Acute myeloid leukemia in the era of precision medicine: recent advances in diagnostic classification and risk stratification

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ABSTRACT
Acute myeloid leukemia (AML) is a genetically heterogeneous myeloid malignancy that occurs more commonly in adults, and has an increasing incidence, most likely due to increasing age. Precise diagnostic classification of AML requires clinical and pathologic information, the latter including morphologic, immunophenotypic, cytogenetic and molecular genetic analysis. Risk stratification in AML requires cytogenetics evaluation as the most important predictor, with genetic mutations providing additional necessary information. AML with normal cytogenetics comprises about 40%-50% of all AML, and has been intensively investigated. The currently used 2008 World Health Organization classification of hematopoietic neoplasms has been proposed to be updated in 2016, also to include an update on the classification of AML, due to the continuously increasing application of genomic techniques that have led to major advances in our knowledge of the pathogenesis of AML. The purpose of this review is to describe some of these recent major advances in the diagnostic classification and risk stratification of AML.

KEYWORDS
Acute myeloid leukemia; myeloid neoplasms; genomics; precision medicine; next-generation sequencing; cytogenetics; mutations

Introduction
The diagnosis and classification of myeloid neoplasms has been described in the revised World Health Organization (WHO) classification of hematopoietic neoplasms, published in 2008\(^1\). The WHO classification was originally published in 2001, and at that time, had represented a paradigm shift by incorporating genetic information into the diagnostic algorithms\(^2,3\). According to the WHO classification, disease entities are defined by a combination of clinical, morphologic, immunophenotypic and genetic features. Myeloid neoplasms include the following major subgroups: (1) acute myeloid leukemias (AML) and related neoplasms; (2) myelodysplastic syndromes (MDS); (3) myeloproliferative neoplasms (MPN); (4) myelodysplastic/myeloproliferative neoplasms; and (5) myeloid/lymphoid neoplasms with eosinophilia and abnormalities of PDGFR\(A\), PDGFR\(B\), or FGFR\(1\). All myeloid neoplasms are considered to be clonal diseases arising from hematopoietic stem cells, with AML diagnosed as an acutely presenting disease with >20% bone marrow or circulating myeloid blasts, and the latter 4 subgroups as chronic diseases. MDS are considered pre-leukemic disorders, characterized by ineffective hematopoiesis, cytopenias, often with detected clonal cytogenetic abnormalities, <20% bone marrow blasts, and a propensity to secondarily progress to AML (secondary AML).

Since 2008, additional major advances in our knowledge of the molecular genetic features of hematologic neoplasms, in particular for myeloid neoplasms, are leading to another paradigm shift in the diagnosis and clinical management of these neoplasms in the era of precision medicine, as was also evident by papers presented at the recent annual meeting of the American Society of Hematology (ASH) in December, 2015. Due to these continuing major advances, a revision of the 2008 WHO classification of hematopoietic neoplasms has been proposed, including for AML and other hematopoietic neoplasms. This classification is due to be released in 2016, for AML and all other hematopoietic neoplasms, as per conference presentations by the WHO classification authors at the Society for Hematopathology session at the March 2015 Annual Meeting of the United States and Canadian Academy of Pathology in Boston, MA\(^4\), and at an educational
session at the recent ASH meeting for the proposed 2016 classification criteria for MDS. The purpose of this review is to describe some of the recent major advances that have occurred due to the impact of genomics in the diagnostic classification and risk stratification of AML.

Acute myeloid leukemia

Acute myeloid leukemia is a genetically heterogeneous disease that occurs primarily in adults, with an increasing incidence in the U.S., due primarily to longer survival, including after treatment for prior non-hematologic malignancies. In 2015, 20,830 new cases of AML were expected to occur in the U.S., including 12,730 in males and 8,100 in females. The original classification of acute leukemias by the French-American-British group was based on morphology and cytochemical stains, followed by the WHO classification in 2001, with revision in 2008 including the categories for AML, as shown in Table 1. The WHO classification recognized distinct clinicopathological entities characterized by the underlying specific cytogenetic or molecular genetic abnormalities. Since the 2001 WHO criteria, the diagnosis of an acute leukemia requires >20% bone marrow or peripheral blood blasts, with >20% myeloid blasts for a diagnosis of AML, or <20% myeloid blasts in the presence of recurrent cytogenetic abnormalities specific for AML. Diagnostic criteria for each of the categories were described in multiple monographs in the 2008 WHO edition, along with the rationale for the categories.

The majority of AML occur de novo, and few occur secondary to another myeloid disorder (secondary AML), or may occur after cytotoxic or other leukemogenic therapy (therapy-related), which are differentiated by clinical history. In AML, clonal chromosomal aberrations detected by cytogenetics at presentation were, in the past, recognized as the most important prognostic variable for AML, with three main (low, intermediate and high) prognostic risk groups, with the intermediate risk cytogenetically normal AML (CN-AML) comprising the largest group. Since then, numerous studies have identified additional molecular abnormalities leading to modification of the previous solely cytogenetic risk groups to now be defined by combined cytogenetic and molecular abnormalities, with only a few cited here. The combined cytogenetic and molecular abnormalities are now incorporated into the National Comprehensive Cancer Network (NCCN) guidelines for AML.

Risk stratification in AML

As per current NCCN guidelines Version 2.2014, the better risk group includes AML with recurrent translocations, including the t(8;21)(q22;q22), inv(16)(p13.1q22) or t(16;16)(p13.1;q22) and t(15;17)(q22;q12). It is important to note that not all AML with recurrent translocations/inversions are in the low risk category, as described in the WHO classification. The t(6;9) and t(3;3) translocations, and inv(3) are in the high risk group, which also includes AML with complex karyotypes (>3 clonal chromosomal abnormalities), monosomal karyotype (at least two autosomal monosomies, or one autosomal monosomy and
In AML leukemogenesis, mutations of several different classes co-operate, with Class I mutations, such as FLT3 and NRAS activating mutations and BCR-ABL fusion, conferring a proliferative advantage, and co-operating with Class II mutations, such as CEBPA, NPM1 and RUNX1 that primarily impair hematopoietic differentiation and subsequent apoptosis. Currently, the molecular markers NPM1, CEBPA and FLT3 are widely used clinically, dividing the large group of CN-AML into two subsets: molecularly favorable (NPM1 mutated without FLT3-ITD, or biallelic CEBPA mutation) and unfavorable (FLT3-ITD with or without mutant NPM1, or wild-type NPM1 without FLT3-ITD), according to the European LeukemiaNet recommendations. Epigenetic regulators are a third category of genes (Class III), mutations in which have emerged as clinically significant co-operators for the pathogenesis of AML, and these genes include DNMT3A, IDH1, IDH2, and ASXL1. TET2 (tet methylcytosine dioxygenase 2), also an epigenetic regulator, is mutated in a subset of CN-AML, but currently, sufficient evidence does not appear to exist for TET2 to be considered a clinically relevant prognostic marker in AML.

The fourth category (Class IV), comprised of tumor suppressor genes, includes the TP53 and WT1 genes, with TP53 aberrant AML having a dismal prognosis. Genes in the fifth category (Class V) are needed for RNA maturation, and include the RNA splicing genes, SF3B1, SRSF2, U2AF1, and ZRSR2. Mutations in these Class V genes currently appear to be more clinically significant in chronic diseases such as MDS and MPNs than in de novo AML.

Due to several next-generation sequencing (NGS) platforms now available to detect hundreds of gene mutations in the same analysis, and with the increasing application of these genomic techniques in the clinical setting, our knowledge about the clinical significance of the detected mutations is continuously increasing. The Cancer Genome Atlas (TCGA) study of 200 de novo AML showed at least one driver mutation in 199 (>99%) cases, with thirteen coding mutations (single nucleotide variants and indels) on average per patient, and recurrent mutations in 23 genes. Human AML is a multi-step genetic process, with a complex clonal structure that evolves much before clinical presentation with AML. Different subtypes of AML appear to have different latency periods. For example, therapy-related AML, which are classified as therapy-related myeloid neoplasms, may have a shorter (25-26 months) latency period after the initiating event. Also, mutation rates have been shown to be different within different subclones within the same AML, indicating that numbers of mutations alone may not correspond to the duration of the latency period. Clonal cell populations...
appear to evolve during the latency period, as suggested by a genomic sequencing study of AML cases at diagnosis and relapse, that showed several clones present at diagnosis of AML. Clinically, increased clonal heterogeneity at diagnosis contributes to resistance to chemotherapy in any cancer, including in AML, with relapse often occurring due to clonal expansion of a previously present resistant subclone. In the whole-genome sequencing study mentioned above, AML relapse originated in 3 of 8 patients from a single major clone present at AML diagnosis.

Clonal abnormalities may occur in normal individuals, and recent studies have shown clonal hematopoiesis in normal individuals without evidence of a blood disorder. Interestingly, abnormalities identified as clonal in normal individuals are age-related, with clonal hematopoiesis identified in 0.8% of individuals under 60, rising to 19.5% in those >90 years of age, in a study of 4,219 individuals with investigation of 15 mutation hotspots in blood DNA using ultra-deep sequencing. In this study, DNMT3A-R882 mutations, which occur often in CN-AML, were most commonly identified, even in age <25 years, but with normally increased prevalence with age. In contrast, mutations of the spliceosome genes (Class V) SF3B1 and SRSF2, which occur commonly in MDS, were identified only in individuals aged >70 years in this study. Further, the 61 of 112 clones with DNMT3A mutations identified in this study had a low variant allele frequency of <3%. Notably, NPM1 mutations were almost completely absent in this cohort of >4,000 individuals, despite a highly sensitive assay designed specifically for their detection in this study. Since NPM1 mutations co-occur frequently with DNMT3A mutations in AML, the investigators suggested that NPM1 mutations appear to be closely related to the evolution of AML, and that DNMT3A mutations may allow the NPM1 mutant clones to be founded and expand towards AML. In addition to explaining the high incidence of these clonal myeloid neoplasms with age, and clarifying the role of mutations in AML, these studies also have implications for the interpretation of mutational analysis findings in AML in clinical management.

Currently, many institutions and groups are analyzing AML samples upfront with multi-gene mutational profiling using NGS at diagnosis of AML, for prognosis, risk stratification and to aid therapeutic decisions. Molecular monitoring of post-therapy disease started initially with quantitative PCR-based assays. Now, multi-gene mutational profiling using genomic techniques is also being increasingly evaluated post-therapy for AML, at remission, for monitoring and at relapse, with clinically important information provided by these studies to aid in clinical management. Persistence of a mutation such as DNMT3A may occur even in clinical remission as would be expected since DNMT3A mutations may be present in normal individuals. Earlier, an analysis of 18 genes in a study of 398 AML patients less than 60 years of age had suggested that mutational profiling could potentially be used for risk stratification, with internal tandem duplication in FLT3 (FLT3-ITD), partial tandem duplication in MLL (MLL-PTD) and mutations in ASXL1 associated with reduced overall survival, while CEBPA and IDH2 mutations were associated with improved survival. Only the IDH2 R140Q mutations had a favorable effect in these patients. Recently, in a study of 28 recurrently mutated genes in 271 patients with de novo AML, complex molecular genetic abnormalities involving three or more genetic mutations were found to be a strong independent prognostic factor, even among patients <65 years of age who were also negative for FLT3-ITD.

**Secondary AML**

In a study of 93 clinically defined secondary AML (AML occurring after MDS or chronic myelomonocytic leukemia), with sequencing of 82 genes that are known to be recurrently mutated in myeloid malignancies, 353 single nucleotide variants and small insertions or deletions affecting 40 genes were found, with at least one mutation detected in 90/93 (97%) cases. Mutations in 8 genes, including 4 spliceosome genes, SF3B1, U2AF1, and ZRSR2, 3 chromatin modifier genes, ASXL1, EZH2, and BCOR, and one cohesion complex gene, STAG2, showed >95% specificity for secondary AML as compared to de novo AML. Since these 8 genes are also commonly mutated in MDS, it was suggested that these mutations may primarily drive the myelodysplastic disease and not overt AML. Correlation with clinicopathologic features of the antecedent MDS (extent of bone marrow dysplasia or lineages involved by cytopenias) was not given in this study. The investigators also identified 3 additional aberrations, NPM1 mutations, MLL/11q23 rearrangements, and core binding factor (CBF) rearrangements, which occurred significantly less in secondary AML as compared with de novo AML, and therefore, called them de novo AML type mutations. In addition, 14 (15%) of their secondary AML cases had TP53 mutations, and these cases had more complex karyotypes and reduced overall survival as compared with their secondary AML cases without TP53 mutations. Further, in their 17 paired studied cases of antecedent MDS followed by AML, they noted that newly acquired mutations leading to the
AML most commonly involved genes encoding myeloid transcription factors or genes in the RAS/tyrosine kinase signaling pathway, in contrast with the mutations in the 8 genes specific for secondary AML, or mutations in TP53, which were present in the MDS but not at the time of development of the secondary AML. They grouped mutations into (1) those occurring as early events, such as DNMT3A, TET2 and TP53, or the secondary type mutations in the 8 genes given above, and (2) the driver mutations (RAS or tyrosine kinase pathway, transcription factors) that led to the emergence of AML, and found that at morphologic remission, the latter (driver) type mutations were preferentially lost, while the former group of mutations persisted.

In another recent study of 76 paired samples from 38 patients with MDS and subsequent progression to secondary AML that were examined by chromosome banding analysis and a 33 gene molecular panel, FLT3-ITD was significantly associated with MDS than with secondary AML (16% vs. 0%, P= 0.025), and while not statistically different, FLT3-TKD, NRAS and KRAS were more often mutant in secondary AML than in MDS. Interestingly, 26 cases acquired mutations and of these, 18 remained cytogenetically normal, including all 12 patients with acquired KRAS or NRAS mutations. In comparing these MDS patients who transformed to AML, with their control cohort of MDS patients from a previous study who did not progress to AML, the investigators found that mutations in ASXL1, ETV6, GATA2, IDH2, NRAS, RUNX1, and SRSF2 genes were more frequent in the MDS cases that progressed to AML, than in the control MDS cohort, suggesting that mutations in these genes predispose to transformation to secondary AML.

### Minimal residual disease detection by molecular approaches in AML

Detection of subclinical levels of leukemic cells by molecular based approaches provides powerful independent prognostic information. Due to the extreme molecular genetic heterogeneity in AML, and due to difficulties in distinguishing normal benign myeloid blast cells from malignant (leukemic) blasts, detection of minimal residual disease (MRD) has been typically restricted to acute lymphoblastic leukemias. Nonetheless, MRD detection is now also being increasingly recognized as an important variable to be assessed in the clinical management of those categories of AML where a molecular abnormality present at diagnosis can be tracked after induction or consolidation therapy, as an early predictor of relapse. Further, risk stratification in AML, which typically has been used at the time of diagnosis for initial treatment of AML, now also includes MRD detection for therapeutic decisions after initial therapy, including for stem cell transplantation.

Quantitative PCR assays are used for AML patients with balanced translocations such as PML-RARA, or for mutations in NPM1. However, the degree of complexity of genetic aberrations in AML, as shown both in The Cancer Genome Atlas study of de novo AML, and in the recent study of targeted sequencing analysis of 194 patients with secondary or therapy-related AML, presents a major challenge in establishing molecular assays for multiple single genetic abnormalities in AML. Therefore, NGS is being increasingly utilized for detecting minimal levels of leukemic cells and can detect resistant disease when PCR assays are not informative. Recently, Klco et al. used whole genome or exome sequencing on paired diagnostic and morphologic remission samples from 50 AML patients to show that the detection of persistent leukemia-associated mutations in at least 5% bone marrow cells at 30-day morphologic remission samples was associated with a significant risk of relapse, and reduced overall survival (19.3 months for persistent mutations versus 46.8 months for cleared mutations, P=0.02). Further, in their study, mutations in DNMT3A, TET2, IDH1 and IDH2 were rarely eradicated by conventional chemotherapy, as would be expected for DNMT3A and TET2 since mutations in these two genes have been shown to occur as early events in clonal hematopoiesis. In a different study, IDH1/IDH2 mutations were found to be suitable targets for the detection of minimal residual disease in AML and for predicting relapse, while mutant DNMT3A persisted in remission reflecting clonal hematopoiesis.

Further, in a study by the UK National Cancer Research Institute Working Group, of 2,569 samples obtained from 346 intensively treated patients with NPM1-mutated AML, and with targeted sequencing of 51 genes performed in 223 diagnostic samples and 49 relapse samples, MRD as assessed by quantitation of NPM1 transcripts by reverse transcriptase PCR provided powerful prognostic information independent of other risk factors. In this study, persistence of NPM1-mutated transcripts in blood after the second cycle of chemotherapy was associated with a greater (82%) risk of relapse after 3 years of follow-up, as compared to the risk (30%) in patients without MRD. Also, the presence of MRD was the only independent prognostic factor for death in multivariate analysis, and relapse was reliably predicted by rising levels of NPM1-mutated transcripts in these patients.
Therapeutic targeting of mutations in AML

The detection of subclinical leukemic cells is significant due to developed therapeutic agents targeting specific genetic mutations in AML. Several such pharmaceutical agents are in clinical trials, with promising early results, for incorporating these novel agents in combination with chemotherapy, with therapies that may make it to single agent therapy in clinical practice in the next five years described in a recent review\(^64\). The potential for therapeutic targeting of the most common mutations in CN-AML, with aberrations of the FLT3, NPM1, DNMT3A, IDH1, IDH2 and TET2 genes, was reviewed in another recent article\(^65\).

Therapeutic targeting of FLT3 mutations is desirable since FLT3 mutations confer a poor prognosis in AML, and several agents have been developed targeting both the FLT3-ITD and the point mutation in codon 835 of the second tyrosine kinase domain of the FLT3 gene\(^66\). A landmark global, prospective study presented at the annual ASH meeting in December 2015 showed the favorable results of a clinical trial using midostaurin, an agent that targets FLT3-ITD and FLT3-TKD in AML patients\(^67\), finally indicating that FLT3 inhibitors (and FLT3 testing) will soon become mandatory for standard of care in AML with FLT3 mutations. In this randomized, placebo-controlled, double-blind trial from 17 countries, 3,279 previously untreated AML patients, age 18-60 (excluding acute promyelocytic leukemia) in 225 sites/17 countries were screened for FLT3 mutations (FLT3-ITD low or high mutant allelic fraction, and FLT3-TKD) at one of 7 academic laboratories subject to extensive assay cross-validation\(^67\). Of critical importance, this effort demonstrated that a prospective trial in a pre-therapy genetically defined subgroup of AML patients was feasible and that the addition of the multi-kinase inhibitor midostaurin to standard chemotherapy and for one year of maintenance therapy significantly improved event-free survival and overall survival in patients whose leukemic blasts had a TKD or ITD (low or high FLT3 mutation burden)\(^67\).

Molecular and clinically distinct classes of AML defined by modeling of genomic structure, towards a personalized approach for every patient with AML

Significantly, a complete analysis of the genomic structure of 1,540 AML patients enrolled in clinical trials of the German-Austrian AML Study Group was presented at the ASH 2015 meeting\(^68\). In this study, in addition to cytogenetic analysis, 5,234 pathogenic lesions across 77 genomic loci were mapped, and \(\geq 1\) driver mutation in 96% patients and \(\geq 2\) in 85% were identified. The investigators subdivided AML into at least 11 molecular and clinically distinct classes defined by t(15;17), t(8;21), inv(16)/t(16;16), t(6;9), inv(3)/t(3;3), AML defined by MLL-rearrangements, CEBPA biallelic mutations, NPM1 mutations, TP53/complex karyotype, AML with chromatin/splicing factor mutations, and provisionally AML with \(<3\) aneuploidies. Approximately 87% of patients in their study with acquired mutations were molecularly classified, and each class was defined by a distinct subset of genetic lesions, with evidence of preferred order in mutation acquisition\(^68\). Of clinical significance, based on comprehensive genomic profiling, the study investigators were able to show that the genetic heterogeneity in AML is not random, and instead, informs towards a personalized approach for every patient for risk stratification, MRD monitoring and combination therapy protocols\(^68\).

Genes commonly mutated in AML with intermediate risk, normal karyotype (CN-AML)

NPM1

The official name for NPM1 is "nucleophosmin (nucleolar phosphoprotein B23, numatrin)", with the gene located on chromosome 5q35.1, from base pair 171,387,648 to base pair 171,410,884. The gene encodes for a nucleolar protein named "nucleophosmin" that shuttles between the nucleus and the cytoplasm, and is thought to play a part in many cellular functions including processes involved in protein formation, DNA replication and the cell cycle\(^69\). Much before its role in AML was discovered in 2005\(^12,70-72\), NPM1 was well-known to have a pathogenetic role in human non-Hodgkin’s lymphomas\(^73\), as the most commonly involved partner gene in the NPM1-ALK fusion in cases of ALK-positive anaplastic large cell lymphomas, which are described separately in the WHO 2008 classification\(^74\). The translocations and mutations involving the NPM1 gene in lymphomas and leukemias were reviewed in detail elsewhere\(^75\). In AML, NPM1 mutations are prognostically favorable in the absence of FLT3-ITD mutations\(^12,70-72\). It has been suggested that the altered nucleophosmin protein is unable to allow the normal tumor suppressor role of the Arf nucleolar protein, and the subsequent reduction in the tumor suppressor function leads to the leukemic proliferation in AML\(^76\).

FLT3

The official name for the gene is "fms-related tyrosine kinase 3", with the gene located on chromosome 13q12. The gene
encoded for a Class III receptor tyrosine kinase with important roles in hematopoietic stem/progenitor cell survival and proliferation. Mutations that result in constitutive activation of this receptor lead to acute leukemia. Mutations of the FLT3 gene are present in about one-third of AML patients. There are two major types of FLT3 mutations that lead to constitutive activation of the FLT3 receptor: internal tandem duplication (ITD) mutations of 3-400 base pairs that map to the juxtamembrane region, which are present in ~23% of AML patients, and point mutations that most frequently involve the kinase domain that occur less frequently than the ITD mutations. In pediatric patients with AML, the overall incidence of ITD mutations is lower than in adults with AML. Notably, the prognostic impact of FLT3 mutations in AML depends on the co-occurrence of other gene mutations. A large study of 1,425 young adult patients with AML showed FLT3-ITD mutations to have a dismal prognosis in the absence of NPM1 mutations, a better prognosis if NPM1 mutations were present without FLT3 mutations, and a prognosis intermediate between the two situations if both FLT3 and NPM1 mutations were present. In contrast, FLT3 tyrosine kinase mutations (TKD) showed a significantly more favorable prognosis than FLT3-ITD mutations in AML. In another large study of 3,082 consecutive AML patients from the German cooperative group trials, FLT3-TKD mutations were shown to have an unfavorable effect if present with an MLL mutation or a t(15;17) translocation, but a favorable prognosis when paired with an NPM1 mutation.

**DNMT3A**

The official name for DNMT3A is "DNA (cytosine-5')-methyltransferase 3 alpha", with the gene located on chromosome 2p23, from base pair 25,232,961 to base pair 25,342,590. In humans, DNA methylation occurs in cytosines that precede guanines, called dinucleotide CpGs, which occur in CpG-rich regulatory regions. DNA methylation occurs at the 5' position of the cytosine ring and leads to silencing of genes and noncoding genomic regions, thereby providing a means for epigenetic regulation. The DNMT3A gene encodes for a DNA methyltransferase enzyme, DNMT3A, which is required for genome-wide de novo methylation and is essential for the establishment of DNA methylation patterns during development, genomic imprinting and X-chromosome inactivation. DNMT3A is critical for the epigenetic silencing of hematopoietic stem cell regulatory genes, and is thus essential for hematopoietic stem cell differentiation. Somatic mutations of DNMT3A were described in AML, and subsequently also in MDS, MPNs and T-acute lymphoblastic leukemias.

In a study of 511 hematologic malignancies (194 de novo AML, 115 MDS, 103 chronic myelomonocytic leukemia and 99 T-acute lymphoblastic leukemias), DNMT3A mutations showed the highest frequency in AML (36% of AML), also with strong association with NPM1, FLT3-ITD, and IDH1 mutations. The majority of DNMT3A mutations are missense, affecting the arginine codon 882, and are predominantly heterozygous. In a different study of de novo adult AML, the clinical significance of DNMT3A mutations was found to be age-dependent, with R882 mutations associated with an adverse prognosis in patients aged >60 years, and non-R882 DNMT3A mutations associated with an adverse prognosis in adult patients <60 years. Interestingly, DNMT3A mutations were rare events in a study of pediatric AML, and this observation, in conjunction with the identification of DNMT3A mutations in normal individuals with increasing age, suggests that DNMT3A mutations may occur in AML primarily due to increasing age, and other mutations that co-occur subsequently lead to the development of AML.

**IDH1 and IDH2**

The official name for IDH1 is "isocitrate dehydrogenase 1 (NADP+)", with the gene located on chromosome 2q33.3, from base pair 208,236,227 to base pair 208,255,143. The official name for IDH2 is "isocitrate dehydrogenase 2 (NADP+), mitochondrial", with the gene located on chromosome 15q26.1, from base pair 90,083,978 to base pair 90,102,554. The IDH1 gene encodes for the isocitrate dehydrogenase 1 enzyme, which is present in the cell cytoplasm and peroxisomes, while the IDH2 gene encodes for the isocitrate dehydrogenase 2 enzyme in mitochondria. Both enzymes convert isocitrate to 2-ketoglutarate to produce NADPH needed for cell metabolism. Somatic gain of function mutations in IDH1 and IDH2 that occur in CN-AML alter the enzymes, which now instead produce a different compound, 2-hydroxylutarate (an oncometabolite) that interferes with the process of cell fate determination such that immature blood cells with mutant IDH1 divide uncontrollably, instead of maturing to normal cells, and lead to CN-AML. Mutations in IDH genes, therefore, lead to markedly altered cellular respiration, and lead to the accumulation of the abnormally produced metabolite, which can serve as a biomarker in cancers with mutant IDH genes. Among human cancers, mutations of IDH1 and IDH2 genes occur predominantly in gliomas and AML, with such mutations found only rarely in other cancer types despite exhaustive sequencing efforts. Similar to gliomas, human...
AML samples with \textit{IDH} mutations also show specific DNA methylation profiles\textsuperscript{92}. In contrast with gliomas, however, where \textit{IDH1} and \textit{IDH2} mutations have a favorable prognosis, \textit{IDH} mutations in AML have been either shown to confer a worse prognosis\textsuperscript{93-95}, or be favorable for overall survival in intermediate-risk AML\textsuperscript{44}.

\textbf{CEBPA}

The official name for \textit{CEBPA} is "CCAAT/enhancer binding protein (C/EBP), alpha", with the gene location on chromosome 19q13.1, from base pair 33,299,934 to base pair 33,302,564\textsuperscript{96}. The gene belongs to a family of genes called basic leucine zipper proteins, and encodes for a transcription factor protein, which is involved in the maturation of hematopoietic cells and is also believed to act as a tumor suppressor. In individuals with an inherited germline mutation in \textit{CEBPA}, the occurrence of an additional somatic mutation in \textit{CEBPA} leads to an AML with biallelic \textit{CEBPA} mutation, which is the entity proposed to be included as a distinct entity in the upcoming WHO 2016 classification\textsuperscript{4}.

\textbf{Proposed 2016 WHO classification for acute myeloid leukemia}

In the session presented by the WHO leading authors for the proposed 2016 classification for hematopoietic neoplasms, at the March, 2015 United States and Canadian Academy of Pathology (USCAP) conference\textsuperscript{4}, Dr. Daniel A. Arber from Stanford University had presented the proposed revision for classification of acute leukemias. Dr. Arber’s presentation at the website\textsuperscript{8} is the source for the information provided in this review about the currently proposed revision that could undergo changes before being published in 2016. Both entities of AML with mutated genes (AML with mutated \textit{NPM1}, and AML with mutated \textit{CEBPA}) that were included as provisional in the 2008 WHO classification were proposed to now be included as distinct clinicopathologic entities. In addition, AML with mutated \textit{RUNX1}, and AML with \textit{BCR-ABL} were proposed as distinct entities. Other gene mutations present in AML are considered important for prognosis and are recommended for analysis, but AML with these other gene mutations do not represent distinct entities.

A diagnostic algorithm for acute leukemias and classification of AML was initially presented by Dr. Daniel Arber after the WHO 2008 classification, incorporating clinical history, morphology, immunophenotyping, cytogenetics and mutations of \textit{NPM1} and \textit{CEBPA}. At the 2015 USCAP meeting, Dr. Arber presented a revised algorithm for the proposed 2016 classification of AML, now incorporating mutations in several genes\textsuperscript{4}. A major change, as compared to the 2008 WHO classification, is that in addition to cytogenetic analysis, which is still necessary upfront, molecular analysis for mutations is now also required upfront in the pathologic diagnostic work-up of an acute leukemia. Similar to the 2008 WHO classification, clinical history and cytogenetics are required for precise classification: if a newly diagnosed AML patient has Down syndrome, or a previous history of cytotoxic or leukemogenic therapy, the classification would be "myeloid proliferation of Down syndrome" or "therapy-related AML" respectively, and, if with cytogenetics, a recurrent genetic abnormality is found, then the classification would be an "AML with recurrent genetic abnormality." However, if \textit{NPM1} is mutant, or \textit{CEBPA} is biallelic mutant, or \textit{RUNX1} is mutant, then regardless of any morphologic dysplasia that may be present, the diagnosis would be "AML with mutated \textit{NPM1}" or "AML with mutated \textit{CEBPA}," or "AML with mutated \textit{RUNX1}," respectively, under the category of AML with recurrent genetic abnormalities, in the absence of a history of MDS or MPN, and in the absence of cytogenetic abnormalities specific for MDS, as described\textsuperscript{97}, since these cytogenetic abnormalities are sufficient to diagnose AML with MDS-related changes when >20% peripheral blood or bone marrow blasts are present. For any of the three AML entities with specific mutated genes (\textit{NPM1}, \textit{CEBPA} or \textit{RUNX1}), if there is a history of previous MDS or MPN, the classification would be an "AML with MDS-related changes", and if there is a history of Down syndrome or previous leukemogenic therapy, the classification would be related to the clinical condition (Down syndrome or therapy-related), and, notan AML with recurrent genetic abnormality. Presence of an MDS-related cytogenetic abnormality, as described in the WHO 2008 classification\textsuperscript{97}, excluding del(9q) if \textit{NPM1} is mutant, would also classify as an AML with MDS-related changes, instead of AML with mutated \textit{NPM1}, or AML with mutated \textit{CEBPA}, or AML with mutated \textit{RUNX1}\textsuperscript{4}.

\textbf{AML with mutated \textit{NPM1}}

This entity was described as one of the two provisional entities of AML with mutated genes, in the 2008 WHO classification\textsuperscript{22}. In AML, the mutations in \textit{NPM1} lead to an altered nucleophosmin protein due to heterozygous mutations that occur almost entirely in exon 12 of the \textit{NPM1} gene, as first described in 2005\textsuperscript{70}. At that time, and with subsequent studies, the prognostic impact of \textit{NPM1} mutations in AML was noted to depend on co-existing \textit{FLT3-ITD} mutations. While the presence of \textit{FLT3-ITD} mutations
NPM1 mutations were present or absent, mutant NPM1 conferring a favorable prognosis in young adult patients with CN-AML if FLT3-ITD mutations were absent\textsuperscript{12,14,71,72}, and a better prognosis even when FLT3-ITD mutations were present, as compared with only FLT3-ITD mutations without mutant NPM\textsuperscript{14}. Although the majority of AML with mutated NPM1 have a normal karyotype, 14.7% (93 of 631) patients were found to have an abnormal karyotype, with the most frequent abnormalities being +8, +4, -Y, del(9q) and +21\textsuperscript{98}. The presence or absence of an abnormal karyotype did not alter the favorable prognosis of the NPM1 mutant and absent FLT3-ITD category of AML\textsuperscript{98}. Accordingly, in the European LeukemiaNet recommendations published in 2010, NPM1 mutant AML with no FLT3-ITD mutations and normal cytogenetics lowered the risk from intermediate to favorable, while FLT3-ITD with wild type NPM1 or with mutant NPM1 and with normal cytogenetics, had an intermediate risk\textsuperscript{17}. The favorable prognostic significance of NPM1 mutant and absent FLT3-ITD is present in AML patients with age 55-65 years, but not in older AML patients > 65 years of age\textsuperscript{99}. Quantitative detection of mutant NPM1 genes or mRNA transcripts is useful in clinical monitoring of AML\textsuperscript{63,100,101}. The molecular subtypes of NPM1 mutations also have varying clinical outcomes in intermediate risk AML, as recently shown\textsuperscript{102}. In that study from the Munich Leukemia Laboratory, 877 (~31%) of 2,859 adult newly diagnosed AML patients showed NPM1 mutations, and 806 of these 877 samples were de novo AML with intermediate risk karyotypes\textsuperscript{102}. Type A NPM1 mutations [insertion of TCTG (thymine, cytosine, thymine and guanine)] were most frequently found in 458 (69%) cases, Type B mutations (insertion of CATG) in 72 (11%) and Type D (insertion of CCTG) in 51 (8%). All other NPM1 mutation types were rarely found in their study. DNMT3A was found to be most frequently co-mutated (n=337, 55%) with NPM1, followed by FLT3-ITD (n=270, 41%), and while FLT3-ITD showed a worse prognosis only with Type A NPM1 co-mutations, DNMT3A mutations conferred a worse prognosis with both Types A and D NPM1 mutations, suggesting that in addition to co-existing FLT3-ITD mutations, DNMT3A mutations should also be examined for predicting prognosis in AML with mutated NPM1\textsuperscript{102}. 

**AML with mutated CEBPA**

This entity was included as provisional in the 2008 WHO classification\textsuperscript{22}. AML with biallelic mutant CEBPA occurs in individuals who have an initial germline monoallelic mutation in CEBPA, followed by a subsequent acquired, somatic mutation in the second CEBPA allele. This AML entity may occur sporadically or in familial form, with the first family harboring a germline CEBPA mutation and showing autosomal dominant of AML reported in 2004\textsuperscript{103}. The germline CEBPA mutation typically occurs in the N-terminal region of the gene, while the somatic, acquired mutation occurs in the C-terminal region. The molecular events underlying disease progression and evolution in familial AML with biallelic mutated CEBPA were described in a recent multi-institutional study of ten families having 24 members with AML\textsuperscript{104}. Germline CEBPA mutations appeared to be highly penetrant, with the majority presenting with AML at an early median (range) age of 24.5 (1.75-46) years. All AML cases were de novo in the absence of preceding cytopenias. Germline mutations, universally located in the N-terminal region of the gene, caused a frameshift preceding the internal p30 start codon, while the somatic mutations were located in the C-terminus in all 18 examined patients, with the majority representing inframe insertions or deletions within the DNA binding and leucine zipper domains\textsuperscript{104}. Interestingly, in contrast with sporadic AML, in these familial AML cases, somatic (acquired) mutations identified at relapse were not the same as the somatic mutations present at diagnosis, and were also confirmed to be absent as a subclone in the initial diagnostic AML, indicating that in familial cases, disease recurrence may be initiated by a completely new clonal leukemic occurrence\textsuperscript{104}. Their study highlighted the critical importance of evaluation of family history, for continued long-term clinical follow-up, and for germline DNA assessment in patients <50 years presenting with AML with biallelic mutated CEBPA\textsuperscript{104}. 

Mutations in CEBPA may be monoallelic, or biallelic, or there may be polymorphisms detected in the gene, in cases of AML\textsuperscript{105}. In large cohorts of patients with CN-AML, mutations of CEBPA were found in 12.8% and 10.6% cases, with biallelic and monoallelic mutations comprising about 60% and 40%, respectively\textsuperscript{105,106}. It is important to remember that whether sporadic or familial, the favorable prognosis of this AML entity is present only with biallelic mutations of CEBPA, and is absent with monoallelic CEBPA gene mutations in AML\textsuperscript{105-110}. Also, the favorable significance of biallelic mutant CEBPA is lost in the presence of a FLT3-ITD mutation, while NPM1 mutations are unlikely to co-occur in AML with biallelic mutant CEBPA\textsuperscript{110}. Mutations in GATA2 may be present in AML patients with CEBPA mutations, but they do not affect the favorable prognosis of the biallelic mutant AML\textsuperscript{111}. 

 led to a worse prognosis in CN-AML regardless of whether NPM1 mutations were present or absent, mutant NPM1 conferring a favorable prognosis in young adult patients with CN-AML if FLT3-ITD mutations were absent\textsuperscript{12,14,71,72}, and a better prognosis even when FLT3-ITD mutations were present, as compared with only FLT3-ITD mutations without mutant NPM\textsuperscript{14}. Although the majority of AML with mutated NPM1 have a normal karyotype, 14.7% (93 of 631) patients were found to have an abnormal karyotype, with the most frequent abnormalities being +8, +4, -Y, del(9q) and +21\textsuperscript{98}. The presence or absence of an abnormal karyotype did not alter the favorable prognosis of the NPM1 mutant and absent FLT3-ITD category of AML\textsuperscript{98}. Accordingly, in the European LeukemiaNet recommendations published in 2010, NPM1 mutant AML with no FLT3-ITD mutations and normal cytogenetics lowered the risk from intermediate to favorable, while FLT3-ITD with wild type NPM1 or with mutant NPM1 and with normal cytogenetics, had an intermediate risk\textsuperscript{17}. The favorable prognostic significance of NPM1 mutant and absent FLT3-ITD is present in AML patients with age 55-65 years, but not in older AML patients > 65 years of age\textsuperscript{99}. Quantitative detection of mutant NPM1 genes or mRNA transcripts is useful in clinical monitoring of AML\textsuperscript{63,100,101}. The molecular subtypes of NPM1 mutations also have varying clinical outcomes in intermediate risk AML, as recently shown\textsuperscript{102}. In that study from the Munich Leukemia Laboratory, 877 (~31%) of 2,859 adult newly diagnosed AML patients showed NPM1 mutations, and 806 of these 877 samples were de novo AML with intermediate risk karyotypes\textsuperscript{102}. Type A NPM1 mutations [insertion of TCTG (thymine, cytosine, thymine and guanine)] were most frequently found in 458 (69%) cases, Type B mutations (insertion of CATG) in 72 (11%) and Type D (insertion of CCTG) in 51 (8%). All other NPM1 mutation types were rarely found in their study. DNMT3A was found to be most frequently co-mutated (n=337, 55%) with NPM1, followed by FLT3-ITD (n=270, 41%), and while FLT3-ITD showed a worse prognosis only with Type A NPM1 co-mutations, DNMT3A mutations conferred a worse prognosis with both Types A and D NPM1 mutations, suggesting that in addition to co-existing FLT3-ITD mutations, DNMT3Amutations should also be examined for predicting prognosis in AML with mutated NPM1\textsuperscript{102}. 

**AML with mutated CEBPA**

This entity was included as provisional in the 2008 WHO classification\textsuperscript{22}. AML with biallelic mutant CEBPA occurs in
Familial predisposition to AML

Hematologic malignancies are usually considered to be sporadic, with very few recognized familial cases. With increasing evaluation of molecular genetics in myeloid neoplasms, there is now increasing appreciation of the need for clinical testing for assessing familial predisposition to MDS/AML. Accordingly, the proposed WHO 2016 classification will include a section on familial myeloid neoplasms. Currently, clinical testing is available for several familial MDS/AML disorders, including the autosomal dominant familial platelet disorder with germ line RUNX1 mutations, with propensity to develop AML, and for familial AML with biallelic mutated CEBPA. Disorders with inherited predisposition to AML have been reviewed elsewhere. Guidelines have been proposed for clinical management of patients and families with predisposition to familial AML, with recommendations including that all carriers of such predisposing genetic mutations undergo a baseline bone marrow biopsy and twice annual physical examination and complete blood counts with differential counts. While 100% of the mutated CEBPA carriers develop AML, albeit with long latency periods, the development of AML in the familial disorder with RUNX1 mutation is variable (20%-60%). Interestingly, in a recent genomic study of familial MDS/AML cases, mutations in genes that are recurrently mutated in de novo AML were underrepresented in familial MDS/AML cases. Also, clonal hematopoiesis was detected in young, asymptomatic carriers of RUNX1 mutations, providing a possible biomarker in these families with high risk for development of AML.

Future directions

The WHO classification from 2008 for hematopoietic neoplasms, which is currently proposed to be revised in 2016, will include a revision of AML categories due to the significant advances in the molecular genetic landscape of AML. Since the classification recognizes only clinicopathological entities that have accumulated sufficient evidence to be classified as distinct, the continuing major advances in the molecular genetic aberrations underlying AML will likely lead to more frequent classification updates in the future. Increased use of molecular profiling at the time of diagnosis, remission and follow-up is expected to become standard of care, with NGS used for multi-gene profiling, instead of single gene tests. With the development of pharmaceutical agents against molecular targets, use of combination chemotherapy for AML is expected to become more frequent. Finally, the rapid pace of our continuously increasing knowledge of AML pathogenesis, which forms the basis of diagnostic classification, risk stratification and clinical management, is currently far greater than that of concurrent advances in the clinical therapy for AML. Nonetheless, these major advances are now actually beginning to seem promising, significantly in contrast with what would have been considered a dream even less than a decade ago, for individually personalized therapy for patients with a malignant disease category characterized by extreme molecular heterogeneity such as AML.

Conflict of interest statement

No potential conflicts of interest are disclosed.

References


66. Levis M. FLT3 mutations in acute myeloid leukemia: what is the


78. Mead AJ, Linch DC, Hills RK, Wheatley K, Burnett AK, Gale RE. FLT3 tyrosine kinase domain mutations are biologically distinct from and have a significantly more favorable prognosis than FLT3 internal tandem duplications in patients with acute myeloid leukemia. Blood. 2007; 110: 1262-70.


95. Schnittger S, Haferlach C, Ulke M, Alpermann T, Kern W, Haferlach T. IDH1 mutations are detected in 6.6% of 1414 AML patients and are associated with intermediate risk karyotype and unfavorable prognosis in adults younger than 60 years and unmutated NPM1 status. Blood. 2010; 116: 5486-96.


