DIGITAL PCR COMPLEMENTS AND CONFIRMS LOW-ABUNDANCE RESISTANCE MUTATIONS IDENTIFIED BY TARGETED NEXT-GENERATION SEQUENCING IN A PRECLINICAL MODEL OF ACQUIRED RESISTANCE USING A NOVEL MUTANT EGFR INHIBITOR

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SUMMARY

- EGFR T790M is a frequent source of acquired resistance to first-line treatment for non-small cell lung cancer (NSCLC).
- A chronically-dosed PC-9 mouse xenograft model was used to investigate mechanisms of resistance to tyrosine kinase inhibitors (TKI) erlotinib and rociletinib. Targeted NGS was used to detect and quantify EGFR T790M, EGFR del746-750, EGFR and MET copy number variations (CNV). Digital PCR (ddPCR) assays designed for target mutations and CNVs confirmed NGS findings.
- Emergence of EGFR T790M contributed to acquired resistance to erlotinib but not rociletinib. Copy gain of MET gene and pathway activation contributed to acquired resistance to rociletinib.
- Targeted NGS and ddPCR are powerful molecular tools to interrogate base-substitution mutations, indels, and CNVs at high analytical sensitivity.

INTRODUCTION

Erlotinib is a reversible tyrosine kinase inhibitor (TKI) for treating EGFR mutant non-small cell lung cancer (NSCLC) patients. In these patients, a frequent source of acquired resistance is the EGFR T790M mutation. Rociletinib (CO-1686) is a novel, oral, irreversible TKI that is effective for T790M tumor. Methods that can assess low-abundance EGFR mutations are needed to evaluate the emergence of resistance and assess the efficacy of new therapies to advance drug development. We utilized targeted next-generation sequencing (NGS) and Droplet Digital™ PCR (ddPCR™) to quantify EGFR mutations, insertion/deletions (indels), and copy number variations (CNV) in order to compare and contrast the preclinical mechanisms of acquired resistance to the two TKIs, rociletinib and erlotinib, respectively.

METHODS

Mice bearing PC-9 (del746-750 EGFR) human NSCLC tumors were chronically dosed with erlotinib or rociletinib or vehicle (10 animals/group). DNA was isolated from tumor tissues collected from vehicle group (N=3), erlotinib resistant (ER) (N=3) and rociletinib resistant (RR) (N=4) at tumor progression. Targeted NGS analysis was conducted using Asuragen's QuantideX® NGS chemistry on a MiSeq (Illumina) instrument. Droplet digital PCR assays for EGFR T790M, EGFR del746-750, and EGFR CNVs were run on a QX200TM ddPCR™ System and analyzed using QuantaSoft™ Software (Bio-Rad).

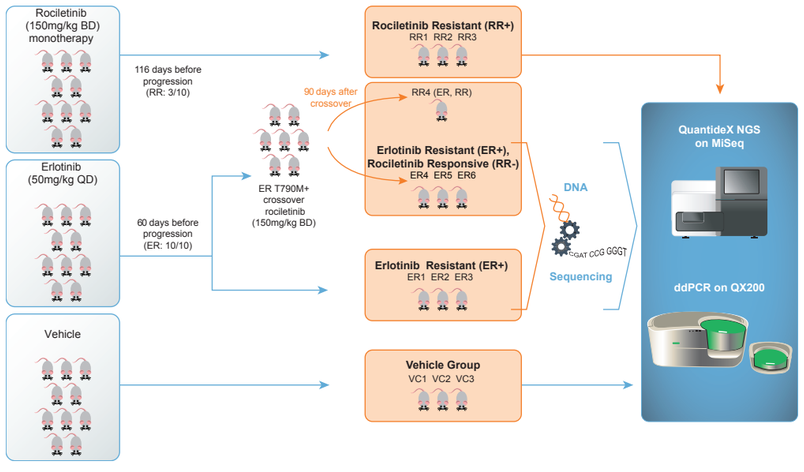

Figure 1. Experiment design and workflow. Mice bearing PC-9 tumors were treated with vehicle, erlotinib, or rociletinib at doses indicated until progression (n=10 per group). At 60 days post-dosing, all 10 animals treated with erlotinib had tumor progression; 7 of the 10 mice on erlotinib were crossed-over to rociletinib treatment and showed response. Three mice with tumor progression in vehicle group (VC1-VC3) and 6 erlotinib-resistant mice were tested for EGFR mutations and CNV (ER1-ER6). 90 days after crossing-over, 1 of the 7 mice development resistance to rociletinib (RR4). Tumor regression was observed in all 10 animals in rociletinib monotherapy group up to 110 days post-dosing. Beginning 116 days after dose initiation, rociletinib resistance emerged in 3 of the 10 mice (RR1, RR2, RR3). The 4 rociletinib-resistant mice (RR1-RR4) were also tested for EGFR mutations and CNV.

RESULTS

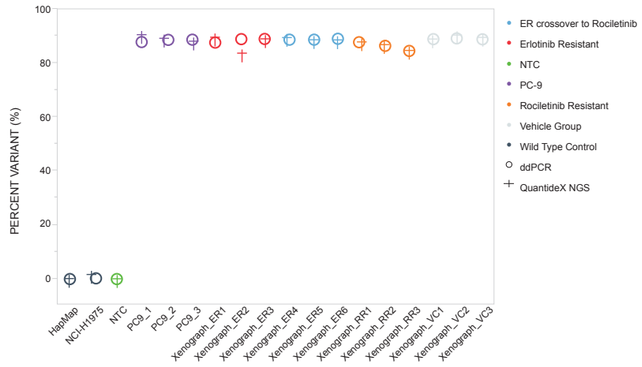

Figure 2. Detection of structural variant (EGFR del746-750) using targeted NGS and digital PCR. EGFR del746-750 was detected concordantly in PC-9 parental cell line and all xenograft samples derived from PC-9 by both targeted NGS (QuantideX NGS) and ddPCR. Average percent variant is 88.3±2.0% measured by NGS, and 88.3±1.5% measured by ddPCR.

		EGFR_T790M PERCENT VARIANT (%)	
Experiment Group	**Sample Description**	**ddPCR Assay**	**QuantideX NGS panel**
T790M Positive Cell Line	NCI-H1975	79.50	78.80
PC-9	PC9_1	0.70	0.51
	PC9_2	0.50	0.31
	PC9_3	0.27	0.34
Vehicle Group Tumor Progression	Xenograph_VC1	0.18	0.15
	Xenograph_VC2	0.51	0.22
	Xenograph_VC3	0.10	0.19
Erlotinib Resistant Tumor Progression	Xenograph_ER1	11.66	11.23
	Xenograph_ER2	3.75	4.81
	Xenograph_ER3	10.70	11.56
ER Crossover to Rociletinib	Xenograph_ER4	8.52	9.31
	Xenograph_ER5	11.17	11.65
	Xenograph_ER6	6.40	6.58
Rociletinib Resistant Tumor Progression	Xenograph_RR1	0.10	0.10
	Xenograph_RR2	0.13	0.17
	Xenograph_RR3	0.08	0.09
	Xenograph_RR4	0.18	0.30
Negative Controls	HapMap	0.0	0.0
	NTC	0.0	0.0

Table 1. Emergence of EGFR T790M mutation in erlotinib-resistant (ER) tumors. EGFR T790M mutation was detected concordantly by ddPCR and QuantideX NGS in all 6 ER tumors, with mutation abundance ranging from ~4% to 12%. PC-9 mouse tissue and vehicle group tumors have low level of T790M (<1%). None of the 4 RR tumors showed significant increase in T790M mutation abundance.

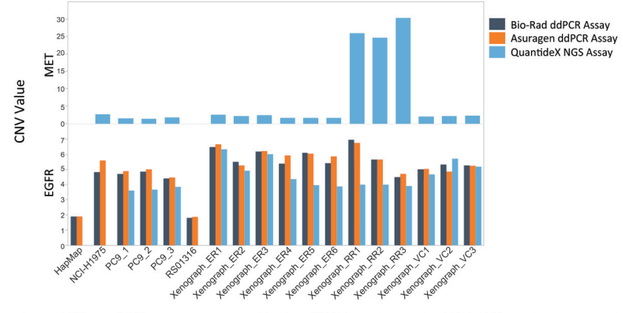

Figure 3. Detection of MET and EGFR copy number amplification. T790M positive cell line NCI-H1975 cell line had approximately 2 fold increase (CNV value=4.9) in EGFR copy number compared to negative control DNA samples (CNV value=~2.0). PC-9 and PC-9 derived xenografts did not show significant EGFR copy number amplification (CNV value range from 3.8~7.0). These results were consistent using both QuantideX NGS assay and two separate ddPCR assays. Interestingly, MET copy number amplification was observed in all 3 RR tumors (Fold change: 11~15) but not in ER tumors.

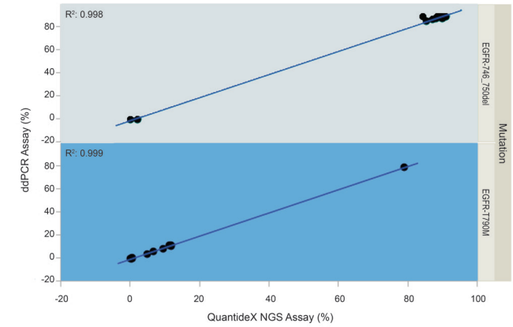

Figure 4. Consistent quantification of EGFR mutation abundance between ddPCR and QuantideX NGS assay. Quantitation of EGFR mutations using ddPCR and QuantideX NGS assay demonstrated high concordance: R^2=0.998 for EGFR-747_750del and R^2=0.999 for EGFR T790M. Similarly high sensitivity was also observed in both methods: EGFR T790M abundance among tested samples range from 0.1% to 79%.

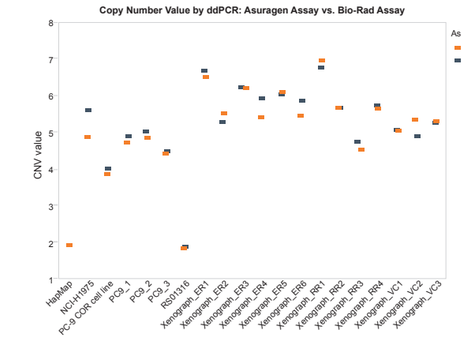

Figure 5. Concordant measurements of EGFR copy number variation (CNV) between different ddPCR assay designs. Two digital PCR assays were utilized to measure EGFR copy number alterations. The Asuragen and Bio-Rad assays target different region of EGFR gene. CNV values measured by the two assays were highly concordant (R^2=0.957).

CONCLUSIONS

- Emergence of EGFR T790M mutation was detected in all ER tumors but not RR tumors. Erlotinib-resistant, T790M+ tumors responded to rociletinib crossover treatment. The delayed development of resistance and lack of T790M in the rociletinib mice suggests that first line treatment with 3rd gen agents should be explored clinically.
- Targeted NGS and ddPCR reported T790M mutations from <1% to 79% abundance; correlation coefficients for variant quantification were >0.99 for both high (>10%) and low-abundance (<10%) variants. In mice with low abundance T790M, rociletinib was still effective, suggesting that such mutation abundance levels are relevant.
- EGFR del746-750 mutations were identified consistent with the known genotype of the PC-9 cell line.
- NGS analysis reported a novel 11-15-fold copy gain of MET observed in RR tumors. Combining EGFR TKIs with c-MET inhibitors may overcome such resistance.
- No significant change in EGFR copy number was detected in ER or RR tumors using NGS, and this finding was confirmed by two distinct ddPCR EGFR CNV assays.

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