Monitoring of Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor-sensitizing and resistance mutations in the plasma DNA of patients with advanced Non-Small Cell Lung Cancer

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In the last years, the possibility to perform somatic mutation profiling on cell-free tumour DNA (cftDNA) extracted from blood sample of cancer patients has encouraged researchers to investigate the potential use of "liquid biopsy" in clinical practice (Perakis S. and Speicher M., 2017). Non small cell lung cancer (NSCLC) patients with EGFR sensitizing mutations (i.e. p.L858R point mutation and deletions in exon 19) benefit from gefitinib, erlotinib and afatinib, the three widely used epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) (Yang Z., 2017). However, most patients experience disease progression that in half of cases is mediated by the p.T790M point mutation of EGFR (Wang Z., 2014). Since monitoring EGFR status with tissue re-biopsy have spatiotemporal limitations, the use of a sensitive method to discriminate low levels of tumour-specific mutations in a large background of germinal DNA in a blood sample could be a novel, less invasive, alternative approach to cancer tissue biopsy (Siravegna G., 2017). Digital droplet PCR (ddPCR) has demonstrated to have suitable clinical sensitivity able to detect somatic point mutations at a sensitivity range of 1% to 0.001% (Perkins G, 2017). The aim of this study was to clarify the role of liquid biopsy and ddPCR in the pharmacogenetics monitoring of EGFR mutations that might be addressed by targeted therapy in patients with advanced NSCLC and to evaluate the early detection of mutations responsible for resistance to treatment.

In this explorative study, 43 patients with histological diagnosis of advance NSCLC susceptible for EGFR targeted therapy have been enrolled. For all patients, tissue biopsy was available before the beginning of the therapy. Serial blood samples were collected at baseline and at each clinical follow up. CftDNA was extracted from 6 ml of plasma with the QIAmp Circulating nucleic acid Kit (Qiagen®, Valencia, CA, USA) and the identification of EGFR sensitising and resistance mutations was performed by using ddPCR (BioRad®, Hercules, CA, USA).

The monitoring of circulating EGFR mutations was performed in 19 NSCLC patients for a follow up of two years. For the other patients enrolled in this study the limited number of sample collected until now prevented us from drawing a firm conclusion about the role of liquid biopsy in the pharmacogenomics monitoring of cancer evolution and resistance acquisition.

Thirteen patients were treated with gefitinib, four with afatinib and two patients were randomized to receive either AZD9291 or EGFR-TKI (Flaura phase III study). Before the beginning of the treatment, liquid biopsy was collected for 17 out of 19 patients: 5 out of 17 patients (30%) resulted positive for p.L858R mutation, 9 out 17 patients (53%) showed a deletion in exon 19, while 3 out of 17 patients (18%) resulted negative for these alterations. The amount of single plasma EGFR mutations showed dynamic and case-specific changes during the treatment and was correlated with clinical response. Interestingly, the appearance of low levels of EGFR sensitizing mutations anticipated the appearance of both high levels of the resistance mutation and clinical

progression occurring several months later. Five patients experienced clinical progression after a median of 9 months after the beginning of the targeted therapy. The identification of p.T790M mutation in all of these patients allow the switch to the AZD9291, the potent, irreversible EGFR-TKI selective for T790M mutation.

In conclusion the pharmacogenetic analysis of cftDNA is useful not only to understand the heterogeneity and to monitor clonal evolution of NSCL cancer cells under the stress of EGFR-TKIs, but also in evaluating treatment responses and choose the more effective therapy in individual patients.

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