



36_ EGFR mutational analysis in ctDNA from patients with NSCLC under TKI therapy

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Aim: Nearly all patients with EGFR mutated non-small cell lung cancer (NSCLC) who initially respond to EGFR Tyrosine kinase inhibitor (TKI) therapy develop resistance. The T790M mutation is the most common mechanism of resistance in advanced *EGFR* mutated NSCLC. This mutation is detectable in almost 60% of tissue biopsies taken after progression. Recently, Osimertinib, a third-generation irreversible *EGFR* TKI, was approved for the treatment of patients with metastatic *EGFR* T790M mutation-positive NSCLC who have progressed on or after *EGFR* TKI therapy. Circulating tumor DNA (ctDNA) has emerged as a specific and sensitive blood-based biomarker for detection of *EGFR* mutations, especially in cancer patients with tumors that cannot be easily biopsied. The aim of this study was to implement *EGFR* mutational analysis, including T790M, in plasma ctDNA from patients with NSCLC under TKIs therapy.

Material and methods: This study includes patients with advanced or recurrent NSCLC carcinoma, presenting an *EGFR* sensitizing mutation, in progression under treatment with TKIs. Blood samples from 14 patients and five paired tumor rebiopsies were obtained. DNA was extracted from plasma and tumor biopsies using QIAamp Circulating Nucleic Acid Kit and Cobas DNA Sample Preparation Kit, respectively. *EGFR* mutation analysis was conducted using the Cobas *EGFR* Mutation Test kit.

Results: Sixty-four percent (9/14) of the patients presented *EGFR* mutations in plasma ctDNA. Of the nine patients with mutation in ctDNA, the T790M mutation was identified in 56% (5/9), whereas in the remaining 44% (4/9) only the sensitizing mutation previously identified in tumor tissue was detected. Regarding the five patients with paired tumor rebiopsies and blood samples, one case was negative in plasma and tissue, one presented the same sensitizing mutation (L858R) in plasma and tissue, and three cases presented *EGFR* mutations in tissue only (two cases with the T790M and a sensitizing mutation, L858R and an exon 19 deletion; one case with the L858R mutation). In the patients in whom the mutation was only detected in the tumor rebiopsy, in one the disease was confined to the lung and other only presented cerebral metastasis. Overall, the T790M mutation was present in 50% (7/14) of the patients.

Conclusion: Over 60% of patients had detectable *EGFR* mutations in plasma samples, indicating that ctDNA can be a new DNA source for tumor genotyping. However, in a subset of samples we only detected *EGFR* mutations in tumor rebiopsies. Our data indicates that ctDNA analysis is a viable first approach to identify the mechanism of resistance to *EGFR* TKI therapy, but that a tissue rebiopsy should be performed when *EGFR* mutations are not identified using ctDNA analysis.