In vivo acquired resistance to the mutant EGFR inhibitor rociletinib (CO-1686) is associated with activation of the c-MET pathway

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BACKGROUND

- Rociletinib (CO-1686) is a novel, oral, irreversible tyrosine kinase inhibitor (TKI) for the treatment of patients with mutant epidermal growth factor receptor (EGFR) non-small cell lung cancer (NSCLC) and has demonstrated efficacy against the activating mutations (L858R and del19) and the acquired primary resistance mutation (T790M), while sparing wild-type EGFR.
- Heavily pretreated T790M+ patients treated with rociletinib at 500 or 625 mg BID demonstrated a 67% objective response rate (n=56) (Soria J *et al.* ENA, 2014).
- Despite promising evidence of activity in patients, acquired resistance to rociletinib is likely to occur.
- To assess the preclinical mechanisms of acquired resistance to rociletinib, mice bearing PC-9 (del19 EGFR) human NSCLC tumors were chronically dosed with erlotinib or rociletinib.

In vivo data demonstrate rociletinib superiority over erlotinib in a front-line PC-9 (del19 EGFR) model of NSCLC

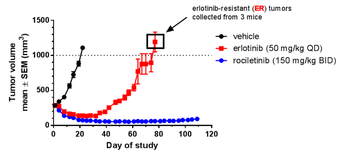


Figure 1. Mice bearing PC-9 tumors were treated with vehicle or erlotinib at the doses indicated until disease progression (*n*=10 animals per group). At 60 days post-dosing, all 10 animals treated with erlotinib had progressed on therapy, whereas tumor regression was observed up to day 110 in all 10 animals treated with rociletinib.

Erlotinib-resistant (ER) tumors harbor EGFR T790M

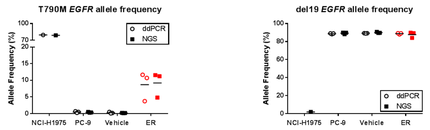


Figure 2. Digital droplet PCR (ddPCR) and next-generation sequencing (NGS) using the SuraSeq 500 panel (Asuragen) were used to assess del19 and T790M *EGFR* allele frequencies in vehicle and ER tumors. Genomic DNA from NCI-H1975 and PC-9 cells were used as positive controls for T790M and del19, respectively.

Erlotinib-resistant, T790M+ tumors respond to rociletinib crossover treatment

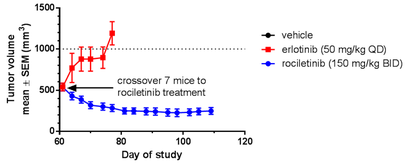
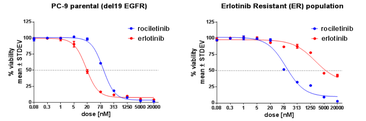


Figure 3. Erlotinib-treated animals from Figure 1 were split into 2 cohorts on day 60. One cohort of mice (*n*=3) continued on erlotinib treatment, while the remaining animals (*n*=7) were crossed-over to treatment with rociletinib.

A cell line derived from a T790M+ ER tumor responds to rociletinib



Treatment	PC-9 parental GI_{50}	PC-9 ER GI_{50}
rociletinib	102 ± 4	98 ± 3
erlotinib	17 ± 2	2141 ± 478

Figure 4. A cell population established from an ER tumor was screened for sensitivity to rociletinib in a 72-hour CellTiter-Glo cell viability assay. ER cells were isolated and maintained in the absence of erlotinib selection. Data reflects three experiments. GI_{50}=half maximal inhibition of cell proliferation.

Acquired resistance to rociletinib is observed following chronic dosing of the PC-9 (del19 EGFR) xenograft model

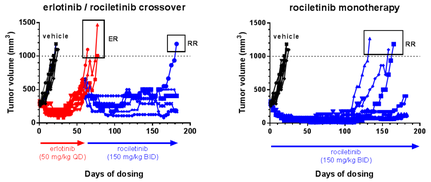


Figure 5. Ninety days after crossing 7 animals over from erlotinib to rociletinib treatment, a rociletinib-resistant (RR) tumor emerged. When rociletinib was used as a monotherapy, resistance to rociletinib emerged in 3/10 animals, beginning 116 days after dose initiation. Boxes indicate ER and RR tumors which were collected for further analysis. RR tumors were all T790M negative by ddPCR and NGS testing (data not shown).

MET amplification and pathway activation was observed in RR tumors

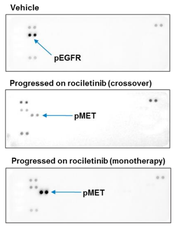


Figure 6a. Vehicle-treated and RR tumors were lysed and exposed to R&D Systems RTK Profiler arrays, which simultaneously detect 42 different phosphorylated receptor tyrosine kinases. Similar levels of phosphorylated MET were detected in 2 additional RR tumors from the monotherapy group.

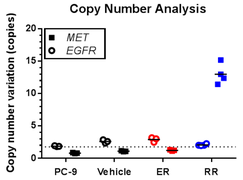
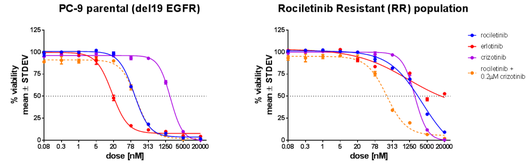


Figure 6b. NGS demonstrates an 11–15 copy gain of *MET* in RR tumors. No change in *MET* copy number was detected in ER tumors, nor were there any changes in *EGFR* copy numbers across the samples tested.

A *MET* amplified tumor-derived cell line responds to a rociletinib and crizotinib combination



Treatment	PC-9 parental GI_{50}	PC-9 RR GI_{50}
rociletinib	102 ± 4	3435 ± 275
erlotinib	17 ± 2	1109 ± 153
crizotinib	1819 ± 86	1881 ± 159
rociletinib + 0.2 µm crizotinib	119 ± 7	199 ± 5

Figure 7. A cell population established from a monotherapy-treated, RR tumor was screened in a 72-hour CellTiter-Glo cell viability assay. RR cells are >30-fold less sensitive to rociletinib than PC-9 parental cells. In addition, RR cells are cross-resistant to the EGFR TKIs erlotinib and AZD-9291 (data not shown). RR cells were isolated and maintained in the absence of rociletinib selection. Data reflects three experiments.

Rociletinib monotherapy (ER cells) or rociletinib + crizotinib (RR cells) are sufficient to suppress downstream signaling

	PC-9 parental				ER population				RR population					
1 µM erlotinib	-	+	-	-	-	+	-	-	-	+	-	-	+	-
1 µM rociletinib	-	-	+	-	-	-	+	-	-	-	+	-	-	+
0.2 µM crizotinib	-	-	-	+	-	-	-	+	-	-	-	+	+	+

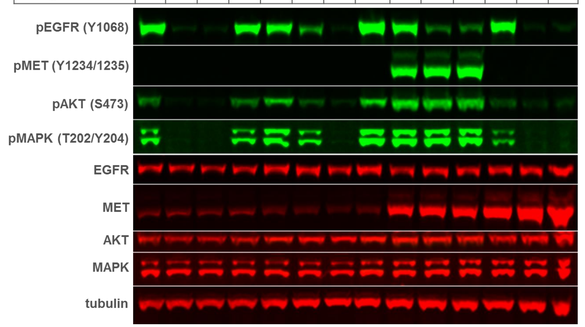


Figure 8. Western blot analysis of PC-9 parental, ER, and RR cell lines following 1-hour incubations with the compounds indicated. A cell population derived from an ER tumor demonstrated strong EGFR pathway inhibition when treated with rociletinib relative to treatment with erlotinib. In a cell population derived from a RR tumor, combining EGFR TKIs with crizotinib enhanced suppression of downstream signaling compared with single agent activity.

Proposed model of acquired resistance to erlotinib and rociletinib in PC-9 xenografts

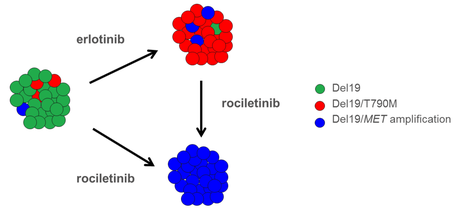
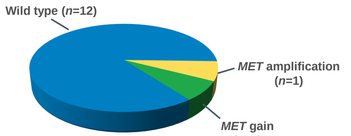
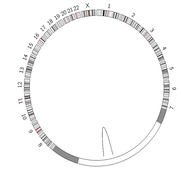


Figure 9. Pre-existing rare subclones are hypothesized to exist within the PC-9 cell line. Chronic treatment with erlotinib results in selection for del19/T790M *EGFR* subclones and, to a lesser extent, del19 *EGFR* + *MET*-amplified subclones. Crossover and monotherapy treatments using rociletinib select for del19 *EGFR* + *MET*-amplified subclones.

MET amplification observed in patients as a potential mechanism of acquired resistance to rociletinib





***MET* amplification Circos plot for patient with acquired resistance to rociletinib. Plasma sample from cycle 20.**

Figure 10. The MetDetect-R™ assay (Personal Genome Diagnostics) was performed on plasma samples from 14 patients with baseline or acquired resistance to rociletinib after 4–20 cycles. *MET* amplification was observed in 1/14 patients, and *MET* gain was observed in a second patient (*MET* copy number increased 1.2-fold; significance of this is unknown). *MET* amplification was also observed in a positive control patient with known baseline *MET* amplification (data not shown).

CONCLUSIONS

- In a chronically-dosed PC-9 mouse xenograft model of frontline mutant del19 EGFR:
  - Rociletinib has a longer time-to-resistance than erlotinib
  - Erlotinib-resistant tumors acquire the T790M EGFR gatekeeper mutation and respond to rociletinib crossover treatment
  - Acquired resistance to rociletinib is associated with *MET* amplification and pathway activation, and can be overcome by combining rociletinib with crizotinib
- Taken together, these data implicate activation of the MET pathway as a potential mechanism of *de novo* and acquired resistance to EGFR TKIs in the clinic.
  - Combining EGFR TKIs with c-MET inhibitors may overcome such resistance.

ACKNOWLEDGEMENTS

- **ddPCR and NGS testing: Asuragen (Austin, TX)**
- **Plasma-testing for focal *MET* amplification: Personal Genome Diagnostics (Baltimore, MD)**