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Measurable residual disease monitoring in patients with acute myeloid leukemia pre- and post-allogeneic hematopoietic cell transplantation to assess risk of relapse using 16 key AML-associated genes

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INTRODUCTION

Measurable Residual Disease (MRD) has become an essential prognostic marker for assessing how patients with Acute Myeloid Leukemia (AML) are responding to therapy. Currently, limitations in MRD testing include the paucity of key mutations for monitoring, as well as a lack of highly sensitive comprehensive assays that can efficiently test many patients in a single workflow. There is an imminent need to increase the number of targets that can be identified and utilized for monitoring progression of disease pre- and post- allogenic hematopoietic cell transplantation (alloHCT) in AML, as many patients cannot be monitored due to lack of mutations currently tracked by MRD. To this end, we have utilized an Ultra-High Sensitivity (UHS) next-generation sequencing (NGS)-based MRD assay to evaluate a retrospective cohort of serially collected bone marrow specimens (post-induction, pre- and post- alloHCT) from AML patients to assess the ability of this assay to better identify risk of relapse.

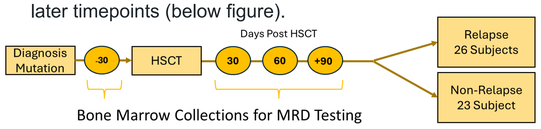
AIM

Utilizing a sensitive and comprehensive NGS-based assay the two key aims of this study are, to first predict risk of recurrence when monitoring pre-alloHCT bone marrow specimens and second to monitor serially collected post-alloHCT bone marrow specimens for identifying recurrence earlier than current methodologies. Both aims focus on the identification of MRD with the future goal of using this assay to modify patient treatment and outcomes.

METHOD

Study Design & Patient Cohorts:

- Retrospective study utilizing archived samples from AML patients who underwent allogeneic hematopoietic stem cell transplantation (HSCT) and were monitored during remission.
- Only subjects with diagnostic mutations represented on the NGS panel were included, enabling a tumor-informed assay. Results were compared to flow cytometry MRD.
- Two cohorts: Relapse: 26 patients who ultimately experienced clinical relapse; Non-relapse: 23 patients who remained disease-free for ≥2 years post-transplant.
- Sampling included measurements at approximately 30 day pre-, 30, 60, and ≥90 days post-transplant, with additional later timepoints (below figure).



Workflow and Panel:

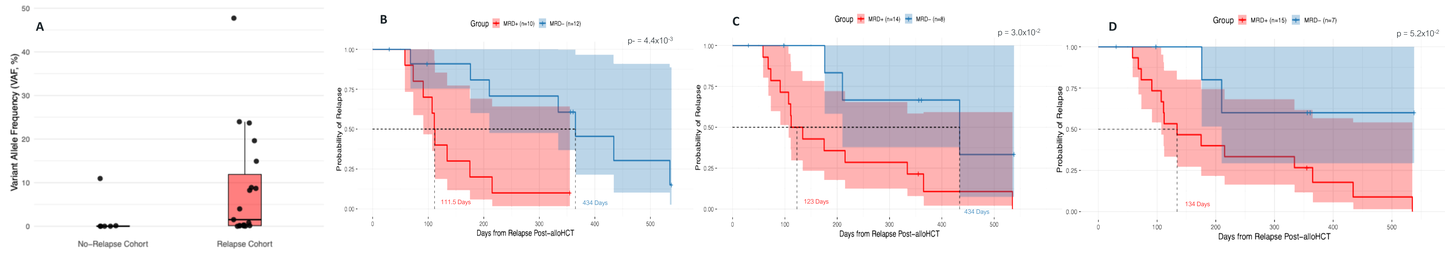
- Libraries were generated using OGT's Ultra low MRD NGS Complete Workflow Solution together with the SureSeq™ Myeloid MRD Plus NGS Panel (Table 1). Sequencing was conducted using 2 x 150 bp reads on an Illumina NextSeq2000. Analysis was performed using OGT's Interpret Software

CALR Exon 9	CEBPA Exon 1	CSF3R Exons 13–17	FLT3 Exons 13–15, 20
IDH1 Exon 4	IDH2 Exons 4–5	JAK2 Exons 12, 14	KIT Exons 2, 8–11, 13, 17
KRAS Exons 2–3	MPL Exon 10	NPM1 Exon 11	NRAS Exons 2–3
RUNX1 Exons 4–8	SF3B1 Exons 13–16	TP53 Exons 2–11	WT1 Exons 7, 9

Table 1: The SureSeq Myeloid MRD Panel targets SNVs, indels and FLT3-ITDs in 16 genes key AML-associated biomarkers

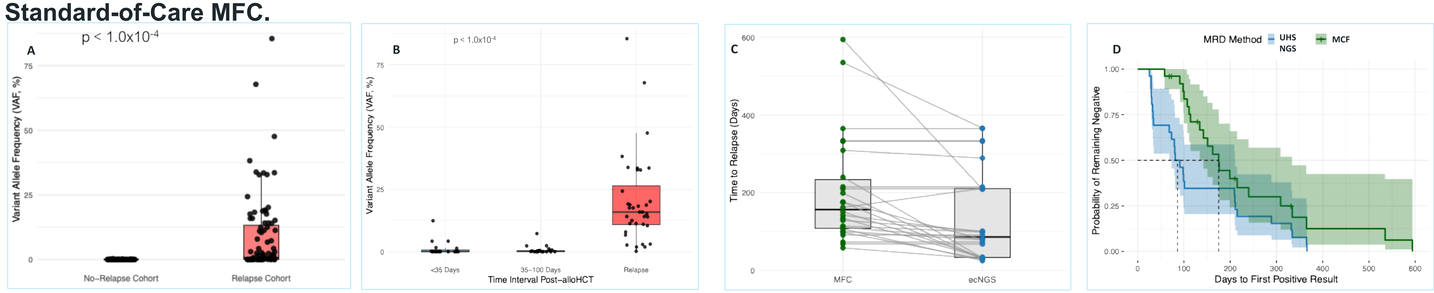
RESULTS

Post-Induction, pre-alloHCT tumor-informed variant MRD detection is associated with increased relapse risk.



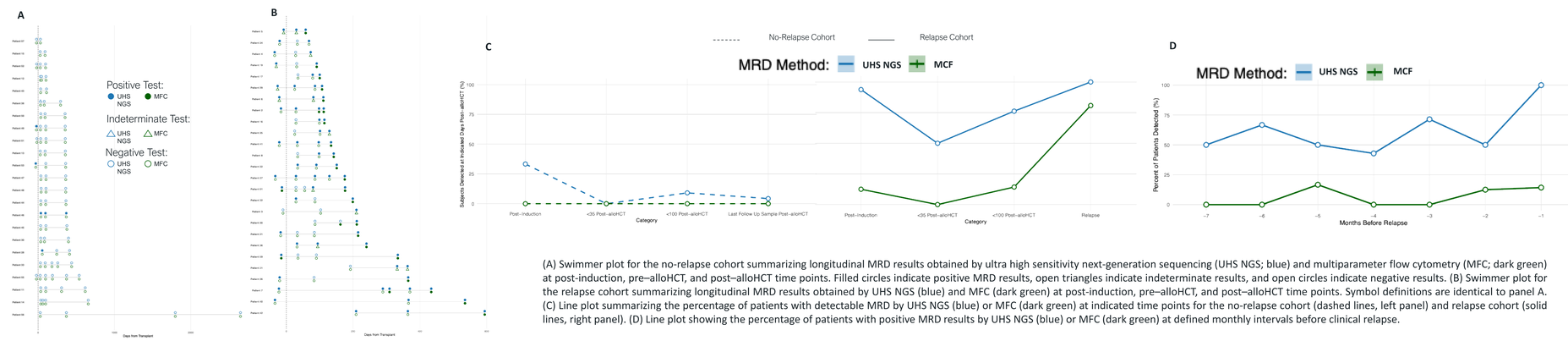
(A) Boxplot showing variant allele frequencies (VAFs, %) detected in bone marrow specimens from the no-relapse and relapse cohorts at the post-induction, pre–alloHCT time point (Mann–Whitney test, p = 0.013). (B) Kaplan–Meier analysis of relapse probability following alloHCT stratified by MRD status using a VAF threshold of >1.0% at the post-induction, pre–alloHCT time point. MRD-positive (MRD+) patients had at least one tumor-informed variant detected with VAF >1.0% (n = 10), whereas MRD-negative (MRD−) patients had no variants detected above this threshold (n = 12) (log-rank p = 0.0044). (C) Kaplan–Meier analysis of relapse probability following alloHCT stratified by MRD status using a VAF threshold of >0.5% at the post-induction, pre–alloHCT time point. MRD+ patients had at least one tumor-informed variant detected with VAF >0.5% (n = 14), while MRD− patients had no variants detected above this threshold (n = 8) (log-rank p = 0.030). (D) Kaplan–Meier analysis of relapse probability following alloHCT stratified by MRD status using a VAF threshold of >0.1% at the post-induction, pre–alloHCT time point. MRD+ patients had at least one tumor-informed variant detected with VAF >0.1% (n = 15), whereas MRD− patients had no variants detected above this threshold (n = 7) (log-rank p = 0.052).

Post-alloHCT Longitudinal Monitoring of Tumor-Informed Variant MRD Dynamics Correlates with Tumor Relapse & Provides a Lead-Time Advantage Over Standard-of-Care MFC.



(A) Boxplot showing variant allele frequencies (VAFs, %) detected in bone marrow specimens from the no-relapse and relapse cohorts across all post–alloHCT time points (Mann–Whitney test, $p < 1.0 \times 10^{-4}$). (B) Boxplot showing tumor-informed VAFs (%) detected in bone marrow specimens from the relapse cohort across specified post–alloHCT time points (one-way ANOVA, $p < 1.0 \times 10^{-4}$). (C) Boxplot depicting paired differences in days to relapse detection comparing UHS NGS with multiparameter flow cytometry (MFC) at the individual patient level (paired t-test, p = 1.5×10^{-3}). (D) Kaplan–Meier analysis comparing time to first positive MRD result post–allo-HCT across UHS NGS and MFC. Median time to MRD positivity was 86 days for UHS NGS and 175 days for MFC (log-rank p = 1.9×10^{-2}).

NGS Identifies MRD Earlier and More Frequently Than MFC Across Longitudinal Patient Trajectories



(A) Swimmer plot for the no-relapse cohort summarizing longitudinal MRD results obtained by ultra high sensitivity next-generation sequencing (UHS NGS; blue) and multiparameter flow cytometry (MFC; dark green) at post-induction, pre–alloHCT, and post–alloHCT time points. Filled circles indicate positive MRD results, open triangles indicate indeterminate results, and open circles indicate negative results. (B) Swimmer plot for the relapse cohort summarizing longitudinal MRD results obtained by UHS NGS (blue) and MFC (dark green) at post-induction, pre–alloHCT, and post–alloHCT time points. Symbol definitions are identical to panel A. (C) Line plot summarizing the percentage of patients with detectable MRD by UHS NGS (blue) or MFC (dark green) at indicated time points for the no-relapse cohort (dashed lines, left panel) and relapse cohort (solid lines, right panel). (D) Line plot showing the percentage of patients with positive MRD results by UHS NGS (blue) or MFC (dark green) at defined monthly intervals before clinical relapse.

CONCLUSIONS

- Ultra-high-sensitivity, target-capture NGS has the potential to predict AML relapse (including pre-transplant risk) significantly earlier than current MRD approaches.
- This NGS assay enables multi-gene analysis at ultra-low variant frequencies, supporting its use for sensitive, comprehensive MRD monitoring.
- Relapse cohort: NGS demonstrated superior sensitivity for detecting recurrent disease compared with FCM with an 89-day median lead time advantage.
- Non-relapse cohort: NGS results showed a strong correlation with sustained MRD negativity, indicating high specificity.
- **Overall, the study confirms that capture-based NGS can reliably detect variants predictive of clinical relapse, supporting its utility for future MRD research and post-transplant surveillance.**

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CONTACT INFORMATION

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