Mutations in AML: prognostic and therapeutic implications

Courtney D. DiNardo and Jorge E. Cortes

Department of Leukemia, University of Texas MD Anderson Cancer Center, Houston, TX

Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy characterized by the proliferation and aberrant differentiation of immature clonal myeloid cells. The prognosis of AML is variable, based on clinical features such as patient age, performance status, and comorbidities, as well as leukemia-specific genetic features including cytogenetics and molecular classification. The modern application of next-generation sequencing technology has uncovered marked heterogeneity and genomic complexity within AML, based on the presence or absence of cooperating mutations within functional categories such as epigenetic regulators, cell signaling and proliferation pathways, and master hematopoietic transcription factors. Although the treatment of AML has hitherto changed little in the past 40 years, the enhanced scientific understanding of AML pathophysiology and leukemogenesis has led to the recent development of multiple targeted and selective treatment approaches, and our increasing awareness of functional AML subsets will be evermore used to inform rational and personalized treatment strategies.

Learning Objectives

- A comprehensive genomic assessment including cytogenetics and molecular annotation is essential for optimal classification and prognosis of patients with AML
- Awareness of functional AML subsets can be used to inform rational treatment strategies

Introduction

Acute myeloid leukemia (AML) accounts for ~25% of all adult-onset leukemias in the Western world, with an incidence of 3 to 5 cases out of every 100 000 adults per year and a median age of 69 years at diagnosis.[1-3] AML is a hematopoietic stem cell (HSC) malignancy characterized by differentiation arrest and uncontrolled clonal proliferation of neoplastic immature precursors, preventing normal bone marrow hematopoiesis. The prognosis of AML is variable, based in part on clinical features such as patient age, medical comorbidities, and performance status, and also on underlying genetic features including both cytogenetic and molecular aberrations.[4,5]

Treatment of AML has changed little in over 40 years; curative therapy remains the nucleoside analog cytarabine, administered in combination with an anthracycline as induction therapy, followed with repeated cycles of high-dose cytarabine and/or an allogenic stem cell transplant (SCT) in patients attaining a complete remission (CR).[6] The administration of intensive cytarabine-based therapy is appropriate regardless of age in "medically fit" older patients[7]; hypomethylating agent (HMA) strategies are also often recommended and used as frontline treatment approaches in elderly patients.[8,9] A recent analysis of treatment patterns and outcomes in elderly AML patients by the Surveillance, Epidemiology and End Results-Medicare database demonstrated survival benefit with the receipt of either intensive or HMA strategies, compared with the 60% of elderly AML patients in the United States who received no leukemia-directed therapy.[10] Current outcomes with these standard AML approaches remain highly unsatisfactory; patients ≤60 years of age demonstrate a long-term disease-free survival (DFS) of only ~40%, whereas DFS in older patients is a sobering 10% or less, with a median overall survival (OS) less than 1 year regardless of therapeutic approach.[11-13] These poor outcomes with traditional therapies, particularly when contrasted with the effectiveness of targeted therapy such as all-trans retinoic acid and arsenic trioxide for the AML subset acute promyelocytic leukemia (M3 AML),[14,15] offers hope that the improved understanding of AML leukemogenesis will lead to personalized and genomically targeted therapeutic approaches, which will improve outcomes in additional AML subsets.

Cytogenetic analysis to detect large structural chromosomal abnormalities provided the first "genetic" prognostication schema in AML, and remains the backbone of current AML genomic classification, partitioning patients based on their pretreatment karyotype into those with favorable, intermediate, or adverse cytogenetics, and correlating with 5-year OS of ~60%, 30% to 40%, and 5% to 10%, respectively.[4,16-18] With the advancement of next-generation sequencing approaches, recurrent mutations in AML have also been characterized, and utilizing current technology >95% of AML cases are confirmed to harbor at least 1 somatic alteration, with approximately a dozen genomic alterations identified per AML sample including an average of 3 driver mutations, and providing particularly useful prognostic information in the majority of AML patients with otherwise intermediate-risk cytogenetics.[19-21] The genetic profile of AML is notably heterogeneous, and only a few mutations (eg, Fms-related tyrosine kinase 3 [*FLT3*], nucleophosmin [*NPM1*], and DNA methyltransferase 3A [*DNMT3A*]) are present in more than a quarter of AML patients,[20] reinforcing the understanding that the clinical diagnosis of AML encompasses a diverse group of genetically distinct malignancies.

Conflict-of-interest disclosure: C.D.D. has received research funding from Novartis, Genentech, Daiichi-Sankyo, Celgene, Agios, and Abbvie, and has consulted for Daiichi-Sankyo and Agios. J.E.C. has received research funding from Novartis, Pfizer, Teva, Arog, Astellas, Ambit, BMS, and Ariad, and has consulted for Novartis, Pfizer, BMS, and Ariad.

Off-label drug use: None disclosed.

Table 1. Examples of recurrent AML mutations by functional group

Functional class	Specific example mutations
Signaling and kinase pathway	*FLT3, KRAS, NRAS, KIT, PTPN11,* and *NF1*
Epigenetic modifiers (DNA methylation and chromatin modification)	*DNMT3A, IDH1, IDH2, TET2, ASXL1, EZH2,* and *MLL/KMT2A*
Nucleophosmin	*NPM1*
Transcription factors	*CEBPA, RUNX1,* and *GATA2*
Tumor suppressors	*TP53*
Spliceosome complex	*SRSF2, U2AF1, SF3B1,* and *ZRSR2*
Cohesin complex*	*RAD21, STAG1, STAG2, SMC1A,* and *SMC3*

*Not discussed in detail within the manuscript.

The focus of this review is to summarize the genomic landscape of AML with a highlight on somatic mutations, underscoring the utility of a comprehensive genomic assessment. Attention to recent clinical advances, including novel molecularly targeted therapies for treatment of AML, will also be highlighted.

The AML genomic landscape

At the time of AML diagnosis, prognostically important and distinct chromosomal translocations and large chromosome gains or losses can be observed on standard karyotype analysis in approximately half of AML patients.[4] Over the past decade, DNA sequencing technology has revealed recurrent AML gene mutations, undetectable by standard cytogenetic analysis, which additionally contributes to AML pathogenesis. Many of these gene mutations have prognostic implications, independently and/or in the presence of certain co-occurring driver mutations (gene-gene interactions), and targeted therapeutic strategies directed at specific mutations and molecular classes are currently under development. Although the following list is not meant to be exhaustive, a summary of the most frequent recurrent genetic abnormalities in AML are described in the sections to follow, and detailed in Table 1. Of note, the presence of mutations within a functional category is largely mutually exclusive, suggesting functional overlap between recurrent mutations within functional groups. Moreover, the individual prognostic information derived from the presence or absence of a specific mutation can be modified by the presence of cooperating co-mutations (ie, *NPM1* with *FLT3*-internal tandem duplications [ITDs] or *DNMT3A* mutations), signifying awareness of the complete AML genomic landscape will be necessary to provide optimal personalized prognostication.

Signaling and kinase pathway mutations (eg, FLT3, KRAS, NRAS, PTPN11, NF1, and KIT)

Present in approximately two-thirds of AML cases, mutations leading to aberrant activation and proliferation of cellular signaling pathways, frequently referred to as type 1 mutations, make up the most common mutational subset in AML. Intriguingly, mutations within this class are frequently identified in subclonal cellular fractions, indicating they are often late clonal events in disease evolution.[21]

FLT3. ITDs and/or activating kinase domain point mutations (D835) in the *FLT3* gene are present in nearly one-third of patients with AML, resulting in constitutive activation and downstream signaling through the RAS/RAF/MEK/mammalian target of rapamycin growth and proliferation pathways, and the phosphatidylinositol 3 kinase (PI3K)/AKT prosurvival pathway. *FLT3-ITD* mutations are more frequent within younger adult patients, those with normal karyotype (NK), and are associated with proliferative AML (eg, higher white blood cell and blast %), and increased risk of relapse leading to decreased OS.[22] In addition to the presence/absence of *FLT3* mutations, the degree of mutational burden as estimated by the *FLT3* variant allele frequency (VAF) is of importance, with AML patients with low FLT3-ITD burden (often considered as a VAF <0.5), especially in the setting of concomitant *NPM1* mutation experiencing relatively improved outcomes,[23] suggesting that both the FLT3-mutational status and FLT3-VAF should be reported in all AML patients for optimal prognostication.

RAS oncogenes. The family of RAS oncogenes is mutated in ~10% to 15% of AML cases. These include activating mutations in *NRAS*, *KRAS*, *PTPN11*, and *NF1*, leading to aberrant proliferative signaling through the RAS/RAF/MEK/extracellular signal-regulated kinase pathway. Although the prognostic impact of *RAS* mutations has been debated, the acquisition or clonal outgrowth of *RAS* mutations, analogously to *FLT3*, can be seen at the time of myelodysplastic syndrome (MDS) progression to AML and portends a poor outcome in this circumstance.[24] The effect of NRAS mutations (specifically those within codon G12/13) was recently described to confer a favorable outcome in the setting of an *NPM1* and *DNMT3A* co-mutated genotype, with minimal prognostic importance in other molecular permutations.[21] Development of directed therapeutic targets for RAS-mutant leukemias, such as MEK, extracellular signal-regulated kinase, PI3K, and mammalian target of rapamycin inhibitors are ongoing, but have been complicated by the rapid development of resistance or activation of bypass pathways in the single-agent setting.[25,26]

KIT. Nearly exclusively identified in AML with core-binding factor (CBF) translocations, mutations in the receptor tyrosine kinase KIT, particularly the D816V missense variant, occur in ~20% of CBF-AML and are frequently associated with an adverse prognosis, although this has not been universally observed.[27,28] The addition of multikinase inhibitors with activity against KIT mutations, such as midostaurin or dasatinib, may be of benefit in this otherwise favorable cohort. In updated results of a phase 2 trial of 7 + 3 chemotherapy in combination with dasatinib 100 mg orally daily (days 8 to 21) for newly diagnosed CBF-AML patients, clinical outcomes were comparable to historical CBF-AML cohorts (90% CR rate and 65% 2-year DFS), with no difference in outcomes between KIT-mutated and wild-type (WT) patients, confirming the safety of this approach.[29]

Multikinase and FLT3 inhibitors

The first generation of "FLT3" inhibitors incorporates several multikinase inhibitors (eg, sorafenib, sunitinib, midostaurin, and lestaurtinib) employed for the treatment of *FLT3*-mutated AML. The lack of *FLT3* selectivity with these multikinase, or "dirty kinase" first-generation inhibitors, has been frequently (and perhaps naively) viewed to represent a suboptimal *FLT3*-targeted therapeutic strategy, and much effort has been devoted to the development of more selective second-generation small molecule FLT3 inhibitors such as quizartinib (AC220), crenolanib, and gilteritinib (ASP-2215).

Sorafenib. A double-blind, placebo-controlled, phase 2 trial of adults aged 18 to 60 years with newly diagnosed AML, randomized to 7 + 3 induction therapy vs 7 + 3 + sorafenib 400 mg twice daily

was recently reported.[30] A total of 267 patients were enrolled, and a significant difference in event-free survival (EFS) with a median EFS of 9 months vs 21 months and a 3-year EFS of 22% vs 40% was demonstrated. A total of 46 enrolled patients (17%) had FLT3-ITD mutations; the EFS benefit was seen regardless of FLT3-ITD mutational status, suggesting that inhibition of kinases other than FLT3 may have provided antileukemic activity in this newly diagnosed, younger AML cohort. Whether this study indicates that sorafenib should be added to the induction regimen of all younger AML patients, regardless of molecular status, is an ongoing debate.

Midostaurin. The landmark results of the phase 3 international prospective RATIFY study of adults (n = 717) aged 18 to 60 years with newly diagnosed FLT3-mutated AML, treated with 7 + 3 + midostaurin vs placebo, were recently reported.[31] The 5-year EFS rate in the midostaurin vs placebo arm of 26.7 vs 19.1 ($P = .004$), and 5-year OS of 50.8 vs 43.1 ($P = .007$), respectively, confirms that pretreatment genetic assessment improves treatment decisions and patient outcome. In February 2016, the US Food and Drug Administration granted midostaurin "breakthrough therapy" designation status for patients with newly diagnosed, FLT3-mutated AML. A global compassionate use program and US Expanded Treatment Protocol are currently in place to enable midostuarin access. Midostaurin inhibits multiple kinases in addition to FLT3, including CKIT, platelet-derived growth factor receptor, vascular endothelial growth factor receptor, and protein kinase C. Thus, whether midostaurin will provide antileukemia activity in FLT3 WT but otherwise class I addicted AML is a valuable question to be evaluated in future trials.

Quizartinib (formerly AC220). Several phase 2 studies have been completed in the relapsed/refractory AML setting, with encouraging single-agent activity including a composite CR rate (CRc) of 44% to 54% and modest median response duration of 11 to 12.7 weeks, due primarily to the development of resistance mutations within the FLT3 tyrosine kinase domain (eg, D835), or gatekeeper mutations (eg, F691) when used in the single-agent setting.[32,33] A phase 3 trial of quizartinib monotherapy vs salvage chemotherapy in relapsed/refractory FLT3-ITD mutant AML is ongoing (#NCT02039726).

Crenolanib. Initially developed as a platelet-derived growth factor receptor inhibitor, crenolanib demonstrates pan-selective FLT3 activity, active against both the FLT3-ITD and FLT3-D835 resistance mutation. Several phase 2 studies of crenolanib in combination with induction chemotherapy, or as post-SCT maintenance, are ongoing (#NCT02283177 and #NCT02400255).

Gilteritinib (formerly ASP2215). Gilteritinib has potent activity against both FLT3-ITD and D835 mutations, and also the gatekeeper mutation F691L. Interim results from the phase 1/2 trial of relapsed/refractory AML were reported last year, with a CRc rate of 43% as a single-agent therapy. Notably different than the first generation of multikinase inhibitors, AML patients with WT FLT3 status receiving gilteritinib derived nominal benefit, with a CRc <10%. A randomized phase 3 trial of gilteritinib vs investigator choice salvage chemotherapy is ongoing for relapsed/refractory FLT3-mutant AML (#NCT02421939), and a randomized phase 3 trial (#NCT02752035) of gilteritinib alone, vs gilteritinib + azacitidine, vs azacitidine alone, in FLT3-mutant newly diagnosed AML, began recruitment in June 2016.

Mutations in epigenetic modifiers: regulation of DNA methylation and chromatin modification

Somatic mutations within key epigenetic regulators are identified in >50% of AML, and are now recognized as a key, and often an inciting, component of leukemogenesis.[21] It is of interest that age-related clonal hematopoiesis, identified in >10% of individuals over age 65, is predominantly defined by the clonal outgrowth of preleukemic clones harboring mutations in one of the genes within this epigenetic class.[34,35] It is likely that mutations in this class of epigenetic modifiers promote clonal outgrowth, but are insufficient to initiate leukemic transformation without subsequent mutational events.

DNMT3A. One of the 3 most common mutations in AML, *DNMT3A*, is recurrently mutated in ~20% of de novo AML. Operating via de novo methylation of cytosines in cytosine guanine dinucleotide islands, *DNMT3A* plays a critical role in regulation via epigenetic silencing of HSC differentiation and self-renewal. The most frequent *DNMT3A* mutation, the R882 missense mutation, has been demonstrated to prevent methyltransferase activity and DNA binding, leading to impaired HSC function, increased self-renewal, and a differentiation block.[36,37] *DNMT3A* mutations frequently occur with advanced age and in conjunction with *NPM1*, *FLT3-ITD*, and/or isocitrate dehydrogenase 1 (*IDH1*) mutations; the prognostic importance of *DNMT3A* mutations by themselves remains unclear, although several series have shown a correlation with resistance to chemotherapy and disease relapse.[38]

Ten-eleven translocation-2 (*TET2*). Inactivating mutations throughout *TET2* occur in 10% to 20% of MDS and AML, with a prominent frequency up to 50% in patients with chronic myelomonocytic leukemia. The resultant decrease in enzymatic *TET2* function in the setting of mutations impedes the breakdown of 5-methylcytosine to 5-hydroxymethylcytosine, leading to increased HSC self-renewal, impaired myeloid differentiation, and a hypermethylated epigenetic signature.[39] The prognosis of patients with *TET2* mutations is variable and likely dependent on the presence of additional cooperating pathogenic events.

Additional sex comb-like 1 (*ASXL1*). Loss-of-function mutations in the chromatin modifier gene *ASXL1* occurs in 10% to 20% of AML and are associated with advanced age, antecedent hematologic malignancy, concurrent runt-related transcription factor-1 (*RUNX1*) and spliceosome mutations, and poor outcomes.[40] ASXL1 is a chromatin-binding protein that interacts with the polycomb repressor complex 2, including the H3K27 methyltransferase *EZH2*; mutant *ASXL1* decreases polycomb repressor complex 2 recruitment and stabilization, and reduces repressive H3K27 trimethylation marks at various loci.[41]

IDH1/IDH2. The IDH family of metabolic enzymes operates within the Krebs cycle, catalyzing the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG). Recurrent "hot spot" mutations within the IDH enzymatic active sites (IDH1-R132, IDH2-R140, and IDH2-R172) occur in ~20% of AML, and are more frequent in intermediate-risk AML, advanced age, and concurrent with *NPM1* mutations.[42] IDH1/IDH2 mutations lead to an alternate reaction which reduces α-KG into the oncometabolite 2-hydroxyglutarate, which then competitively inhibits α-KG–dependent reactions, including *TET2*-dependent DNA hydroxymethylation, histone demethylase activity, B-cell lymphoma 2 (BCL2)-dependent mitochondrial respiration, and the activation of the hypoxia-inducible factor-1α pathway via prolyl hydrolase inhibition.[43] Of interest, mutations in *IDH1*, *IDH2*, and

TET2 appear to be nearly mutually exclusive, suggestive of a unique epigenetic mutational class leading to a global hypermethylation signature. IDH2-R172 mutations in particular appear to result in a unique gene expression profile with more profound metabolic aberrations, and are suggested to identify a distinct subgroup of 1% of AML patients with fewer co-mutations and a favorable prognosis with intensive chemotherapy.[21]

Epigenetic therapies

Targeted small molecule inhibitors of *IDH2* (enasidenib, formerly AG221), *IDH1* (AG120, IDH305, and FT-2102), or pan-IDH1/IDH2 (AG881) are currently in clinical development, with encouraging early results. Interim results of the phase 1 study of single-agent enasidenib in refractory hematologic malignancies show an overall response rate (ORR) (defined as CR/CR with incomplete blood count recovery [CRi]/morphologic leukemic-free state/partial remission) of 41% in 198 patients, with 18% CR rate in a primarily relapsed/refractory AML population.[44,45] Interval results of the phase 1 study of AG120 (#NCT02074839) describes similar efficacy in a smaller (n = 65) patient population, with an ORR of 36% and a CR rate of 18%.[46] Several additional clinical trials with AG120 and enasidenib are ongoing, including 7 + 3 + enasidenib or AG120 (#NCT02632708) and azacitidine + enasidenib or AG120 (#NCT02677922), both as frontline therapy for newly diagnosed AML populations, and a randomized phase 3 "IDHENTIFY" trial of enasidenib vs investigator's choice for relapsed/refractory AML with IDH2 mutation (#NCT02577406). Preliminary results of the phase 1 (#NCT02381886) study of the IDH1-inhibitor IDH305 are anticipated by the end of 2016.

Small molecule inhibitors of bromodomain-containing "reader" proteins, which recognize acetylated lysine residues and direct chromatin remodeling, include the bromodomain and extraterminal domain family and bromodomain inhibitors OXT015, CPI-0610, TEN-010, GSK525762, and INCB054329. These inhibitors have demonstrated formidable preclinical activity, and several are currently under evaluation in phase 1 AML studies (#NCT02158858, #NCT02308761, #NCT01943851, and #NCT02308761).

In view of the epigenetic instability introduced by mutations in epigenetic modifier genes, the HMAs such as azacitidine, decitabine, and guadecitabine (SGI-110) are frequently recommended for the treatment of AML patients harboring these mutations, particularly given the more advanced age of AML patients with epigenetic mutations. HMAs inhibit DNA methyltransferase-1 and are known to induce hypomethylation, although the mechanisms responsible for antileukemia activity remain poorly understood and there has been no confirmed relationship between the presence or absence of epigenetic mutations, degree of demethylation with HMA treatment, and patient outcome.[47,48] Of interest among MDS patients, the combination of mutant *TET2* with WT *ASXL1* demonstrated an improved HMA response rate (60% vs 43%) although without OS impact,[49] further indicating that evaluation of gene-gene interactions, and not only single mutations, will be essential to better identify treatment strategies and provide improved prognostication.

Mutations in nucleophosmin

NPM1. Along with *FLT3* and *DNMT3A*, *NPM1* is one of the 3 most frequent driver mutations in AML. *NPM1* mutations, occurring almost exclusively within exon 12 of the gene, occur in approximately one-third of adults with AML, and in more than 50% of NK-AML, frequently in the context of mutations in epigenetic modifiers such as *DNMT3A*, *TET2*, *IDH1*, or *IDH2* mutations. The *NPM1* gene encodes for the nuclear chaperone protein NPM, which shuttles between the nucleus and cytoplasm and plays a role in diverse cellular functions, including protein formation, ribosome biogenesis, DNA replication, and the cell cycle. *NPM1* mutations are typically stable throughout the disease course, and are identified in nearly all leukemic cells and impart a distinct expression profile.[50] *NPM1*-mutant AML, specifically those without *FLT3-ITD* mutations, consistently demonstrates augmented chemosensitivity to standard therapy and overall favorable outcomes, even among older patients.[51,52] However, *NPM1* mutations in the setting of mutant *DNMT3A,* particularly in the setting of *FLT3-ITD,* confer a markedly poor prognosis.[21,53] Some reports suggest distinctive activity of all-trans retinoic acid and actinomycin D in AML with mutant *NPM1*, although this observation has not been systematically confirmed.[54,55]

Mutations in transcription factors and master regulators

Approximately 20% to 25% of adults with AML have mutations involving the myeloid transcription factors *RUNX1*, CCAAT/enhancer binding protein α (*CEBPA*), and/or *GATA2*. To date, no targeted or otherwise specific therapeutic options exist for this prognostically disparate molecular subgroup.

CEBPA. *CEBPA* is a key hematopoietic transcription factor involved in lineage-specific myeloid differentiation. Mutations in the single exon *CEBPA* gene occur in ~10% of AML, predominantly in younger patients and NK, or otherwise intermediate-risk karyotypes. Pathogenic *CEBPA* mutations occur within 2 discrete gene regions: *N*-terminal domain frameshifts directing an alternate 30-kDa protein, and C-terminal domain insertions and deletions, which disrupt DNA-binding and dimerization. Originally included in the 2008 World Health Organization (WHO) AML classification system, AML with mutated *CEBPA* has been confirmed as a unique pathologic entity within the 2016 revised classification schema, with the important distinction that biallelic mutations (encompassing 2/3 of *CEBPA*-mutant patients) are associated with this favorable prognosis.[56-58]

RUNX1. *RUNX1* is a critical transcription factor, essential during embryogenesis in HSC generation, and during adulthood in the regulation of HSC differentiation and homeostasis. Various missense and frameshift mutations in *RUNX1* are identified in AML patients at a frequency of ~10% to 15%, and are particularly frequent in patients with antecedent hematologic disorders or secondary AML, advanced age, increased cytopenias at diagnosis, shorter OS, and chemoresistance to standard therapy.[59] Mutations in *RUNX1* are thought to establish a compromised stem cell phenotype characterized by early HSC exhaustion.[60] The 2016 revised WHO AML classification system has added AML with mutated *RUNX1* as a provisional entity, owing to the biologically distinct molecular signature and poor prognosis with this molecular AML subtype.[59,61]

GATA2. The *GATA2* gene encodes a zinc-finger transcription factor with a major role in myeloid hematopoiesis, via cooperation within a network of transcription factors in a tightly regulated and dose-dependent manner. Assorted gene-wide mutations resulting in *GATA2* haploinsufficiency include truncating, missense, and non-coding variants within the regulatory GATA2 region.[62] *GATA2*-mutated AML represents ~5% of all patients, although notably *GATA2* mutations are seen as a cooperating event in ~25% of *CEBPA*-mutated AML patients and do not appear to impact the favorable outcome of *dmCEBPA*.[63]

Germ line predispositions to malignancy. Although mutations in myeloid malignancies are nearly always presumed to be somatic

Table 2. Emerging targets and selected AML targeted agents

Targets	Emerging targeted agents
Tyrosine and signaling kinases	Midostaurin, sorafenib, crenolanib, quizartinib, gilteritinib, and pacritinib (FLT3)
	BGB324 (AXL)
	BKM120, BYL719, and TGR-1202 (PI3K)
	Trametinib (RAS)
	GSK2141795 (AKT)
Cell cycle regulators	Alisertib and AZD1152 (aurora kinases)
	Rigosertib (PLK1)
	MK-1775 (WEE1)
	Palbociclib, LEE011, and FLX-925 (CDK4/6)
DNA methylation	AG120, IDH305, AG881, and FT-2102 (IDH1)
	AG221 and AG881 (IDH2)
Histone methylation	EPZ-5676 (MLL)
	GSK2879552 (LSD1)
BETs/epigenetic "readers"	OTX-015, CPI-0610, TEN-010, GSK525762, and INCB054329
WNT pathway	PRI-724
TP53	ONC201
Apoptosis regulators	Venetoclax (BCL2)
	Birinapant (IAP)
	DS3032b, RO5503781, AMG232, and HDM201 (MDM2)

Selected agents are provided as examples; this is not an exhaustive list. Monoclonal antibodies and immunotherapies are outside the scope of this review.
BET, bromodomain and extraterminal domain family.

events, recent evidence suggests that a portion of hematologic cancers, similar to solid tumors, have an underlying hereditary cancer predisposition syndrome.[64] Because germ line samples in patients with hematologic malignancies are not typically obtained, standard sequencing technology is unable to distinguish whether a mutation is germ line or somatic in nature. The germ line inheritance of mutations in the transcription factor genes *CEBPA*, *RUNX1*, and *GATA2* lead to well-described, autosomal dominant, familial predispositions to AML, which are notably now outlined within the 2016 WHO AML guidelines.[56] Attention to the medical and family history, and consideration of germ line testing must be considered when mutations in the above genes are identified.[65]

Approximately 10% of patients with biallelic *CEBPA* mutations are predicted to have an *N*-terminal germ line mutation, with a secondary somatic C-terminal *CEBPA* mutation identified at the time of AML diagnosis. The favorable prognosis of biallelic *CEBPA* mutations is not affected by whether both mutations are somatic or one is of germ line inheritance.[66] Notably, there are no prodromal signs, symptoms, or antecedent cytopenias described with this syndrome that would help augment clinical suspicion. Individuals with germ line *RUNX1* mutations are diagnosed with the syndrome "familial platelet disorder with propensity to myeloid malignancies," which is characterized by variable degrees of life-long thrombocytopenia and aspirin-like platelet dysfunction. The long-term outcome of "familial platelet disorder with propensity to myeloid malignancies" is variable, with an ~35% lifetime risk of hematologic malignancy at an average age at diagnosis of 33 years.[67] In addition to increased lifetime risk of MDS/AML, germ line *GATA2* mutations are further characterized by dramatic immunodeficiency related to profound B lymphocyte, NK, and dendritic cell deficiencies, frequent atypical and viral infections, and other nonhematologic symptoms including pulmonary, rheumatologic, lymphatic, and vascular pathology comprised within the "MonoMAC Syndrome."[68]

RNA splicing factor mutations

Frequently mutated in MDS and myeloproliferative neoplasms, mutations in splicing factors (*SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2*) are identified in ~10% of patients with AML and are associated with older age, less proliferative disease, poor rates of response to standard treatment, and decreased survival. Spliceosome mutations are postulated to promote malignancy through missplicing of various genes involved in epigenetic regulation, transcription, and genome integrity.[69] Current evidence posits spliceosome mutations are mutually exclusive of each other and always co-expressed with the WT allele, suggesting that one functional allele is required for cellular integrity. Thus, a synthetically lethal approach of splicing inhibitor therapy for patients with preexisting splicing factor mutations has been postulated to be a uniquely effective targeted therapy for this genetically defined AML subset.[70] Preclinical and phase 1 studies are ongoing, including a phase 1 trial of the selective small molecule splicing modulator (H3B-8800), specifically for patients with hematologic malignancies (#NCT02841540).

Tumor protein 53 (TP53) tumor suppressor

TP53. Termed "the guardian of the genome," TP53 is a key tumor suppressor with protean functions related to maintenance of genomic stability, including regulation of cellular senescence, apoptosis, metabolism, and DNA repair. Although uncommon in de novo AML, *TP53* mutations occur in ~15% of therapy-related AML or AML with MDS-related changes, and are predominantly associated with complex cytogenetics, advanced age, chemoresistance, and poor survival.[71,72] Irrespective of age or treatment modality, *TP53* mutations in AML portend lower response rates and inferior outcomes compared with *TP53* WT AML patients,[73] including high relapse rates after SCT. The optimal treatment strategy for *TP53*-mutant patients thus remains a critical area of unmet need.

Selected small molecule therapeutic strategies

With activity extending outside of a specific molecular group, BCL2 inhibitors, along with other inhibitors of antiapoptotic proteins such as MCL1, are promising small molecule approaches in AML therapy (Table 2). Despite modest single-agent activity in the relapsed/refractory AML setting with an ORR of 19%, the BCL2 inhibitor venetoclax (formerly ABT-199) has demonstrated impressive early responses in the frontline treatment of elderly AML patients unfit for standard chemotherapy, with a CR/CRi rate of 71% and median time to CR/CRi of 29.5 days.[74] Elegant preclinical work has demonstrated striking synthetic lethality of the IDH-mutant AML to BCL2 inhibition owing to the aberrant oncometabolite 2-hydroxyglutarate elevation in IDH mutants, rendering these leukemic cells particularly dependent on BCL2.[75] Clinical responses have encouragingly been seen across AML subgroups, and an analysis of BCL2 protein sensitivity index at presentation may provide a useful predictive biomarker for response.[76]

The MDM2 protein, which functions in complex with TP53 and is frequently overexpressed in TP53 WT AML, can be inhibited by small molecule MDM2 inhibitors, leading to TP53 stabilization and restoration of TP53 activity. The MDM2 inhibitor RG7112 demonstrated modest single-agent activity in TP53 WT patients, with baseline MDM2 expression levels predictive of response.[77] The efficacy and tolerability of novel MDM2 inhibitors, particularly in synergistic combinations, is keenly awaited.

Improved AML classification at diagnosis

Patients with AML have traditionally been characterized within 1 of 3 risk groups based on the presence of various cytogenetic abnormalities,

with the majority of patients historically falling within the NK or otherwise intermediate-risk group. For the first time, in 2010, recurrent somatic mutations, specifically mutations in *NPM1*, *FLT3*, and *CEBPα*, were incorporated to further refine prognostic information.[18] It is now estimated that two-thirds of the variation in AML outcome is based on genomic features, including fusion genes, copy-number alterations, point mutations, and gene-gene interactions.[21] The current 2016 revision to the WHO AML classification reinforces the favorable prognostic importance of AML with mutated *NPM1* (without concurrent *FLT3* mutation), and AML with biallelic *CEBPA* mutations.[56] Similar to patients with CBF AML, patients with *NPM1*-mutant and *dmCEBPA* AML appear to be particularly chemosensitive to anthracycline and cytarabine-based combination chemotherapy approaches, and allogeneic SCT is not recommended at the time of first CR.[50,78] Thus, in addition to CBF AML patients, patients with *NPM1*-mutant or *dmCEBPA* AML, regardless of age, should receive intensive induction and consolidation therapy when possible. Furthermore, the addition of FLT3-inhibitor therapy at diagnosis for patients with *FLT3-ITD*–mutated AML has demonstrated significant survival benefit and this should now be considered the standard of care. New to the 2016 WHO classification, AML with mutated *RUNX1* has been incorporated as a provisional entity, due to the apparent biologically distinct *RUNX1*-mutated AML subgroup, demonstrating both a poor response to standard cytotoxic therapy and poor overall outcomes.[59] The best treatment algorithm for *RUNX1*-mutated patients, as well as patients with other high-risk genetic lesions such as chromatin-spliceosome mutations, *TP53* mutations, or complex cytogenetics has not been established, and well-designed clinical trials remain the most appropriate option.

A unique AML classification schema has been described among patients with secondary or therapy-related AML; secondary defining AML arising from an antecedent hematologic disorder (secondary AML) and therapy-related AML (tAML) characterizing patients having previously received leukemogenic therapies for nonmyeloid disorders. The presence of mutations within 8 genes (*ASXL1*, *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, *EZH2*, *BCOR*, and *STAG2*) demonstrated >95% sensitivity in defining secondary AML compared with de novo AML, notably all genes commonly mutated in MDS and involved in chromatin modification, cohesion complex, and the spliceosome.[71] In patients with tAML, the presence of *TP53* mutations in 15% was associated with a highly complex karyotype, chemoresistance, and poor survival. A separate cohort of tAML patients was identified who were genetically classified as "de novo AML," with the presence of genetic events such as *NPM1* mutations and CBF rearrangements; these patients had outcomes consistent with de novo AML, including favorable response to standard cytotoxic chemotherapy and improved EFS. These results advocate that the evaluation of genetic "ontogeny" in AML may provide more prognostic and therapeutic information than the traditional classification.

AML classification during therapy and at relapse

The importance of the identification of recurrent mutations in AML extends beyond the initial treatment decision. Evaluation of somatic mutations can be followed over time, especially as reproducible and standardized methods of molecular minimal residual disease (MRD) assessment monitoring continue to improve (see Ossenkoppele, in this book[79]). This is particularly important in the cohort of NK-AML patients, for whom cytogenetic and fluorescence in situ hybridization methods of MRD analysis are not routinely available. For example, a "favorable risk" NK-AML patient in CR1 with *NPM1*-mutant, *FLT3*-WT status, yet with persistent mutant *NPM1* detection by MRD assessment after cycle 2 of therapy, is associated with a higher risk of relapse, and an SCT in CR1 would be indicated based on real-time molecular MRD results, despite the favorable original prognostic score.[80]

In relapsed/refractory AML, few standard treatment options exist, and patient outcome remains poor. An enhanced understanding of the patient-specific mutational AML landscape at relapse can inform salvage treatment programs, and repeat cytogenetic and molecular classification at the time of relapse is warranted to determine whether patients are eligible and/or appropriate for investigational or alternative treatment options. Patients with favorable-risk AML may benefit most from re-induction cytarabine-based strategies. An evaluation for *FLT3*, *IDH1*, and *IDH2* mutations should be universal at relapse, given the promising results with the respective targeted inhibitors available in late-phase clinical trials. In cytarabine-refractory AML patients with poor-risk molecular or cytogenetic features, clinical trial options incorporating hypomethylating-agent combinations, novel targeted therapeutics, or other treatment modalities (ie, antibody-based and/or immunotherapy approaches) are appropriate strategies.

Conclusion

Given the extraordinary recent progress made in the understanding of cancer biology and mechanisms of leukemogenesis, we are now uniquely poised to see our research-based innovations translate directly into the care of patients with AML. Encouraging single-agent efficacy has been demonstrated with targeted small molecule inhibitors such as *FLT3*, *IDH1*, and *IDH2*, and the early evidence of synergistic activity of the BCL2 inhibitor venetoclax in combination with HMA, speaks to the importance of rational AML combinations. The ultimate realization of this recent sequencing era will be to organize the diverse genomic information into a distinct framework to inform our prognostic accuracy and provide personalized treatment decisions for all.

Correspondence

Courtney D. DiNardo, Department of Leukemia, University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 0428, Houston, TX 77030; e-mail: cdinardo@mdanderson.org.

References

1. Dores GM, Devesa SS, Curtis RE, Linet MS, Morton LM. Acute leukemia incidence and patient survival among children and adults in the United States, 2001-2007. *Blood*. 2012;119(1):34-43.
2. Deschler B, Lübbert M. Acute myeloid leukemia: epidemiology and etiology. *Cancer*. 2006;107(9):2099-2107.
3. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin*. 2013;63(1):11-30.
4. Grimwade D, Hills RK, Moorman AV, et al; National Cancer Research Institute Adult Leukaemia Working Group. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood*. 2010;116(3):354-365.
5. Fröhling S, Schlenk RF, Kayser S, et al; German-Austrian AML Study Group. Cytogenetics and age are major determinants of outcome in intensively treated acute myeloid leukemia patients older than 60 years: results from AMLSG trial AML HD98-B. *Blood*. 2006;108(10):3280-3288.
6. Yates JW, Wallace HJ Jr, Ellison RR, Holland JF. Cytosine arabinoside (NSC-63878) and daunorubicin (NSC-83142) therapy in acute nonlymphocytic leukemia. *Cancer Chemother Rep*. 1973;57(4):485-488.

7. Juliusson G, Antunovic P, Derolf A, et al. Age and acute myeloid leukemia: real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry. *Blood*. 2009;113(18):4179-4187.
8. Kantarjian HM, Thomas XG, Dmoszynska A, et al. Multicenter, randomized, open-label, phase III trial of decitabine versus patient choice, with physician advice, of either supportive care or low-dose cytarabine for the treatment of older patients with newly diagnosed acute myeloid leukemia. *J Clin Oncol*. 2012;30(21):2670-2677.
9. Fenaux P, Mufti GJ, Hellström-Lindberg E, et al. Azacitidine prolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia. *J Clin Oncol*. 2010;28(4):562-569.
10. Medeiros BC, Satram-Hoang S, Hurst D, Hoang KQ, Momin F, Reyes C. Big data analysis of treatment patterns and outcomes among elderly acute myeloid leukemia patients in the United States. *Ann Hematol*. 2015; 94(7):1127-1138.
11. Shah A, Andersson TM, Rachet B, Björkholm M, Lambert PC. Survival and cure of acute myeloid leukaemia in England, 1971-2006: a population-based study. *Br J Haematol*. 2013;162(4):509-516.
12. Appelbaum FR, Gundacker H, Head DR, et al. Age and acute myeloid leukemia. *Blood*. 2006;107(9):3481-3485.
13. Bloomfield CD, Lawrence D, Byrd JC, et al. Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. *Cancer Res*. 1998; 58(18):4173-4179.
14. Lo-Coco F, Avvisati G, Vignetti M, et al; Gruppo Italiano Malattie Ematologiche dell'Adulto; German-Austrian Acute Myeloid Leukemia Study Group; Study Alliance Leukemia. Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. *N Engl J Med*. 2013;369(2):111-121.
15. Ravandi F, Estey E, Jones D, et al. Effective treatment of acute promyelocytic leukemia with all-trans-retinoic acid, arsenic trioxide, and gemtuzumab ozogamicin. *J Clin Oncol*. 2009;27(4):504-510.
16. Byrd JC, Mrózek K, Dodge RK, et al; Cancer and Leukemia Group B (CALGB 8461). Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). *Blood*. 2002;100(13): 4325-4336.
17. Grimwade D, Walker H, Harrison G, et al; Medical Research Council Adult Leukemia Working Party. The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. *Blood*. 2001;98(5):1312-1320.
18. Döhner H, Estey EH, Amadori S, et al; European LeukemiaNet. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood*. 2010;115(3):453-474.
19. Patel JP, Gönen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med*. 2012; 366(12):1079-1089.
20. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia [published correction appears in *N Engl J Med*. 2013;369(1):98]. *N Engl J Med*. 2013; 368(22):2059-2074.
21. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med*. 2016;374(23): 2209-2221.
22. Ravandi F, Kantarjian H, Faderl S, et al. Outcome of patients with FLT3-mutated acute myeloid leukemia in first relapse. *Leuk Res*. 2010;34(6): 752-756.
23. Pratcorona M, Brunet S, Nomdedéu J, et al; Grupo Cooperativo Para el Estudio y Tratamiento de las Leucemias Agudas Mieloblásticas. Favorable outcome of patients with acute myeloid leukemia harboring a low-allelic burden FLT3-ITD mutation and concomitant NPM1 mutation: relevance to post-remission therapy. *Blood*. 2013;121(14):2734-2738.
24. Badar T, Patel KP, Thompson PA, et al. Detectable FLT3-ITD or RAS mutation at the time of transformation from MDS to AML predicts for very poor outcomes. *Leuk Res*. 2015;39(12):1367-1374.
25. Burgess MR, Hwang E, Firestone AJ, et al. Preclinical efficacy of MEK inhibition in Nras-mutant AML. *Blood*. 2014;124(26):3947-3955.
26. Borthakur G, Popplewell L, Boyiadzis M, et al. Activity of the oral mitogen-activated protein kinase kinase inhibitor trametinib in RAS-mutant relapsed or refractory myeloid malignancies. *Cancer*. 2016; 122(12):1871-1879.
27. Pollard JA, Alonzo TA, Gerbing RB, et al. Prevalence and prognostic significance of KIT mutations in pediatric patients with core binding factor AML enrolled on serial pediatric cooperative trials for de novo AML. *Blood*. 2010;115(12):2372-2379.
28. Klein K, Kaspers G, Harrison CJ, et al. Clinical impact of additional cytogenetic aberrations, cKIT and RAS mutations, and treatment elements in pediatric t(8;21)-AML: results from an international retrospective study by the International Berlin-Frankfurt-Münster Study Group. *J Clin Oncol*. 2015;33(36):4247-4258.
29. Marcucci G, Geyer S, Zhao W, et al. Adding KIT inhibitor dasatinib (DAS) to chemotherapy overcomes the negative impact of KIT mutation/over-expression in core binding factor (CBF) acute myeloid leukemia (AML): results from CALGB 10801 (Alliance). *Blood*. 2014; 124(21):8.
30. Röllig C, Serve H, Hüttmann A, et al; Study Alliance Leukaemia. Addition of sorafenib versus placebo to standard therapy in patients aged 60 years or younger with newly diagnosed acute myeloid leukaemia (SORAML): a multicentre, phase 2, randomised controlled trial. *Lancet Oncol*. 2015;16(16):1691-1699.
31. Stone RM, Mandrekar S, Sanford BL, et al. The multi-kinase inhibitor midostaurin (M) prolongs survival compared with placebo (P) in combination with daunorubicin (D)/cytarabine (C) induction (ind), high-dose C consolidation (consol), and as maintenance (maint) therapy in newly diagnosed acute myeloid leukemia (AML) patients (pts) age 18-60 with *FLT3* mutations (muts): an international prospective randomized (rand) P-controlled double-blind trial (CALGB 10603/RATIFY [Alliance]) [abstract]. *Blood*. 2015;126(23). Abstract 6.
32. Daver N, Cortes J, Ravandi F, et al. Secondary mutations as mediators of resistance to targeted therapy in leukemia. *Blood*. 2015;125(21): 3236-3245.
33. Stein EM. Molecularly targeted therapies for acute myeloid leukemia. *Hematology Am Soc Hematol Educ Program*. 2015;2015:579-583.
34. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014;371(26): 2488-2498.
35. Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med*. 2014;371(26):2477-2487.
36. Challen GA, Sun D, Jeong M, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat Genet*. 2011;44(1):23-31.
37. Mayle A, Yang L, Rodriguez B, et al. Dnmt3a loss predisposes murine hematopoietic stem cells to malignant transformation. *Blood*. 2015; 125(4):629-638.
38. Eriksson A, Lennartsson A, Lehmann S. Epigenetic aberrations in acute myeloid leukemia: early key events during leukemogenesis. *Exp Hematol*. 2015;43(8):609-624.
39. Moran-Crusio K, Reavie L, Shih A, et al. Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. *Cancer Cell*. 2011;20(1):11-24.
40. Schnittger S, Eder C, Jeromin S, et al. ASXL1 exon 12 mutations are frequent in AML with intermediate risk karyotype and are independently associated with an adverse outcome. *Leukemia*. 2013;27(1):82-91.
41. Abdel-Wahab O, Adli M, LaFave LM, et al. ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. *Cancer Cell*. 2012;22(2):180-193.
42. DiNardo CD, Ravandi F, Agresta S, et al. Characteristics, clinical outcome, and prognostic significance of IDH mutations in AML. *Am J Hematol*. 2015;90(8):732-736.
43. Inoue S, Lemonnier F, Mak TW. Roles of IDH1/2 and TET2 mutations in myeloid disorders. *Int J Hematol*. 2016;103(6):627-633.
44. Stein EM, DiNardo C, Altman JK, et al. Safety and efficacy of AG-221, a potent inhibitor of mutant IDH2 that promotes differentiation of

myeloid cells in patients with advanced hematologic malignancies: results of a phase 1/2 trial [abstract]. *Blood*. 2015;126(23). Abstract 323.
45. Stein EM, Altman JK, Collins R, et al. AG-221, an oral, selective, first-in-class, potent inhibitor of the IDH2 mutant enzyme, induces durable remissions in a phase I study in patients with IDH2 mutation positive advanced hematologic malignancies. *Blood*. 2014;124(21):115.
46. DiNardo C, de Botton S, Pollyea DA, et al. Molecular profiling and relationship with clinical response in patients with IDH1 mutation-positive hematologic malignancies receiving AG-120, a first-in-class potent inhibitor of mutant IDH1, in addition to data from the completed dose escalation portion of the phase 1 study [abstract]. *Blood*. 2015; 126(23). Abstract 1306.
47. Voso MT, Santini V, Fabiani E, et al. Why methylation is not a marker predictive of response to hypomethylating agents. *Haematologica*. 2014; 99(4):613-619.
48. Im AP, Sehgal AR, Carroll MP, et al. DNMT3A and IDH mutations in acute myeloid leukemia and other myeloid malignancies: associations with prognosis and potential treatment strategies [published correction appears in *Leukemia*. 2015;29(2):516]. *Leukemia*. 2014;28(9):1774-1783.
49. Bejar R, Lord A, Stevenson K, et al. TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. *Blood*. 2014;124(17):2705-2712.
50. Becker H, Marcucci G, Maharry K, et al. Favorable prognostic impact of NPM1 mutations in older patients with cytogenetically normal de novo acute myeloid leukemia and associated gene- and microRNA-expression signatures: a Cancer and Leukemia Group B study. *J Clin Oncol*. 2010; 28(4):596-604.
51. Döhner K, Schlenk RF, Habdank M, et al. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. *Blood*. 2005;106(12):3740-3746.
52. Falini B, Mecucci C, Tiacci E, et al; GIMEMA Acute Leukemia Working Party. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med*. 2005;352(3):254-266.
53. Alpermann T, Schnittger S, Eder C, et al. Molecular subtypes of NPM1 mutations have different clinical profiles, specific patterns of accompanying molecular mutations and varying outcomes in intermediate risk acute myeloid leukemia. *Haematologica*. 2016;101(2):e55-e58.
54. Falini B, Brunetti L, Martelli MP. Dactinomycin in NPM1-mutated acute myeloid leukemia. *N Engl J Med*. 2015;373(12):1180-1182.
55. Burnett AK, Hills RK, Green C, et al. The impact on outcome of the addition of all-trans retinoic acid to intensive chemotherapy in younger patients with nonacute promyelocytic acute myeloid leukemia: overall results and results in genotypic subgroups defined by mutations in NPM1, FLT3, and CEBPA. *Blood*. 2010;115(5):948-956.
56. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-2405.
57. Wouters BJ, Löwenberg B, Erpelinck-Verschueren CA, van Putten WL, Valk PJ, Delwel R. Double CEBPA mutations, but not single CEBPA mutations, define a subgroup of acute myeloid leukemia with a distinctive gene expression profile that is uniquely associated with a favorable outcome. *Blood*. 2009;113(13):3088-3091.
58. Pabst T, Eyholzer M, Fos J, Mueller BU. Heterogeneity within AML with CEBPA mutations; only CEBPA double mutations, but not single CEBPA mutations are associated with favourable prognosis. *Br J Cancer*. 2009; 100(8):1343-1346.
59. Gaidzik VI, Teleanu V, Papaemmanuil E, et al. RUNX1 mutations in acute myeloid leukemia are associated with distinct clinico-pathologic and genetic features [published online ahead of print June 10, 2016]. *Leukemia*.2016.
60. Jacob B, Osato M, Yamashita N, et al. Stem cell exhaustion due to Runx1 deficiency is prevented by Evi5 activation in leukemogenesis. *Blood*. 2010;115(8):1610-1620.
61. Mendler JH, Maharry K, Radmacher MD, et al. RUNX1 mutations are associated with poor outcome in younger and older patients with cytogenetically normal acute myeloid leukemia and with distinct gene and microRNA expression signatures. *J Clin Oncol*. 2012;30(25): 3109-3118.
62. Wlodarski MW, Hirabayashi S, Pastor V, et al; EWOG-MDS. Prevalence, clinical characteristics, and prognosis of GATA2-related myelodysplastic syndromes in children and adolescents. *Blood*. 2016;127(11): 1387-1397, quiz 1518.
63. Green CL, Tawana K, Hills RK, et al. GATA2 mutations in sporadic and familial acute myeloid leukaemia patients with CEBPA mutations. *Br J Haematol*. 2013;161(5):701-705.
64. DiNardo CD, Bannon SA, Routbort M, et al. Evaluation of patients and families with concern for predispositions to hematologic malignancies within the Hereditary Hematologic Malignancy Clinic (HHMC). *Clin Lymphoma Myeloma Leuk*. 2016;16(7):417-428.e2.
65. DiNardo CD. Getting a handle on hereditary CEBPA mutations. *Blood*. 2015;126(10):1156-1158.
66. Pabst T, Eyholzer M, Haefliger S, Schardt J, Mueller BU. Somatic CEBPA mutations are a frequent second event in families with germline CEBPA mutations and familial acute myeloid leukemia. *J Clin Oncol*. 2008;26(31):5088-5093.
67. Owen CJ, Toze CL, Koochin A, et al. Five new pedigrees with inherited RUNX1 mutations causing familial platelet disorder with propensity to myeloid malignancy. *Blood*. 2008;112(12):4639-4645.
68. Spinner MA, Sanchez LA, Hsu AP, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. *Blood*. 2014; 123(6):809-821.
69. Dvinge H, Kim E, Abdel-Wahab O, Bradley RK. RNA splicing factors as oncoproteins and tumour suppressors. *Nat Rev Cancer*. 2016;16(7): 413-430.
70. Lee SC, Dvinge H, Kim E, et al. Modulation of splicing catalysis for therapeutic targeting of leukemia with mutations in genes encoding spliceosomal proteins. *Nat Med*. 2016;22(6):672-678.
71. Lindsley RC, Mar BG, Mazzola E, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood*. 2015;125(9): 1367-1376.
72. Devillier R, Mansat-De Mas V, Gelsi-Boyer V, et al. Role of ASXL1 and TP53 mutations in the molecular classification and prognosis of acute myeloid leukemias with myelodysplasia-related changes. *Oncotarget*. 2015;6(10):8388-8396.
73. Rücker FG, Schlenk RF, Bullinger L, et al. TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome. *Blood*. 2012;119(9):2114-2121.
74. DiNardo C, Pollyea D, Pratz K, et al. A phase 1b study of venetoclax (ABT-199/GDC-0199) in combination with decitabine or azacitidine in treatment-naive patients with acute myelogenous leukemia who are ≥ to 65 years and not eligible for standard induction therapy. *Blood*. 2015; 126(23):327.
75. Chan SM, Thomas D, Corces-Zimmerman MR, et al. Isocitrate dehydrogenase 1 and 2 mutations induce BCL-2 dependence in acute myeloid leukemia. *Nat Med*. 2015;21(2):178-184.
76. Konopleva M, Pollyea DA, Potluri J, et al. Efficacy and biological correlates of response in a phase II study of venetoclax monotherapy in patients with acute myelogenous leukemia [published online ahead of print August 12, 2016]. *Cancer Discov*. 2016.
77. Andreeff M, Kelly KR, Yee K, et al. Results of the phase I trial of RG7112, a small-molecule MDM2 antagonist in leukemia. *Clin Cancer Res*. 2016;22(4):868-876.
78. Büchner T, Berdel WE, Haferlach C, et al. Age-related risk profile and chemotherapy dose response in acute myeloid leukemia: a study by the German Acute Myeloid Leukemia Cooperative Group. *J Clin Oncol*. 2009;27(1):61-69.
79. Ossenkoppele G, Schuurhuis GJ. MRD in AML: does it already guide therapy decision-making? *Hematology Am Soc Hematol Educ Program*. 2016;2016:356-365.
80. Ivey A, Hills RK, Simpson MA, et al; UK National Cancer Research Institute AML Working Group. Assessment of minimal residual disease in standard-risk AML. *N Engl J Med*. 2016;374(5):422-433.