### 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 PIKCA in up to 40% of HR+ breast cancer, which leads to activation of phosphoinositide 3-kinase alpha (PI3Ka), appears to be a key driver of resistance. Therefore, PI3Ka inhibitors combinations with CDK4/6 inhibitors and/or fulvestrant are of high interest to prevent and overcome therapeutic resistance in HR+. PI3Ka mutant breast cancer. The therapeutic index of active site (orthosteric) inhibitors of PI3Ka has been limited by the dual issues of insufficient orthosteric meaningful selectivity for mutant versus wild-type (WT) PI3Ka and off-target inhibitory activity. Abaliptin, the only approved orthosteric PI3Ka inhibitor, is embolic in the class with toxicity related to inhibition of wild-type PI3Ka and other PI3K isoforms resulting in sub-optimal inhibition of mutant PI3Ka, frequent discontinuation, and challenges in combining with CDK4/6 inhibitors. We solved the full-length cryo-EM structure of PI3Ka, performed large-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 PI3Ka.

### Background

PI3Ka is the most frequently mutated kinase in cancer. PI3Ka3A is one of the most frequently mutated genes in cancer across different indications. (A) The hotspots mutations are distal to the orthostatic site, where all clinical inhibitors bind. (B) PI3Ka signaling is critical to growth and metabolism and its inhibition disrupts glucose homeostasis. (C).

### Results

RLY-2608 inhibits phosphoAKT (pAKT) in PI3Ka mutant cancer cell lines independent of the hotspot mutation (A). Upon longer incubation (3 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 orthostatic inhibitors (B).

RLY-2608 synergizes with standard of care therapies in ER+/HER2- breast cancer cell lines. Co-treatment of RLY-2608 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 treatment) or CyQUANT® (abemaciclib combination) colony-formation assays were generated with GeneSieve Scissors using loess’s additivity method.

### Conclusions

- RLY-2608 is the first allosteric, pan-mutant PI3Ka inhibitor.
- RLY-2608 achieves maximum efficacy in PI3Ka mutant in vivo xenograft models and minimally impacts insulin levels compared to orthostatic inhibitors.
- RLY-2608 combines with standard of care therapies to drive regressions in both kinase and helical domain PI3Ka mutant patient derived xenograft models.
- Results validate differentiated mechanism of mutant PI3Ka inhibition and provide strong rationale for clinical testing, with the first-in-human study anticipated to start in 2022.

### References