Chapter 2
Acute Myeloid Leukemia with Recurrent Genetic Abnormalities, Part II: Mutations Involving *CEBPA*, *NPM1*, and *RUNX1*

Ryan S. Robetorye

Introduction

Recurrent chromosomal structural abnormalities identified by conventional cytogenetic studies and fluorescence in situ hybridization (FISH) analysis have a well-established role in the classification and risk stratification of acute myelogenous leukemia (AML). For example, patients with cytogenetic alterations associated with favorable risk, such as inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB-MYH11*, t(15;17)(q22;q12);*PML-RARA*, and t(8;21)(q22;q22);*RUNX1-RUN1T1*, have relatively good responses to chemotherapy-based regimens. Patients with unfavorable risk profiles, including monosomies of chromosomes 5, 7, and 17, deletions of chromosomes 5q and 7q, 11q23 abnormalities other than t(9;11), 17p abnormalities, complex karyotypes (>3 abnormalities), and other specific chromosomal rearrangements such as inv(3)/t(3;3) and t(6;9), often require allogeneic stem cell transplantation to improve their prognosis. However, approximately one-half of AML cases have an intermediate cytogenetic risk associated with a normal karyotype and cannot be further subclassified using cytogenetics [1–3]. During the past decade, numerous molecular genetic tests and gene sequencing approaches have been employed to identify specific gene mutations that could be used to further divide cytogenetically normal AML (CN-AML) cases into clinically relevant prognostic subsets. The most frequently mutated genes in CN-AML occur in the *NPM1* (~50–60% of cases), *FLT3* and *DNMT3A* (~30% of cases), *TET2* (23%), *IDH2* (19%), *IDH1* (14%), *CEBPA* (10–18%), *NRAS* (13%), *ASXL1* (~10%), *WT1* (~7–13%, with increased mutation frequency in younger patients), and *RUNX1* (~6–26%, with increased mutation frequency in elderly individuals) genes [4–19].

R.S. Robetorye (✉)
Department of Laboratory Medicine & Pathology, Mayo Clinic Arizona, 5777 E Mayo Blvd, Phoenix, AZ, USA
e-mail: Robetorye.Ryan@mayo.edu

C.-C. Chang, R.S. Ohgami (eds.), *Precision Molecular Pathology of Myeloid Neoplasms*, Molecular Pathology Library, DOI 10.1007/978-3-319-62146-3_2

The discovery of such gene mutations in CN-AML resulted in inclusion of the provisional diagnostic entities "AML with mutated *CEBPA*" and "AML with mutated *NPM1*" in the "Acute myeloid leukemia with recurrent genetic abnormalities" category in the 2008 WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues [1]. However, a recent revision of the 2008 WHO Classification changed these two provisional gene mutation entities to distinct diagnostic entities and also added "AML with mutated *RUNX1*" as a new provisional diagnostic entity [20, 21]. Further refinement of CN-AML cases within the AML classification will improve the identification of molecularly defined subsets of AML patients with different risk categories and will also provide the possibility of minimal residual disease monitoring using such mutations in specific quantitative molecular assays [22, 23]. Among the most frequent CN-AML-associated mutations, those affecting the *CEBPA*, *NPM1*, and *RUNX1* genes are associated with distinct biological and clinical features and gene expression profiles [5, 24–28]. Therefore, this chapter will focus on the characteristic clinicopathologic features of AML with mutations in the *CEBPA*, *NPM1*, and *RUNX1* genes.

AML with Biallelic Mutations of CEBPA

The *CEBPA* gene on chromosome 19q13.1 has a GC-rich coding region (greater than 70%) contained within a single exon and encodes for the CCAAT/enhancer binding protein alpha, a basic region leucine zipper transcription factor important for regulation of cell proliferation and differentiation of myeloid precursors [29–31]. *CEBPA* is transcribed as a single mRNA that is translated into two isoforms due to an alternative start site, resulting in a full-length 42 kDa protein and shorter 30 kDa N-terminal truncated isoform. Both CEBPA isoforms share a transactivation domain that mediates antimitotic effects through interactions with the cyclin dependent kinase inhibitor p21 [32] and regulates proliferation through interaction with the chromatin remodeling complex SWI/SNF [33] and inhibition of the cyclin dependent kinases CDK2 and CDK4 [34]. In *CEBPA* knockout mice, there is a selective block in neutrophil differentiation at the myeloblast stage that resembles the arrest in maturation observed in human AML patients [35]. Given the phenotype of the *CEBPA*-mutant mice, Pabst et al. looked for *CEBPA* mutations in a large collection of human AML and were the first to report the presence of these mutations in a subset of AML cases [36]. *CEBPA* mutations occur in 5–10% of de novo AML, but are most commonly found in CN-AML (10–18%) or AML with 9q deletion [19, 37]. Although there are no specific morphological characteristics of *CEBPA* mutation-positive leukemias, AML patients with these mutations tend to have higher hemoglobin levels, higher peripheral blood WBC and blast counts, lower platelet counts, and are less likely to present with lymphadenopathy or extramedullary leukemia [38].

Two major types of *CEBPA* mutations have been identified in AML that predominantly affect the N-terminal and C-terminal regions of the protein [39].

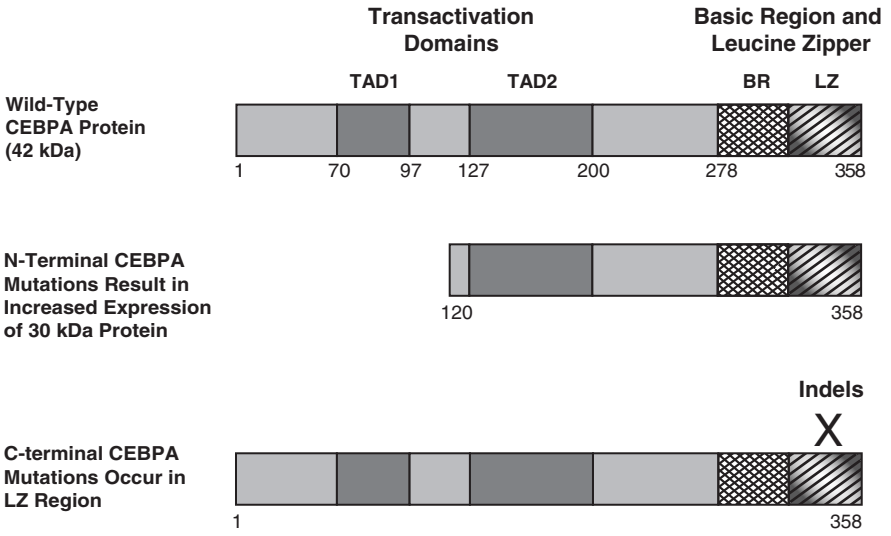

Fig. 2.1 CEBPA protein structure. The wild-type CEBPA 42 kDa protein consists of two transactivation domains (*TAD1* and *TAD2*), a basic region (*BR*), and a leucine zipper region (*LZ*). The protein is translated as two isoforms due to an alternative start site, resulting in full-length 42 kDa and truncated 30 kDa isoforms (amino acid numbering is shown below each isoform). N-terminal mutations occur as frameshift mutations that cause premature termination of the 42 kDa protein and result in increased expression of the 30 kDa isoform that also acts as a dominant-negative inhibitor of wild-type *CEBPA*. C-terminal mutations occur as in-frame insertions or deletions in the *LZ* region and result in decreased DNA binding and/or dimerization activity

N-terminal mutations occur as frame-shift mutations that cause premature termination of the full-length 42 kDa protein and result in formation of a nonfunctional truncated 20 kDa protein, and increased expression of the 30 kDa isoform that also acts as a dominant-negative inhibitor of wild-type CEBPA (Fig. 2.1) [36]. C-terminal mutations occur as in-frame insertions or deletions in the leucine zipper region and result in decreased DNA binding and/or dimerization activity (Fig. 2.1). About two-thirds of AML with *CEBPA* mutations have two mutations (biallelic; also called double mutations), with the majority of cases (approximately 90%) being compound heterozygotes, harboring an N-terminal mutation on one allele and a C-terminal mutation on the second allele, and the rest of the cases being homozygous, with both mutations comprising either N-terminal or C-terminal mutations. Consequently, *CEBPA* double mutation cases lack detectable expression of wild-type 42 kDa CEBPA protein. The remaining one-third of AML with *CEBPA* mutations carry a single CEBPA mutation (single allele; also called single mutations) [40].

The role of *CEBPA* mutations in leukemogenesis was further clarified by Bereshchenko et al. using a mouse model for the spectrum of *CEBPA* mutations occurring in human AML [41]. They found that N-terminal and C-terminal *CEBPA*

mutations had different effects on hematopoietic stem cell expansion, homeostasis, and myeloid differentiation. The most efficient leukemogenesis occurred when pre-malignant hematopoietic stem cell expansion induced by the presence of a C-terminal *CEBPA* mutation was combined with maintenance of myeloid lineage commitment by the presence of a coexisting N-terminal *CEBPA* mutation. These findings in the mouse model are consistent with the prevalence of *CEBPA* double mutations in AML patients.

Germline *CEBPA* mutations have also been described in familial AML. These cases are typically heterozygous N-terminal frame-shift mutations that lead to preferential expression of the 30 kDa dominant-negative isoform of the CEBPA protein, suggesting that decreased CEBPA function might play a role in the subsequent development of AML [42–44]. Familial AML associated with mutated *CEBPA* is inherited in an autosomal dominant fashion and is highly penetrant, with most of the patients inheriting the mutated gene going on to develop AML. No genotype-phenotype correlations have been associated with germline *CEBPA* mutations, and most of these cases have a normal karyotype [45]. The majority of cases present at an early age and are associated with development of de novo disease without preceding cytopenias or myelodysplasia [46]. Most of the AML patients develop an additional C-terminal in-frame *CEBPA* mutation on a separate allele at diagnosis, with the C-terminal mutation subsequently disappearing on remission [47, 48]. Approximately 5–10% of *CEBPA*-mutated AML harbor germline mutations. Thus, germline N-terminal *CEBPA* mutations may predispose to the development of a subsequent somatic C-terminal mutation and progression to AML after a long latency period [47, 49]. Patients with familial *CEBPA*-mutated AML may also be at increased risk of developing additional leukemia clones after treatment of their initial leukemia and may acquire new *CEBPA* mutations that are different from those in the original leukemia [45]. Germline *CEBPA* mutations comprise a novel leukemia predisposition syndrome, with development of de novo AML without prodromal cytopenias. Genetic counseling is a critical component of management, and a thorough family history and germline DNA analysis of patients is recommended in patients over 50 years of age that present with double-mutated *CEBPA* in order to adequately identify families with germline *CEBPA* mutations requiring long-term surveillance [46].

Although more than 600 *CEBPA* mutations have been reported [37], several studies have also documented that the *CEBPA* gene can be inactivated (silenced) by methylation. Hypermethylation of the *CEBPA* core promoter (−141 to +103 from the transcription start site) was first reported in 2002 in two out of 70 (2.8%) AML cases, both of whom had AML-M2 morphology [50]. A correlation was also found by Wouters et al. between silenced *CEBPA* and core promoter hypermethylation in six out of 285 (1.4%) AML patients [51]. These cases also exhibited an expression profile that was similar to AML with mutated *CEBPA*, even though they lacked such mutations. However, Hackanson et al. observed that methylation of the distal *CEBPA* promoter region (1422 to −896 upstream from the transcription start site) was much more common than the core promoter, occurring in 20 out of 39 (51%) of AML patients, and that aberrant DNA methylation was strongly associated with

samples carrying the cytogenetically favorable inv(16) and t(15;17) abnormalities [52]. An evaluation of the *CEBPA* core, proximal (−1121 to −896), and distal promoter regions in 193 unselected patients with de novo AML by Lin et al. also found heterogeneous methylation in the distal promoter region, but not in the core or proximal promoter regions [53]. Hypermethylation of the distal *CEBPA* promoter was associated with decreased *CEBPA* expression, younger age, and the (15;17) translocation. Overall, AML patients with high *CEBPA* methylation also exhibited better response to induction chemotherapy than AML patients with low *CEBPA* methylation and showed longer disease-free and overall survival; however, the effects of *CEBPA* hypermethylation on patient prognosis have not been reproduced [54].

Initially, *CEBPA* single mutation and double mutation cases were considered to be a homogeneous group of AML with a favorable prognosis and precipitated the inclusion of these cases as a new provisional entity in the 2008 Classification of Tumours of Haematopoietic and Lymphoid Tissues [1]. However, numerous recent studies have clearly demonstrated that only AML harboring *CEBPA* double mutations represents a distinct entity with a favorable outcome, whereas AML with a *CEBPA* single mutation is associated with survival similar to that of AML with wild-type *CEBPA* [40, 49, 55–59]. Several clinical trials have demonstrated that the presence of a *CEBPA* double mutation is an independent prognostic factor for favorable outcome in AML cases [22], and this finding has also been biologically substantiated by the distinct gene expression signature associated with AML with *CEBPA* double mutation [40] and by the mouse experiments modeling the mutations by knock-in mutagenesis performed by Bereshchenko et al. [41]. Multilineage dysplasia can also occur in AML with mutated *CEBPA*, but this does not influence the favorable prognosis of these cases, further supporting the classification of these cases as a unique entity [60]. Interestingly, AML cases that harbor *CEBPA* double mutations usually exhibit a characteristic immunophenotype that includes aberrant expression of CD7 on the leukemic cells [61]. Therefore, with regard to disease classification and risk stratification, only AML cases with biallelic *CEBPA* mutations should be regarded and as a separate entity associated with a favorable prognosis. These findings are reflected in the decision to change the provisional category of "AML with mutated *CEBPA*" to the distinct diagnostic entity "AML with biallelic mutations of *CEBPA*" in the recent 2016 revision of the WHO classification of myeloid neoplasms and acute leukemia [21].

AML with Mutated NPM1

The *NPM1* gene on chromosome 5q35 contains 12 exons and encodes for a 32 kDa nucleolar protein that shuttles continuously between the nucleus and the cytoplasm [37, 62–65]. Nucleophosmin (NPM) is a multifunctional protein that acts as a molecular chaperone to facilitate multiple protein-protein interactions and has both proliferative and growth suppressive roles [66, 67]. NPM is involved in critical cell functions, including ribosome biogenesis and export and regulation of centrosome

duplication [68–70], and also contributes to genomic stability by controlling DNA repair mechanisms [71, 72]. NPM is also involved in the control of cellular proliferation and apoptosis through interactions with the important regulatory proteins TP53, RB, $p19^{ARF}$, and HDM2 [73–77]. *NPM1* knockout mice show abnormal organogenesis and exhibit embryonic lethality due to severe anemia resulting from defects in primitive hematopoiesis. However, heterozygous *NPM1* knockout mice are viable and appear to thrive, but show unrestricted centrosome duplication and genetic instability, resulting in a hematologic syndrome with features similar to those observed in human patients with a myelodysplastic syndrome [78]. Heterozygous mice also show increased susceptibility to development of hematologic malignancies of both myeloid and lymphoid origin, with myeloid malignancies displaying the highest incidence [79]. The discovery of *NPM1* mutations in AML resulted from the observation that many cases of AML showed an aberrant expression of nucleophosmin protein in the cytoplasm of the leukemic cells [4]. Cytoplasmic NPM was detected in 208 out of 591 (35%) primary AML specimens, but not in 135 secondary AML or in 980 hematopoietic or extrahematopoietic neoplasms other than AML. This immunohistochemical finding led to sequencing of the *NPM1* gene and the identification of exon 12 mutations in nearly all of the AML cases with cytoplasmic NPM. There are no specific morphological characteristics exhibited by these leukemias; however, cytoplasmic/*NPM1*-mutated AML is associated with distinctive clinicopathological features, including a normal karyotype, female sex, strong expression of CD33, lack of CD34 expression in the leukemic cells (more than 95% are CD34-negative), hypercellular bone marrow with multilineage involvement, high white blood cell count at presentation, high blast percentage at diagnosis, high incidence of extramedullary dissemination (mostly confined to gingival hyperplasia and lymphadenopathy), and good response to induction chemotherapy [4, 80]. Cytoplasmic NPM is also readily detectable by immunohistochemistry in routinely-fixed paraffin-embedded tissue samples and can be used as a surrogate for molecular *NPM1* testing, making this technique potentially useful in the diagnosis of *NPM1*-mutated AML and myeloid sarcoma [81, 82].

NPM1 mutations are the most common genetic abnormality in adult AML, comprising approximately 30% of all AML and 50–60% of CN-AML [19]. *NPM1* mutations are usually restricted to frameshift mutations in exon 12 and are characteristically heterozygous with a retained wild type allele [83]. Approximately 55 molecular variants of *NPM1* exon 12 mutations have been identified so far [84], and only rare cases with a mutation occurring outside exon 12 have been reported, including a splice-site donor mutation in exon 9 [85] and an 8 base pair insertion in exon 11 [86]. Greater than 95% of *NPM1* mutations occur as a 4 base pair insertion at nucleotide position 960. A tandem duplication of TCTG accounts for approximately 80% of cases (so-called “mutation A”), with CATG (“mutation B”) and CCTG (“mutation D”) tetranucleotide duplications accounting for an additional 10% and 5% of cases, respectively. Other mutations (so-called “mutation C” [CGTG], “mutation E” [CTCTTGCCC], and “mutation F” [CCCTGGAGA]) are extremely rare [4, 84]. All *NPM1* exon 12 mutation variants cause similar alterations in the C-terminus of the mutant proteins and result in the creation of a new

nuclear export motif. As a consequence, the NPM protein mutants aberrantly accumulate in the cytoplasm of leukemic cells [84]. *NPM1* mutations are highly stable and may be detected at AML relapse many years after initial diagnosis, in patients with more than one relapse, and even in relapses that occur at extramedullary sites [87–89]. Uncommon cases with loss of *NPM1* mutations at leukemia relapse may be associated with development of new cytogenetic abnormalities [90, 91]. *NPM1* mutations appear to be mutually exclusive of other recurrent cytogenetic abnormalities in AML [92]. A small number of *NPM1*-mutated AML also carry a *CEBPA* mutation, but analysis of *NPM1*/*CEBPA* mutated cases has shown that this rare association only occurs with *CEBPA* single mutation cases and is mutually exclusive of *CEBPA* double mutations [93].

The pathogenic role played by *NPM1* mutation in the development of AML is still not completely understood. *NPM1* can function as an oncogene as well as a tumor suppressor depending on gene dosage, expression level, interacting proteins, and cell localization, with many of the functions of NPM1 requiring continuous shuttling between the cytoplasm, nucleoplasm, and nucleoli [80, 93]. However, because the *NPM1* mutation always results in aberrant cytoplasmic localization of the mutant protein, this event appears to be critical for leukemogenesis. The *NPM1* mutation likely affects multiple cellular pathways by a combination of loss of function of the nucleophosmin nucleolar-interacting proteins that are delocalized by movement of the mutant protein into the leukemic cell cytoplasm, and gain of function resulting from the deregulated shuttling of the mutant protein between the nucleus and the cytoplasm [94]. Additional insights regarding the role of *NPM1* mutation in leukemogenesis have recently been provided by Vassiliou et al. [95]. These authors used a strategy of introducing a humanized *NPM1* mutation allele into the endogenous *NPM1* locus in a knock-in mouse model, thus keeping *NPM1* expression under the control of the endogenous promoter and mimicking the process of human AML development. Hematopoietic stem cells derived from these mice exhibited overexpression of homeodomain-containing transcription factor (*HOX*) genes (a characteristic also found in *NPM1*-mutated human AML [27, 28]), enhanced self-renewal, and expanded myelopoiesis. In addition, one-third of the mice also developed delayed-onset AML, demonstrating that the *NPM1* mutation can act as an AML-driving lesion [96].

The presence of *NPM1* mutation has emerged as an important favorable prognostic factor in AML patients. AML with mutated *NPM1* are highly responsive to induction chemotherapy [4, 80], and patients with CN-AML and mutated *NPM1* also exhibit higher rates of complete remission, disease-free survival, and overall survival compared to CN-AML with wild type *NPM1* [24]. However, the prognostic importance of the *NPM1* mutation in AML is also dependent upon the mutation status of the fms-like tyrosine kinase-3 (*FLT3*) gene [6]. Internal tandem duplication (ITD) mutations in the *FLT3* gene are found in approximately 40% of patients with AML and mutated *NPM1* compared with approximately 14% of the *NPM1* wild type cases [97]. The *FLT3*-ITD mutation in AML is usually associated with more aggressive disease, high white blood cell counts, early relapses, and poor survival [23]. Therefore, *NPM1* mutations confer a favorable prognosis in CN-AML

only in the absence of a concomitant *FLT3*-ITD mutation, and testing for both gene mutations should be performed together to provide the most accurate prognostic information [2, 80]. The favorable prognostic effects of *NPM1* mutations also appear to be dominant over other secondary AML features such as chromosomal abnormalities and multilineage dysplasia, which may be present in up to 15% and 23% of *NPM1*-mutated AML cases, respectively [98, 99].

The distinct biological properties of *NPM1*-mutated AML have also been confirmed by a number of gene expression profiling studies that showed upregulation of several *HOX* and transcription activator-like effector (*TALE*) gene family members [28, 100, 101]. These genes are known to be important for hematopoietic cell development and stem cell maintenance, supporting the possibility that *NPM1*-mutated AML is derived from a multipotent hematopoietic progenitor. However, the mechanism by which *NPM1* mutation leads to aberrant expression of *HOX* genes is unclear. Mutation of *NPM1* could directly influence the expression of *HOX* genes, or perhaps, *NPM1* mutation might cause arrested development of hematopoietic cell precursors at a primitive stage when the expression of *HOX* genes is elevated. It has also been suggested that microRNAs (miRNAs) could play a key role in the upregulation of *HOX* genes in *NPM1*-mutated AML. A unique miRNA signature was found in *NPM1*-mutated cases, with upregulation of *miR-10a*, *miR-10b*, *miR-196a*, *miR-196b*, and three families of tumor suppressor miRNAs, including *miR-15-a*/*miR-16-1*, *mir-29s* (*a/b/c*), and *let-7* family members (*7a/7b/7f*) [27, 102]. Several other miRNAs were found to be downregulated, including *miR-204* and *miR-128a*. Interestingly, miRNAs *10a*, *10b*, *196a*, and *196b* are all located within the genomic cluster of *HOX* genes, and *miR-204* has been shown to target the expression of the *HOXA10* and *MEIS1* genes, suggesting that *HOX* gene dysregulation in *NPM1*-mutated AML could be the result of altered miRNA expression. These findings are consistent with the possibility of an aberrant regulatory network including *NPM1*, *HOX* genes, and miRNAs that might be engaged in the arrest of cellular differentiation of hematopoietic precursors and development of AML with mutated *NPM1* [84].

Patients with AML that are found to have minimal residual disease after induction chemotherapy or before stem cell transplantation are more prone to relapse disease and show decreased overall survival [103–105]. The high incidence of the *NPM1* mutation in AML (present in approximately 30% of all AML and 50–60% in CN-AML), and the stability of the *NPM1* mutation in AML during disease evolution, makes this mutation a useful marker for the detection of submicroscopic levels of leukemia (minimal residual disease detection) after therapy. Numerous studies have now shown that assessment of *NPM1* mutation status in AML can serve as an important tool for prognosis prediction and therapy guidance [106–111]. A recent publication by Ivey et al. also clearly indicates that assessment of minimal residual disease in AML patients (based on the detection and quantitation of *NPM1*-mutated transcripts) provides prognostic information that is independent of other risk factors [112]. Patients who developed morphologic remission after chemotherapy and showed evidence of minimal residual disease in peripheral blood, as compared to patients with no evidence of minimal residual disease, had a significantly greater

risk of relapse (82% versus 30%) and a lower rate of survival (24% versus 75%). Multivariate analysis showed that the presence of minimal residual disease, as evidenced by persistence of *NPM1*-mutated transcripts, was the only significant prognostic factor for relapse and survival [112]. The ability to reclassify standard-risk or low-risk patients as high risk based on persistence of *NPM1*-mutated transcripts may help to appropriately stratify patients who would benefit from stem cell transplant rather than chemotherapy alone, and bone marrow transplant might be also be appropriately avoided in high-risk patients that have no evidence of minimal residual disease after therapy [113].

AML with Mutated RUNX1

In 1973, Dr. Janet Rowley identified a reciprocal translocation between chromosomes 8 and 21 in two patients with AML [114]. Eighteen years later, the Acute Myeloid Leukemia 1 (*AML1*) gene located at the breakpoint of the (8;21)(q22;q22) translocation on chromosome 21q22.12 was cloned and later renamed as the Runt-related Transcription Factor 1 (*RUNX1*) gene (named after the Drosophila RUNT protein, the first member of this gene family to be cloned) [115, 116]. The *RUNX1* gene contains 10 exons and encodes a sequence-specific DNA binding protein characterized by an N-terminal RUNT homology domain (RHD; exons 3–5) that mediates DNA binding and interaction with core-binding factor beta (CBFB), and a C-terminal transactivation domain (TAD; exons 6–8) [117]. *RUNX1* is widely expressed in hematopoietic cells and is required for the establishment of definitive hematopoiesis. In mouse models, *RUNX1* is absolutely required for embryogenesis and hematopoiesis, and lack of the *RUNX1* gene in homozygous mutants results in midgestation embryonic death due to necrosis and extensive hemorrhage in the central nervous system [118]. Because germline deletions are lethal, conditional *RUNX1* deletion strategies have been necessary to determine the role of this gene in adult hematopoiesis. Disruption of the *RUNX1* gene in adult hematopoietic stem cells using an inducible gene-targeting method causes inhibition of megakaryocytic maturation, increased hematopoietic progenitor cells, and defective B-lymphocyte and T-lymphocyte development [119]. Interestingly, loss of *RUNX1* function in hematopoietic stem cells does not result in AML, but rather establishes a preleukemic state that predisposes to AML following acquisition of additional mutations [117].

In human acute leukemia, the *RUNX1* gene is involved in a number of recurrent chromosomal translocations, including t(8;21)(q22;q22); *RUNX1-RUNX1T1* and t(3;21)(q26.2;q22); *MECOM(EVI1)-RUNX1* in AML, and t(12;21)(p13;q22); *ETV6-RUNX1* in B-lymphoblastic leukemia. Recurrent intragenic mutations have also been identified in AML, myelodysplastic syndromes (MDS), AML after MDS, therapy-related MDS and AML, radiation-exposed patients with MDS/AML, chronic myelomonocytic leukemia, and T-lymphoblastic leukemia [8, 120]. In AML, *RUNX1* mutations are found in both of the functional domains of the protein

(N-terminal RHD and C-terminal TAD), with the majority of mutations occurring in the RHD [5, 8, 14, 15, 26, 120]. Most *RUNX1* mutations are mono-allelic, and the mutational spectrum includes N-terminal missense mutations and C-terminal truncating mutations that result in deletion of the TAD of the protein. Both missense and truncating mutations cause loss of normal *RUNX1* function and also appear to have a dominant negative effect on the transactivation activity of wild type *RUNX1* in a dose-dependent fashion [121].

Rare germline mutations in the *RUNX1* gene have also been reported that are associated with an autosomal dominant disorder known as familial platelet disorder with predisposition to acute myeloid leukemia (FPD/AML). In FPD/AML families, there is thrombocytopenia with normal platelet size and platelet dysfunction with abnormal aggregation and secretion responses. Most FPD/AML patients exhibit mucocutaneous bleeding symptoms that include easy bruising, epistaxis, and bleeding after minor surgical or dental procedures. Up to 35% of these individuals eventually develop AML [122]. Among the more than 30 families described with FPD/AML, most carriers have a hemizygous *RUNX1* gene mutation. However, within individual FPD/AML families there is variable penetrance, with carriers of the same *RUNX1* mutations displaying heterogeneity in the degree of thrombocytopenia, platelet dysfunction, and bleeding. In addition, some family members develop myeloid malignancies, though not always of the same type, while other family members may remain unaffected. Different families also exhibit varying risks of progression to myeloid malignancy, likely due to the unique *RUNX1* mutations in each family group. The specific mechanisms underlying progression to acute leukemia in *RUNX1* mutation carriers are unknown, but could include haplosufficiency, dominant negative effects on normal *RUNX1* function, acquisition of another *RUNX1* mutation in the nonmutated germline allele, or acquisition of additional cooperating mutations [45]. Familial clustering of myelodysplastic syndromes and acute myeloid leukemia (MDS/AML) is also seen in patients with inherited disorders such as Fanconi anemia and congenital neutropenia, and these patients have also frequently been shown to carry *RUNX1* mutations [120, 123]. Since not all family members with familial *RUNX1* mutations exhibit clinical findings, testing for germline *RUNX1* mutations is recommended when more than two individual family members have been diagnosed with a myeloid malignancy [124].

RUNX1 mutations in AML are almost entirely mutually exclusive of the recurrent genetic abnormalities recognized in the WHO classification and complex abnormal karyotypes, but are frequently associated with trisomy 13 and monosomy 7/del(7q) [8, 120, 125]. Somatic alterations of the second *RUNX1* allele can also be found in a small percentage of AML cases, suggesting a role as a classical tumor suppressor gene [125, 126]. AML with *RUNX1* mutation has been associated with specific clinical and pathological features, including male gender, older age, more immature morphology (M0 French-American-British subtype), and secondary AML evolving from MDS [15, 121, 127]. These parameters also have well-recognized adverse effects on clinical outcome. In numerous studies, the *RUNX1* mutation has been shown to be a significant predictor of resistance to standard induction therapy and for inferior survival rate [5, 8, 14, 15, 25]. In univariate analy-

ses, *RUNX1* mutations are associated with refractory disease and inferior rates of event free survival, relapse free survival, and overall survival [120]. Gene expression profiling analysis has also provided evidence that *RUNX1*-mutated AML shares distinct biological features with other high-risk AML, with enrichment of *RUNX1*-mutated cases in complex karyotype AML and cytogenetic adverse-risk cases with monosomy 7, del(7q), inv(3), and t(3;3) [5]. *RUNX1*-mutated AML also exhibits upregulation of genes normally expressed in primitive hematopoietic cells and B-cell progenitors, and downregulation of promoters of myelopoiesis [26]. These cases also exhibit characteristic microRNA expression signatures, with downregulation microRNAs normally expressed in definitive myeloid progenitors (*miR-223*) and distinctly myeloid AML blasts (*miR-99a* and *miR-100*) [26]. The poor outcome of patients with the *RUNX1*-mutated AML suggests that novel therapeutic approaches should be used, including allogeneic stem cell transplant in younger patients, and investigational therapies in older patients [15, 120].

Future Perspectives and Conclusions

Emerging data from newer molecular techniques such as next generation sequencing (NGS) have revealed that CN-AML harbor numerous gene mutations, with most of them representing background mutations, and only a limited number having important prognostic and therapeutic implications [22]. The evaluation of these mutations as prognostic and predictive markers is a highly active research area, and some of these findings have been incorporated in a recent revision of the 2008 WHO Classification that changed two provisional gene mutation entities "AML with mutated *CEBPA*" and "AML with mutated *NPM1*" to clinically distinct diagnostic entities and also added "AML with mutated *RUNX1*" as a new provisional diagnostic entity [21]. Currently, there are three main genes (*NPM1*, *CEBPA*, and *FLT3*) routinely analyzed for molecular mutations that are used in clinical practice for the diagnosis and management of AML [128]. However, the addition of "AML with mutated *RUNX1*" as a provisional diagnostic entity will also require adding mutational analysis of this gene to current practice. The characteristic clinical and biological features of AML with *CEBPA*, *NPM1*, *RUNX1* gene mutations are summarized in Table 2.1. Evaluation for additional molecular mutations in other genes consistently associated with inferior outcomes, such as *ASXL1* and *TP53*, are also likely to be included in updated recommendations [20, 129]. The prognostic importance of other genes commonly mutated in AML such as *DNMT3A*, *TET2*, *IDH1*, *IDH2*, *NRAS*, *WT1* is less clear, but mutation testing for some of these genes may also be indicated due to the existence of therapies targeting specific gene mutations or combinations of mutations.

Within the past decade, nearly a dozen adult-onset inherited myelodysplastic syndrome and leukemia predisposition syndromes have also been identified. Individuals with inherited forms of hematologic malignancies are currently underdiagnosed due to the low frequency of cases and low level of clinician awareness of

Table 2.1 Characteristic clinical and biological features of acute myeloid leukemia with gene mutations

	AML with biallelic *CEBPA* mutations	AML with mutated *NPM1*	AML with mutated *RUNX1*
Frequency in cytogenetically normal AML	10–18%	50–60%	6–26%; increased frequency in elderly individuals
Clinical characteristics	High hemoglobin levels High WBC and blast counts Low platelet counts Low extramedullary involvement	Female sex High WBC and blast counts High extramedullary involvement	Male sex Older age Less blast differentiation (FAB M0)
Surface ntigen expression characteristics	Aberrant expression of CD7	Strong CD33 expression Lack of CD34 expression (>95% CD34-negative)	None
Germline mutations	10–15%	No	Rare
Response to chemotherapy	Good	Good	Poor
Distinct gene expression profile	Yes	Yes	Yes
Distinct microRNA expression profile	Yes	Yes	Yes
Gene mutation results in abnormal protein localization	No	Yes (aberrant cytoplasmic expression)	No
Prognosis	Good	Good	Poor

these syndromes. However, these individuals are increasingly likely to be encountered in clinical practice with wider adoption of NGS-based testing for the detection of prognostically significant or targetable genomic alterations in hematologic malignancies [130]. FDP/AML due to inherited *RUNX1* mutations was the first hereditary myeloid malignancy syndrome (HMMS) to be defined in 1999, followed by familial AML with *CEBPA* mutation in 2004 (both discussed earlier in this chapter). Expanding use of NGS has also contributed to the rapid identification of several additional HMMS, including familial MDS/AML with *GATA2* mutation, thrombocytopenia 2 (*ANKRD26*), myeloid neoplasms with germline predisposition (*ATG2B/GSKIP*), familial MDS/AML with mutated *DDX41*, thrombocytopenia 5

(*ETV6*), familial aplastic anemia/MDS with *SRP72* mutation, and an adult-onset inherited bone marrow failure/telomere syndrome with familial MDS/AML (*TERC/TERT*) [130, 131]. Clinicians must increasingly recognize the possibility that mutations identified in some genes, like *CEBPA* and *RUNX1* and others listed above, may represent pathogenic germline mutations and initiate appropriate follow-up germline genetic testing. The increasing importance of recognition of germline mutations is evidenced by the inclusion of a new category in the 2016 revision of the WHO classification of myeloid neoplasms and acute leukemia designated "Classification of myeloid neoplasm with germline predisposition" [21].

Given the growing number of gene mutations that are considered prognostically and therapeutically important in MDS/AML, there is no doubt that NGS testing will be showing increased use in conjunction with standard methods such as morphology and flow cytometry for diagnostic purposes. A major advantage of NGS-based assays is that they can detect and monitor multiple mutations simultaneously in a single cost-effective assay. This capability will also allow the possibility of NGS-based minimal residual disease monitoring assays in the future for detection of small clonal populations and low-frequency somatic mutations associated with MDS/AML [132].

Acknowledgment Thanks to Dr. Fiona Craig for critically reviewing this chapter.

References

1. Swerdlow SH, Campo E, Harris NL, Jaffee ES, Pileri SA, Stein H, Thiele J, Vardiman JW. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: International Agency for Research on Cancer; 2008.
2. Wang ML, Bailey NG. Acute myeloid leukemia genetics: risk stratification and implications for therapy. Arch Pathol Lab Med. 2015;139(10):1215–23.
3. Estey EH. Acute myeloid leukemia: 2014 update on risk-stratification and management. Am J Hematol. 2014;89(11):1063–81.
4. Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med. 2005;352(3):254–66.
5. Gaidzik VI, Bullinger L, Schlenk RF, Zimmermann AS, Rock J, Paschka P, et al. RUNX1 mutations in acute myeloid leukemia: results from a comprehensive genetic and clinical analysis from the AML study group. J Clin Oncol. 2011;29(10):1364–72.
6. Schlenk RF, Dohner K, Krauter J, Frohling S, Corbacioglu A, Bullinger L, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med. 2008;358(18):1909–18.
7. Schnittger S, Schoch C, Kern W, Mecucci C, Tschulik C, Martelli MF, et al. Nucleophosmin gene mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. Blood. 2005;106(12):3733–9.
8. Schnittger S, Dicker F, Kern W, Wendland N, Sundermann J, Alpermann T, et al. RUNX1 mutations are frequent in de novo AML with noncomplex karyotype and confer an unfavorable prognosis. Blood. 2011;117(8):2348–57.
9. Metzeler KH, Becker H, Maharry K, Radmacher MD, Kohlschmidt J, Mrozek K, et al. ASXL1 mutations identify a high-risk subgroup of older patients with primary cytogenetically normal AML within the ELN Favorable genetic category. Blood. 2011;118(26):6920–9.

10. Marcucci G, Maharry K, YZ W, Radmacher MD, Mrozek K, Margeson D, et al. IDH1 and IDH2 gene mutations identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. J Clin Oncol. 2010;28(14):2348–55.
11. Hou HA, Kuo YY, Liu CY, Chou WC, Lee MC, Chen CY, et al. DNMT3A mutations in acute myeloid leukemia: stability during disease evolution and clinical implications. Blood. 2012;119(2):559–68.
12. Metzeler KH, Maharry K, Radmacher MD, Mrozek K, Margeson D, Becker H, et al. TET2 mutations improve the new European LeukemiaNet risk classification of acute myeloid leukemia: a Cancer and Leukemia Group B study. J Clin Oncol. 2011;29(10):1373–81.
13. Schnittger S, Eder C, Jeromin S, Alpermann T, Fasan A, Grossmann V, 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.
14. Greif PA, Konstandin NP, Metzeler KH, Herold T, Pasalic Z, Ksienzyk B, et al. RUNX1 mutations in cytogenetically normal acute myeloid leukemia are associated with a poor prognosis and up-regulation of lymphoid genes. Haematologica. 2012;97(12):1909–15.
15. Tang JL, Hou HA, Chen CY, Liu CY, Chou WC, Tseng MH, et al. AML1/RUNX1 mutations in 470 adult patients with de novo acute myeloid leukemia: prognostic implication and interaction with other gene alterations. Blood. 2009;114(26):5352–61.
16. Becker H, Marcucci G, Maharry K, Radmacher MD, Mrozek K, Margeson D, et al. Mutations of the Wilms tumor 1 gene (WT1) in older patients with primary cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. Blood. 2010;116(5):788–92.
17. Virappane P, Gale R, Hills R, Kakkas I, Summers K, Stevens J, et al. Mutation of the Wilms' tumor 1 gene is a poor prognostic factor associated with chemotherapy resistance in normal karyotype acute myeloid leukemia: the United Kingdom Medical Research Council Adult Leukaemia Working Party. J Clin Oncol. 2008;26(33):5429–35.
18. Gaidzik VI, Schlenk RF, Moschny S, Becker A, Bullinger L, Corbacioglu A, et al. Prognostic impact of WT1 mutations in cytogenetically normal acute myeloid leukemia: a study of the German-Austrian AML Study Group. Blood. 2009;113(19):4505–11.
19. Marcucci G, Haferlach T, Dohner H. Molecular genetics of adult acute myeloid leukemia: prognostic and therapeutic implications. J Clin Oncol. 2011;29(5):475–86.
20. Dohner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med. 2015;373(12):1136–52.
21. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
22. Martelli MP, Sportoletti P, Tiacci E, Martelli MF, Falini B. Mutational landscape of AML with normal cytogenetics: biological and clinical implications. Blood Rev. 2013;27(1):13–22.
23. Falini B, Martelli MP. Impact of genomics in the clinical management of patients with cytogenetically normal acute myeloid leukemia. Best Pract Res Clin Haematol. 2015;28(2–3):90–7.
24. Marcucci G, Maharry K, Radmacher MD, Mrózek K, Vukosavljevic T, et al. Prognostic significance of, and gene and microRNA expression signatures associated with, CEBPA mutations in cytogenetically normal acute myeloid leukemia with high-risk molecular features: a Cancer and Leukemia Group B Study. J Clin Oncol. 2008;26(31):5078–87.
25. Becker H, Marcucci G, Maharry K, Radmacher MD, Mrozek K, Margeson D, 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.
26. Mendler JH, Maharry K, Radmacher MD, Mrozek K, Becker H, Metzeler KH, 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):3109–18.
27. Garzon R, Garofalo M, Martelli MP, Briesewitz R, Wang L, Fernandez-Cymering C, et al. Distinctive microRNA signature of acute myeloid leukemia bearing cytoplasmic mutated nucleophosmin. Proc Natl Acad Sci U S A. 2008;105(10):3945–50.

28. Alcalay M, Tiacci E, Bergomas R, Bigerna B, Venturini E, Minardi SP, et al. Acute myeloid leukemia bearing cytoplasmic nucleophosmin (NPMc+ AML) shows a distinct gene expression profile characterized by up-regulation of genes involved in stem-cell maintenance. Blood. 2005;106(3):899–902.
29. Antonson P, Xanthopoulos KG. Molecular cloning, sequence, and expression patterns of the human gene encoding CCAAT/enhancer binding protein alpha (C/EBP alpha). Biochem Biophys Res Commun. 1995;215(1):106–13.
30. Tenen DG. Disruption of differentiation in human cancer: AML shows the way. Nat Rev Cancer. 2003;3(2):89–101.
31. Ohlsson E, Schuster MB, Hasemann M, Porse BT. The multifaceted functions of C/EBPalpha in normal and malignant haematopoiesis. Leukemia. 2016;30(4):767–75.
32. Timchenko NA, Harris TE, Wilde M, Bilyeu TA, Burgess-Beusse BL, Finegold MJ, et al. CCAAT/enhancer binding protein alpha regulates p21 protein and hepatocyte proliferation in newborn mice. Mol Cell Biol. 1997;17(12):7353–61.
33. Muller C, Calkhoven CF, Sha X, Leutz A. The CCAAT enhancer-binding protein alpha (C/EBPalpha) requires a SWI/SNF complex for proliferation arrest. J Biol Chem. 2004;279(8):7353–8.
34. Wang H, Iakova P, Wilde M, Welm A, Goode T, Roesler WJ, et al. C/EBPalpha arrests cell proliferation through direct inhibition of Cdk2 and Cdk4. Mol Cell. 2001;8(4):817–28.
35. Zhang DE, Zhang P, Wang ND, Hetherington CJ, Darlington GJ, Tenen DG. Absence of granulocyte colony-stimulating factor signaling and neutrophil development in CCAAT enhancer binding protein alpha-deficient mice. Proc Natl Acad Sci U S A. 1997;94(2):569–74.
36. Pabst T, Mueller BU, Zhang P, Radomska HS, Narravula S, Schnittger S, et al. Dominant-negative mutations of CEBPA, encoding CCAAT/enhancer binding protein-alpha (C/EBPalpha), in acute myeloid leukemia. Nat Genet. 2001;27(3):263–70.
37. Ziai JM, Siddon AJ. Pathology consultation on gene mutations in acute myeloid leukemia. Am J Clin Pathol. 2015;144(4):539–54.
38. Fröhling S, Schlenk RF, Stolze I, Bihlmayr J, Benner A, Kreitmeier S, et al. CEBPA mutations in younger adults with acute myeloid leukemia and normal cytogenetics: prognostic relevance and analysis of cooperating mutations. J Clin Oncol. 2004;22(4):624–33.
39. Koschmieder S, Halmos B, Levantini E, Tenen DG. Dysregulation of the C/EBPalpha differentiation pathway in human cancer. J Clin Oncol. 2009;27(4):619–28.
40. 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):3088–91.
41. Bereshchenko O, Mancini E, Moore S, Bilbao D, Mansson R, Luc S, et al. Hematopoietic stem cell expansion precedes the generation of committed myeloid leukemia-initiating cells in C/EBPalpha mutant AML. Cancer Cell. 2009;16(5):390–400.
42. Smith ML, Cavenagh JD, Lister TA, Fitzgibbon J. Mutation of CEBPA in familial acute myeloid leukemia. N Engl J Med. 2004;351(23):2403–7.
43. Sellick GS, Spendlove HE, Catovsky D, Pritchard-Jones K, Houlston RS. Further evidence that germline CEBPA mutations cause dominant inheritance of acute myeloid leukaemia. Leukemia. 2005;19(7):1276–8.
44. Pabst T, Mueller BU. Complexity of CEBPA dysregulation in human acute myeloid leukemia. Clin Cancer Res. 2009;15(17):5303–7.
45. Nickels EM, Soodalter J, Churpek JE, Godley LA. Recognizing familial myeloid leukemia in adults. Ther Adv Hematol. 2013;4(4):254–69.
46. Tawana K, Wang J, Renneville A, Bödör C, Hills R, Loveday C, et al. Disease evolution and outcomes in familial AML with germline CEBPA mutations. Blood. 2015;126(10):1214–23.
47. 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):5088–93.

48. Nanri T, Uike N, Kawakita T, Iwanaga E, Mitsuya H, Asou N. A family harboring a germline N-terminal C/EBPalpha mutation and development of acute myeloid leukemia with an additional somatic C-terminal C/EBPalpha mutation. Genes Chromosomes Cancer. 2010;49(3):237–41.
49. Taskesen E, Bullinger L, Corbacioglu A, Sanders MA, Erpelinck CA, Wouters BJ, et al. Prognostic impact, concurrent genetic mutations, and gene expression features of AML with CEBPA mutations in a cohort of 1182 cytogenetically normal AML patients: further evidence for CEBPA double mutant AML as a distinctive disease entity. Blood. 2011;117(8):2469–75.
50. Chim CS, Wong AS, Kwong YL. Infrequent hypermethylation of CEBPA promotor in acute myeloid leukaemia. Br J Haematol. 2002;119(4):988–90.
51. Wouters BJ, Jordà MA, Keeshan K, Louwers I, Erpelinck-Verschueren CA, Tielemans D, et al. Distinct gene expression profiles of acute myeloid/T-lymphoid leukemia with silenced CEBPA and mutations in NOTCH1. Blood. 2007;110(10):3706–14.
52. Hackanson B, Bennett KL, Brena RM, Jiang J, Claus R, Chen SS, et al. Epigenetic modification of CCAAT/enhancer binding protein alpha expression in acute myeloid leukemia. Cancer Res. 2008;68(9):3142–51.
53. Lin TC, Hou HA, Chou WC, DL O, SL Y, Tien HF, et al. CEBPA methylation as a prognostic biomarker in patients with de novo acute myeloid leukemia. Leukemia. 2011;25(1):32–40.
54. Fasan A, Alpermann T, Haferlach C, Grossmann V, Roller A, Kohlmann A, et al. Frequency and prognostic impact of CEBPA proximal, distal and core promoter methylation in normal karyotype AML: a study on 623 cases. PLoS One. 2013;8(2):e54365.
55. 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):1343–6.
56. Dufour A, Schneider F, Metzeler KH, Hoster E, Schneider S, Zellmeier E, et al. Acute myeloid leukemia with biallelic CEBPA gene mutations and normal karyotype represents a distinct genetic entity associated with a favorable clinical outcome. J Clin Oncol. 2010;28(4):570–7.
57. Green CL, Koo KK, Hills RK, Burnett AK, Linch DC, Gale RE. Prognostic significance of CEBPA mutations in a large cohort of younger adult patients with acute myeloid leukemia: impact of double CEBPA mutations and the interaction with FLT3 and NPM1 mutations. J Clin Oncol. 2010;28(16):2739–47.
58. Shen Y, Zhu YM, Fan X, Shi JY, Wang QR, Yan XJ, et al. Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia. Blood. 2011;118(20):5593–603.
59. Fasan A, Haferlach C, Alpermann T, Jeromin S, Grossmann V, Eder C, et al. The role of different genetic subtypes of CEBPA mutated AML. Leukemia. 2014;28(4):794–803.
60. Bacher U, Schnittger S, Macijewski K, Grossmann V, Kohlmann A, Alpermann T, et al. Multilineage dysplasia does not influence prognosis in CEBPA-mutated AML, supporting the WHO proposal to classify these patients as a unique entity. Blood. 2012;119(20):4719–22.
61. Lin LI, Chen CY, Lin DT, Tsay W, Tang JL, Yeh YC, et al. Characterization of CEBPA mutations in acute myeloid leukemia: most patients with CEBPA mutations have biallelic mutations and show a distinct immunophenotype of the leukemic cells. Clin Cancer Res. 2005;11(4):1372–9.
62. Chan WY, Liu QR, Borjigin J, Busch H, Rennert OM, Tease LA, et al. Characterization of the cDNA encoding human nucleophosmin and studies of its role in normal and abnormal growth. Biochemistry. 1989;28(3):1033–9.
63. Li XZ, McNeilage LJ, Whittingham S. The nucleotide sequence of a human cDNA encoding the highly conserved nucleolar phosphoprotein B23. Biochem Biophys Res Commun. 1989;163(1):72–8.
64. Chang JH, Olson MO. Structure of the gene for rat nucleolar protein B23. J Biol Chem. 1990;265(30):18227–33.
65. Borer RA, Lehner CF, Eppenberger HM, Nigg EA. Major nucleolar proteins shuttle between nucleus and cytoplasm. Cell. 1989;56(3):379–90.

66. Dumbar TS, Gentry GA, Olson MO. Interaction of nucleolar phosphoprotein B23 with nucleic acids. Biochemistry. 1989;28(24):9495–501.
67. Grisendi S, Mecucci C, Falini B, Pandolfi PP. Nucleophosmin and cancer. Nat Rev Cancer. 2006;6(7):493–505.
68. Tarapore P, Okuda M, Fukasawa K. A mammalian in vitro centriole duplication system: evidence for involvement of CDK2/cyclin E and nucleophosmin/B23 in centrosome duplication. Cell Cycle. 2002;1(1):75–81.
69. Maggi LB Jr, Kuchenruether M, Dadey DY, Schwope RM, Grisendi S, Townsend RR, et al. Nucleophosmin serves as a rate-limiting nuclear export chaperone for the Mammalian ribosome. Mol Cell Biol. 2008;28(23):7050–65.
70. Yu Y, Maggi LB Jr, Brady SN, Apicelli AJ, Dai MS, Lu H, et al. Nucleophosmin is essential for ribosomal protein L5 nuclear export. Mol Cell Biol. 2006;26(10):3798–809.
71. Lee SY, Park JH, Kim S, Park EJ, Yun Y, Kwon J. A proteomics approach for the identification of nucleophosmin and heterogeneous nuclear ribonucleoprotein C1/C2 as chromatin-binding proteins in response to DNA double-strand breaks. Biochem J. 2005;388(Pt 1):7–15.
72. MH W, Chang JH, Yung BY. Resistance to UV-induced cell-killing in nucleophosmin/B23 over-expressed NIH 3T3 fibroblasts: enhancement of DNA repair and up-regulation of PCNA in association with nucleophosmin/B23 over-expression. Carcinogenesis. 2002;23(1):93–100.
73. Li J, Zhang X, Sejas DP, Bagby GC, Pang Q. Hypoxia-induced nucleophosmin protects cell death through inhibition of p53. J Biol Chem. 2004;279(40):41275–9.
74. Takemura M, Sato K, Nishio M, Akiyama T, Umekawa H, Yoshida S. Nucleolar protein B23.1 binds to retinoblastoma protein and synergistically stimulates DNA polymerase alpha activity. J Biochem. 1999;125(5):904–9.
75. Bertwistle D, Sugimoto M, Sherr CJ. Physical and functional interactions of the Arf tumor suppressor protein with nucleophosmin/B23. Mol Cell Biol. 2004;24(3):985–96.
76. Colombo E, Bonetti P, Lazzerini Denchi E, Martinelli P, Zamponi R, Marine JC, et al. Nucleophosmin is required for DNA integrity and p19Arf protein stability. Mol Cell Biol. 2005;25(20):8874–86.
77. Kurki S, Peltonen K, Latonen L, Kiviharju TM, Ojala PM, Meek D, et al. Nucleolar protein NPM interacts with HDM2 and protects tumor suppressor protein p53 from HDM2-mediated degradation. Cancer Cell. 2004;5(5):465–75.
78. Grisendi S, Bernardi R, Rossi M, Cheng K, Khandker L, Manova K, et al. Role of nucleophosmin in embryonic development and tumorigenesis. Nature. 2005;437(7055):147–53.
79. Sportoletti P, Grisendi S, Majid SM, Cheng K, Clohessy JG, Viale A, et al. Npm1 is a haploinsufficient suppressor of myeloid and lymphoid malignancies in the mouse. Blood. 2008;111(7):3859–62.
80. Falini B, Nicoletti I, Martelli MF, Mecucci C. Acute myeloid leukemia carrying cytoplasmic/mutated nucleophosmin (NPMc+ AML): biologic and clinical features. Blood. 2007;109(3):874–85.
81. Falini B, Martelli MP, Bolli N, Bonasso R, Ghia E, Pallotta MT, et al. Immunohistochemistry predicts nucleophosmin (NPM) mutations in acute myeloid leukemia. Blood. 2006;108(6):1999–2005.
82. Falini B, Lenze D, Hasserjian R, Coupland S, Jaehne D, Soupir C, et al. Cytoplasmic mutated nucleophosmin (NPM) defines the molecular status of a significant fraction of myeloid sarcomas. Leukemia. 2007;21(7):1566–70.
83. Falini B, Nicoletti I, Bolli N, Martelli MP, Liso A, Gorello P, et al. Translocations and mutations involving the nucleophosmin (NPM1) gene in lymphomas and leukemias. Haematologica. 2007;92(4):519–32.
84. Rau R, Brown P. Nucleophosmin (NPM1) mutations in adult and childhood acute myeloid leukaemia: towards definition of a new leukaemia entity. Hematol Oncol. 2009;27(4):171–81.
85. Mariano AR, Colombo E, Luzi L, Martinelli P, Volorio S, Bernard L, et al. Cytoplasmic localization of NPM in myeloid leukemias is dictated by gain-of-function mutations that create a functional nuclear export signal. Oncogene. 2006;25(31):4376–80.

86. Albiero E, Madeo D, Bolli N, Giaretta I, Bona ED, Martelli MF, et al. Identification and functional characterization of a cytoplasmic nucleophosmin leukaemic mutant generated by a novel exon-11 NPM1 mutation. Leukemia. 2007;21(5):1099–103.
87. Falini B, Martelli MP, Mecucci C, Liso A, Bolli N, Bigerna B, et al. Cytoplasmic mutated nucleophosmin is stable in primary leukemic cells and in a xenotransplant model of NPMc+ acute myeloid leukemia in SCID mice. Haematologica. 2008;93(5):775–9.
88. Meloni G, Mancini M, Gianfelici V, Martelli MP, Foa R, Falini B. Late relapse of acute myeloid leukemia with mutated NPM1 after eight years: evidence of NPM1 mutation stability. Haematologica. 2009;94(2):298–300.
89. Bolli N, Galimberti S, Martelli MP, Tabarrini A, Roti G, Mecucci C, et al. Cytoplasmic nucleophosmin in myeloid sarcoma occurring 20 years after diagnosis of acute myeloid leukaemia. Lancet Oncol. 2006;7(4):350–2.
90. Suzuki T, Kiyoi H, Ozeki K, Tomita A, Yamaji S, Suzuki R, et al. Clinical characteristics and prognostic implications of NPM1 mutations in acute myeloid leukemia. Blood. 2005;106(8):2854–61.
91. Chou WC, Tang JL, Lin LI, Yao M, Tsay W, Chen CY, et al. Nucleophosmin mutations in de novo acute myeloid leukemia: the age-dependent incidences and the stability during disease evolution. Cancer Res. 2006;66(6):3310–6.
92. Falini B, Mecucci C, Saglio G, Lo Coco F, Diverio D, Brown P, et al. NPM1 mutations and cytoplasmic nucleophosmin are mutually exclusive of recurrent genetic abnormalities: a comparative analysis of 2562 patients with acute myeloid leukemia. Haematologica. 2008;93(3):439–42.
93. Federici L, Falini B. Nucleophosmin mutations in acute myeloid leukemia: a tale of protein unfolding and mislocalization. Protein Sci. 2013;22(5):545–56.
94. Falini B, Martelli MP, Bolli N, Sportoletti P, Liso A, Tiacci E, et al. Acute myeloid leukemia with mutated nucleophosmin (NPM1): is it a distinct entity? Blood. 2011;117(4):1109–20.
95. Vassiliou GS, Cooper JL, Rad R, Li J, Rice S, Uren A, et al. Mutant nucleophosmin and cooperating pathways drive leukemia initiation and progression in mice. Nat Genet. 2011;43(5):470–5.
96. Sportoletti P, Varasano E, Rossi R, Mupo A, Tiacci E, Vassiliou G, et al. Mouse models of NPM1-mutated acute myeloid leukemia: biological and clinical implications. Leukemia. 2015;29(2):269–78.
97. Thiede C, Koch S, Creutzig E, Steudel C, Illmer T, Schaich M, et al. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). Blood. 2006;107(10):4011–20.
98. Haferlach C, Mecucci C, Schnittger S, Kohlmann A, Mancini M, Cuneo A, et al. AML with mutated NPM1 carrying a normal or aberrant karyotype show overlapping biologic, pathologic, immunophenotypic, and prognostic features. Blood. 2009;114(14):3024–32.
99. Falini B, Macijewski K, Weiss T, Bacher U, Schnittger S, Kern W, et al. Multilineage dysplasia has no impact on biologic, clinicopathologic, and prognostic features of AML with mutated nucleophosmin (NPM1). Blood. 2010;115(18):3776–86.
100. Mullighan CG, Kennedy A, Zhou X, Radtke I, Phillips LA, Shurtleff SA, et al. Pediatric acute myeloid leukemia with NPM1 mutations is characterized by a gene expression profile with dysregulated HOX gene expression distinct from MLL-rearranged leukemias. Leukemia. 2007;21(9):2000–9.
101. Andreeff M, Ruvolo V, Gadgil S, Zeng C, Coombes K, Chen W, et al. HOX expression patterns identify a common signature for favorable AML. Leukemia. 2008;22(11):2041–7.
102. Jongen-Lavrencic M, Sun SM, Dijkstra MK, Valk PJ, Löwenberg B. MicroRNA expression profiling in relation to the genetic heterogeneity of acute myeloid leukemia. Blood. 2008;111(10):5078–85.
103. Loken MR, Alonzo TA, Pardo L, Gerbing RB, Raimondi SC, Hirsch BA, et al. Residual disease detected by multidimensional flow cytometry signifies high relapse risk in patients with de novo acute myeloid leukemia: a report from Children's Oncology Group. Blood. 2012;120(8):1581–8.

104. Walter RB, Buckley SA, Pagel JM, Wood BL, Storer BE, Sandmaier BM, et al. Significance of minimal residual disease before myeloablative allogeneic hematopoietic cell transplantation for AML in first and second complete remission. Blood. 2013;122(10):1813–21.
105. Othus M, Wood BL, Stirewalt DL, Estey EH, Petersdorf SH, Appelbaum FR, et al. Effect of measurable ('minimal') residual disease (MRD) information on prediction of relapse and survival in adult acute myeloid leukemia. Leukemia. 2016;30(10):2080–3.
106. Gorello P, Cazzaniga G, Alberti F, Dell'Oro MG, Gottardi E, Specchia G, et al. Quantitative assessment of minimal residual disease in acute myeloid leukemia carrying nucleophosmin (NPM1) gene mutations. Leukemia. 2006;20(6):1103–8.
107. Chou WC, Tang JL, SJ W, Tsay W, Yao M, Huang SY, et al. Clinical implications of minimal residual disease monitoring by quantitative polymerase chain reaction in acute myeloid leukemia patients bearing nucleophosmin (NPM1) mutations. Leukemia. 2007;21(5):998–1004.
108. Schnittger S, Kern W, Tschulik C, Weiss T, Dicker F, Falini B, et al. Minimal residual disease levels assessed by NPM1 mutation-specific RQ-PCR provide important prognostic information in AML. Blood. 2009;114(11):2220–31.
109. Krönke J, Schlenk RF, Jensen KO, Tschürtz F, Corbacioglu A, Gaidzik VI, et al. Monitoring of minimal residual disease in NPM1-mutated acute myeloid leukemia: a study from the German-Austrian acute myeloid leukemia study group. J Clin Oncol. 2011;29(19):2709–16.
110. Shayegi N, Kramer M, Bornhäuser M, Schaich M, Schetelig J, Platzbecker U, et al. The level of residual disease based on mutant NPM1 is an independent prognostic factor for relapse and survival in AML. Blood. 2013;122(1):83–92.
111. Hubmann M, Köhnke T, Hoster E, Schneider S, Dufour A, Zellmeier E, et al. Molecular response assessment by quantitative real-time polymerase chain reaction after induction therapy in NPM1-mutated patients identifies those at high risk of relapse. Haematologica. 2014;99(8):1317–25.
112. Ivey A, Hills RK, Simpson MA, Jovanovic JV, Gilkes A, Grech A, et al. Assessment of minimal residual disease in standard-risk AML. N Engl J Med. 2016;374(5):422–33.
113. Burke MJ. Minimal residual disease in NPM1-mutated AML. N Engl J Med. 2016;374(5):481–2.
114. Rowley JD. Identificaton of a translocation with quinacrine fluorescence in a patient with acute leukemia. Ann Genet. 1973;16(2):109–12.
115. Miyoshi H, Shimizu K, Kozu T, Maseki N, Kaneko Y, Ohki M. t(8;21) breakpoints on chromosome 21 in acute myeloid leukemia are clustered within a limited region of a single gene, AML1. Proc Natl Acad Sci U S A. 1991;88(23):10431–4.
116. van Wijnen AJ, Stein GS, Gergen JP, Groner Y, Hiebert SW, Ito Y, et al. Nomenclature for Runt-related (RUNX) proteins. Oncogene. 2004;23(24):4209–10.
117. Mangan JK, Speck NA. RUNX1 mutations in clonal myeloid disorders: from conventional cytogenetics to next generation sequencing, a story 40 years in the making. Crit Rev Oncog. 2011;16(1–2):77–91.
118. Wang Q, Stacy T, Binder M, Marin-Padilla M, Sharpe AH, Speck NA. Disruption of the Cbfa2 (AML1) gene causes necrosis and hemorrhaging in the central nervous system and blocks definitive hematopoiesis. Proc Natl Acad Sci U S A. 1996;93(8):3444–9.
119. Ichikawa M, Asai T, Saito T, Seo S, Yamazaki I, Yamagata T, et al. AML-1 is required for megakaryocytic maturation and lymphocytic differentiation, but not for maintenance of hematopoietic stem cells in adult hematopoiesis. Nat Med. 2004;10(3):299–304.
120. Gaidzik VI, Teleanu V, Papaemmanuil E, Weber D, Paschka P, Hahn J, et al. RUNX1 mutations in acute myeloid leukemia are associated with distinct clinico-pathologic and genetic features. Leukemia. 2016;30(11):2160–8.
121. Harada Y, Harada H. Molecular pathways mediating MDS/AML with focus on AML1/RUNX1 point mutations. J Cell Physiol. 2009;220(1):16–20.
122. Lambert MP. Update on the inherited platelet disorders. Curr Opin Hematol. 2015;22(5):460–6.
123. Churpek JE, Pyrtel K, Kanchi KL, Shao J, Koboldt D, Miller CA, et al. Genomic analysis of germ line and somatic variants in familial myelodysplasia/acute myeloid leukemia. Blood. 2015;126(22):2484–90.

124. Churpek JE, Lorenz R, Nedumgottil S, Onel K, Olopade OI, Sorrell A, et al. Proposal for the clinical detection and management of patients and their family members with familial myelodysplastic syndrome/acute leukemia predisposition syndromes. Leuk Lymphoma. 2013;54(1):28–35.
125. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368(22):2059–74.
126. Osato M, Asou N, Abdalla E, Hoshino K, Yamasaki H, Okubo T, et al. Biallelic and heterozygous point mutations in the runt domain of the AML1/PEBP2alphaB gene associated with myeloblastic leukemias. Blood. 1999;93(6):1817–24.
127. Silva FP, Swagemakers SM, Erpelinck-Verschueren C, Wouters BJ, Delwel R, Vrieling H, et al. Gene expression profiling of minimally differentiated acute myeloid leukemia: M0 is a distinct entity subdivided by RUNX1 mutation status. Blood. 2009;114(14):3001–7.
128. Döhner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK, et al. 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):453–74.
129. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374(23):2209–21.
130. DiNardo CD, Bannon SA, Routbort M, Franklin A, Mork M, Armanios 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):417–28.
131. Churpek JE, Godley LA. How I diagnose and manage individuals at risk for inherited myeloid malignancies. Blood. 2016;pii:blood-2016-05-670240. [Epub ahead of print].
132. Duncavage EJ, Tandon B. The utility of next-generation sequencing in diagnosis and monitoring of acute myeloid leukemia and myelodysplastic syndromes. Int J Lab Hematol. 2015;37(Suppl 1):115–21.