

CO-1686, a novel mutant-selective EGFR inhibitor, overcomes T790M-mediated resistance in Non-Small Cell Lung Cancer (NSCLC)

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Abstract

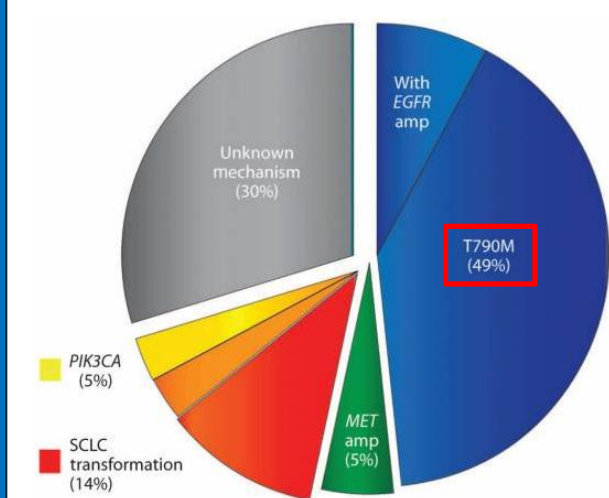
Introduction: Non-small cell lung cancer (NSCLC) patients with activating EGFR mutations initially respond well to the EGFR tyrosine kinase inhibitors (TKIs) erlotinib and gefitinib. However, clinical efficacy is limited by the development of resistance. The most common mechanism of resistance is a second site mutation within exon 20 of EGFR (T790M), observed in ~50% of cases. CO-1686 is an irreversible kinase inhibitor that targets the mutant forms of EGFR by inhibiting the common activating mutations (L858R, delE746-A750) and the gatekeeper mutation (T790M) but not the wild-type receptor. Therefore, CO-1686 has the potential to effectively treat first- and second-line NSCLC patients with EGFR mutations without causing the dose limiting toxicities associated with approved EGFR kinase inhibitors and those in clinical development.

Experimental procedures: Using structure-based drug design, CO-1686, a covalent, irreversible small molecule, which selectively inhibits mutant EGFR, was identified. CO-1686 potency was assessed against four common EGFR mutations (L858R, delE746-A750, L858R/T790M and delE746-A750/T790M) as well as wild-type EGFR using *in vitro* biochemical and cell-based assays. Antitumor activity of CO-1686 as a single agent was assessed in NSCLC xenograft models harboring EGFR mutations. Pharmacodynamic studies were performed to evaluate effects on cell survival and EGFR signaling.

Results: *In vitro* and *in vivo* pharmacology studies were conducted to evaluate CO-1686 potency in four EGFR mutations common in NSCLC patients: L858R, delE746-A750, L858R/T790M and delE746-A750/T790M. CO-1686 was shown to be active against all four EGFR mutants. Effects of CO-1686 on cell proliferation and EGFR signaling were evaluated in HCC827 cells (delE746-A750) and its erlotinib-resistant clone, HCC827-EPR harboring the second site mutation T790M (delE746-A750/T790M). CO-1686 inhibited cell proliferation in both cell lines equally. In mouse xenograft studies, oral dosing of CO-1686 in double mutant (L858R/T790M) and in single mutant (delE746-A750) models caused tumor shrinkage as a single agent in a dose-dependent manner. Different dosing schedules were explored.

Conclusions: Our results establish CO-1686 as a potent, mutant-selective EGFR inhibitor with excellent *in vivo* activity in mice bearing tumors with activating EGFR mutations as well as the resistance mutation T790M. These data suggest that treatment with CO-1686 as a single agent may overcome T790M-mediated drug resistance in NSCLC. Initially, clinical development will focus on NSCLC patients with mutant EGFR.

T790M mutation is the most common mechanism of resistance in NSCLC



Sequist LV *et al.*, Sci. Transl. Med., 2011

Acquired resistance to erlotinib/gefitinib

- All patients on erlotinib (Tarceva®) and gefitinib (Iressa®) will eventually develop acquired drug resistance.
- In approximately 50% of cases, resistance is attributed to the second site T790M mutation.
- T790M is the “gatekeeper” residue and located within the ATP binding site of the EGFR catalytic domain.

CO-1686 potentially inhibits mutant EGFR including T790M

CO-1686 is an irreversible kinase inhibitor that targets the mutant forms of EGFR - it inhibits the common activating mutations (L858R, del19) and the gatekeeper mutation (T790M) more potently than wild-type. The proposed initial indication for CO-1686 is for the treatment of patients with mutant EGFR NSCLC who have received prior EGFR-directed therapy and have T790M-mediated resistant NSCLC.

Biochemical IC₅₀ (nM)

Compound	EGFR ^{L858R/T790M}	EGFR ^{WT}	WT / T790M ratio
CO-1686	<0.51	6±2	> 10
erlotinib	209±17	<0.51	0.002

Binding constants K_d (nM)

Compound	EGFR ^{L858R/T790M}	EGFR ^{WT}	WT / T790M ratio
CO-1686	7	180	25

Biochemical activity and selectivity of CO-1686.

Top panel. In an *in vitro* kinase assay the IC₅₀ was determined by the OMNIA assay with recombinant EGFR and EGFR^{L858R/T790M} proteins.

Bottom panel. Binding constants (K_d values) were determined for recombinant EGFR and EGFR^{L858R/T790M} proteins.

CO-1686 inhibits proliferation and signaling in EGFR-mutant cells

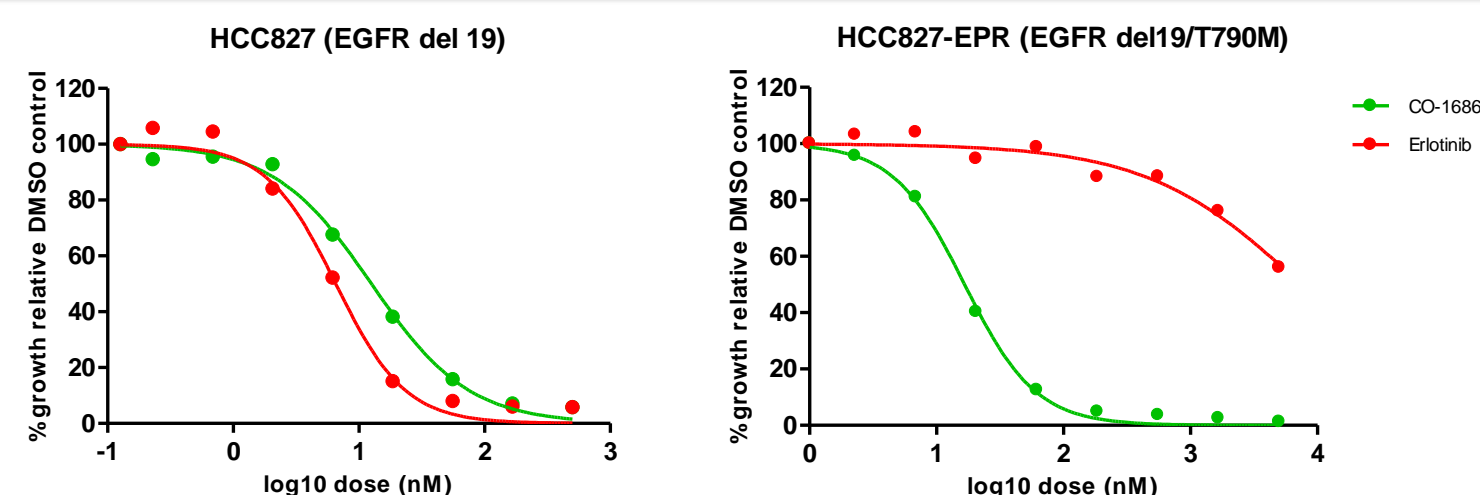
Summary of CO-1686 activity against common EGFR mutations in NSCLC including T790M

NSCLC* cell lines	EGFR status	EGFR mutation	Other mutation	erlotinib proliferation GI50 [nM]	erlotinib pEGFR IC50 [nM]	CO-1686 proliferation GI50 [nM]	CO-1686 pEGFR IC50 [nM]
H1975	mutant	L858R/T790M		>5000	>5000	32±5	62±34
HCC827	mutant	Del 19		7±1	<14	15±5	187±88
HCC827-EPR	mutant	Del 19/T790M		>3393	ND	20±11	180±55
A431 (epidermoid)	wild-type	---		139±34	<7	547±80	> 4331
H1299	wild-type	---	N-Ras	>5000	ND	4030	>2000
H358	wild-type	---	K-Ras	1154	ND	1905	>2000

* except for A431 cells, which are of epidermoid origin

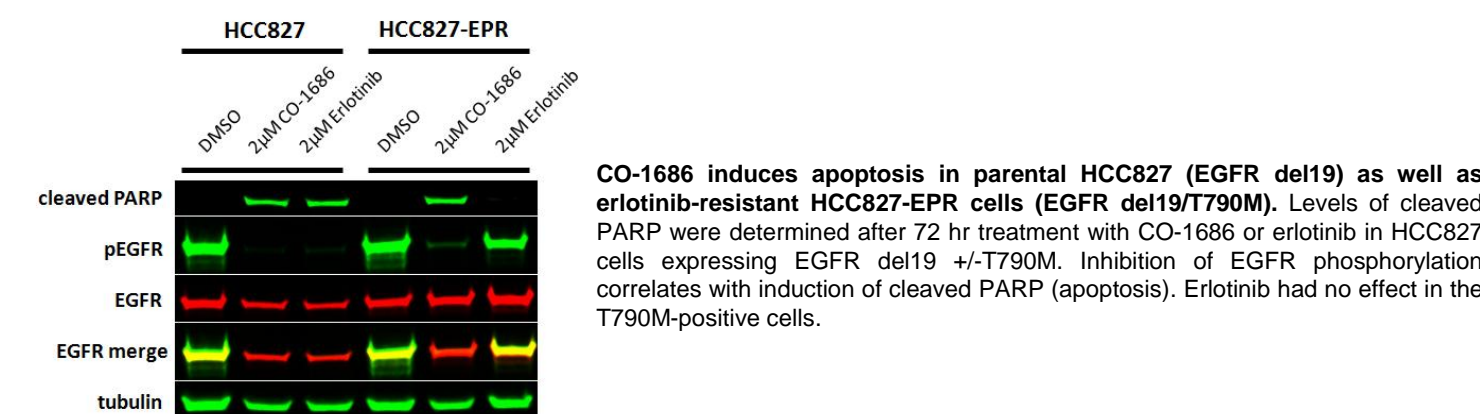
CO-1686 inhibits cell proliferation and EGFR signaling in cell lines expressing mutant EGFR. *In vitro* potency of CO-1686 in comparison to erlotinib was determined in six cancer cell lines. Cell proliferation assays were performed with increasing concentrations of compounds for 72 hrs using CellTiterGlo. For immunoblot analysis cells were treated with increasing concentrations of compounds for 1 hour. Immunoblots were probed for pEGFR and total EGFR. IC₅₀ and GI₅₀ (nM) values were determined by GraphPad software.

CO-1686 inhibits cell viability in cells +/- T790M



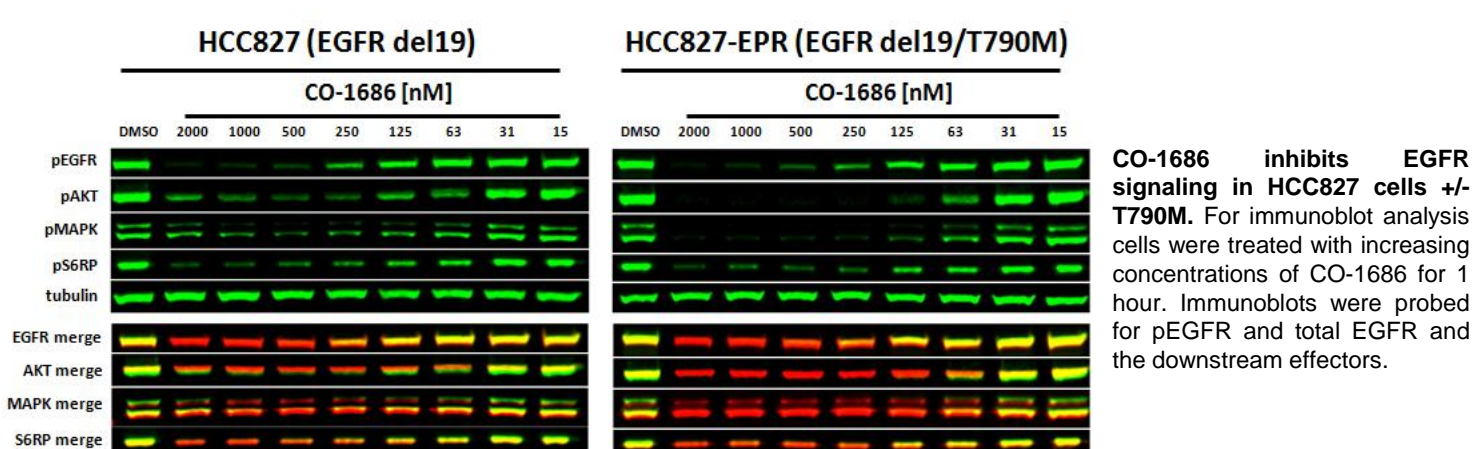
CO-1686 inhibits cell proliferation in parental HCC827 (EGFR del19) and erlotinib-resistant HCC827-EPR cells (del19/T790M) equally. *In vitro* potency of CO-1686 in comparison to erlotinib was determined in HCC827 cells expressing EGFR del19 +/-T790M. HCC827-EPR cells were kindly provided by Dr. Mitsudomi (Suda *et al.*, 2010). Cell proliferation assays were performed with increasing concentrations of compounds for 72 hrs using CellTiterGlo. GI₅₀ (nM) values were determined by GraphPad software.

CO-1686 induces apoptosis in cells +/- T790M



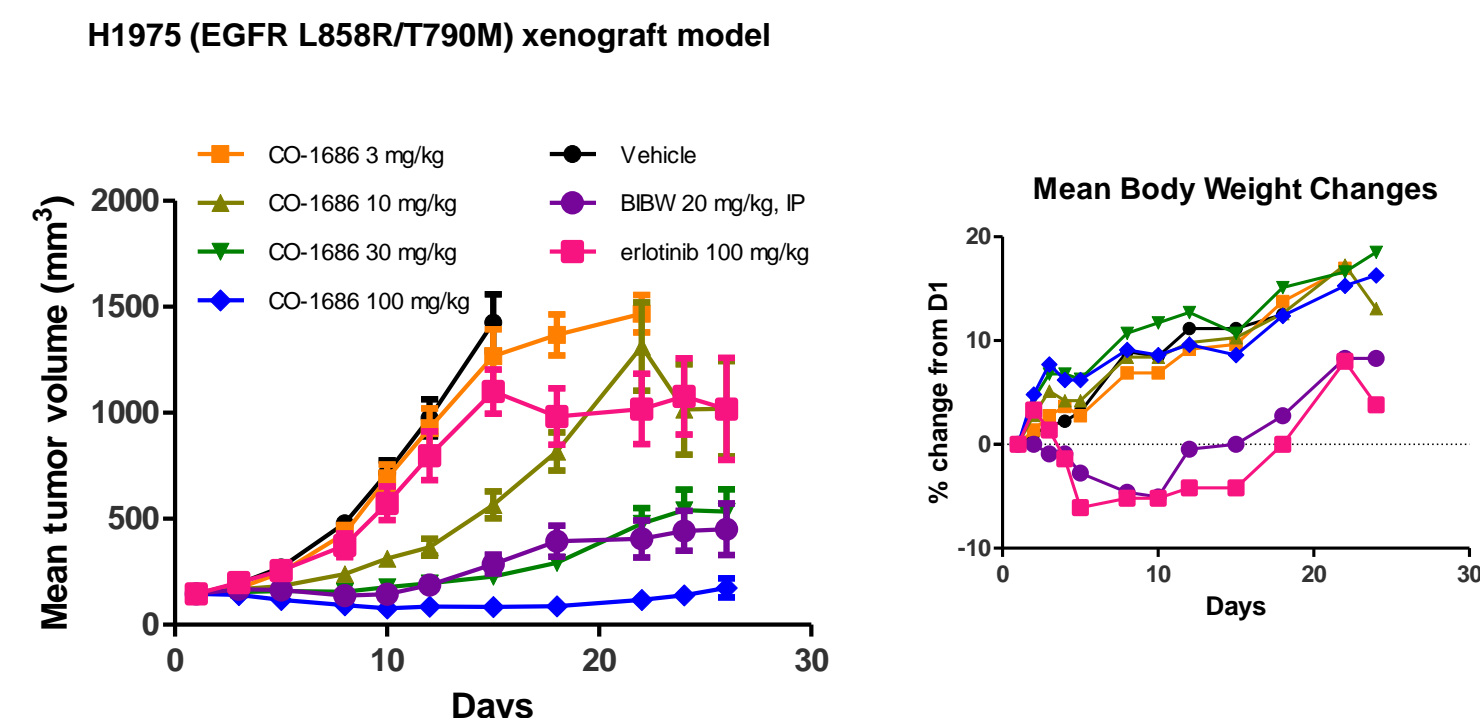
CO-1686 induces apoptosis in parental HCC827 (EGFR del19) as well as erlotinib-resistant HCC827-EPR cells (EGFR del19/T790M). Levels of cleaved PARP were determined after 72 hr treatment with CO-1686 or erlotinib in HCC827 cells expressing EGFR del19 +/-T790M. Inhibition of EGFR phosphorylation correlates with induction of cleaved PARP (apoptosis). Erlotinib had no effect in the T790M-positive cells.

CO-1686 inhibits the EGFR signaling pathway in cells +/- T790M

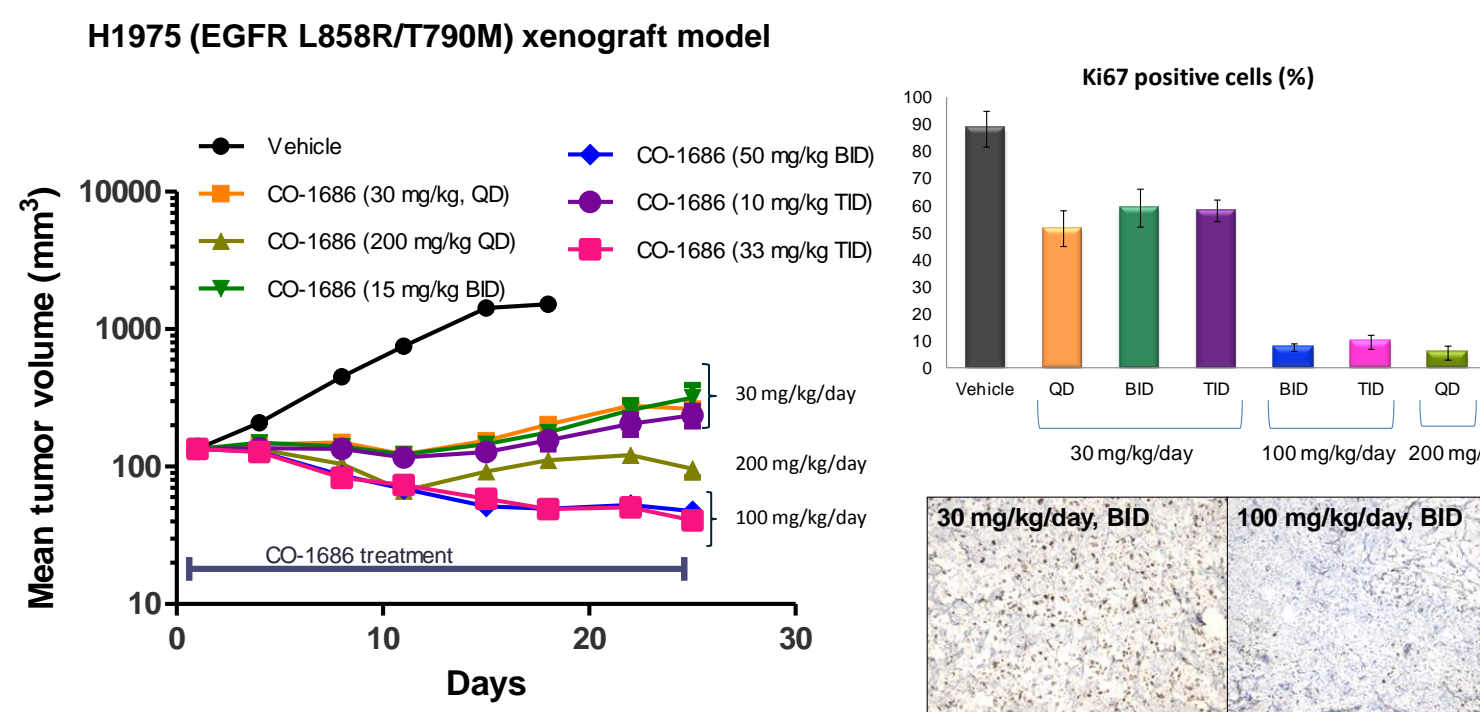


CO-1686 inhibits EGFR signaling in HCC827 cells +/- T790M. For immunoblot analysis cells were treated with increasing concentrations of CO-1686 for 1 hour. Immunoblots were probed for pEGFR and total EGFR and the downstream effectors.

CO-1686 causes tumor shrinkage in T790M-positive NSCLC as single agent

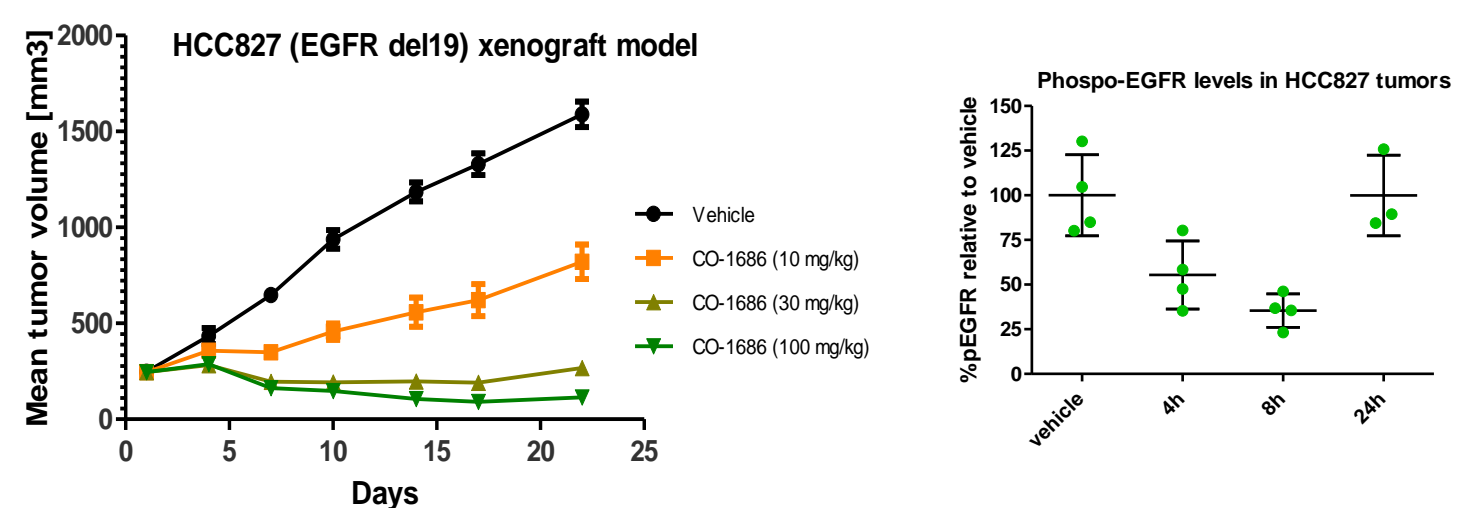


CO-1686 causes dose-dependent tumor growth inhibition in the H1975 NSCLC xenograft model as single agent. CO-1686 was administered orally (PO), daily (QD) for 24 days at 3, 10, 30 and 100 mg/kg. CO-1686 caused significant tumor growth inhibition (TGI) at all dosing schedules indicating that potency is not C_{max}-driven *in vivo* (graph on the left). Quantification of Ki67-positive cells in tumors (n=2) for all groups, determined as the percentage of cells stained positive for Ki67 within a microscope field (10X), revealed no difference among dosing schedules (QD, BID, TID). The means and ranges are plotted (right graph). Ki67 positive staining decreased with dose (IHC, bottom right).



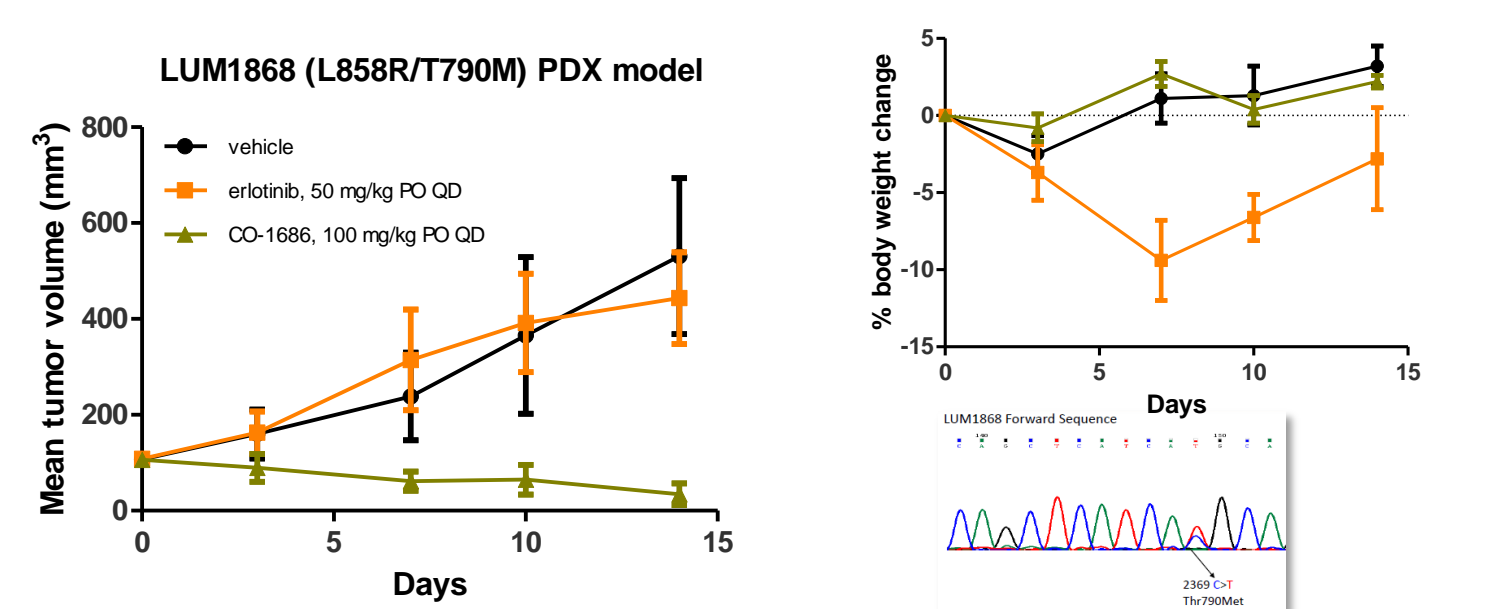
CO-1686 causes tumor growth inhibition in the H1975 NSCLC xenograft model at different dosing schedules. CO-1686 was administered orally (PO), daily (QD), twice daily (BID) or three daily (TID) for 24 days at various concentrations. CO-1686 caused significant tumor growth inhibition (TGI) at all dosing schedules indicating that potency is not C_{max}-driven *in vivo* (graph on the left). Quantification of Ki67-positive cells in tumors (n=2) for all groups, determined as the percentage of cells stained positive for Ki67 within a microscope field (10X), revealed no difference among dosing schedules (QD, BID, TID). The means and ranges are plotted (right graph). Ki67 positive staining decreased with dose (IHC, bottom right).

CO-1686 causes tumor shrinkage in NSCLC with EGFR exon 19 deletion



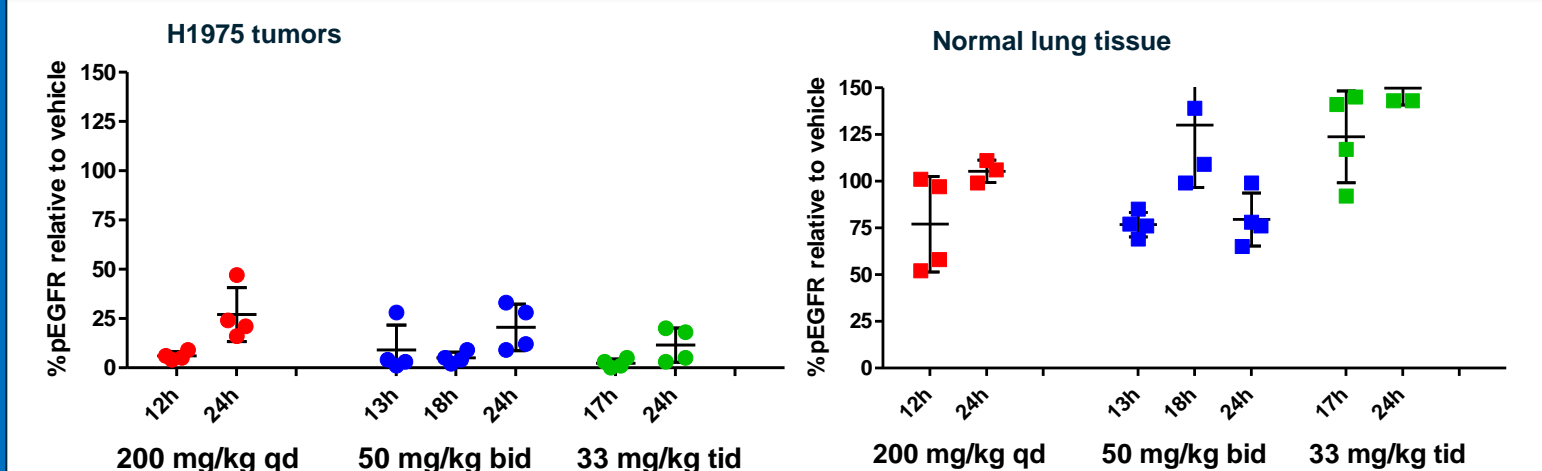
CO-1686 causes partial tumor regression in the HCC827 NSCLC xenograft model. CO-1686 was administered orally (PO), daily (QD) for 21 days at 10, 30 and 100 mg/kg/day. At 30 and 100 mg/kg/day, CO-1686 significantly inhibited tumor growth. In seven out of ten animals, partial regressions (PRs) occurred in the group treated with 100 mg/kg/day. Phospho-EGFR signaling was inhibited in tumor tissues at 4 and 8 h after the last dose of 100 mg/kg/day (graph on the right).

CO-1686 causes tumor shrinkage in T790M+ patient-derived NSCLC model



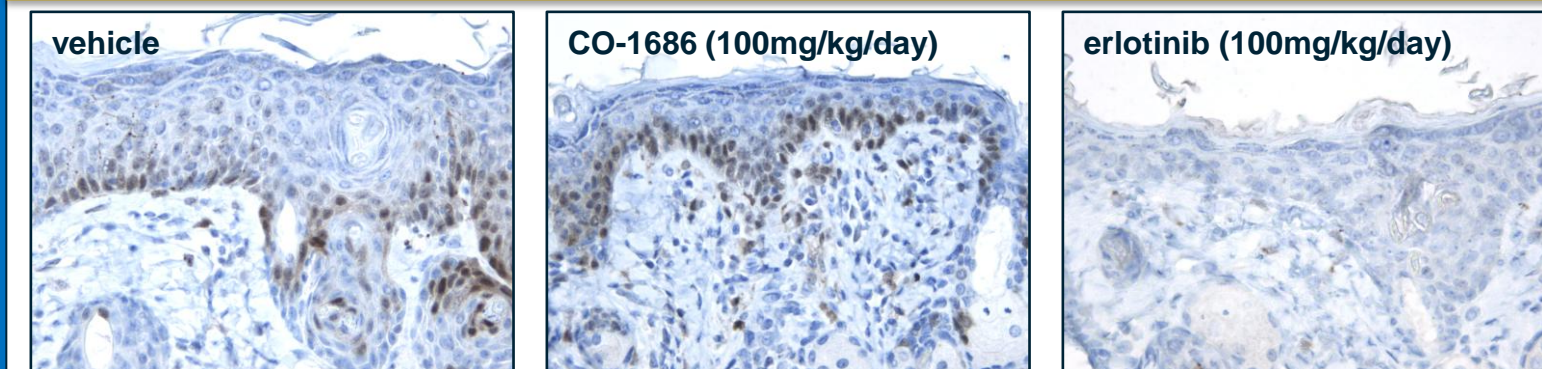
CO-1686 causes tumor regression in the T790M-positive(+) patient-derived NSCLC xenograft model (LUM1868) as a single agent. CO-1686 and erlotinib were administered orally (PO), daily (QD) for 14 days at 50 or 100 mg/kg. CO-1686 caused significant tumor growth inhibition (TGI) while erlotinib was inactive in the LUM1868 PDX model expressing T790M (Crown Bioscience, Inc.). The presence of the T790M mutation was confirmed by DNA sequencing (bottom right). CO-1686 did not cause body weight loss (graph on the upper right).

CO-1686 spares WT EGFR signaling in normal lung tissue



CO-1686 has no effect on wild-type EGFR in normal lung tissues at doses which potentially inhibit mutant EGFR in tumor tissue. CO-1686 was orally administered at 200 mg/kg QD, 50 mg/kg BID or 33 mg/kg TID. Tumor or lung tissues were collected at various time points post first dose and subjected to immunoblot analysis. Each treatment group consists of four mice. Phospho-EGFR levels are plotted relative to the vehicle treated group (100%).

CO-1686 spares WT EGFR signaling in normal skin tissue



WT EGFR signaling is spared by CO-1686 treatment, but not by erlotinib, in skin tissues. Representative immunohistochemical pMAPK staining in normal skin tissues from mice administered with vehicle, CO-1686 (100mg/kg), and erlotinib (100mg/kg). For CO-1686 and erlotinib, mice were orally administered QD x 5 and skin tissues were collected 6 hr post last dose. Each treatment group consists of four mice. Normal dorsal skin tissues collected from nude mice from the H1975 xenograft experiment.

Conclusions

- CO-1686 is a potent, mutant-selective EGFR inhibitor with excellent *in vivo* activity in mice bearing tumors with activating EGFR mutations as well as the resistance mutation T790M.
- CO-1686 causes tumor shrinkage as a single agent in NSCLC tumor models (H1975 and patient-derived xenografts) that are T790M-positive. Erlotinib demonstrates no effect in these models.
- CO-1686 causes tumor shrinkage as a single agent in a NSCLC model (HCC827) with a single activating EGFR mutation (del19).
- CO-1686 inhibits mutant EGFR signaling in tumor tissue but has no inhibitory effect towards WT EGFR signaling in normal lung tissue at potent doses.
- CO-1686 phase 1 clinical trial is underway.