Measurable Residual Mutated *NPM1* Before Allogeneic Transplant For Acute Myeloid Leukemia

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INTRODUCTION

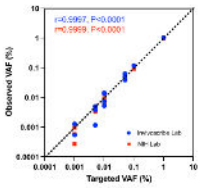
- *NPM1* mutations, seen in up to 30% of adults with acute myeloid leukemia (AML), often co-occur with *FLT3* internal tandem duplication (*FLT3*-ITD).
- Measurable residual disease (MRD) testing before allogeneic hematopoietic cell transplant (alloHCT) for persistence of mutated *NPM1* and/or *FLT3*-ITD can identify patients with AML in complete remission (CR) at highest risk of relapse and death.
- Quantitative RT-PCR assays are currently recommended for *NPM1* MRD testing, while *FLT3*-ITD MRD testing is DNA-based using next generation sequencing (NGS). DNA-based NGS assays for mutated *NPM1* MRD testing may have several advantages but require validation.

AIM

- To examine the association between pre-alloHCT *NPM1* MRD level with relapse and death post-alloHCT in adults with AML in CR1.
- To evaluate the generalizability of DNA-based *NPM1* MRD detection using orthogonal approaches.

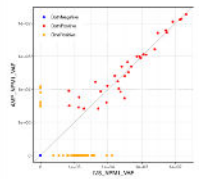
METHOD

- 190 patients aged ≥18 from the Pre-MEASURE study[1] who received alloHCT for *NPM1* mutated AML during CR1 at Center for International Blood and Bone Marrow Transplant Research (CIBMTR) reporting sites in the US between 2013-2019 were included.
- *NPM1* MRD was evaluated on pre-alloHCT blood using a targeted NGS assay (IVS, Invivoscribe, Inc.) following clinical testing standards validated to detect a variant allele fraction (VAF) down to 0.005%.
- *NPM1* MRD results were compared to results previously reported using an anchored multiplex PCR-based (AMP) targeted NGS assay (detection limit 0.01%) and *FLT3*-ITD MRD results, where applicable.[1,2]

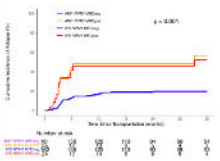


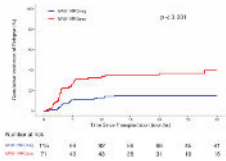
Validation of the IVS *NPM1* MRD assay. *NPM1* competency standards with various types (A,B,D, and other) and targeted VAFs ranging from 1-0.0001% were evaluated using the IVS *NPM1* MRD assay at the Invivoscribe and National Institutes of Health (NIH) laboratories. Graphical representation of expected versus observed VAF of the *NPM1* variants is shown with the Pearson correlation in the graph inset.

RESULTS

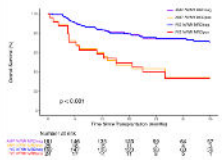


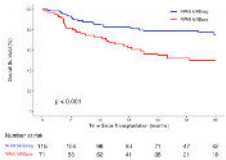
NPM1 NGS MRD testing was successful for 186 of 190 eligible (98%) patients. The IVS assay detected *NPM1* insertion variants in 71 (38%) patients with a median VAF of 0.0026% (range:0.0002-2.1%). The **concordance between IVS and AMP assays was 96%** for a VAF ≥ 0.01%.



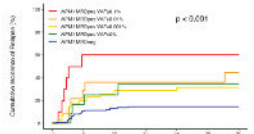


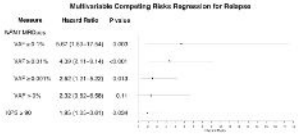
Utilizing **0.01% VAF cutoff**, patients testing *NPM1* MRD positive had **increased relapse** and **decreased overall survival (OS)** by IVS; very comparable to those previously reported by AMP[1].



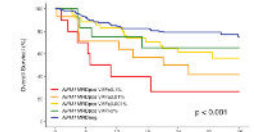


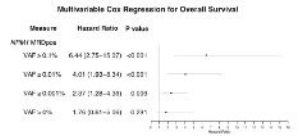
VAF>0% identified 47 additional patients, increasing residual *NPM1* positivity rate to 38%. *NPM1* positivity at any level was associated with poor clinical outcomes.



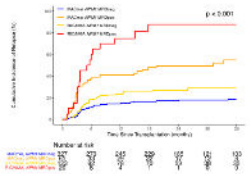


Stratifying patients by the level of residual *NPM1* revealed an **increased risk** of relapse and death for those with **higher disease burden.**

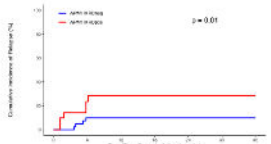




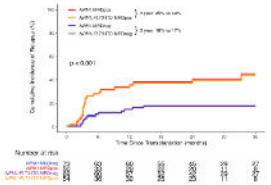
In multivariable analyses, residual *NPM1* was the variable most associated with relapse and OS with a **dose-dependent effect of disease burden.**



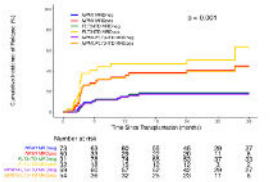
Patients with residual *NPM1* receiving **MAC or Melphalan had decreased rates of relapse and death** compared to those receiving RIC or NMA.



Residual *NPM1* was associated with increased risk of relapse **regardless of baseline *FLT3*-ITD** mutational status. Results shown for *FLT3* wild-type patients.



Defining MRD by the presence of residual *NPM1* *vs* *NPM1* and/or *FLT3*-ITD revealed **equivalent rates of relapse and OS.**



***NPM1* MRD testing alone identified 6 additional relapse events**, versus 1 by *FLT3*-ITD MRD testing alone, with equivalent rates of relapse for the two tests.

CONCLUSIONS

- This study provides strong justification for DNA-based NGS AML MRD clinical testing for persistent *NPM1* prior to alloHCT.
- Residual *NPM1* in the blood of adults in CR1 from AML prior to alloHCT is associated with increased risk of relapse and death in a dose-dependent manner.
- Residual *NPM1* is associated with poor outcomes regardless of baseline *FLT3*-ITD mutational status.
- In patients co-mutated for both *FLT3*-ITD and *NPM1* at diagnosis, *NPM1* MRD testing provides equivalent results and should be prioritized as a target for NGS-MRD if only one test is available.
- Transplant intensification, an intervention not available to all, may help mitigate some of this risk.

ACKNOWLEDGEMENTS

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REFERENCES

[1]Dillon, Gui *et al.* "DNA Sequencing to Detect Residual Disease in Adults With Acute Myeloid Leukemia Prior to Hematopoietic Cell Transplant." JAMA. 2023 Mar 7;329(9):745-755.

[2]Dillon, Gui *et al.* "Measurable Residual *FLT3* Internal Tandem Duplication Before Allogeneic Transplant for Acute Myeloid Leukemia." JAMA Oncol. 2024 Aug 1;10(8):1104-1110.

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