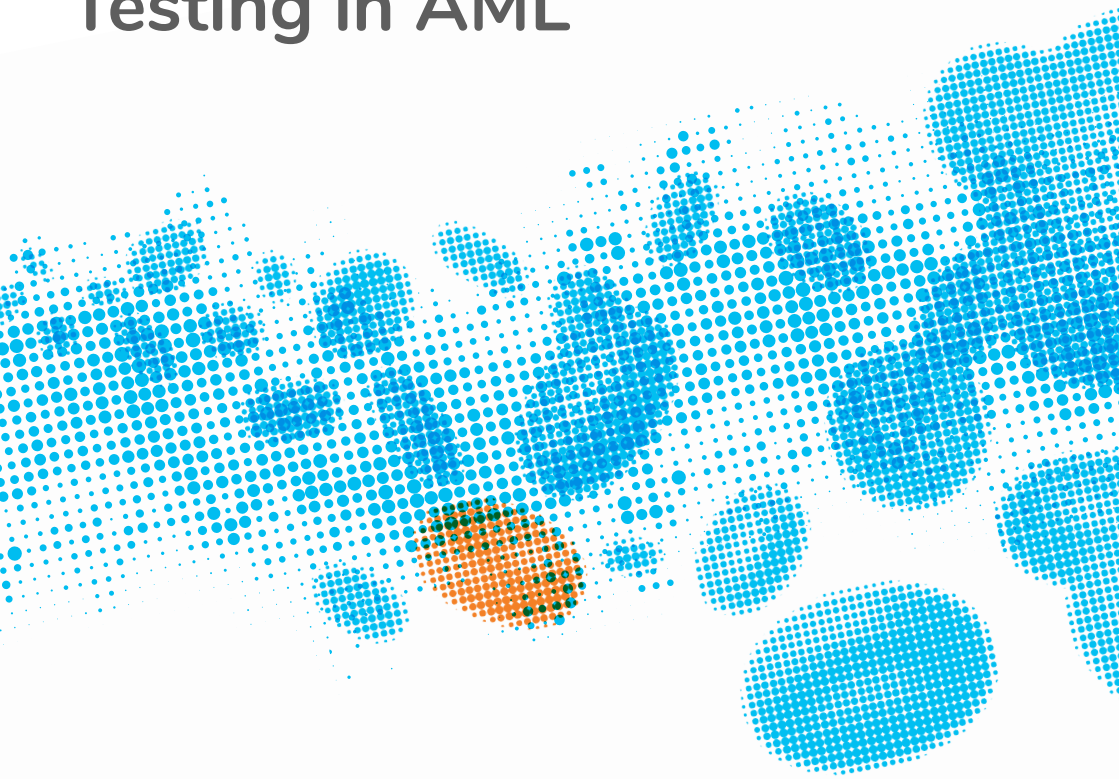
Prima
Cancer Genetics

MEDGENOME

FLT3

Testing in AML



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Introduction:

- FLT3-mutated AML is frequently found in patients with cytogenetically normal AML and predicts poor prognosis in these patients.
- FLT3 gene, which encodes a type II, transmembrane tyrosine kinase receptor, occur in approximately 30% of adult and 12% of paediatric patients with denovo AML. Recurrent oncogenic driver Mutations in FLT3 gene includes SNP and Insertions/Internal Tandem Duplications that constitutes 14% and 20-30% respectively.

FLT3-ITD

- FLT3-ITD is located in Exon 14-15 also called juxtamembrane domain. Inframe insertions in this domain leads to constitutive activation of FLT3 receptor, that leads to uncontrolled cellular proliferation, survival, and differentiation.
- These insertions consist of duplicated coding sequence derived from the juxtamembrane domain inserted in tandem.
- They are in-frame, range from 3 to <200 bp in length and result in a disruption of the autoinhibitory function of this domain.
- The prognostic impact of the ITD in FLT3 depends on the allelic ratio.

FLT3-TKD

- Mutations within the tyrosine kinase domain (TKD) are the second most common type of FLT3 mutation in AML, occurring in up to 14% of adult patients with AML.
- Mutations within the TKD are primarily point mutations within the activation loop, exon 20 (e.g. residues D835, I836, and Y842) of the TKD2 and, to a lesser extent, within the TKD1 (e.g. residues N676 and F691).
- Other point mutations and smaller insertions/deletions have also been identified within the TKD and other domains (e.g. extracellular and juxtamembrane domains), occurring in approximately 2% of patients with AML.
- The prognostic significance of FLT3-TKD mutations in the overall AML population and the impact of the FLT3-TKD mutations are still debatable

FLT3 inhibitors

- FDA approved Midostaurin for the treatment of adult patients with newly diagnosed AML with FLT3 mutation, in combination with standard cytarabine and daunorubicin induction and cytarabine consolidation [1].
- The outcomes of the RATIFY study showed that Midostaurin in combination with standard 7+3 chemotherapy in 717 patients with newly diagnosed AML (aged 18–59 years) with FLT3 mutations significantly improved event-free survival and overall survival [2].
- As per the findings of RATIFY trial, a signal ratio of >0.05, on fragment analysis is important for considering the patient's eligibility for Midostaurin [2]. FLT3-ITD wild type allelic burden will be calculated as the ratio of the area under the curve of mutant and wild-type alleles (FLT3-ITD/FLT3-wild) [3].

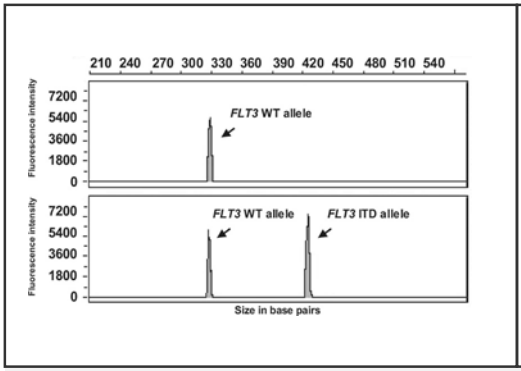

Fig 1: FLT3-ITD detection by fragment analysis

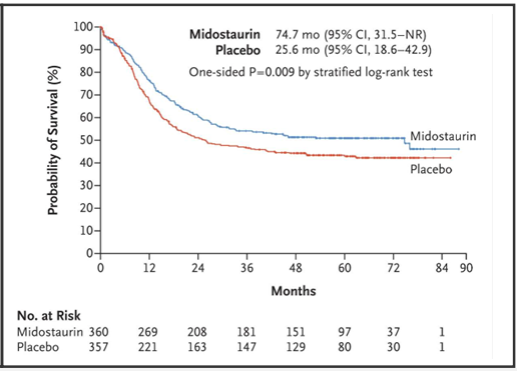

Fig 2: Kaplan-Meier curve for overall survival between Midostaurin and placebo arms [Stone, Richard M., et al. "Midostaurin plus chemotherapy for acute myeloid leukemia with a FLT3 mutation." New England Journal of Medicine 377.5 (2017): 454-464]

- FDA has recently approved Gilteritinib for adult patients who have relapsed or refractory AML with a FLT3 mutation based on a study (NCT02421939) on 252 adults with relapsed or refractory AML [4, 5].

Advantages of NGS in detecting FLT3 mutation evolution and MRD:

- NGS-based Minimal Residual Disease (MRD) is widely applicable to AML patients, highly predictive of relapse and survival, and help refining transplant and posttransplant management in AML patients.
- FLT3-ITDs are clinically useful biomarker for MRD monitoring, particularly post Allo-HSCT or during the course of induction chemotherapy.
- Other clinically useful applications of NGS for FLT3-ITD include:
  - Mutational shifts between diagnosis and relapse
  - Multiclonality at presentation
  - Outgrowth of a clone at relapse different from that dominant at diagnosis
  - Variable insertion sites and lengths
  - Assess clonal dominance during the course of the disease
- Multiple measurable parameters of FLT3-ITD that includes: insertion site, insertion length, number of individual clones, and allelic ratio of insertions can be detected by NGS in a single analysis with a high sensitivity to a Limit of Detection as low as 1%.

MedGenome offers	Test Code	Assay platform	Sample requirements	TAT
FLT3-ITD mutant allele burden analysis	MGM1018	Fragment analysis	 Peripheral blood OR	7 working days
FLT3 gene analysis (includes ITD and D835) NPM1 gene analysis CEBPA gene sequencing	MGM557	Fragment analysis & Sanger sequencing		14 working days
AML Basic panel [PML/Rara, bcr/abl, AML/ETO, FLT3, NPM1, Inv16]	MGM1498	RT-PCR		5 working days
[AML Advance panel PML/Rara, bcr/abl, AML/ETO, FLT3, NPM1, Inv16, C-KIT]	MGM1499	RT-PCR	 Bone marrow aspirate OR	5 working days
[Combo] FLT3 gene internal tandem duplication analysis and D835 point mutation analysis	MGM1009	Fragment analysis & Sanger sequencing		14 working days
FLT3 D835 point mutation analysis	MGM193	Sanger sequencing	 Purified genomic DNA	14 working days
[Combo] AML risk stratification gene panel and FLT3 gene internal tandem duplication analysis	MGM1453	Fragment analysis & NGS		14 working days

References:

1. Medinger M, Lengerke C, Passweg J. Novel Prognostic and Therapeutic Mutations in Acute Myeloid Leukemia. Cancer Genomics Proteomics. 2016 09-10;13(5):317-29.Review.
2. Stone, Richard M., et al. "The addition of midostaurin to standard chemotherapy decreases cumulative incidence of relapse (CIR) in the international prospective randomized, placebo-controlled, double-blind trial (CALGB 10603/RATIFY [Alliance]) for newly diagnosed acute myeloid leukemia (AML) patients with FLT3 mutations." (2017): 2580-2580.
3. Oran, Betül, et al. "Allogeneic Transplantation in First Remission Improves Outcomes Irrespective of FLT3-ITD Allelic Ratio in FLT3-ITD–Positive Acute Myelogenous Leukemia." Biology of Blood and Marrow Transplantation 22.7 (2016): 1218-1226.
4. https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm627045.htm
5. Perl, Alexander E., et al. "Selective inhibition of FLT3 by gilteritinib in relapsed or refractory acute myeloid leukaemia: a multicentre, first-in-human, open-label, phase 1–2 study." The Lancet Oncology 18.8 (2017): 1061-1075.

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