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RLY-2608: the first allosteric mutant- and isoform-selective inhibitor of PI3Kα, is efficacious as a single agent and drives regressions in combination with standard of care therapies in *PIK3CA* mutant breast cancer models

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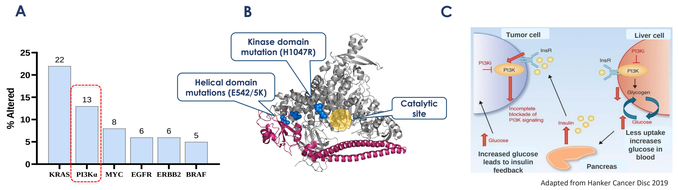
INTRODUCTION

Inhibition of CDK4/6 combined with the estrogen receptor (ER) degrader fulvestrant significantly improves progression free survival and overall survival in advanced hormone receptor positive (HR+) breast cancer patients and is now the standard of care in this disease. However, this combination is not curative, and resistance limits the duration of clinical benefit. Mutation of *PIK3CA* in up to ~40% of HR+ breast cancer[1], which leads to activation of phosphoinositide 3-kinase alpha (PI3Kα), appears to be a key driver of resistance[2]. Therefore, PI3Kα inhibitor combinations with CDK4/6 inhibitors and/or fulvestrant are of high interest to prevent and overcome therapeutic resistance in HR+, *PIK3CA* mutant breast cancer. The therapeutic index of active site (orthosteric) inhibitors of PI3Kα has been limited by the dual issues of no clinically meaningful selectivity for mutant versus wild-type (WT) PI3Kα and off-isoform inhibitory activity. Alpelisib, the only approved orthosteric PI3Kα inhibitor, is emblematic of the class with toxicity related to inhibition of wild type PI3Kα and other PI3K isoforms resulting in sub-optimal inhibition of mutant PI3Kα, frequent discontinuation, and challenges in combining with CDK4/6 inhibitors[3]. We solved the full-length cryo-EM structure of PI3Kα, performed long time-scale molecular dynamic simulations to elucidate conformational differences between WT and mutant forms, and leveraged these insights to enable the design of RLY-2608, the first allosteric, mutant and isoform-selective inhibitor of PI3Kα.

BACKGROUND

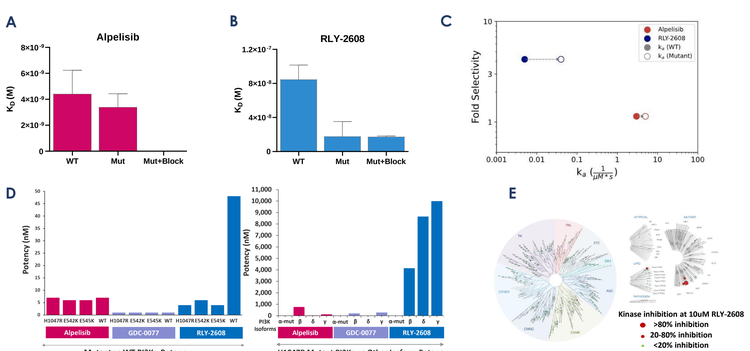


Adapted from Hanker Cancer Disc 2019

PI3Kα is the most frequently mutated kinase in cancer. *PIK3CA* is one of the most frequently mutated genes in cancer across different indications (**A**). The hotspot mutations are distal to the orthosteric site, where all clinical inhibitors bind (**B**). PI3Kα signaling is critical to growth and metabolism and its inhibition disrupts glucose homeostasis (**C**).

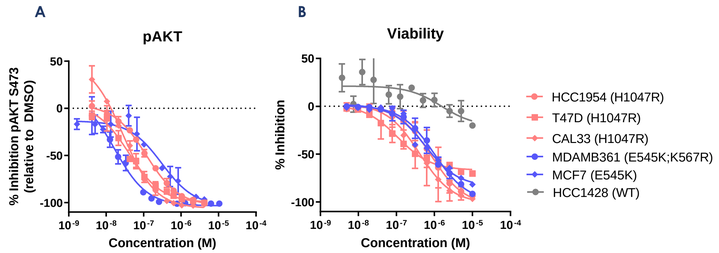
RESULTS

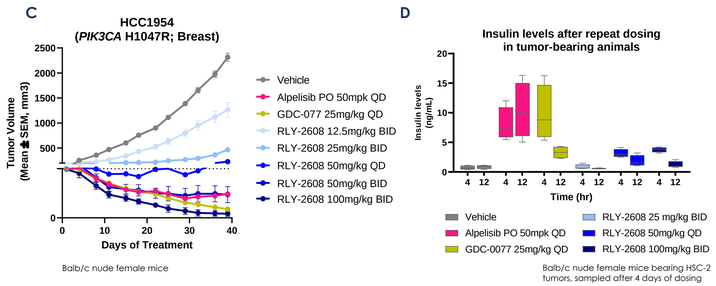
RLY-2608 is the first allosteric PI3Kα mutant- and isoform-selective inhibitor



RLY-2608 binds to mutant protein preferentially (as assessed via surface plasmon resonance (SPR)). Binding is not abrogated by buparlisib, an orthosteric site inhibitor. (**A** and **B**). RLY-2608 binds faster to the mutant protein (**C**). RLY-2608 demonstrates biochemical selectivity for mutant PI3Kα over WT and other family member isoforms (**D**) in addition to exquisite selectivity over the rest of the kinome (**E**).

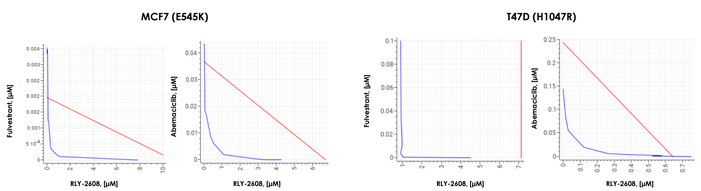
RLY-2608 inhibits kinase- and helical domain- mutant signaling with minimal impact on WT signaling





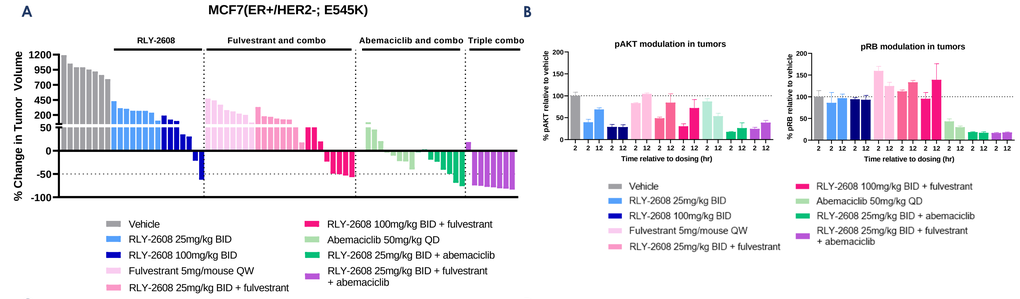
RLY-2608 inhibits phosphorylated AKT (pAKT) in *PIK3CA* mutant cancer cell lines independent of the hotspot mutation (**A**). Upon longer incubation (5 days), RLY-2608 inhibits their proliferation (**B**). RLY-2608 demonstrates potent tumor growth inhibition in a dose-dependent manner leading to full regressions at doses that have a reduced impact on insulin levels compared to orthosteric inhibitors (**D**).

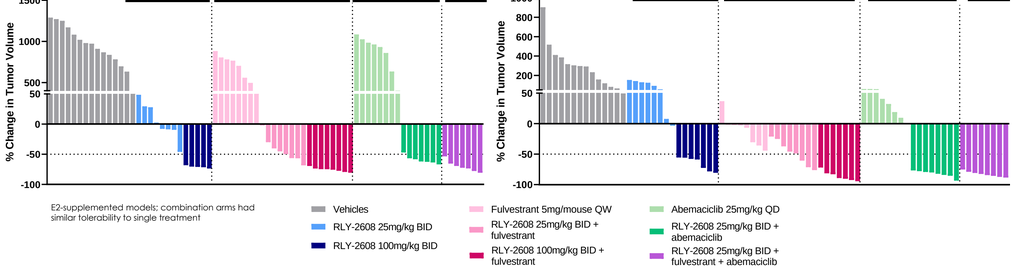
RLY-2608 synergizes with standard of care therapies in ER+/HER2- breast cancer cell lines



Co-treatment of ER+/HER2- cell lines (MCF7 or T47D) with RLY-2608 and fulvestrant or abemaciclib leads to synergy in viability effects as visualized by the difference between the blue line (synergy) and red line (additivity). Proliferation was measured by CellTiter-Glo® (Fulvestrant combinations) or CyQUANT® (abemaciclib combination). Isobolograms were generated by GeneData Screener using Loewe's additivity method.

RLY-2608 combines with standard of care therapies to drive regressions in ER+/HER2- breast cancer models





Oral administration of RLY-2608 in combination with fulvestrant and abemaciclib led to improved efficacy compared to either agent alone, with regressions observed in the combination arms at all doses. Superior efficacy was observed in the triple combination (**A**). Combination efficacy is underlined by concomitant inhibition of each respective pathway in repeat dose-treated MCF7 tumors (**B**). RLY-2608 drives regressions as a single agent or at a lower dose in combination with fulvestrant and abemaciclib across *PIK3CA* mutant patient derived xenografts.

CONCLUSIONS

- RLY-2608 is the first allosteric, pan-mutant PI3Kα inhibitor
- RLY-2608 achieves maximum efficacy in *PIK3CA* mutant in vivo xenograft models and minimally impacts insulin levels compared to orthosteric inhibitors
- RLY-2608 combines with standard of care therapies to drive regressions in both kinase and helical domain *PIK3CA* mutant patient derived xenograft models
- Results validate differentiated mechanism of mutant PI3Kα inhibition and provide strong rationale for clinical testing, with the first-in-human study anticipated to start in 1H22

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