Amplicon-Based Next-Generation Sequencing (NGS) of Plasma Cell-Free DNA (cfDNA) for Detection of Driver and Resistance Mutations in NSCLC

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Purpose
While several studies have evaluated hybrid-capture NGS for cfDNA genotyping, amplicon-based NGS is an attractive alternative with the potential to be faster and less expensive. We performed a blinded evaluation of this approach for the characterization and monitoring of the molecular profile of advanced NSCLC during genotype-directed therapy.

Experimental Design
Plasma samples from patients with advanced NSCLC and a known targetable genotype (EGFR, BRAF, MET, HER2 mutations; ALK, ROS1 rearrangements) were collected and analyzed, blinded to tumor genotype, with IRB approval. Up to 4 specimens were collected for each patient: baseline, initial 2 follow-ups, and progression. Plasma NGS was performed using enhanced tagged amplicon sequencing of hotspots and coding regions from 36 genes. A novel approach was used to detect ALK/ROS1 fusions using amplicon sequencing in cfDNA. Diagnostic accuracy was compared to plasma ddPCR and tumor genotype (including NGS when available).

Results
A total of 146 specimens from 49 patients were studied. Testing was completed for 115 specimens at the time of analysis. Matched plasma NGS and ddPCR were available across 95 samples and revealed high concordance of allelic fraction (AF). At baseline, sensitivity of plasma NGS for the detection of the driver was 100% (26/26) for EGFR (88.5% ddPCR sensitivity). Sensitivity for the detection of ALK/ROS1 fusions was 89% (6/7 ALK, 2/2 ROS1). Rare instances of plasma NGS-positive/tissue NGS-negative discordance were seen across 13 cases with match tumor NGS (3/442 genes sequenced) and appear related to resistance heterogeneity, clonal hematopoiesis, and low tumor content of biopsy. Among patients with acquired T790M and available specimens at osimertinib resistance (n=21), 11 resistance mechanisms could be detected including tertiary EGFR mutations (e.g. C797S), mutations in BRAF, PIK3CA, or KRAS, and amplification of MET, HER2, or FGFR1. 4 were detected pre-osimertinib.

Conclusion
This blinded analysis demonstrates the ability of amplicon-based plasma NGS to detect a full range of targetable genotypes in NSCLC. This approach has attractive sensitivity and specificity and deserves further study as an alternative to hybrid capture approaches. Serial plasma NGS can detect competing resistance mutations in patients with TKIs resistance, highlighting the pitfalls of PCR-based plasma assays in patients with heterogeneous resistance and paving the way towards combination therapies.