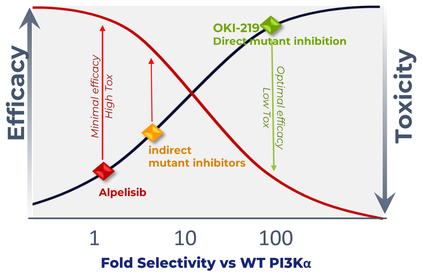
# Discovery and characterization of OKI-219, an orally bioavailable H1047R-mutant-selective inhibitor of PI3Kα

Abstract 33778

Molly A. Taylor, Qian Zhao, David Mareska, Maria Hoh, Yevgeniy Izrayelit, Kevin Litwiler, Mark L. Boys, Rich Woessner, Duncan Walker, Jennifer Diamond, James D. Winkler
OnKure, Inc., 6707 Winchester Circle, Suite 400, Boulder, CO 80301.

## Mutant selective inhibition of PI3Kα H1047R provides greater target coverage without on-target toxicity




- PIK3CA is the most frequently mutated oncogene in cancer- found in approximately 13% of human cancers[1].
- There are 3 hotspot mutations in PI3Kα, with the H1047R mutation in the catalytic domain being the most common, representing approximately one-third of all PI3Kα mutations[2].
  - On-target toxicity associated with inhibition of PI3Kα$^{WT}$ significantly limits efficacy
  - Directly targeting the PI3Kα$^{H1047R}$ mutation has potential to achieve greater target coverage while sparing inhibition of WT-PI3K, thus improving efficacy without on-target toxicity

## OKI-219 is a potent and highly selective inhibitor of PI3Kα H1047R

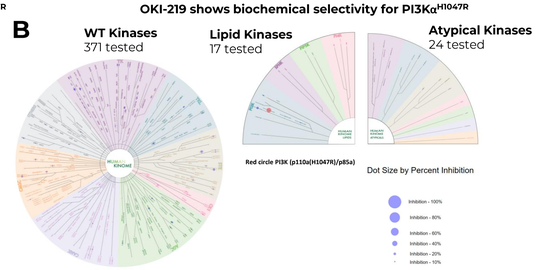
OKI-219 is highly selective for PI3Kα$^{H1047R}$

A

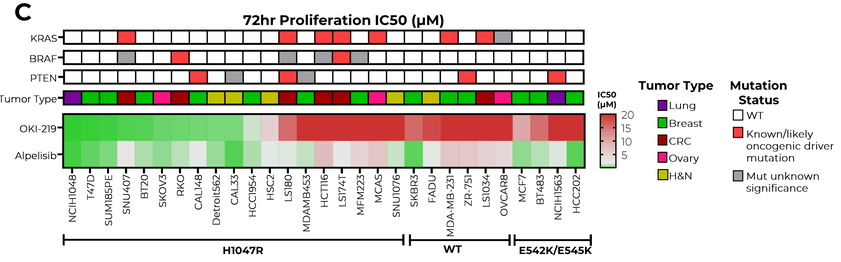
| Assay | OKI-219 | Alpelisib |
|---|---|---|
| pAKT Selectivity (H1047R/WT) | 106X | 1X |
| T47D pAKT IC50 (nM) | 81 | 111 |
| Proliferation Selectivity (H1047R/WT) | 159X | 1X |
| T47D Proliferation IC50 (nM) | 97 | 551 |

OKI-219 shows biochemical selectivity for PI3Kα$^{H1047R}$

B




C

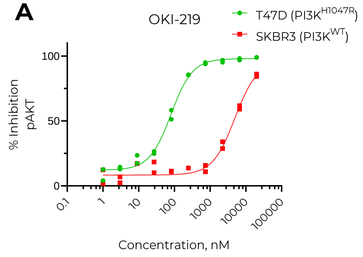



**PI3Kα$^{H1047R}$ cell lines are sensitive to OKI-219, while PI3Kα$^{WT}$ or PI3Kα$^{E542K/E545K}$ lines are not.**

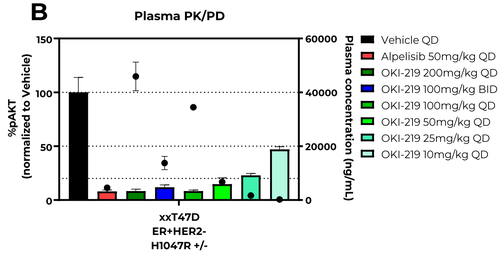
**A)** OKI-219 is highly selective (>100 X) for PI3Kα$^{H1047R}$ over PI3Kα$^{WT}$ in both pAKT (HTRF) and proliferation (Cell Titer Glo) assays.
**B)** OKI-219 shows biochemical selectivity for PI3Kα$^{H1047R}$ in a 412-kinase panel (Reaction Biology), with no other kinases showing >30% inhibition at 1μM.
**C)** Cell lines endogenously expressing PI3Kα$^{H1047R}$, PI3Kα$^{WT}$, or PI3Kα$^{E545K/E542K}$ were treated with a concentration range of OKI-219 or alpelisib and 50% inhibition concentration (IC50) was determined using the CellTiter-Glo luminescent cell viability assay after a 72 hour incubation. Cell lines containing an H1047R mutation are sensitive to OKI-219, while those containing WT and E545K/E542K are not.

## OKI-219 demonstrates target engagement *in vitro* and *in vivo* in PI3Kα H1047R models

A




B

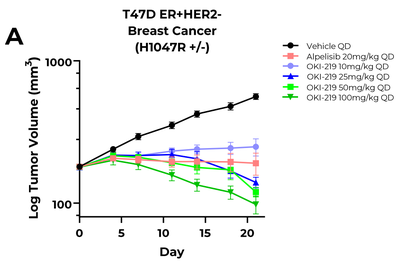



**A)** *In vitro*, OKI-219 selectivity inhibits phosphorylation of AKT in T47D PI3Kα$^{H1047R}$ cells, displaying a <100nM pAKT inhibitory activity compared to SKBR3 PI3Kα$^{WT}$ cells, with >5,000nM pAKT inhibitory activity using a pAKT(S473) HTRF assay. A representative curve is shown.

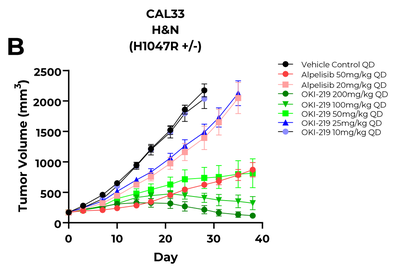
**B)** *In vivo*, OKI-219 inhibits phosphorylation of AKT in a dose dependent manner in xxT47D tumors treated for 4 days, measured 2 hours post last dose.

## OKI-219 demonstrates monotherapy activity *in vivo* without inducing metabolic changes

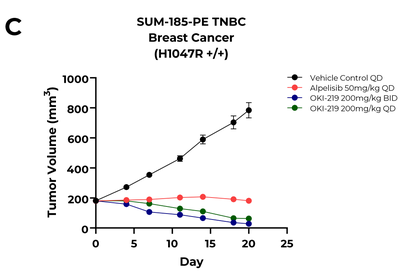
A



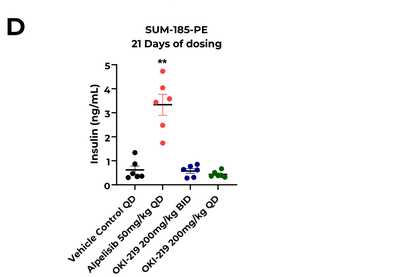

B




C



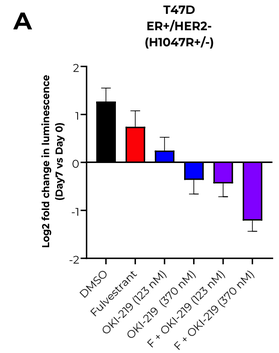

D



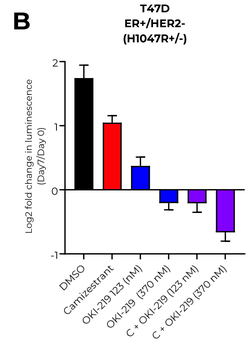

**A)** Dose dependent anti-tumor responses in T47D tumors after treatment with OKI-219 compared to alpelisib dosed at the clinically relevant dose.
**B)** Dose dependent anti-tumor response in CAL33 tumors after treatment with OKI-219 compared to alpelisib dosed at the clinically relevant dose (20mg/kg) and at ~2X the clinically relevant dose (50mg/kg).
**C)** Tumor regressions in SUM-185-PE tumors after treatment with indicated doses of OKI-219 compared to alpelisib dosed at ~2X the clinically relevant dose.
**D)** Plasma insulin levels measurement by ELISA assay in samples taken from SUM-185-PE tumor-bearing mice on day 21 of treatment, 2 hours post dose indicate that OKI-219 does not induce insulin changes, while alpelisib does. ** p<0.01 by ANOVA.

## OKI-219 has combination activity with SERDs

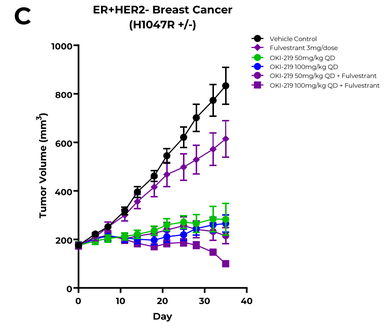
A



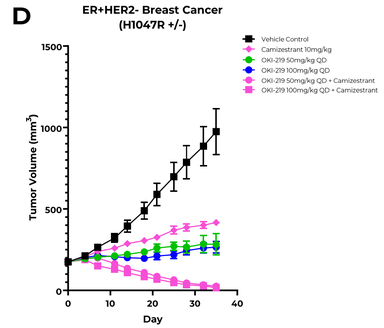

B




C




D




**A)** Combination of OKI-219 and fulvestrant (F), 14nM leads to net cell death in an in vitro 7-day proliferation assay as measured by Cell Titer Glo.
**B)** Combination of OKI-219 and next generation SERD, camizestrant (C) 41nM, leads to net cell death in an in vitro 7-day proliferation assay as measured by Cell Titer Glo.
**C)** In vivo, xxT47D tumors show greater regressions with the combination of OKI-219 and fulvestrant than with either monotherapy.
**D)** In vivo, xxT47D tumors show complete regressions with the combination of OKI-219 and camizestrant.

## Summary

- OKI-219 is greater than 100X fold selective for PI3Kα$^{H1047R}$ over PI3Kα$^{WT}$.
- OKI-219 blocks PI3Kα signaling *in vitro* and *in vivo* in PI3Kα$^{H1047R}$ mutant models.
- OKI-219 is safe and active as a single agent in breast and other tumor models that have PI3Kα$^{H1047R}$.
  - Active at low doses in xenograft models (<25 mg/kg per day)
  - Active in tumors heterozygous for PI3Kα$^{H1047R}$
- High selectivity in vivo: No change in markers of on-target toxicity at doses >15X above those that drive tumor regressions.
- OKI-219 shows combination activity with SERDs in models of ER+ breast cancer.
- Planning to initiate Phase 1 study for OKI-219 in early 2024

[1] Millis SZ et al. JAMA Oncol 2016;2(12):1565-73 . [2] Tate JG et al. Nucleic Acids Res 2018;47(D1):D941-D947.