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CROSS-METHOD COMPARISON FOR BRAF V600 MUTATION CFDNA TESTING IN MELANOMA: BRAFI STUDY

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Background: The BRAF p.V600 mutation is the most frequent driver in melanoma. Detection of BRAF mutations in circulating-free DNA (cfDNA) reflects the shedding of tumor DNA and offers a non-invasive biomarker for disease monitoring and prognosis. However, the lack of standardized methodologies and inter-assay variability hinders its clinical implementation.

Methods: We conducted a prospective study to assess the concordance of eight BRAF mutation detection assays across four laboratories. The presence of BRAF V600 mutation in the purified cfDNA was evaluated in pretreatment plasma samples from 51 stage IV BRAF positive melanoma patients using three digital PCR-based assays (ddPCR, Bio-Rad; Absolute Q, ThermoFisher Scientific), three RT-PCR based assays (Idylla®, Cobas®, PNA-Q-PCR) and two NGS based assays using Oncomine and Illumina Platforms.

Results: Overall, BRAF mutation distribution in tissue was 56.86% p.V600E, 5.89% p.V600K, 1.96% p.V600R and 35.29% other/unknown. The median number of metastatic sites was 2 (1-6), 17.6% of patients had high LDH, and 64.7% of patients were treatment naïve. Regarding comparative analysis between different techniques, two digital PCR methods and Cobas demonstrated the highest detection rates (50.98%), followed by NGS Illumina (47.06%), Oncomine NGS / PNA-Q-PCR (43.14%) and Idylla® (35.29%). Patients with visceral metastases, multiple metastatic sites, and elevated LDH exhibited higher BRAF detection rates in cfDNA. Both NGS platforms and NGS Illumina with PNA-Q-PCR techniques showed near-perfect agreement (Kappa = 0.92), while strong agreement was observed among other assay pairs (Kappa = 0.84-0.85). NGS Illumina with ddPCR demonstrated the highest MAF concordance (ICC = 0.99), while most other assay comparisons had a high grade of concordance.

Conclusions: Our study demonstrates substantial concordance among multiple cfDNA BRAF mutation detection methods, particularly NGS and digital PCR assays. These findings support the utility of ctDNA BRAF testing as a biomarker in melanoma management.