Added Value in Detecting Actionable Mutations for Matched Plasma-Based Vs. Tissue Next-Generation Sequencing in Advanced NSCLC: A Retrospective Analysis

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Background: Liquid biopsy (LB) is increasingly used to detect actionable mutations in newly diagnosed advanced non-small cell lung cancer (aNSCLC) patients, though tissue biopsy (TB) remains the gold standard. The value of systematically combining LB and TB next-generation sequencing (NGS) for genomic profiling in this patient group remains uncertain. **Methods**: This single-center retrospective study assessed 102 LB samples collected at diagnosis from aNSCLC patients. Four circulating-free DNA (cfDNA) NGS assays (1–4) were compared for performance and concordance with matched TB NGS in detecting ESCAT I/II genetic alterations. Additionally, cfDNA droplet digital PCR methylation (ddPCR-met) testing estimated tumor fraction to refine the interpretation of wild-type (wt) results. **Results**: Out of 102 patients, 13 (13%) had stage IIIB disease, and 11 (11%) presented with brain-only metastases. Adenocarcinoma was the predominant subtype (84%). Ninety LB samples yielded interpretable results across all assays. Positive percent agreement with TB ranged from 56% (assay 1) to 79% (assay 4), with high concordance, particularly for single-nucleotide variants (SNVs). Hybrid capture-based assays (3 and 4) detected eight and seven gene fusions, respectively, while amplicon-based assays (1 and 2) detected only two each. Assay 3 uniquely identified 12 *MET* amplifications, five of which were confirmed by fluorescence in situ hybridization (FISH) but missed by TB NGS. ddPCR-met testing found 5 of 6 negative cfDNA samples to be wt across all assays. The plasma-first approach added incremental value, peaking at 21% for assay 4. Amplicon-based assays were faster and required less DNA for analysis. Patients with stage IIIB aNSCLC or brain-only metastases were significantly more likely to have negative cfDNA ddPCR-methylation results. Lower levels of cfDNA ddPCR-met, linked to better overall survival, are found in patients with stage IIIB disease. **Conclusions**:

LB-based NGS demonstrated high concordance with TB in newly diagnosed aNSCLC, particularly for SNV detection. Hybrid capture assays showed superior performance in identifying gene fusions and *MET* amplifications. The incremental benefit of a plasma-first strategy was minimal. Thus, LB NGS should be seen as a complementary tool to TB NGS or an alternative when tissue samples are unavailable. Additionally, cfDNA methylation analysis enhances diagnostic accuracy in wt cases.