Acute Myeloid Leukemia (AML)

Order GTC Hematology Profile (for Fusions, Order Plus)

AML is heterogenous disease. The underlying molecular abnormalities in AML can determine the clinical course, prognosis and therapy strategy. While cytogenetic studies remain the corner stone for any molecular evaluation of AML, molecular studies are essential for proper characterization of the disease. Most common translocations, such as t(15;17), t(8;21) and inv(16) are detected by conventional cytogenetic studies, but some cryptic gene fusions can be missed, for example NUP98-NSD1, CBFA2T3-GLIS2 and MNX1-ETV6. In addition, some t(15;17) and Inv(16) can also be missed by conventional cytogenetic studies when rearrangements are complex. Next generation sequencing can detect these fusion transcripts and can be performed on FFPE tissue. More importantly, thorough NGS testing is essential to provide information on mutations (indels and SNV) that are necessary for subclassification of AML, particularly in cases with intermediate-risk cytogenetics. Structural copy number variations, such as del(5q) and monosomy can also be detected by NGS testing, but the reliability of this detection depends on the percentage of the leukemic cells in the tested sample.

Determining the molecular abnormalities in myeloid neoplasms can help distinguishing advanced MDS from de novo AML and from secondary AML. The presence of FLT3 or NPM1 mutations is a strong indication of AML disease irrespective of blast count. Similary, the presence of mutations in genes typically mutated in MDS (spliceosome genes and ASXL1) is an indication of secondary AML arising on a background of MDS. The presence of TP53 mutation is typically associated with complex cytogenetic abnormalities. One of the most important information that NGS testing can provide is the level of intraclonal heterogeneity. This is becoming more important as more targeted therapy is being developed for treating AML. When a targeted mutation is present in a subclone, therapy strategy should consider that targeting a subclone may not eradicate the disease. Relapse should always be checked for the selection for specific mutations or the development of new additional mutations. Below are the most commonly mutated genes in AML based on our database. Structural abnormalities and translocations are not discussed:

**FLT3**

Frequency: 34%

Prognosis: Adverse

Action: FLT3 tyrosine kinase inhibitors: midostaurin (approved), sorafenib, quizartinib, crenolanib, gilteritinib, lestaurtinib, ponatinib.

Most (27%) of FLT3 mutations are ITD (internal Tandem Repeat) and approximately 7% are SNV in the tyrosine kinase domain (TKD). Some studies suggest that TKD mutations are not associated with more aggressive disease as the case with ITD. The presence of bi-allelic mutations in FLT3 is an indication of significantly more aggressive disease. The recent NCCN and ELN classification recommend distinguishing between low and high FLT3 mutation. This should be interpreted with caution and depends on the percentage of the leukemic cells in the analyzed sample, determining if it is in a subclone or biallelic. Frequently (44%) FLT3 mutations are associated with NPM1 mutations and this modify the prognosis into significantly less aggressive. FLT3 mutations when detected in core-binding factor leukemia may not affect prognosis significantly.

**NPM1**

Frequency: 34%

Prognosis: Favorable

Action: No specific therapy, high dose of Daunorubicin was reported to be effective.

The presence of NPM1 mutation is associated with significantly good prognosis similar to that seen in corebinding factor AML. However, frequently (50%), NPM1 mutations are associated with FLT3 mutations and this modify the prognosis into more aggressive disease.

**RUNX1**

Frequency: 6%

Prognosis: Adverse

Action: RUNX1 Inhibitors

RUNX1 is a transcription factor, also called core-binding factor subunit alpha-2 (CBFA2).

**ΚΙΤ**

Frequency: 4%

Prognosis: Adverse

Action: TKIs: imatinib, dasatinib, ponatinib, sorafenib, sunitinib, quizartinib

KIT mutations are more common in AML with Inv(16) and t(8;21) and associated with more aggressive disease.

**CEBPA**

Frequency: 10% (double mutation: 3%)

Prognosis: Neutral for single mutation and favorable for double mutations

Action: No targeted therapy

Single mutation in CEBPA may not have any impact on prognosis, but double (bi-allelic) mutations are strongly associated with better prognosis and better response to therapy).

**WT1**

Frequency: 9%

Prognosis: Adverse

Action: No targeted therapy

WT1 expression has been reported to be significantly higher in AML cell as compared with normal BM cells. CAR-T cell and vaccines targeting WT1 are being developed.

**TET2**

Frequency: 8%

Prognosis: Neutral (some studies suggest poor prognosis)

Action: Hypomethylating agents, Vitamin C may activate TET2 function

Most of TET2 mutations lead to termination and loss of function and expression. Loss of function in TET2 leads to DNA hypermethylation and silencing the expression of important genes.

**DNMT3A**

Frequency: 20%

Prognosis: Adverse

Action: Hypomethylating agents

Mutations in DNMT3A lead to significant hypermethylation.

**ASXL1**

Frequency: 5%

Prognosis: Adverse

Action: bromodomain and extraterminal (BET) Inhibitors

ASXL1 gen is involved in chromatin modification. ASXL1 mutations can be gain of function or truncation and perhaps loss of function. lead to loss of function and significant hypermethylation.

**SRSF2**

Frequency: 2%

Prognosis: Adverse

Action: Spliceosome modifiers (H3B-8800, sudemycins, spliceostatin A, and meayamycin)

SRSF2 gene is involved in RNA splicing.

**SF3B1**

Frequency: 1 2%

Prognosis: Favorable

Action: Spliceosome modifiers (H3B-8800, sudemycins, spliceostatin A, and meayamycin)

SF3B1 gene is involved in RNA splicing and almost always associated with ring sideroblasts. When detected with JAK2, CALR or MPL, MDS/MPN-RARS-T should be considered.

**TP53**

Frequency: 8%

Prognosis: Adverse

Action: “MDM2 inhibitors, Hypomethylation agents, Wee1 inhibitors, Chk1 inhibitors, kevetrin, APR-246, nutlins, gene therapy"

TP53 mutations imply defect checkpoint/cell cycle mechanism and indicate very aggressive disease even between patients within poor-risk cytogenetic group.

**NRAS**

Frequency: 10%

Prognosis: Neutral

Action: MEK Inhibitors

Presence of NRAS mutation is an indication of monocytic lineage involvement and acute myelomonocytic or monocytic leukemia rather than pure AML.

**KRAS**

Frequency: 5%

Prognosis: Neutral

Action: MEK Inhibitors

Presence of KRAS mutation is an indication of monocytic lineage involvement and acute myelomonocytic or monocytic leukemia rather than pure AML.

**U2AF1**

Frequency: 2%

Prognosis: Adverse

Action: Spliceosome modifiers (H3B-8800, sudemycins, spliceostatin A, and meayamycin)

U2AF1 is involved in RNA splicing

**IDH2**

Frequency: 10%

Prognosis: Neutral

Action: IDH2 inhibitors: Enasidenib (approved), AG-881

IDH2 is involved in DNA methylation

**PHF6**

Frequency: 3%

Prognosis: Adverse

Action: No targeted therapy

PHF6 is a transcription repressor binds to double stranded DNA.

**SETBP1**

Frequency: 5%

Prognosis: Adverse

Action: No targeted therapy

SETBP1 protein is a nuclear oncoprotein forms a complex involved in cell proliferation.

**ZRSR2**

Frequency: 5%

Prognosis: Adverse

Action: Spliceosome modifiers (H3B-8800, sudemycins, spliceostatin A, and meayamycin)

ZRSR2 is located on X chromosome and more frequently mutated in males.

**IDH1**

Frequency: 4%

Prognosis: Neutral

Action: IDH1 inhibitors: Ivosidenib (approved), IDH-305, BAY1436032, FT-2102, AG-881 IDH1 mutations lead to hypermethylation.

Specimen Requirements:

-Liquid Biopsy: 5 mL. Peripheral blood in EDTA tube.

-Bone Marrow Cells: 2 mL. EDTA tube.

-Peripheral Blood Cells: 5 mL. EDTA tube.

-FFPE: 1 H&E slide and 6-8 unstained slides, 5-7 microns of BM clot or tissue

Shipping:

Ship using cold pack. The cold pack should not directly contact Blood or BM specimen. Ship As soon as sample collected with overnight delivery.

Turn Around Time:

5-7 days