Review Article

Molecular Mutations and Their Cooccurrences in Cytogenetically Normal Acute Myeloid Leukemia

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1. Introduction

Acute myeloid leukemia (AML) clinically is a disparate disease that requires intensive treatments ranging from chemotherapy alone to allogeneic hematopoietic cell transplantation (allo-HCT). Historically, cytogenetic analysis has been a useful prognostic tool to classify patients into favorable, intermediate, and unfavorable prognostic risk groups. However, the intermediate-risk group, consisting predominantly of cytogenetically normal AML (CN-AML), itself exhibits diverse clinical outcomes and requires further characterization to allow for more optimal treatment decision-making. The recent advances in clinical genomics have led to the recategorization of CN-AML into favorable or unfavorable subgroups. The relapsing nature of AML is thought to be due to clonal heterogeneity that includes founder or driver mutations present in the leukemic stem cell population. In this article, we summarize the clinical outcomes of relevant molecular mutations and their cooccurrences in CN-AML, including \( \text{NPM1} \), \( \text{FLT3} \) \(_{\text{ITD}} \), \( \text{DNMT3A} \), \( \text{NRAS} \), \( \text{TET2} \), \( \text{RUNX1} \), \( \text{MLL} \) \(_{\text{PTD}} \), \( \text{ASXL1} \), \( \text{BCOR} \), \( \text{PHF6} \), \( \text{CEBPA} \) biallelic, \( \text{IDH1} \), \( \text{IDH2} \) \(_{\text{R140}} \), and \( \text{IDH2} \) \(_{\text{R170}} \), with an emphasis on their relevance to the leukemic stem cell compartment. We have reviewed the available literature and TCGA AML databases (2013) to highlight the potential role of stem cell regulating factor mutations on outcome within newly defined AML molecular subgroups.
AML, which comprises up to 40% to 50% of AML patients [12, 13]. Level of MRD correlates with the chemotherapy increases with the increased MRD, a condition which can be assessed by immunophenotypical detection of leukemic cells and/or leukemic stem cells (LSC) after chemotherapy alone. For patients with unfavorable risk, such as monosomies, 17p deletion, or complex abnormalities, there is a very low likelihood of cure with chemotherapy alone and consolidation with allo-HSCT is pursued if possible. Intermediate-risk patients include CN-AML, which comprises up to 40% to 50% of AML patients [6]. The clinical outcome of CN-AML patients varies widely and cannot be predicted based solely on cytogenetics.

The focus on improving our understanding of CN-AML prognosis and outcomes leads to the identification additional molecular markers of clinical significance. Mutations in nucleophosmin 1 (NPM1), fetal liver tyrosine kinase 3 (FLT3), and CCAAT/enhancer binding protein \( \alpha \) (CEBPA) have been shown to have clinically significant prognostic value [7]. The FLT3 internal tandem duplication (ITD) mutation (\( \text{FLT3}^{\text{ITD}} \)) is present in nearly one-third of AML cases and has been associated with adverse clinical outcomes including increased relapse risk and decreased overall survival (OS) [8]. \( \text{FLT3}^{\text{ITD}} \) presence in CN-AML identified a subgroup of patients with more adverse outcome, particularly patients with a high mutant allelic frequency [9]. In addition to providing prognostic information, the \( \text{FLT3}^{\text{ITD}} \) is a therapeutic target as well. Sorafenib, a tyrosine kinase inhibitor targeting \( \text{FLT3}^{\text{ITD}} \) mutations, has been shown to increase event-free survival (EFS) and relapse-free survival (RFS) when added to both induction and consolidation therapies, although there was no OS benefit in the three years of follow-up in the newly diagnosed AML patients aged 60 years or younger [10]. NPM1 mutation has also been recently validated as a molecular marker of minimal residual disease (MRD) in NPM1 mutation positive patients and the presence of MRD was shown to be the only independent prognostic factor for death in multivariate analysis [11].

Consolidative chemotherapy is utilized to eliminate residual leukemia cells and/or leukemic stem cells (LSC) after induction chemotherapy to reduce the chance of relapse. Risk of a relapse after induction chemotherapy and consolidation chemotherapy increases with the increased MRD, a condition which can be assessed by immunophenotypical detection of leukemia cells [12, 13]. Level of MRD correlates with the amount of leukemic stem cells and predicts outcome in AML [14–17]. Therefore, it is imperative to keep leukemic stem cells in mind when clinicians stratify patients for treatment purposes.

Advances in clinical genomics have identified an expanding array of recurrent molecular lesions in AML that will add layers of complexity to prognostic stratification needed to guide treatment and provide needed targets for new AML therapies. The evolving challenge is to incorporate these molecular abnormalities and their combinatorial effect on AML prognosis and in turn treatment strategies. The availability of this new AML data has created a requirement of a new classification system based on both cytogenetics and additional molecular lesions, which will be pivotal in establishing new clinical treatment guidelines. Furthermore, new classifications based on molecular abnormalities may help clinical trial design to develop targeted therapies to specific subgroups of AML patients. Recently a new AML classification system has been proposed by Papaemmanuil et al. [18]. Here the authors classified AML based on the presence of one or more driver mutations \(+/-\) other mutations into 11 different subgroups and correlated with clinical outcomes. This new classification system has provided insight regarding the effects of specific driver mutations and the additive effect seen when they are found in combination. In this paper, we summarize the significance of the most clinically relevant molecular mutations, cooccurrences of these mutations, and their functional role on leukemic stem cell population in relation to clinical outcomes based on this newly developed classification system.

### 2. The New AML Classification System

The proposed new AML classification system is based on a retrospective genomic analysis of 1540 AML patients in three prospective trials of the German-Austrian AML Study Group [18]. Patients received induction chemotherapy with idarubicin, cytarabine, and etoposide (ICE) with or without ATRA; high-risk patients were offered allo-HCT; intermediate-risk patients were offered a matched related donor allo-HCT, if a matched sibling was available; low risk patients received chemotherapy alone. The median follow-up period was 5.9 years.

In addition to cytogenetic analyses, III candidate driver genes were sequenced and 5234 somatic driver mutations were identified across 76 genes or genomic regions. Nearly all AML patients (96%) had at least one mutation and 86% patients were found to have two or more mutations. Statistical analysis of mutational patterns was utilized to define 11 mutually exclusive AML subtypes including three novel genetic subgroups that have not been described in the World Health Organization (WHO) classification in 2008 [19]. These novel subgroups are, namely, (1) AML with mutations in genes encoding chromatin, RNA-splicing regulators, or both (18% of patients); (2) AML with \( \text{TP53} \) mutations, chromosomal aneuploidies, or both (13%); and (3) AML with \( \text{IDH2}^{R172} \) mutations (1%). Many of the mutations used to define the novel subgroups involve genes which have roles in stem cell functions. Of note, only 48% of patients were classifiable
Table 1: Molecular classification of CN-AML and clinical outcomes.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Clinical Outcomes</th>
</tr>
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<tbody>
<tr>
<td>NPM1+</td>
<td>Better EFS and OS [29–32]; better CRR, high RR with EFS or OS benefits [33]; better OS [34]</td>
</tr>
<tr>
<td>NPM1/DNMT3A</td>
<td>Worse OS [41]</td>
</tr>
<tr>
<td>NPM1/DNMT3A/FLT3&lt;sup&gt;ITD&lt;/sup&gt;</td>
<td>Worse OS [18]</td>
</tr>
<tr>
<td>NPM1/DNMT3A/NRAS&lt;sup&gt;Gi2/13&lt;/sup&gt;</td>
<td>Better OS [18]</td>
</tr>
<tr>
<td>NPM1/TET2</td>
<td>Worse CRR, EFS, DFS, and OS [50]; no impact on outcomes [41]</td>
</tr>
<tr>
<td>NPM1/IDH1* or NPM1/IDH2&lt;sup&gt;R140&lt;/sup&gt;*</td>
<td>Worse OS [55]; better OS [27, 32]</td>
</tr>
<tr>
<td>RUNX1</td>
<td>Worse EFS, DFS [59, 61, 62]; worse OS [59–62]</td>
</tr>
<tr>
<td>MLL&lt;sup&gt;PTD&lt;/sup&gt;</td>
<td>Worse EFS [60, 66, 67]; worse OS [27, 41, 60, 67]</td>
</tr>
<tr>
<td>ASXL1</td>
<td>Worse outcomes [27, 60, 75]; worse CR, EFS, DFS, and OS [77]</td>
</tr>
<tr>
<td>BCOR</td>
<td>Worse EFS and OS [81]</td>
</tr>
<tr>
<td>PHF6&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Worse OS [27]</td>
</tr>
<tr>
<td>NPM1/biallelic mutation</td>
<td>Better EFS and OS [86]</td>
</tr>
<tr>
<td>CEBPA&lt;sup&gt;biallelic&lt;/sup&gt;</td>
<td>Worse OS [89]</td>
</tr>
<tr>
<td>CEBPA&lt;sup&gt;biallelic&lt;/sup&gt;/TET2</td>
<td>Worse OS [89]</td>
</tr>
<tr>
<td>CEBPA&lt;sup&gt;biallelic&lt;/sup&gt;/GATA2</td>
<td>Better OS [89]</td>
</tr>
<tr>
<td>IDH2&lt;sup&gt;R172&lt;/sup&gt; mutation</td>
<td>Worse RR and OS [52, 90]; better OS [18]</td>
</tr>
</tbody>
</table>

EFS: event-free survival; OS: overall survival; CRR: complete remission rate; RR: relapse rate; DFS: disease-free survival.

* Without FLT3<sup>ITD</sup>.

Based on the current WHO guidelines, whereas 80% of patients could be allocated into this novel classification system. Only 8% of patients had either no detected driver mutations (4%) or ≥2 genomic subgroups (4%).

Clinical outcomes, such as OS of AML patients with genetic mutations, were found to be significantly altered by the presence or absence of other driver mutations as has been described by others [20]. For instance, NPM1-mutated AML, as the largest subgroup in this novel classification, had variable clinical outcomes influenced by the presence of concurrent mutations such as FLT3, DNMT3A, NRAS, IDH, PTPN11, or chromatin-spliceosome mutations. We focused on the clinical outcomes of the most significant single or concurrent molecular mutations based on this novel classification (Table 1) and the significance of concurrent or mutually exclusive alterations in genes of interest (Table 2). We did not include the effect of traditional cytogenetic abnormalities in this study.

3. AML with NPM1 Mutation

Nucleophosmin is a protein encoded by the NPM1 gene in humans. Nucleophosmin has multiple functions in various processes including histone chaperones, ribosome biogenesis and transport, genomic stability and DNA repair, control of centrosome duplications, and regulation of the ARF-p53 tumor suppressor pathway [21]. All these functions have a part in leukemic stem cell self-renewal and limited differentiation. Indeed, stem cell/progenitor cell surface marker CD34+ cells from NPM1-mutated AML patients are able to recapitulate leukemia in immunodeficient mice [22]. Alteration of the NPM1 gene was found to be present at a high frequency in AML patients, ranging from 25% to 53% in all AML and 46% to 67% in CN-AML. NPM1-mutated AML consists of 27% of all AML and therefore forms the largest subgroup in this novel classification [18]. The identification of NPM1 mutation in AML is important for both disease prognosis and the subsequent treatment decision-making regarding consolidation with chemotherapy alone or an allo-HCT treatment. Furthermore, a recent study demonstrated the importance of MRD analysis in NPM1-mutated AML [11]. Since high MRD correlates positively with high stem cell frequency in AML [16], persistence of NPM1-mutated transcripts in blood was associated with a greater risk of relapse after 3 years of follow-up.

AML with NPM1 mutation is a clinically heterogeneous group likely due to the prevalence of concurrent mutations: 54% DNMT3A, 39% FLT3<sup>ITD</sup>, 19% NRAS, 16% TET2, and 15% PTPN11. NPM1 is usually a secondary or downstream mutation, whereas mutations in DNMT3A, ASXL1, IDH1/2, and TET2 occur very early during clonal evolution but are typically not sufficient to cause leukemia on their own. Therefore, the analysis of comutation patterns in this group has become crucial in predicting disease prognosis.

3.1. NPM1 and FLT3<sup>ITD</sup>. FLT3<sup>ITD</sup> represents one of the most frequent genetic alterations with a 20% frequency in adult AML, 28–34% in cytogenetically normal AML [23], and 39% in NPM1-mutated AML [18]. FLT3<sup>ITD</sup> activates STAT5 to maintain survival of leukemic stem cell population in AML [24]. It was not a surprise that FLT3<sup>ITD</sup>-positive AML patients had higher relapse incidence and lower DFS [25, 26] as well as OS [27]. These observations have validated FLT3 as a therapeutic target in AML and FLT3 inhibitors have shown promising results when combined with standard therapy [10, 28].

With regard to NPM1 and FLT3<sup>ITD</sup>, several studies have shown that AML with NPM1 mutation, but without FLT3<sup>ITD</sup> mutation, is associated with significantly better OS and EFS.
Table 2: Cooccurrent or mutually exclusive alterations and their significance in genes of interests.

<table>
<thead>
<tr>
<th>Gene A</th>
<th>Gene B</th>
<th>P value</th>
<th>Odds ratio</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASXL1</td>
<td>IDH2</td>
<td>0.003</td>
<td>2.241</td>
<td>Tendency towards coocurrence Significant</td>
</tr>
<tr>
<td>IDH1</td>
<td>DNMT3A</td>
<td>0.005</td>
<td>1.266</td>
<td>Tendency towards coocurrence Significant</td>
</tr>
<tr>
<td>TET2</td>
<td>DNMT3A</td>
<td>0.01</td>
<td>1.156</td>
<td>Tendency towards coocurrence Significant</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>FLT3</td>
<td>0.035</td>
<td>0.713</td>
<td>Tendency towards coocurrence Significant</td>
</tr>
<tr>
<td>KMT2A</td>
<td>DNMT3A</td>
<td>0.042</td>
<td>−1.169</td>
<td>Tendency towards mutual exclusivity Significant</td>
</tr>
<tr>
<td>IDH2</td>
<td>KMT2A</td>
<td>0.056</td>
<td>1.052</td>
<td>Tendency towards coocurrence Marginal</td>
</tr>
</tbody>
</table>

Database used for analysis is TCGA, NEJM 2013 [94]. The database contains all 166 complete tumors of AML. Query was performed on 10 genes which include ASXL1, BCOR, TET2, IDH1, IDH2, RUNX1, PHF6, KMT2A (MLL), DNMT3A, and FLT3. The query results contain 23 gene pairs with mutually exclusive alterations (1 significant) and 22 gene pairs with cooccurrent alterations (4 significant, 1 marginal).

[29–32]; one study demonstrated that NPM1 mutation with or without FLT3ITD was only favorable in achieving complete remission but was associated with a high relapse rate with no OS and EFS benefits [33]. The German-Austrian AML Study Group conducted a study to evaluate genetic mutations and clinical outcomes in 872 adults younger than 60 years of age and again demonstrated that NPM1 mutation without FLT3ITD was associated with lower risk of relapse and death [34]. The majority of these earlier studies showed that NPM1 mutation without FLT3ITD is associated with better clinical outcomes, and allo-HCT conferred no benefit in this patient group [34] similar to the core binding-factor leukemia patient group [35]. It is apparent that all these studies pointed to a worse clinical outcome when NPM1 mutation and FLT3ITD mutation coexisted in AML. However, the most recent study argued against NPM1 and FLT3ITD mutations being the sole determinants in AML prognosis, and another mutation, DNMT3A, must also be taken into consideration in the decision-making process of the treatment of NPM1-mutated AML [18].

3.2. NPM1 and DNMT3A. DNMT3A (DNA methyltransferase 3A) is an enzyme that catalyzes the transfer of methyl groups to specific CpG structures in DNA and hence plays an essential role in DNA methylation and gene silencing regulatory processes [36]. DNMT3A is important in normal hematopoietic stem cell differentiation and self-renewal [37] and its mutation produces a reservoir of preleukemic stem cells which can evolve into AML [38]. DNMT3A mutations were found in 22.1% of all AML and 33.7% of AML with intermediate-risk cytogenetic profile and were independently associated with a poor outcome regardless of age [39]. DNMT3A mutations tend to cooccur with FLT3, TET2, or IDH1 in AML (Table 2). The combination of DNMT3A mutation with FLT3, TET2, or IDH1 tends to have an adverse effect on disease-free survival in AML compared to wild-type group (Figure 1). Interestingly, hypomethylating agents, such as decitabine and 5-azacitidine, have a higher clinical remission rate in DNMT3A-mutated AML [40].

Patients with DNMT3A, TET2, ASXL1, PHF6, or MLLITD mutations who were in the WHO intermediate group had an adverse outcome compared to those with other genotypes [27]. DNMT3A mutation was found to be an adverse prognostic factor in cytogenetically normal AML with mutated NPM1 without FLT3ITD in terms of OS [41]. However, this finding was not confirmed by the most recent study [18]. Instead, it was reported that patients with both NPM1 and DNMT3A mutations but without FLT3ITD showed much better outcomes than those with FLT3ITD. Therefore, triple-mutated AML (NPM1/DNMT3A/FLT3ITD) yields the worst prognosis and the consolidation with allo-HCT should be considered, although prospective study is needed to confirm these results.

3.3. NPM1 and NRASG12/13. NRAS belongs to the RAS GTPass family of genes. It plays important roles in the regulation of proliferation, differentiation, and apoptosis in AML and is a fairly common mutation in AML ranging from 11% to 30% [42]. NRAS mutation consists of 19% of NPM1-mutated AML [18]. The prognostic impact of NRAS mutation has been reported to be insignificant for OS, EFS, and disease-free survival (DFS) [42]. However more recently mutations in NPM1 and DNMT3A in the presence of NRASG12/13 in AML patients were associated with a more favorable outcome [18].

3.4. NPM1 and TET2. TET2 (ten-eleven translocation) protein is an epigenetic modifier that converts methylcytosine to 5-hydroxymethylcytosine and plays a role in DNA methylation and myelopoiesis. Normal expression and function of TET2 are essential in maintaining the hematopoietic stem cell (HSC) pool and in controlling HSC differentiation [43]. Studies using conditional knockout mouse models have revealed that complete loss of TET2 (TET2−/−) or TET2 haploinsufficiency (TET2+/−) impaired hematopoietic stem cell differentiation, held cells in a more immature state, and initiated aberrant hematopoiesis both in vitro and in vivo [44–46]. TET2 expression is tightly regulated by the master stem cell transcription factors Oct4 and Sox2 [44]. TET2 mutations are present in 5–25% of adult AML cases, with the highest frequency in the elderly [47, 48]. TET2 mutations are significantly correlated with NPM1 (16%) in this most recent study [18] and were found to be mutually exclusive with MLLITD [48] and IDH1/2 mutations [48, 49]. TET2 mutation resulted in a lower complete remission rate, shorter EFS and DFS in patients with mutated NPM1 without FLT3ITD [47], and shorter OS in patients with mutated NPM1 without FLT3ITD [47, 50] and with mutated NPM1 [50]. However, TET2 mutations were also reported to have no impact on the
clinical outcomes of de novo AML [48, 49], CN-AML [49, 50], and CN-AML with mutated NPM1 or CEBPA without FLT3[ITD] [41]. The full clinical impact of TET2 mutations has yet to be fully understood.

3.5. NPM1 and IDHI/IDH2[140]. IDHI and IDH2 (isocitrate dehydrogenases 1 and 2) are enzymes that catalyze the interconversion of isocitrate and alpha-ketoglutarate and appear to play an epigenetic role in histone and possibly DNA
methylations. IDH1 or IDH2 mutations confer a hypermethylating phenotype in leukemia and inhibit hematopoietic stem cell differentiation [51]. These phenotypic characteristics are shared by TET2 loss-of-function mutations [46]. The most common IDH1 mutation is in the arginine residue at position 132 (IDH1R132H), occurring in 6–9% of adult AML, while IDH2 mutations occur in 9–19%, predominantly IDH2R140 in 8–12% [18, 27, 52–54]. IDH1 and IDH2 mutations are mutually exclusive in AML. Furthermore, IDH1 and IDH2R140 are strongly associated with NPM1 mutations [18]. IDH1 [53, 54] and IDH2 [53] mutations have been reported to carry an unfavorable prognosis with regard to survival in normal karyotype AML lacking NPM1 and FLT3ITD mutations. In patients with cooccurring DNMT3A and IDH2R140 mutations, the OS was significantly poorer than those with wild-type or a single mutation [18].

An earlier study demonstrated that the IDH1 and IDH2 mutations constitute poor prognostic factors in cytogenetically normal AML with NPM1 mutation without FLT3ITD [55]. In some other studies, however, patients with mutated IDH1 or IDH2R140 had good prognoses for OS in AML patients with the NPM1 mutation without FLT3ITD [27, 52], and it was further concluded that the favorable effect of NPM1 mutations was restricted to patients with cooccurring NPM1 and IDH1 or IDH2R140 mutations [27].

4. AML with Mutated Chromatin, RNA-Splicing Genes, or Both

This chromatin-spliceosome group is the second largest subgroup in this new classification [18]. This is also an extremely heterogeneous group, consisting of genes regulating RNA splicing (SRFS2, SF3B1, U2AF1, and ZRSR2), chromatin (ASXL1, STAG2, BCOR, MLLPTD, EZH2, and PHF6), and transcription (RUNXI). Functional proteins encoded by these genes have functions in hematopoietic stem cell self-renewal and differentiation. Using the European LeukemiaNet (ELN) guidelines, the majority (84%) of the patients in this new chromatin-spliceosome group would be classified as having intermediate prognostic risk. However, this new subgroup demonstrated resistance to induction chemotherapy and inferior long-term outcomes [18] suggesting a reclassification of AML patients with chromatin-spliceosome mutations as an adverse prognostic group. Nearly all of the genetic mutations in this subgroup have been previously reported to be adverse prognostic markers.

4.1. RUNXI. The RUNXI (runt-related transcription factor 1, formerly known as AML1) gene encodes the alpha subunit of the core binding factor involved in transcription and is required for definitive hematopoiesis [56]. RUNXI protein also plays an essential role in mesenchymal stem cell proliferation and promotes cell survival in AML [57, 58]. RUNXI mutations are present in 5% to 18% of AML [59–62]. They are associated with ASXL1 [59], MLLPTD [62], and IDH1/IDH2 mutations [62] and are essentially mutually exclusive of NPM1 mutations [59, 62]. RUNXI mutations were found to be associated with resistance to chemotherapy, inferior DFS, EFS [59, 61, 62], and OS [59–62]. More importantly, RUNXI mutations were deemed to be an independent prognostic marker for shorter EFS in multivariable analysis [62]. An explorative subgroup analysis demonstrated that RUNXI-mutated AML patients benefited from allo-HSC in terms of RFS [62].

4.2. MLLPTD. The MLL (Mixed Lineage Leukemia) gene, located on chromosome 11q23, is frequently involved in translocations that recur in AML and have been classified into an individual subgroup, AML with MLL fusion genes, t(x;11)(x;23) [18]. MLL fusion proteins are capable of transforming normal hematopoietic stem cells into malignant leukemic stem cells [63]. A MLL partial tandem duplication (MLLPTD), the result of a tandem duplication of an internal portion of the MLL gene that spans either exons 2 to 6 or exons 2 to 8, is present in approximately 10% of CN-AML [64, 65]. MLLPTD mutation has been identified as a poor prognostic factor for EFS [60, 66, 67] and OS [27, 41, 60, 67]. Furthermore, it is clear that the OS was shortened when the intermediate-risk group patients had mutated MLLPTD regardless of the presence of FLT3ITD [27].

4.3. ASXL1. The ASXL1 (additional sex combs like-1) gene is a human homologue of the Drosophila additional sex combs (Asx) gene, which is highly conserved across multiple species. The ASXL1 protein functions in both epigenetic activation and repression of gene transcription [68–70]. Its regulation of histone modification affects hematopoietic stem cell pool maintenance and its loss causes severe defects in HSC development [71, 72]. ASXL1 mutations are more common in the aberrant karyotypes, the elderly, and MDS-associated and secondary AML [73–77], while 9–12% of ASXL1 mutations are detected in cytogenetically normal AML [75–77]. NPM1 and ASXL1 mutations appear to be mutually exclusive [76–78]. Several studies have shown that AML patients with ASXL1 mutations had worse outcomes when compared to those without these mutations [27, 60, 75]. Specifically in CN-AML, ASXL1 mutations were associated with inferior complete remission, DFS, OS, and EFS [77].

4.4. BCOR. The BCOR (BCL6 corepressor) gene is located on chromosome X and encodes a nuclear protein that is a key transcriptional regulator of hematopoiesis [79]. Studies demonstrated that normal BCOR retains hematopoietic stem cells in a quiescent, undifferentiated state and loss-of-function BCOR mutations result in enhanced HSC cell proliferation and differentiation [80]. BCOR mutations occur in 3.8% of CN-AML, and DNMT3A mutations are detected in 43.5% of these patients. BCOR mutations tend to be associated with an inferior EFS and OS [81].

4.5. PHF6. The PHF6 (plant homeodomain finger 6) gene, also located on chromosome X, plays a key role in chromatin remodeling [82]. PHF6 mutations are found in approximately 3% of adult AML and confer worse OS in intermediate-risk patients that are FLT3ITD negative [27].
5. AML with CEBPA^biallelic^ Mutation

The CEBPA gene is located on chromosome 19 band q13.11 and encodes a 42 kDa size protein that is a member of the basic region leucine zipper transcription factor family [83]. Normal function of CEBPA is crucial in maintaining adult hematopoietic stem cell in a quiescent state and CEBPA gene knockout in mice results in impaired HSC differentiation [84]. The CEBPA protein is expressed in myelomonocytic cells and is critical for neutrophil development [83]. Mutated CEBPA regulates Sox4 expression which affects self-renewal of leukemic stem cells [85]. The frequency of CEBPA mutations is reported to range from 7% to 22% in patients with AML [86] and 15% to 18% in CN-AML [87, 88]. About two-thirds of CEBPA-mutated patients are biallelic-mutated (CEBPA^biallelic^), and the remaining one-third carry a single mutation (CEBPA^monoallelic^). In a meta-analysis of 10 clinical studies covering 6219 patients, CEBPA^biallelic^ mutation was found to be associated with favorable clinical outcomes with regard to EFS and OS in patients with AML or CN-AML; conversely, no significant difference was found between CEBPA^monoallelic^ mutation and wild-type CEBPA in patients with AML or CN-AML [86]. In a long-term follow-up study (median follow-up time of 9.8 years), patients with CEBPA^biallelic^ mutations showed longer OS, longer relapse-free survival, and a lower cumulative incidence of relapse compared to those with CEBPA^monoallelic^ mutation. The ten-year OS rate for patients ≤60 years and with CEBPA^biallelic^ mutation was 81%. CEBPA^biallelic^-mutated AML was associated with TET2 mutation in 34% of the patients, and the combination resulted in significantly worse OS, whereas it was associated with GATA2 (GATA binding protein 2, a transcription factor) mutation, found in 21% of CEBPA^biallelic^-mutated AML, resulting in improved OS [89].

6. AML with IDH2^R172^ Mutation

IDH2^R172^ is a distinct IDH2 mutation that occurs in AML with a frequency of 1–3% [18, 52, 90]. Unlike IDH2^R140^ that significantly correlates with NPM1 mutation, IDH2^R172^ is generally not associated with other molecular mutations. The gene expression and DNA methylation profiles of IDH2^R172^-mutated AML differ from those of other IDH mutations and lead to more severe aberrations in metabolic activity [91, 92]. Thus, IDH2^R172^ mutation has been defined as a unique subgroup in the new classification scheme [18]. In previous studies, IDH2^R172^ mutation was associated with a higher relapse rate and lower OS that were comparable with those of the adverse-risk cytogenetics patients [52, 90]. In this most recent study, however, the presence of IDH2^R172^ mutation was associated with a favorable prognosis with regard to OS, similar to patients with NPM1-mutated AML [18].

7. Conclusions and Future Directions

The progress in AML risk stratification using next-generation sequencing technologies over the past decade has been truly remarkable. As an example, the identification of NPMI- or CEBPA^biallelic^-mutated CN-AML to have favorable risk has significantly impacted the clinical management of these patient groups. The novel AML classification system proposed by Papaemmanuil et al. has been especially valuable in organizing the growing array of AML mutations in terms of additive effects on prognosis. As an example, the subgroup “AML with NPM1 mutation” is the largest subgroup and has a large number of comutations. The different comutation combinations do not have a strictly additive effect on clinical outcomes indicating further risk stratification in this group is necessary. Conversely, the subgroup “AML with mutated chromatin, RNA-splicing genes, or both” is more consistent as an adverse prognostic group, at least among the five genetic mutations we reviewed here: RUNX1, MLL^PTD^, ASXL1, BCOR, and PHF6. A central theme of this adverse-risk group is that the majority of these genes have roles in maintaining normal HSC quiescence by their epigenetic regulation and their mutations result in transformation of HSC into leukemic stem cells. These malignant stem cells, in turn, are thought to be the wellsprings of leukemia cell expansion, likely directly responsible for the relapsing nature of AML. Of note, mutations in epigenetic modifiers or regulators such as DNMT3A, TET2, and IDH1/2 alter normal HSC quiescent state and prime HSC to a preleukemic state [93]. These epigenetic factors function as stem cell regulators and impact DFS (Figure 1). This observation may be true beyond AML and therapies targeting epigenic modifiers or stem cell regulating factors may hold promise in improving disease-free survival of patients with other hematologic malignancies. These observations of recurrent AML mutations and comutation patterns await validation in larger prospective clinical trials. Regardless, this new classification strategy is an important step forward in understanding the molecular complexity of AML and has the potential to yield many new therapeutic targets to be exploited to someday eradicate this aggressive disease.

Competing Interests

The authors declare that they have no competing interests.

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