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Discovery and Characterization of Firmonertinib, a novel *EGFR* Inhibitor with Broad Activity Against both *EGFR* Classical and Exon 20 Mutations

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Background

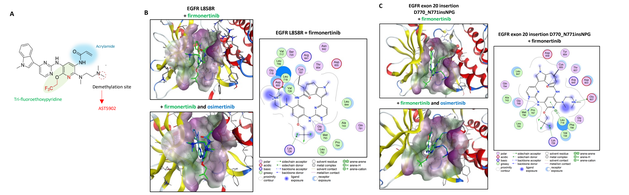
- Firmonertinib (or furmonertinib) is a broadly active, mutant selective, irreversible epidermal growth factor receptor (EGFR) inhibitor which structurally includes a tri-fluoroethoxypyridine side chain with the potential to stabilize its interactions within the drug binding pocket of EGFR mutant proteins
- Firmonertinib is approved in China for first-line advanced non-small-cell lung cancer (NSCLC) with *EGFR* exon 19 deletion or L858R mutations based on progression-free survival (PFS) benefit observed in a Phase 3 study (FURLONG), as well as for second-line or later patients with *EGFR* exon 20 insertion mutations
- Firmonertinib is an investigational agent in a global Phase 3 trial for first-line NSCLC patients with *EGFR* exon 20 insertion mutations (FURVENT; NCT05607550), a global Phase 3 trial for first-line NSCLC patients with *EGFR* P-loop and αC-helix compression (PACC) mutations (ALPACCA; NCT07185997) and in a global Phase Ib trial evaluating firmonertinib in patients with activating *EGFR* or *HER2* mutations, including uncommon *EGFR* mutations (FURTHER; NCT05364043)
- Firmonertinib received US FDA Breakthrough Therapy Designation for the treatment of patients with previously untreated, locally advanced or metastatic non-squamous NSCLC with EGFR exon 20 insertion mutations
- *EGFR* classical mutations occur in the exons encoding the kinase domain of EGFR including exon 19 deletions (ex19del) and L858R mutations and lead to constitutive activation of EGFR which promotes cell proliferation
- *EGFR* exon 20 insertion mutations are uncommon *EGFR* mutations that activate EGFR and result in the addition of 1 or more amino acids within the EGFR kinase domain, which alters the drug binding pocket to impede binding by drugs such as osimertinib
- About 75% of the most pathogenic *EGFR* exon 20 insertion mutations are observed in the near loop following the C-helix while the far loop following the C-helix harbors about 18% of the pathogenic mutations. Generally, far loop insertion mutations have been resistant to treatment
- Here, we describe the discovery and preclinical characterization of firmonertinib, an irreversible EGFR TKI with high selectivity for mutant over wild-type *EGFR*. Our data demonstrate the high potency inhibition of *EGFR* harboring ex20ins mutations using structural, biochemical, cell line, and xenograft data. Findings from this study support the structural mechanism of mutant EGFR binding and inhibition by firmonertinib, eliciting strong anti-tumor activity in multiple *in vitro* and *in vivo* models harboring *EGFR* ex20ins mutations

Methods

- The EGFR L858R mutant and firmonertinib model was constructed from a tether minimized EGFR L858R:osimertinib complex model structure. The EGFR L858R mutant and osimertinib model was constructed using the RCSB PDB structure 7K1H. The EGFR D770_N771insNPG insertion mutant protein and firmonertinib structure was created using an internally generated x-ray structure (structure to be deposited). Molecular modeling work was performed with Molecular Operating Environment (MOE) software (Chemical Computing Group)
- A431 and HCC-827 were purchased from the Cell Bank of Shangai Institutes for Biological Sciences, Chinese Academy of Sciences. NCI-H1975 was purchased from American Type Culture Collection (ATCC). HCC-827 and NCI-H1975 were cultured in RPMI1640 medium containing 10% fetal bovine serum (FBS) and A431 was cultured in 10% FBS in RPMI1640/DMEM medium. Mouse Ba/F3 cell line was transfected with different EGFR ex20ins mutations
- *In vitro* drug potency was evaluated in a CellTiter-Glo® luminescent cell assay and GraphPad PRISM was used to calculate the IC_{50} values. The graphical curves were fitted using a nonlinear regression model with a sigmoidal dose response
- *In vivo* xenograft mouse studies were conducted following subcutaneous inoculation of either tumor cells or PDX tumor fragments. Mice were administered either vehicle or indicated drug treatments at indicated dose levels once daily via oral gavage. Tumor size was measured 2-3 times per week in 2 dimensions using calipers. Tumor growth inhibition from start of treatment was assessed by comparison of the mean change in tumor volume for the control and treated groups. Statistical significance was evaluated using a one-way analysis of variance (ANOVA)

Results

Figure 1. Firmonertinib can adapt and bind to EGFR mutant proteins via contact points with the tri-fluoroethoxypyridine side chain



- **The tri-fluoroethoxy group provides flexibility within the drug binding pocket of classical *EGFR* mutants but it was unknown whether this potential flexibility would enhance binding to *EGFR* exon20ins mutants**
- **In comparison to EGFR L858R mutant protein, EGFR ex20ins D770_N771insNPG mutant protein causes a conformational shift that locks the C-helix in an inward position which blocks access to the ATP-binding pocket**
- **Despite the conformational shift, the tri-fluoro moiety of firmonertinib makes a halogen bond with the terminal amine of Lys728 which likely results in increased binding compared to osimertinib (blue). The distance between the terminal lysine nitrogen atom and the carbon of the tri-fluoro group is 3.89 Å which is a short distance suggesting a direct interaction between these atoms**
- **These structural findings in EGFR classical and ex20ins D770_N771insNPG mutant proteins highlight the tri-fluoroethoxy group of firmonertinib as a unique attribute differentiating it from other EGFR TKIs by providing flexibility and increased direct interactions within the drug binding pocket of EGFR**

(A) Description of firmonertinib chemical and structural attributes; (B) X-ray structure and structural 2D model of firmonertinib binding mode to L858R mutant EGFR protein and binding differentiation in comparison with osimertinib; (C) X-ray structure and structural 2D model of firmonertinib binding mode to EGFR ex20ins D770_N771insNPG protein and binding differentiation in comparison with osimertinib

Figure 2. Firmonertinib and its active metabolite AST5902 strongly inhibit cell proliferation of cancer models harboring *EGFR* exon 20 insertion alterations *in vitro*

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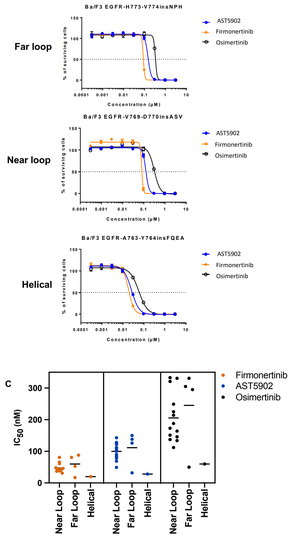
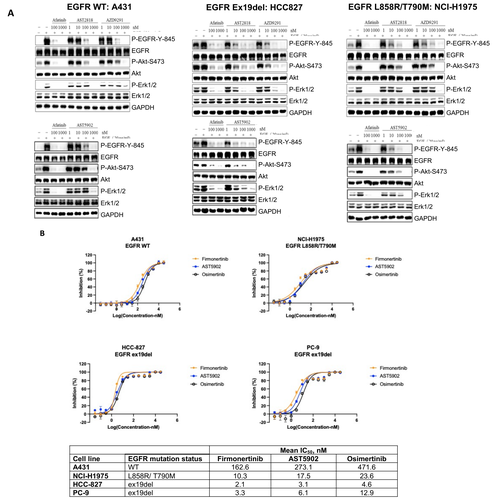
	EGFR activating Exon 20 insertion mutations	IC_{50} (nM) Firmonertinib	AST5902	Osimertinib
Far loop	V774-C775insHV	17	32	50
	H773-V774insAH	53	126	295
	H773_V774insNPH	88	150	331
Near loop	D770-N771insNPG	31	49	112
	N771 del insGY	37	69	151
	N771-P772insH	38	78	137
	P772-H773insNP	40	90	249
	N771-P772insN	41	83	134
	D770 del insGY	42	112	188
	D770-N771insY	42	103	173
	A767-S768insTLA	43	85	146
	D770-N771insG	43	109	164
	D770-N771insGT	46	122	225
	D770-N771insSVD	48	100	214
	D770-N771insGF	61	143	333
	V769-D770insGE	66	128	331
	V769-D770insASV	81	126	321
Helical	A763-Y764insFQEA	20	28	60
	Median	43	103	188
	Mean	48	98	206



- **Firmonertinib and its active metabolite AST5902 demonstrate high potency across a panel of models expressing various *EGFR* ex20ins mutations within the far loop, near loop, and helical domain**
- **In comparison, osimertinib shows much higher IC_{50} values across all *EGFR* ex20ins mutations with a fold difference up to 6- fold higher IC_{50} values overall compared to firmonertinib and a higher average IC_{50} value of 206 nM compared to firmonertinib which had an average IC_{50} of 48 nM**
- **Most importantly, firmonertinib and AST5902 treatments are both highly potent against *EGFR* exon 20 far and near- loop insertion mutations**

(A) Inhibition of cell proliferation by firmonertinib or AST5902 compared with osimertinib, in Ba/F3 cell lines expressing *EGFR* ex20ins mutations spanning mutations that alter the C-helix, the near-loop and far-loop following the helix. IC_{50} (nM) values are shown for all *EGFR* ex20ins mutations evaluated. (B) Inhibition of cell proliferation by firmonertinib or AST5902 compared with osimertinib, in Ba/F3 cell lines expressing *EGFR* ex20ins mutations encompassing helical, near and far- loop insertions. Individual IC_{50} values (dot) and mean (bar) are shown. Mean values for firmonertinib, AST5902, and osimertinib are 48, 98 and 206 nM, respectively. Representative graphs are shown for ex20ins mutations that alter the C-helix (A763-Y764insFQEA), the near-loop (V769-D770insASV) and far-loop (H773-V774) following the helix. (C) Grouped IC_{50} values (orange, blue, black dots) and mean (bar) for ex20ins mutations in the C-helix, the near and far-loops are shown for each treatment.

Figure 3. Firmonertinib and its active metabolite AST5902 strongly inhibit EGFR phosphorylation, downstream signaling, and cell proliferation of cancer models harboring *EGFR* classical mutations *in vitro*



Cell line	EGFR mutation status	Mean IC_{50}, nM Firmonertinib	AST5902	Osimertinib
A431	WT	162.6	273.1	471.6
NCI-H1975	L858R/ T790M	10.3	17.5	23.6
HCC-827	ex19del	2.1	3.1	4.6
PC-9	ex19del	3.3	6.1	12.9

- **Both firmonertinib and AST5902 strongly inhibit EGFR phosphorylation and downstream signaling at concentrations as low as 10 nM in models harboring *EGFR* classical mutations and do not inhibit downstream signaling in an *EGFR* wild-type model**
- **In line with their effects on EGFR phosphorylation, firmonertinib and AST5902 are highly potent in models harboring *EGFR* classical mutations and show limited activity in a wild-type *EGFR* model**

(A) Western blot of phosphorylated and total EGFR and of downstream signaling pathway proteins including phosphorylated and total Akt and ERK following treatment with firmonertinib or AST5902, compared with afatinib and osimertinib, in cell lines expressing either wild-type or classical *EGFR* mutations (ex19del or L858R/T790M). (B) Inhibition of cell proliferation by firmonertinib or AST5902 compared with osimertinib, in cell lines expressing wild-type or classical *EGFR* mutants (L858R, ex19del).

Figure 4. Firmonertinib profoundly inhibits the *in vivo* tumor growth of xenograft and patient-derived cancer models harboring *EGFR* ex20ins mutations

- **Ba/F3 xenograft *in vivo* models were used to evaluate the effects of firmonertinib treatment in models representing the three most prevalent near and far loop *EGFR* ex20ins mutations in patients including near-loop insertions V769_D770insASV and D770_N771insSVD as well as far-loop insertion H773_V774insNPH**
- **Overall, all firmonertinib treatments were generally well tolerated as demonstrated by the body weight measurements**

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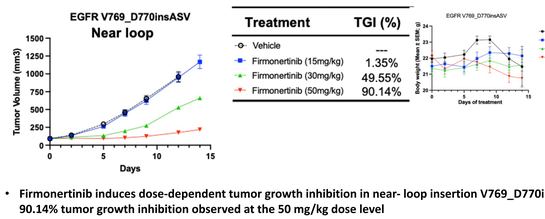
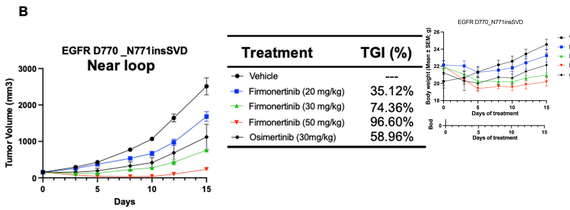


Treatment	TGI (%)
Vehicle	---
Firmonertinib (15mg/kg)	1.35%
Firmonertinib (30mg/kg)	49.55%
Firmonertinib (50mg/kg)	90.14%

- **Firmonertinib induces dose-dependent tumor growth inhibition in near- loop insertion V769_D770insASV with 90.14% tumor growth inhibition observed at the 50 mg/kg dose level**

B



Treatment	TGI (%)
Vehicle	---
Firmonertinib (20 mg/kg)	35.12%
Firmonertinib (30 mg/kg)	74.36%
Firmonertinib (50 mg/kg)	96.60%
Osimertinib (30mg/kg)	58.96%

- **In near- loop insertion D770_N771insSVD, firmonertinib induces dose-dependent tumor growth inhibition with 35.12%, 74.36% and 96.6% tumor growth inhibition at the 20, 30, and 50 mg/kg dose levels, respectively**
- **In comparison, osimertinib induces a partial response of 58.96% tumor growth inhibition at the high dose of 30 mg/kg**

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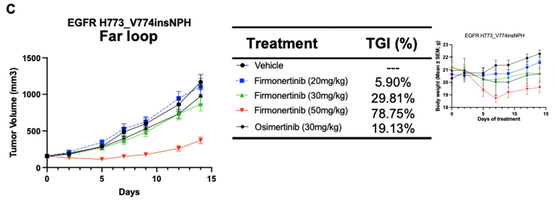

Treatment	TGI (%)
Vehicle	---
Firmonertinib (20mg/kg)	5.90%
Firmonertinib (30mg/kg)	29.81%
Firmonertinib (50mg/kg)	78.75%
Osimertinib (30mg/kg)	19.13%

- **Far- loop insertion H773_V774insNPH also demonstrates strong tumor responses to firmonertinib treatment and dose-dependent tumor growth inhibition of 29.81% and 78.75% at the 30 and 50 mg/kg dose levels**
- **In comparison, osimertinib has minimal effects on tumor growth with 19.13% tumor growth inhibition at the high dose of 30 mg/kg**

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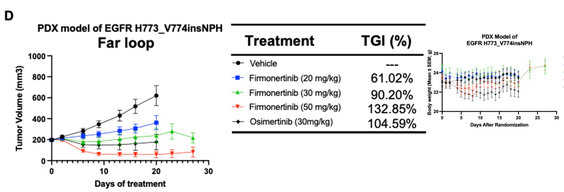

Treatment	TGI (%)
Vehicle	---
Firmonertinib (20 mg/kg)	61.02%
Firmonertinib (30 mg/kg)	90.20%
Firmonertinib (50 mg/kg)	132.85%
Osimertinib (30mg/kg)	104.59%

- **In a patient-derived xenograft (PDX) model of *EGFR* ex20ins mutation H773_V774insNPH, firmonertinib induces dose-dependent tumor growth inhibition of 61.02% and 90.20% at the 20 and 30 mg/kg dose levels and deep regressions of 132.85% tumor growth inhibition at the 50 mg/kg dose level**
- **Overall, our findings demonstrate that firmonertinib is a highly potent inhibitor of tumor cell proliferation in models harboring both near and far loop *EGFR* ex20ins mutations**

(A) *In vivo* tumor growth inhibition by firmonertinib in a xenograft model harboring *EGFR* ex20ins V769_D770insASV and mean body weight measurements in grams. (B) *In vivo* growth inhibition by firmonertinib or osimertinib in xenograft model harboring *EGFR* ex20ins D770_N771insSVD and mean body weight measurements in grams. (C) *In vivo* growth inhibition by firmonertinib or osimertinib in xenograft model harboring *EGFR* ex20ins H773_V774insNPH and mean body weight measurements in grams. (D) *In vivo* growth inhibition by firmonertinib or osimertinib of a lung cancer patient-derived model harboring *EGFR* ex20ins H773_V774insNPH and mean body weight measurements in grams. Firmonertinib doses of 15, 20, 30, and 50 mg/kg approximate to 40, 80, 160, and 240 mg clinical doses, respectively, based on exposure matching. Based on published data for osimertinib, a 10-15 mg/kg in mice would approximately match exposures achieved in patients treated with an 80mg dose. Osimertinib dose of 30 mg/kg is higher than the approved dose of 80mg.

Figure 5. Firmonertinib also profoundly inhibits the *in vivo* tumor growth of xenograft models harboring *EGFR* classical mutations

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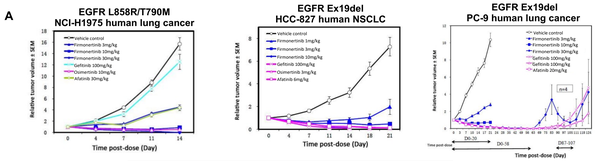

Treatment	NCI-H1975	HCC-827	PC-9
	TGI (%)		
Firmonertinib (1 mg/kg)	NA	83	NA
Firmonertinib (3 mg/kg)	75	157	81
Firmonertinib (10 mg/kg)	115	200	128
Firmonertinib (30 mg/kg)	200	NA	175
Gefitinib (100 mg/kg)	19	200	185
			182
Osimertinib (3 mg/kg)	147	185	NA
Afatinib[c]	76	185	190

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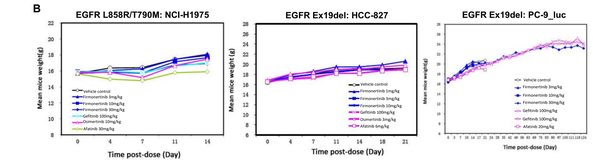

- **The *in vivo* activity of firmonertinib is evaluated in comparison to other EGFR TKIs in lung xenograft tumor models harboring classical *EGFR* L858R and T790M mutations (NCI-H1975) and *EGFR* ex19del (HCC-827 and PC-9)**
- **Firmonertinib daily oral dosing induces dose-dependent tumor growth inhibition in all three tumor models evaluated while osimertinib and afatinib treatments demonstrate lower tumor growth inhibition**
- **Overall, our findings demonstrate that firmonertinib is a highly potent inhibitor of tumor cell proliferation in tumors harboring classical *EGFR* mutations**

(A) *In vivo* tumor growth inhibition by firmonertinib, gefitinib, osimertinib, or afatinib, in xenograft models harboring *EGFR* classical mutations: L858R/T790M (H1975) and ex19del (PC9, HCC827). (B) Mean body weight measurements in grams of subcutaneous xenograft models harboring classical *EGFR* mutations L858R/T790M or ex19del

Conclusions

- While non-small cell lung cancers harboring classical *EGFR* oncogenic mutations are generally sensitive to approved EGFR therapeutics, tumors harboring *EGFR* ex20ins mutations have limited treatment options
- In comparison to osimertinib, firmonertinib demonstrates unique features including improved mutant selectivity, *in vitro* safety profile, and overall high efficacy in tumors harboring *EGFR* ex20ins mutations, all contributing to an optimal drug profile critical to achieving superior efficacy and tolerability in the clinic
- Our study of firmonertinib-EGFR co-crystals of ex20ins mutation D770_N771insNPG highlights the tri-fluoroethoxy group of firmonertinib as a unique and differentiated attribute providing mixed hydrophobic/hydrophilic clefts and halogen bond interactions resulting in stable and increased binding to EGFR ex20ins mutant proteins
- In cancer cell lines, firmonertinib and its active metabolite AST5902 potently inhibit all models harboring *EGFR* ex20ins mutations compared to osimertinib and demonstrate similar IC_{50} values for near and far-loop insertion mutations indicating broad inhibitory activity across the spectrum of *EGFR* ex20ins mutations
- Our *in vivo* findings show that firmonertinib suppresses the growth of tumors harboring *EGFR* ex20ins mutations in a dose-dependent manner. Deep regressions are observed across models at dose levels equivalent to a 240 mg human dose which demonstrates a manageable safety profile in clinical studies
- Firmonertinib has demonstrated promising anti-tumor activity and a favorable safety profile in first-line NSCLC patients with ex20ins mutations and is currently being clinically evaluated in a global Phase 3 study for first-line NSCLC patients with *EGFR* P-loop and αC-helix compression (PACC) mutations (ALPACCA; NCT07185997)

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