Abstract # 236

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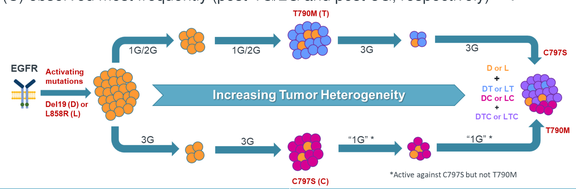
Preclinical characterization of THE-349, a CNS-active, mutant-selective fourth-generation EGFR inhibitor with potent activity against single, double, and triple mutant EGFR variants including T790M and C797S

Sen Zhang, Wei-Sheng Huang, Sara Nadworny, Emily Ye, Narayana Narasimhan, Charles J. Eyermann, David C. Dalgarno, Victor M. Rivera, William C. Shakespeare.
Theseus Pharmaceuticals, Inc., Cambridge, MA, USA

Introduction

- EGFR activating mutations are observed in 10-50% of NSCLC patients and the common mutations (L858R [L] and exon 19 deletions [D]) are initially sensitive to first-, second-, and third-generation EGFR inhibitors (eg erlotinib [1G], afatinib [2G], and osimertinib [3G])[1,2,3].
- However, on-target resistance is observed in a substantial percentage of patients, with T790M (T) and C797S (C) observed most frequently (post-1G/2G and post-3G, respectively) [4, 5].



Problem	Our Solution
EGFR mutational heterogeneity increases during treatment with 1G/2G and 3G inhibitors	Potent activity against all major EGFR mutant variants (ie, single-, double-, and triple-mutant variants)
Inhibition of wild type (WT) EGFR causes dose-limiting toxicities	Substantial selectivity over WT EGFR
EGFR-mutant NSCLC commonly metastasizes to the brain	Strong activity in the central nervous system (CNS)
Addressing off-target resistance mechanisms requires combination with a non-EGFR inhibitor	Single compound likely more combinable

Methods

- **In vitro cellular assay**: Compound potency against EGFR mutants was assessed by measuring effects on viability of Ba/F3 cells expressing EGFR mutant variants. Potency against WT EGFR was assessed by measuring Tyr1068 phosphorylation (pEGFR) in EGFR-amplified A431 cells stimulated with 25 ng/mL EGF.
- **In vivo efficacy**: Anti-tumor activity was evaluated in mice implanted with engineered Ba/F3 cells, human NSCLC cell lines or patient-derived xenografts (PDX; LUPF-104 [LD1-0025-200717]) expressing EGFR mutant variants. Animals were randomized by tumor volume or bioluminescence and dosed once daily, beginning Day 0, for the number of days indicated in each study (10 – 75 days). Log rank Mantel Cox test was used to assess statistical significance ± where indicated. All dose levels were well-tolerated with no adverse clinical signs observed.
- **Pharmacokinetics (PK) /pharmacodynamics (PD)**:After a single compound dose, tumor pEGFR levels (AlphaLisa) and plasma compound concentrations (LC-MS/MS) were determined. Least squares regression or robust regression analysis of all PK/PD data was used to calculate the concentration required to inhibit pEGFR by 50%.

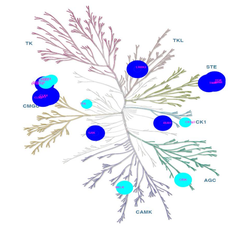
Results

THE-349 potently inhibits EGFR single-, double-, and triple-mutant variants in biochemical assays with broad kinome selectivity

- Including 3 C797 variants - C797S, C797A and C797G
- THE-349 has a high degree of kinome selectivity
  - S-score (50) = 0.07 (23/330 kinases inhibited by >50% at 0.1 µM)

EGFR variant	Biochemical IC_{50} (nM)		
	Erlotinib	Osimertinib	THE-349
L	0.2	0.6	0.7
LT	207	0.2	0.1
LC	0.3	746	0.6
L-C797G	0.5	824	0.8
LTC	345	360	0.4
D	0.3	0.6	0.6
DC	0.6	418	0.9
D-C797A	2.8	1258	3.2

- Kinase activity measured in the presence of 10 µM ATP except for LT which was measured at 20 µM ATP
- Purple indicates IC_{50} > 100 nM against mutant EGFR



Kinome map: THE-349

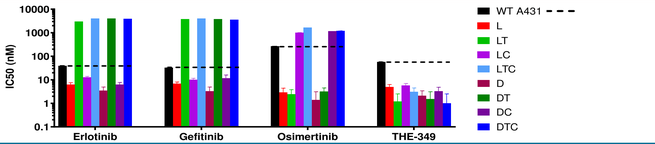
≥ 80% inhibition 50-79% inhibition

THE-349 potently, and selectively, inhibits all major EGFR single-, double-, and triple-mutant variants in cellular assays

- Selectivity of THE-349 for all 8 mutant variants versus WT EGFR (10-56X) exceeds that of erlotinib (6-12X) and gefitinib (5-10X) against D and L (approved indications)

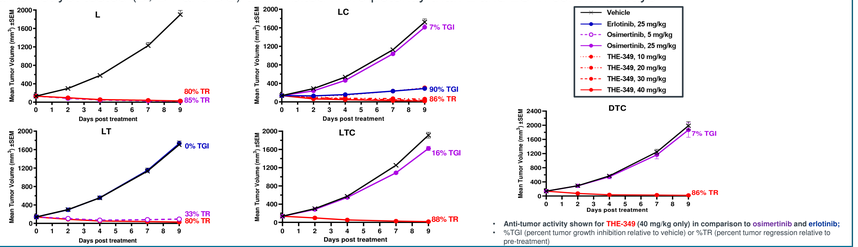
EGFR Variant:	Ba/F3 viability, IC_{50} (nM)								A431 pEGFR IC_{50} (nM)
	L	LT	LC	LTC	D	DT	DC	DTC	WT
Erlotinib	6.3	>2960	12.7	>4000	3.5	>4000	6.3	>3921	42
Gefitinib	6.9	>3819	10.2	>4000	3.3	>4000	11.8	>3569	33
Osimertinib	3.0	2.6	1010	1684	1.5	3.4	1169	1190	327
THE-349	5.0	1.2	5.8	3.1	2.1	1.5	3.3	1.0	57

- Purple indicates IC_{50} > 1000 nM against mutant EGFR



THE-349 induces deep regressions, at well-tolerated doses, in Ba/F3 tumor models expressing EGFR single-, double-, and triple-mutant variants

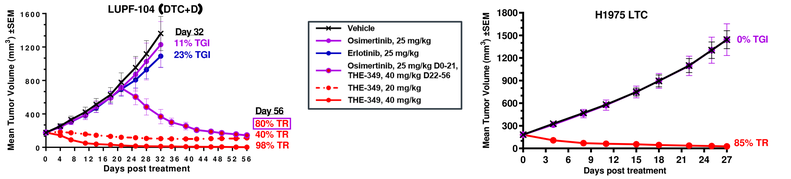
- THE-349 (40 mg/kg, QD) induced ≥80% tumor regression (TR) against all 5 variants tested in aggressive Ba/F3 models
  - Variants not yet tested (D, DT and DC) all inhibited more potently than L and LC in cellular assays



- Anti-tumor activity shown for THE-349 (40 mg/kg only) in comparison to osimertinib and erlotinib;
- %TGI (percent tumor growth inhibition relative to vehicle) or %TR (percent tumor regression relative to pre-treatment)

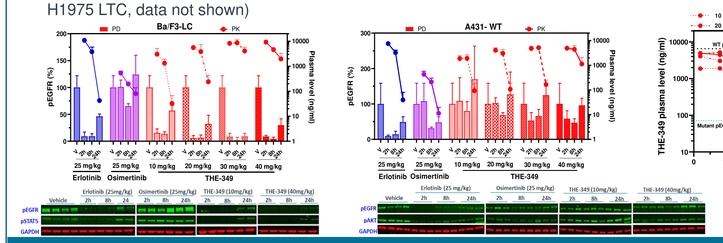
THE-349 induces deep, durable regressions in osimertinib-resistant PDX and NSCLC tumor models

- LUPF-104 is a model developed from a patient with NSCLC after failure of >5 lines of therapy (including erlotinib and osimertinib)
  - RNAseq/WES analysis suggests presence of both EGFR-DTC and -D (T and C mutants detected *in cis* at ~16%; D mutant detected at ~37%)
- Single agent THE-349 (40 mg/kg) induced long-term tumor regression (98% TR), with complete responses in 9/10 mice
  - In a cross-over arm, following treatment with osimertinib for 22 days, THE-349 treatment for the next 35 days induced 80% TR
- THE-349 induced 85% TR in an H1975 LTC model developed by CRISPR knock-in of C797S into H1975 (LT) cells



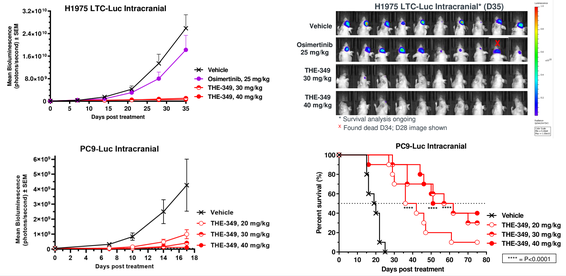
A single dose of THE-349 leads to sustained *in vivo* inhibition of mutant EGFR (Ba/F3-LC), but not WT EGFR (A431)

- PK/PD analysis suggests a wide selectivity window *in vivo*, with THE-349 exposure at efficacious dose levels in mice (10-40 mg/kg) substantially exceeding the IC_{50} for inhibition of mutant pEGFR, while remaining below the IC_{50} for inhibition of WT EGFR
  - Strong, sustained inhibition of mutant pEGFR also observed in 2 triple mutant models (Ba/F3-DTC and H1975 LTC, data not shown)



THE-349 is active in CNS mouse models

- THE-349 demonstrates significant anti-tumor activity in H1975 LTC and PC9 (D) intracranial models in mice, with increased survival highlighting CNS activity/penetration
- CNS activity reflects balance of strong potency and ~9% brain:plasma ratio (Kp) in mice (similar in rats)



- Kp was calculated as follows: AUC_{brain}/AUC_{plasma}
- Since both brain and plasma protein binding are ~99.9% (in mice and rats), Kp approximates Kp,uu

Conclusions

- EGFR mutational heterogeneity in patients who develop osimertinib resistance is complex
- THE-349 is a reversible, potent, and selective 4th generation EGFR inhibitor specifically designed to target this complexity **as a single agent**
- THE-349 potently inhibits **all major EGFR single-, double-, and triple-mutant variants** with excellent WT- and broad kinome-selectivity, potentially resulting in a streamlined development path and greater combinability (with non-EGFR agents)
- THE-349 demonstrates strong tumor regressions against osimertinib-resistant C797S double- (LC) and triple-mutants (LTC and DTC) to address clinical failure after 1st and 2nd line use, respectively
- THE-349 induces deep/complete tumor regression in an osimertinib-resistant patient derived model (PDX) harboring heterogeneous EGFR mutant variants (D and DTC)
- THE-349 exhibits substantial CNS activity, extending survival in intracranial models
- IND-enabling studies are ongoing with submission expected in H2 2023

References

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