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CNX-2006, a Novel Irreversible Epidermal Growth Factor Receptor (EGFR) Inhibitor, Selectively Inhibits EGFR T790M and Fails to Induce T790M-Mediated Resistance In Vitro

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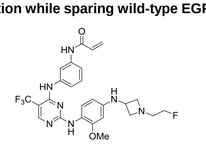
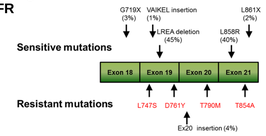
Background

- EGFR-mutant lung cancers are highly sensitive to first generation EGFR tyrosine kinase inhibitors (TKIs; gefitinib and erlotinib), but resistance eventually develops
- In the majority of patients, such acquired resistance (AR) is associated with development of a second-site T790M "gatekeeper" mutation
- Second generation TKIs (e.g. afatinib (BIBW2992), dacomitinib, neratinib) are more potent but have minimal efficacy as single agents in patients with AR
- Unfortunately, second generation TKIs inhibit wild-type EGFR, limiting dose escalation, and they still select for T790M-mediated resistance in *in vitro* models

Approach

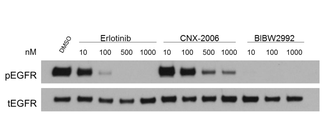
- In this study, we characterize properties of CNX-2006, a novel irreversible EGFR TKI developed specifically to inhibit activating mutations of EGFR as well as the T790M mutation while sparing wild-type EGFR

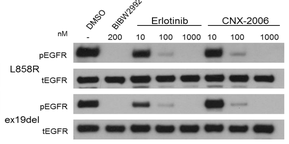


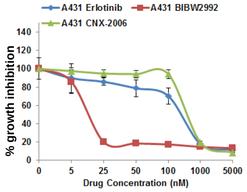


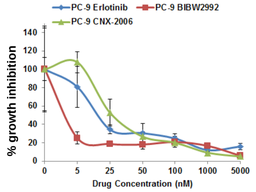
- CNX-2006 is the prototype for CO-1686, which is currently in a Phase I clinical trial for the treatment of EGFR-mutant lung cancer
- General methods:
  1. Human EGFR mutant lung adenocarcinoma cell lines were treated with drugs in standard growth inhibition assays
  2. Cells with endogenous or transiently transfected mutant EGFRs (293 cells) were treated with inhibitors for 6 hours and then corresponding lysates were extracted and analyzed by immunoblotting
  3. H1975 cell xenografts were treated with CNX-2006

Effect of CNX-2006 on Wildtype/Mutant EGFR

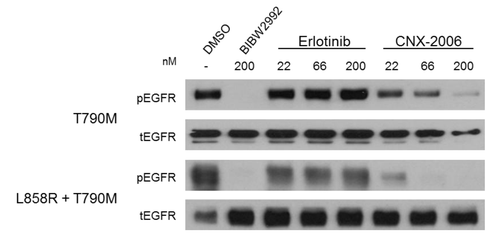


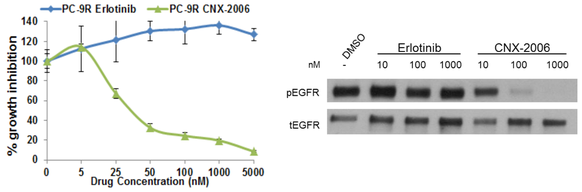




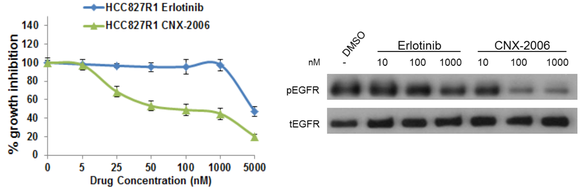


Effect of CNX-2006 on EGFR T790M

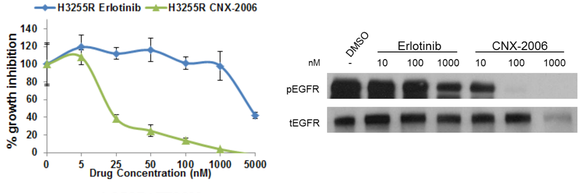




19del+T790M

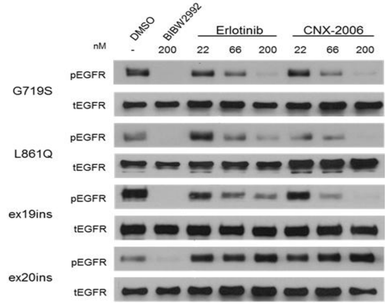


19del+T790M



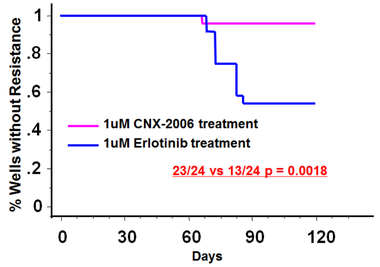
L858R+T790M

Effect of CNX-2006 on Rarer EGFR Mutants



Ex19ins: I744-K745insKIPVAI); Ex20ins: H773-V774HVdup

Acquired Resistance to CNX-2006



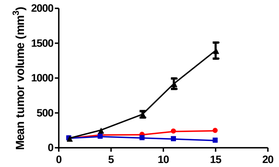
PC-9 cells were plated into 24-well plates and treated for 120 days with either erlotinib (1 μM) or CNX-2006 (1 μM). Medium was changed every 3 days. When cell confluence reached more than 50%, the cells were counted as having "progressed".

	T790M pre	T790M post
PC-9	N	N
PC-9/ER	Y	Y
HCC827	N	N
HCC827/R1	Y	N

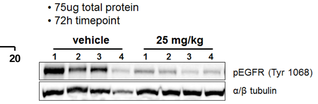
EGFR mutant cells were cultured in escalating doses of CNX-2006 in standard assays (Ohashi et al *PNAS* '12) and then examined for the presence of *EGFR T790M* and other recurrent lung cancer mutations by a SNaPshot assay (Su et al *JMD* '11). Parental drug-sensitive cells did not develop T790M-mediated acquired resistance.

Effect of CNX-2006 on EGFR T790M In Vivo

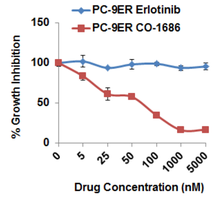
H1975 (EGFR L858R/T790M) xenograft model

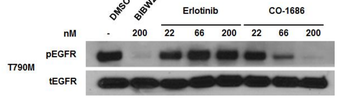


dosing 17 x QD, IP



Effect of CO-1686 on EGFR T790M





293 transfectants; EGFR T790M

Summary

- Compared to erlotinib, CNX-2006 is
  - less active against WT EGFR ("wild-type sparing" e.g. see A431 data)
  - as active against the major drug-sensitive mutants L858R and ex19del
  - more active against the major drug-resistant mutant T790M
  - as or more active against minor mutants G719S, L861Q, ex19ins
  - as inactive against ex20ins
- In *in vitro* modeling of acquired resistance,
  - Continuous exposure of drug-sensitive EGFR mutant cells to CNX-2006 leads to resistance more slowly than to erlotinib
  - Dose escalation with CNX-2006 leads to differential effects in different lines, but does not select for T790M-mediated resistance
- CO-1686 appears effective at overcoming T790M-mediated resistance in *in vitro* models

Acknowledgements

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