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Is liquid biopsy a good molecular monitoring tool? Detection of L858R and T790M EGFR mutations in circulating tumor DNA derived from NSCLC patient treated with gefitinib (case study)

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BACKGROUND

Liquid biopsy is the most innovative and noninvasive diagnostic method that detects circulating tumor DNA (ctDNA) in blood and monitors cancer disease progression. ctDNA is a challenge to detect in blood samples due to its low amount in blood, short half-life and the possibility of sample contamination with genomic DNA derived from leukocytes. If an EGFR somatic mutation is detected in tumor cells, a patient is qualified for targeted therapy with Tyrosine Kinase Inhibitors (TKIs). Unfortunately some patients have serious medical contraindications for operational treatment or biopsy. This results in a lack of tumor tissue for assessing the mutational status in EGFR gene. Analysis of ctEGFR from blood can overcome such a difficulties.

STUDY AIM:

The aim of this study was to evaluate ctEGFR analysis in the context of TKI monitoring.

MATERIAL & METHODS

The study was conducted on 20 plasma samples derived from 7 patients previously diagnosed with non-small cell lung cancer (NSCLC) receiving erlotinib, gefitinib or afatinib (TKIs). DNA was extracted from FFPE samples (collected before chemotherapy) using FFPE QIAamp DNA Tissue Kit (QIAGEN). EGFR mutations were detected with CE-IVD EGFR Mutation Analysis Kit (EntroGen). 4 ml of blood samples were collected during routine visits at least every 6 months between 2015 and 2016. Blood samples were immediately centrifugated for plasma collection. ctDNA was isolated using QIAamp Circulating Nucleic Acid Kit (QIAGEN). The presence of the most common EGFR mutations in exon 19, 20, 21 and the inhibiting T790M mutation in ctDNA was assessed using the highly multiplexed ctEGFR Mutation Detection CE-IVD kit (Entrogen) on the Light Cycler 480 (Roche).

RESULTS (1&2)

1. 20/20 DNA samples derived from liquid biopsies passed internal quality control for circulating tumor EGFR mutation analysis. The plasma volume used for ctDNA isolation ranged from 1050 -2400 µl.

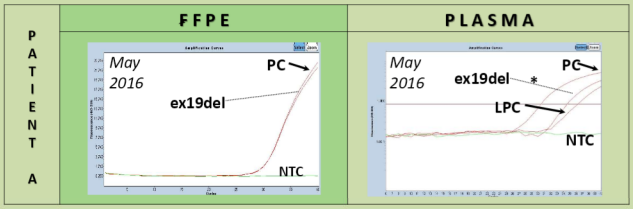

Fig.1. Concordance of the most common EGFR deletion in exon 19 between FFPE tissue and plasma samples collected within 2 weeks. Plasma amount used for ctDNA isolation: 1050 µl. PC - Positive Control, LPC – Low Positive Control, NTC – No Template Control, ex19del – patient A FFPE sample.

2. During 15 months of plasma collection and ctEGFR mutation monitoring we observed correlation between stabilization of patient condition and the lack of T790M mutation detection in ctEGFR during TKI's treatment (5/7 patients).

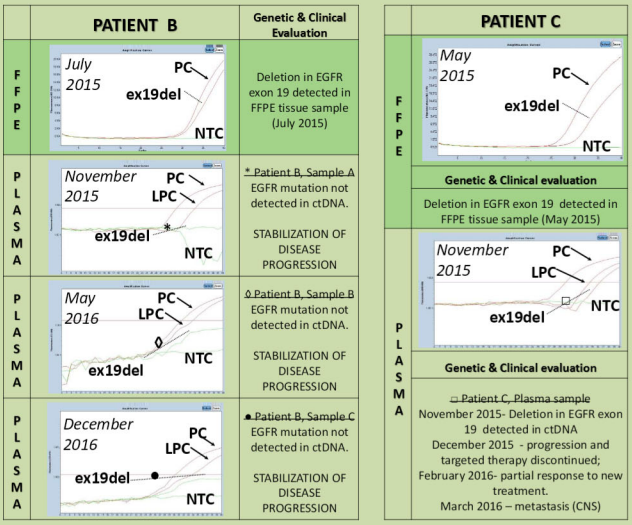

Figure 2. Results of mutation analysis in *EGFR* gene in DNA isolated from FFPE tissue sample and ctDNA. Patient A&B were treated with TKI – afatinib.
Left panel: Patient B. Absence of exon 19 deletion in ctEGFR from November 2015 until December 2016 correlates with disease stabilization confirmed clinically using a broad range of medical imagining techniques.
Right panel: Patient C. The most common EGFR mutation (ex19 deletion) detected in FFPE tissue and plasma sample collected 6 months later. Partial response to new treatment determined in February 2016.

RESULTS (3)

3. We observed correlation between disease progression and T790M mutation detection in ctEGFR (2/7 cases)

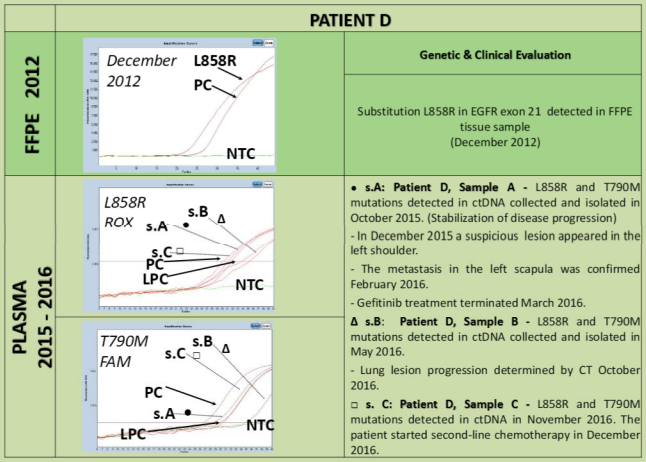

Fig. 3. Results of EGFR mutation analysis using DNA isolated from archival material (2012) and ctDNA (2015-2016) from Patient D. Substitution L858R detected in EGFR exon 21. Patient was treated with gefitinib. Three plasma samples A, B, C, were collected during gefitinib treatment in October 2015, May 2016 and November 2016, respectively. **Additional T790M mutations were detected in all plasma samples.** Amount of plasma used for ctDNA isolation were 1700 µl, 1400 µl, 1450 µl, respectively. PC - Positive Control, LPC – Low Positive Control, NTC – No Template Control. Each EGFR mutation is detected on different channel (FAM – T790M, ROX – L858R, Cy5 – ex19del).

Despite clinical stabilization (Patient D), T790M mutation was detected in October 2015 (2 months before a suspicious lesion in the left shoulder appearance and 4 months before clinical confirmation of metastasis in scapula).

RESULTS (4)

4 .Patient E (Fig. 4) was treated with gefitinib and had 35 months progression free survival. Additional inhibiting T790M mutation was detected two months before the progression of lung lesions.

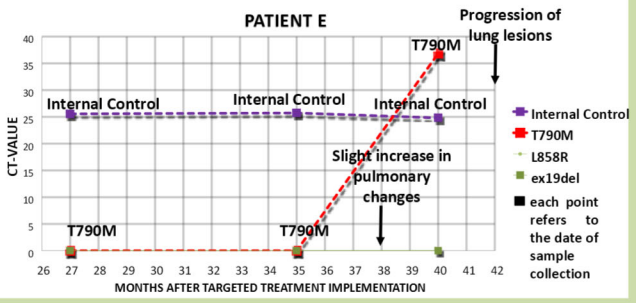

Fig. 4: T790M mutation detected in plasma sample collected 2 months before clinical progression. Plasma samples were collected 27, 35 and 40 months after implementing targeted treatment. Amount of plasma used for isolating ctDNA were 1550 µl, 1250 µl, and 1500 µl, respectively.

Conclusions & Future Directions:

1. ctDNA EGFR mutation analysis can be considered not only as alternative tool for qualifying NSCLC patients for TKIs therapy but also as monitoring one.

2. In order to decrease potential false negative results it is important to start the diagnostic procedure with proper blood collection, immediate centrifugation and high volumes of plasma for ctDNA isolation. CE-IVD tests designed for ctDNA in human plasma only (not a combined test for FFPE and ctDNA) have significantly better limits of detection and detect lower numbers of mutant copies.

3. Recent publications indicate that the existence of T790M neighboring rs1050171 reduces the sensitivity of the ARMS-based T790M mutation detection assay and produces a 14.3% false-negative rate (*Sanpeng Xu et all, Oncol Lett. 2016 Nov*). Therefore it is important to use a CE-IVD test that was designed to account for T790M SNP mutation (Q787Q) for detecting EGFR mutants in ctDNA

4. Research on larger homogenous groups of patients receiving TKIs should be performed to evaluate the potential of a) somatic mutation screening in ctDNA for residual disease detection and b) determining complete or major molecular response to targeted treatment.