Comparative analysis of cfDNA liquid biopsy and tumor-based next-generation sequencing (NGS) approaches

Introduction
NGS techniques can detect molecular alterations in cell-free DNA (cfDNA) isolated from plasma samples for biomarker discovery, diagnostics, and disease monitoring. We previously reported a 0.2% variant allele frequency (VAF) limit of detection (LOD) for single nucleotide variants (SNVs) and insertions and deletions (Indels) with 99% and 99% accuracy and 52% and 83% sensitivity, respectively, for our cfDNA assay. There are limited numbers of studies with small cohorts comparing gene alterations found by tumor-based NGS assays and cfDNA liquid biopsy (Loi). To assess the clinical applications of our developed cfDNA assay, we compared findings of matched tissue and blood samples for 10 patients.

Methods
A CLIA-certified panel of 216 clinically actionable genes, including exonic and intronic regions, was used in the cfDNA-based LB assay. Commercial references and human model cell lines were used for analytical validation. A bioinformatics variant calling pipeline was developed and applied to analyze sequenced clinical samples (Figure 1). WeIS and RNAm-seq of tumor biopsies, and targeted sequencing of cfDNA were performed for 10 patients with different solid cancers to compare results and investigate discrepancies. Somatic SNV/Indel results were compared between tumor WES and target cfDNA sequencing. Fusion calling from tumor RNA-seq was compared to structural variant calling from cfDNA data.

Results
We detected 295 and 217 somatic mutations in 97/70 and 44/70 patients by WES and cfDNA, respectively (Figure 2). Due to heterogeneity of the tumor types in the cohort, we did not observe any biologically relevant groups of genes for cancer. The cfDNA assay identified an additional 66.2% and 31.4% SNVs and Indels, respectively, within the clinically actionable gene panel compared to WES (Figure 3). Nevertheless, groups of frequently mutated genes (TP53, APC, PIK3CA) remained constant between assays. Intersection was observed between traditional WES analysis and the developed LB assay for 36.8% (23/57) patients (Figures 3, 4). WES results were concordant with the cfDNA assay for a subset of mutations from the targeted gene panel for SNVs (sensitivity = 0.23) and Indels (sensitivity = 0.29), for clinically actionable mutations, we found a slightly stronger relationship (sensitivity = 0.35). For structural variant calling with a LOD of 1% circulating tumor DNA (ctDNA) fraction, 86% sensitivity was observed. Notably, for 3 patients with negative cfDNA status after point mutation analysis, structural variant findings corrected assay outcome (Table 2).

Conclusions
While WES and cfDNA analyses had low concordance, previous reports noted discordance in tumor DNA and ctDNA analyses due to tumor heterogeneity and other biological factors, such as cfDNA half-life and variations in shedding among clones. Our cfDNA panel also included genes from intronic regions and detected additional alterations not found by WES of tumors. Therefore, each assay identified alterations that were missed by the other. These findings highlight the potential of the cfDNA-based assay to guide clinical decision-making. The low concordance between tumor-based NGS assays and the cfDNA LB assay indicates that these two approaches for genomic profiling of the patient are considered complementary to each other, but not interchangeable.

Table 1. Comparison of structural variant detection by tumor RNA-seq based fusion calling and cfDNA assay.

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Figure 1. Design of the developed LB cfDNA assay

Figure 2. Mutation landscape of studied patients

Figure 3. Intersection of mutated genes with identified somatic SNV/Indel between cfDNA and WES assays

Figure 4. Intersection of high and low VAFs between cfDNA and WES assays

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