Poster #3342

AACR 2022

Discovery of potent and selective next-generation EGFR inhibitors with activity against single, double, and triple mutant EGFR variants including T790M and C797S

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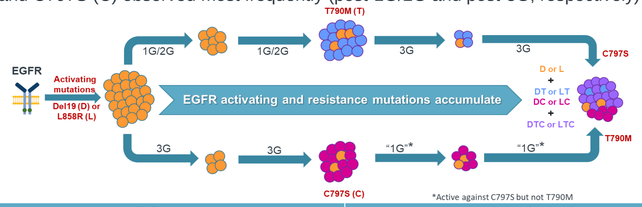
Introduction

- 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 pan-EGFR mutant activity (i.e., activity against single-, double-, and triple-mutant variants)
Inhibition of wild type (WT) EGFR causes dose-limiting toxicities	Selectivity over WT EGFR
EGFR-mutant NSCLC commonly metastasizes to the brain	Ability to penetrate the central nervous system (CNS)
Addressing off-target resistance mechanisms requires combination with a non-EGFR inhibitor	Single compound likely more combinable

Methods

Compounds: All compounds were synthesized internally or purchased from Selleck or MedChemExpress.
Kinase assay: Kinase inhibition assays to determine IC_{50}s against EGFR mutant variants were conducted with 3-fold serial dilutions using ATP concentrations at their respective Km.
In vitro cellular viability assay: Cellular potency was evaluated in Ba/F3 cells expressing EGFR mutant variants. Parental Ba/F3 cells were cultured in the presence of 10 ng/mL IL3. Cell viability (IC_{50}) was assessed using CellTiter Glo (Promega) after 72-hour treatment.
In vitro cellular kinase activity: Potency against WT EGFR was assessed by measuring levels of EGFR phosphorylated at Tyr1068 (pEGFR) in EGFR-amplified A431 cells stimulated with 25 ng/mL EGF.
In vivo efficacy: Anti-tumor activity was evaluated in Ba/F3 cells expressing EGFR mutant variants. When tumor sizes reached ~130 mm^3, mice were randomized and sorted by tumor volume, and vehicle or compound was administered by oral gavage once daily for 10 days. All dose levels were well-tolerated with no adverse clinical signs observed.
Pharmacokinetics (PK) /pharmacodynamics (PD): Tumor-bearing mice were treated with a single dose of vehicle or compound. Tumor samples were collected post-dose and analyzed by AlphaLisa (PerkinElmer) and western blot. Compound concentrations in plasma were determined by LC-MS/MS.
Measure of CNS penetrance: Rats were dosed orally at 10 mg/kg/day. After the 4th dose, blood was collected and brain was harvested from 2 rats at multiple time points. Compound concentrations in plasma and brain homogenates were determined by LC-MS/MS.

Results

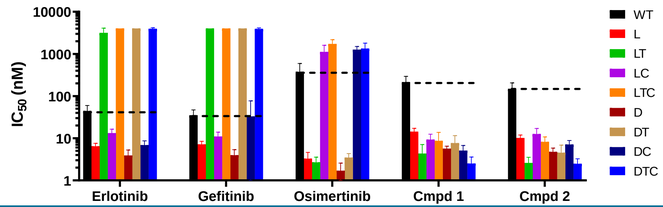
Cmpd 1 and Cmpd 2 potently inhibit EGFR single-, double-, and triple-mutant variants in biochemical assays

	EGFR kinase activity, IC_{50} (nM)		
Compound	L	LT	LTC
Erlotinib	0.1	207	345
Gefitinib	0.1	240	612
Osimertinib	0.4	0.2	360
Cmpd 1	3.4	0.1	0.3
Cmpd 2	0.6	0.1	0.7

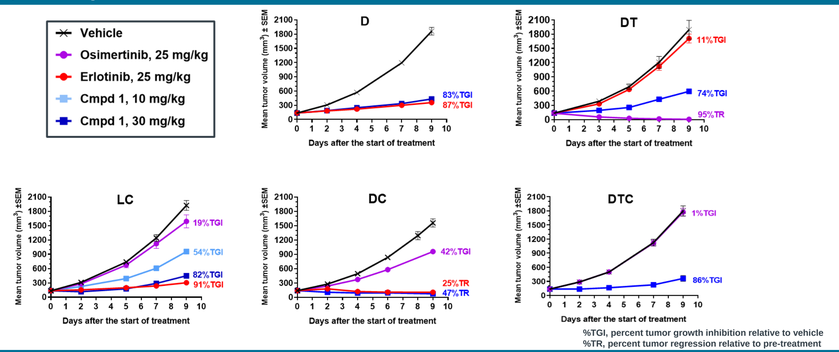
Cmpd 1 and Cmpd 2 potently inhibit all major EGFR single-, double-, and triple-mutant variants in cellular assays

– **Selectivity over WT EGFR exceeds that of erlotinib and gefitinib**

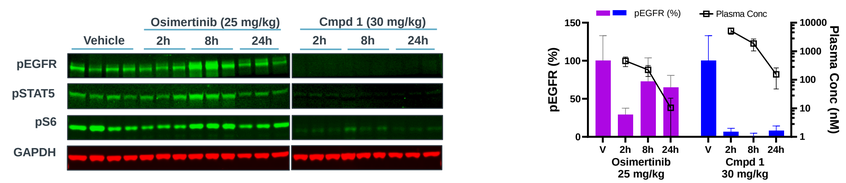
	Ba/F3 viability, IC_{50} (nM)									A431 pEGFR IC_{50} (nM)
Compound	L	LT	LC	LTC	D	DT	DC	DTC	Parental	WT
Erlotinib	6.4	3039	12.9	>4000	3.7	>4000	6.7	>4000	>4000	42
Gefitinib	7.0	>4000	10.7	>4000	3.8	>4000	15.5	>4000	>4000	33
Osimertinib	3.0	2.6	1010	1684	1.5	3.4	1234	1234	1563	327
Cmpd 1	13.9	3.4	8.9	7.7	5.6	6.8	4.9	2.3	1213	202
Cmpd 2	10.0	2.4	11.9	7.9	4.6	4.0	6.9	2.4	3159	136



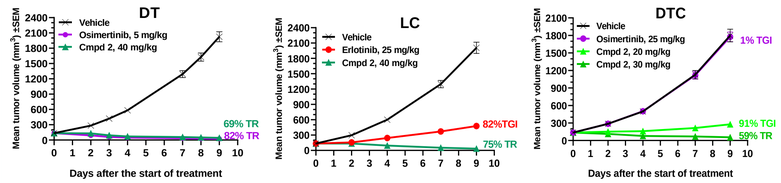
Cmpd 1 is highly efficacious in aggressive Ba/F3 tumor models expressing EGFR single-, double-, and triple-mutant variants at well-tolerated doses



A single dose of Cmpd 1 leads to sustained inhibition of EGFR signaling in a Ba/F3 triple-mutant model (DTC)



Cmpd 2 has superior efficacy in Ba/F3 tumor models expressing EGFR double- or triple-mutant variants at well-tolerated doses



Cmpd 2 penetrates the CNS and has favorable drug-like properties

- **Rat brain:plasma ratio predicts CNS activity in patients**
- **Favorable ADME and large animal PK properties predict good human PK**
- **High degree of kinome selectivity: S-score (50) = 0.07 (23/330 kinases inhibited by >50% at 0.1 μM)**

Compound	Brain:Plasma Ratio	Target	CNS–Active in Patients
Cmpd 2	0.30	EGFR	N/A
Erlotinib	0.24	EGFR	Yes[6,7]
Osimertinib	11	EGFR	Yes[7]
Lorlatinib	0.54	ALK	Yes[8]
Brigatinib	0.04	ALK	Yes[9]
Avapritinib	0.67	PDGFRA	Yes[10]

Conclusions

- Data strongly support that pan-variant EGFR inhibition, with selectivity over WT, is achievable with a single molecule, potentially resulting in greater safety and combinability and a streamlined development path
- Cmpds 1 & 2 both have pan-EGFR inhibitory profiles with activity against mutant variants resistant to 1st, 2nd, and 3rd generation EGFR inhibitors
  - Cmpd 1
    - Potent cellular activity with good selectivity over WT EGFR (15- to 88-fold)
    - Significant anti-tumor efficacy in models harboring both activating and resistance mutations
  - Cmpd 2
    - Demonstrated tumor regressions against variants associated with both 1st and 2nd line osimertinib clinical failure – i.e., C797S double (LC) and triple (DTC) mutants
    - Demonstrated brain permeability – expected to be active against CNS metastatic disease
- Additional studies to further characterize lead compounds are underway

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