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Discovery and characterization of a PI3Kα^{H1047X} mutant selective inhibitor with a best-in-class profile

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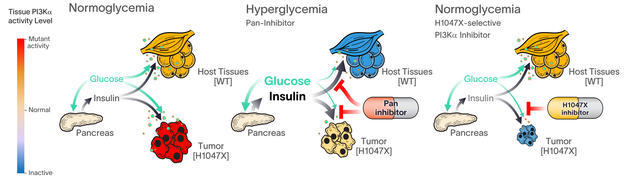
Introduction

PI3Kα is highly mutated in cancer with the most prevalent mutation, H1047R, occurring in approximately 14% of breast cancers[1]. This mutation causes hyperactivation of lipid kinase activity and downstream AKT signaling. Therapeutic proof-of-concept for targeting PI3Kα mutations was established with alpelisib, an alpha-selective PI3K inhibitor that is equipotent against wild-type and mutant forms[2]. However, the therapeutic benefit of alpelisib is limited by the inhibition of wild-type PI3Kα in normal tissues, resulting in dose-limiting toxicities including hyperglycemia. We hypothesize that selective targeting of mutant PI3Kα^{H1047X} will improve anti-tumor activity with reduced toxicity. To test this, we have identified ST-814 an allosteric, CNS-penetrant, mutant selective PI3Kα^{H1047X} inhibitor, which has excellent drug-like properties and exceptional kinome and PI3K isoform selectivity. ST-814 has potent activity in cell-based target engagement and tumor cell growth assays and demonstrates robust anti-tumor activity in PI3Kα^{H1047X} xenografts. ST-814 efficacy was superior to alpelisib at a dose level that exceeds the clinically relevant exposure. Importantly, ST-814 lacked metabolic dysfunction observed with alpelisib which caused profound insulin resistance.

Selective targeting of mutant PI3Kα improves efficacy



Results

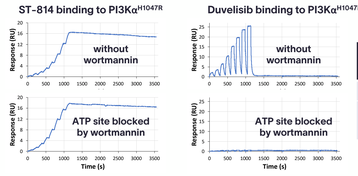
ST-814 is a potent PI3Kα H1047X-Selective Kinase Inhibitor

PI3Kα Mutation		alpelisib IC_{50} (nM)	ST-814 IC_{50} (nM)
Wildtype		7.1	130.7
H1047X	R	3.9	9.4
	L	6.1	4.4
	Y	9.9	17.1

Kinase	alpelisib[2] IC_{50} (nM)	ST-814 IC_{50} (nM)
PI3Kβ	>1000	50,000
PI3Kγ	250	>100,000
PI3Kδ	290	9700
Broad Kinome Profiling	11/254 kinases <10,000	372 kinases >10,000

Recombinant PI3Kα mutants were tested for ATPase activity using ADP-Glo (Promega). ST-814 kinome profiling was conducted at Km for ATP (Eurofins).

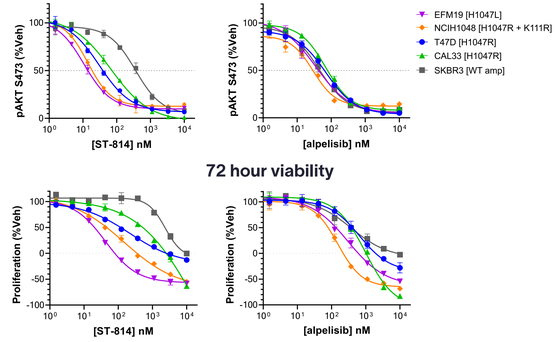
ST-814 binds directly to a PI3Kα H1047R allosteric site



Compound	K_D (nM), H1047R, without wortmannin	K_D (nM), with wortmannin
ST-814	2.9	0.88
duvelisib (ATP-competitive inhibitor)	44	no binding

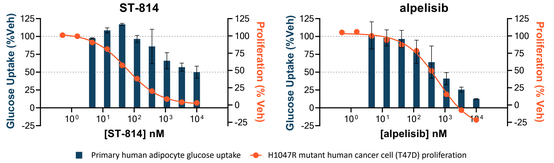
Biacore 8K instrument and Sensor Chip SA were used with biotinylated protein pretreated with/without wortmannin and 90 seconds association and 2,400 seconds dissociation times.

ST-814 selectively reduces tumor cell PI3Kα signaling and proliferation



Effect of compounds on target engagement and cell growth was assessed in a panel of PI3Ka mutant and wild-type cell lines grown in media with 10% FBS. Target engagement was measured after 1 hour compound treatment using pAKT S473 HTRF (Perkin Elmer). Cell growth was assessed after 72 hours of treatment using CTG assay (Promega).

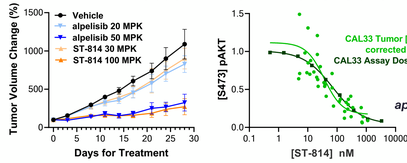
ST-814 preserves insulin-mediated glucose uptake in human primary adipocytes



Insulin-dependent ^{3}H-2-deoxyglucose uptake in 1^0 human subcutaneous adipocytes (N=2, Zen-Bio) was assessed following 1 hour compound treatment and insulin stimulation. Cells were washed, lysed, scintillant added, and counted. Insulin-dependent glucose uptake was calculated using average CPM treatment minus non-specific uptake in cytochalasin B treated cells.

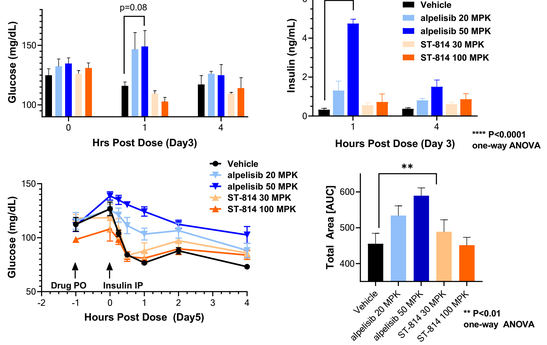
ST-814 demonstrates superior anti-tumor efficacy vs. alpelisib

Efficacy of ST-814 in CAL33 xenografts with robust in vitro: in vivo correlation



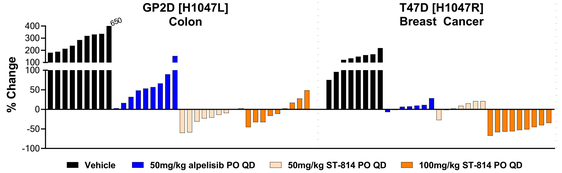
Efficacy and PK/PD studies were carried out using CAL33 xenografts in Balb/c nude mice. Tumor volumes are shown (left). PK/PD was performed on dosing day 3, tumor drug levels and relative pAKT S473 / AKT was measured by quantitative western blot (light green) vs. the in vitro CAL33 HTRF data (dark green).

ST-814 does not induce metabolic dysfunction



Blood glucose and insulin were measured at 1 and 4 hours after the third dose in the CAL33 study (top). Insulin sensitivity was measured by ITT in Balb/c nude mice (bottom). Food was removed 5 hours prior to drug treatment, and IP insulin.

ST-814 treatment provides superior efficacy with tumor regressions

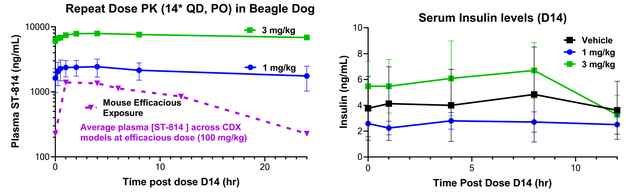


GP2D human tumor cells were implanted SC in Balb/c nude mice. T47D cells were implanted SC in female NSG mice with 90-day release 17-β-estradiol tablets. Treatment was initiated when tumors were ~150 mm^3 for 28 (GP2D) or 21 (T47D) days.

ST-814 Has Excellent Drug-Like Properties

Assay Parameter	ST-814 Key Characteristics
P_{app} (A-B) (10^{-6}, cm/s)| Pgp efflux ratio	High permeability and low efflux → Good bioavailability and distribution
Mouse $K_{p,uu}$	CNS exposure → Likely suitable to treat brain tumors/metastases
HLM $Cl_{int,u}$ | hHep $Cl_{int,u}$ (L/h/kg)	Low Cl → Low dose & peak to trough, improved safety
CYP inhibition 3A4 | 2D6 (μM)	Low inhibition of CYPs → Potential for combinations
hERG IC_{50} (μM)	Low hERG inhibition → Low risk for CV safety

Dog exposure (3 mg/kg, 14*QD) exceeds efficacious plasma AUC (mouse CDX) by 10-fold with no effect on serum insulin levels



Conclusions

- ST-814 is a potent and H1047X mutant-selective, allosteric PI3Kα inhibitor
- It has exceptional selectivity against PI3K isoforms and the kinome
- In cell assays, ST-814 selectively inhibits downstream signaling (pAKT) and tumor cell growth of H1047X mutant cell lines
- ST-814 is active across a spectrum of PI3Kα^{H1047X} CDX models and tumor types, while sparing metabolic dysfunction and achieving efficacy that is superior to alpelisib at clinically relevant exposures
- PK in higher species predicts low QD human dose with a long half-life and minimal peak-to-trough plasma concentrations, this is expected to further support a favorable therapeutic index
- ST-814 has the potential to provide a best-in-class profile to improve outcomes in patients harboring tumors with prevalent H1047X mutations and is currently in IND enabling studies

References / Acknowledgements

[1] Martínez-Sáez 2020 , Breast Cancer Research 22, 45
[2] Fritsch 2014, Molecular Cancer Therapeutics 13, 1120
[3] NDA/BLA Multi-disciplinary Review and Evaluation {NDA 212526} Document {Piqray, Alpelisib}

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