Toward Individualized Therapy in Acute Myeloid Leukemia
A Contemporary Review

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Acute myeloid leukemia (AML) is a heterogeneous disease in its clinical presentation, response to therapy, and overall prognosis. For decades, pretreatment karyotype evaluation has served to identify subgroups for risk-adapted postremission therapy, but the initial treatment approach has been largely unchanged. With continued advances in the genetic and epigenetic characterization of AML, we have discovered even more diversity and are starting to understand the biological underpinnings of these multiple disease entities. Newer therapies are being developed to address the pathophysiology within these individual AML subsets. This review categorizes AML into biologically defined groups based on currently available data and describes the evolving treatment approaches within these groups.

Cytogenetic and Molecular Classification

The prognostic relevance of recurrent cytogenetic abnormalities on AML outcome is well established.1,2 For about 2 decades, pretreatment cytogenetic abnormalities have been used to help classify AML into prognostic categories and direct treatment strategies. Those with favorable karyotypes are treated with curative intent using high-dose chemotherapy as postremission consolidation. Those with adverse karyotypes are known to have much lower cure rates and should be referred for allogeneic stem cell transplantation (SCT), investigational approaches, and long-term maintenance strategies. While there is good consensus for the categorization of favorable and adverse karyotypes in AML, there is inconsistency in defining the prognostic impact of several less common, but recurrent cytogenetic abnormalities that make up the intermediate-risk group. In addition, the implications of recurrent mutations in a number of genes such as NPM1, FLT3, and CEBPA in cytogenetically normal AML (CN-AML) are still being refined.4,5 Currently, 2 major classifications are commonly used to categorize AML into prognostic subsets: the United Kingdom Medical Research Council (MRC-C) and European Leukemia Net (ELN-C) systems (Table 1).2,3

The MRC-C is based on the outcomes of about 6000 patients younger than 60 years and divides patients into 3 categories by pretreatment cytogenetics: favorable, intermediate, and adverse.3 Owing to its large cohort size, the MRC-C had statistical power to refine the previous systems and individually report the outcomes of most of the recurrent cytogenetic abnormalities seen in AML, including those previously overlooked owing to small numbers. The ELN-C prognostic classification system expands on existing knowledge of cytogenetic subgroups by including the prognostic influence of recurrent somatic mutations in AML.2 Incorporating the mutation status of NPM1, FLT3, and CEBPA in patients with CN-AML, the ELN-C pro-
may help delineate further subsets of AML and provide more insight into disease biology. The top 10 genes mutated at higher than 5% frequency include FLT3, NPM1, DNMT3a, IDH1, IDH2, TET2, RUNX1, p53, NRAS, CEBPa, and WT1.

• Early recognition of pretreatment karyotype is an important prognostic and predictive factor and has led to improved outcomes in subsets of patients with core-binding factor AML and acute promyelocytic leukemia.

The roles of many of these genes in AML pathogenesis are still being investigated. Their prognostic significance is also not yet clear, often confounded by small numbers and the heterogeneity of patient characteristics and treatment approaches. The challenge will be to study the implications of each genetic abnormality in the context of uniform treatment, alone and integrated with other known mutations. Studies by Schlenk et al and Patel et al, for example, provide a framework to gain further insight into newer mutations in a systematic fashion. Herein we highlight our current understanding of treatment subsets in AML, organized by underlying biology and therapeutic strategy.

### Acute Promyelocytic Leukemia

Acute promyelocytic leukemia (APL) is defined by the t(15;17) cytogenetic abnormality, which leads to the PML-RARA (promyelocytic leukemia–retinoic acid receptor alpha) fusion gene and oncoprotein. The PML-RARA protein acts as a dominant negative inhibitor of the wild-type retinoic acid receptor, resulting in differentiation block and the clinicopathologic picture of APL.

The treatment of APL, which has evolved from nonspecific chemotherapy to select nonchemotherapy-based regimens, represents one of the first examples of individualized therapy targeted to a genetically defined AML subset. This has led to an improvement in cure rates from 30% to 90%.

The major breakthroughs in APL therapy were the discovery of the activity of all-trans-retinoic acid (ATRA) and the activity of arsenic trioxide (ATO) in APL. ATO overcomes the differentiation arrest in APL. ATO binds to PML, accelerates the degradation of PML-RARA, and is likely the most active single agent in APL. An additional important observation is the high expression of CD33 on APL and the significant clinical activity of the anti-CD33 antibody-drug conjugate, gemtuzumab ozogamicin (GO). Based on these findings, combination studies of ATRA, ATO, and chemotherapy were developed.

Investigators at our institution developed a nonchemotherapy-based regimen combining ATRA and ATO for remission induction, followed by ATRA and ATO consolidation. Patients with leukocytosis at diagnosis or following therapy, and those who remained molecularly positive for PML-RARA 3 months into consolidation or later received...
additional GO therapy at 6 mg/m².16 The combination demonstrated a complete remission (CR) rate of 92% and a 3-year survival rate of 85%. This study was the basis of a European consortium randomized study comparing the nonchemotherapy ATRA-ATO regimen with standard AIDA regimen (combination of ATRA and idarubicin). Among 162 patients with low- and intermediate-risk APL treated, the CR rate was 100% with ATRA-ATO and 95% with AIDA. The 2-year event-free survival was 97% vs 86% (P = .02). The ATRA-ATO regimen was also associated with significantly better survival (P = .02), lower incidences of hematologic toxic effects, and fewer infections.17 This represents a major advance in the individualized treatment of patients with APL and can be considered the new standard of care in newly diagnosed low-risk APL.

Although cure rates have increased significantly with the introduction of ATRA and ATO, rates of early mortality remain high, particularly in patients younger than 55 years and those treated outside major academic centers.20 Life-threatening hemorrhage from severe coagulopathy, delay of ATRA-based therapy, and complications of differentiation syndrome account for the majority of induction deaths. Better supportive care, early recognition of APL, and better education of “first responders” such as primary care physicians and emergency department personnel are needed to increase awareness and provide disease-modifying therapy. The development of guidelines and their dissemination through a partnered network of academic and community hospitals can help reduce rates of early death and has been previously pioneered in developing countries.21

Core-Binding Factor Leukemia

The 2 subtypes of core-binding factor (CBF) AML include the t(8; 21) cytogenetic abnormality, resulting in the formation of the RUNX1/ RUNX1T1 fusion gene and inversion of chromosome 16 (inv 16) (and its variant translocation t(16;16)), which results in the formation of the CBFB/MYH11 fusion gene. Treatment of CBF AML was associated with high CR rates (80%-90%) and cure rates of 40% to 60% in earlier studies using 3 or 4 cycles of high-dose cytarabine consolidations.22

Recognizing the sensitivity of CBF AML to intensive chemotherapy has led to the refinement of the standard chemotherapy regimens with more potent combinations (fludarabine, high-dose ara-C [cytarabine], [HiDAC], idarubicin, and GO), improved schedules, and more consolidations, leading to cure rates of 80% to 90%.23-29 Bradstock et al24 reported 1 of the first studies to demonstrate the benefit of HiDAC-based induction followed by HiDAC consolidation, which led to relapse-free survival and overall survival rates of 76% and 88%, respectively, among patients with CBF AML.24 At our institution, the use of fludarabine and HiDAC (FLAG) with GO or with idarubicin resulted in CR rates of 90% and estimated 5-year survival rates of 80%.29 These results were recently confirmed by the AML15 trial,30 which found an 8-year overall survival rate of 95% in patients with CBF AML treated with FLAG and idarubicin. Furthermore, in separate analyses, the addition of GO was found to be the most significant factor associated with improved survival.25-26 The survival benefit of GO among a subset of patients with CBF AML was also confirmed by a Southwest Oncology Group study in the United States.28 These studies demonstrate a steady improvement in long-term overall survival with the incorporation of higher doses of ara-C and fludarabine in induction and consolidation. Patients with CBF AML should therefore be offered high-dose therapy with curative intent, and allogeneic SCT should be reserved for second CRs and beyond.

While GO has shown significant benefit in CBF AML, its lack of commercial availability limits its use to compassionate use protocols in the United States. However, the effective activity of GO has prompted exploration of monoclonal antibodies for use in treatment of this disease and has led to the development of newer antibody constructs for treatment. A new anti-CD33 antibody-drug conjugate, SGN-33a, is currently undergoing early clinical investigation in patients with AML.31 In an ongoing phase 1 dose-escalation study of SGN-33a, patients with relapsed or minimally pretreated AML have been treated with SGN-33a, 5 to 60 μg/kg intravenously every 3 weeks without yet reaching a maximum tolerated dose.32 Among 38 evaluable patients, 16 (42%) have had clearance of bone marrow blasts.

Radioimmunotherapy using an actinium isotope-labeled monoclonal antibody to CD33 is also under investigation.33 A new bi-specific T-cell engager (BiTE) antibody construct is engineered to specifically recognize CD3 on an immune-effector T lymphocyte as well as CD33 on AML blasts. Engagement of both antigens by a BiTE monoclonal antibody brings an AML blast in close proximity with an immune-effector cell, triggers the formation of an immune synapse thus inducing T-cell activation and lysis of the target cell. Following on the success of the CD3-CD19 BiTE, blinatumumab,34 in acute lymphoblastic leukemia, the CD3-CD33 BiTE AMG330 is currently in preclinical development. Other AML-specific antigens such as CD123 are also being explored with different monoclonal antibody approaches.

Among patients with CBF AML, several studies have suggested that the presence of c-KIT mutation or persistence of minimal residual disease (MRD) may be associated with a higher incidence of relapse and with worse outcome.35-37 Studies of dasatinib, a KIT inhibitor, in c-KIT-mutated and wild-type CBF AML are ongoing.38 In cases of CR, MRD is monitored routinely, and treatment is adjusted if MRD remains positive. Among such patients, allogeneic SCT may be beneficial.39,40

NPM1-Mutated and CEBPa-Mutated AML

The NPM1 gene encodes for nucleophosmin, a nuclear phosphoprotein that shuttles between the nucleus and cytoplasm.41 NPM1 mutations lead to aberrant cytoplasmic localization of the protein and are found in 50% of patients with normal karyotype AML and in 60% of patients with FLT3 internal tandem duplication (ITD) mutations.42 In the absence of concurrent FLT3 ITD mutations, NPM1 mutations confer a favorable prognosis.8,41-43 Patients with normal karyotype AML and NPM1 mutation without an FLT3 mutation are now classified as having a favorable prognosis and should be treated accordingly.44 Those with NPM1 mutations and either IDH1 or IDH2 mutations, without FLT3 mutations may have a particularly favorable prognosis.9 Patients with both NPM1 and FLT3 ITD mutations have a worse prognosis, reflecting the adverse effect of FLT3 ITD.

The CEBPa gene encodes for the CCAAT-enhancer binding protein α, a transcription factor. Loss-of-function mutations in CEBPa occur in about 10% of patients with AML and normal karyotype and
were reported to be associated with a favorable prognosis compared with wild-type CEBPa. More recent analyses have indicated that a biallelic loss of CEBPa is important in conferring this favorable prognosis.

The recent World Health Organization (WHO) classification of myeloid neoplasms considers these subtypes as provisional distinct entities. FLT3, NPM1, FLT3, and CEBPa mutations should now be routinely tested in patients with AML to define prognosis and to choose appropriate therapy. Our approach to patients with NPM1-mutated or CEBPa-mutated AML (in the absence of FLT3 mutations) is similar to that in CBF AML, to pursue induction and consolidation using HiDAC-based regimens with curative intent.

**FLT3-Mutated AML**

The FMS-like tyrosine kinase 3 (FLT3) and its ligand are important for the normal proliferation of hematopoietic precursors. Activating mutations in the FLT3 gene occur in about 30% of patients with AML. Internal tandem duplications of the juxtamembrane domain, and point mutations in the tyrosine kinase domain (TKD) affecting amino acid D835, lead to ligand-independent constitutive activation of FLT3 signaling. FLT3 ITD–mutated AML is associated with inferior survival compared with wild-type FLT3; the prognostic significance of FLT3 D835 is less clear.

In addition to the FLT3 mutation type, the allelic ratio of FLT3-mutant genes (ratio of mutant FLT3 allele to wild-type FLT3 allele by polymerase chain reaction) may affect prognosis. The exact threshold and prognostic relevance of the FLT3-mutant allelic ratio needs to be refined; but for now, it appears that a high FLT3-mutant allelic ratio may characterize an AML subtype that is dependent on FLT3 signaling and potentially more sensitive to FLT3-inhibitor therapy.

Several FLT3 tyrosine kinase inhibitors (midostaurin, sorafenib, and quizartinib) are in clinical development. These are mostly active in FLT3 ITD AML, but not in FLT3 TKD mutants. Early studies suggest that development of an FLT3 TKD mutation may be a resistance measure or escape mechanism in patients being treated with these drugs.

The available FLT3 inhibitors are being evaluated, alone or in combination with chemotherapy. The combination of sorafenib with idarubicin and HIDAC in newly diagnosed AML demonstrated a CR rate of 95% in FLT3-mutated disease vs 84% in FLT3 wild type AML (P = .23). There were no significant differences in survival or disease-free survival between the 2 groups. Stone et al evaluated treatment with midostaurin combined with daunorubicin and cytarabine. The CR rate was 80% overall and 92% in FLT3-mutated AML. Two-year survival was 62% in FLT3-mutated AML. Similar outcomes between FLT3 ITD and wild-type FLT3 cohorts in these studies suggest that the FLT3 inhibitors may negate the adverse effect of the FLT3 mutation.

In a study conducted by a German AML study group (SORAML study), 276 younger patients (<=65 years) were randomized to receive treatment with daunorubicin and cytarabine with or without sorafenib. After a median follow-up of 3 years, the addition of sorafenib was found to be associated with a significant prolongation of 3-year event-free survival (40% vs 22%; P = .01) and a trend for better 3-year overall survival (63% vs 56%; P = .38), particularly among patients with FLT3 ITD. In contrast, Serve et al investigated this same treatment approach in 201 older patients with AML and reported trends for a lower CR rate (48% vs 60%, P = .12), a higher early death rate (17% vs 7%; P = .05), and no improvement in event-free survival or overall survival with sorafenib.

These studies suggest an improvement in outcome with the addition of an FLT3 inhibitor in AML therapy, with the possible exception of increased toxic death rate in older patients receiving the combination. Lower-intensity therapy in combination with an FLT3 inhibitor may be a better option for this subset of patients. For instance, a phase 2 trial combining sorafenib with azacytidine in patients with multiply relapsed FLT3 ITD–mutated AML demonstrated an overall response rate of 46%. This regimen was well tolerated and is under evaluation in newly diagnosed older patients with FLT3 ITD–mutated AML.

Newer, more selective FLT3 inhibitors are in development. A potential FLT3 inhibitor, quizartinib (AC220), was studied in relapsed or refractory AML cases, and CR or CR with incomplete blood count recovery rates of 54% were observed in patients with FLT3 ITD and 32% in patients without FLT3 ITD. The median survival in patients with FLT3 ITD–positive AML was 25 weeks. These encouraging results with single-agent quizartinib have prompted ongoing combination studies. Studies with next-generation inhibitors such as crenolanib, which have activity against FLT3 TKD mutations, are ongoing.

The longer-term impact of FLT3 inhibitors on overall survival in patients with FLT3-mutated AML will need to be determined. In a historical comparison with previous non–FLT3 inhibitor regimens at our institution, the addition of FLT3 inhibitors to chemotherapy since 2006 appears to have improved survival in both front-line and salvage AML settings. Whether their use can obviate the role of allogeneic SCT in this setting remains to be seen, but their clinical activity is clear, and their optimal utilization is being studied. Our approach in patients with FLT3-mutated AML with higher allelic burdens (>0.25) is to offer enrollment in clinical trials of FLT3 inhibitors, when available, to monitor allelic ratios during treatment and to refer eligible patients for allogeneic SCT in first remission. As we obtain more data on the safety and activity of newer FLT3 inhibitors in AML and develop combinations to overcome resistance, we can expect FLT3 inhibitors to become part of standard regimens for long-term remission maintenance after consolidation and also after allogeneic SCT to improve outcomes.

**RAS-Activated AML**

RAS is a guanosine triphosphate (GTP)-dependent second messenger protein that couples signals from receptor tyrosine kinases with downstream signaling networks. Mutations in RAS affect its inherent GTPase function and lead to aberrant, constitutive downstream signaling. RAS mutations are present in 10% to 25% of patients with AML and are overrepresented in those with the inv(16) karyotype. There does not appear to be an independent prognostic role for RAS mutations in AML, but recent data suggest that patients with these mutations may benefit from postremission consolidation with high-dose ara-C. Mutated RAS leads to dysregulated downstream signaling through Mek (mitogen activated protein kinase [MAPK] kinase) and may serve as a driver in AML. Inhibition of this pathway may be therapeutic. In a phase I/2 trial of...
patients with relapsed or refractory AML, the Mek inhibitor trame
tinib produced an overall response rate of 28%, including 12% CRs in a cohort enriched for patients with activating RAS mutations.66

Proteomic analysis of patients with RAS-mutated AML has dem-
strated simultaneous upregulation of the RAS-MAPK pathway as well as the phosphatidylinositol 3-kinase-protein kinase B (PI3K-
AKT) signaling pathway, providing a rationale for dual-pathway inhibition.64 Studies combining Mek inhibitors with PI3K and AKT inhibitors in RAS-mutated AML are in progress (NCT01907815). In a recent report examining myeloid malignancies with karyotypic abnormalities affecting the EVI1 locus (inv(3), t(3;3)), 98% of the cases were found to have mutations that led to activated RAS or receptor-

Epigenetically Targeted Therapy in AML

Epigenetics refers to biochemical modifications to chromatin such as DNA methylation, histone methylation, or histone acetylation that do not alter the primary DNA sequence but play an important role in genomic regulation at the level of gene transcription. Acute myeloid leukemia is an epigenetically regulated disease. This is evidenced empirically by the clinical activity of hypomethylating agents in this disease and directly by the identification of recurrent somatic mutations in genes of epigenetic modifiers.

Hypomethylating agents such as 5-azacytidine (5-AZA) and decitabine have shown significant activity in AML, providing an impor-
tant option for older patients who are not candidates for intensive chemotherapy. In a subset analysis of a phase 3 randomized study of older patients with AML (20%-30% bone marrow blasts),68 5-AZA was found to be associated with an improvement in survival compared with conventional care (24.5 vs 16 months; \( P = .001 \)). The follow-up trial,69 which randomized 488 patients 65 years or older with AML and greater than 30% bone marrow blasts to 5-AZA vs conventional care, showed improved survival with 5-AZA (median survival 10.4 vs 6.5 months; \( P = .08 \)).69 Similarly, in a randomized phase 3 trial of 435 patients (median age, 73 years) with AML randomized to decitabine or standard treatment choice,70 decitabine treatment was associated with an improvement in survival (median, 7.7 months vs 5 months; \( P = .037 \)), an effect that was maintained in patients with higher bone marrow blast counts. While treatment with hypomethyl-

Evolution of the contribution of epigenetic abnormalities to the pathogenesis of AML is supported by the discovery of recurrent somatic mutations in genes of proteins involved in DNA methylation and histone modification. The enzyme DNA methyltransferase (DNMT) is responsible for catalyzing the addition of a methyl group to cytosine, leading to DNA methylation. Mutations in DNMT3a are among the most common mutations in de novo AML.6,71,72 While aberrant DNA methylation may lead to AML pathogenesis, the functional significance of DNMT3a mutations and their correlation with response to hypomethylating-agent therapy is still being determined. A small study of patients with newly diagnosed AML treated with decitabine suggested improved CR rates in patients with DNMT3a mutations,73 but this has not been further confirmed.

Mutations in TET2 are also common in AML and have fur-
thered our understanding of the epigenome. TET2 catalyzes the oxida-
tion of 5-methyl cytosine to 5-hydroxymethylcytosine, eventually leading to loss of methylation.74,75 A loss-of-function mutation for TET2 would therefore predict for a net increase in DNA methylation and a potential role for hypomethylating agents.76

IDH-Mutated AML and IDH-Directed Therapies

Mutations in IDH1 and IDH2 create a neomorphic enzyme activity that leads to the aberrant production of the oncometabolite 2-hydroxyglutarate (2-HG), which leads to the inhibition of enzymes involved in epigenetic function and may be sufficient in causing leukemia.77-79 Strategies to target IDH-mutant AML are being investigated with promising results. A small-molecule inhibitor of IDH2, AG-221, has demon-

Targeting the Chromatin-Modification Machinery

Posttranslational modification of histone H3 within the chromatin is involved in the regulation of gene transcription. This is coordi-
nated by proteins that can add, remove, and “read” the posttransla-
tional modifications—which may include methylation or acetylation.7 Changes in the pattern of chromatin modification can alter entire programs of gene expression that may be involved in growth and differentiation. Recurrent abnormalities within several of the genes encoding these proteins highlight their important role in AML pathogenesis. The MLL gene, frequently involved in AML chromosomal translocations, is a histone 3 lysine 4 methyltransferase. By virtue of its translocations, MLL, within the fusion protein, retains its DNA-binding capacity but loses its normal histone 3 lysine 4 methyltransferase activity.71,72 Instead, it gains the ability to recruit an associated histone 3 lysine 79 methyltransferase,
DOT1L. The MLL fusion proteins can thereby recruit DOT1L to MLL target genes and direct an aberrant gene expression program that drives leukemogenesis. Small-molecule inhibitors of DOT1L showed promising activity in preclinical models and are currently in clinical trials in AML (NCT01684150). EZH2, another recurrently mutated gene in AML, encodes a histone 3 lysine 27 methyltransferase and aberrant methyltransferase activity is associated with unfavorable prognosis. EZH2 may have an anti-apoptotic function that may underlie its role in leukemogenesis. EZH2 is a component of the PRC2 complex and interacts with MLL fusion proteins of MLL t(11;19), which are associated with an intermediate prognosis. BET inhibitors may be active in MLL-translocated leukemia.

Many of the discoveries of aberrant proteins may not yield obviously druggable targets. Innovative methods such as RNA interference screens and synthetic lethal approaches may be used to find vulnerabilities that can be exploited. For example, one such RNA interference screen targeting known chromatin regulators led to the discovery of BET bromodomain–containing protein 4 (BRD4) as an important protein required for AML disease maintenance. The BRD4 protein functions as an epigenetic reader of acetylated histones and activates transcription of large genetic programs, most prominently of MYC.
and robust antileukemic activity.\textsuperscript{8,8} This may be important in several subsets of AML cases, particularly those with mutations in MLL or p53.\textsuperscript{8,9,10} More recent data have implicated the BET proteins in a broader role, as regulators of a core transcriptional program that initiates leukemogenesis in several non-MLL subsets, including those with mutated NPM1.\textsuperscript{91} The role of BRD4 inhibitors as targeted antileukemic therapy will likely expand and can be the subject of a more in-depth review. Currently, small-molecule inhibitors of BRD4 based on JQ1 are in clinical trials in AML and have preliminarily demonstrated responses in heavily pretreated patients (NCT01943851 and NCT01713582).\textsuperscript{92}

Conclusions

As we gain further insight into its biology, AML appears to be a highly heterogeneous group of diseases with different drivers and different vulnerabilities (Table 2). The challenge going forward is to identify these differences and develop therapies that address the drivers and vulnerabilities. Through collaborative science and intense focus on each individual subtype, we can continue to develop tailored therapies for specific targets and have a smaller fraction of patients treated with the “1 size fits all” anthracycline and cytarabine approach that has defined AML therapy for decades.
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