IVIS Spectrum imager to measure bioluminescence
- At day 109–110 (study endpoint), mice were sacrificed, and excised organs were imaged ex vivo
  - Tissue samples were collected for hematoxylin and eosin (H&E) and immunohistochemistry (IHC) staining
  - Due to the unexpected death of 5/6 mice in the fulvestrant + palbociclib cohort by day 108, the ex-vivo data for this cohort are not shown
- 8 treatment groups
  - Vehicle subcutaneously (SC), 5 days/week
  - OP-1250 3 mg/kg gavage, 5 days/week
  - OP-1250 10 mg/kg gavage, 5 days/week
  - Fulvestrant 5 mg/mouse SC, 1 day/week
  - Palbociclib 70 mg/kg gavage, 5 days/week
  - OP-1250 3 mg/kg gavage + palbociclib 70 mg/kg gavage, 5 days/week
  - OP-1250 10 mg/kg gavage + palbociclib 70 mg/kg gavage, 5 days/week
  - Fulvestrant 5 mg/mouse SC, 1 day/week + palbociclib 70 mg/kg gavage, 5 days/week
- Antibodies for IHC
  - Ki-67 (Thermo Scientific #RM-9106-s, clone: SP6)
  - Anti-mitochondria human (Abcam #ab92824, clone 112-1)

Figure 1. Study Design



References

1. Noone AM, et al. *Cancer Epidemiol Biomarkers Prev*. 2017;26(4):632–641. **2.** American Cancer Society. *Breast Cancer Facts & Figures 2019-2020*. Atlanta: American Cancer Society, Inc. 2019. **3.** Burstein HJ, et al. *J Clin Oncology*. 2018;37(5):423-438. **4.** Spoerke JM, et al. *Nat Commun*. 2016;7:11579. **5.** Jeselsohn R, et al. *Clin Cancer Res*. 2014;20(7):1757–1767. **6.** Robinson DR, et al. *Nat Genet*. 2013;45(12):1446–1451. **7.** Toy W, et al. *Nat Genet*. 2013;45(12):1439–1445.

Acknowledgments

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Disclosures

Muriel Lainé: Nothing to disclose. **Marianne E. Greene:** Nothing to disclose. **Tiffany Leng:** Nothing to disclose. **Sophia Li:** Nothing to disclose. **Gopinath S. Palanisamy:** Olema Oncology: Salary, Ownership interest. **Cyrus Harmon:** Olema Oncology: Salary, Ownership interest, IP rights; IDbyDNA: Ownership interest; Primary Diagnostics: Ownership interest. **Leslie Hodges-Gallagher:** Olema Oncology: Salary, Ownership interest. **Peter J. Kushner:** Olema Oncology: Salary, Ownership interest. **Geoffrey L. Greene:** Sermonix: Consulting, sponsored research, and options; Olema: Stock, sponsored research, and member of SBA.

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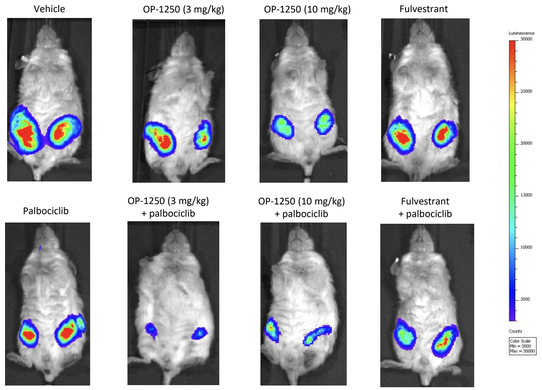
Results

OP-1250 at both doses inhibits primary tumor growth, with greater effect observed at 10 mg/kg

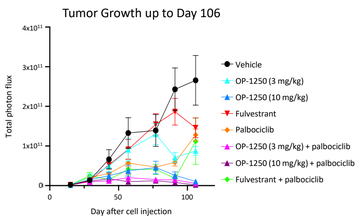


IVIS images showing luciferin total photon flux at day 106; 1 representative mouse for each treatment group.

OP-1250 alone and in combination with palbociclib substantially slows primary tumor growth in an endocrine-resistant breast cancer model with ERα Y537S mutation

- OP-1250 at 10 mg/kg is almost 100% effective at inhibiting primary tumor growth vs vehicle, fulvestrant, and palbociclib
- Adding palbociclib improves OP-1250 at 3 mg/kg but does not improve OP-1250 at 10 mg/kg because the single agent is maxed out

A



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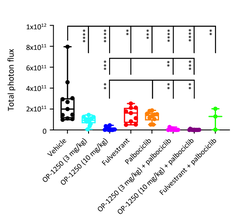

A. Quantification of total flux of the mammary glands over time measured with an Xenogen IVIS
B. Quantification of the gland total flux at day 106 showing that OP-1250 alone and in combination with palbociclib significantly slows primary tumor growth in comparison to vehicle and fulvestrant treatment
[a]Statistical significance is shown for compound treatments against vehicle, fulvestrant, and palbociclib.
Statistics: 2-way analysis of variance (ANOVA) with **p<0.01, ***p<0.001, ****p<0.0001.

OP-1250 at 10 mg/kg alone and in combination with palbociclib substantially reduces tumor cell proliferation (Supplemental Table 1)

- OP-1250 at 10 mg/kg is effective vs all single agent therapies
- Adding palbociclib to OP-1250 improves inhibition of Ki-67 vs OP-1250 alone
- Fulvestrant inhibition does not improve with the addition of palbociclib

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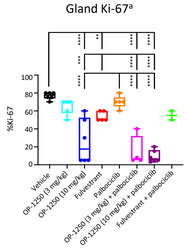

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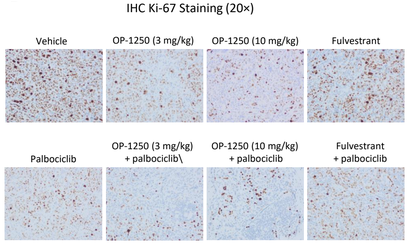
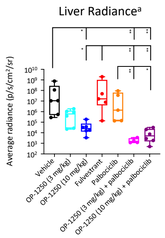


A. Quantification of Ki-67 IHC staining of the mammary gland for each treatment showing that OP-1250 (10 mg/kg) alone and in combination with palbociclib slows tumor proliferation
B. One representative image of Ki-67 IHC staining for each treatment group
[a]Statistical significance is shown for compound treatments against vehicle, fulvestrant, and palbociclib.
Statistics: 2-way ANOVA with **p<0.01, ***p<0.001, ****p<0.0001.

OP-1250 at 10 mg/kg alone and in combination with palbociclib substantially reduces metastasis to the liver (A and B below) and lungs (Supplemental Figures 1 and 2)

- There is a clear benefit to adding palbociclib to either dose of OP-1250, suggesting that this effect is driven by palbociclib

A



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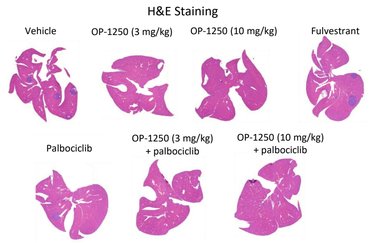
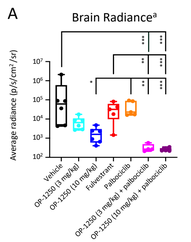


A. Quantitation of ex vivo liver average radiance measure with the Xenogen IVIS showing that OP-1250 alone and in combination with palbociclib reduces metastasis growth to the liver in comparison to vehicle and fulvestrant
B. H&E staining of livers, one representative image for each treatment
[a]Statistical significance is shown for compound treatments against vehicle, fulvestrant, and palbociclib.
Statistics: 2-way ANOVA with *p<0.05, **p<0.01.

OP-1250 at 10 mg/kg alone and in combination with palbociclib substantially reduces metastasis to the brain

- OP-1250 at 10 mg/kg shows superior efficacy against vehicle and fulvestrant
- OP-1250 in combination with palbociclib shows superior efficacy against any single agent

A



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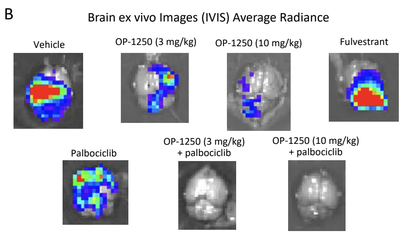
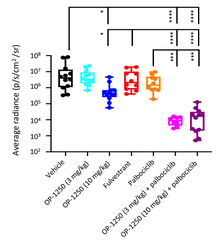


A. Quantitation of average radiance of the brain measured ex vivo on a Xenogen IVIS showing that OP-1250 (10 mg/kg) alone and the combination of OP-1250 with palbociclib significantly reduced brain metastases
B. Ex vivo images of photon flux observed in the brain. One representative image per group
[a]Statistical significance is shown for compound treatments against vehicle, fulvestrant, and palbociclib.
Statistics: 2-way ANOVA with *p<0.05, **p<0.01, ***p<0.001.

OP-1250 at 10 mg/kg alone and in combination with palbociclib substantially reduces metastasis to the bone

- OP-1250 in combination with palbociclib shows superior efficacy against any single agent



Quantitation of the bone average radiance measured ex vivo on a Xenogen IVIS showing that OP-1250 (10 mg/kg) alone and the combination of OP-1250 with palbociclib significantly reduced bone metastases
[a]Statistical significance is shown for compound treatments against vehicle, fulvestrant, and palbociclib.
Statistics: 2-way ANOVA with *p<0.05, ***p<0.001, ****p<0.0001.

Metastasis to liver, lung, and brain occurs less frequently in mice treated with OP-1250 in combination with palbociclib (Supplemental Table 2)

Conclusions

- **OP-1250 alone or in combination with palbociclib substantially inhibits primary tumor growth in an endocrine-resistant breast cancer model with ERα Y537S mutation**
  - OP-1250 at 10 mg/kg is almost 100% effective at inhibiting primary tumor growth vs vehicle, fulvestrant, and palbociclib
- **OP-1250 in combination with palbociclib substantially:**
  - Reduces tumor cell proliferation
  - Decreases metastasis to liver, lung, brain, and bone
- **OP-1250 in combination with palbociclib is a promising therapy for MBC**