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POSTER ABSTRACTS

803.EMERGING TOOLS, TECHNIQUES AND ARTIFICIAL INTELLIGENCE IN HEMATOLOGY

A Novel Comprehensive Tumor-Informed Plasma cfDNA Assay to Monitor Minimal Residual Disease for Hematological and Solid Malignancies

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Background:
Cell-free DNA (cfDNA) in peripheral blood plasma is a promising tool for monitoring minimal residual disease (MRD) in hematologic and solid malignancies. Unlike conventional biopsy methods, cfDNA extraction is non-invasive, and the short turnaround time enables the analysis of patient samples across multiple time points over an extended period to monitor disease progression. Currently available tests are difficult to scale because they detect only a limited number of mutations, require patient- and mutation-specific customization, and demand constant review by experts.
Here, we describe a novel and comprehensive cfDNA testing platform encompassing all major clinically reliable genetic hotspots that we validated using plasma samples from a cancer patient cohort. We also analyzed plasma samples collected from a follicular lymphoma (FL) patient at multiple time points to illustrate disease relapse before clinical manifestation and to show the applicability of our assay in MRD testing.
Methods:
We constructed a panel of 216 clinically actionable genes, including exonic and intronic regions, capable of tracking point somatic mutations (SNVs/indels), structural variants, and microsatellite instability (MSI). To identify mutational events, we used deep sequencing (median exon coverage of at least 2000×) and a tumor-informed variant calling algorithm developed in-house. Next, we determined the limit of detection (LOD), sensitivity, and specificity of our assay using plasma samples from 74 patients with various solid cancer diagnoses. We tested our platform on standard reference dilutions (Seraseq® ctDNA Complete™ Mutation Mix 0.1%, 0.25%, 0.5%, 1%, 2.5%; Twist cfDNA Pan-cancer Reference Standard 0.1%, 0.25%, 0.5%, 1%, 2%) to validate our results. Our assay was validated by sequencing samples from the internal cohort (n = 74) in replicates and by repeat sequencing of the reference materials in various input amounts in triplicate. Finally, we analyzed plasma samples from an FL patient at five timepoints to showcase our ability to detect mutations in disease recurrence before clinical manifestation.
Results:
We established the capability of our assay using plasma samples from a cancer patient cohort (n = 74) and two sets of dilutions of reference materials. With an LOD > **0.05% variant allele frequency (VAF)** and sequencing depth of more than **2000x** our assay achieved a **sensitivity of 93-97%** and a **specificity of 98-99%**. It also detected a clonal hematopoiesis of indeterminate potential (CHIP) mutation in one plasma sample, implying that it was more sensitive than variant calling from solid tumors and may allow us to distinguish true somatic mutations from CHIP mutations. VAF comparison between two plasma replicates showed high correlation (Pearson's $r > 0.99$, $p < 0.0001$), indicating that our assay is robust and suitable for comparing samples from the same patient across multiple time points. Using different amounts of the reference cfDNA as the starting material for library preparation (15 ng, 25 ng, 50 ng), we assessed the variability of VAF to determine the error range associated with each amount used. By identifying the appropriate amount of starting material needed in order to maintain the LOD at 0.05% VAF across all samples analyzed, we were able to account for variations in cfDNA levels in plasma samples collected at different time points from the same patient. As such, it ensured the reliability of our test across all analyzed samples.
We used our assay to analyze plasma samples from a patient diagnosed with stage III FL in 2014 who underwent six cycles of therapy with bendamustine and rituximab. The five plasma samples analyzed were collected in 2021, 2022, and 2023 (**Figure 1**). Our assay detected clinically relevant mutations in samples from Mar 2021, which was 14 months earlier than mutation calling from blood cells.
Conclusions:

At a LOD of 0.05% VAF and with all performance metrics greater than 95%, our assay meets the requirements for MRD testing. Analysis of plasma samples from an FL patient also shows that our assay can detect clinically relevant mutations in plasma samples much earlier than mutation calling from blood cells. We will further validate our assay with more clinical samples and expand our scope to include structural variants and MSI, to further lower the LOD.

Disclosures Yudina: *BostonGene:* Current Employment, Current equity holder in private company, Current holder of *stock options* in a privately-held company. **Danchurova:** *BostonGene:* Current Employment. **Alekseeva:** *BostonGene:* Current Employment, Current equity holder in private company, Current holder of *stock options* in a privately-held company. **Efremov:** *BostonGene:* Current Employment, Current equity holder in private company, Current holder of *stock options* in a privately-held company. **Starikov:** *BostonGene:* Current Employment, Current equity holder in private company, Current holder of *stock options* in a privately-held company. **Sookiasian:** *BostonGene:* Current Employment, Current equity holder in private company, Current holder of *stock options* in a privately-held company. **Nuzhdina:** *BostonGene:* Current Employment, Current equity holder in private company, Current holder of *stock options* in a privately-held company, Patents & Royalties: patents. **Podsvirova:** *BostonGene:* Current Employment, Current equity holder in private company, Current holder of *stock options* in a privately-held company. **Chasse:** *BostonGene:* Ended employment in the past 24 months. **Conroy:** *BostonGene:* Current Employment, Current equity holder in private company, Current holder of *stock options* in a privately-held company. **English:** *BostonGene:* Current Employment, Current equity holder in private company, Current holder of *stock options* in a privately-held company. **Tabakov:** *BostonGene:* Current Employment, Current equity holder in private company, Current holder of *stock options* in a privately-held company. **Yong:** *BostonGene:* Current Employment. **Baisangurov:** *BostonGene:* Current Employment, Current equity holder in private company, Current holder of *stock options* in a privately-held company. **Tazearslan:** *BostonGene:* Current Employment, Current equity holder in private company, Current holder of *stock options* in a privately-held company. **Fowler:** *BostonGene:* Current Employment, Current equity holder in private company, Current holder of *stock options* in a privately-held company; *CelGene:* Consultancy, Research Funding; *Roche:* Consultancy, Research Funding; *Gilead:* Consultancy, Research Funding. **Bagaev:** *BostonGene:* Current Employment, Current equity holder in private company, Current holder of *stock options* in a privately-held company, Patents & Royalties: patents.

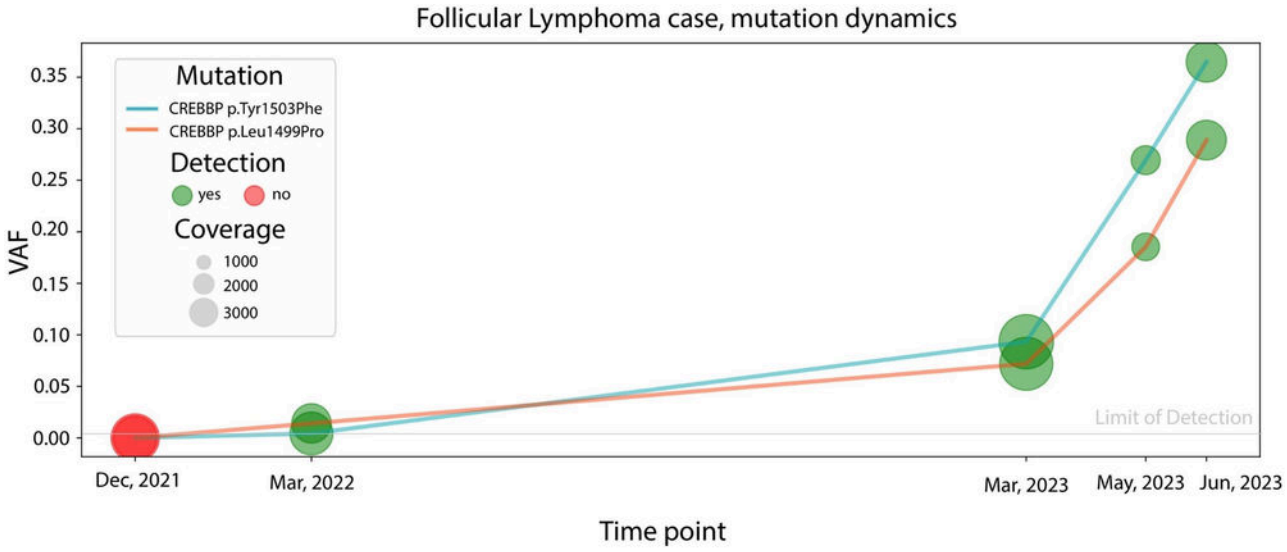

Figure 1. Variant allele frequency (VAF) dynamics across five time points for a clinical case of follicular lymphoma. The red dot marks the absence of mutation in the sample. The green dots represent mutations detectable above all required thresholds. The diameter of the dots represents depth of coverage for the mutation analyzed.

Figure 1

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