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POSTER ABSTRACTS

617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Development of Circulating Tumor DNA (ctDNA) for Molecular Measurable Residual Disease (MRD) in Acute Myeloid Leukemia (AML)

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Introduction: Despite advances in therapeutic options, the majority of AML patients experience relapsed or refractory disease. Standard-of-care (SOC) methods for MRD detection (multiparametric flow cytometry and single gene or structural variant molecular assays) are hampered by (1) inadequate lower limits of detection (LOD), (2) paucity of recurrent mutations across AML genomes, (3) insufficient numbers of identified mutations to characterize clonal evolution, and/or (4) need for invasive bone marrow biopsy. Herein we develop and apply non-invasive circulating tumor DNA (ctDNA) liquid biopsies using a customized Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq) approach to simultaneously quantify MRD and characterize clonal dynamics in AML.

Methods: To increase the number of tumor-specific mutations per case for MRD detection and improve the LOD for ctDNA-MRD, we performed whole exome sequencing (WES) on purified blasts and paired T cells (latter as germline control) from 24 patients with AML (n = 20 previously untreated, n = 4 with relapsed/refractory disease) and designed personalized hybrid capture panels. We combined the personalized panels with a shared hybrid capture panel of 58 recurrently mutated ('canonical') AML genes covering 225kB as a composite approach to generate patient-specific AML-CAPP-Seq assays. We applied AML-CAPP-Seq to track single nucleotide variants (SNVs) in 468 biological samples from the 24 AML patients and 21 healthy control subjects. We compared the performance of MRD detection in cellular (bone marrow mononuclear cell/BMMC, peripheral blood mononuclear cell/PBMC) and cell-free (BM-ctDNA, PB-ctDNA) compartments against SOC clinical MRD assays as well as patient specific clinical outcomes.

Results: We observed a median of 30 SNVs (IQR 16.5-40.25) per patient from WES, compared to only 2 (IQR 1-3.25) if using the AML-focused canonical panel alone ($p < 0.0001$) (**Fig. 1A**). This number of variants enabled sensitive molecular disease monitoring in 96% (23/24) of patients with a LOD of < 0.01% tumor fraction from typical quantities of cell-free DNA. In contrast, when using the canonical panel alone, only 83% (20/24) of patients had 1 or more SNV to allow MRD detection. Notably, only 12.5% (3/24) of patients in this cohort had a feature amenable to SOC molecular MRD methods.

Upon comparing quantitative levels of MRD in each biological compartment, we found that tumor burden in PB-ctDNA, BM-ctDNA and BMMC samples were highly correlated ($r > 0.92$, $p < 0.0001$) with similar amounts of disease. Interestingly, PB-ctDNA contained quantitatively more tumor DNA than corresponding PBMC samples ($p = 0.0002$), most pronounced at low tumor burdens (median 8.2-fold enrichment when mean tumor allele fraction in PBMCs was < 1%). These results suggest that PB-ctDNA effectively captures total body BM disease and mutational burden in AML and serves as a superior analyte for MRD monitoring compared to PBMCs.

When evaluating the performance of AML-CAPP-Seq against clinical SOC methods, our assay correctly detected molecular disease in 99.6% (237/238) of samples known to contain measurable disease, including 98.2% (54/55) of samples at timepoints of clinical MRD positivity during clinical remission (CR). However, restricting trackable SNVs to only 'canonical' genes led to

significant degradation of performance. Specifically, only 69.1% (38/55) of samples with positive MRD during CR by clinical SOC assay were detected by ctDNA if canonical genes alone were employed ($p = 0.0001$). Intriguingly, when using the complete gene panel, AML-CAPP-Seq also identified molecular MRD in 37.5% (30/80) of samples with undetectable MRD by SOC assays.

Finally, stratifying patients who achieved CR after 1st treatment line based on PB-ctDNA MRD significantly predicted relapse free survival ($p = 0.046$, hazard ratio (HR) = 4.5). Moreover, attaining an MRD negative CR in PB-ctDNA at any timepoint was strongly prognostic of overall survival ($p = 0.0004$, HR = 8.5) (**Fig. 1B**).

Conclusions: ctDNA is a promising biomarker for ultrasensitive detection and tracking of MRD in AML without need for invasive bone marrow assessment. Tracking mutations beyond canonical AML genes improves performance of such assays. ctDNA analysis has numerous areas of clinical utility, including response and relapse prediction, evaluation of clonal dynamics, and exploration of mechanisms of therapy resistance.

Disclosures Sworder: *Foresight Diagnostics:* Consultancy. **Mannis:** *BMS/Celgene:* Consultancy; *Astellas:* Consultancy; *Macrogenics:* Honoraria; *Agios:* Consultancy; *Abbvie:* Consultancy; *Genentech:* Consultancy; *Stemline:* Consultancy. **Majeti:** *Orbital Therapeutics:* Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees; *kodikaz Therapeutic Solutions:* Membership on an entity's Board of Directors or advisory committees; *858 Therapeutics:* Membership on an entity's Board of Directors or advisory committees; *Pheast Therapeutics:* Current equity holder in private company; *MyeloGene:* Current equity holder in private company. **Khodadoust:** *Nutcracker Theraputics:* Research Funding; *Daiichi Sankyo:* Membership on an entity's Board of Directors or advisory committees; *CRISPR Theraputics:* Research Funding. **Kurtz:** *Foresight Diagnostics:* Consultancy, Current equity holder in private company, Current holder of *stock options* in a privately-held company, Patents & Royalties: Patents Pertaining to circulating tumor DNA licensed to Foresight Diagnostics. **Zhang:** *Abbvie:* Consultancy; *Rigel:* Consultancy; *Servier:* Consultancy; *Bristol Myers Squibb:* Research Funding; *Stanford University:* Current Employment.

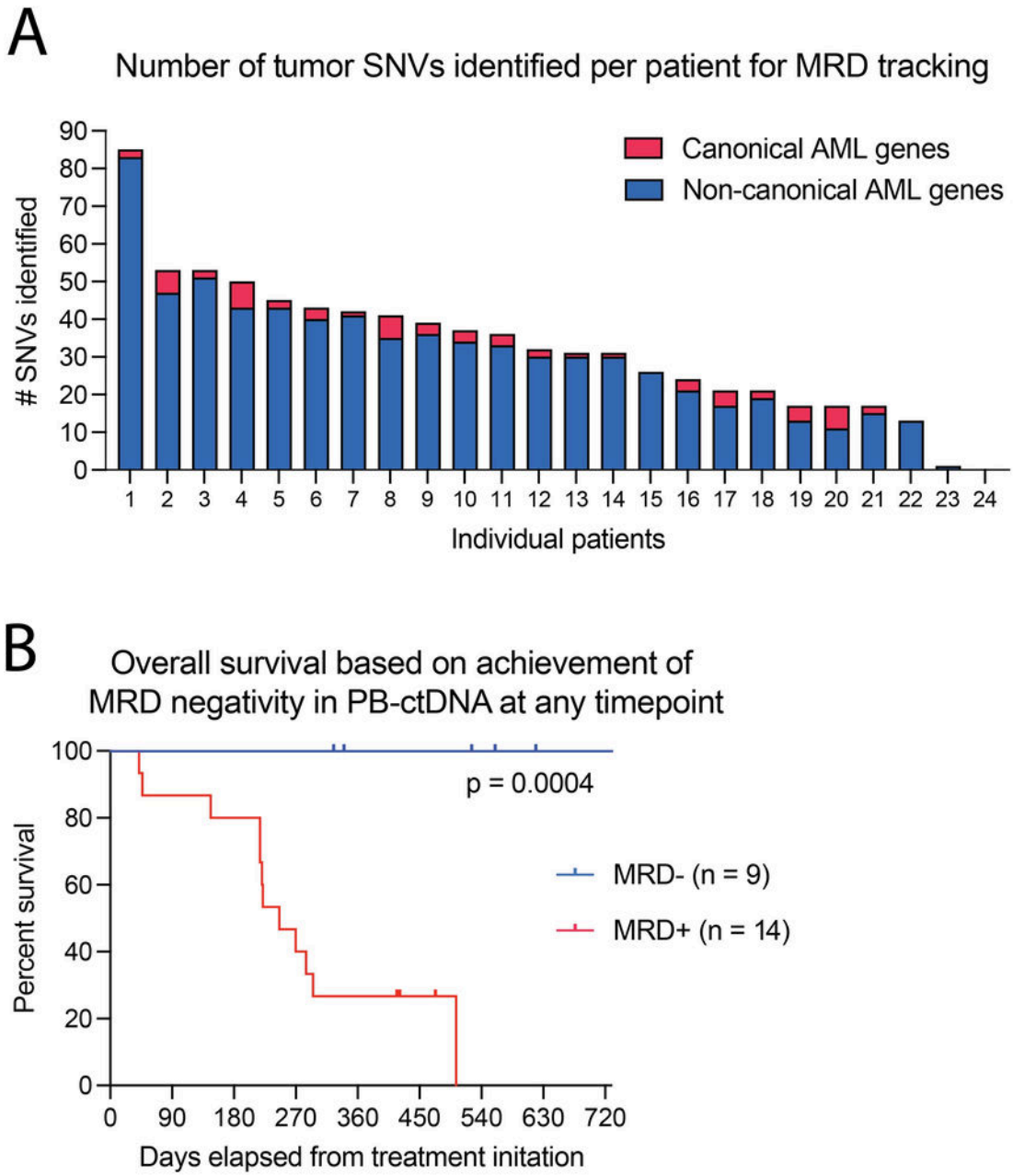

Figure 1. (A) Bar graph depicting the total number of SNVs per AML patient (n = 24) identified by WES and used for MRD tracking, with the fraction of SNVs from 'canonical' AML genes shown in red and the remainder shown in blue. (B) Kaplan-Meier curve illustrating overall survival among AML patients stratified based on achievement of MRD negativity in peripheral blood ctDNA by AML-CAPP-Seq at any timepoint.

Figure 1

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