Detection of EGFR Mutations in Plasma cfDNA and Paired CTCs of NSCLC Patients before and after Osimertinib Therapy Using Crystal Digital PCR

Aliki Ntzifa[1], Athanasios Kotsakis[2], Vassilis Georgoulias[3], Evi Lianidou[1]

1 Analysis of Circulating Tumor Cells Lab, Lab of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, 15771 Athens, Greece
2 Department of Medical Oncology, General University Hospital of Larissa, 41110 Larissa, Greece
3 Hellenic Oncology Research Group (HORG), 11471 Athens, Greece

Background: Circulating tumor DNA (ctDNA) analysis has clinical utility in EGFR mutant NSCLC. Circulating tumor cells (CTCs) constitute a unique source of information at the cellular level. Digital PCR (dPCR) is a valuable tool for accurate and valid mutation analysis in liquid biopsy. The aim of the current study was to detect EGFR mutations in plasma cfDNA and paired CTC-fractions of NSCLC patients before the initiation of osimertinib therapy and at progression of disease using crystal digital PCR (cdPCR).
Patients and Methods: Forty-eight patients with EGFR mutated lung adenocarcinomas resistant to 1st or 2nd generation EGFR TKIs were treated with osimertinib in the context of a multicenter Phase II clinical study (NCT02771314). 2 mL of plasma were used for cfDNA extraction using the IDXtract kit (ID-Solutions, France). Crystal dPCR (naica® system, Stilla Technologies, France) was used to quantify mutation allele frequencies (MAF) of EGFR mutations in 91 cfDNA samples before and after osimertinib. 80 identical plasma samples were also analyzed with the FDA-approved cobas® technology. In parallel, EGFR mutations were detected with cdPCR in 64 matched CTC-fractions enriched by Parsortix™ (ANGLE plc, UK). Quality control steps were followed both at the preanalytical and analytical level.
Results: Direct comparison between cdPCR and cobas® revealed high concordance rates for all EGFR mutations; however, in case of T790M, cdPCR proved to be more sensitive. Patients with higher %MAFs at PD presented significantly lower PFS compared to those with lower levels or without EGFR mutations. Direct comparison of EGFR genotyping between primary tissue and baseline plasma cfDNA samples revealed high concordance rates, too. During EGFR mutation analysis in paired CTC-derived gDNA, 11 samples were found positive for EGFR mutations with %MAF ranging from 0.2 to 2.25%.
Conclusions: Crystal dPCR combines the unique benefits of sensitivity and accuracy with the multiplexing capacity for the detection of multiple EGFR mutations in plasma cfDNA samples and CTCs. High concordance rates were observed between cdPCR and cobas® assay. For the first time, cdPCR was applied to detect EGFR mutations in CTC-derived gDNA of NSCLC patients under osimertinib. Discordance between plasma and CTC-fractions might be indicative of tumor heterogeneity and predictive for acquired resistance.