

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

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**Background:** 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. Unfortunately, disease progression occurs after a median period of 9-10 months of treatment with crizotinib. Several mechanisms of resistance have been identified and include other mutations in ALK gene, ALK amplification, activation of bypassing signaling pathways involving EGFR, KRAS and c-KIT. 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 cell-free DNA (cftDNA) has a promising role for identification of mechanisms of resistance to targeted therapy.

**Patients and Methods:** Twelve patients progressing during crizotinib were enrolled. After tumor progression, blood was collected and plasma isolated by centrifugation. DNA was extracted from plasma using QIAamp circulating nucleic acid kit (Qiagen®) and tested for ALK secondary mutations, KRAS exon 12 mutations and BRAF V600E, using a Digital Droplet PCR (BioRad®).

**Results:** Twelve patients were studied. Of them, 8 were female and 4 were male, 6 were never-smokers and 6 former-smokers. Median age was 49 yrs (range 40-81) and all patients were stage IV adenocarcinoma. Eleven patients received crizotinib and only 1 ceritinib. ALK-TKIs was administered mainly as second-line, in 2 cases as first-line and in the remaining as third-line therapy. Best response was partial in 10 patients and stable disease in 2. Median PFS was 16.9 months. In 10 patients brain was a site of progression while on ALK-TKIs. Only 5 patients presented a tumor site that could potentially undergo re-biopsy. ALK secondary point mutations were identified in 3 patients, all female, never-smokers and treated with crizotinib. The first showed p.L1196M and p.G1269A ALK mutations; their plasma levels decreased after the 2 months of therapy with second generation ALK-TKI, in association to tumor response. The second presented p.L1196M while the third, initially wild-type, showed p.F1174L after initiation of second generation ALK-TKI. In a total of 9 patients, including those with secondary ALK point mutations, the KRAS mutation G12D or G12V appeared in blood samples at the time of resistance to TKI.

**Conclusion:** 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. Moreover, the development of KRAS mutations may play a role in resistance to ALK-TKIs.