AML – *FLT3* ITD MRD Assay

Measurable Residual Disease Detection of *FLT3* ITD Mutations

Clinical Information

Measurable residual disease (MRD) detection in patients with leukemia has proven to be useful in the clinical management of disease and can facilitate the development of new therapies. Mutations in the fms-like tyrosine kinase 3 (*FLT3*) gene are the most prevalent mutations found in acute myeloid leukemia (AML)[1] and are characterized by an aggressive phenotype with a high prevalence of relapse. Internal tandem duplication (ITD) mutations within the juxtamembrane domain are the most common mutations of *FLT3*.[2] The development of a sensitive and specific assay for *FLT3* ITD mutations represents a significant advancement in guiding treatment decisions.

LabPMM's *FLT3* ITD MRD test is an NGS-based, targeted, deep-sequencing assay that detects ITDs ranging from 9 bp to 252 bp in size. Once a specific ITD (length and sequence) has been identified in a primary sample, it can easily be tracked in subsequent samples at a sensitivity of $5x10^{-5}$, provided sufficient DNA quantity is tested.

The treatment of AML has become a paradigm for precision medicine. This MRD assay is at least two orders of magnitude more sensitive than other commercially available *FLT3* assays. It detects the persistence of a driver mutation, *FLT3* ITD, in patients with no overt evidence of disease, allowing clinicians to identify those patients that can benefit from continuation or modification of treatment.[3]

MRD detection by Next-Generation Sequencing has demonstrated utility in predicting clinical outcomes and in generating clinically actionable results, allowing early intervention, confirmation of disease status prior to transplant, and increased confidence in remission status.

Workflow Overview

- **Sample Receipt** Peripheral blood, bone marrow aspirate, or DNA
- **NGS Testing, CLIA-Validated Assay** Sensitivity $5x10^{-5}$
- **Turnaround Time** 7 to 10 Business Days
- **Results Report** with Scientific Interpretation

Indications for Testing

- Post-treatment monitoring
- Stratify risk for disease recurrence
- Identify tumor-specific markers for post-treatment for monitoring

Interpretation

An interpretive report will be issued indicating whether *FLT3* ITD MRD was detected.

Specimen Requirements

- 1–3 mL of peripheral blood in EDTA
- 0.25–1 mL of bone marrow in EDTA
- 1 µg of previously isolated DNA

Turnaround Time

7 to 10 business days

Shipping Conditions

- Ambient or Cool; do not freeze **(peripheral blood or bone marrow)**
- Ambient or frozen on dry ice **(isolated DNA)**

Specimen Stability

2–8 °C up to 7 days **prior to testing**

CPT Codes

PLA Code: 0049U

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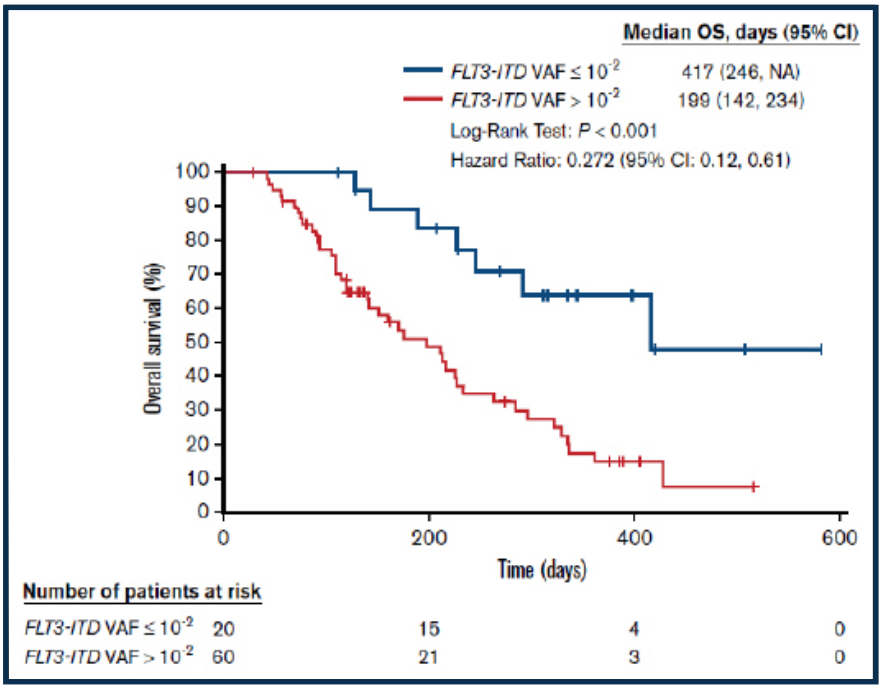

FIGURE 1: SUBJECTS, OVERALL SURVIVAL STRATIFIED BY MOLECULAR RESPONSE, USING THE INTERNATIONALLY-HARMONIZED *FLT3* ITD MRD ASSAY (10^{-2})

Molecular response	Achieved a molecular response: n	Achieved a molecular response: Median OS (95% CI), d	Did not achieve a molecular response: n	Did not achieve a molecular response: Median OS (95% CI), d	*P*
ITD VAF $\leq 10^{-2}$	20	417 (246–NA)	60	199 (142–234)	<.001
ITD VAF $\leq 10^{-3}$	18	417 (228–NA)	62	213 (143–264)	.003
ITD VAF $\leq 10^{-4}$ (MRD negative)	13	417 (228–NA)	67	213 (144–264)	.002

TABLE 1: SUBJECTS, OVERALL SURVIVAL

Comparison between patients achieving a molecular response (*FLT3* ITD VAF $\leq 10^{-2}$, $\leq 10^{-3}$, or negative as defined by ITD VAF $\leq 10^{-4}$) by the MRD assay and those not achieving a molecular response by the MRD assay. The P values were determined by the log-rank test.

FLT3 ITD MRD Assay as Predictor of Molecular Response

FLT3 ITD mutated patients enrolled in the CHRYSALIS study, who were treated with *FLT3*-inhibitory oral doses of 120mg/day or 200 mg/day gilteritinib, had their molecular response assessed from bone marrow aspirates obtained at baseline and at ≥1 additional time point. *FLT3* ITD and total *FLT3* alleles were quantified using the Invivoscribe *FLT3* ITD MRD assay and used to determine molecular response[3]. A Cox regression model of overall survival (OS) by Kaplan-Meier estimation was used to evaluate the impact of ITD variant allele frequency (VAF) on overall survival. Molecular response was defined as follows:

- Molecular response = ITD VAF (*FLT3* mutant reads: *FLT3* total reads) of $\leq 10^{-2}$ point.
- Major molecular response = ITD VAF of $\leq 10^{-3}$
- Negative MRD status = ITD VAF of $\leq 10^{-4}$

As shown in Table 1 and Figure 1, patients with molecular response had longer overall survival than those without a molecular response. This is the first demonstration of molecular response to a *FLT3* inhibitor in AML[3].

References

1. Ley, T. J. et al. (2013) *N Engl J Med.* 368: 2059–2074.
2. Konig, H. et al. (2015) *Expert Opin Ther Targets* 19:37-54.
3. Levis, M. J. et al. (2018) *Blood Advances*, 2: 825-831.

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