Acute Myeloid Leukemia: Biologic, Prognostic, and Therapeutic Insights

We review here the state of the art of diagnosis and treatment of AML and provide insights into the emerging novel biomarkers and therapeutic agents that are anticipated to be useful for the implementation of personalized medicine in AML.

Introduction

In 2015, approximately 20,000 new cases of acute myeloid leukemia (AML) and 10,000 AML-related deaths occurred in the United States alone.[1] The median age at diagnosis is 67 years, and the incidence of the disease increases with age. While recurrent acquired genetic abnormalities have been found in leukemia blasts, the direct causes of AML are unknown for the majority of patients.[2] However, for some patients, risk factors are readily identifiable. Hereditary or congenital conditions (eg, Down syndrome, ataxia telangiectasia, Bloom syndrome, Fanconi anemia, congenital neutropenia) and germline mutations (eg, CEBPA, RUNX1, TP53, ANKRD26, DDX41, ETV6, GATA2, SRP72, TERC, and TERT) have been linked to a higher predisposition to AML.[3,4] Patients with prior exposure to chemotherapeutics are at risk for developing therapy-associated AML.[5,6] Similarly, patients with clonal hematologic disorders, including myelodysplastic syndrome (MDS) and myeloproliferative neoplasms (MPNs), may progress to “secondary” AML. In general, patients with therapy-associated AML and secondary AML have worse outcomes than those with primary (de novo) AML.

The World Health Organization (WHO) classification has established a blast cutoff of 20% to distinguish AML from MDS—except for certain genetic abnormalities pathognomonic for AML, which require < 20% blasts, ie, t(8;21), inv(16), t(15;17).[2] The WHO classification also recognizes a variety of categories that reflect the disease’s clinical and biologic heterogeneity, including AML with certain genetic abnormalities, AML with myelodysplasia-related changes, AML related to previous chemotherapy or radiation, and AML not otherwise specified. The revised 2016 WHO classification is expected to incorporate additional emerging biologic and molecular information. While the WHO classification does not provide prognostic information, other classifications utilize cytogenetic and molecular features for treatment guidance (eg, to decide between chemotherapy and allogeneic hematopoietic stem cell transplantation [alloHSCT]), according to the risk of relapse.[7-9]

Cytogenetic and Molecular Risk

The mainstream approach to defining prognostic risk in AML patients is to conduct cytogenetic and molecular analyses at diagnosis. Generally, patients with recurrent cytogenetic aberrations (~45%) have been grouped into favorable-, intermediate-, and poor-risk categories.[10,11] The favorable cytogenetic groups include patients with t(15;17), t(8;21), or inv(16)/t(16;16). Individuals are classified as poor risk if aberrations constitute a complex karyotype (CK; defined here as at least three chromosomal abnormalities that are not included in the WHO category of “AML with recurrent abnormalities”); if they have t(6;9), inv(3)/t(3;3), 11q23 translocations, or ~7; or if they have a monosomal karyotype (defined as the occurrence of at least two autosomal monosomies [loss of chromosomes besides Y or X] or a single autosomal monosomy with additional structural abnormalities in the absence of t(15;17), t(8;21), or inv(16)/t(16;16)).[12] Patients with cytogenetic abnormalities that are not classifiable as belonging in any of the aforementioned groups, those who have t(9;11), and those who are cytogenetically normal (CN; ie, lacking a cytogenetic abnormality (~55%)) are considered to be intermediate risk. Cytogenetics alone cannot accurately predict outcome for any of these prognostic groups. However,
burgeoning technological developments have recently allowed for extended assessment of molecular biomarkers useful for outcome prediction; these new biomarkers include gene mutations, gene and noncoding RNA expression signatures, and DNA methylation profiles associated with distinct cytogenetic groups.[13-15] Still, to date only a few of these biomarkers have been incorporated into outcome-risk classifications. The European LeukemiaNet (ELN) classification, for example, incorporates *NPM1* and *CEBPA* mutations and *FLT3* internal tandem duplication (ITD) in an integrated cytogenetic-molecular classification that separates AML patients into four genetic groups: favorable, intermediate I, intermediate II, and adverse.[7,8] The favorable group includes patients with the *NPM1* mutation in the absence of *FLT3*-ITD, and those with *CEBPA* mutations. The intermediate I group is composed of CN patients with *FLT3*-ITD. The intermediate II and adverse groups comprise those with intermediate and poor cytogenetic risk, respectively. The National Comprehensive Cancer Network (NCCN) treatment guidelines also use *KIT* mutations.[9] As new molecular aberrations are discovered, these biomarkers may be grouped according to biologic and/or functional criteria useful for the selection of corresponding molecularly targeted drugs.[14,15]

**Receptor tyrosine kinase mutations**

Mutations occurring in the Fms-related tyrosine kinase 3 gene (*FLT3*), a gene that encodes a member of the class III receptor tyrosine kinase family, often lead to aberrant tyrosine kinase activation and, in turn, to rapid blast proliferation.[16-18] These mutations either appear within the juxtamembrane domain of the gene as an ITD or within the tyrosine kinase domain (TKD). *FLT3*-ITD mutations occur in 25% to 35% of patients with CN-AML, and they are linked to an increased risk of relapse and mortality.[8] The ratio of mutant-to-wild-type alleles influences the prognostic effect of the ITD mutation, since the absence of the *FLT3* wild-type allele is associated with a more dismal prognosis. The prognostic significance of *FLT3* TKD mutations (seen in ~7% of AML) is unknown, although a recent large study has shown a potentially favorable prognostic effect.[19] *FLT3* mutations may also occur in the favorable genetic risk subgroups, including in patients with core binding factor (CBF) AML—ie, AML harboring t(8;21)(q22;q22) or inv(16)(p13.1q22)/t(16;16)(p13.1;q22)—and in acute promyelocytic leukemia (not reviewed here), but the prognostic impact of these mutations in distinct subsets remains to be fully elucidated.[20,21] *KIT*, like *FLT3*, encodes a member of the class III receptor tyrosine kinase family. *KIT* mutations are present in ~20% of CBF AML patients and have been tied to relapse and worse outcomes, especially in patients with t(8;21).[8,22]

**NPM1 mutations**

Nucleophosmin (*NPM1*) is a nucleolar phosphoprotein that normally shuttles between the nucleus and the cytoplasm to maintain cellular processes.[23] Frameshift mutations at the C-terminus of the protein are relatively common in CN-AML patients (occurring in 45% to 60%).[24] A significant prognostic interaction has been reported between the *NPM1* mutation and *FLT3*-ITD.[8,25] Patients who had the combination *NPM1*-mutated/*FLT3* wild-type AML were found to have higher remission rates and better outcomes compared with patients who had *NPM1* wild-type or *NPM1*-mutated/*FLT3*-ITD disease. In older CN-AML patients treated with intensive induction chemotherapy, *NPM1* mutations predict for excellent disease response and better survival.[26]

**Transcription factors**

CCAAT/enhancer binding protein α (*CEBPA*) is a Basic Leucine Zipper (bZIP) transcription factor that is required for myeloid differentiation. Frameshift mutations within the N-terminus affect the transactivating domain, and insertions or deletions within the C-terminus affect the DNA-binding domain. *CEBPA* mutations occur in 10% to 15% of patients with CN-AML, and it is accepted now that only patients with *CEBPA* mutations that are biallelic (ie, each allele carries mutations) have a favorable prognosis.[27] The runt-related transcription factor 1 (*RUNX1*) is a transcription factor that regulates the expression of genes that are essential for hematopoietic growth and differentiation. Point mutations result in a loss of normal hematopoietic transcription factor activity, resulting in impaired cellular differentiation and altered mechanisms of apoptosis, thereby promoting leukemogenesis. Acquired *RUNX1* mutations have been identified in ~13% of AML patients; the frequency of the mutations in younger patients is less than that in older patients. These mutations have been associated with lower complete remission (CR) rates and shorter median disease-free survival (DFS) and overall survival (OS).[28] *RUNX1* mutations and favorable mutations (such as those of *NPM1* and *CEBPA*) appear to
be mutually exclusive.
A loss of normal function in the tumor suppressor gene TP53—as a result of mutations, deletions, or both—results in genetic instability and in general promotes tumorigenesis. In AML, TP53 mutations are often associated with CK-AML and are correlated with worse outcomes.[29]

Epigenetic modifiers

DNA cytosine methylation silences gene expression by epigenetically modifying structurally normal DNA. At least three isoforms of the DNA methyltransferase (DNMT) enzyme—DNMT1, DNMT3A, and DNMT3B—are recognized as responsible for establishing or maintaining DNA methylation. Aberrant DNA methylation can result in the silencing of tumor suppressor genes and thus in leukemia growth. In AML, mutations are relatively frequent in DNMT3A. [30-35] Approximately 60% of these mutations are identified as missense mutations at codon R882, whereas the remaining are nonsense or frameshift mutations that result in premature truncation of the protein; the latter mutations have been found across different domains of the gene.[31] In an AML Study Group report, DNMT3A mutations were identified in 17.8% of younger AML patients.[32] The majority of these mutations occurred in CN-AML patients (27.2%) and were linked with a lower CR rate and shorter OS. Within the NPM1 wild-type/FLT3-ITD subset, patients who also harbored the DNMT3A mutation had a lower CR rate, shorter OS (0.85 vs 4.41 years; P < .001), and shorter relapse-free survival than patients without the mutation. Additionally, Alliance reported a differential effect of DNMT3A mutations on different age groups.[33] In younger patients, DNMT3A R882 mutations had no impact on outcome, whereas non-R882 mutations predicted for worse outcome compared with patients without the mutation. Among older patients, on the other hand, those with R882 mutations had both a shorter OS and DFS, whereas non-R882 mutations did not influence outcomes. The predictive effect of DNMT3A mutations with regard to the therapeutic response has also been evaluated. Patients with DNMT3A mutations who were randomly assigned to receive dose-intensified daunorubicin as part of induction therapy seemed to have a better OS.[34-36] In a more restricted analysis, patients with DNMT3A mutations appeared to have a better chance of achieving CR when treated with the hypomethylating agent (HMA) decitabine than did patients with wild-type DNMT3A, but this finding requires confirmation.[37]

Isocitrte dehydrogenase (IDH), a member of the β-decarboxylating dehydrogenase family of enzymes, normally catalyzes the oxidative decarboxylation of 2,3-isocitrate during the Krebs cycle, generating 2-oxoglutarate and carbon dioxide.[38,39] In AML, missense mutations occur in the two isoforms, IDH1 (ie, R132 mutations) and IDH2 (ie, R140 or R172 mutations).[40-42] Mutant IDHs reduce α-ketoglutarate to 2-hydroxyglutarate via nicotinamide adenine dinucleotide phosphate (NADPH) oxidation, promoting tumorigenesis via a mechanism that has yet to be clarified but that likely includes epigenetic changes (eg, DNA hypermethylation) through inhibition of TET2 demethylase activity.[39] Although the prognostic value of IDH mutations in AML remains unknown because of differences in reported studies, the clinical utility of detecting these mutations lies more in their ability to predict response to treatment with IDH inhibitors.

TET proteins are iron- and α-ketoglutarate-dependent proteins that lead to the demethylation of DNA via the conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine. Mutations in the TET2 isoform lead to impaired hydroxylation of 5mC; consequently, genes necessary for normal cellular function remain hypermethylated and silenced. These mutations have been identified in AML, as well as in MDS and MPNs. CN-AML patients with TET2 mutations who have ELN favorable genetic risk (but not in those with ELN intermediate-I risk) have been reported to have a worse CR rate and shorter event-free survival (EFS) and OS.[43] Similarly, Patel et al[35] reported an association of TET2 mutations with poorer outcomes in patients with NPM1 wild-type/FLT3 wild-type AML, while other studies have failed to demonstrate a prognostic impact for TET2 mutations.[40,44]

The additional sex combs-like 1 gene (ASXL1) encodes a potential tumor suppressor that regulates gene transcription. ASXL1 mutations have been identified in approximately 15% of older patients with AML, MDS, and MPNs.[46] Similar to TET2 mutations, the negative prognostic impact of ASXL1 mutations seems to be restricted to patients in the ELN favorable group.[45]

The mixed-lineage leukemia gene (MLL) encodes a histone methyltransferase considered to be a positive global regulator of gene transcription. Chromosomal translocations involve the MLL gene, located at chromosomal band 11q23, in both AML and acute lymphoblastic leukemia; the prognostic significance of these translocations depends on the fusion partner. In AML, patients with t(9;11) have an improved outcome compared with patients who have other 11q23 translocations, and the former are usually classified in the intermediate cytogenetic risk group. An internal rearrangement (partial tandem duplication [PTD]) of the MLL gene without any contributing partner was one of the first
molecular markers described in CN-AML patients and was initially associated with worse outcomes, although the negative prognostic impact may be overcome with more intensive consolidation treatments (eg, autologous stem cell transplantation).[46]

The Wilms tumor 1 gene \( (WT1) \) encodes a transcription factor that plays a significant role in normal cellular growth and development. A functional epigenetic interplay between WT1 and TET2 has been recently recognized. Although the prognostic impact of \( WT1 \) mutations in patients with AML is not universally agreed upon, most studies report an inferior outcome.[47,48]

**Treatment of AML**

**Induction treatment**

The conventional approach for a patient with newly diagnosed AML is induction chemotherapy followed by consolidation or intensification treatment. The goal in induction remission chemotherapy is to reduce the number of leukemic cells in the blood, bone marrow, and extramedullary sites to undetectable levels and to restore normal hematopoiesis. At the time of remission, however, lower levels of leukemic cells are likely to persist and can lead to disease relapse if additional treatment with high-dose chemotherapy and/or alloHSCT is not administered.

**To Put That Into Context**

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**Where Has There Been Progress in AML—and Where Has Progress Lagged?**

Acute myeloid leukemia (AML) is a disease of older adults, with an incidence rate that is rising each year as a result of the aging of the population, and as more patients are treated successfully for other cancers, with a subset of these survivors developing therapy-related myeloid neoplasms. We have become increasingly sophisticated in how we classify AML by risk, starting with basic morphology (which enables us to distinguish acute promyelocytic leukemia from all the other subtypes), moving to cytogenetics, and now including molecular profiling. It has been a source of embarrassment that the basic treatment for AML—remission induction with “7+3” chemotherapy with cytarabine and an anthracycline—has changed little in 4 decades.

**What Promising Developments Has Genomic Analysis Led to?**

Finally, the genetic underpinnings of the disease have lent us a hand, as we have steered patients identified as having higher-risk AML, even in the setting of a normal karyotype, toward earlier hematopoietic stem cell transplantation—and hopefully to a better outcome than they would have experienced with chemotherapy alone. Even more recently, patients with abnormalities such as the \( FLT3 \) mutation are now able to receive specific agents that have demonstrated a survival advantage when combined with cytotoxic approaches, and those with \( IDH \) mutations are treated in studies with well-tolerated drugs that, as single agents, have resulted in appreciable remission rates. For younger adults, the therapeutic landscape is characterized by new pathways to better survival.

**For What Patient Population Is Progress Still Sorely Needed?**

For older adults, however, while lower-dose therapies have proven well tolerated, we have yet to budge the survival needle.

**Financial Disclosure:** Dr. Sekeres serves on an advisory board for Celgene and on the steering committee for Daiichi Sankyo.

With intensive remission induction chemotherapy regimens, 60% to 85% of younger patients (< 60 years) (Figure 1), and 40% to 60% of older patients (> 60 years) are expected to achieve a CR. These regimens consist of combination chemotherapy with standard-dose cytarabine (100–200
mg/m²) for 7 days, and an anthracycline (idarubicin or daunorubicin) for 3 days. After one “7+3” cycle, if persistence of leukemia is detected on day 14, the patient may be re-treated with the same agents on a “5+2” schedule. Idarubicin is commonly given at a dose of 12 mg/m²/day,[49] whereas daunorubicin is administered at doses of 45, 60, or 90 mg/m²/day. Although the highest dose of daunorubicin has been reported to be more effective than the lowest dose (45 mg/m²/day), more recent studies have shown that an intermediate dose (60 mg/m²/day) yields similar outcomes but with less toxicity than the 90-mg/m²/day dose.[36,50-52] High-dose cytarabine–based regimens have also been successful in achieving high CR rates, but it remains to be fully determined whether this approach yields better results than standard-dose cytarabine without worsening toxicity.[53]

Although clinical benefit has been achieved as a result of the dose-optimization studies just mentioned, it is likely that we have maximized the antileukemic activity of chemotherapy, and we anticipate that future substantial progress will more likely be achieved by integrating molecularly targeted therapies and/or immunotherapeutics into the therapeutic armamentarium for AML patients rather than by relying on chemotherapy dose escalation. Thus, we take the position that, if possible, all AML patients should first be evaluated for clinical trials involving novel molecularly targeted therapies incorporated into established chemotherapy regimens.

In older patients, outcomes are generally poorer than in younger patients, likely due to the increased frequency of negative clinical, cytogenetic, and molecular prognosticators in older patients, and because of greater treatment-related morbidity and mortality.[54] These patients should be considered for clinical trials (Figure 2). Alternatively, older patients can be induced with a 7+3 regimen if they are physically fit and if they are candidates for subsequent intensification with alloHSCT (unless they are in the ELN favorable genetic group). Older patients who are unwilling or unable to undergo intensive remission induction may be considered for single-agent therapies (Figure 3). Low-dose cytarabine induces responses in 15% to 20% of such patients, although median survival is only 5 to 6 months.[55] HMAS, including 5-azacytidine or decitabine, may also be an effective approach in older patients, as demonstrated by two large randomized trials.[56,57] We have pioneered a 10-day regimen of decitabine, 20 mg/m², in previously untreated older AML patients and demonstrated an excellent 47% CR rate and 64% overall response (OR) rate.[58] These very encouraging results have been recently confirmed by other investigators. Second-generation oral HMAS are also being developed, with excellent results.[59]

Consolidation therapy

Standard post-remission strategies include high-dose chemotherapy and/or alloHSCT. A patient’s leukemic genetic risk profile, which predicts the risk of relapse, and/or other clinical risk factors can be successfully used to decide whether or not to proceed to alloHSCT. Younger patients (see Figure 1) with a favorable ELN genetic risk (eg, CBF) can be treated with chemotherapy alone, with a cure rate of ~50% to 60%; the most appropriate dose and number of consolidation chemotherapy cycles remain to be determined—although there is convincing evidence that doses of 2,000 to 3,000 mg/m² lie beyond the plateau of the maximal therapeutic effect.[8,60] For patients with intermediate or adverse cytogenetic/molecular ELN risk, alloHSCT may confer relatively greater chances of long-term survival (see Figure 1).[61,62] Optimization studies of conditioning regimens (myeloablative and reduced-intensity), incorporation of novel therapeutics into transplant treatment programs, and better infection and graft-vs-host disease prophylaxis may improve these results.

Although cytogenetic and molecular features in diagnostic samples serve reasonably well for the purpose of risk stratification of patients to chemotherapy vs alloHSCT, monitoring of minimal residual disease (MRD) by flow cytometry may also be informative.[63-65] Patients positive for MRD, who would otherwise be destined to receive chemotherapy only, may be switched to treatment studies designed to achieve MRD negativity, and may eventually receive alloHSCT. Furthermore, an attempt to achieve MRD negativity should be considered in patients already selected for alloHSCT, as this may increase their chance for cure.[66] Clinical decisions made based on MRD status are challenging, however, since they involve numerous complex factors, including the choice of cutoff levels of MRD considered to be clinically informative, the sensitivity of the assay used, and the time points at which MRD assessment is performed (see Figures 1 and 2). For individuals with specific molecular markers, highly sensitive quantitative real-time reverse transcription polymerase chain reaction (RT-PCR)–based assays are a suitable alternative to flow cytometry.[63-65] For CBF AML, recent studies showed that failure to achieve a more than 3-log reduction of the fusion transcripts [eg, RUNX1/RUN1T1 for t(8;21) and CBFB/MYH11 for inv(16)] early in the course of treatment, after induction or first consolidation chemotherapies, is predictive of disease relapse independently of other unfavorable molecular features.[67] Similar to findings in CBF leukemia, it has been shown that
assessment of MRD in patients with NPM1 mutations can stratify them into markedly different levels of risk of relapse on the basis of response kinetics.[68] Therefore, it is possible that combining log reduction at early time points and subsequently measuring MRD levels at regular time intervals (eg, every 3 to 6 months) may provide an optimal approach for predicting impending relapse. Much progress is needed in the treatment of older AML patients (see Figure 2). Although randomized trials have compared the effectiveness of high-intensity and low-intensity consolidation chemotherapy, results so far have been inconclusive.[69] At present, it is recommended that older patients with a favorable-risk ELN genetic profile and good performance status receive consolidation with an intermediate-dose cytarabine-based regimen, while for the remaining patients, in whom cure rates are estimated to be < 10% to 15%, investigational treatments, with the possible inclusion of new maintenance therapies and/or alloHSCT, should be offered.[62]

**Treatment of relapsed and primary refractory AML**

Between 20% and 30% of AML patients may be refractory to initial remission induction chemotherapy. Furthermore, disease recurrence occurs within 3 years after diagnosis for most patients with AML. A short duration of remission (ie, < 6 months), adverse genetic factors, prior alloSCT, older age, and poor general health status are the main negative determinants of outcome after relapse. There have been few controlled trials to provide data regarding the best salvage regimen for AML patients with refractory or relapsed disease.[70] Commonly used intensive salvage regimens are designed to achieve CR and to enable the patient to proceed to alloHSCT, in order to maximize the chances of a durable remission. Patients who are physically unable to receive intensive salvage therapy or to undergo alloHSCT should be considered for clinical trials.

**Targeting Distinct Cytogenetic and Molecular Subsets of AML**

Next-generation sequencing technology has provided fertile ground for the development of novel treatment approaches based on individual patients’ clinical, cytogenetic, and molecular features (ie, “personalized medicine”). The whole genome is a potential source of information on pathways that drive leukemogenesis in individual patients and that can be successfully targeted. A seminal Cancer Genome Atlas Research Network study delineated the mutational landscape of genes and drew correlations with DNA methylation and specific gene and microRNA expression in a large group of patients with AML.[14] Over 97% of patients have at least one of the most frequently found mutations, and new molecular subsets of patients are being defined by patterns of cooperation and mutual exclusivity among these mutations.[71] Furthermore, these molecular markers may provide information regarding the temporal evolution and growth of minor clones that may be responsible for resistant or relapsed disease, and which therefore signal a need for timely switching to different treatments.[72] Several of these molecular features are already being used to guide treatment.

**CBF AML**

Both younger and older CBF patients are consistently sensitive to 7+3 induction chemotherapy, demonstrating 80% to 90% CR rates (see Figures 1 and 2).[73] Multiple cycles of high-dose cytarabine chemotherapy have been established as an effective consolidation regimen for CBF AML patients. However, despite optimal chemotherapy treatment, only ~50% to 60% of adult CBF patients are cured.[60] Therefore, further treatment optimization is needed. Gemtuzumab ozogamicin, a monoclonal anti-CD33 antibody conjugated to the cytotoxic agent calicheamicin, was initially approved for AML treatment but was subsequently withdrawn from the market because of increased toxicity and lack of substantial clinical benefit. However, it is reported that a lower dose of this agent in combination with chemotherapy may be beneficial, especially in the favorable (eg, CBF) and intermediate cytogenetic risk groups; reconsideration of the availability of this agent for AML treatment may be warranted.[74,75] Because KIT mutations are frequently harbored by and are nearly exclusive to CBF blasts, Alliance and the German AML Study Group have investigated the addition of the dual kinase inhibitor dasatinib to a cytarabine/daunorubicin induction regimen and high-dose cytarabine consolidation, followed by maintenance with dasatinib as a single agent. Early promising results included a relatively low relapse rate and longer duration of survival, which have been demonstrated in both younger and older patients, with or without KIT mutations.[76] A multi-institutional randomized trial of chemotherapy with or without dasatinib is now underway (ClinicalTrials.gov identifier: NCT00850382). AlloHSCT is not given as part of the initial therapeutic program for CBF AML; it is reserved for patients who are high risk, such as those with KIT mutations.[73] Nevertheless, if
relapse occurs, alloHSCT is beneficial for patients who are given salvage chemotherapy and who subsequently achieve a CR.

**FLT3-mutated AML**

Several small-molecule FLT3 tyrosine kinase inhibitors are in development and have been studied as single agents or in combination with salvage chemotherapy. More recently, these agents have been incorporated into upfront standard induction and consolidation chemotherapy regimens (see Figures 1 and 2).[77] Sorafenib is a multikinase inhibitor of BRAF, FLT3, c-KIT, vascular endothelial growth factor, and platelet-derived growth factor receptor. Recently, the SORAML trial of newly diagnosed younger AML patients reported that the incorporation of sorafenib into chemotherapy regimens significantly prolonged EFS compared with chemotherapy alone.[78] However, in another report, no demonstrable benefit from the addition of sorafenib to standard chemotherapy was noted in older AML patients.[79] A recent international phase III study of patients with previously untreated FLT3-mutated AML demonstrated a benefit of adding midostaurin, another multikinase inhibitor, to chemotherapy in both FLT3-ITD and FLT3-TKD patients, irrespective of whether they underwent alloSCT.[80] Other FLT3 inhibitors, including quizartinib and crenolanib (which is active in a subset of patients with FLT3-ITD/D835 mutants who are resistant to quizartinib), as well as ASP2215, have shown activity in clinical trials as single agents; the testing of these agents in combination with chemotherapy or HMAs is underway.[81-83]

**IDH1- and IDH2-mutated AML**

AG-221 is a reversible inhibitor of mutant IDH2. Research has been valuable in demonstrating the effects of inhibiting mutant IDH2 in patients with AML. Over 170 patients with untreated AML, relapsed/refractory AML, MDS, and chronic myelomonocytic leukemia have been enrolled in ongoing trials.[84] The preliminarily reported OR rate and CR rate were 40% and 16.5%, respectively. Another reversible inhibitor, AG-120, which targets mutant IDH1, has also recently entered into clinical trials. AG-221 and AG-120 are expected to be combined with chemotherapy and HMAs, as upfront therapy and in the relapse setting (see Figures 1 and 2).

**Newer Treatments for AML**

**CPX-351**

CPX-351 is a formulation of cytarabine and daunorubicin in a 5:1 molar ratio packaged within 100 nm–diameter liposomes.[85,86] Investigators recently reported a parallel-arm comparison of CPX-351 with conventional 7+3 chemotherapy in older patients with newly diagnosed, previously untreated AML. Patients receiving CPX-351, particularly those with adverse cytogenetics, had higher response rates.

**Vosaroxin**

Vosaroxin is a quinolone derivative that inhibits topoisomerase II. However, to date it has not yet demonstrated an overall clinical benefit in randomized trials.[87,88]

**Polo-like kinase inhibitors**

Volasertib is an adenosine triphosphate–competitive kinase inhibitor of polo-like kinase 1 (PLK1). This agent leads to the formation of abnormal, monopolar mitotic spindles in dividing cells; as a result, the cell cycle is blocked at the G2-M phase, and apoptosis eventually follows from the prolonged mitotic arrest. In a phase II trial of newly diagnosed AML patients who were randomly assigned to receive either volasertib with low-dose cytarabine or low-dose cytarabine alone, patients who received both drugs had significantly higher OR rates (31.0% vs 13.3%).[89] These results are being confirmed in larger randomized trials and in studies combining volasertib with HMAs.

**KPT-330**

Chromosome region maintenance protein 1 (CRM1; also known as exportin-1 [XPO1]) is a nuclear protein export receptor involved in transporting important tumor suppressor proteins and cell-cycle regulators, including p53 and p27.[90] Inhibitors in the selective inhibitor of nuclear export (SINE) class bind to CRM1 and inhibit its function. Selinexor (KPT-330), a slowly reversible oral SINE, has demonstrated antileukemic activity as a single agent in AML, and combination studies of selinexor
MLN4924

MLN4924 is a small-molecule inhibitor of NEDD8-activating enzyme, a cullin-dependent E3 ligase controlling ubiquitination and hence affecting cellular growth, proliferation, and apoptosis. A phase I trial using various schedules and doses of MLN4924 as a single agent have been conducted in patients with relapsed or refractory AML/MDS, and a recommended phase II dose has been determined for further studies.[92] Trials of MLN4924 in combination with HMAs are now underway.

Epigenetic modulators

HMAs, including decitabine and 5-azacytidine, are commonly considered as potential therapies for patients with previously untreated AML, especially individuals who are not suitable candidates for induction chemotherapy. SGI-110, a second-generation HMA, is a dinucleotide of decitabine with a longer half-life. In a randomized phase II trial of patients with relapsed or refractory AML, and of older patients with previously untreated AML who were not fit for induction chemotherapy, the CR rate in those patients with relapsed/refractory AML was 16%, while the CR rate in the previously untreated group was 53%.[59] A phase III study to definitively investigate the efficacy of SGI-110 will be conducted.

EPZ-5676 is a small-molecule inhibitor of DOT1L, a histone methyltransferase that is crucial for the development and maintenance of MLL-rearranged leukemias. In a phase I clinical trial of EPZ-5676, 2 of 28 patients who were evaluable for antileukemic activity achieved CR, and 2 additional patients had resolution of leukemia cutis.[93]

Bromodomain and extraterminal (BET) family proteins are also attractive targets, since inhibitors can be directed against epigenetic regulators that maintain aberrant chromatin states commonly associated with AML. Both preclinical studies, as well as initial clinical trials with the BET inhibitor OTX015, have shown promise.[94]

Lastly, histone deacetylases (HDACs) are a well-known class of enzymes that affect gene expression via the removal of acetyl groups on core lysine groups in nucleosomal histones and other chromatin proteins.[95] HDAC inhibitors alter the landscape of acetylation, thereby influencing cell-cycle arrest, growth inhibition, and apoptosis. Leukemic cells are one of the many cell lines affected by HDAC inhibitors. HMAs and HDACs have been combined, with the rationale that they would have a possible synergistic effect on aberrant epigenetic changes in AML blasts that would restore the expression of silenced tumor suppressor genes. However, no definitive clinical benefit of the combination has been shown after more than 10 years of study. More recently, newer HDAC inhibitors (eg, pracinostat, panobinostat) are in the process of being investigated in clinical trials, alone or in combination with chemotherapy, HMAs, or other novel molecularly targeted compounds.[95]

ABT-199

ABT-199 (GDC-0199) is a highly selective antagonist of the antiapoptotic BCL-2 protein (which may mediate chemoresistance). In a recent trial, patients with refractory/relapsed AML, and patients who were treatment-naive but not able to undergo intensive therapy, were given ABT-199, with excellent responses, especially in patients with IDH mutations.[96] ABT-199 is now being tested in combination with HMAs or chemotherapy.

Immunotherapeutics

SGN-CD33A is a humanized CD33 antibody conjugated to a pyrrolobenzodiazepine (PBD) dimer that is a highly potent DNA binding agent. Early results from phase I clinical trials have recently been presented. In one such trial—of SGN-CD33A monotherapy in patients who had CD33-positive AML in relapse or who had declined intensive induction chemotherapy—a dose of 40 μg/kg led to a 60% OR rate in treatment-naive patients.[97] In another phase I trial—in treatment-naive CD33-positive AML patients who were not eligible for or who declined conventional intensive chemotherapy—combining SGN-CD33A and an HMA was determined to be tolerable, with an OR rate (CR + CR with incomplete blood count recovery) of 65%.[98]

Chimeric antigen receptors (CARs) that redirect T-cell specificity towards antigens that are enriched on the surface of “bulk” blasts and/or leukemic stem cells have also recently begun to be explored in clinical trials.[99] A phase I trial of CD123 CAR T cells in patients with refractory or relapsed disease (including individuals who have already undergone alloHSCT) is ongoing at our institution. Whether these immunotherapeutic approaches will benefit from being used in combination with the
emerging immune checkpoint inhibitors is an exciting area of intensive clinical research.

**Conclusions**

Emerging biological insights and new molecular and immunotherapeutic approaches are generating exciting results for AML patients, and while preliminary, these may result in improvement in outcome, in particular for those patients most in need: older patients and those with high-risk disease. The challenge is how to match molecular and clinical information with emerging compounds in order to select the best treatment for individual patients. The success of personalized approaches in AML is likely to depend on our ability to readily attain molecular information and on access to new drugs, in addition to several other key factors. The latter include a critical revision of more traditional clinical trial designs in favor of novel approaches that are more likely to identify drugs’ early biologic and clinical activities; increased referral of patients to tertiary centers by community oncologists; and close collaboration among cancer center networks, oncology cooperative groups, pharmaceutical sponsors, and regulatory authorities.

**Financial Disclosure:** The authors have no significant financial interest in or other relationship with the manufacturer of any product or provider of any service mentioned in this article.