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FLT3 ITD MRD Assay

RUO Kit - Now Available for MiSeq™

Background

Stratifying acute myeloid leukemia (AML) disease according to molecular genetic alterations, such as those in the fms related tyrosine kinase 3 (*FLT3*) gene, aids in prognosis[1]. The most frequent and clinically significant type of *FLT3* mutation is an internal tandem duplication (ITD) in the juxtamembrane domain[2]. The *FLT3*-ITD mutation occurs in about 25% of newly diagnosed AML patients and is associated with an increased risk of relapse and lower overall survival rate[1]. Unlike flow cytometry assays which require fresh sample and are highly subjective, the *FLT3* ITD MRD Assay, a targeted, deep sequencing assay can be used with previously isolated DNA to detect ITD mutations at an allelic sensitivity level of $5x10^{-5}$.

Invivoscribe's *FLT3* ITD MRD Assay includes 24 unique dual-indices enabling the ability to multiplex multiple samples. This kit configuration provides laboratories the flexibility to scale testing for variable AML MRD research needs. To further simplify your workflow, our *FLT3* ITD MRD Software can easily be integrated into down-stream reporting systems and/or LIMS to automate data analysis.

Key Benefits

- **Bring MRD testing in-house for faster, cost-effective results**
- **Streamlined workflow reduces errors**
- **Flexibility to multiplex samples**
- **Use previously isolated DNA enabling sample batching**
- **Dockerized software enables highly portable, flexible and efficient sample analysis**
- **Standardized for international collaboration**

Principle of the Procedure

FLT3 ITD or length mutations are caused by duplication and insertion of a portion of the *FLT3* gene that includes the region in and around the juxtamembrane (JM) region. Next-generation sequencing of the PCR products is used to identify DNA sequences specific to previously identified mutations and estimate variant read frequencies (VRF). The *FLT3* ITD MRD Software provides an objective variant call in a TSV output file.

Workflow

01 DNA PREPARATION

02 PCR AMPLIFICATION (ITD) & PURIFICATION

03 NORMALIZING & MULTIPLEXING AMPLICONS

04 SEQUENCING ON THE ILLUMINA® MISEQ™ PLATFORM

05 *FLT3* ITD MRD SOFTWARE

06 OUTPUT FILE WITH VARIANT CALL

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ORDERING INFORMATION

Catalog #	Product	Quantity
14120019	*FLT3* ITD MRD Assay (MiSeq™)	96 reactions
14120029	*FLT3* ITD MRD Software (MiSeq™)	1 Dockerized Application

REAGENTS INCLUDED IN THE KIT

Controls	Quantity
FLT3 ITD Positive Control	500 µL tube x 2 each
FLT3 ITD Negative Control	500 µL tube x 2 each
Master Mixes	**Quantity**
FLT3 ITD Master Mixes	75 µL tube x 24 each

VAF READ DEPTH

FLT3 ITD mutated subjects enrolled in the CHRYSALIS study, who were treated with *FLT3*-inhibitory oral doses of 120mg/day or 200mg/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:

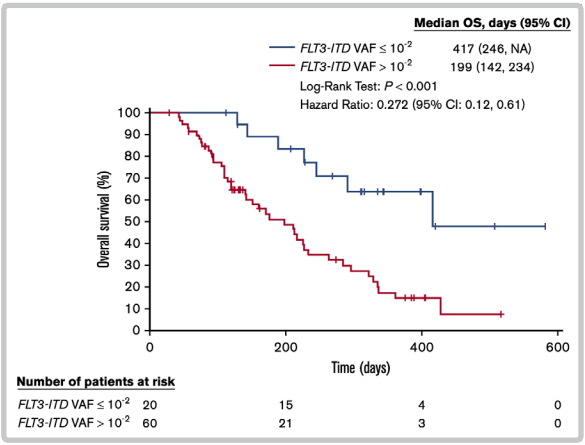
Molecular response = ITD VAF (*FLT3* mutant reads: *FLT3* total reads) of $\leq 10^{-2}$ point.

Major molecular response = ITD VAF of $\leq 10^{-3}$

Negative MRD status = ITD VAF of $\leq 10^{-4}$

As shown in Table 1.0 and Figure 1.0, subjects with molecular response had longer overall survival than those without a molecular response[3]. This demonstrates that when evaluating MRD, achieving a sensitivity > 10^{-2} may be unnecessary when evaluating VAF for OS.

Figure 1.0



Mark J. Levis, et al. (2018) *Blood Adv.* 2(8):825-831.

Table 1.0

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 1.0 Overall Survival of AML Subjects with *FLT3* ITD

Comparison between subjects achieving a molecular response (*FLT3* ITD VAF > 10^{-2}) 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.

Figure 1.0 Subjects, Overall Survival Stratified by Molecular Response, using the Internationally-Harmonized *FLT3* ITD MRD Assay.

REFERENCES

1. Naval Daver, et al. (2019) *Leukemia.* 33:299-312.
2. Heiko Konig, Mark Levis, (2015) *Expert Opin. Ther. Targets.* 19(1): 37-54.
3. Mark J. Levis, et al. (2018) *Blood Adv.* 2(8):825-831.

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