Discovery of novel heterobifunctional degraders of mutant EGFR proteins for NSCLCs harboring various EGFR mutations

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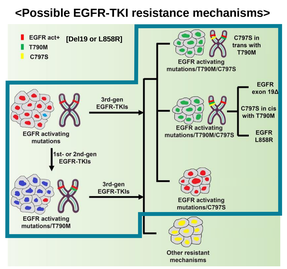
Introduction

The development of EGFR-targeted tyrosine kinase inhibitors (TKIs) over a span of several decades has exhibited remarkable progress in the treatment of NSCLC patients with EGFR mutant proteins. However, these patients remain at significant risk of mortality due to drug resistance arising from the continuous emergence of EGFR mutations.

Targeted protein degradation (TPD) technologies, such as PROteolysis Targeting Chimeras (PROTACs), have recently emerged as a promising alternative modality to address the issue of drug resistance. PROTACs are small molecules that induce the degradation of target proteins through the proteasome pathway, and they can potentially provide a more effective treatment option compared to traditional small molecule inhibitors.

In this study, we present the results of *in vitro* and *in vivo* experiments of selective and potent, and orally bioavailable degraders of various EGFR mutant proteins.

<Possible EGFR-TKI resistance mechanisms>

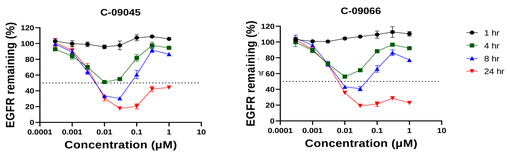


Modified from Jingyi He et al. *Int J Oncol.* 2021 Nov;59(5):90

Coverage of the EGFR mutant-specific bifunctional degrader

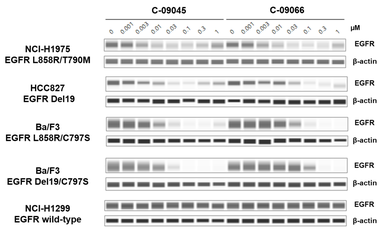
Mutant EGFR-selective degradation

❑ EGFR degradation kinetics in the NCI-H1975 HiBiT assay



Compound ID	C-09045	C-09066
HiBiT H1975 DC_{50} (nM) / D_{max} (%)	2.05 / 82	2.72 / 79

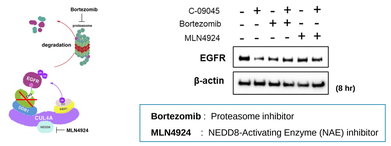
❑ Mutant EGFR-selective degradation



➢ **Our EGFR mutant selective BiFx degraders degrade various EGFR mutant types in a dose- and time-dependent manner**

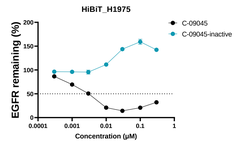
Validation of the ubiquitin-proteasome dependent EGFR degradation

❑ Proteasome or NEDD inhibitor treatment

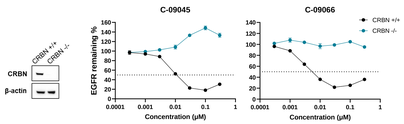


Bortezomib : Proteasome inhibitor
MLN4924 : NEDD8-Activating Enzyme (NAE) inhibitor

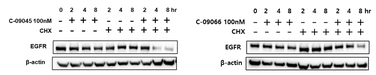
❑ Inactive BiFx degrader treatment



❑ CRBN WT vs KO (H1975 HiBiT)



❑ mRNA translation inhibitor treatment



➢ **The EGFR mutant selective BiFx degraders, C-09045 and C-09066, degrade various types of EGFR mutants using a ubiquitin-proteasome system**

In vitro cell viability

❑ IC_{50} values in the Ba/F3 cells harboring various EGFR mutations

Compound	IC_{50} (nM)							
	HFL-1	Ba/F3 EGFR_WT	Ba/F3 EGFR_DC	Ba/F3 EGFR_LC	Ba/F3 EGFR_DTC	Ba/F3 EGFR_LTC	H1975 EGFR LTC	Ba/F3 EGFR_Ex20ins_H773NPHV774
C-09045	359	63	2	7	6	10	30	31
C-09066	200	125	4	9	13	16	25	46
C-13951	ND	113	7	23	19	29	490	36
Gefitinib	-	-	4	20	-	-	-	-
Osimertinib	-	-	-	-	ND	ND	-	121

[Cell line information] HFL-1: Human fetal lung fibroblasts

❑ IC_{50} values in the patient derived NSCLC cells, YU-1097

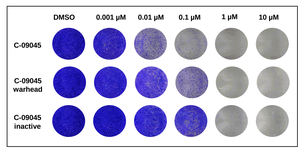
IC_{50} (nM)	Osimertinib	BLU-945	C-06354	C-09045	C-09066
H1666	621	3113	552	918	746
MRC-5	3874	ND	663	1229	162
YU-1097	2155	208	42	33	14

[Cell line information] H1666: EGFR wild-type NSCLC cell line, **MRC-5:** Human fetal lung fibroblast, **YU-1097**: NSCLC PDC EGFR Del19/T790M/C797S; gefitinib resistant; osimertinib resistant

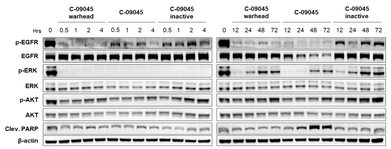
➢ **Our BiFx degraders effectively decrease the viability of Ba/F3 cells that contain different types of EGFR mutations, as well as PDCs that are resistant to 1st and 3rd generation EGFR TKIs**

❑ Comparison of cell growth inhibitory potency between BiFx degrader and its warhead

▪ **NCI-H1975 14-day clonogenic assay**

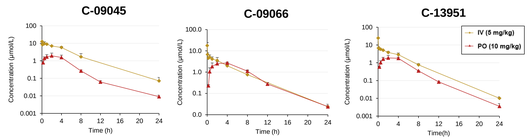


▪ **EGFR downstream signaling in NCI-H1975 cells (100 nM treated)**



➢ **C-09045 exhibits the most potent cell-killing activity compared to its warhead and inactive form, which may be attributed to the delayed reactivation of p-ERK**

Pharmacokinetic properties

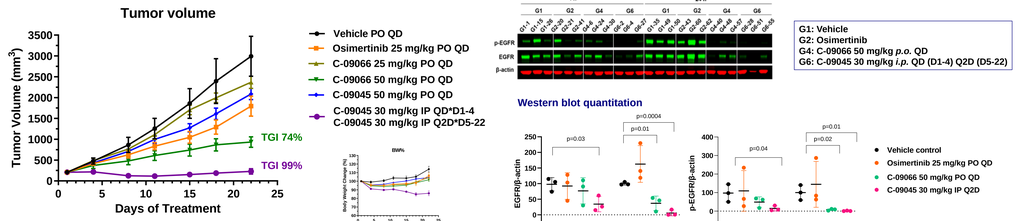


CYRS ID	C-09045	C-09066	C-13951
Mouse [IV/PO mpk]	[5/10]	[5/10]	[5/10]
CL (mL/min/kg)	1.77	4.07	3.25
Vdss (L/kg)	0.47	0.97	0.70
AUCinf (μM•h)	10.2	19.2	10.9
Cmax (μM)	2.02	2.79	2.00
%F	9.9	41	20

➢ **Our BiFx degraders show decent exposure in mice allowing for once-daily oral dosing in *in vivo* efficacy studies**

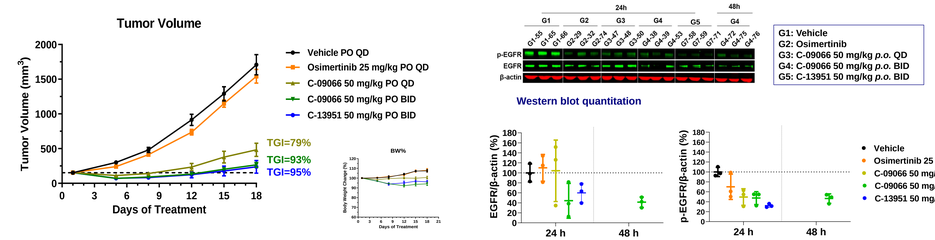
In vivo tumor growth inhibition

❑ The engineered NCI-H1975 EGFR L858R/T790M/C797S xenograft model



* Mutant frequency:
Endogenous L858R 0.43/T790M 0.32; Exogenous L858R 0.37/T790M 0.58/C797S 0.58

❑ The Ba/F3 EGFR Del19/C797S allograft model



➢ **Our BiFx degraders demonstrate excellent tumor growth inhibition efficacy in both mouse models, which correlates with the results of tumor pharmacodynamics analysis after discontinuation of treatment**

Summary

- Mutant EGFR-selective degraders are identified using the HiBiT assay system and those BiFx degraders show strong viability inhibition in cells harboring various types of mutant EGFRs
- The degradation of mutant EGFR proteins by our BiFx degraders occurs through the ubiquitin-proteasome system
- One of our BiFx degraders, C-09045 exhibits the most potent cell-killing activity compared to its warhead and inactive form, which may be attributed to the delayed reactivation of p-ERK
- Orally available BiFx degraders targeting mutant EGFR proteins are discovered showing potent *in vivo* TGI efficacy in the xenograft and allograft mouse models

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