Zovegalisib (RLY-2608), a Novel, Mutant-selective, PI3Kα Inhibitor, Induces Lesion Regression, with Minimal Hyperinsulinemia, in Murine Models of *PIK3CA*-mutant Vascular Malformations

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Disclosure slide

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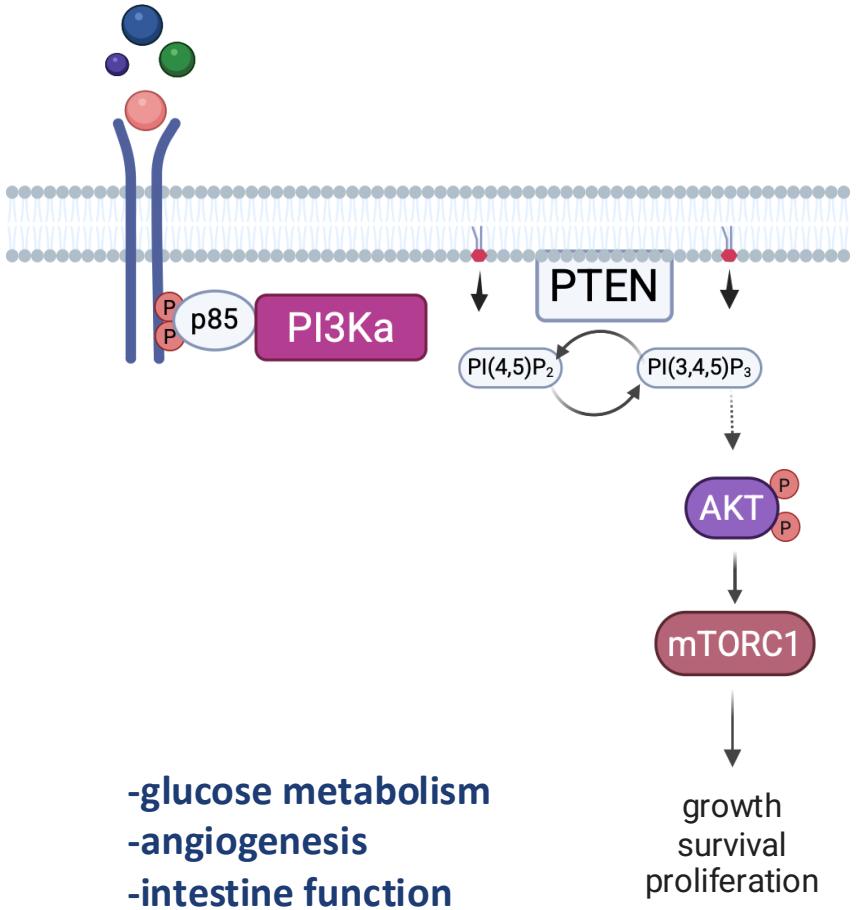
Activating *PIK3CA* Mutations Lead to Vascular Overgrowth but Wildtype PI3Kα Inhibition Impairs Glucose Metabolism and Normal Cellular Processes

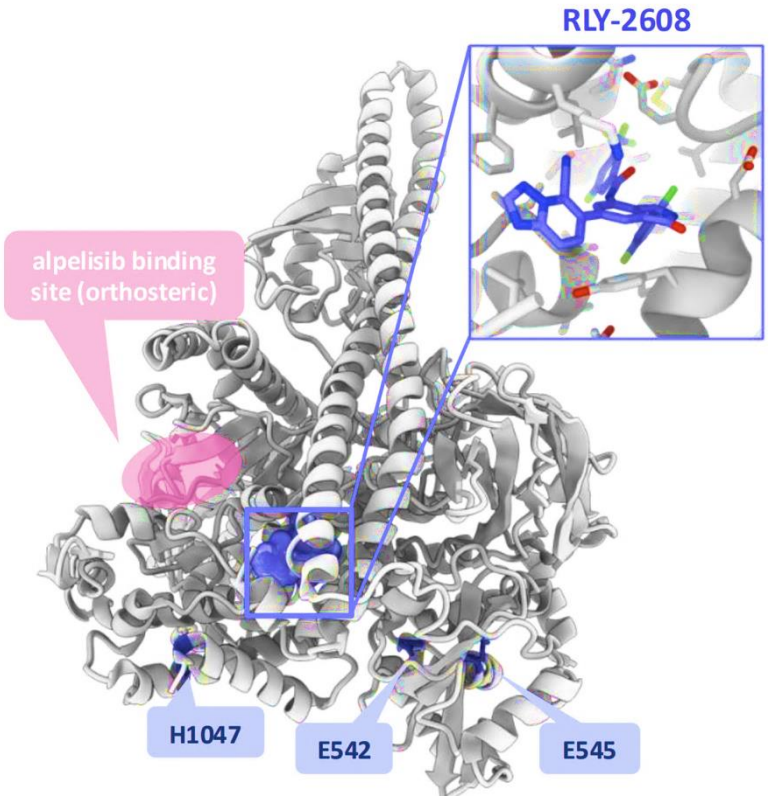


- Activating somatic mutations in *PIK3CA* cause nearly all cases of *PIK3CA*-related Overgrowth Spectrum (PROS)[1], 80% of lymphatic malformations[2] and 20% of venous malformations[3]
- *PIK3CA* mutations are present in a low allelic frequency, primary in mesoderm derivates
- Non-mutant selective PI3Kα pathway inhibitors like alpelisib have severe toxicities including hyperglycemia, rash, stomatitis[4] and growth retardation[5] which limit dose and efficacy

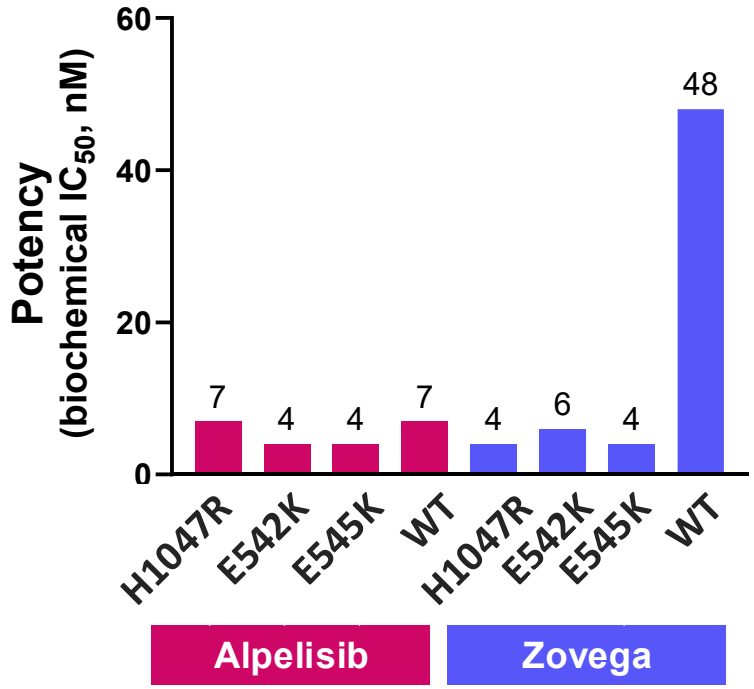
[1] Angulo-Urarte et al. Cardiovasc Res 2022; [2]Keppler-Noreuil K et al, Am J Med Gene A 2025; [2]Luks VL et al, J Pediatr. 2015; Osborn AJ et al, Hum Mol Genet. 2015; [3]Castillo SD et al, Sci Transl Med. 2016; [4]Vijoice USPI, [5]Triana et al. JoVA 2025

Zovegalisib (RLY-2608) is a Novel, Oral, Allosteric Mutant-Selective PI3Kα Inhibitor

Differentiated mechanism from other PI3Kα inhibitors



Potent, mutant-selective PI3Kα inhibition



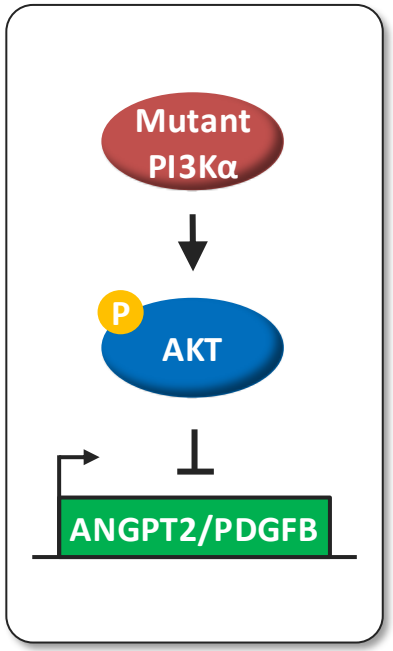
- Binds a **novel allosteric pocket** preferentially formed in mutated, hyperactivated PI3Kα[1]
- Demonstrated **pan-mutant selectivity** over wildtype PI3Kα and other PI3K isoforms
- **FDA Breakthrough Designated** in HR+ HER2- breast cancer and in pivotal Phase 3 development
- Activity of zovegalisib was characterized in xenograft and genetic vascular malformation models prior to development

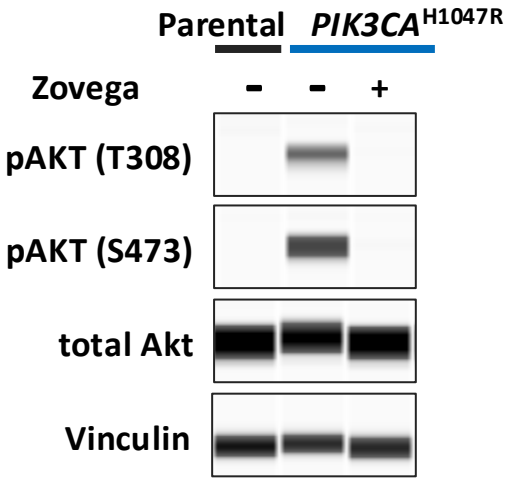
[1]Varkaris et al. Cancer Dis 2024

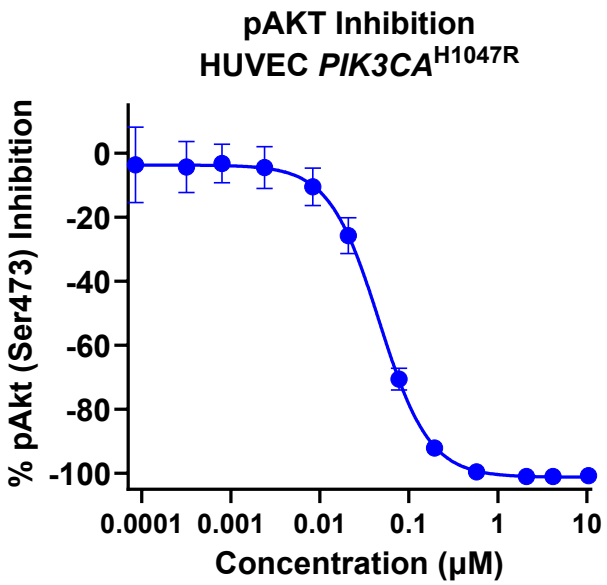
Zovegalisib Fully Suppresses Aberrant AKT Phosphorylation and Restores Expression of the Endothelial Genes ANGPT2 and PDGFB to Physiological Levels

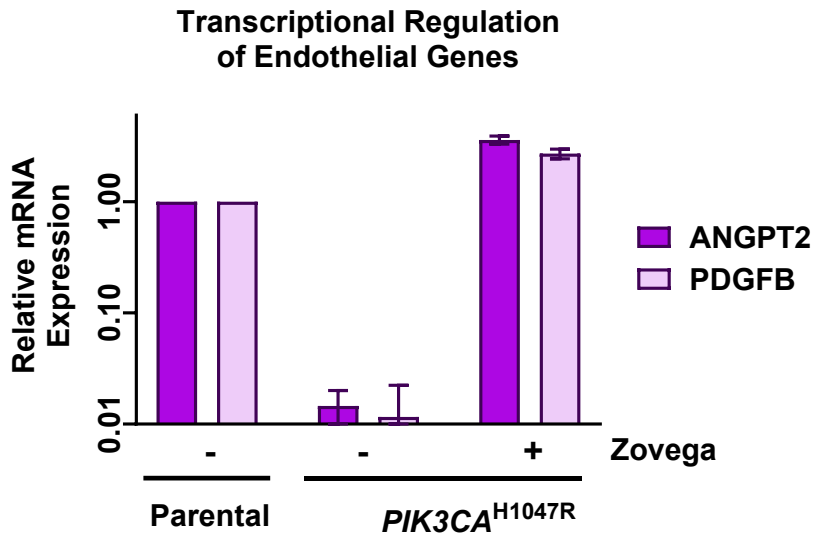
Zovega fully inhibits pAKT in *PIK3CA*^{H1047R} HUVEC cells…

…and restores endothelial genes transcriptional regulation



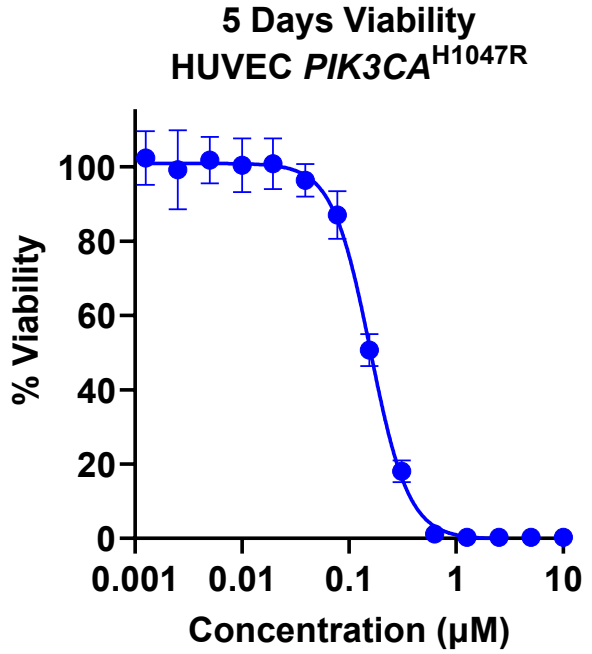




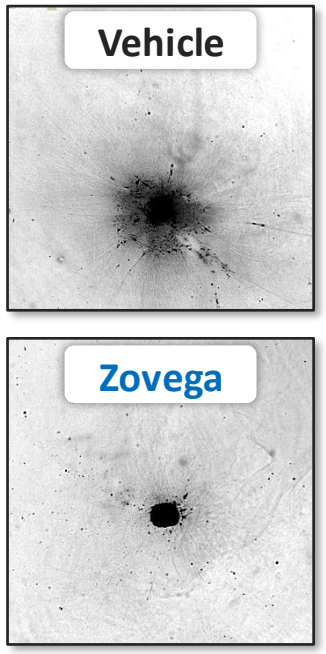


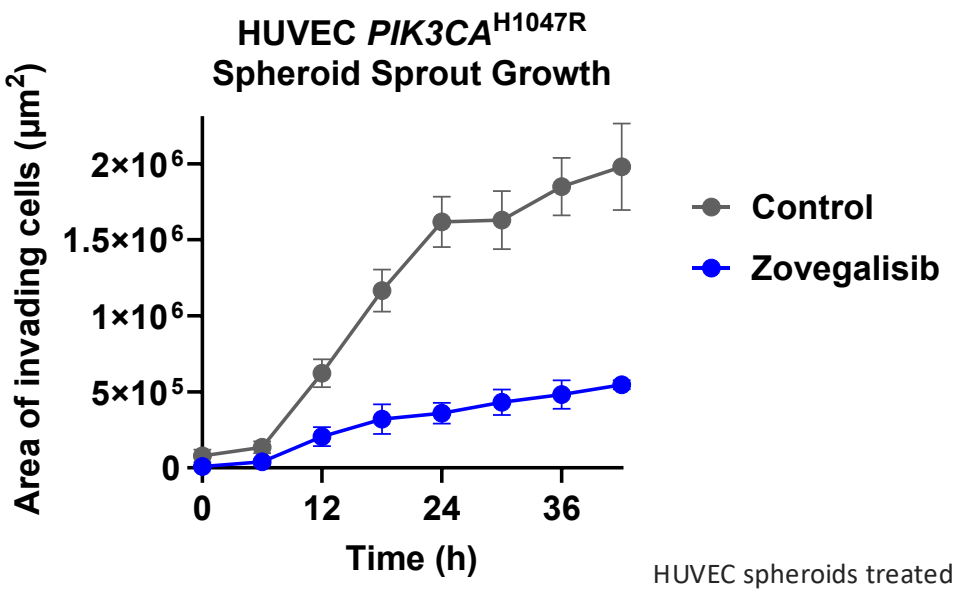
Zovegalisib Inhibits Mutant *PIK3CA* Endothelial Cell Proliferation and Invasiveness

Proliferation



Spheroid Sprout Growth





HUVEC spheroids treated with 2.5 µM of zovegalisib

Zovegalisib treatment reverts mutant phenotypes in HUVEC cells

Development of a *PIK3CA*^{H1047R} HUVEC Xenograft Model to Assess WT and Mutant PI3Kα Inhibition *In Vivo*

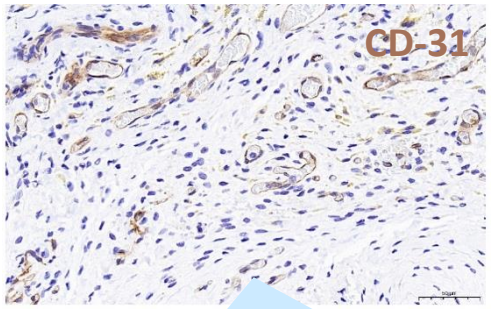
PIK3CA^{H1047R} *HUVEC xenograft (~ 14 days)*

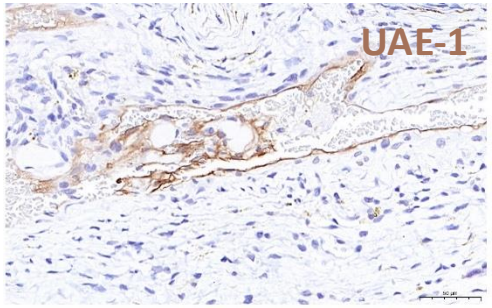
Daily oral dosing (42 days)

- Lesion growth (measurement of mutant PI3Kα inhibition)
- Insulin production (measurement of WT PI3Kα inhibition)

Highly Vascularized Lesions

Histopathology (Untreated Animals)



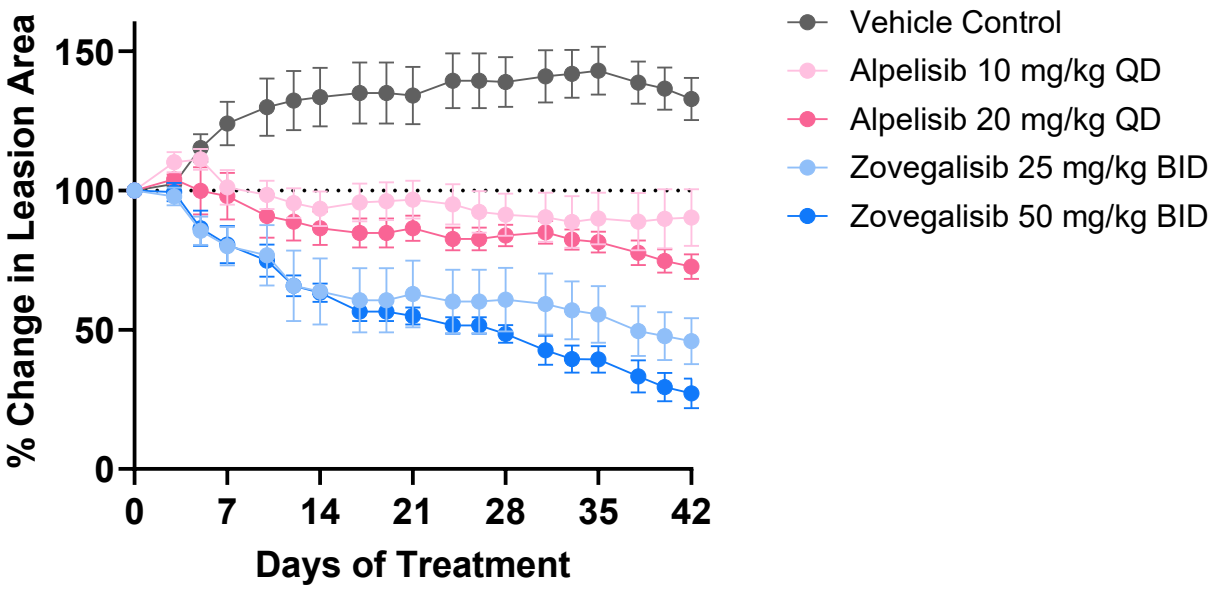


Human Endothelial Markers

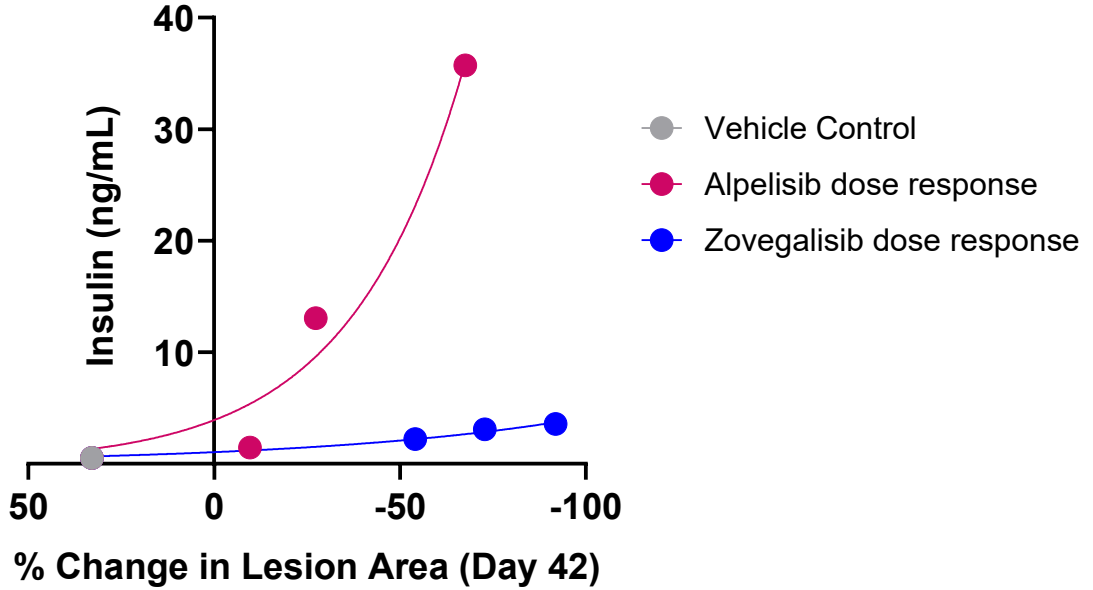
PIK3CA^{H1047R} HUVECs Populate Only a Fraction of the In Vivo Xenograft Lesions

Zovegalisib Achieves Superior Efficacy with More Selectivity Over WT Signaling

Lesion Regression at Clinically Relevant Doses

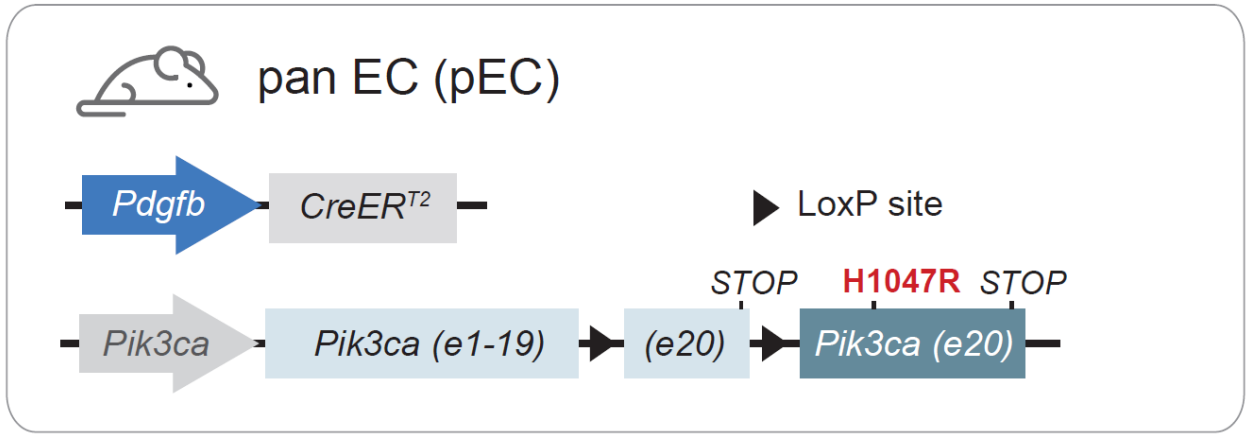


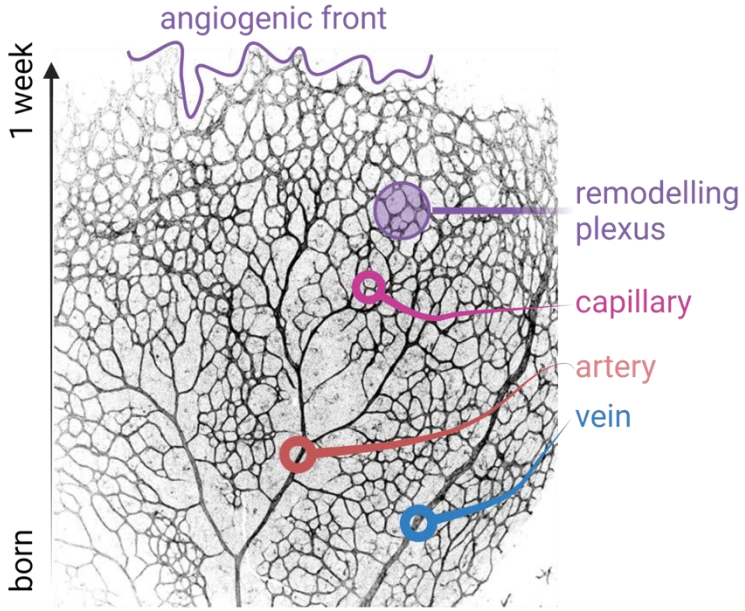
Efficacy vs. Insulin Induction



Mouse Dose		Est. Human Dose
Alpelisib 20 mg/kg QD	~	250-300 mg QD
Zovega 25 mg/kg BID	~	200 mg BID
Zovega 50 mg/kg BID	~	400 mg QD

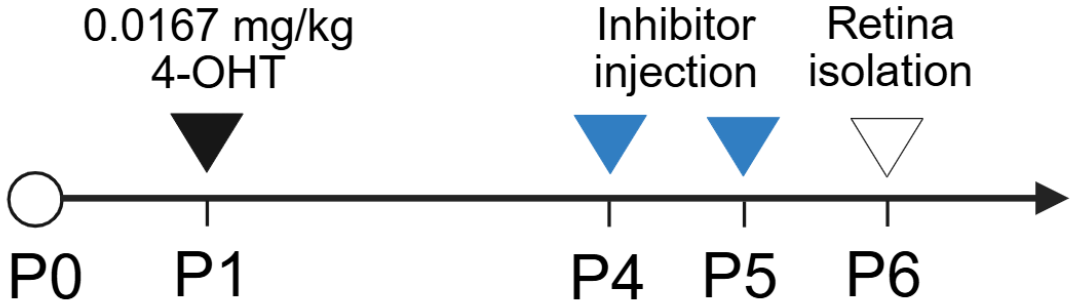
The Mouse Retinal Vasculature Recapitulates All Traits of *PIK3CA*-related Blood Vascular Malformation



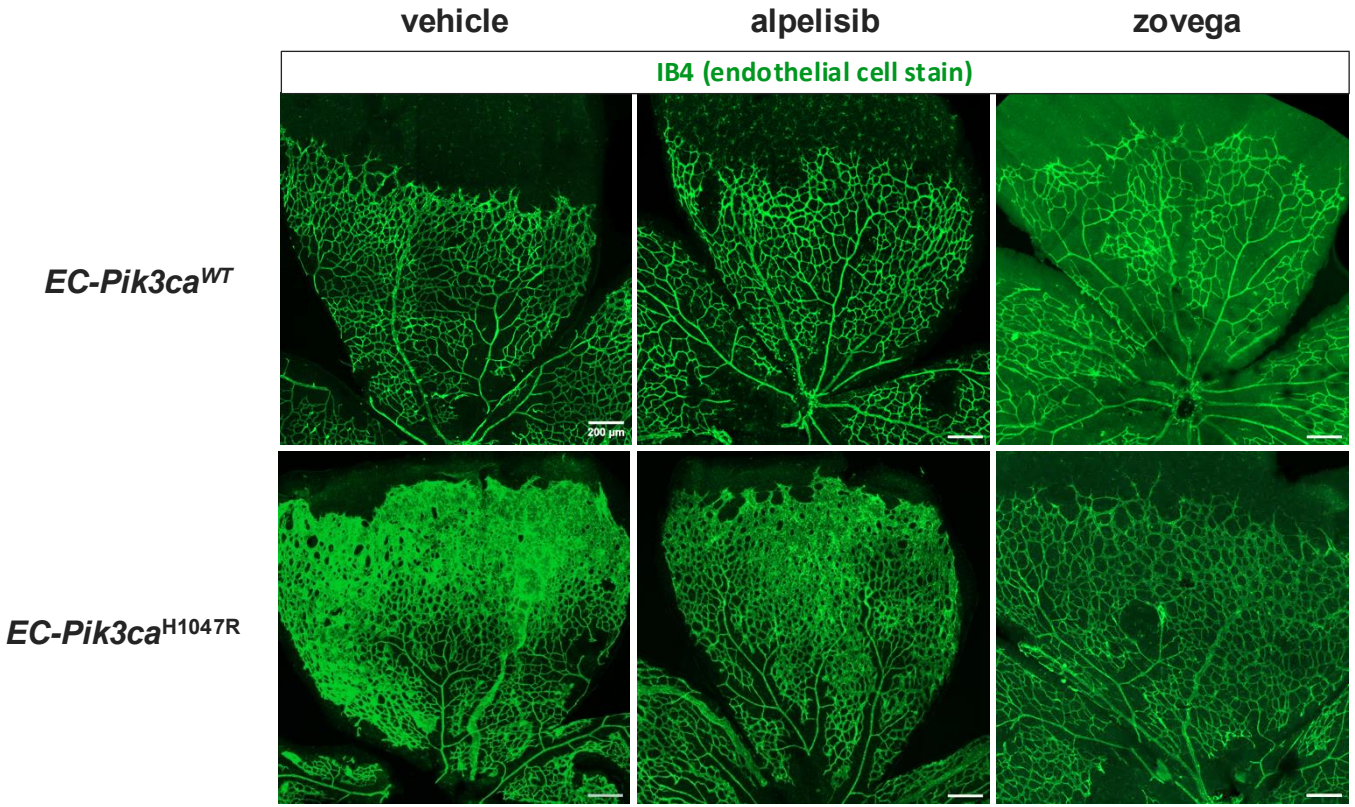


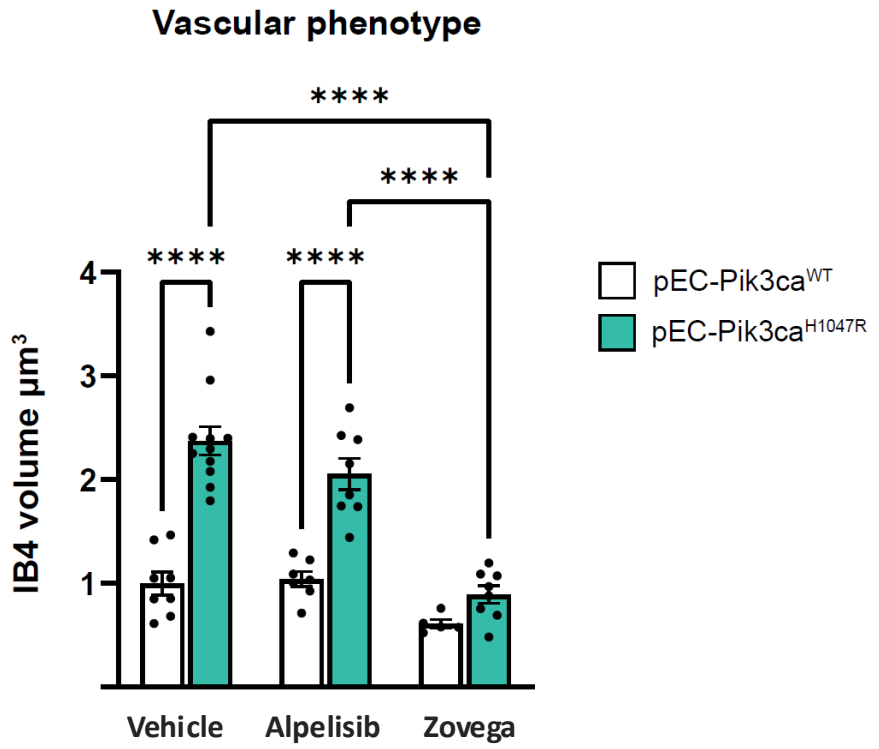
alpelisib: 20 mg/kg

zovegalisib: 50 mg/kg



Zovegalisib Leads to Reversion of Abnormal Vascular Phenotype, Unlike Alpelisib, In a *PIK3CA*-Driven Retinal Vascular Malformation Model





Zovegalisib Is A Novel, Allosteric Mutant-selective PI3Kα Inhibitor

- Zovegalisib potently inhibits aberrant PI3Kα signaling driven by expression of *PIK3CA*^{H1047R} and restores gene expression to a physiological non-disease state in endothelial cells
- Zovegalisib efficiently blocks endothelial cell invasion and proliferation
- *In vivo*, zovegalisib induces greater lesion regression than alpelisib at clinically relevant concentrations due to sustained PI3Kα inhibition and pathway suppression
- Zovegalisib induces less insulin production than alpelisib at efficacious and clinically relevant concentrations
- In more physiological genetic models of vascular malformations, zovegalisib leads to reversion of the abnormal vascular retinal phenotype driven by *PIK3CA* mutation, in contrast to alpelisib

Acknowledgments

We would like to thank the study investigators, sub-investigators, and research staff

ReInspire: A Phase 2 Study of Mutant-selective PI3Kα Inhibitor, RLY-2608, in Adults and Children with PIK3CA Related Overgrowth Spectrum (PROS) and Malformations Driven by *PIK3CA* Mutation

The Phase 2 ReInspire Trial is global trial evaluating RLY-2608 in children (>2yr old), adolescents and adults with PIK3CA-related Overgrowth Spectrum and PIK3CA mutated Vascular Malformations.

Clincaltrials.gov Link: https://clinicaltrials.gov/study/NCT06789913