Preclinical Evaluation of CFT8919 as a Mutant Selective Degrader of EGFR with L858R Activating Mutations for the Treatment of Non-Small Cell Lung Cancer

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Introduction

The L858R activating mutation in Exon 21 of Epidermal Growth Factor Receptor (EGFR) accounts for approximately 40% of EGFR-mutant non-small cell lung cancer (NSCLC)[1]. EGFR tyrosine kinase inhibitors (TKIs) provide significant clinical benefit. However, patients ultimately develop resistance, often by acquisition of a secondary resistance mutation in EGFR. T790M is the most prevalent resistance mutation after first-generation and second-generation EGFR TKIs[2,3]. A third-generation covalent EGFR inhibitor, osimertinib, can overcome this resistance and is approved in the first line, but acquired resistance remains an issue. Patients who progress after osimertinib lack effective treatment options, and EGFR C797S mutation is the most common on-target resistance mechanism[4,5]. Furthermore, patients with L858R mutation have inferior clinical outcomes with osimertinib treatment compared to patients with exon 19 deletion, which accounts for ~45% of EGFR-mutant NSCLC[1] (median progression-free survival 14.4 vs 21.4 months)[6]. We reasoned that a degrader, by eliminating rather than inhibiting mutant EGFR, may induce deeper and more durable responses in patients with L858R mutation and improve upon the suboptimal outcomes achieved with osimertinib. We identified and optimized a heterobifuntional degrader exploiting an allosteric EGFR binding site that is created in the presence of L858R mutation[7]. CFT8919 was developed as a highly mutant selective degrader targeting EGFR L858R and remains active against known resistant mutations in the orthosteric binding site acquired upon EGFR TKI treatment (e.g. T790M, C797S, T790M-C797S).

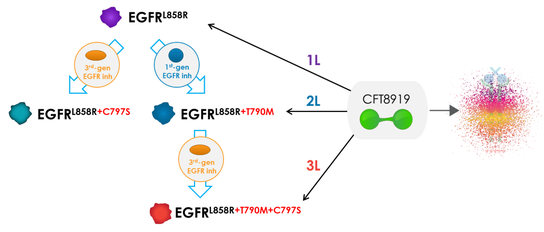
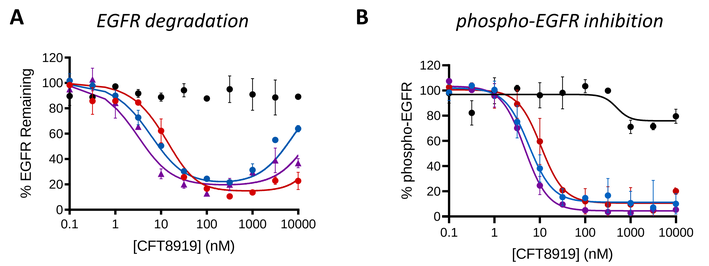


Figure 1: CFT8919 is optimized to overcome resistance to approved 1st/2nd/3rd-generation frontline EGFR inhibitors in EGFR-L858R mutant-driven lung cancer

CFT8919 Selectively Targets EGFR-L858R in Human Cancer Cell Lines



C

	Cell line	EGFR genotype	Degradation (DC50, nM) CFT8919	Inhibition (IC50, nM) CFT8919	Osimertinib 3rd gen	Erlotinib 1st gen
Selectivity	A431	wild-type	>10000	>10000	901	>10000
1L	H3255	L858R	5	5	32	142
2L	H1975	L858R-T790M	8	9	54	>10000
3L	H1975-CS*	L858R-T790M-C797S	14	13	2285	>10000

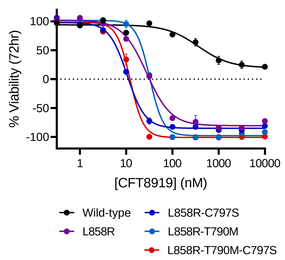
Color key: Potency fold change (FC) relative to L858R

Selectivity against wild-type	Activity against resistant mutations
25 < FC	FC < 5
5 ≤ FC ≤ 25	5 ≤ FC ≤ 25
FC < 5	25 < FC

Figure 2: Cellular activity of CFT8919 on the degradation (**A**) and inhibition (**B**) of EGFR. Effect of CFT8919 on EGFR phosphorylation (phospho-EGFR) and total EGFR protein levels were evaluated in L858R-mutant and wild-type EGFR human lung cancer cell lines. EGFR degradation and phospho-EGFR inhibition were evaluated after 6 hours of compound treatment using HTRF® (Perkin Elmer) assays. (**C**) DC50 (concentration at which 50% of protein is degraded) and IC50 (concentration required for 50% of inhibition) were calculated by 4 parameter logistic fit analysis.

CFT8919 is Active in Ba/F3 Models Expressing Secondary Mutations Resistant to 1st and 2nd line EGFR Inhibitors

A *Viability of Ba/F3 cells expressing the indicated EGFR variant*



B *Ba/F3 cell growth inhibition (GI50, nM)*

	EGFR genotype	CFT8919	Osimertinib 3rd gen	Erlotinib 1st gen
Selectivity	wild-type	486	12	200
1L	L858R	16	3	8
	L858R-T790M	16	6	5951
2L	L858R-C797S	7	2753	not determined
	L858R-L718Q	23	1206	1033
	L858R-L792H	8	314	142
	L858R-T790M-C797S	8	2671	6605
3L	L858R-T790M-L718Q	36	1280	>10,000
	L858R-T790M-L792H	17	385	>10,000

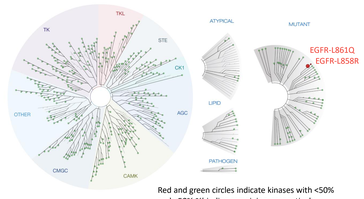
Color key: Potency fold change (FC) relative to L858R

Selectivity against wild-type	Activity against resistant mutations
25 < FC	FC < 5
5 ≤ FC ≤ 25	5 ≤ FC ≤ 25
FC < 5	25 < FC

Figure 3: Viability changes in response to CFT8919 were evaluated in Ba/F3 cells ectopically expressing human EGFR wild-type or mutant following 72 hours of treatment using CellTiter-Glo® (Promega) assay. (**A**) Effect of viability within the indicated dose range of CFT8919 in Ba/F3 cells expressing the indicated representative EGFR variant. (**B**) GI50 (concentration at which 50% of cell growth is inhibited) were calculated by 4 parameter logistic fit analysis.

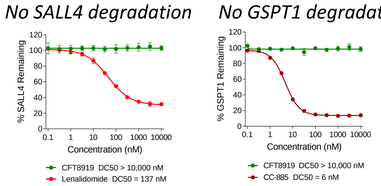
CFT8919 is Highly Selective

A *Kinome binding specificity*



Red and green circles indicate kinases with <50% and >50% %binding remaining, respectively

B *Evaluation against known CRBN neo-substrates*



C *Global proteomic evaluation*

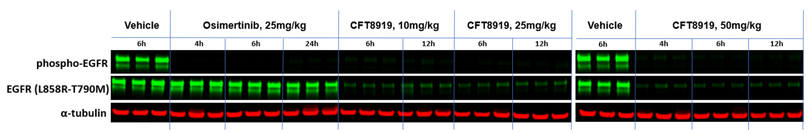
Cell line	EGFR genotype	# of proteins detected	# of proteins with >50% protein level decrease*
A431	Wild-type	9190	0
H1975	L858R-T790M	8853	2 (EGFR, CCND1*)

*p-value < 0.001
*Likely due to the biological effect of EGFR suppression; similar change observed upon osimertinib treatment

Figure 4: (**A**) Kinome binding specificity measured against 468 kinases from KINOMEscan (DiscoverX) using 100 nM of CFT8919. The size of circles mapped onto the kinase phylogenetic tree utilizing DiscoverX TREEspot using <50% of % control as a cutoff. (**B**) *In vitro* degradation of SALL4 (left) and GSPT1 (right) levels within the indicated dose response of CFT8919 as compared to lenalidomide and CC-885, respectively. SALL4 protein level was measured by luminescence using Nano-Glo HiBiT® Lytic assay (Promega) in KELLY cells engineered to endogenously express HiBiT tagged SALL4 via CRISPR-Cas9. GSPT1 protein level was measured in 293T cells CRISPR-edited to endogenously express HiBiT tagged GSPT1. (**C**) Proteomic changes following 6 hours of 300 nM CFT8919 treatment. The adjusted Benjamini-Hochberg p value < 0.001 used as a cutoff.

CFT8919 Degrades and Inhibits Mutant EGFR in Tumors Upon Oral Administration

A *Tumor PD in H1975 EGFR-L858R-T790M xenograft model*



B

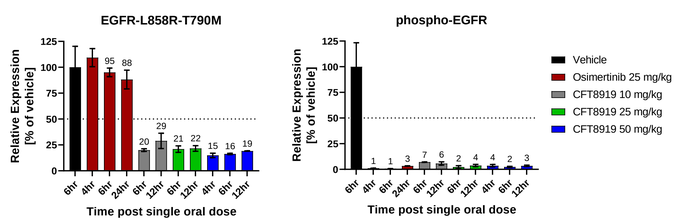


Figure 5: (**A**) *In vivo* degradation and inhibition of mutant EGFR in NCI-H1975 (EGFR-L858R-T790M) NSCLC xenograft model upon single oral dose administration of CFT8919. Tumor tissue was harvested at the indicated time points, lysed, and probed for total mutant EGFR protein level and phospho-EGFR level by Western blot. Antibody specific to L858R mutant-containing EGFR was used to detect EGFR mutant protein level without detecting wild-type. α-Tubulin was used as a loading control. (B) Quantification of Western blot bands was performed using Image Studio™ software (Licor). EGFR-L858R-T790M protein levels and phospho-EGFR levels were quantified and normalized relative to the vehicle control samples.

CFT8919 Induces Tumor Regression in Mouse Models Resistant to 1st and 3rd generation EGFR Inhibitors

A *1st-generation EGFRi resistant H1975 (L858R-T790M) xenograft*

B *3rd-generation EGFRi resistant Ba/F3 (L858R-T790M-C797S) allograft*

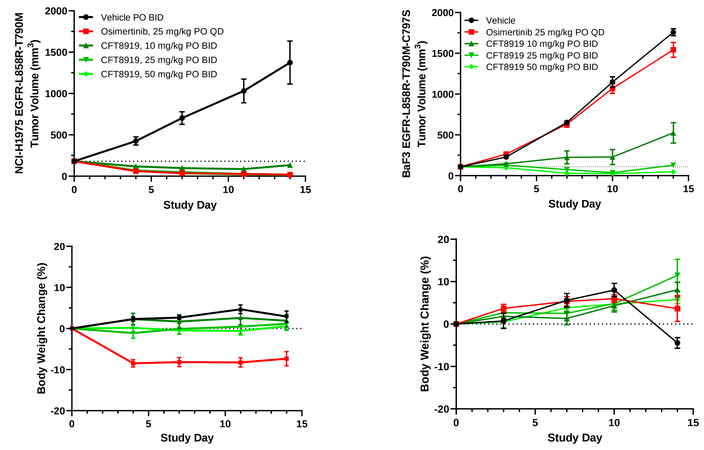
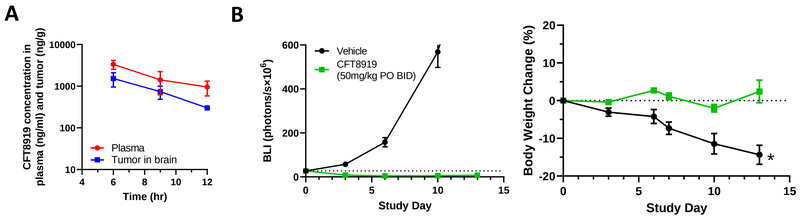


Figure 6: (**A**) Dose dependent efficacy (top), body weight change (bottom) in NCI-H1975 (EGFR-L858R-T790M) NSCLC xenograft model. (**B**) Dose dependent efficacy (top), body weight change (bottom) in Ba/F3 (EGFR-L858R-T790M-C797S) allograft model.

CFT8919 Demonstrates Activity in H1975-LUC (EGFR-L858R-T790M) Brain Metastasis Model



Plasma clearance $t_{1/2}$ = 3.1 hrs

**BWL due to tumor burden*

Figure 7: (**A**) Mean plasma and tumor concentration time profile of CFT8919 following a single oral dose at 50 mg/kg. Female BALB/c nude mice were injected intracranially with NCI-H1975 (EGFR-L858R-T790M) luciferase-expressing cells and administered a single oral dose of CFT8919. Plasma and tumors were harvested at the indicated time points and injected into the LC/MS/MS system for quantitative analysis. (**B**) *In vivo* efficacy (left) and body weight change (right) in the treatment of female BALB/c nude mice bearing intracranial NCI-H1975-luciferase expressing NSCLC tumors. Bioluminescence signal (BLI) was measured in tumor cells to monitor tumor growth over time.

Summary

- CFT8919 is a potent and mutant-selective degrader of EGFR L858R
- CFT8919 is active *in vitro* and *in vivo* in models with secondary mutations (such as T790M, C797S, T790M-C797S) that cause acquired resistance to 1st-, 2nd-, and 3rd-generation EGFR inhibitors
- CFT8919 demonstrates intracranial activity indicating potential to treat brain metastases in patients with EGFR L858R-driven tumors
- CFT8919's preclinical profile warrants clinical evaluation in patients with EGFR L858R driven NSCLC who have progressed on prior EGFR inhibitors and suggests that the molecule would be active in the 1L setting as single agent

References
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Keystone – Targeted Protein Degradation, Virtual Meeting June 2021