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Clinical implications of molecular markers in acute myeloid leukemia

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Abstract

The recently updated World Health Organization (WHO) Classification of myeloid neoplasms and leukemia reflects the fact that research in the underlying pathogenic mechanisms of acute myeloid leukemia (AML) has led to remarkable advances in our understanding of the disease. Gene mutations now allow us to explore the enormous diversity among cytogenetically defined subsets of AML, particularly the large subset of cytogenetically normal AML. Despite the progress in unraveling the tumor genome, only a small number of recurrent mutations have been incorporated into risk-stratification schemes and have been proven to be clinically relevant, targetable lesions. We here discuss the utility of molecular markers in AML in prognostication and treatment decision making, specifically highlighting the aberrations included in the current WHO classification.

Keywords

acute myeloid leukemia; molecular markers; prognostic impact

1 | INTRODUCTION

In acute myeloid leukemia (AML), recurrent cytogenetic abnormalities are established diagnostic and prognostic markers, suggesting that acquired genetic abnormalities play an essential role in leukemogenesis.[1] The prognosis for patients with AML is determined to a large degree by the biology of the disease. Roughly 45% of the patients harbor a normal karyotype as detected by conventional cytogenetics at diagnosis, yet a somatic mutation can be identified in 97.3% of the cases.[2] Targeted sequencing has identified several mutations

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CONFLICT OF INTEREST

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that carry prognostic information, including mutations in *FLT3, NPM1, KIT, CEBPA,* and *TET2*.[3] In addition, massively parallel sequencing led to the discovery of recurrent mutations in *DNMT3A* and *IDH*.[4,5]

The recently updated World Health Organization (WHO) classification of myeloid neoplasms and leukemia reflects the fact that research in the underlying pathogenic mechanisms of AML has led to remarkable advances in our understanding of the disease.[5] Currently, the WHO classification includes eight AML categories defined by recurrent genetic abnormalities (including AML with t(9;22)(q34;q11.2)/*BCR-ABL1* as a provisional entity) as well as three categories defined by gene mutations, that is, AML with *NPM1*, AML with biallelic mutated *CEBPA,* and the provisional entity AML with mutated *RUNX1*. Consistently, AMLs defined by recurrent genetic abnormalities are associated with distinctive clinicopathological features and impact prognosis. AML with *FLT3,* however, is not included as a separate entity, because it occurs across multiple subtypes. However, the WHO classification acknowledges that *FLT3* should be tested in all AML cases.[5]

Although new molecular analysis techniques, such as ultra-deep sequencing, has helped to identify numerous recurrent genetic abnormalities, to date, however, only a limited number have been incorporated into risk-stratification schemes, such as the National Comprehensive Cancer Network or European LeukemiaNet (ELN) Guidelines (Table 1).[6] In addition, increasing evidence indicates that the presence of minimal residual disease (MRD), measured either molecularly or by multiparameter flow cytometry, identifies patients at particularly high risk of relapse and provides powerful prognostic information beyond pretreatment characteristics such as cytogenetic or molecular abnormalities.[7] Nonetheless, in adult AML MRD as a tool to fine-tune risk assessment during postremission therapy with adaption of treatment strategy is lagging behind acute lymphoblastic leukemia (ALL), acute promyelocytic leukemia (APL), or chronic myeloid leukemia (CML), in which MRD is now routinely used to guide treatment decisions at predefined check-points during therapy.[8–12] Here, we review genetic abnormalities that should be used for treatment stratification in AML and their clinical implications.

2 | CORE-BINDING FACTOR LEUKEMIA

In this category, AMLs characterized by the balanced translocation t(8;21)(q22;q22.1) and with the pericentric inversion inv(16) (p13.1q22) or the less frequent balanced translocation t(16;16) (p13.1;q22) are grouped and considered as AML regardless of bone marrow blast cell counts at diagnosis.[6] Both t(8;21), involving *RUNX1-RUNX1T1,* and inv(16)/t(16;16), involving *CBFB-MYH11,* harbor chimeric fusions involving genes of the core-binding factor (CBF) complex, a major regulator of hematopoiesis, providing the common designation CBF-AML. Patients with CBF-AML are considered to have a favorable prognosis as compared to other genetic risk groups.[13] After an anthracycline- and cytarabine-based induction chemotherapy, about 90% of CBF-AML patients achieve a complete remission (CR)[14,15] and repeated cycles of postremission chemotherapy with high-dose cytarabine (HiDAC) (usually 3–4) have emerged as preferred treatment of CBF-AML.[16–18] The relapse risk may not exceed 20–35% in 3–5 years with repeated courses of intensive consolidation therapy.[15,19–22] Consequently, these patients have, on average, no

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survival advantage with allogeneic stem cell transplantation (allo-SCT) while in first remission because the transplant-related mortality is greater than the decrease in relapse rates afforded by the transplant.[23,24]

Recently published data from the Medical Research Council (MRC) AML15 trial in younger adult patients with favorable-risk AML indicated an improved survival after therapy with fludarabine, cytarabine, granulocyte colony-stimulating factor, and idarubicin (FLAG-Ida) followed by two cycles of HiDAC consolidation with an 8-year survival rate of 95%.[25] In addition, the combination of gemtuzumab ozogamicin (GO) with chemotherapy has been shown to improve overall survival (OS) in subgroup analysis of CBF-AML,[26–28] suggesting that higher intensity regimens may lead to deeper log reductions after the first course of chemotherapy as has been shown for the addition of GO to intensive chemotherapy[20] as well as for anthracycline dose intensification during intensive induction chemotherapy.[29] Patients who received daunorubicin of 90 mg/m^2 showed a faster and deeper MRD reduction and achieved a higher proportion of complete molecular responses (at least 3 log reduction as compared to diagnosis as measured by real-time quantitative polymerase chain reaction (RT-qPCR) with at least 10^{-4} sensitivity) that translated into a reduced relapse rate as compared to those patients receiving 60 mg/m^2. As a consequence, the combination of FLAG-GO (GO 3 mg/m^2 intravenously on day 1 during induction and two of six postremission cycles) has been evaluated as front-line therapy in 45 younger adult patients (median age, 48 years) with CBF-AML.[30] This regimen resulted in a high CR rate of 95% with 5% induction deaths. The OS and relapse-free survival (RFS) probability at 3 years were 78% and 85%, respectively, suggesting that a deeper molecular remission could be achieved as compared to the standard HiDAC approach, though no head-to-head comparison of the reduction in measurable disease (MRD) according to treatment strategy had been performed. However, even though MRD-positive CBF-AML patients have higher relapse rates, it has only been shown that therapy intensification based upon the presence of either MRD or pretreatment mutations like *KIT* improves event-free survival (EFS), but not OS, which is in part reflected by a high response rate to salvage chemotherapy.[31] Taken together, CBF-AML is a very chemo-responsive disease so, in this case, more chemotherapy (whichever we choose—HiDAC, FLAG-Ida, dose-intensified daunorubicin, or FLAG-GO) in patients who can tolerate it will lead to a higher cure rate.

In addition, several variables associated with worse outcome have been recognized in at least some studies, including a high white blood cell count (WBC) and the presence of *KIT* or *FLT3* mutations at diagnosis.[15,32–36] In CBF-AML, *KIT* mutations occur in about 20–30% of the patients and have been associated with an adverse prognostic impact on survival.[33,34] However, based on a previous report on AML with inv(16)/t(16,16), the unfavorable impact on relapse rate did not translate into an inferior survival.[37] In contrast, AML with inv(16)/t(16,16) harboring additional *FLT3* mutations was associated with a strong negative impact in multivariable analysis on OS.[37] In a study by Boissel et al[35] who evaluated 103 pediatric and adult patients with CBF-AML, *KIT* mutations occurred in 17% and were associated with a shorter EFS and RFS ($P = 0.002$ and $P = 0.003$) in t(8;21) but not inv(16) patients. Again, *FLT3* mutations (1% ITD; 7% TKD835) were significantly associated with a shorter EFS and OS ($P < 0.0001$ and $P = 0.0002$), owing to an excess of early events. In addition, Allen et al[38] noted that the relative *KIT* mutant level should be taken into account, since in

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their analysis on 354 younger adult patients with CBF-AML, only *KIT* mutations with a mutant level greater than 25% increased the risk of relapse. Thus, due to the inconsistencies in the available data, cooperating gene mutations in CBF-AML should currently not be used to guide treatment decisions.

In addition, the MRD level seems to be an important prognostic factor. Recent studies have highlighted the heterogeneity of the disease by identifying subsets of patients with distinct risks of disease recurrence based on the degree of reduction in *RUNX1-RUNX1T1/CBFB-MYH11* transcripts.[20–22] Currently available evidence suggests that optimal outcomes are achieved when patients with CBF-AML obtain either a molecular remission by RT-qPCR or very significant reductions in *RUNX1-RUNX1T1/CBFB-MYH11* transcripts with induction and postremission therapy (defined as at least a > 3 log reduction of transcript levels from baseline after consolidation therapy);[20,22,39] higher intensity regimens may lead to deeper log reductions after chemotherapy.[20,22,40] Moreover, emerging evidence from a study by Jourdan et al[39] suggests that information from post-treatment *RUNX1-RUNX1T1* transcript levels may be preferable over high WBC or *KIT*/*FLT3* mutational status to identify patients with high-risk t(8;21)(q22;q22) AML, as only MRD but not the other factors had a significant prognostic impact in multivariate analyses.

3 | ACUTE PROMYELOCYTIC LEUKEMIA

Acute promyelocytic leukemia (APL) with the balanced translocation t(15;17)(q22;q12) involving *PML-RARA* accounts for 5–8% of AML cases with a lower incidence in elderly patients and is considered as AML regardless of bone marrow blast cell counts at diagnosis.[6] In APL, molecular assessment of disease response has become standard practice, and MRD-directed therapy quite plausibly improves outcome, particularly in patients with high-risk disease.[41,42] The chemotherapy-free regimen with arsenic trioxide and all-trans retinoic acid has proven to be highly effective in de novo APL and has become standard first-line therapy in younger adult, non-high-risk (pretreatment WBC 10×10^9/L) patients, providing the first paradigm of a molecularly targeted treatment.[43] Nevertheless, early death is still a major issue in APL, particularly in older patients, emphasizing the need of rapid diagnostics and supportive care together with immediate access to ATRA-based therapy. To date, data on the impact of additional chromosomal or genetic aberrations including *FLT3* mutations on outcome in APL patients are still conflicting.[43–52] In addition, there seems to be an association of higher WBC at diagnosis and *FLT3*-ITD mutations in patients with APL as compared to *FLT3* wild-type patients,[48–52] which may affect outcome. However, recently published data by Cicconi et al[49] indicate that *FLT3*-ITD mutations have no prognostic impact in APL patients treated with ATO/ATRA. Taken together, due to the inconsistencies in the available data and due to its high cure rate, there is currently no convincing evidence that *FLT3* mutations should currently be used to guide treatment decisions in APL.

4 | AML WITH t(6;9)(p23;q34.1)/*DEK-NUP214*

The translocation t(6;9)(p23;q34.1), which results in formation of the *DEK-NUP214* chimeric fusion gene, was first described in AML in 1976[53] and acts as an aberrant transcription factor.[54] It alters the nuclear transport by binding soluble transport factors[55]

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and has been reported to enhance protein synthesis in myeloid cells.[56,57] In AML, t(6;9) accounts for only a very small subgroup (1–2%) and has been associated with a poor prognosis with 5-year OS rates of 28% reported in children and only 9% in adults.[58] Overall, outcome in both pediatric and adult AML with t(6;9) is poor[58–62] and adult patients with this translocation are assigned to the adverse-risk group.[13] Of note, up to 90% of AML patients with t(6;9) are described to harbor a concomitant *FLT3*-ITD,[58–60] whereas secondary cytogenetic abnormalities occur in roughly 20%.[58,61] Currently, the impact of a concurrent *FLT3*-ITD is controversial. While results of a meta-analysis of 50 adult AML patients indicated an association between *FLT3*-ITD and an inferior outcome in t(6;9) AML,[63] others were inconclusive due to the low number of *FLT3*-ITD-unmutated patients,[58] or have not found a significant adverse impact.[60,61]

However, allo-SCT may improve survival if applied early during first CR.[58,64] In a matched-pair analysis of de novo AML using data from the Japanese allo-SCT data registry, the outcome of 57 patients with t(6;9) was compared to that of 171 patients with normal karyotype.[64] All patients received an allo-SCT between 1996 and 2007, either in first or second CR (CR1 and CR2, n = 116), or as salvage therapy (n = 112). In this matched-pair analysis in patients with t(6;9), the 5-year OS (45% vs 40%), disease-free survival (42% vs 33%), cumulative incidence of relapse (42 vs 45%), and non-relapse mortality (16% vs 22%) were not different as compared to normal karyotype AML.[64] In addition, we have recently evaluated the impact of allo-SCT on outcome in n = 123 adult patients with t(6;9) in a large, international collaborative analysis.[65] In our cohort, we observed a high CR rate of 79% including sixteen patients, who required an intensive salvage treatment cycle with HiDAC. Five-year OS rates after allo-SCT (n = 51) were very encouraging (55% vs 18%; $P < 0.001$) as compared to patients who received consolidation chemotherapy (n = 44) regardless of *FLT3*-ITD status, additional cytogenetic abnormalities, or timing of transplant. These results suggest that allo-SCT may overcome the adverse impact of t(6;9) in AML patients, rendering outcome comparable to patients with normal cytogenetics.

5 | AML WITH inv(3)(q21.3q26.2) OR t(3;3) (q21.3;q26.2); *GATA2, MECOM*

AML with inv(3) or t(3;3) accounts for 1–2% of all AML and occurs most commonly in adults.[6] AML with inv(3)/t(3;3) is most frequently associated with monosomy 7 or complex karyotype (3 chromosomal abnormalities in the absence of one of the WHO designated recurring translocations or inversions) and confers an unfavorable outcome.[66] Genetically, inv(3)/t(3;3) involves *MECOM* (also termed *EVI1*) at 3q26.2 and repositions the GATA2 enhancer to activate MECOM expression, thereby inducing GATA2 haploinsufficiency.[67] Gröschel et al[68] showed that 98% of inv(3)/t(3;3) myeloid malignancies harbor mutations in genes activating RAS/receptor tyrosine kinase (RTK) signaling pathways. This high incidence of RAS/RTK signaling pathways may provide a target for a rational treatment strategy in this high-risk patient group. In addition, the authors could show that hemizygous mutations in *GATA2*, as well as heterozygous alterations in *RUNX1*, *SF3B1,* and genes encoding epigenetic modifiers, frequently co-occur with inv(3)/t(3;3) aberrations.[68] Within their analysis, neither mutational patterns nor gene expression profiles differed across inv(3)/t(3;3) AML, CML, or myelodysplastic syndrome cases. The authors therefore suggested that inv(3)/t(3;3) myeloid malignancies should be regarded as a single disease entity irrespective

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of blast count.[68] From a clinical aspect, however, there is currently no specific treatment available that has shown convincing improvement of outcome of this high-risk disease with only about one-third of patients with inv(3)(t(3;3) achieving a CR despite intensive induction therapy and few, if any, long-term survivors.[66] Thus, investigational therapy within a clinical trial should be considered in these patients, whenever possible.

6 | AML (MEGAKARYOBLASTIC) WITH t(1;22)(p13.3;q13.1)/*RBM15-MKL1*

Acute megakaryoblastic leukemia (AMKL) with t(1;22)(pp13.3;q13.1) is a subtype of AML in which the cells morphologically resemble abnormal megakaryoblasts. While AMKL is very rare in adults (~1%),[69] it accounts for 4–15% of newly diagnosed childhood AML.[69,70] In addition, congenital cases have been described[71] as well as the occurrence in patients with Down syndrome.[6] In most cases, t(1;22) (p13.3;q13.1) is the sole abnormality, leading to the fusion gene *RBM15-MKL1*.[6] This fusion gene seems to be involved in modulation of chromatin organization, HOX-induced differentiation, and extra-cellular signaling pathways.[72]

Regarding outcome, data are contradictory with some reports indicating that childhood patients with t(1;22)(p13.3;q13.1) may respond well to intensive chemotherapy,[73–77] but other studies indicated that this entity seems to be associated with a high-risk and inferior survival as compared to patients without t(1;22) (p13.3;q13.1).[78,79] This might be attributable to the high rate of early deaths in patients with t(1;22)(p13.3;q13.1), particularly due to their very young age at diagnosis.[78] Interestingly, in the publication by Schweitzer et al[79] allo-SCT in first CR did not provide a significant survival benefit. Recently, de Rooij et al[80] have evaluated cooperating mutations in 75 pediatric and 24 adult patients with non-Down syndrome AMKL by RNA and exome sequencing analysis to gain further insights into the genomic alterations that lead to non-Down syndrome AMKL. They identified chimeric oncogenes in a substantial number, including *CBFA2T3-GLIS2* (18.6%), *KMT2A* gene rearrangements (17.4%), *NUP98-KDM5A* (11.6%), and *RBM15-MKL1* (10.5%). They could show that outcome was based on chimeric oncogenes and cooperating mutations in epigenetic and kinase signaling genes, such as *GATA1* (13.3%), *Cohesin* or *CTCF* genes (18.1%), *JAK/STAT* genes (16.9%), and *RAS* pathway genes (15.7%). Of note, all *GATA1*-mutated cases which lacked a fusion gene were cured, mimicking the excellent outcomes observed in Down syndrome AMKL.[81] In contrast, patients with *CBFA2T3-GLIS2*, *KMT2A* gene rearrangements, and *NUP98-KDM5A* were associated with a poor prognosis and should therefore be candidates for allo-SCT.[80]

7 | AML WITH *BCR-ABL1*

BCR-ABL1-mutated AML again is a rare subtype (<1%) that is now included as a provisional entity in the current WHO classification.[6] Since a clear distinction between de novo *BCR-ABL*-mutated AML and CML in blast crisis is challenging in many cases, the existence of de novo *BCR-ABL*-mutated AML has been a matter of debate for a long time. However, increasing evidence suggests that *BCR-ABL*-mutated AML is in fact a distinct subgroup of AML.[82,83] Preliminary data suggest that deletion of antigen receptor genes

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(immunoglobulin heavy chain and T-cell receptor), *IKZF1*, and/or *CDKN2A* may support a diagnosis of AML rather than CML blast phase.[84]

The prognosis of *BCR-ABL*-mutated AML seems to depend on the cytogenetic and/or molecular background rather than on *BCR-ABL* itself.[84] A therapy with tyrosine kinase inhibitors (TKIs) such as imatinib, dasatinib, or nilotinib is reasonable,[85] but their use cannot be routinely recommended in first-line therapy due to a lack of systematic clinical data. However, intensive chemotherapy in combination with TKI[86,87] and/or as a bridge to allo-SCT seems to be a feasible approach.

8 | AML WITH GENE MUTATIONS

8.1 | AML with mutated *NPM1*

Frameshift mutations of the *NPM1* gene are one of the most frequent molecular abnormalities in AML, particularly in patients with a normal karyotype[88] with a high incidence in both young and older AML patients.[89–91] *NPM1* mutations result in cytoplasmic accumulation, regulating the ARF-p53 tumor suppressor pathway, thus controlling proliferation and apoptosis.[92] In contrast to other molecular aberrations (eg, *FLT3*), *NPM1* mutations are typically stable during the course of the disease, which supports the notion that they are an early pathogenetic lesion in AML.[93] To date, more than 50 different *NPM1* mutations have been reported; however, the subtypes A, B, and D comprise 90% of all variants.[94] These three mutation subtypes have been shown to be reliable markers for MRD detection with high sensitivity.[7,95] The same assay can be adapted for cases with rare *NPM1* mutation variants by replacing mutation-specific primers, but case-specific RT-qPCRs need to be carefully established to avoid non-specific background amplification from the wild-type *NPM1* allele.[96]

Concurrent mutations occur typically in *FLT3, DNMT3A, IDH1/2,* or *TET2*.[88] In several studies, it has been shown that the prognostic impact of *NPM1* should be interpreted in the context of a cooperating *FLT3*-ITD mutation, which is present in approximately 45% of this patient population with normal karyotype.[97–99] In particular, in younger adult *NPM1*-mutated patients with high *FLT3*-ITD allelic ratio (0.5)[13,100–102] the favorable prognostic effect of *NPM1* is mitigated or even abolished as compared to patients with a low allelic ratio.[21,101,102] In comparison, patients with mutated *NPM1* without *FLT3*-ITD or *FLT3*-ITD with a low allelic ratio (< 0.5) are associated with a somewhat better outcome.[13,102] These data have recently been confirmed in a large cohort of intensively treated adult AML patients.[103] However, in patients with mutated *NPM1* without *FLT3*-ITD two reports from cooperative study groups showed a negative impact of cooperating IDH1/2 mutations on RFS and OS.[104,105] In contrast, Patel et al[2] reported on a favorable impact of mutated *NPM1* without *FLT3*-ITD only if cooperating *IDH1/2* mutations were present. Such opposed effects of genotypes on outcome highlight statistical shortcomings of retrospective molecular studies.

Similar to the findings in CBF-AML, RT-qPCR assessment of MRD can distinguish patients at high risk of relapse: In a study on 245 adult patients with *NPM1*-mutated AML, relevant MRD check-points could be defined.[106] Achievement of RT-qPCR negativity after two

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courses of induction therapy identified patients with a low CIR (6.5% after 4 years) as compared to RT-qPCR-positive patients (53% after 4 years; $P < 0.001$), translating into significant differences in OS (90% vs 51%, respectively; $P = 0.001$). After completion of therapy, CIR was 15.7% in RT-qPCR-negative patients as compared to 66.5% in RT-qPCR-positive patients ($P < 0.001$).[106] These data are extended by the study of Hubmann et al[107] in whom a *NPM1* mutation cutoff level of 0.01 after induction therapy as measured by RT-qPCR (with a sensitivity of 10^{-6}) was associated with a CIR after 2 years of 77.8% for patients with ratios above as compared to 26.4% for those with ratios below the cutoff. Within the ALFA-0701 trial, *NPM1* MRD positivity as measured by RT-qPCR (quantitative detection limit of 0.1%) after one course of standard induction chemotherapy according to the 7 + 3 scheme with or without GO was associated with an increased cause-specific hazard of relapse of 3.66 ($P = 0.035$). Of note, *NPM1*-MRD was predictive for response to therapy since more MRD-negative results were obtained in patients treated in the GO arm as compared to those treated in the control arm after induction therapy (39% vs 7%; $P = 0.006$) as well as at the end of treatment (91% vs 61%; $P = 0.028$).[108] This is one of the first randomized studies indicating that MRD assessment may serve as a surrogate for survival endpoints for the treatment under investigation. Additionally, in a retrospective analysis performed by the German Study Alliance Leukemia, increasing levels of *NPM1* MRD were predictive of an impending relapse after chemotherapy (MRD increase >1% *NPM1*mut/ABL1) or allo-SCT (MRD increase >10% *NPM1*mut/ABL1).[109] Importantly, MRD status has been found to be a better predictor of the relapse risk than *FLT3*-ITD in *NPM1*-mutated AML.[110] Besides, different studies have convincingly demonstrated that MRD positivity by RT-qPCR before allo-SCT is independently associated with a significantly increased risk of relapse and inferior survival.[111,112] Assuming that a further reduction of MRD levels optimizes outcome after allo-SCT, this relationship would justify risk-stratified treatment allocation, including the use of additional pretransplant chemotherapy. However, as MRD might simply reflect reduced sensitivity of leukemia cells to chemotherapy, the presence of residual disease might only mark those patients who are unlikely to be cured with subsequent similar-type therapies, even if disease levels are brought temporarily below the level of detection. Therefore, another approach could be pre-emptive immune[113] or antibody therapy (ClinicalTrials.gov Identifier: NCT02789254) in MRD-positive patients, which has successfully been demonstrated in childhood AML with mixed chimerism after allo-SCT,[114] or by post-transplant application of demethylating agents, such as azacitidine, to prevent imminent relapse in MRD-positive patients.[115]

8.2 | AML with biallelic mutation of *CEBPA*

The CCAAT/enhancer binding protein alpha (CEBPA) gene encodes a transcription factor for granulocyte differentiation. *CEBPA* mutations prevent DNA binding, resulting in a lack of granulocyte differentiation.[116] *CEBPA* mutations have been reported in roughly 10% of AML patients and half of them comprise biallelic mutations, generally involving both the N- and C-terminal domains.[117] The frequency of biallelic *CEBPA* mutations seems to be age-dependent and decreases with increasing age.[118–121] Several studies have shown convincingly that AML with biallelic mutation of *CEBPA* can be distinguished from AML with single mutated *CEBPA* with respect to biological and prognostic features and that the favorable prognostic impact of mutated *CEBPA* can be attributed to the subtype of AML

with biallelic *CEBPA* mutations.[118–121] In this subgroup of patients, CR rates of 85% to 92% have been reported; however, these results are mainly based on younger adults.[118–121] Therefore, the current WHO classification has included AML with biallelic mutated *CEBPA* as a separate entity.[6] Concurrent mutations most frequently include *TET2* (34%), followed by *GATA2* (21%), *WT1* (13.7%), *DNMT3A* (9.6%), *ASXL1* (9.5%), *RAS* mutations (*NRAS*, 8.4%; *KRAS,* 3.2%), *IDH1/2, and FLT3*-ITD (6.3%, each).[122] Regarding the prognostic impact, concurrent *TET2* mutations were associated with an unfavorable impact on OS as compared to *TET2* wild-type patients ($P = 0.035$), whereas *GATA2* mutations had a favorable impact ($P = 0.032$). The favorable prognostic impact of biallelic mutated *CEBPA* gets lost in the presence of *FLT3*-ITD.[119,120,122] Given the high CR rate after reinduction therapy in younger adult relapsed AML patients with biallelic mutated *CEBPA* of 83% (n = 35/42) and favorable outcome after allo-SCT in CR2,[123] we recommend HiDAC-based consolidation chemotherapy in patients with biallelic mutated *CEBPA* in CR1.

8.3 | AML with mutated *RUNX1*

The Runt-related transcription factor 1 (*RUNX1*) gene encodes a transcription factor critical for hematopoiesis and was associated with embryonic lethality in a murine model.[124] In AML, *RUNX1* mutations have been reported to occur with an incidence of 5.6%15 to 13.2%,[125] predominantly in patients with intermediate-risk cytogenetics. In patients with cytogenetically normal AML, the incidence seems to increase with higher age, with an incidence of 8% in younger patients compared with 16% in older patients.[126] Interestingly, *RUNX1* mutations are almost mutually exclusive of other disease-defining genetic aberrations such as *NPM1,* biallelic mutated *CEBPA, CBFB-MYH11, RUNX1-RUNX1T1,* and *PML-RARA.*[126–128] In addition, *RUNX1* mutations are characterized by a distinct gene expression pattern,[125,127] and monoallelic germline mutations have been reported in rare cases of familial platelet disorder with predisposition to AML,[128] further supporting the idea of a separate disease entity. Therefore, the new provisional entity "AML with mutated *RUNX1*" (excluding cases with myelodysplasia-related changes) was added to the current WHO classification.[6] Approximately two-thirds of *RUNX1* mutations are found in cytogenetically normal AML and have been associated with a very unfavorable prognosis in both young and elderly patients.[126,127] Thus, *RUNX1* was added to the adverse-risk group.[13]

8.4 | AML with *TP53*

The tumor protein p53 (TP53) encodes a transcription factor, which is involved in cell cycle arrest and apoptosis.[129] TP53 mutations occur in roughly 12% of AML patients,[130] predominantly in therapy-related or secondary AML as well as in elderly patients.[131] Moreover, TP53 alterations are found in roughly 70% of AML patients with a complex karyotype.[132] TP53 alterations predict for very low CR rates (less than 30%) and were shown to be an independent poor prognostic factor among the subgroup of AML with complex karyotype.[132] Interestingly, TP53 could be identified in hematopoietic stem and progenitor cells (HSPC) in chemotherapy-naïve controls and in therapy-related or secondary AML patients years prior to development of overt disease, suggesting that HSPC carrying TP53 may be chemotherapy-resistant and expand after treatment.[133] Individuals with clonal hematopoiesis with indeterminate potential have a 13-fold increased risk of developing a hematologic malignancy, and the data by Wong et al[133] suggest that this risk is increased in

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the context of cytotoxic therapy, at least if a *TP53* mutation is present. Recently published data suggest that treatment with decitabine at a dose of 20 mg/m^2 per day for 10 consecutive days in monthly cycles may improve the dismal outcome of AML with TP53 alterations.[134] Although these responses were not durable, they resulted in OS rates that were similar to those among patients with AML who had an intermediate-risk cytogenetic profile and who also received serial 10-day courses of decitabine.[134] Recent data from 2 phase I trials suggest a high response rate after the combination therapy of venetoclax, a BCL2 inhibitor, with either decitabine, azacitidine (ClinicalTrials.gov identifier: NCT02203773),[135] or low-dose cytarabine[136] in newly diagnosed elderly (60 years) AML patients not eligible for intensive chemotherapy, a group in whom a high incidence of TP53 mutations would be suspected. After venetoclax and low-dose cytarabine, the overall response rate was 61% with 54% in patients achieving CR/CRi.[136] Venetoclax and low-dose cytarabine were shown to be active across a wide range of cytogenetic mutations and patient profiles (overall response rate: 70% in patients 75 years; 52% in secondary AML; 47% in patients with adverse karyotypes; 53% in patients previously treated with hypomethylating agents). The OS was estimated to be 79% at 6 months and 70% at 12 months, with the median not reached.[135] Based on these encouraging data, venetoclax received breakthrough designation by the Food & Drug Administration (FDA) in combination with cytarabine for the treatment of elderly patients with treatment-naïve AML not eligible for intensive chemotherapy and a large, international randomized phase III study evaluating azacitidine with or without venetoclax (planned inclusion number: n = 400; ClinicalTrials.gov identifier: NCT02993523) is currently recruiting patients. In addition, a large, international phase III randomized trial of venetoclax in combination with low-dose cytarabine vs low-dose cytarabine alone in treatment-naïve AML patients ineligible for intensive chemotherapy has started recruiting (ClinicalTrials.gov Identifier: NCT03069352). Moreover, venetoclax is currently evaluated at the MD Anderson in a phase Ib/II study in combination with intensive chemotherapy with FLAG-IDA in patients with newly diagnosed or relapsed/refractory AML (ClinicalTrials.gov Identifier: NCT03214562).

8.5 | *FLT3*-mutated AML

Activating *FLT3* mutations are one of the most frequently affected genetic abnormalities in AML and are present in about 30% of newly diagnosed patients.[3] As a member of the type III receptor tyrosine kinase subfamily, including c-KIT, c-FMS, and PDGFR-α/β, it is involved in proliferation and differentiation of myeloid progenitor cells.[137] Mutations of the *FLT3* gene lead to ligand-independent activation and dysregulation of downstream pathways such as PI3K/AKT, MAPK/ERK, and STAT5.[138–140] Clinically, *FLT3* mutations are associated with high WBC count and myeloid blast cells in bone marrow and peripheral blood and a more frequent diagnosis of de novo rather than secondary AML.[141] In cytogenetically normal AML, *FLT3*-ITD confers an unfavorable prognosis due to a high relapse rate and very limited options after relapse with conventional salvage regimens, whereas the prognostic impact of point mutations within the tyrosine kinase domain (*FLT3*-TKDs) remains controversial.[141] Nevertheless, *FLT3*-TKDs can occur after treatment with FLT3 TKIs as a mechanism of resistance, thus implicating an adverse prognosis.[142] Besides cytogenetically normal AML, *FLT3*-ITD is frequently associated with t(6;9)(p23;q34)[58–60] as well as with t(15;17)(q22;q12) in APL.[3]

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The incidence of *FLT3*-ITD is associated with age: Whereas it can only rarely be found in children, its incidence is highest in young adults up to the age of 60 years and declines in the elderly.[143] Regarding specific ITD characteristics, the size of these duplications varies widely, typically ranging from 3 to over 100 base pairs (bps) with a median of 48 bps.[141] In addition, size and ITD insertion site in the *FLT3* gene seem to be correlated in that the more 3' the insertion site within the *FLT3* gene, the longer the ITD.[144] The impact of the size on outcome is still unclear with some publications stating no impact on outcome,[145,146] whereas one publication found that short ITDs may impart an unfavorable outcome.[147] Nevertheless, most publications stated that longer ITDs correlate with lower CR rates and shorter OS and EFS.[148–150] In addition, the ITD insertion site within the *FLT3* gene has been shown to be an important prognostic factor and was associated with an inferior prognosis.[144] About one-third of all *FLT3*-ITDs occur within the tyrosine kinase domain 1 (TKD1) of the *FLT3* gene, in particular in the beta1-sheet.[144,151] In cell culture analyses, a prototypic *FLT3*-ITD with insertion site in the β2-sheet of the TKD1 (*FLT3*-ITD627E) mediated phosphorylation of FLT3 and STAT5, suggesting that non-JMD *FLT3*-ITD mutations confer constitutive activation of the receptor.[151] Additionally, *FLT3*-ITD627E induced transformation of hematopoietic 32D cells and led to a lethal myeloproliferative disease in a syngeneic mouse model. Insertions in the beta 1-sheet of TKD1 may introduce a greater instability into the protein structure and may therefore be associated with a pronounced adverse prognosis.[151] Besides the insertion site, further prognostic and predictive impact has been shown for the allelic ratio,[100,102] which is quantified by GeneScan analysis using DNA fragment analysis. A high allelic ratio is associated with an unfavorable impact on OS and EFS.[100,102,144] The prognostic impact of *FLT3*-ITD is also affected by concurrent mutations, such as *NPM1* and *DNMT3A*. In normal karyotype AML with *NPM1* mutation, *FLT3*-ITDs are present in about 45% of patients.[97,99] Recent recommendations from the ELN include a revised version of the risk stratification according to genetics including the *FLT3*-ITD allelic ratio (Table 1).[13] It should be noted, however, that no internationally standardized method of determining the allelic ratio has been established, and it is not routinely reported by many or even most commercial diagnostic laboratories. This is problematic for a practitioner attempting to managing these patients according to these ELN guidelines.

In addition, *NUP98/NSD1* has been described to have a further independent prognostic impact in *FLT3*-ITD-mutated AML.[152,153] In 1421 patients from six Children Oncology Group/Southwest Oncology Group trials, *NUP98/NSD1* was detected in 15% (37 of 253) of *FLT3*-ITD and 7% (26 of 367) of cytogenetically normal AMLs. Patients with *FLT3*-ITD and *NUP98/NSD1* (82% of *NUP98/NSD1* patients) had a CR rate of 27% as compared to 69% in *FLT3*-ITD without *NUP98/NSD1* ($P < 0.001$). The corresponding 3-year OS rate was 31% as compared to 48% ($P = 0.011$), respectively.[154] Moreover, *WT1* mutations were enriched in patients with *FLT3-ITD* and *NUP98/NSD1*, with a prevalence of 31% as compared to 17% in those with *FLT3-ITD* only ($P = 0.047$). The authors suggest that additional genetic lesions (ie, *WT1+*) might further impact response to therapy and outcome of patients harboring *NUP98/NSD1* and *FLT3-ITD*. However, this finding needs to be confirmed in an independent cohort.

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Pretherapeutic molecular testing for *NPM1* and *FLT*3 is considered standard of care to determine the best treatment option. Whereas *NPM1* has been shown to be a reliable marker for MRD detection with high sensitivity,[94–97] the suitability of *FLT3*-ITD for MRD detection has been questioned. First, *FLT3*-ITD mutations display substantial heterogeneity in terms of size, number of clones per patient, allelic ratio, and insertion site within the *FLT3* gene and second, its proposed instability (reported on about 25% of paired diagnosis-relapse samples) during the course of treatment.

Current methods used to determine *FLT3*-ITD mutations have limited sensitivity and are not suitable for MRD detection. Newer techniques, such as RT-qPCR with patient-specific primers, aim to improve the sensitivity of *FLT3*-ITD.[155] Nevertheless, this approach has limitations, since each *FLT3*-ITD mutation needs a clone-specific primer/probe set, which is time-consuming and may not be possible in every case. In addition, direct sequencing may be hampered due to low allelic ratio since the wild-type sequence is competitively amplified. Recently, another PCR-based assay for *FLT3*-ITD MRD was reported.[156,157] This assay employed primers oriented in the opposite direction; hence, amplification occurred only if a *FLT3*-ITD was present. Again, this approach has limitations since short *FLT3*-ITDs (less than 30–40 bases) are not detected due to insufficient primer annealing space, which may apply to roughly 25% of all *FLT3*-ITD cases. Both approaches are therefore not ready to be implemented in clinical routine care. Next-generation sequencing (NGS) is potentially useful[158,159] since it can identify clonal populations but generates complex data, which is still expensive and requires considerable expertise to interpret. Additionally, NGS gives only an estimate on the allelic ratio. In patients with a concurrent *NPM1* mutation, however, MRD can be assessed by analysis of *NPM1*-mutated transcripts. In summary, *FLT3* mutational testing should be mandatory in all AML patients at diagnosis as well as at relapse for prognostic purposes and for guiding therapeutic decisions. At present, it has little utility for MRD monitoring until different methodologies can be standardized.

Regarding postremission therapy in *FLT3*-ITD AML, allo-SCT has been shown to improve outcomes particular in patients with a high allelic ratio.[160–162] Nevertheless, recent studies indicate that AML patients with *NPM1* mutation and low *FLT3*-ITD allelic ratio may have a more favorable prognosis and should therefore not routinely be assigned to allo-SCT.[103,163,164] In contrast, an ITD insertion site in the TKD1 remained an unfavorable prognostic factor regardless of the applied therapy.[102]

Currently, midostaurin (Rydapt®, Novartis Pharmaceuticals, Inc.) is the first approved TKI in combination with standard intensive chemotherapy for adult patients without age restriction with newly diagnosed *FLT3*-mutated AML in the United States and Europe. The approval of midostaurin was based on the positive results from the large, international randomized phase III trial.[163] The combination of midostaurin with intensive chemotherapy significantly improved OS in younger adults with *FLT3*-mutated AML translating into a median OS of 74.7 months for the midostaurin arm (range, 31.5 months-not reached) as compared to 25.6 months for the placebo arm (range, 18.6–42.9 months), respectively.[163] Interestingly, this improvement was regardless of the *FLT3* mutational status (either ITD or TKD) or the *FLT3*-ITD allelic ratio. Furthermore, patients receiving an allo-SCT in first CR had better outcome if they were treated with midostaurin during induction therapy ($P =$

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0.08), suggesting that the optimal treatment strategy in *FLT3*-mutated AML would be to move on to allo-SCT early in first CR. Given the remarkable difference in survival after allo-SCT early in first CR in patients treated with midostaurin as compared to those treated with placebo, it is tempting to speculate that the combination of midostaurin with intensive chemotherapy results in deeper remissions.

Nevertheless, a significant proportion of patients within the CALGB 10603/RATIFY trial still relapsed within the first two years, even in the midostaurin arm,[163] raising the question as to whether or not TKIs with higher FLT3 selectivity would be more efficient. Currently, various other, more selective FLT3 inhibitors, such as quizartinib,[164,165] crenolanib,[166] and gilteritinib,[167] are in clinical evaluation. Overall, these second-generation inhibitors are significantly more potent and selective with respect to FLT3 inhibition as compared to midostaurin. Quizartinib is an oral selective FLT3 inhibitor currently in phase III development for relapsed/refractory (QuANTUM-R; ClinicalTrials.gov identifier: NCT02668653) and newly diagnosed (QuANTUM-First; ClinicalTrials.gov identifier: NCT02668653) *FLT3*-ITD AML in the United States, EU, and Japan, and phase II development for relapsed/refractory *FLT3*-ITD AML in Japan. Recently, the large, randomized international phase III trial QuANTUM-R evaluating quizartinib monotherapy vs salvage chemotherapy in relapsed/refractory *FLT3*-ITD-positive AML patients (age >18 years) has reached its planned inclusion number of 367 patients.[168] Allowed salvage chemotherapy regimens were low-dose cytarabine; mitoxantrone, etoposide, and intermediate-dose cytarabine (MEC); or FLAG-IDA. Up to 2 cycles of MEC or FLAG-IDA were permitted; quizartinib and low-dose cytarabine were given until lack of benefit, unacceptable toxicity, or allo-SCT. Quizartinib significantly prolonged OS as a single agent compared to salvage chemotherapy in patients with relapsed/refractory *FLT3*-ITD AML (27 weeks vs 20.4 weeks).[168] Based on these results, quizartinib was granted breakthrough therapy designation by the FDA on 1 August 2018. Quizartinib has also been granted fast track designation by the FDA for the treatment of relapsed/refractory AML. Additionally, a large, randomized, international phase III trial (QuANTUM-First) tests quizartinib as compared to placebo for newly diagnosed *FLT3*-ITD AML patients in the United States and Europe (age range: 18–75 years, planned inclusion number: n = 536). The trial design is similar to the CALGB 10603/RATIFY trial: Quizartinib or placebo is administered in combination with standard intensive induction chemotherapy and up to four cycles of consolidation with cytarabine and/or allo-SCT including up to 12 months of maintenance therapy. Recruitment was initiated in 09/2016 and is ongoing.

In addition, gilteritinib has been granted fast track designation by the FDA for the treatment of adult patients with relapsed/refractory *FLT3*-mutated AML as detected by an FDA-approved test based on the randomized phase III ADMIRAL trial (ClinicalTrials.gov identifier: NCT02421939). The trial design is comparable to the QuANTUM-R trial; currently, the trial has reached its planned inclusion number (n = 371). Both quizartinib and gilteritinib are under FDA review and likely will become available for therapy in relapsed/refractory AML.

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9 | ISOCITRATE DEHYDROGENASE (IDH)

Mutations in *IDH1* and *IDH2* are detected in about 8% and 12% of patients with AML, respectively.[88] *IDH1* mutations almost exclusively occur at R132 while *IDH2* involve substitutions at R140 or R172.[169] These mutations tend to occur early in disease pathogenesis[170] and affect the active site of the IDH enzymes that exist in the cytoplasm (IDH1) and the mitochondria (IDH2). Functionally, *IDH* mutations result in arrest of hematopoietic differentiation due to increased levels of the oncometabolite 2-hydroxyglutarate leading to DNA hypermethylation via inhibition of histone demethylation.[169,171,172] *IDH* mutations are associated with higher platelet counts,[173,174] lower median WBC counts,[104] intermediate-risk, or normal karyotype cytogenetics, and less frequently occur in therapy-related AML.[173,174] Genetically, *IDH* mutations are associated with *NPM1* mutations,[105] but less frequently co-occur with *TET2* or *WT1* mutations, presumably because all three classes of mutations affect DNA methylation.[2,175]

Regarding outcome in *IDH*-mutated AML, data are conflicting. For instance, in AML exhibiting the genotype mutated *NPM1* with unmutated *FLT3*-ITD two reports from cooperative study groups showed a negative impact of cooperating *IDH1/2* mutations on relapse-free survival and OS.[104,105] In contrast, Patel et al[2] reported on a favorable impact of the genotype mutated *NPM1* with unmutated *FLT3*-ITD only if cooperating *IDH1/2* mutations were present. The effects on survival are likely distinct for each of the *IDH* mutations, with the presence or absence of other mutations also affecting outcomes. Additionally, such opposed effects of genotypes on outcome highlight statistical shortcomings of retrospective molecular studies.

The prognosis and treatment of *IDH*-mutated AML may also be changed by IDH inhibitors, which have been associated with responses in the relapsed setting and are now being added to induction chemotherapy in upcoming trails. Enasidenib, a potent IDH2 inhibitor (formerly known as AG-221/CC-90007), received regulatory approval in the United States on 1 August 2017 for the treatment of relapsed/refractory AML with an *IDH2* mutation as detected by an FDA-approved test. The recommended dose of enasidenib is 100 mg orally once daily until disease progression or unacceptable toxicity. Analysis of paired diagnosis/relapse samples did not identify second-site mutations in *IDH2* at relapse.[176] Instead, relapse arose by clonal evolution or selection of terminal or ancestral clones, thus highlighting multiple bypass pathways that could potentially be targeted to restore differentiation arrest.

In addition, ivosidenib (Tibsovo®; formerly known as AG120-C-001) has received FDA approval on 20 July 2018 for the treatment of adult patients with relapsed/refractory *IDH1*-mutated AML. The approval was based on an open-label, single-arm, multicenter clinical trial (ClinicalTrials.gov identifier: NCT02074839) that included 179 adult patients with relapsed or refractory *IDH1*-mutated AML.[177] Ivosidenib was given orally at a starting dose of 500 mg daily until disease progression, unacceptable toxicity, or allo-SCT. The rate of CR or CR with incomplete hematologic recovery was 30.4%, and the overall response rate was 41.6%. The median treatment and response duration were 4.1 months and 8.2 months, respectively.[177] Both inhibitors are currently evaluated in several clinical trials in newly diagnosed as well as relapsed/refractory AML, as single agent or in combination with

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chemotherapy (ClinicalTrials.gov identifier: NCT02074839, NCT02073994, NCT02632708, NCT02677922). Other IDH1[178] and combined IDH1/2 inhibitors (such as AG-881, ClinicalTrials.gov identifier: NCT02492737) have also entered clinical development. Finally, Debarri et al[179] have evaluated MRD monitoring of *IDH1/2* mutations by NGS in 31 AML patients. Within their analysis, IDH1/2 mutations were reliable MRD markers that allowed the prediction of relapse in the majority of patients.

10 | LYSINE-SPECIFIC METHYLTRANSFERASE 2A (*KMT2A*)

Chromosomal rearrangement of the *KMT2A* (formerly known as mixed lineage leukemia, *MLL*) gene at 11q23 has been reported in approximately 10% of acute leukemias.[180] This rearrangement results in AMLs with predominantly monocytic or myelomonocytic phenotypes. *KMT2A* rearrangement is also associated with therapy-related myeloid neoplasm, specifically following topoisomerase II inhibitors.[181] *KMT2A* encodes a histone methyltransferase, which regulates homeobox genes affecting hematopoiesis.[182] A subtype is characterized by internal partial tandem duplication in the *KMT2A* gene (*KMT2A*-PTD). *KMT2A*-PTD often occurs in elderly patients and consists of 3%-5% of de novo AML.[183,184] *KMT2A*-PTD is generally believed to act as an oncogenic driver by modulating expression of *HOX* genes. However, mice carrying *KMT2A*-PTD alone do not develop spontaneous leukemia,[185] unless they are crossed with those harboring another major leukemogenic driver (eg, *FLT3*-ITD),[186] suggesting that by itself *KMT2A*-PTD is not sufficient to transform hematopoietic cells. Clonality analysis suggested that *KMT2A*-PTD is acquired after initiating mutations (ie, *IDH1/2, DNMT3A, TET2,* and *U2AF1*), but prior to proliferation mutations (such as *FLT3* and *RAS*).[187] *KMT2A*-PTD seems to be mutually exclusive with *DNMT3A*[2] and *NPM1* mutations.[187] However, 25% of patients harbor a *FLT3*-ITD, which may contribute to the poor prognosis.[188] Outcome of patients with acute leukemia and a translocation or PTD of the *KMT2A* gene is poor. In a study of 1897 patients with AML treated within German AML Cooperative Group trials between 1992 and 1999, 2.8% of patients were found to have a rearrangement involving 11q23 and OS rate at 3 years was 12.5%.[189]

Elegant in vitro and in vivo experiments demonstrated that a key mediator of *KMT2A*-rearranged leukemia is the histone methyltransferase DOT1L.[190–194] Similarly, preclinical studies of DOT1L inhibition in acute leukemia associated with translocations involving the *KMT2A* gene have shown remarkable effectiveness.[195,196] Translating these results to patients, however, has been more difficult. Inhibition of DOT1L with the small molecule inhibitor EPZ-5676 resulted in a CR in only two of 34 patients with a *KMT2A* rearrangement or partial tandem duplication.[197] In cell culture analysis using a *DNMT3A*-mutated cell line, inhibition of DOT1L resulted in inhibition of proliferation, induction of apoptosis, and terminal differentiation.[198] Again, the DOT1L inhibitor EPZ5676 was effective in a xenograft model as well as primary patient samples with *DNMT3A*-mutated AML, suggesting that DOT1L could be a therapeutic target.[198]

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11 | *DNMT3A* MUTATIONS

Awareness of the complexity of the leukemic genome has further been highlighted by the discovery of mutations in genes important for epigenetic regulation of gene transcription. DNA methyltransferase 3 alpha (DNMT3A) belongs to the family of DNA methyltransferase enzymes that catalyzes the addition of a methyl group to the cytosine of CpG dinucleotides, thereby regulating gene transcription. *DNMT3A* mutations can be found in 15%-25% of AML patients, particularly in AML with normal cytogenetics,[5,199–201] and are thought to be a "founder" mutation since they are present in early preleukemic hematopoietic stem cells.[202] Approximately 60% of all *DNMT3A* mutations affect residue R882,[5,199] and seem to be age-dependent with a higher incidence in elderly patients.[203] Several studies evaluated the stability of *DNMT3A* mutations in paired diagnosis and relapse material.[93] In the largest analyses, Hou et al[204] studied sequentially 316 samples from 138 patients, including 35 patients with distinct *DNMT3A* mutations and 103 patients without mutations at diagnosis. At relapse, all *DNMT3A*-mutated patients who had available samples for serial study regained the same mutations, whereas all 103 patients without *DNMT3A* mutation at diagnosis remained *DNMT3A* negative at relapse.[204] Due to its high stability, the authors claimed *DNMT3A* mutations to be a potential marker for MRD monitoring. Ploen et al[201] who developed a multiplex allele-specific quantitative PCR assay for the sensitive detection of *DNMT3A* mutations affecting residue R882 questioned the suitability as a MRD marker. Analysis of DNA from 298 diagnostic AML samples revealed *DNMT3A* mutations in 45 AML patients (15%); the mutation was stable in 12 of 13 patients presenting with relapse or secondary myelodysplastic syndrome, but was also found in remission samples from 14 patients (at allele frequencies of <1–50%) up to 8 years after initial AML diagnosis, despite the loss of all other molecular AML markers.[201] Nine of the 14 patients relapsed within a median time of 9.5 months (range 4–104 months); the five remaining patients were reported to be in continuous CR and had a long-term survival at a median time of 53 months (range 34–100 months). Due to its long-term persistence without relapse, the authors question *DNMT3A* mutations as a suitable biomarker for AML patient management.[201]

12 | CONCLUSIONS

Progress in deciphering the molecular pathogenesis of pediatric and adult AML[205] and the identification of the genetic determinants of response to treatment have been impressive, and translation of these findings into the clinical decision making has been increasing in recent years. The availability of the molecular profile enables a targeted-based treatment. Thus, evaluation of the genetic profile at diagnosis, but also at relapse, is of utmost importance. Besides the achievement of a morphological remission as a prerequisite of cure, the MRD level gives further insights into the remission status and determines kinetics of disease response as well as enables to detect an impending hematologic relapse. However, non-uniform definitions of MRD-positive/negative results, interobserver variability using flow cytometric MRD detection, sampling error from marrow to blood, and lack of uniformly agreed standardization upon molecular targets, methods, and sampling timing have hampered the transition into routine clinical practice. Thus, there is a high need for dedicated diagnostic standards to avoid wrong, ineffective, and expensive targeted treatment

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approaches. Therefore, a common international attempt to move forward standardization of genetic diagnostics including cryptic fusions (eg, *CBF-GLIS2, NUP98/NSD1*) as well as immunophenotypic abnormalities (eg, RAM phenotype) leading to the recommendation of standardized assays is mandatory for future use of targeted treatment approaches.

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TABLE 1

2017 European LeukemiaNet risk stratification by genetics[13]

Risk category	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD low[b]
	Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD high[b]
	Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD low[b] (w/o adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*[c]
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214*
	t(v;11q23.3); *KMT2A* rearranged
	t(9;22)(q34.1;q11.2); *BCR-ABL1*
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM(EVI1)*
	−5 or del(5q); −7; −17/abn(17p)
	Complex karyotype,[d] monosomal karyotype[a]
	Wild-type *NPM1* and *FLT3*-ITD high[b]
	Mutated *RUNX1*[e]
	Mutated *ASXL1*[e]
	Mutated *TP53*[f]

[a]Modified according to Ref.[13] Defined by the presence of one single monosomy (excluding loss of X or Y) in association with at least one additional monosomy or structural chromosome abnormality (excluding core-binding factor AML).[13]

[b]Low, low allelic ratio (<0.5); high, high allelic ratio (>0.5); as determined by GeneScan analysis.

[c]The presence of t(9;11)(p21.3;q23.3) takes precedence over rare, concurrent adverse-risk gene mutations.

[d]Three or more unrelated chromosome abnormalities in the absence of one of the World Health Organization-designated recurring translocations or inversions, that is, t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3); AML with *BCR-ABL1*.

[e]These markers should not be used as an adverse prognostic marker if they co-occur with favorable-risk AML subtypes.

[f]*TP53* mutations are significantly associated with AML with complex and monosomal karyotype.[132]