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*Review Article*

# Acute myeloid leukemia: novel mutations and their clinical implications

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**Abstract:** Acute myeloid leukemia (AML) is a heterogenous and challenging hematological malignancy with suboptimal outcomes. The implications of advanced technologies in the genetic characterization of AML have enhanced the understanding of individualized patient risk, which has also led to the development of new therapeutic strategies. A comprehensive study of novel mutations is essential to moderate the complicacies in patient management and achieve optimal outcomes in AML. In this review, we summarized the clinical relevance of important novel mutations, including *TET2, ETV6*, *SATB1*, *EZH2*, *PTPN11*, and *U2AF1*, which impact the prognosis of AML. *TET2* mutation can lead to DNA hypermethylation, and gene fusion, and mutation in *ETV6* disrupts hematopoietic transcription machinery, *SATB1* downregulation aggravates the disease, and *EZH2* mutation confers resistance to chemotherapy. *PTPN11* mutation influences the RAS-MAPK signaling pathway, and *U2AF1* alters the splicing of downstream mRNA. The systemic influence of these mutations has adverse consequences. Therefore, extensive research on novel mutations and their mechanism of action in the pathogenesis of AML is vital. This study lays out the perspective of expanding the apprehension about AML and novel drug targets. The combination of advanced genetic techniques, risk stratification, ongoing improvements, and innovations in treatment strategy will undoubtedly lead to improved survival outcomes in AML.

**Keywords:** Acute myeloid leukemia, genetic mutations, next-generation sequencing, risk-stratification, targeted therapy, survival

## Introduction

Acute myeloid leukemia is a myeloid dysplastic malignancy of hematopoietic stem cells. This disease is presented in children and adults with considerable diversity in molecular pathogenesis and disease outcomes [1]. Cytogenetic abnormalities are more common in pediatric AML (p-AML) than in adult AML, with some of them appearing exclusively in newborns and young children. Additionally, there are significant differences between the epigenetic landscapes of pediatric and adult AML in terms of the frequency and type of mutations in epigenetic modulators [2]. Despite the great degree of heterogeneity, many gene fusions and point mutations are recurrent and have been employed in risk stratification for the past three decades. However, the implication of advanced techniques like karyotyping, chromosomal microarray analysis (CMA), and next-generation sequencing (NGS) has revealed newer mutations, which have diagnostic and prognostic significance.

Acute myeloid leukemia constitutes 15-20% of childhood leukemia and approximately 35% of adult leukemia [3]. The survival rate of AML remains dismal, and there is insufficient understanding of the basis of poor outcomes in AML. However, chromosomal abnormalities like AML-ETO t(8;21), PML-RARA t(15;17), CBFB-MYH11 t(16;16), *FLT3*-ITD and mutations in *CEBPA*, *KIT*, *NPM1,* and *ASXL*1 are commonly reported in p-AML and is used in risk stratification on the basis of overall survival and relapse rate. The implication of advanced molecular techniques in diagnosis has revealed new mutations that may have prognostic value.

**Table 1.** Genetic mutations in acute myeloid leukemia (AML)

| Common mutations | Incidence | | References | Novel mutations | Incidence | | References |
|---|---|---|---|---|---|---|---|
| | Adult | Pediatric | | | Adult | Pediatric | |
| *NPM1* | 35% | 8-10% | [13] | *CTNNB1* | 22% | 1.8% | [14, 15] |
| *DNMT3A* | 20% | 2.1% | [16, 17] | *SRSF2* | 12.5% | <1% | [18], TARGET |
| *RUNX1* | 10-15% | ~2.8% | [5, 19] | *PTPN11* | 4% | 6.9% | [20, 21] |
| *CEBPA* | ~10% | 18% | [5, 22] | *PHF6* | 3% | 2% | [23, 18] |
| *TP53* | 10% | 2.1% | [24, 25] | *U2AF1* | 3.4% | <1% | [26], TARGET |
| *GATA2* | ~5% | 2.6% | [5] | *ETV6* | 1.35% | 2.2% | [27, 28] |

Recently, there have been significant advancements in the diagnostics of AML and myelodysplastic syndromes (MDS), including the integration of NGS strategies into establishing diagnostic algorithms, classification and risk stratification systems, and detection of minimal residual disease (MRD). With the availability of more specific treatments for AML (such as FLT3 or IDH1/IDH2 inhibitors), prompt and thorough genetic mutation screening has become a necessary practice [4].

## Genetics and mutations in AML

Traditionally, classifying patients with AML into favorable, intermediate, and adverse risk categories entail cytogenetic markers. However, due to the complexity of the disease genetics in patients with normal karyotypes, risk stratification and treatment decision become difficult. Hence high-throughput techniques are implied to identify clinically relevant mutations [5]. The two-hit mutation model proposed by Gilliland and Griffin in 2001 classifies the key oncogenic mutations [6]. It is hypothesized that AML, is a repercussion of a collaboration between at least two broad classes of mutations, wherein Class I mutations confer proliferative, and survival advantages and Class II mutations affect the processes of cell differentiation and apoptosis. Some mutations, mainly epigenetic modifiers, are not identified in these two classes. World Health Organization (WHO), proposed a new classification which uses clinical, morphological, and genetic features to classify different subgroups: AML with recurrent genetic abnormalities, AML with myelodysplastic-related changes, AML therapy-related myeloid neoplasms, and AML without any other specification, which generally is classified on the basis of the FAB [7].

Some of the primarily mutated genes are *KIT, FLT3, NPM1, CEBPA, RAS, WT1, BAALC, ERG, MN1, DNMT, TET2, IDH, ASXL1, PTPN11* and *CBL*. Amongst these, *FLT3, NPM1,* and *CEBPα* genes are well studied to be associated with treatment response and disease progression (**Table 1**) [8-10]. Epigenetic modifiers include DNA methyltransferases: *DNMT1, DNMT3A* and *DNMT3B*; methylcytosine dioxygenase: *TET1, TET2* and *TET3* convert 5mC to 5-hydroxymethylcytosine (5hmC) [11, 12].

## Profound mutations in AML

The advent of techniques and their implication in clinical diagnostics has led to the revelation of the most common mutations causing the disease. Risk stratification and estimation of drug response are dependent on these established genetic factors. The favorable group comprises mutations in *NPM1* (30% in adults) [29] and *CEBPA* (10%) [30]. Mutations in *ASXl1* (6.5%) and *TP53* (8%) [31] present with poor prognosis [32]. A few other mutations, such as *PTPN11*, *NRAS*, *KRAS*, *NF1*, *GATA2*, *TET2,* and *DNMT3A,* are frequently observed in AML, but the prognosis of these mutations is still undefined [5]. The recent guidelines by European LeukemiaNet (ELN), 2022 have included mutations in *BCOR, EZH2, SF3B1, SRSF2, U2AF1,* and/or *ZRSR2* under the category of adverse risk (**Table 2**).

The development of disease and its response to the therapy is a repercussion of the altered gene function and its following molecular pathway. Various molecular pathways play a crucial role in maintaining cellular processes; any aberration in these pathways leads to the dysregulation of cellular differentiation and division, ultimately causing malignancy like AML [33]. Signal transducers, epigenetic modifiers, transcription factors, splicing factors, and cohesion complexes are directly or indirectly involved in the disease pathogenesis [34]. A profound understanding of the disease is re-

**Table 2.** Characterization of recurrent genetic mutations according to the recommendation by ELN, 2022

| Risk group | Mutations |
|---|---|
| Favourable | • Mutated *NPM1*<br>• bZIP in-frame mutated *CEBPA* |
| Intermediate | • Mutated *NPM1* with *FLT3*-ITD<br>• Wild-type *NPM1* with *FLT3*-ITD (without adverse-risk genetic lesions)<br>• Cytogenetic and/or molecular abnormalities not classified as favorable or adverse |
| Adverse | • Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2*, *U2AF1*, and/or *ZRSR2* (in absence of favourable risk subtype)<br>• Mutated *TP53* |

quired to improve the prognosis of AML, which necessitates the further elaboration of consequences linked to mutations with lower prevalence and undefined significance.

In this study, we have performed a comprehensive survey of the literature available on AML (pediatric and adults). We have also, utilized the TARGET database for sequencing data of pediatric patients. Several unexplored and uncategorized mutations were recognized, which lacked prognosis analysis. *MYO18, PHF6, SRSF2, CTNNB1, CCND2, EPOR, SF3B1, SMC3,* and *SETB1* are genes reported in various research studies prevailing in patients of AML. The nature of this disease is heterogenous with high mortality and relapse rate. Despite the availability of risk stratification criteria, the disease outcome remains variable primarily for the intermediate risk class, which is a poorly understood heterogeneous group. Hence, the need for understanding uncommon recurrent mutations is indispensable. Here, we have presented a comprehensive review of such potential mutations, which may have ramifications for improving clinical practices and finding novel drug targets.

## Current diagnostic and treatment strategies for AML

The diagnosis of AML is confirmed by the presence of ≥10% blast cells in peripheral blood or bone marrow with the presence of most common cell surface markers CD33, CD34, CD13, and HLA-DR and recurrent genetic abnormality. Core binding factor (CBF) AML is the most common subtype of AML as t(8;21) (*RUNX1-RUNX1T1*) and inv(16) (*CBFB-MYH11*) constitutes 25% of pediatric AML and 15% adult AML cases [35]. Other cytogenetic abnormalities such as t(6;9) (*DEK-NUP214*) and t(9;22) (*BCR-ABL*) have relatively lower incidence rates but are associated with poor outcomes of the disease (**Figure 1**). The genetic aberration in *RUNX1, ASXL1,* and *TP53* are associated with adverse risk [32].

The therapy of AML includes a combination of daunorubicin and cytosine arabinoside (7+3) or cytosine arabinoside, daunorubicin, and etoposide (10+3+5) chemotherapeutics. Acute promyelocytic leukemia (APML) comprises 5-10% of AML cases demarked by the presence of t(15;17) and is treated with all-trans-retinoic acid (ATRA) and arsenic trioxide with a high remission rate (80-90%) [36]. Although these treatment approaches have the potential to reduce the burden of leukemia, the disease's prognosis is still not optimal because of poor tolerance, a higher risk of induction mortality in patients with concomitant conditions, unfavorable cytogenetics, and molecular mutations. The prognosis of AML varies with age, as adults typically have a worse outcome as compared to pediatric AML. The OS in adults is suboptimal at 40-45%. Whereas, the pediatric population has a better OS and EFS (70-75% and 60-65%, respectively). Complete remission (CR) rates in paediatric AML are 85-90% and 60-70% in adults. The relapse rates within three years account for 40% of pediatric AML and 60% of adult AML [37-39].

The FDA's recent approval of therapeutics in 2017-18 encouraged the development of novel targeted compounds with therapeutic potential. AML with an FMS-like tyrosine kinase 3 (FLT3) mutation that was newly diagnosed has been demonstrated to respond effectively to the multi-kinase inhibitor midostaurin. It cannot be overstated how important Smoothened (SMO), a component of the hedgehog (Hh) signaling system, is for the growth, and mainte-

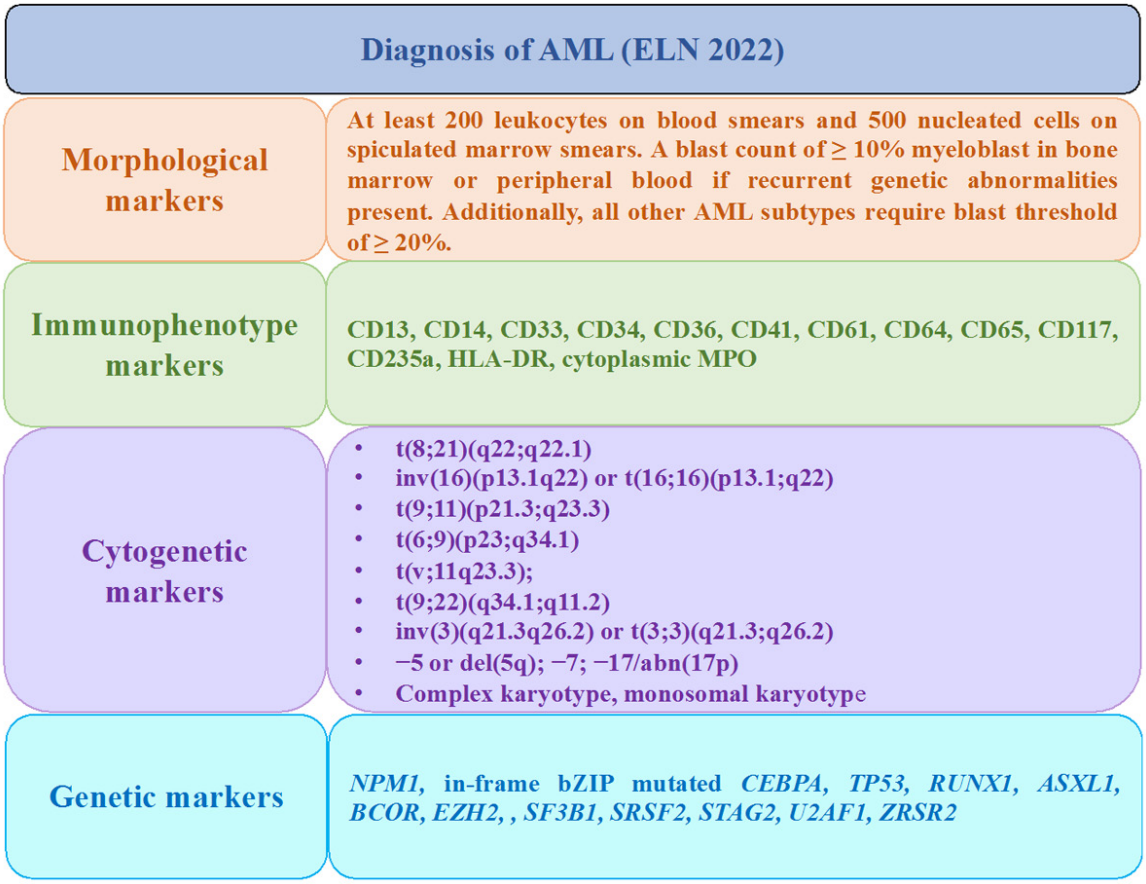


**Figure 1.** AML detection markers recommended by European LeukemiaNet (ELN), 2022.

nance of leukemic stem cells (LSC). LSCs offer resistance to chemotherapy and raise the likelihood of relapse. Glasdegib effectively targets this pathway to increase the survival outcome [40].

### Mutations with unknown significance

It has been summarised in **Table 3**.

*TET2*

TET2 protein catalyzes the conversion of 5-methylcytosine to 5-hydroxymethylcytosine and somatic loss of mutations in the Ten-Eleven Translocation (*TET2*) gene supposedly alter hematopoietic stem cell functions and development by epigenetic modifications [41, 42]. *TET2* mutation is observed in 23% of patients, and according to the European LeukemiaNet (ELN) classification of AML, *TET2* mutations are associated with a favorable-risk group but inferior outcome [43]. Figueroa et al. (2010) [44] and Dang et al. (2009) [45] described the mutual exclusion of *TET2* mutations with the mutation in *IDH1* and *IDH2*. Contextually, a mutation in *IDH1* deregulates the conversion of isocitrate to α-ketoglutarate; instead, NADPH-dependent reduction of α-ketoglutarate to 2-hydroxyglutarate is catalyzed, which inhibits TET2 activity [44-46]. Exon 3 and exon 11 are reported to be more labile to mutations in AML patients. In contrast, the density of mutations is highest in exon 5-9 [41], and it leads to overall DNA hypermethylation in the enhancer region [47].

*ETV6*

*ETV6* or *TEL* encodes for transcription repressor and has been reported to be a gene fusion partner with more than 30 translocation oncogenes [48]. It has a crucial role in embryonic development and hematopoiesis [49]. *ETV6* has three domains: C-terminal DNA binding (ETS) domain, central regulatory domain, and N-terminal pointed (PNT) domain which provides oligomerization motif for fusion with other partners, especially with kinases and leads to

**Table 3.** Novel mutations and their association with prognosis in AML

| Study (references) | Functional category | Novel mutations | Study Population | EFS (HR) | OS (HR) | Overall Prognosis |
|---|---|---|---|---|---|---|
| Wang et al., (2019) [96]; Langemeijer et al., (2010) [97]; Kaburagi et al., (2019) [98] | Methylcytosine dioxygenase | *TET2* | Adult | HR: 1.594 (P=0.002) | HR: 1.386 (P<0.001) | Poor |
| | | | Pediatric | 54.5% P=0.907 | 77.9% vs. 75.9% P=.688 | Poor |
| Haferlatch et al., (2012) [99] | Transcription Factor | *ETV6* | Adult | 4.0 vs. 15.4 months | 26.3 vs. 62.2 months | Poor |
| | | | Pediatric | - | - | |
| | Wnt signaling Pathway, cell adherens junctions | *CTNNB1* | | | P<0.05 | Poor |
| | Chromatin remodeling factor | *SATB1* | | | | Poor |
| Stasik et al., 2021 [21]; Loh et al., 2004 [100] | Signal transducer (RAS/MAPK pathway) | *PTPN11* | Adult | HR: 1.52; P=0.013 | HR: 1.75; P<0.001 | Poor |
| | | | Pediatric | No change | No change | |
| Ohgami et al., (2015) [42]; Li et al., [101] | Splicing factor | *U2AF1* | Adult | P<0.0001 | Median 3 months vs. 7 months | Poor |
| | | | Pediatric | - | - | |
| Patel et al., (2012) [93] | Transcription Factor | *PHF6* | Adult | P=0.006 | | Poor |

constitutive activation of gene fusion transcription factor [50]. Doorn-Khosrovani et al. described that despite the presence of wild-type *ETV6*, low protein level was transcribed with no relation to mRNA expression level [51]. In *de novo* pediatric AML, 2.2% of patients had mutations altering ETV6 amino acid sequence, deletion was observed in 1.5% and 9.2% of patients had *MNX1/ETV6* translocation. *MNX1-ETV6* t(7;12)(q36;p13) translocation is reported to be associated with poor prognosis and is enriched in the infantile group [28]. *ETV6* aberrations result in poor outcomes of disease in children and a higher risk of relapse [52].

*CTNNB1*

*CTNNB1,* which encodes for β-catenin, is a central role player in cell development and defining differentiation fate during embryogenesis through the Wnt signalling pathway and cell-cell adhesion [53, 54]. Wnt signalling pathway is primarily involved in the regulation of developmental processes, cell growth, and differentiation [55, 56], which can be canonical (β-catenin dependent) or non-canonical (β-catenin independent). Upon activation of the canonical Wnt pathway, β-catenin is hypophosphorylated and stabilized. The canonical pathway is shown to lead to hematopoiesis failure associated with loss in a commitment of myeloid lineage at the granulocyte-macrophage progenitor stage (**Figure 2**); moreover, it blocks erythrocyte differentiation and lymphoid development facilitating loss of repopulating stem cell activity hence causing hematopoietic crisis [57, 58]. β-catenin is evidently linked to clonogenicity of blast cells in AML [59]. Quantitative analysis of CTNNB1 mRNA depicted up to 100-fold upregulation in AML patients which is highly suggestive of its role in disease-related myeloproliferation [53]. In the Chinese population, lower overall survival was observed with high CTNNB1 expression [57]. According to TARGET, deep deletion in CTNNB1 is prevalent in 1.8% of the pediatric population. Griffiths et al. showed β-catenin as an independent prognostic factor and indicator of poor event-free survival (EFS) and OS [15].

*SATB1*

Special AT-rich sequence-binding protein 1 (SATB1), is a chromatin remodeller which modulates gene expression in different types of cancer. Upregulation of *SATB1* is reported to be linked with the progression of the tumor, metastatic potential, and poor prognosis. Luo et al., identified reduced expression of *SATB1* in AML patients which consecutively the expression increased in CR ($P$=0.03), indicative of its role in disease progression and potential as a biomarker for drug response. In this study, adult AML patients (n=52) were categorized into $SATB1_{high}$ and $SATB1_{low}$, with CR rates of 76.5% and 56.1%, respectively. Additionally, they suggested SATB1 regulates various genes involved in hematopoietic cell differentiation and development [60]. There are studies that suggest the role of *SATB1* as a regulator of lymphocyte differentiation and additionally, its contribution is predicted to regulate Wnt signalling genes through interaction with β-catenin [60]. Bachas et al., reported *SATB1* deregulation in AML relapse (95.7%; 22 out of 23 cases) [61].

*PTPN11*

The tyrosine-protein phosphatase nonreceptor type 11 (*PTPN11*) has two N-terminal Src homology 2 (SH2) domains, a protein tyrosine phosphatase (PTP) catalytic domain, and a COOH terminus [62]. It encodes for cytoplasmic phosphatase SHP2, which is a crucial role player in cell growth, and cell differentiation and serves as a signaling component to positively regulate RAS/MAPK signaling pathways [63, 64]. RAS signaling molecules are frequently observed to be mutated with AML. In a study by Stasik et al., mutation analysis using NGS revealed *PTPN11* mutations in 106 of 1529 (6.93%) patients (median VAF: 24%) in dominant (36%) and subclonal (64%) configuration. As per their study, *PTPN11* mutations presented with *NPM1* (63%), *DNMT3A* (37%), and *NRAS* (21%) and had a higher rate of co-occurrence of favorable cytogenetics (57.8% vs. 39.1%; $P$<0.001) with adverse effect in patients with subclonal *PTPN11* mutations (HR: 2.28; $P$< 0.001) but not found with dominant *PTPN11* mutations (HR: 1.07; $P$=0.775). Patients with *PTPN11* mutations had poor OS (HR: 1.75; $P$<0.001), relapse-free survival (RFS) (HR: 1.52; $P$=0.013), and a lower rate of CR (odds ratio: 0.46; $P$=0.008) [21]. Somatic gain-of-function mutations in N-SH2/PTP domains prevent the autoregulation of SHP2 catalytic activity during leukemogenesis. Upregulation of SHP2 was found to cause leukemic transformation by increasing hematopoietic progenitor cells' sen-

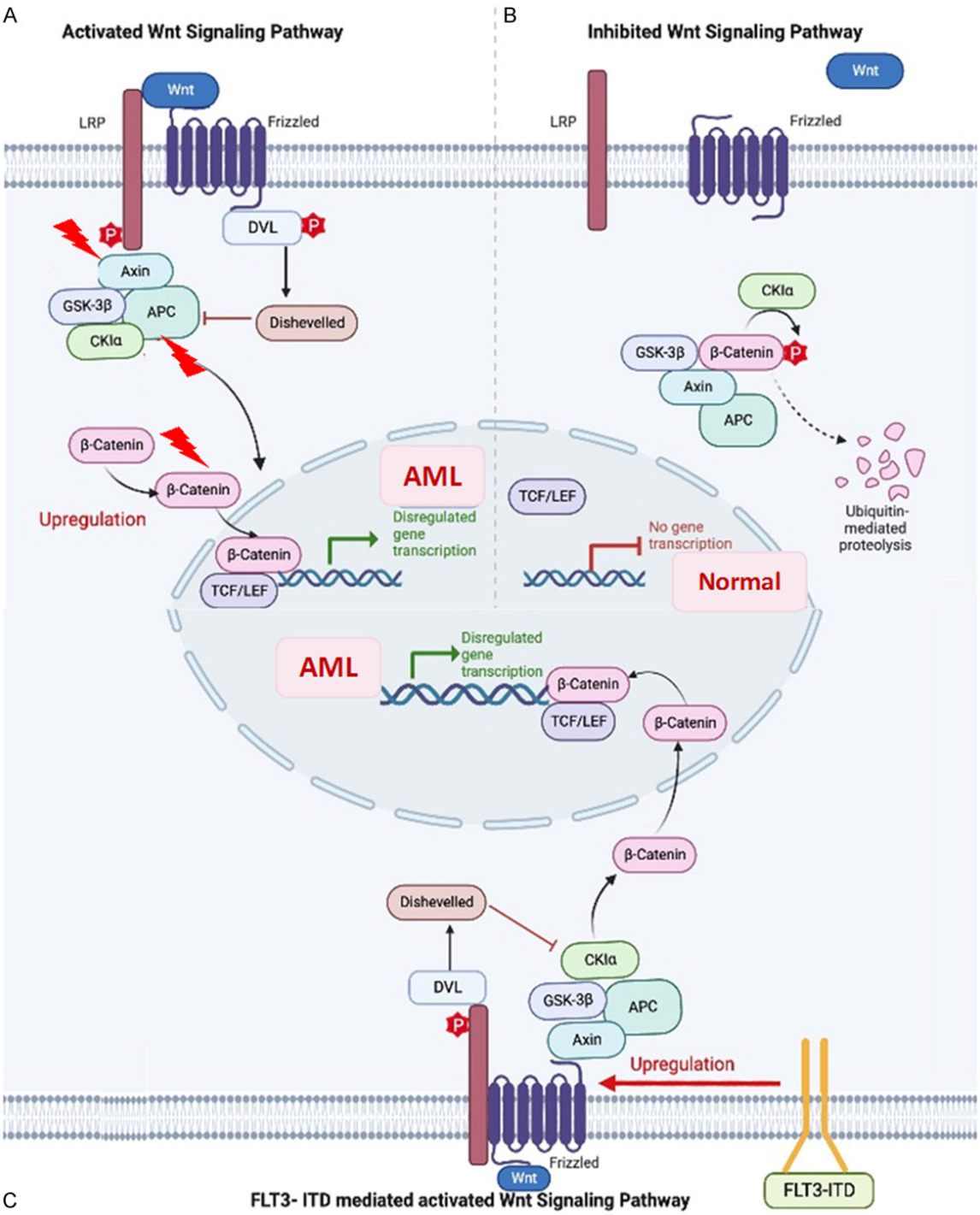


**Figure 2.** β-catenin stabilization mechanism leading to abnormal hematopoietic stem cell (HSC) proliferation. A. Wnt binding to Frizzeled (fzd) receptors and with Lipoprotein receptor-related proteins 5/6 (LRP5/6) co-receptors, activates the signalling pathway leading to the stabilization of β-catenin additionally, the mutations in Axin, adenomatous polyposis coli (APC), and β-catenin genes can impair the downregulation of β-catenin which leads to dysregulated gene transcription and abnormal HSC differentiation. B. Normal gene transcription by phosphorylation of β-catenin and its subsequent ubiquitin-mediated proteolysis. C. Pathway of AML pathogenesis, in the presence of FLT3-ITD (Internal Tandem Duplication) mutations induced FZD expression and increased β-catenin nuclear localization.

sitivity to granulocyte-macrophage colony-stimulating factor (GM-CSF) and the RAS signaling axis [65-67]. Patients with juvenile myelomonocytic leukemia (JMML) frequently have somatic PTPN11 mutations, and certain other hematologic malignancies have also been associated with this mutation [68] but its association with pediatric AML is not well understood [69]. Alfayez M et al. studied adult patients with AML (N=1406), 112 (8%) had PTPN11mut, which showed results similar to other published studies [21]. They showed an association of PTPN11mut with lower CR rates (54% vs. 40%; P=0.04), and shorter OS (median 13.6 vs. 8.4 months; P=0.008) [70].

*U2AF1*

Mutations of the splicing machinery are recurrent and specific to myelodysplastic diseases, therapy-related AML, or AML with myelodysplasia-related changes (25.8%), but comparatively uncommon in de novo AML (6.6%) [71]. A noncanonical function of U2 small nuclear RNA auxiliary factor 1 (U2AF1) is reported, it directly binds to mature mRNA in the cytoplasm to negatively regulate mRNA translation. Mutation in U2AF1 affects the splice site selection. The most frequent change in S34F region, alters a conserved nucleic acid-binding domain, recognition of the 3' splice site, and alternative splicing of many mRNAs. One functional repercussion of alteration in this splicing-independent role is increased synthesis of the secreted chemokine interleukin 8, which promotes metastasis, inflammation, and the growth of cancer in mice and humans [72].

Somatic mutations of the *U2AF1* gene have recently been discovered in myelodysplastic syndrome (MDS) and AML. Qian et al. investigated U2AF1 mutations in Chinese patients with myeloid neoplasms (n=452). In this study, mutations in *U2AF1* were recognized in 2.5% (7/275) of AML, which were heterozygous missense mutations in two highly conserved amino acid positions S34 or Q157 [73]. The OS of AML patients affected by mutation was shorter than those without mutation (median seven months) (*P*=0.035). This study projected that *U2AF1* mutation is a recurrent but less frequent event. Patients with *U2AF1* mutations had an increased probability of progression of MDS to AML. MDS is reported to progress to AML (MDS/AML) within a few months [74, 75]. High-throughput sequencing was implied to identify mutations in 58 genes with known clinical significance in 99 patients with *de novo* MDS or MDS/AML, and *U2AF1* was found to be the most frequently mutated gene (13.6%; 29/214) [76]. *U2AF1* mutation has a poor prognosis in AML [42]. A study suggested that alterations in the U2AF1 gene are an uncommon event in pediatric AML, implying that the driver effect of its mutation is unlikely in myeloid leukemogenesis [77].

In a study by Venkatasubramanian et al., "*U2AF1*-covarying" or "*SRSF2*-covarying" (CV) had an independent occurrence of splicing-factor mutations, which are principally linked to mis-splicing rather than differential gene expression. U2AF1-CV splicing events are linked to canonical rather than changed U2AF1 binding specificity, in contrast to patients with U2AF1-S3F mutations. In both adult and pediatric cohorts, *U2AF1*-CV splice events, resulting from an inclination toward longer protein isoforms, have significantly worse outcomes (poor survival and increased relapse). Similar outcomes are observed during relapse in adults [78].

*EZH2*

The histone lysine N-methyltransferase (EZH2) is the enzymatic component of the polycomb repressive complex 2 (PRC2) that regulates stem cell maintenance and differentiation [79, 80]. Various studies have defined the heterogeneous nature of EZH2 with a prevalence of 1-5% in AML patients [21, 81-83]. Mutations in the gene can be frameshift, nonsense, or missense mutation, whereas truncating mutations are spread throughout the gene. Missense mutations are most prevalent in evolutionarily highly conserved residues in domain II and the CXC-SET domain [84]. In myeloid disorders, EZH2 mutations are found to be inactivating, which suggests the essentiality of the balance of polycomb activity for normal stem cell activity [85]. The correlation of low EZH2 protein levels with poor prognosis in AML patients and failure of consolidation therapy (P=0.004) is suggested in various studies [21, 82, 86, 87]. Göllner et al. showed that a reduction in EZH2 level and histone H3K27 trimethylation led to resistance to tyrosine kinase inhibitors (TKIs) and cytotoxic drugs in AML. Loss of EZH2 protein occurred in about 45% of relapsed AML

patients in this study. Various other mechanisms have been mentioned in the literature wherein a decrease in EZH2 mRNA and/or protein levels is associated with the deletion of 7/7q chromosome. Furthermore, dysfunction of splicing by mutations in spliceosomal genes such as U2AF1 or SRSF2 has been related to reduced EZH2 mRNA expression in 10-25% of AML patients [50, 88, 89]. EZH2 protein expression, analyzed by immunohistochemical staining, showed a significant correlation with poor OS (P=0.008), poor EFS (P=0.005), and poor RFS (P=0.047) [82]. Furthermore, a significant association between the most mutations in E640 K (P=0.049) and E644 K (P=0.047) and chemotherapy resistance after the consolidation phase has been reported [87].

Contrastingly, overexpression of EZH2 has been reported with a high risk in MDS, MDS-derived AML, and AML patients [90]. In a parallel study, 13 out of 714 (1.8%) AML patients were diagnosed with EZH2 mutation and significant association with low blast percentage (21-30%) in bone marrow (P<0.0001) and -7/del(7q) (P=0.025). However, no variance was observed in CR, EFS, or OS between patients with and without EZH2 mutation (P>0.05) [81]. In a different cohort of EZH2-mut AML patients, significantly greater co-occurrence rates were found with RUNX1 (25%), ASXL1 (22%), and NRAS (25%), and comparable results were reported by Kempf et al. [83]. The shorter median OS (12.55 vs. 15.61 months) and RFS (8.15 vs. 17.29 months) were observed for patients with homozygous mutations in comparison to heterozygous mutations. EZH2 mutations are recurrent alterations in patients with AML. However, data implicated the poor potential of EZH2 mutations as an independent prognostic factor in AML [88].

*PHF6*

An X-linked gene, PHD-finger protein 6 (*PHF6*), is a tumor suppressor gene that encodes a plant homeodomain (PHD) protein. PHD contains four nuclear localization signals and two imperfect PHD zinc finger domains. It has a suggested role in transcriptional regulation and/or chromatin remodelling [91]. Jalnapurkar SS et al. reported *PHF6* mutation in 10 out of 353 AML patients (3%). This gene is reported to be mutated in 3.2% of *de novo* AML, 4.7% of chronic myelomonocytic leukemia (CMML), 3% of MDS, and 1.6% of chronic myeloid leukemia (CML) patients. Frameshift and nonsense mutations constitute two-thirds of somatic gene lesions in PHF6, resulting in loss of PHF6 protein. Also, point mutations are clustered in ePHD2 (extended PHD) with unknown functional consequences. A chromatin-binding protein PHF6, binds to key myeloid transcription factors through ePHD domains and restricts AML progression. R274Q mutation in PHF6 enables it to regulate downstream signaling describing a vital role in hematopoiesis [23, 92].

*PHF6* mutations were identified in 2% of pediatric AML patients, majorly affecting the male population. In a different study, the pediatric *de novo* AML cohort was enriched in FAB-M0, M1, and M2 lower *PHF6* mRNA expression, and the prevalence was persistent as former study. Accordingly, the presence of loss-of-function mutations in *PHF6* in pediatric AML indicates its role in leukemogenesis. Mutations in *PHF6* are associated with reduced OS (P=0.006) [93]. Investigation of concomitant mutations with *PHF6* mutation revealed significant association with *RUNX1, U2AF1, SMC1A, ZRSR2, EZH2*, and *ASXL1* [94, 95].

The cytogenetic analysis serves as the basis for AML classification into the favorable, intermediate, or adverse group associated with 5-year OS of ~60%, 30% to 40%, and 5% to 10%, respectively [5]. The advent of NGS approaches, has deciphered a new depth of information, >95% of AML cases are reported to possess at least one somatic mutation, which provides prognostic information of otherwise intermediate-risk cytogenetics [93, 102]. The novel mutations bring a new opportunity for researchers and clinicians to treat the disease with a personalized approach. A synthetic molecule-based splicing inhibitor therapy for targeting splicing inhibitors has been postulated for increasing treatment efficacy [103]. Small molecule splicing modulator (H3B-8800) is undergoing a phase 1 trial specifically for patients with hematologic malignancies (#NCT02841-540). The aberration in Hedgehog (Hh) signaling pathway affects the differentiation of leukemia stem cells, and its upregulation imparts resistance to chemotherapy in AML cells. Glasdegib acts as an oral inhibitor that interacts with SMO in the Hh pathway. In its randomized phase 2 study, the combination of low-dose

cytosine arabinoside (LDAC) and Glasdegib is administered to *de novo* AML or high-risk MDS patients and has shown to improve OS (8.3 months vs. 4.3 months, HR 0.51; 80% CI, 0.39-0.67, *P*=0.0004) and CR (15% vs. 2.3%) [104]. Likewise, C-82 mediated disruption of Wnt/β-catenin signaling suppressed growth, induced apoptosis, and overcame stromal protection of cancerous and stem/progenitor cells [105].

*Authors' view:* Although the treatment procedure has been improved over the years, there is still a significant gap between treatment and improving survival. As a result, the adoption of improved diagnostic tools and targeted medications with minimal toxicity and off-site specificity is becoming progressively vital for early care. Furthermore, more precise risk stratification is required, including mutations and differential expression of pathways. The novel mutations might not have a high prevalence, but the recurrence influences the outcome with or without any other genetic abnormality.

Albeit, AML is a heterogenous malignancy with a dismal outcome, its pathogenesis and biology are poorly understood; however, advancement in technologies has made risk stratification-based treatment to decrease treatment-related toxicity. There is a lot of research that needs to be done to gain knowledge for the novel targeted therapy to make easy access to every needy patient and to make improvements in the outcome of AML. The persistence of epigenetic factor mutations may lead to clonal evolution in chemorefractory cells and leading to chemo-resistance and recurrence of AML. A deeper comprehension of the molecular mechanisms driving chemotherapy resistance must serve as the foundation for the development of revolutionary therapeutic approaches for pediatric AML. Integrative genomic investigations that combine DNA sequencing, DNA copy number analysis, transcriptional profiling, and functional genetic techniques show considerable promise for uncovering other anomalies in AML that are essential for leukemogenesis and can be employed therapeutically. In the future, it may be possible to increase the survival rate in AML patients by using pathogenesis-focused drug combinations.

## Conclusion

AML is a rigorous malignancy of hematopoietic stem cells (HSC) characterized by differentiation arrest and uncontrolled clonal proliferation of precursor cells. In traditional clinical practice, history, cell morphology, immunophenotype, cytogenetic studies, and molecular analyses play integral roles in creating diagnosis and risk classification. The exploitation of high-throughput data can provide more targeted solutions to disease complications and prevent secondary recurrence events. Identification of the pathogenic potential of recurrent mutations like *TET2, SATB1, PTPN11, U2AF1* and *EZH2* lays out provisions for novel drug targets which can be used in combination with common drugs. Refractory disease or relapse highlights the intricacy of AML, and the therapy has its own complicacies. Consequently, additional studies with the discovery of novel drug targets are necessary to achieve new milestones in clinical practice.

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## Disclosure of conflict of interest

None.

## Abbreviations

AML, Acute myeloid leukemia; CI, Confidence interval; CR, Complete remission; CTNNB1, Catenin Beta 1; ETV6, ETS Variant Transcription Factor 6; CMA, Chromosomal microarray analysis; EFS, Event free survival; EZH2, Enhancer of zeste 2 polycomb repressive complex 2; HR, Hazard risk; NGS, Next-generation sequencing; PTPN11, Protein tyrosine phosphatase non-receptor type 11; PHF6, PHD-finger protein 6; OS, Overall survival; RFS, Relapse free survival; SATB1, Special AT-rich sequence-binding protein 1; TET2, Tet methylcytosine dioxygenase 2; U2AF1, U2 small nuclear RNA auxiliary factor 1.

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