EAI-432, a mutant-selective allosteric EGFR inhibitor for L858R-mutant lung cancer

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Introduction

- About a third of all non-small cell lung cancer (NSCLC) patients have EGFR mutations. By far the most common mutations are exon19 deletions and the L858R point mutation, which account for approximately 45% and 40%, respectively, of EGFR-mutant NSCLC.
- The 3rd generation EGFR TKI osimertinib is standard of care therapy for patients with these mutations, and is also effective against the treatment-acquired T790M mutation that confers resistance to 1st generation EGFR TKIs gefitinib and erlotinib.
- Like all 3rd generation EGFR TKIs, osimertinib relies on formation of a covalent bond with Cysteine 797 in EGFR. Mutation of this cysteine residue (C797S, C797A) is an important mechanism of treatment-acquired resistance (e.g. L858R/C797S and L858R/T790M/C797S).
- Other mutations in the EGFR ATP-site also confer resistance to ATP-site inhibitors (L718X, L792F, G796S). Together with C797X (7 to ~12%), these on-target mutations in EGFR account for up to ~15% of osimertinib resistance, representing a major unmet need.[1]
- In addition, most of the benefit of osimertinib vs. 1st gen EGFR TKIs is in exon19del patients. It offers little improvement in overall survival in L858R patients.
- Due to the prevalance of CNS metastases, brain penetration is important for new therapeutics in this space.
- Our development candidate EAI-432 is specifically designed to address these unmet needs. It is a highly mutant-selective allosteric inhibitor effective against EGFR L858R, L858R/C797S, and L858R/T790M/C797S.

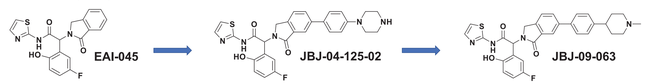
Discovery of allosteric EGFR inhibitors at DFCI

Collaboration between the Eck, Gray, and Jänne Labs

Overcoming EGFR(T790M) and EGFR(C797S) resistance with mutant-selective allosteric inhibitors *Nature* 2016 (ref. 2)

Single and Dual Targeting of Mutant EGFR with an Allosteric Inhibitor *Cancer Discovery* 2019 (ref. 3)

An allosteric inhibitor against the therapy resistant mutant forms of EGFR in non-small cell lung cancer *Nature Cancer* 2022 (ref. 4)



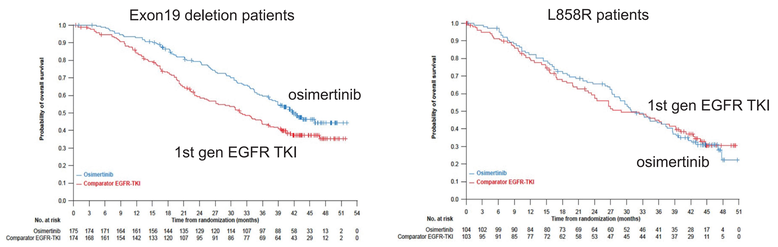
This isoindolinone series was the starting point for an industry collaboration that led to our development candidate EAI-432.

Development path for EAI-432

- Conduct initial trial and seek approval in EGFR L858R+ patients who develop resistance to osimertinib due to treatment-acquired mutations in EGFR (C797S, L718Q, G719S, etc).
- Expansion to first line for EGFR L858R+ lung cancer as combination with osimertinib or other compatible 3rd generation EGFR TKI..
- Combination with our allosteric inhibitor is relevant to ~40-50% of the total osimertinib patient population.

Opportunity for front-line impact in L858R+ patients

FLAURA study showed that osimertinib does not improve overall survival in L858R patients relative to 1st generation EGFR TKIs (Ramalingam et al. *NEJM* 2020)[5]



EAI-432 co-binds EGFR with osimertinib. Will "double-drugging" the mutant receptor with EAI-432 plus osimertinib improve outcomes for L858R+ patients?

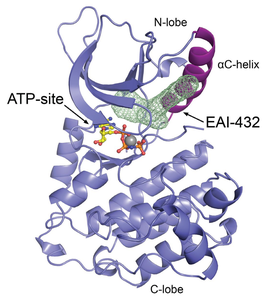
EAI-432 is potent across all EGFR L858R mutants

Inhibition of proliferation in L858R mutant Ba/F3 cell lines and A431 cells (WT EGFR).

Cpd	L858R Cell IC_{50} (μM)	L858R/T790M Cell IC_{50} (μM)	L858R/C797S Cell IC_{50} (μM)	L858R/T790M/C797S Cell IC_{50} (μM)	EGFR wt Cell IC_{50} (μM)	A431 Cell IC_{50} (μM)
EAI045	>10	3.82	>10	1.89	>10	>10
JBJ-09-063	0.013	0.008	0.028	0.007	2.68	3.14
EAI-432	0.010	0.004	0.014	0.004	1.75	1.87
gefitinib	0.011	>10	0.033	>10	0.11	1.32
osimertinib	0.005	0.013	>10	>10	2.89	0.31
BLU945	0.051	0.003	0.126	0.004	1.54	>10

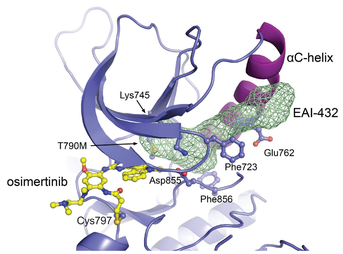
EAI-432 co-binds with osimertinib

The allosteric site occupied by EAI-432 is adjacent to the ATP-site



Co-crystal structure of EAI-432 and an ATP analog bound to T790M-mutant EGFR. EAI-432 is shown in a surface representation (green mesh).

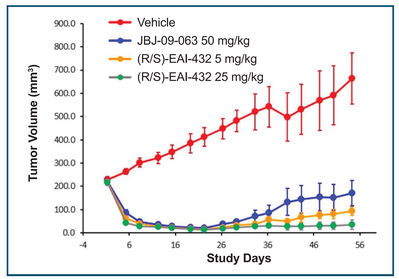
EAI-432 and osimertinib can bind simultaneously to mutant EGFR



Co-crystal structure of EAI-432 and osimertinib bound to T790M-mutant EGFR. EAI-432 locks the kinase in an inactive conformation with the C-helix (purple) displaced from the active site.

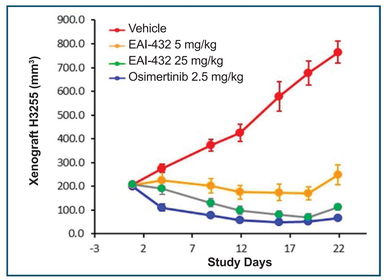
EAI-432 has good efficacy in mouse xenograft models

PDX DFCI52 (L858R/T790M) + C797S



Mice were dosed PO for 21 days QD with the indicated compounds. EAI-432 was dosed as a racemate in this study. The C797S mutation was introduced in the patient-derived DFCI52 cell line using CRISPR.

H3255 NSCLC xenograft (L858R)



Mice were dosed PO for 21 days QD with the indicated compounds. EAI-432 was dosed as the active (*R*) enantiomer.

Advantages of our allosteric approach

- EAI-432 is specifically developed for L858R EGFR and its resistance variants. It spares WT EGFR.
- EAI-432 can co-bind with osimertinib.
- The co-binding mechanism is an occupancy and efficacy multiplier. With two ATP-site drugs, one must unbind to allow the other to bind, leaving an *active* receptor in the interim. With the allosteric plus ATP-site approach, both can bind simultaneously, and if one dissociates the receptor remains *inhibited*.
- EAI-432 should delay or prevent emergence of resistance due to second-site mutations in the receptor. Because of its distinct binding site, different mutations are required to confer resistance as compared with ATP-site inhibitors.
- It is difficult to combine two ATP-site kinase inhibitors due to overlapping off-target toxicities. The exquisite kinome-wide selectivity of EAI-432 recommends it as a combination agent.

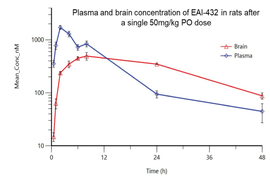
EAI-432 has good oral PK and is brain penetrant

Hepatocyte stability	Mouse	Rat	Dog	Monkey	Human
In vitro CLint (μL/min/million cells)	4.3	15.6	11	<2	4.3

Species	IV Cl (ml/min/kg)	T1/2 (hr)	Vd (L/kg)	F%
Rat (IV 1mpk, PO 5mpk)	19	4.5	5.6	55
Dog (IV 1mpk, PO 5mpk)*	19	11.7	16	43
Monkey (IV 0.5mpk, PO 3mpk)	2.9	22.6	5.0	51

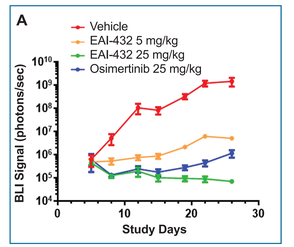
*Dog PK was measured with the racemate, all other PK data with the R enantiomer

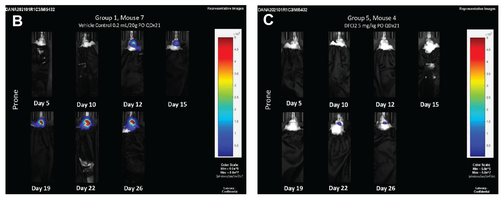
Rat Kp,uu = 0.49



EAI-432 is effective in intracranial H1975-luc model

H1975 (L858R/T790M) luciferized intracranial implant, 21 days oral dosing, QD





EAI-432 is effective in an intracranial H1975-luc NSCLC brain metastasis model. A, Intracranial anti-tumor efficacy of EAI-432 and osimertinib was monitored by bioluminescence imaging. B and C, Representative images of mice treated with vehicle control, and 5mg/kg EAI-432

Summary

- EAI-432 is a potent and mutant-selective allosteric EGFR inhibitor for L858R-driven NSCLC.
- EAI-432 has good oral PK and is brain-penetrant.
- EAI-432 has efficacy in multiple L858R+ tumor models at low doses and is well-tolerated *in vivo*.
- Our allosteric EGFR inhibitors have exquisite kinase selectivity and minimal activity on WT EGFR.
- IND-enabling studies are in progress, to be completed 2H 2024

Acknowledgments

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