Dynamics of EGFR Mutational Load in Urine and Plasma Correlates with Treatment Response in Advanced NSCLC

Abstract ID # 5529

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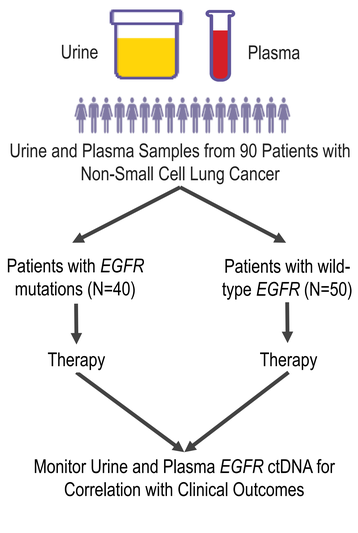
Introduction

- Non-small cell lung cancer (NSCLC) is a heterogeneous and dynamic disease where testing for key mutations is essential.
- With the emergence of clonal resistance, obtaining serial biopsies to assist in the real-time treatment decision-making has proven challenging.
- Noninvasive urinary circulating tumor (ct) DNA-based liquid biopsy approach can be used to detect and track cancer driver mutations for rapid diagnosis and disease monitoring.
- Here, using a highly sensitive ctDNA mutation detection platform, we demonstrate detection of *EGFR* mutations in urine and plasma samples obtained from patients with NSCLC.

Clinical Study Design

- This is a prospective observational study of patients with non-squamous, tissue-confirmed *EGFR*, *KRAS* or *ALK*-mutant NSCLC preparing to receive a systemic treatment regimen.
- The primary endpoints are correlation between ctDNA and tumor-based molecular results, and measurable change in ctDNA with response by RECIST v1.1.
- Urine and blood specimens are collected from patients at baseline, on treatment, and at progression for ctDNA analyses.
- This study has enrolled 34 patients, and presented here is an interim analysis of 20 NSCLC patients with *EGFR*-positive tumors



Patient demographics	N=34
Age Median	**66 (45-78)**
Females	**15 (44.2%)**
Males	**19 (56.1%)**
ECOG Status	
0	**6 (17.7%)**
1	**28 (82.5%)**
Race	
White	**26**
Black	**4**
Asian/PI	**4**
Unknown	**0**
Ethnicity	
Hispanic	**4**
Non-Hispanic	**30**
Smoking	
Current	**4**
Former	**15**
Never	**15**
Mutation	
EGFR Mutant	**25***
KRAS Mutant	**8***
ALK Mutant	**2**

*One patient with co-occurring EGFR and KRAS mutation.

Results

EGFR Mutation Enrichment NGS Assay

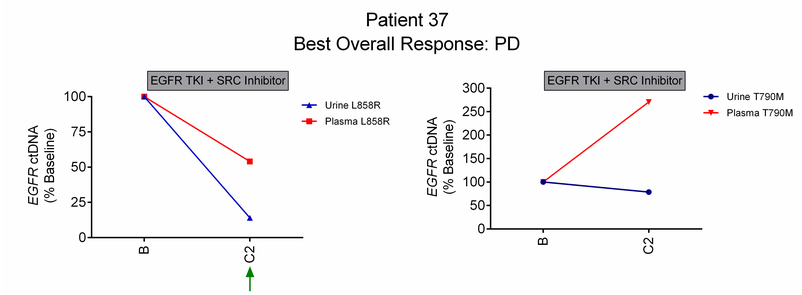
- Mutant allele-enrichment PCR followed by NGS, utilizes a short footprint amplicon (42-46 bp) and selectively amplifies mutant *EGFR* DNA fragments.
- Quantitation of mutant DNA is achieved by including a set of standard samples in addition to clinical samples and controls to each single multiplexed NGS run.
- Mutation enrichment results in approximately 3000-fold increase in ratio of mutant over wild-type signal for low copy number inputs.
- Lower limit of detection: 1 copy in 18,181 WT GEq (0.006%) for *EGFR* Ex19del and L858R assays; and 2 copies in 18,181 GEq (0.01%) for *EGFR* T790M assay.

Correlation of ctDNA *EGFR* with Therapeutic Response

- 17 of 20 (85%) NSCLC patients with *EGFR*-positive tumors had detectable concordant *EGFR* mutation in pre-treatment (baseline) urine and/or plasma.
- 9 of 11 patients with **matched serial ctDNA samples** demonstrated detectable *EGFR* mutation signal in pre-treatment urine and/or plasma samples.
- Of 9 patients, 3 received single EGFR TKI, 3 received combination TKIs, 1 received chemotherapy, 1 received immune checkpoint inhibitors alone, and 1 received immune checkpoint inhibitors in combination with TKI.
- In all 9 patients, changes in ctDNA levels from baseline to every cycle on therapy were examined, and correlated with best response by RECIST v1.1.

Longitudinal Monitoring of ctDNA *EGFR* in Non-Responders

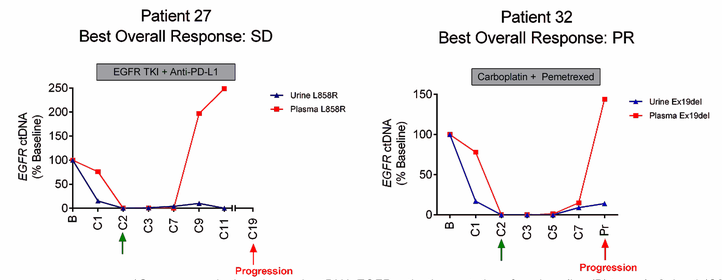
Representative case showing dynamics of urine and plasma ctDNA *EGFR* in NSCLC patient with progressive disease



*Green arrow depicts change in ctDNA *EGFR* activating mutations from baseline (B) to cycle 2 day 1 (C2).

Longitudinal Monitoring of ctDNA *EGFR* in Responders

Representative cases showing dynamics of urine and plasma ctDNA *EGFR* in NSCLC patients responding to therapy (stable disease or partial response)



*Green arrow depicts change in ctDNA *EGFR* activating mutations from baseline (B) to cycle 2 day 1 (C2).

Summary

- In both responders and non-responders, change in ctDNA *EGFR* activating mutations from baseline (B) to cycle 2 day 1 (C2, depicted by green arrow) correlated with best response by RECIST v1.1.
- A 100% decrease in urine and plasma ctDNA *EGFR* activating mutations was observed in patients with partial response (PR, n=3) or stable disease (SD, n=3).
- A <90% decrease or an increase in urine and plasma ctDNA *EGFR* activating mutations was observed in patients with progressive disease (PD, n=3).
- Data presented here suggest that monitoring urine and plasma ctDNA for changes in levels of *EGFR* activating mutations during therapy may reflect tumor dynamics and correlate with patient outcomes.

Conclusions

- Mutation enrichment NGS testing by urine and plasma ctDNA correctly identified *EGFR* activating mutations in 85% of patients.
- Monitoring *EGFR* levels in urine/plasma enabled accurate assessment of response in advance of radiographic evaluation and regardless of therapy type in 100% of patients, with a cut-off of 90% decrease in *EGFR* load, discriminating between patients with disease control (PR or SD) and patients with disease progression (PD).
- Analysis of urine and plasma for *EGFR* mutations may be a viable approach for therapeutic monitoring of patients with advanced cancers.

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