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ETX-636, a Potential Best-In-Class, Oral, Small Molecule, Allosteric Pan-Mutant-Selective PI3Kα Inhibitor and Degrader

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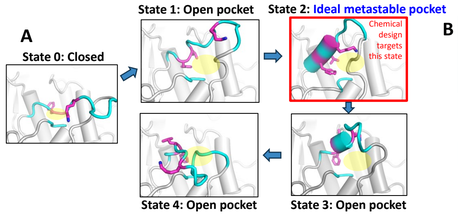
Introduction

- Phosphatidylinositol 3 kinase alpha (PI3Kα) is a frequently mutated oncogene whose activity is important for tumor growth. Up to 40% of hormone receptor (HR)-positive breast tumors are PI3Kα mutant.
- Orthosteric inhibitors, alpelisib and inavolisib, which inhibit both WT and mutant PI3Kα, are approved in combination regimens for treating *PIK3CA*-mutant, HR-positive, HER2-negative, advanced or metastatic breast cancer. However, because PI3Kα is a critical component of the insulin signaling pathway, these non-selective inhibitors which target both the WT and mutant PI3Kα often cause severe hyperglycemia, limiting their clinical utility [1,2,3].
- ETX-636 is an allosteric, pan-mutant-selective PI3Kα inhibitor and degrader, designed leveraging our Kinetic Ensemble® platform for optimal binding properties. Compared to other allosteric, pan-mutant-selective PI3Kα inhibitors (i.e. RLY-2608 and STX-478), ETX-636 has stronger target binding affinity, better on-target potency in biochemical and cellular pharmacodynamic and viability assays, and demonstrates superior anti-tumor activity *in vivo*. Mechanistically, ETX-636 selectively induces proteasome-dependent degradation of mutant p110α protein.
- At efficacious doses, ETX-636 has significantly less effect on blood glucose in mice compared to orthosteric inhibitors, demonstrating that ETX-636 can achieve potent anti-tumor activity by selectively targeting mutant PI3Kα protein without disrupting glucose homeostasis.

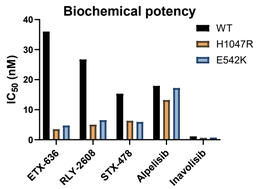
Figure 1. ETX-636 Has Superior Binding Affinity, Potency, and Selectivity Compared to Other Allosteric Pan-Mutant-Selective PI3Kα Inhibitors



B

		Biophysical (Surface Plasmon Resonance)					
		ETX-636		RLY-2608		STX-478	
		PI3Kα WT	PI3Kα H1047R	PI3Kα WT	PI3Kα H1047R	PI3Kα WT	PI3Kα H1047R
K_D	(nM)	13.8	<1	35.9	2.8	49.8	6.7
k_{on}	($M^{-1}s^{-1}$) x 10^3	1.3	11	1.1	12	13.7	76
k_{off}	(S^{-1}) x 10^{-5}	1.8	<1 *	3.9	3.4	68.2	51

*Reached machine detection limit



Mode of inhibition	Compound	Biochemical Selectivity ratio	
		WT/H1047R	WT/E542K
Pan Mutant Allosteric	**ETX-636**	**10.4**	**7.8**
	RLY-2608	5.4	4.1
	STX-478	2.5	2.6
ATP Competitive Orthosteric	Alpelisib	1.4	1.0
	Inavolisib	1.9	1.6

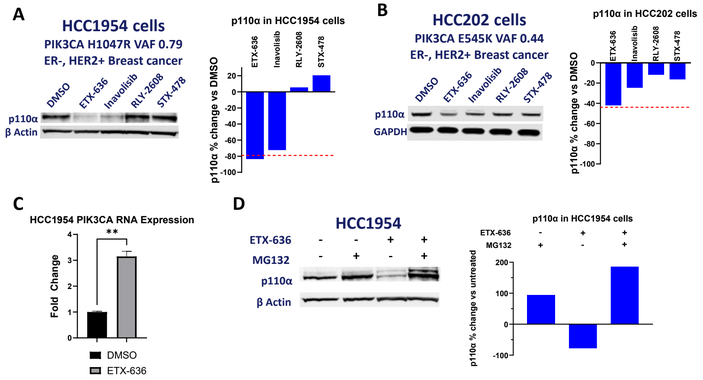
D

pAKT and Cell Proliferation IC_{50} (nM)										
	ETX-636		RLY-2608		STX-478		Alpelisib		Inavolisib	
Cell lines (PIK3CA Status)	pAKT IC_{50} (nM)	CTG IC_{50} (nM)	pAKT IC_{50} (nM)	CTG IC_{50} (nM)	pAKT IC_{50} (nM)	CTG IC_{50} (nM)	pAKT IC_{50} (nM)	CTG IC_{50} (nM)	pAKT IC_{50} (nM)	CTG IC_{50} (nM)
HCC1954 (H1047R)	31	78	87	860	59	1920	135	928	15	74
GP2D (H1047L)	8	57	23	415	23	353	33	790	6	89
MDA-MB-361 (E545K, K567R)	31	206	97	1160	87	838	46	724	11	149
AGS (E453K, E545A)	17	76	90	954	75	811	43	507	6	40
NCIN87 (WT)	260	514	633	1385	535	2607	181	550	23	123

A and B. ETX-636 was designed to target the most stable inhibitory A-loop conformation, resulting in very strong binding affinity as determined by SPR.

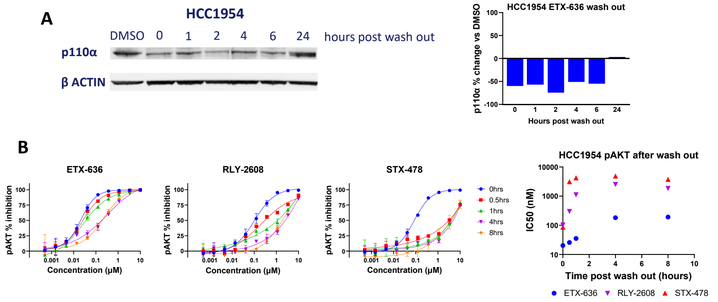
C and D. ETX-636 is more potent and mutant-selective than other allosteric pan-mutant inhibitors in biochemical and cell assays. Biochemical assays **(C)** were run with full length proteins and 2 hours pre-incubation with compound. Cellular levels of pAKT **(D)** were measured by HTRF and included 2 hours of incubation with compound (incubation >2hr results in higher potency for ETX-636 but not other compounds). CellTiter-Glo (CTG) assay for evaluating effect on Cell proliferation **(D)** was run after cells were incubated for 7 days with compound. Absolute IC_{50} values are shown for CTG viability assays.

Figure 2. ETX-636 Selectively Induces Degradation of Mutant p110α Protein



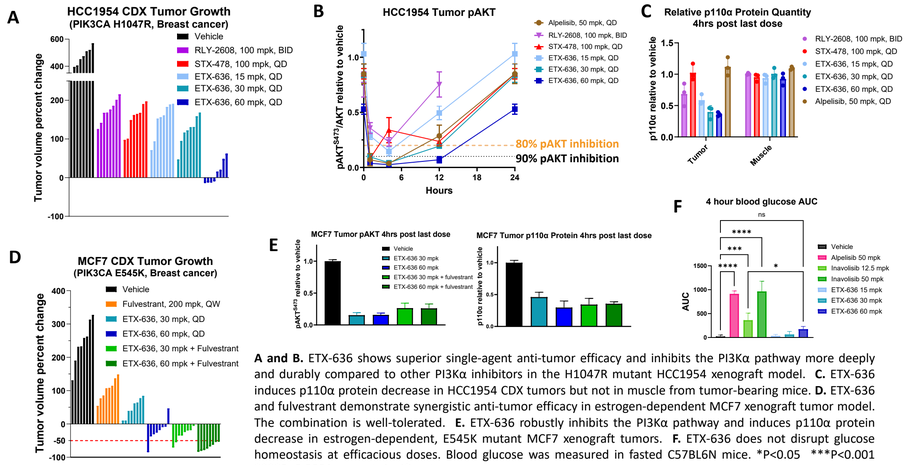
A and B. ETX-636 induces degradation of H1047R and E545K mutant p110α. Protein decrease that corresponds to mutant variant allele frequency for each cell line is marked by a dashed red line on the graphs. Cells were incubated with 1μM of compound for 24hrs. HCC1954 graph corresponds to the western blot shown. HCC202 blot is representative, and the graph is an average of two independent experiments. **C.** Incubation with ETX-636 increases PIK3CA mRNA expression as measured by qPCR (n=2) **P<0.01, consistent with other PI3Kα inhibitors[4]. **D.** ETX-636-induced p110α protein decrease is prevented by the proteasome inhibitor MG132. **E.** Immunoprecipitation for ubiquitin followed by western blot for p110α shows treatment with ETX-636 induces p110α ubiquitination. Compounds were incubated at 1μM for 18-24 hours for *in vitro* experiments.

Figure 3. ETX-636-induced Mutant p110α Decrease Corresponds to Durable Pathway Inhibition



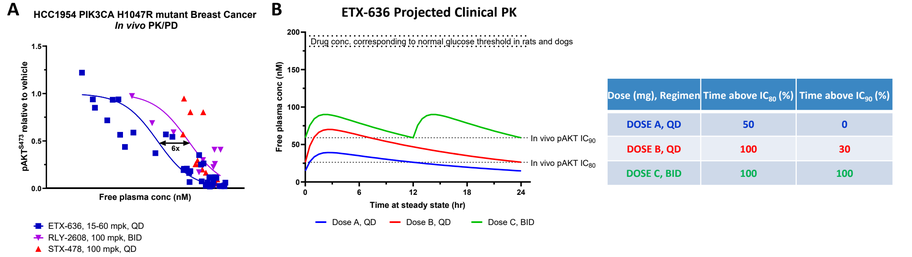
A. Following washout of ETX-636, p110α protein requires greater than 6 hours to return to baseline levels. Cells were incubated with 1μM ETX-636 for 24 hours and then cultured in fresh media without compound for the indicated durations of time. **B.** PI3Kα pathway inhibition is better sustained after wash out of ETX-636 compared to other allosteric pan-mutant selective PI3Kα inhibitors.

Figure 4. ETX-636 Achieves Superior Anti-tumor Efficacy and Pathway Inhibition Without Disrupting Glucose Homeostasis



A and B. ETX-636 shows superior single-agent anti-tumor efficacy and inhibits the PI3Kα pathway more deeply and durably compared to other PI3Kα inhibitors in the H1047R mutant HCC1954 xenograft model. **C.** ETX-636 induces p110α protein decrease in HCC1954 CDX tumors but not in muscle from tumor-bearing mice. **D.** ETX-636 and fulvestrant demonstrate synergistic anti-tumor efficacy in estrogen-dependent MCF7 xenograft tumor model. The combination is well-tolerated. **E.** ETX-636 robustly inhibits the PI3Kα pathway and induces p110α protein decrease in estrogen-dependent, E545K mutant MCF7 xenograft tumors. **F.** ETX-636 does not disrupt glucose homeostasis at efficacious doses. Blood glucose was measured in fasted C57BL6N mice. *P<0.05 ***P<0.001 ****P<0.0001 ns= not significant

Figure 5. ETX-636 is Projected to Achieve Deep Pathway Inhibition Without Causing Hyperglycemia



Dose (mg), Regimen	Time above IC_{80} (%)	Time above IC_{90} (%)
DOSE A, QD	50	0
DOSE B, QD	100	30
DOSE C, BID	100	100

A. *In vivo* PK-PD relationship indicates ETX-636 has superior intrinsic potency. **B.** ETX-636 is projected to achieve clinical exposures sufficient for deep, sustained pathway inhibition without causing hyperglycemia. pAKT IC_{80} and IC_{90} values were calculated from panel A. Drug concentration thresholds corresponding to normal glucose were determined in rat and dog toxicology studies.

Conclusions

- ETX-636 is a potential best-in-class allosteric, pan-mutant-selective PI3Kα inhibitor with superior biophysical, biochemical, and cellular profiles
- ETX-636 induces degradation of mutant p110α *in vitro* and *in vivo*, but spares wildtype p110α - a unique mechanism of action among allosteric pan-mutant-selective PI3Kα inhibitors
- ETX-636 demonstrates regressions in both kinase and helical domain PI3Kα mutant tumors, synergizes with fulvestrant, and shows concordant deep and durable PI3Kα pathway inhibition in preclinical xenograft models
- ETX-636 has the potential to achieve drug exposures to continuously cover the pAKT IC_{90} in the clinic without hyperglycemia and is entering first-in-human clinical trials in the first half of 2025

References
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3. Rugo, H. S. et al. *Ann. Oncol.* **31**, 1001–1010 (2020).
4. Song, K.W. et al. Cancer Discov. 12(1), 204-219 (2022)