CO-1686, an orally available, mutant-selective inhibitor of the epidermal growth factor receptor (EGFR), causes tumor shrinkage in Non-Small Cell Lung Cancer (NSCLC) with T790M resistance mutations

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Abstract

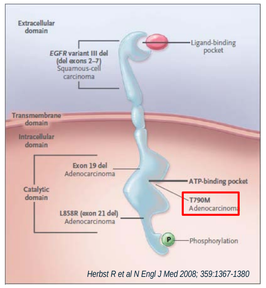
Introduction: Non-small cell lung cancer (NSCLC) patients with activating epidermal growth factor receptor (EGFR) mutations initially respond well to EGFR tyrosine kinase inhibitors (TKI). However, clinical efficacy is limited by the development of resistance. The most common mechanism of resistance is a second site mutation within exon 20 of EGFR (T790M), observed in ~50% of cases. Our goal was to develop a mutant-selective EGFR inhibitor that potently inhibits activating EGFR mutations as well as the T790M resistance mutation while sparing wild-type EGFR for the treatment of NSCLC patients. Such a drug 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 or those in clinical development.

Experimental procedures: Using structure-based drug design, we identified CO-1686, a covalent, irreversible small molecule, which selectively inhibits mutant EGFR. We assessed antitumor activity of CO-1686 both *in vitro* and *in vivo* in two NSCLC cell lines harboring EGFR mutations: H1975 (EGFR L858R/T790M) and HCC827 (EGFR delE746-A750). We evaluated inhibition of EGFR phosphorylation and downstream signaling by immunoblot analysis in cells and tissue samples. IHC staining on skin samples was performed to address effects on wild-type EGFR.

Results: CO-1686 is a potent inhibitor of cell proliferation and EGFR signaling in NSCLC cells harboring the single activating mutation EGFR delE746-A750 as well as the double mutation EGFR L858R/T790M. When administered orally, CO-1686 (3 - 100 mg/kg) significantly suppresses tumor growth of H1975 cells (L858R/T790M) in a dose-dependent manner causing tumor regressions at the highest dose (100 mg/kg) without affecting body weight. Erlotinib at the same dose exhibits no effect against H1975 xenografts. In HCC827 (delE746-A750) xenografts, both agents cause tumor shrinkage. In both NSCLC mouse models, inhibition of EGFR phosphorylation in tumors correlate with the observed anti-tumor activity, while no effect on EGFR signaling is observed in normal lung or skin tissues with CO-1686 treatment, confirming that CO-1686 does not inhibit wild-type EGFR.

Conclusions: Our results establish CO-1686 as a potent, mutant-selective EGFR inhibitor with excellent *in vivo* efficacy against tumors with activating EGFR mutations as well as the resistance mutation T790M. These data suggest that treatment with CO-1686 as a single agent can overcome T790M-mediated drug resistance in NSCLC. This hypothesis will be tested clinically.

T790M is the most common resistance mutation in NSCLC



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Acquired resistance to erlotinib/gefitinib

- T790M within ATP binding site of EGFR
- Approximately 50% of resistant patients have T790M

T790M is the most common drug resistance mutation in EGFR. 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 gatekeeper mutation.

- Exon 19 deletion and L858R, both in the catalytic domain, are the most common EGFR activating mutations
- Approximately 90% of EGFR TKI sensitive patients have del19 or L858R mutations

CO-1686 potently 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). 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 IC_{50} (nM)

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

Binding constants Kd (nM)

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

Biochemical activity and selectivity of CO-1686.
Top panel. In an *in vitro* kinase assay the IC_{50} was determined by the OMNIA assay with recombinant EGFR and $EGFR^{L858R/T790M}$ proteins.
Bottom panel. Binding constants (Kd values) were determined for recombinant EGFR and $EGFR^{L858R/T790M}$ proteins.

CO-1686 inhibits most common activating EGFR mutations in NSCLC

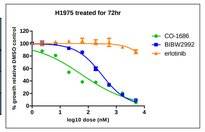
Summary of CO-1686 activity against common EGFR mutations in NSCLC including T790M

EGFR Genotype	EGFR-TKI erlotinib/gefitinib	CO-1686
L858R	sensitive	sensitive
DelE746-A750	sensitive	sensitive
L858R / T790M	resistant	sensitive
DelE746-A750 / T790M	resistant	sensitive

CO-1686 inhibits proliferation of EGFR-mutant cells

Cell Proliferation (Mean GI_{50}, nM)

Cell Lines	EGFR Genotype	erlotinib	CO-1686
H1975	L858R/T790M	>5000	48±17
HCC827	DelE746-A750	12±6	14±8
A431	WT	297±143	544±170



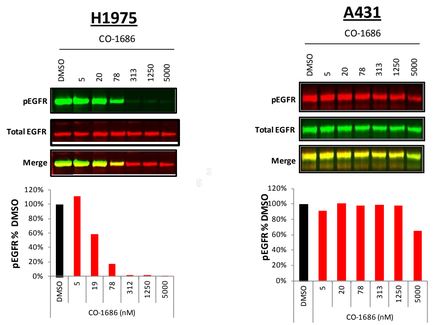
CO-1686 inhibits cell proliferation of cell lines expressing mutant EGFR. Cell proliferation assays were performed with increasing concentrations of compounds for 72 hrs using CellTiterGlo. GI_{50} (nM) values were determined by GraphPad software. N=4-5 assays/cell line; Values are mean ± SD. An example of the GI_{50} curves is shown on the right. CO-1686 inhibits proliferation of the T790M-positive H1975 cells more potently than erlotinib (Tarceva®) or BIBW2992 (afatinib).

CO-1686 selectively targets mutant EGFR in cells, while sparing WT

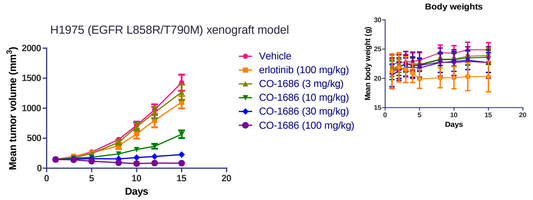
EGFR Signaling (IC_{50}, nM)

Cell Lines	EGFR Genotype	pEGFR	pAkt	pS6K	pS6rp
H1975	L858R/T790M	62±34	55±12	44±41	28±22
HCC827	DelE746-A750	187±88	80±49	53±36	74±48
A431	WT	> 4331	>4307	>3156	>4841

CO-1686 inhibits the EGFR signaling pathway. Inhibition of phosphorylation of EGFR leads to inhibition of its downstream effectors p-Akt, p-p70-S6K and p-S6rp. Cells were treated with increasing concentrations of CO-1686 for 1 hour. Immunoblots were probed for pEGFR, total EGFR, pAkt, total Akt, P-p70S6K, total p70-S6K, pS6R and total S6R. All antibodies were obtained from Cell Signaling. Table showing IC_{50} (nM) values in A431, H1975 and HCC827 cells. IC_{50} values were determined by GraphPad software.

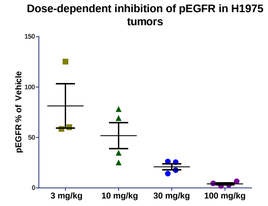


CO-1686 causes tumor shrinkage in NSCLC with T790M as single agent



CO-1686 causes dose-dependent tumor growth inhibition in H1975 NSCLC xenograft model as single agent. CO-1686 was administered orally (PO), daily (QD) for 15 days at 3, 10, 30 and 100 mg/kg. CO-1686 caused significant tumor growth inhibition (TGI) at doses ranging from 10-100 mg/kg/day (graph shown at day 15). Partial tumor regressions were observed at 100 mg/kg/day after 24 days of dosing. Erlotinib at the high dose of 100 mg/kg/day was inactive in this H1975 model. CO-1686 did not cause body weight loss (graph on the right).

CO-1686 inhibits pEGFR in H1975 tumors in a dose dependent manner

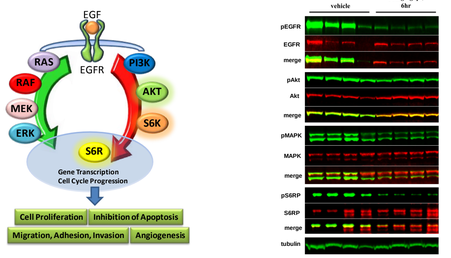


CO-1686 dose (mg/kg)	pEGFR inhibition (%)
3	0
10	48
30	79
100	96

CO-1686 causes dose-dependent inhibition of pEGFR in tumor tissue in the H1975 NSCLC xenograft model. CO-1686 was administered orally (PO), daily (QD) for 5 days at 3, 10, 30 and 100 mg/kg. Tumor tissues were harvested 6 hours after the last dose. Immunoblots were probed for pEGFR and total EGFR and imaged using the Odyssey imager (Li-Cor). All antibodies were obtained from Cell Signaling. N=4 animals per group.

CO-1686 inhibits EGFR signaling (pAKT, pS6rp, pMAPK) in H1975 tumors

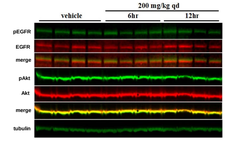
T790M-EGFR signaling in H1975 tumors is inhibited by CO-1686

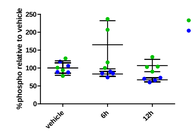


CO-1686 inhibits the EGFR signaling pathway in H1975 tumors. Inhibition of phosphorylation of EGFR leads to inhibition of its downstream effectors p-Akt, p-MAPK and p-S6rp. CO-1686 was administered PO at 50 mg/kg QD x 2 days and tumors were harvested at 6 h after the last dose. Immunoblots were probed for pEGFR, total EGFR, pAkt, total Akt, p-MAPK, total MAPK, pS6RP and total S6RP and imaged using the Odyssey Fc imager (Li-Cor). All antibodies were obtained from Cell Signaling. N=4 animals per group.

CO-1686 spares WT EGFR signaling in normal lung and skin tissues

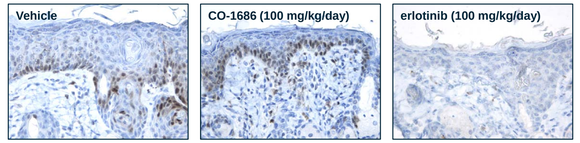
Wild-type EGFR signaling in normal lung tissue is not inhibited by CO-1686





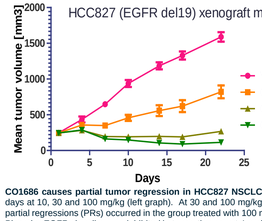
CO-1686 has no effect on wild-type EGFR in normal lung tissues. CO-1686 was orally administered QD x 2 at 200 mg/kg/day and lung tissues were collected at 6 and 12 hours post last dose. Each treatment group consists of four mice.

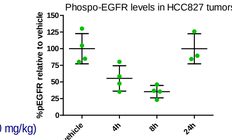
Wild-type EGFR signaling in normal skin is not affected by CO-1686



WT EGFR signaling is spared by CO-1686 treatment, but not by erlotinib, in skin tissues. Representative immunohistochemical p-MAPK staining in normal skin tissues from mice administered with vehicle, CO-1686 (100 mg/kg), and erlotinib (100 mg/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 were collected from nude mice from the H1975 xenograft experiment.

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





CO1686 causes partial tumor regression in HCC827 NSCLC xenograft model. CO-1686 was administered orally (PO), daily (QD) for 21 days at 10, 30 and 100 mg/kg (left graph). 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).

Conclusions

- CO-1686 is a potent, mutant-selective EGFR inhibitor with excellent *in vivo* efficacy against tumors with activating EGFR mutations as well as the resistance mutation T790M
- CO-1686 causes tumor shrinkage as a single agent in a T790M-positive NSCLC xenograft model (H1975). Erlotinib at the same dose demonstrates no effect
- CO-1686 causes tumor shrinkage as a single agent in a NSCLC model (HCC827) with a single activating mutation (del19)
- CO-1686 inhibits mutant EGFR signaling in tumor tissue in a dose-dependent manner
- While very potent against the mutant forms of EGFR, CO-1686 has little inhibitory potency towards WT EGFR
- CO-1686 has no inhibitory effect towards WT EGFR signaling in normal lung or skin tissue at potent doses