Tumour biology and pathology

1PD
Differences in expression of predictive biomarkers between primary and metastatic non-small cell lung cancer tumors

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Background: Metastatic non-small cell lung cancer (NSCLC) carries especially poor prognosis. Recently developed targeted therapies and predictive value of their biomarkers, coupled with tumor heterogeneity, dictate thoughtful profiling of tumor samples in order to achieve maximum therapeutic response.
Methods: We analyzed 10,764 profiled samples of NSCLC (Caris Life Sciences, Phoenix, AZ), and categorized them into primary tumors, lymph node and distant organ metastases, in order to detect site-specific actionable targets (biomarkers). 20 patients had matched primary and metastatic tumors. Biomarkers were detected using immunohistochemistry (IHC), in-situ-hybridization (ISH) and sequencing methods (Sanger and Next Generation Sequencing).
Results: Numerous biomarkers of targeted biological therapies [e.g. 2.4% ALK and 1.0% ROS1 rearrangement, 2.9% HER2 and 4.0% cMET amplification; EGFR: 49.2% overexpression, 29.5% gene amplification and 12.3% mutations) and immune checkpoints inhibitors (25% PD-L1 expression), as well as chemotherapeutic agents (e.g. BRCA1 and 2, ERCC1, TUBB3, RRM1, TOPO1, TS) were detected in both squamous cell and adeno-carcinomas. Lymph-node metastases (LNM) of lung adenocarcinomas had significantly higher ALK (8% vs. 1%), EGFR (50% vs. 42% for IHC; 39% vs. 28% for ISH), PD-L1 (36% vs. 25%) and ROS-1 (3% vs. 1%) detection rate than primary tumors. Distant organ metastases also exhibited higher cMET amplification (7% vs. 3%) than primary tumors. Squamous carcinomas (SCC) showed higher ALK expression in lymph node metastases (10%) than in the primary site (1%). Similarly, SCC PD-L1 expression was higher in LNM (42%) than in primary tumor (33%). Trends observed in unmatched cohort were also confirmed in patient-matched tissues cohort. Both, gains (e.g. PD-L1 expression, cMET amplification, TP53 mutations) and losses (e.g. KRAS mutations) were observed.
Conclusions: Comprehensive molecular profiling data of NSCLC identified important differences between primary and metastatic sites (up to 87% of matched samples for some biomarkers). These findings highlight the importance of extent and timing of the tissue sampling for the purpose of molecular profiling.
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Monitoring of secondary drug resistance mutations in circulating tumor DNA of patients with advanced ALK positive NSCLC

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Background: Disease progression in ALK positive NSCLC patients treated with crizotinib occurs after a median of 9–10 months of treatment. Several mechanisms of resistance were identified and include ALK gene mutations and amplification and activation of bypassing signaling pathways like EGFR, KRAS or c-KIT. Second-generation ALK-TKIs demonstrated an enhanced spectrum of activity in crizotinib-resistant patients. However, re-biopsy in NSCLC patients represents a critical issue and analysis of circulating cell-free DNA (cfDNA) has a promising role for the identification of mechanisms of resistance.
Methods: Sixteen patients progressed during ALK-TKI were enrolled. After progression, blood was collected and DNA was extracted from plasma using QIAamp circulating nucleic acid kit (Qiagen®) and tested for ALK secondary mutations and KRAS exon 12 mutations using the Digital Droplet PCR (ddPCR – BioRad®).
Results: All patients were stage IV adenocarcinoma; 11 female and 5 male. Nine were never-smokers and 7 former-smokers. Median age was 53 yrs (range 40–81). Fifteen patients received crizotinib and 1 ceritinib. ALK-TKIs was administered mainly as second-line, in 2 cases as first and in the remaining as third-line therapy. Twelve patients had partial response, 3 stable disease, one progressed. Median PFS was 8 months. In 12 cases brain was a site of progression and only 5 patients had a tumor site that could potentially undergo re-biospy. ALK secondary mutations were identified in 4 patients. One showed both p.L1196M and p.G1269A mutations which levels decreased after 2 months of therapy with second generation ALK-TKI, along with tumor response. The second and the third patient had p.L1196M and p.G1269A, respectively. The 4th patient showed p.F1174L after initiation of second generation ALK-TKI. A total of 9 patients KRAS mutations p.G12D or p.G12V appeared in cfDNA at the time of resistance to TKI, 3 of them presented both ALK and KRAS mutations.
Conclusions: ddPCR can detect resistance mutations in cfDNA of ALK+ NSCLC and is an effective alternative to re-biopsy. The assessment of mutant allele burden could be used for response monitoring during treatment. Moreover, KRAS mutations may play a role in resistance to ALK-TKIs.
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