Detection of ALK and KRAS mutations in circulating tumor DNA associated with drug resistance in patients with advanced ALK+ NSCLC

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Introduction: ALK translocation is present in about 5% of advanced NSCLC and is a predictive factor of response to ALK Tyrosine Kinase Inhibitors (TKI), such as crizotinib. In patients with ALK-positive non-small cell lung cancer (NSCLC) disease progression occurs after a median of 9-10 months of crizotinib treatment. Several mechanisms of resistance have been identified and include other mutations in ALK gene, ALK amplification, activation of bypassing signaling pathways [1]. Second-generation ALK-TKIs demonstrated an enhanced spectrum of activity in crizotinib-resistant ALK mutants. However, re-biopsy in NSCLC patients represents a critical issue and analysis of circulating free tumor DNA (cftDNA) has a promising role for the identification of mechanisms of resistance to targeted therapy.

Patients and Methods: Sixteen patients with avanced ALK-positive NSCLC were enrolled after progression to crizotinib; blood was collected and plasma isolated by centrifugation. cftDNA was extracted from plasma using QIAamp circulating nucleic acid kit (Qiagen®) and was analysed by digital droplet PCR (ddPCR, BioRad®, Hercules, CA, USA) for ALK secondary mutations (p.L1196M, p.G1269A and p.F1174L) KRAS (codons 12 and 13) and BRAF (p.V600E) mutations.

Results: Sixteen patients were studied. Median age was 55 yrs (range 40-81) and all patients were stage IV adenocarcinoma. After treatment with ALK-TKIs, best responseto was partial in 11 patients, stable disease in 3 and progression disease in 2. Median PFS was 8 months. ALK secondary mutations (p.L1196M and p.G1269A) were identified in 4 patients. One patient had two ALK mutations p.L1196M and p.G1269A. Regarding KRAS, overall 8 patients presented KRAS point mutation; of these, 6 presented p.G12D and 2 p.G12V mutations, respectively. In 4 patients KRAS was associated with other mutations: ALK in 2 patients and B-RAF mutations in other 2. cftDNA was monitored during the treatment with second generation ALK-inhibitors and we saw that ALK or KRAS mutations decreased along with tumor response.

Conclusion: ALK mutations are associated with acquired resistance to crizotinib in ALK-positive NSCLC. Acquired mutations can be detected in plasma and could represent tumor marker to monitor responses. ddPCR can detect resistance mutations in cftDNA of ALK+ NSCLC and may represent an effective alternative to re-biopsy. Moreover, the assessment of mutated allele burden could be used for response monitoring during treatment.

Reference

[1] Doebele RC, Pilling AB, Aisner DL, et al. Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. Clin Cancer Res 2012; 18: 1472-82.