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Original contribution

Classification of acute myeloid leukemia by the revised fourth edition World Health Organization criteria: a retrospective single-institution study with appraisal of the new entities of acute myeloid leukemia with gene mutations in *NPM1* and biallelic *CEBPA*☆,☆☆

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Summary The 2016/2017 World Health Organization (WHO$^{2016/2017}$) classification for acute myeloid leukemia (AML) includes new entities with gene mutations in *NPM1* (AML-*NPM1*^{mut}) and biallelic *CEBPA* (AML-bi*CEBPA*^{mut}). To retrospectively identify and study these new molecularly defined WHO$^{2016/2017}$entities, we reviewed clinicopathologic data and pretherapy archived pathologic materials at diagnosis for 143 consecutive AML cases (55.2% male, median age 62 [range 18-89] years) and classified all cases by the 2008 WHO (WHO^{2008}) and revised WHO$^{2016/2017}$ criteria. By WHO^{2008}, cases included 21 (15%) with recurrent genetic abnormalities (52.3% male, median age 54 [range 18-82] years), 54 (38%) with myelodysplasia-related changes (57.4% male, median age 65 [range 32-84] years), 3 (2%) therapy related (100% male, median age 66 [range 32-84] years), and 65 (45%) not otherwise specified (52.3% male, median age 61 [range 19-89] years). Twenty-two (15.4%) cases (21 AML, not otherwise specified; 1 AML with myelodysplasia-related changes by WHO^{2008}) reclassified by WHO$^{2016/2017}$ as AML-*NPM1*^{mut} showed female predominance (54.5%), and median (range) values were as follows: age 60.5 (23-84) years, hemoglobin 8.6 (5.6-12.9) g/dL, total leucocytes 30.1 (2.58-241.84) × 10^9/L, monocytes 1.65 (0-49.34) × 10^9/L, neutrophils 1.96 (0-29.79) × 10^9/L, platelets 55 (11-320) × 10^9/L, blasts (peripheral blood 41% [2%-98%], bone marrow 66% [17%-97%]), with myeloblasts^{CD34neg} (17 [77%]/21), cytogenetics^{normal} (20 [91%]/22), *FLT3*-ITD^{pos} (9 [41%]/22), *FLT3*-ITD^{neg}*FLT3*-TKD^{pos} (5 [23%]/22), *FLT3*-ITD^{neg}*FLT3*-TKD^{neg} (8 [36%]/22), and extramedullary involvement (6 [27%]/22), including 1 novel cutaneous presentation. Notably, presenting features among AML-*NPM1*^{mut} included those of anemia (22 [100%]) and thrombocytopenia (20 [91%]/22). This is also the first report of 4 [18%]/22 AML-*NPM1*^{mut} (including 3 [75%]/4 nonsmokers) with a family history of leukemia and one 74-year-old with familial AML-bi*CEBPA*^{mut}. This study validates the application of the WHO$^{2016/2017}$ classification criteria by retrospectively identifying AML-*NPM1*^{mut} and

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AML-bi*CEBPA*^{mut} cases using single-gene molecular analyses. Additional studies are needed to characterize the complete spectrum of WHO$^{2016/2017}$-defined AML-bi*CEBPA*^{mut} and for familial AML including AML-*NPM1*^{mut}.

1. Introduction

Acute leukemias were first classified by the French-American-British (FAB) criteria based on cytomorphologic and cytochemical features, with lineage assigned as myeloid by the presence of Auer rods or ≥3% myeloperoxidase-positive blasts. By FAB criteria, acute leukemia diagnosis required 30% blasts in peripheral blood (PB) or bone marrow (BM), with that arbitrary cutoff proposed to distinguish from myelodysplastic syndromes (MDSs) because chemotherapy was not considered then for MDSs [1]. Based on the principles of the Revised European-American Lymphoma Classification [2], the World Health Organization (WHO) classification of hematopoietic neoplasms, developed in 1999, defined distinct disease entities by a combination of morphologic, immunophenotypic, genetic, and clinical features, with the relative importance of each dependent upon the entity [3]. In 2001, the WHO classification represented a paradigm shift by incorporating genetic information into diagnostic algorithms and recognized 4 main subgroups of myeloid neoplasms [4,5]: (1) acute myeloid leukemia (AML), with a lower cutoff of ≥20% PB or BM myeloblasts, in the absence of 3 chromosomal translocations (t[15;17], t[8;21], inv[16] or t[16;16]), each of which was sufficient to diagnose AML with <20% myeloblasts; (2) MDS, with <20% myeloblasts; (3) myeloproliferative neoplasms (MPNs); and (4) myelodysplastic/myeloproliferative neoplasms (MDSs/MPNs). The major 2001 WHO AML categories were (1) AML with recurrent genetic abnormalities (AML-RGA), including t(15;17), t(8;21), inv(16) or t(16;16), and 11q23 abnormalities; (2) AML with multilineage dysplasia, diagnosed by either of the 2 following criteria: (*a*) if there was a history of MDS or MDS/MPN or (b) if dysplasia, as assessed by morphologic review, was present in ≥50% cells in ≥2 hematopoietic lineages; (3) therapy-related MDS and AML (t-AML); and (4) AML, not further specified (AML-NOS), with the category then introduced for cases nonclassifiable into the above 3 specific categories [4].

By the 2008 WHO (WHO^{2008}) classification [6,7], myeloid/lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA, PDFRB*, or *FGFR1* were added as a new subgroup of myeloid neoplasms, and t(9;11), t(6;9), inv(3) or t(3;3), and t(1;22), with ≥20% myeloblasts, were recognized as distinct entities among AML-RGA [6,7]. AML-NOS subclassification by FAB criteria was no longer required; categories of myeloid sarcoma and myeloid proliferations related to Down syndrome were added. AML cases with myelodysplasia-related changes (AML-MRC) were then recognized to also include, as an independent diagnostic criterion, specific cytogenetic abnormalities that were considered sufficient to diagnose AML-MRC with ≥20% PB or BM myeloblasts [6].

Advances in our knowledge in myeloid neoplasms led to a proposed revision of the WHO^{2008} classification in 2015 [8] and were previously reviewed [9]. The revised WHO criteria for myeloid neoplasms were published in 2016 [10], with the revised fourth edition in 2017 [11]. The revised 2016/2017 WHO (WHO$^{2016/2017}$) followed the same WHO principles of integrating clinicopathologic and genetic features to recognize distinct disease entities and retained the major categories of AML while eliminating nonerythroid blast counts, in addition to including a new category of myeloid neoplasms with germline predisposition [11]. Among AML-RGAs, 2 distinct entities with gene mutations were recognized: AML with mutated *NPM1* (hereafter designated as AML-*NPM1*^{mut}) and AML with biallelic mutations of *CEBPA* (designated as AML-bi*CEBPA*^{mut}) [11]. The nucleophosmin gene (*NPM1)* is the most frequently mutated gene in cytogenetically normal AMLs, which comprise ~45%-50% of all AML cases, with prognostic significance in AML in conjunction with mutations in other genes [12]. In AML-*NPM1*^{mut}, mutations that occur typically as insertions in *NPM1* lead to abnormal localization of the nucleophosmin protein in the cytoplasm [13], with maintenance of that aberrant localization required to maintain the leukemic state [14]. The *CEBPA* gene encodes for the transcription factor CCAAT/enhancer binding protein *alpha* (CEBPA), and the gene function is crucial for granulocytic differentiation [15]; only double, and not single, *CEBPA* gene mutations are included in the WHO$^{2016/207}$-defined AML-bi*CEBPA*^{mut} entity [10,11].

The objective of this single-institution study was to retrospectively classify consecutive, newly diagnosed cases of AML by the WHO$^{2016/2017}$ criteria with integration of all clinicopathologic and genetic features available at diagnosis; to identify, as a pathologist, the new molecularly defined categories of AML-*NPM1*^{mut} and AML-bi*CEBPA*^{mut}; and to assess the clinical application of the WHO$^{2016/2017}$ classification for the diagnosis and classification of these entities among AML cases diagnosed and treated at a tertiary care medical center, which primarily served the patient population for the surrounding geographical region (with *tertiary care* referring to the level of care available for hematologic oncology patients at the medical center).

2. Materials and methods

2.1. Study design and case selection

The study was approved by the Institutional Review Board (IRB) of Pennsylvania State University Hershey Medical Center (Study #00003377). Consecutive cases of newly diagnosed AML in adults (age ≥18 years) were retrospectively identified by a search of the database in the Department of Pathology, with archived materials for cases during the 4-year period prior to October 2015 retrieved for review. All cases were previously diagnosed by experienced board-certified hematopathologists, with clinical information available in the electronic medical records. Cases were classified as secondary AML if there was prior history of cytotoxic therapy or antecedent MDS or MDS/MPN, and as de novo if no such history or preceding cause was identified. Cases were included if archived pathologic glass slides and reports for the necessary diagnostic components (including cytogenetics) were available for review. Cases with an initial diagnosis of AML but with subsequent confirmation of any other antecedent myeloid neoplasm except MDS or MDS/MPN were excluded.

2.2. Review of clinical, pathologic, and genetic data for all cases

One hundred and forty-three newly diagnosed, retrospectively identified, consecutive adult AML cases were included. For all cases, the archived clinical data were reviewed from electronic medical records, including history of prior cytotoxic therapy, prior myeloid neoplasm, any genetic disorder, social and occupational history, family history, clinical presenting features including blood counts at diagnosis (hemoglobin values, total and absolute leucocyte counts, platelet counts), and clinical follow-up, including the follow-up time from diagnosis, and patient status (alive or dead) at last follow-up.

In all cases, the archived pathologic materials reviewed were from pretherapy diagnostic specimens including the BM biopsies and PB processed using standard methods. In each case, the diagnosis of AML was confirmed by morphologic review of Wright Giemsa (WG)–stained PB and BM aspirate smears, and hematoxylin-eosin (H&E)–stained trephine core biopsy sections, in conjunction with confirming the myeloid lineage of the leukemic cells by review of prior flow cytometric immunophenotypic (FCI) findings. Blast counts by visual inspection were 200 cell counts in PB smears and 500 cell counts in BM aspirate smears; multilineage dysplasia was noted if ≥2 hematopoietic lineages included ≥50% cells with dysplastic features, which were defined in the granulocytic, erythroid, and megakaryocytic lineages as described by WHO criteria [6].

FCI analyses were previously performed using BM aspirate or PB specimens on a BD FACSCanto 1 flow cytometer (BD BioSciences, San Jose, CA) using a panel of antibodies for lymphoid and myeloid lineages including the surface antigens CD13, CD33, CD34, and CD45. Positivity or negativity of surface antigens on leukemic blasts was determined by examining the surface antigen expression on blasts in comparison with isotype-matched negative controls. Any case with an initial differential diagnosis including AML but with a final diagnosis of a mixed-phenotype acute leukemia was excluded [6,11].

In all cases, cytogenetic analyses including karyotype and fluorescence in situ hybridization (FISH) studies were processed, analyzed, and reported from pretherapy diagnostic BM aspirate samples by extramural Clinical Laboratory Improvement Amendments (www.cms.gov) (CLIA) CLIA-certified reference diagnostic laboratories (performed at Mayo Clinic Laboratories, Rochester, MN). The FISH studies usually included an AML FISH panel to confirm or exclude specific recurrent chromosomal translocations/inversions in the AML-RGA group, unless specimen was unavailable for cytogenetic study. The cytogenetic findings were reported by the reference laboratories using the International System for Human Cytogenetic Nomenclature. For each case in the study, the integrated karyotypic and FISH results were recorded as (1) normal cytogenetics, (2) abnormal cytogenetics, and (3) the presence of a complex karyotype comprised of ≥3 clonal abnormalities.

Molecular genetic analyses were previously performed in a subset of cases; all molecular assays were also performed, analyzed, and reported in all cases by extramural CLIA-certified diagnostic reference laboratories from pretherapy diagnostic BM aspirate or PB specimens sent to those laboratories. The results were determined predominantly from qualitative single-gene assays including fragment length polymerase chain reaction analysis for mutations in exon 12 of *NPM1*, FMS-like tyrosine kinase 3 (*FLT3)* internal tandem duplication (*FLT3*-ITD) mutations in the coding region for the intracellular juxtamembrane domain of *FLT3* and for *FLT3* tyrosine kinase domain (*FLT3*-TKD) mutations, and polymerase chain reaction amplification followed by bidirectional Sanger sequencing for mutations in the entire *CEBPA* coding sequence. Results of molecular tests were recorded as positive or negative for mutations in the respective examined genes, including the presence or absence of biallelic (double) *CEBPA* mutations.

2.3. Excluded cases (n = 6)

These included 2 cases with AML presentation but antecedent *JAK2*-mutated, *BCR-ABL*–negative MPN; 1 case of a mixed-phenotype acute leukemia, B/myeloid by FCI and the t(11;19)(q23;p13.1) translocation with *MLL* rearranged by FISH [6,11]; and 2 cases of myeloid/lymphoid neoplasms with AML presentation but with a chromosomal translocation t(4;6)(q12;p25) in one case and t(4;12)(q12;p13) in the other case, with *CHIC2* gene abnormalities identified at the

4q12 locus in both cases by FISH studies [11]. One possible case of an AML with the t(15;17) translocation was excluded because of the absence of clinical history (whether prior cytotoxic therapy was given or not) and the absence of cytogenetics reports.

2.4. Classification by WHO^{2008} criteria

For each case, the pathologic, immunophenotypic, and genetic findings were integrated with clinical history including after excluding Down syndrome, if prior cytotoxic therapy or prior MDS or MDS/MPN was present [6]. If there was history of prior cytotoxic therapy, the classification was t-AML. The presence of myeloblasts ≥20% in PB or BM was required for AML diagnosis in all except the AML-RGA cases with an unequivocal cytogenetic diagnosis of t(15;17), or t(8;21), or inv(16) or t(16;16). After excluding AML-RGA and t-AML, cases were classified as AML-MRC by WHO^{2008} if either or any combination of the following 3 criteria was present: (1) antecedent MDS or MDS/MPN; (2) dysplasia in ≥50% cells in ≥2 lineages by morphologic review of WG-stained marrow aspirate and peripheral blood smears, Prussian blue (iron)–stained marrow aspirate smears and sections, and H&E-stained core biopsy sections; or (3) cytogenetic abnormalities were present sufficient to diagnose AML-MRC with ≥20% PB or BM myeloblasts [6,7]. Dysplastic features in the granulocytic elements primarily included hyposegmented or hypersegmented nuclei and hypogranular cytoplasm in maturing granulocytes; dysplastic features in erythroid precursors primarily included irregular nuclear contours or nuclear budding (instead of round nuclear contours), nuclear fragmentation or karyorrhexis, or multiple nuclear lobes, megaloblastoid features, and the presence of ringed sideroblasts in iron stains; dysplasia in megakaryocytes primarily included small forms (micromegakaryocytes) or forms with hypolobated or disjointed nuclear lobes. After classifying in the definitive categories, the cases were classified as AML-NOS, with further precise FAB subclassification precluded by the absence of cytochemically stained smears.

2.5. Classification by WHO$^{2016/2017}$ criteria

The cases were reclassified to identify AML-*NPM1*^{mut} and AML-bi*CEBPA*^{mut} cases by WHO$^{2016/2017}$ criteria [11], as follows: (1) if there was antecedent MDS or if cytogenetics showed specific MDS-related abnormalities (in Table 8.04, page 151) [11], excluding del(9q) as defined, classified as AML-MRC; (2) if no antecedent MDS and no specific MDS-related cytogenetic abnormalities and no prior cytotoxic therapy, and no AML-RGA–specific chromosomal abnormality, and if positive for *NPM1* mutation, classified as AML-*NPM1*^{mut} or, with the same exclusions as for AML-*NPM1*^{mut}, if instead positive for biallelic *CEBPA* mutations, classified as AML-bi*CEBPA*^{mut}; (3) if a mutation of *NPM1* or a monoallelic *CEBPA* mutation was present with antecedent MDS or with the presence of any specific MDS-related cytogenetic abnormalities (in Table 8.04, page 151) [11], and not including del(9q), classified as AML-MRC.

For the AML-*NPM1*^{mut}, AML-bi*CEBPA*^{mut}, and remaining AML-NOS cases after reclassification, absolute monocytosis was noted with absolute monocyte counts ≥1.0 × 10^9/L (reference 0-1.0 × 10^9/L), BM cellularity determined by morphologic review of H&E-stained core biopsy sections, and the presence of monocytic features in the leukemic (blast) cells by review of WG-stained smears, including promonocytes and monoblasts in blast counts.

2.6. Statistical comparison for selected covariables among AML-*NPM1*^{mut} cases

The Fisher exact test with 2-way contingency tables was used for categorical variables, and the Mann-Whitney test was used for continuous variables [16]; $P < .05$ was considered significant.

3. Results

3.1. Distribution of cases in the entire cohort by WHO^{2008} and reclassified cases by WHO$^{2016/2017}$ criteria

Table 1 provides a distribution of all 143 cases with overall characteristics including patient demographics, presenting hematologic counts and cytogenetics, and clinical follow-up in the major AML categories by WHO^{2008} and the reclassified WHO$^{2016/2017}$ criteria. Overall, anemia was present in all (100%), with thrombocytopenia in 91% cases, high white blood cell (WBC) counts in almost half of the cohort, and decreased WBC in 36% but with absolute neutrophil counts (ANC) <2 × 10^9/L in 64% cases. The AML-MRC category comprised the highest percentage with leukopenia with ANC <2 × 10^9/L in 76% cases. In contrast, the WHO^{2008} AML-NOS cohort included only 20% with leukopenia but with decreased ANC in 62% cases.

Among the WHO^{2008}-classified 54 AML-MRC cases, *NPM1* mutations were present in 5/31 cases examined for *NPM1* mutations. Of those 5 cases, only 1 was classified as an AML-MRC by WHO^{2008} criteria based only on the single criterion of dysplasia in ≥50% cells in ≥2 lineages [6], in the absence of either of the 2 criteria of antecedent MDS or any of the WHO$^{2016/2017}$-defined, MDS-specific cytogenetic abnormalities [11], either or both of which were present as the diagnostic criteria for AML-MRC in the remaining 4/5 cases with a positive *NPM1* mutation. That single case, with trilineage dysplasia in a young female with pancytopenia and normal cytogenetics, was classified as an AML-*NPM1*^{mut} due to the presence of an *NPM1* mutation in the absence of any

Table 1 Characteristics of all 143 AML cases classified by 2008 WHO and of the new AML entities after reclassification by 2016/2017 WHO criteria

WHO category of AML	Demographics			Hematologic laboratory values for blood counts at diagnosis					Results of cytogenetic analyses, [a] as normal or abnormal, including if complex (≥3 abn)			Clinical follow-up time from diagnosis, and if patients alive or dead at last follow-up		
	n (%)	Age, [b] y, median (range)	M:F, n (higher %)	↓HgB n (%) cases	↑Total WBC count n (%)	↓Total WBC count n (%)	↓ ANC <2.0 × 10^9/L, n (%)	↓Plt count, n (%)	Normal, n (%) cases	Abn, n (%) cases	≥3 Abn, n (%)	Time, median (range), in mo	Alive, n (%)	Dead, n (%)/NA n (%)
2008 WHO														
AML-RGA [c]	21 (15%)	54 (18-82)	11:10 (52.3 males)	21 (100%)	10/21 (48%)	5/21 (24%)	9/21 (43%)	18/21 (86%)	0 (0%)	21/21	0 (0%)	12 (0.1-33)	13 (62%)	8 (38%)
AML-MRC [d]	54 (38%)	65 (32-84)	31:23 (57.4 males)	54 (100%)	14/54 (26%)	33/54 (61%)	41/54 (76%)	47/54 (87%)	13/54 (24%)	39/54 (72%); 2 NA	18/54 (33%)	9 (0.03-33)	15 (27%)	38 (70%)/ 1 (2%)
t-AML [e]	3 (2%)	66 (32-84)	3:0 (100 males)	3 (100%)	3/3 (100%)	0 (0%)	1/3 (33%)	3/3 (100%)	0 (0%)	3/3 (100%)	0 (0%)	4 (0.1-7)	1 (33%)	2 (66%)
AML-NOS [f]	65 (45%)	61 (19-89)	34:31 (52.3 males)	65 (100%)	41/65 (63%)	13/65 (20%)	40/65 (62%)	61/65 (94%); 1 NA	53/65 (82%)	11/65 (17%); 1 NA	0 (0%)	10 (0.06-45)	27 (42%)	35 (54%)/ 3 (4%)
Total cases by 2008 WHO	143 (100%)	62 (18-89)	79:64 (55.2 males)	143 (100%)	68/143 (48%)	51/143 (36%)	91/143 (64%)	129/143 (90%); 1 NA	66/143 (46%)	74/143 (52%); 3 NA	18/ 143 (13%)	9 (0.03-45)	56 (39%)	83 (58%)/ 4 (3%)
2016/2017 WHO														
AML-*NPM1*^{mut}	22 (15.4%)	60.5 (23-84)	10:12 (54.5 females)	22 (100%)	17/22 (77%)	1/22 (4%)	11/22 (50%)	20/22 [g] (91%)	20/22 (91%)	2/22 (9%)	0 (0%)	13 (0.3-32)	12 (55%)	8 (41%)/ 2(9%)
AML-bi*CEBPA*^{mut}	2 (1.4%)	51.5 (29-74)	1:1 (50 females)	2 (100%)	1/2 [h] (50%)	1/2 [h] (50%)	2/2 [h] (100%)	1/2 [h] (100%)	2/2 (100%)	0 (0%)	0 (0%)	14 (7-20)	1 (50%)	1 (50%)
Combined cases AML with gene mutations	24 (16.8%)	60.5 (23-84)	11:13 (54.1 females)	24 (100%)	18/24 (75%)	2/24 (8%)	13/24 (54%)	21/24 (88%)	22/24 (92%)	2/24 (8%)	0 (0%)	13 (0.3-32)	13/24 (54%)	9(38%)/ 2(8%)

Abbreviations: M:F, male-female ratio; ↓HgB, decreased hemoglobin <13.8 g/dL in men (reference range 13.8-17.2 g/dL) or <12.1 g/dL in women (reference range 12.1-15.1 g/dL); ↑WBC, increased total white cell count >10.0 × 10^9/L (reference range, 4.5-10.0 × 10^9/L); ↓WBC, decreased total white cell count <4.5 × 10^9/L; ↓ANC, absolute neutrophil count, if <2 × 10^9/L (reference range 2.0-7.7 × 10^9/L); ↓Plt, decreased platelet count <150 × 10^9/L(reference range 150-450 × 10^9/L); abn, abnormal; ≥3 Abn, complex karyotype, with ≥3 clonal abnormalities, as per cytogenetic nomenclature; mo, months; NA, value or information not available.

[a] Cytogenetics results after integrating karyotype and FISH studies.

[b] Patient age at AML diagnosis.

[c] AML-RGA included only chromosomal translocations/inversions, with n cases, all de novo, as follows: t(8;21)(q22;q22); *RUNX1-RUNX1T1* (n = 4); inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* (n = 5); t(15;17)(q22;q12); *PML-RARA* (n = 6); t(9;11)(p22;q23); *MLLT3-MLL* (n = 4); t(6;9)(p23;q34); *DEK-NUP214* (n = 1); inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1* (n = 1); these cases remained the same with 2016/2017 WHO.

[d] AML-MRC included 10 cases with preceding myelodysplastic syndrome; 1 case was reclassified by 2016/2017 WHO as AML-*NPM1*^{mut}.

[e] A history of prior cytotoxic therapy was present in only these 3/143 cases; these cases remained the same with 2016/2017 WHO.

[f] AML-NOS, all de novo cases, with 23 cases reclassified by 2016/2017 WHO as 21 AML-*NPM1*^{mut} and 2 AML-bi*CEBPA*^{mut}.

[g] Both AML-*NPM1*^{mut} cases with normal platelet counts had anemia and overt leukemia in PB, in conjunction with high WBC in one and a nonelevated WBC (close to upper normal limit) but with ANC <1.5 × 10^9/L in the other case; the only case with leukopenia was reclassified from WHO^{2008} AML-MRC.

[h] Thrombocytopenia with WBC >100 × 10^9/L due to >90% PB blasts and absolute monocytosis was present in one AML-bi*CEBPA*^{mut} case, whereas leukopenia due to neutropenia with >30% PB and 25% BM blasts were present in the other case with the platelet count within normal limits.

Table 2 Characteristics of 22 AML-*NPM1*^{mut} classified by WHO$^{2016/2017}$ criteria and of the 42 remaining AML-NOS cases

Characteristics	AML with mutations in *NPM1* by WHO$^{2016/2017}$ classification				Remaining AML-NOS
	Total n = 22	Male, n = 10	Female, n = 12	Family history of leukemia, n = 4 [a]	Total n = 42
Median age, [b] y (range)	60.5 (23-84)	62 (42-73)	49 (23-84)	68 (49-84)	61 (19-89)
M:F, n	10: 12	Not applicable	Not applicable	1:3	23: 19
Median hemoglobin value, g/dL (range)	8.6 (5.6-12.9)	8.5 (7.3-12.9)	8.6 (5.8-11.9)	9.2 (8.5-12.9)	8.6 (5.5-11.2)
Median platelet count, × 10^9/L (range)	55 (11-320)	55 (16-320)	50.5 (11-171)	33 (11-59)	55 (5-135); 1 NA
Median WBC, ×10^9/L, (range)	30.1 (2.58-241.84)	22.75 (7.9-241.84)	30.1 (2.58-137.78)	30.30 (5.8-113)	18 (0.3-194)
Median ANC, ×10^9/L (range)	1.96 (0-29.79); 1 NA (in high WBC)	2.34 (0.16-13.54)	1.84 (0-29.79); 1 NA	6.37 (0.97-10.47); 1 NA	1.01 (0-50.62); 3 NA (included 2 with WBC ≤0.9) [c]
Median absolute monocyte count, ×10^9/L	1.65 (0-49.34); 3 NA (all with high total WBC)	3.46 (0-49.34); 1 NA	1.5 (0-14.72); 2 NA	0.59 (0-0.97); 1 NA	0.50 (0-26.67); 5 NA (included 4 with high WBC) [d]
Median PB blasts, % (range)	41 (2-98); 1 NA	41.5 (6-77)	24 (2-98); 1 NA	61 (2-94)	39 (0-94)
Median BM blasts, % (range)	66 (17-97)	63.5 (17-90)	66 (33-97)	45.5 (36-84)	66.5 (20-95)
Median BM core biopsy cellularity, % (range) [e]	95 (70-100); 1 NA	95 (70-100)	95 (70->95); 1 NA	95 (90 to >95)	90 (30 to >95)
Normal cytogenetics, n (%)	20 (91)	8 (80)	12 (100)	3 (75)	32 (76); 1 NA
Abnormal cytogenetics, n (%)	2 (9) [f]	2 (20)	0 (0%)	1 (25)	9 (21)
CD34^{neg} myeloid blasts by FCI, n (%)	17 (77); 1 NA	8 (80)	9 (75); 1 NA	4 (100)	2 (5); 16 NA
CD34^{pos} myeloid blasts by FCI, n (%)	4 (18); 1 NA	2 (20)	2 (17)	0 (0)	24 (57)
FLT3-ITD^{pos}, n (%)	9 (41)	3 (30)	6 (50)	2 (50)	19 (45)
FLT3-ITD^{neg} *FLT3*-TKD^{pos}, n (%)	5 (23)	4 (40)	1 (8)	0 (0)	2 (5)
Both *FLT3*-ITD^{neg} and *FLT3*-TKD^{neg}, n (%)	8 (36)	3 (30)	5 (42)	2 (50)	15 (36); 6 NA

Abbreviations: CD34^{pos}, CD34 positive; CD34^{neg}, CD34 negative; *FLT3*-ITD^{pos}, positive for *FLT3* internal tandem duplication mutation; *FLT3*-ITD^{neg} *FLT3*-TKD^{pos}, negative for *FLT3*-ITD and positive for *FLT3* tyrosine kinase domain mutation; *FLT3*-TKD^{pos}, positive for *FLT3*-TKD.

[a] Three (75%) of 4 with nonsmoking clinical history, with history of leukemia in a first-degree female relative in all 3 (100%)/3 with nonsmoking history; among all 22 AML-*NPM1*^{mut}, 8/22 with nonsmoking history and 11 (50%) with history of smoking, including 4/11 with history of having quit smoking at least 14 years prior to AML diagnosis in all 4 (individually, 14, 20, 30, and at least 30 years prior).

[b] Patient age at AML diagnosis.

[c] Median value provided for ANC in 41 AML-NOS including the 2 cases with WBC ≤0.9, with ANC <1.5 × 10^9/L in 24 (57%) or <2.0 × 10^9 in 25 (60%) in 41 AML-NOS cases; 1 AML-NOS case with ANC value NA in high total WBC count.

[d] Eighteen of 37 AML-NOS cases with known values had absolute monocyte counts >1.0 × 10^9/L, with median value provided for known values; 5 NA included 4 with high total WBC counts.

[e] BM cellularity % based on review of H&E-stained core biopsy sections.

[f] Trisomy 8 in both AML-*NPM1*^{mut} cases.

CEBPA mutation [11]. The remaining 23 cases reclassified by WHO$^{2016/2017}$ criteria were from the WHO^{2008} AML-NOS category, which, after the reclassification comprised 29% of total cases, correspondingly with an increase for AML-RGA to 32% of the cohort. Although the study provides an overall distribution of the major WHO-defined AML subtypes, because only a subset of cases was molecularly examined for mutations, the percentages for AML-*NPM1*^{mut} and AML-bi*CEBPA*^{mut} were the minimum comprised by these 2 categories. The median age for AML-*NPM1*^{mut} was closer to that for AML-NOS than for AML-RGA with chromosomal abnormalities. The combined reclassified AML categories with gene mutations showed female predominance, in contrast with male predominance in the AML-NOS category and in the entire AML cohort.

3.2. Characteristics of 22 AML-*NPM1*^{mut} and the remaining 42 AML-NOS cases by WHO$^{2016/2017}$ criteria

3.2.1. Characteristics of 22 AML-*NPM1*^{mut} cases classified by the WHO$^{2016/2017}$ criteria

Table 2 provides characteristics for all AML-*NPM1*^{mut} cases, including as separated by sex of the patient and by a positive family history of leukemia, shown in comparison with the remaining 42 AML-NOS cases

3.2.1.1. AML-NPM1^{mut} cases (n = 22). Interestingly, women <50 years of age comprised 7 (31.8%) of all 22 cases or 58% of all women with AML-*NPM1*^{mut}. However, the apparent difference in ages between men and women was not significant (P = .2466). Among all AML-*NPM1*^{mut}, platelet counts were $<50 \times 10^9$/L in 10 (45.5%)/22, $>50-<100 \times 10^9$/L in 6 (27%)/22, and $>100-<150 \times 10^9$/L in 4 (18%)/22, often with high WBC counts due to increased PB blasts and ANC $<1.5 \times 10^9$/L in 8 (36%)/22, including 2 with mild (ANC $>1.0-<1.5 \times 10^9$/L), 1 with moderate ($>0.5-<1.0 \times 10^9$/L), and 5 cases with severe neutropenia ($<0.5 \times 10^9$/L). PB blasts were >20% in 14 (64%) and ≤3% in 2 (9%) cases. The latter 2 cases had nonelevated WBC counts but with absolute monocytes close to 1×10^9/L in one and $>1-<2 \times 10^9$/L in the other case, suspicious for acute leukemia after PB smear examination.

BM biopsies showed cellularity ≥95% with morphologic features for leukemic cells (blasts) including promonocytes and monoblasts in at least 13 (59%) cases and nuclear membrane invaginations ("cuplike" nuclei) in at least 2 additional cases, both of which were also positive for *FLT3*-ITD. By FCI analysis, myeloblasts expressed CD13, CD33, and dim CD45 in all cases, with absent CD34 expression in 17 (77%)/21 and variably positive CD34 in 4 (18%) cases.

3.2.1.2. AML-NPM1^{mut} according to FLT3-ITD and FLT3-TKD mutation status. Table 3 shows features of AML-*NPM1*^{mut} cases according to *FLT3*-ITD and *FLT3*-TKD mutation status. Females comprised 66.6% of AML-*NPM1*^{mut} with *FLT3*-ITD^{pos} (P = .4149) and 62.5% of the *FLT3*-ITD^{neg} *FLT3*-TKD^{neg} group (not given in the table). In comparison, the entire *FLT3*-ITD^{neg} group (including the *FLT3*-TKD^{pos} cases) showed a tendency for lower median age, moderate thrombocytopenia instead of severe, and less elevated WBC counts than in the *FLT3*-ITD^{pos} group.

3.2.1.3. Presenting clinical features in AML-NPM1^{mut} cases. These were available in 19 AML-*NPM1*^{mut} cases reclassified from the WHO^{2008} AML-NOS category and included primarily symptoms of anemia and thrombocytopenia with an acute onset (few days to 3-4 months). At least 9 patients presented with fatigue or decreased exercise tolerance and were referred for abnormal hematology laboratory values, including 1 with significant weight loss. In 3 additional cases, easy bruising was the presenting feature in 2 and heavy vaginal bleeding in 2. One presented with dizziness/fall, another with shortness of breath and fever, and another with shortness of breath and symptoms attributed to leukostasis. Features of extramedullary involvement were present in at least 27% (n = 6) including bleeding/swollen gums in at least 2, radiologically reported abdominal mass in 1, diffuse lymphadenopathy in 1, and fever with neck swelling/pain due to lymphadenopathy in 2 cases.

One unique previously unreported presentation included a rapidly spreading (thoracic, abdominal, upper limbs) papular skin rash with fatigue and concern for blasts in the PB smear. Anemia and WBC $>10-<20 \times 10^9$/L in the absence of absolute neutropenia but with absolute monocytosis ($>1-<2.2 \times 10^9$/L) were present with a normal platelet count, <10% PB blasts, and >95% BM cellularity, including >60% myeloblasts with monocytic morphologic features and absent CD34 expression by FCI, normal cytogenetics, and positive *FLT3*-ITD. Relapse occurred in that case only as a cutaneous rash in the absence of BM involvement and with menorrhagia, and the clinical course led to scheduling for a bone marrow transplant at follow-up.

3.2.2. Family history of leukemia in 4 (18%) of 22 AML-*NPM1*^{mut} cases

A family history of leukemia was noted from the clinical records in 4 AML-*NPM1*^{mut} cases. The provided family history included death due to a familial leukemia at a young age in 1 and included history of familial leukemia in first-degree female relatives in 3 of those 4, with unspecified sex for the fourth case. Three additional cases had reported family histories of chronic lymphocytic leukemia (n = 1), von Willebrand disease (n = 1), and a female relative with thrombocytosis (n = 1).

As shown in Table 2, women predominated (75%) among the 4/22 AML-*NPM1*^{mut} cases with history of leukemia. Platelet counts were in the severe thrombocytopenia range ($<50 \times 10^9$/L) in 3 (75%) and with elevated WBC in all except 1 case with a nonelevated WBC and absolute monocyte count close to 1×10^9/L. PB blasts ranged from <3% to >90%, but BM biopsies allowed the overt diagnosis of leukemia with >95% marrow cellularity, with monocytic features in leukemic blasts in at least 2/4 (50%) and "cuplike" nuclear

Table 3 Characteristics of 22 AML-*NPM1*^{mut} according to *FLT3*-ITD and *FLT3*-TKD status

Characteristics	AML-*NPM1*^{mut} All n = 22	*FLT3*-ITD^{pos} n = 9	*FLT3*-ITD^{neg} All n = 13	*FLT3*-ITD^{neg} *FLT3*-TKD^{pos} n = 5
Median age,[a] y (range)	60.5 (23-84)	64 (34-84)	54 (23-73)	52 (23-62)
M/F, n	10:12	3:6	7:6	4:1
Median hemoglobin value, g/dL (range)	8.6 (5.6-12.9)	7.9 (5.8-11.9)	8.7 (7.1-12.9)	8.3 (7.3-11)
Median platelet count, $\times 10^9$/L (range)	55 (11-320)	29 (11-171)	64 (17-320)	64 (34-128)
Median WBC, $\times 10^9$/L, (range)	30.1 (2.58-241.84)	32.4 (7.9-137.8)	12.46 (2.58-53.41)	12.46 (5.53-53.41)
Median ANC, $\times 10^9$/L (range)	1.96 (0-29.79); 1 NA	5.18 (0-13.66); 1 NA	1.62 (0.15-29.79)	1.06 (0.15-8.85)
Median absolute monocyte count, $\times 10^9$/L	1.65 (0-49.34) 3 NA	2.13 (0-49.34) 2 NA	1.31 (0-20.7) 1 NA	8.61 (1.65-20.72); 1 NA
Median peripheral blood blasts, % (range)	41 (2-98) 1 NA	66 (9.4-98)	27 (2-77) 1 NA	30 (3-77)
Median BM blasts, % (range)	66 (17-97)	77.5 (44-90)	53 (17-97)	63 (17-97)
Median BM core biopsy cellularity, % (range)[b]	95 (70-100) 1 NA	>95 (70 to >95)	95 (70-100) 1 NA	95 (70 to >95)
Monocytic features in leukemic cells,[c] n (%)	13 (59)	4 (44%)	9 (69%)	4 (80%)
Normal cytogenetics, n (%)	20 (91)	8 (89)	12 (92)	5 (100)
Abnormal cytogenetics, n (%)	2 (9)	1 (11)	1 (8%)	0 (0)
CD34^{neg} myeloid blasts by FCI, n (%)	17 (77); 1 NA	8 (89)	9 (69); 1 NA	2 (40)
CD34^{pos} myeloid blasts by FCI, n (%)	4 (18)	1 (11)	3 (23)	3 (60)
Chemotherapy/BMT; not given CT, n	18 CT/4 BMT; 1 no CT; 3 NA	6 CT /2 BMT; 1 no CT; 2 NA	12 CT/2 BMT; 1 NA	5 CT/ 2 BMT
DOD or DWD, n, at median (range) LFU in mo	8 DOD or DWD, at 1.0 (0.5-11); 1 time NA	1 DOD, 3 DWD, 1 dead (cause NA), at 0.7(0.5-1.0); 1 time NA	2 DOD, 1 DWD, at 9 (1-11)	2 DOD[d]
Alive/for BMT at LFU, n	12 alive (2 NA)/2 for BMT	3 alive (1 NA)/2 for BMT	9 alive (1 NA)	3 alive, 1 after BMT

Abbreviations: AML-*NPM1*^{mut}, AML with mutated *NPM1*; *FLT3*-ITD, *FLT3* internal tandem duplication mutation; *FLT3*-TKD, *FLT3* tyrosine kinase domain mutation; *FLT3*-ITD^{pos}, positive for *FLT3*-ITD; *FLT3*-ITD^{neg}, negative for *FLT3*-ITD, 13 cases in column included 8 negative for *FLT3*-TKD and 5 positive for *FLT3*-TKD; *FLT3*-TKD^{pos}, positive for *FLT3*-TKD; BMT, bone marrow transplant; CT, chemotherapy; chemotherapy/BMT, n received BMT after CT; DOD, died of disease; DWD, died with disease; LFU, last follow-up; alive/for BMT, n alive, of which n scheduled for BMT at LFU.

[a] Age at diagnosis of AML.

[b] BM cellularity % based on H&E-stained core biopsy sections.

[c] Monocytic included myelomonocytic and monocytic.

[d] Both relapsed, including one after BMT, and DOD.

invaginations in at least 1 case, and with too few cells in the smears examined for evaluation of morphologic features in the fourth case. The myeloblasts were negative for CD34 by FCI in all 4 cases.

Notably, 3 (75%)/4 were nonsmokers by clinical history, and those 3/3 (including male and female) were positive for the clinical history of a first-degree female relative with leukemia. Presenting features included those of cytopenia in 2, symptoms attributed to leukostasis in 1, and a mass with pain attributed to leukemic infiltration in the fourth AML-*NPM1*^{mut} case with a family history of leukemia. Three of 4 were not considered to be candidates for chemotherapy and died with disease, whereas the youngest patient showed a good response to induction chemotherapy at follow-up.

3.2.3. The remaining 42 AML-NOS cases

As shown in Table 2, men predominated, comprising 54.7% of the remaining 42 AML-NOS cases. The abnormal cytogenetics included trisomy 8 (n = 2) and tetrasomy 8 (n = 2), with 3 (75%) of those 4 cases negative for *NPM1* mutations including 1 case also negative for *CEBPA* mutations; MDS-specific cytogenetic abnormalities were not present in any of the remaining 5 AML-NOS cases with abnormal cytogenetics. *NPM1* mutations were absent in 12/42 (28.5%), and *CEBPA* mutations were absent in 4/42(9.5%)

Table 4 Characteristics of total AML cohorts and of AML with mutant *NPM1* in prior studies with AML-*NPM1*^{mut} in this study

Characteristics	Falini et al [13]	Verhaak et al [17]	Dohner et al [18]	Suzuki et al [19]	Thiede et al [20]	Boissel et al [21]	Gale et al [22]	Becker et al [23]	This study
Year publication, study location	2005, Italy	2005, The Netherlands	2005, Germany	2005, Japan	2006, Germany	2005, France	2008, UK	2010, USA	2019, USA
Pathologic classification	FAB	FAB	FAB	FAB	FAB	FAB	FAB	FAB	WHO 2008 & 2016/2017
Total AML cohorts									
Total AML, n	726	275	300	257	1485	106	1425	148	143
Median age, y (range)	NA (16-60)	44 (15-78)	< 49 (16-60)	NA	< 60 (17-87)	44 (17-65)	43 (16->60) [a]	< 69 (60-83)	62 (18-89)
Total M:F, n	NA for all	135: 140	131: 169	NA	673: 812	57:49	711: 714	82: 66	79: 64
AML de novo, n (%)	591 (81.5)	275 (100)	257 (86)	257 (100)	1221 (82)	106 (100)	1307 (92)	148 (100)	130 (91)
AML^{sec}, n (%)	135 (18.5)	0 (0)	24 (8)	0 (0)	244 (16)	0 (0)	116 (8)	0 (0)	13 (9)
Total AML^{NK}, n (%)	230 (32) in 726	116 (42)	300 (100)	97 (38)	709 (48) in 1395	106	574 (40) in 1425	148 (100)	66 (46)
AML, complex karyotype, n (%)	NA	11 (4)	0 (0)	NA	185 (12.45)	0 (0)	< 139 (<9.7)	0 (0)	18/143 (12.58)
AML with *NPM1* mutations									
Total number, n (% of total AML)	208 (28.6)	95 (35)	131 + 14 = 145 (48)	64 (25) of 257	408 (27.5)	50 (47)	503 (41) in 1217	83 (56)	22 (15)
n (% of all AML^{NK})	142 (60) of 230	74 (64) of 116	145 (48) of 300	46 (47) of 97	324 (46) of 709	50 (47) of 106	340 (59) of 574	83 (56)	20 (30) of 66
n (% of all de novo AML)	208 (35) of 591	95 (35)	131 (51) of 257	64 (25) of 257	373 (31) of 1221	50 (47)	478 (37) in 1307	83 (56)	22 (17) of 130
Median age (range), y	51.8 (range NA)	Mean 47.3 ± 10.7 y	Per *FLT3* [b]	58 (15-77)	Per *FLT3* [b]	43 (20-65)	46 (range NA)	67 (60-81)	60.5 (23-84)
Total M:F, n	NA for all	40: 55	52: 93	NA	171: 237 [c]	28: 22	211: 292	43: 40	10: 12
Median Hgb, g/dL (range)	NA	NA	Per *FLT3*	NA	NA	NA	NA	9.4 (6.0-15.0)	8.6 (5.6-12.9)

Median platelet count, $\times 10^9$/L (range)	NA	NA	Per *FLT3*	NA	Per *FLT3*	NA	NA	59.5 (17-356)	55 (11-320)
Median WBC, $\times 10^9$/L, (range)	Included high WBC	42% above 20 × 10^9/L	Per *FLT3*	52.2 (1.0-372)	Per *FLT3*	69 (1-453)	35.4 median	26.2 (1.0-249.3)	30.1 (2.58-241.84)
Median PB blasts, % (range)	NA	NA	Per *FLT3*	In 10^9/L; see note [d]	NA	NA	NA	44.5 (0-97)	41 (2-98)
Median BM blasts, % (range)	NA	NA	Per *FLT3*	NA	Per *FLT3*	NA	NA	66 (11-93)	66 (17-97)
Extramedullary inv	NA	NA	25 (17)	NA	NA	NA	NA	20 (25)	6 (27)
Cytogenetics normal, n (%)	142 (85.5) of 166	74 (64)	145 (100)	46 (72) of 64	182 + 142 = 324 (79)	50 (100)	340/574 (59)	83 (100)	20 (91)
Cytogenetics abnormal, n (%)	24 (14.5) of 166	+8 in 5 (21); no complex	0 (0)	7 (11) of 64	58 (14); 4 complex [c]	0 (0)	61 (12); ≤4 complex	0 (0)	2 (9); +8 in both
CD34-negative blasts, n (%)	Present, significant[c]	NA	Present, significant [c]	NA	NA	NA	NA	NA	17 (77); 1 NA
CD34-positive blasts, n (%)	Present; 12 (7.5) /159	NA	Present	NA	NA	NA	NA	NA	4 (18)
FLT3-ITD^{pos}, n (%)	26 (53)/ 49 *FLT3* mutated;	47 (60)	59 (41)	35 (54) /64	164 (40) /408	19 (38)	208 (41)	33 (40)	9 (41) [e]
FLT3-TKD^{pos}, n (%)	specific NA	14 (44)	21 (14.8); 4 NA	4 (6) /64	39 [f]/337 (11.5)	NA	NA	10(12)	5 (22.7) [e]
FLT3-ITD^{neg}, n (%)	NA	NA	86 (59)	25 (39) *FLT3*	244 (60) [g]	NA	295 (59)	50 (60)	13 (59)
FLT3-TKD^{neg}, n (%)	NA	NA	120 (82)	wild type	298 (88.4)	NA	NA	73 (88)	17 (77.2)

Abbreviations: AML^{sec}, secondary AML; AML^{NK}, AML with normal karyotype; inv, involvement; *FLT3*-ITD^{pos}, positive for *FLT3* internal tandem duplication mutation; *FLT3*-ITD^{neg}, negative for *FLT3*-ITD; *FLT3*-TKD^{pos}, positive for *FLT3* tyrosine kinase domain mutation; *FLT3*-TKD^{neg}, negative for *FLT3*-TKD.

[a] Greater than >60 years age comprised 35 (2%) of total AML cases in that cohort [22];

[b] Per *FLT3* indicates that values were provided according to *FLT3* mutation status; those values are provided in Table 5 in comparison with this study;

[c] $P < .001$ for female predominance, low number of complex cytogenetic karyotypes, and absent CD34 expression.

[d] PB blasts [19] 29.6 (0.093-357) $\times 10^9$ /L.

[e] All *FLT3*-ITD^{pos} were *FLT3*-TKD^{neg}, and all *FLT3*-TKD^{pos} were *FLT3*-ITD^{neg}.

[f] Included [20] 8 *FLT3*-ITD^{pos}.

[g] Included [20] 173/204 *FLT3*-TKD^{neg}.

examined AML-NOS cases; therefore, the possibility of additional cases classifiable as AML-*NPM1*^{mut} or AML-bi*CEBPA*^{mut} could not be excluded. The absence of comprehensive mutational analysis precluded identification of any possible case of the provisional entity AML-*RUNX1*^{mut}. A family history of leukemia, including AML, was present in 2/42, a family history of a hematologic malignancy in 1/42, and family history of chronic lymphocytic leukemia in a fourth AML-NOS case.

Both AML-NOS cases with absent CD34 expression on leukemic myeloblasts by FCI showed normal cytogenetics and monocytic morphologic features; in 1 case with high WBC, chemotherapy could not be initiated, whereas the other (with the youngest age in this study cohort) presented with a sore throat, swollen gums and neck glands and, interestingly, a family history of leukemia (included in preceding paragraph) and was found to have abnormal blood counts. A good response to induction chemotherapy was present in the latter case with familial history of leukemia, notably also with a nonsmoking clinical history. Although overall features suggested AML-*NPM1*^{mut}, including in the latter familial case, the retrospective classification could not be confirmed without molecular tests. Notably, other AML-NOS cases, including those that were negative for *NPM1* mutations, also showed monocytic features in blasts on smears, indicating that morphologic features alone cannot be relied upon to reclassify AML-NOS as AML-*NPM1*^{mut}.

3.3. Features of both AML-bi*CEBPA*^{mut} cases

In addition to features provided in Table 1, both cases showed overt leukemia by PB smear examination, and myeloblasts positive for CD13, CD33, CD34, and dim CD45 by FCI. A family history of leukemia was present in 1 (50%)/2 AML-bi*CEBPA*^{mut} cases with age in the seventh decade. Features in that familial case included multiple leukemic cells with Auer rods and subsequent relapsed AML. The pathologic features were nonspecific in the other (sporadic) case, with morphologic dysplastic features in 10%-15% cells.

3.4. Notable findings in 2 excluded cases

A strong family history of MPN was present in 1 of 2 excluded MPNs. Furthermore, in the case with t(4;6)(q12;p25) and *CHIC2-PDGFRA* loci abnormalities, an *NPM1* mutation was present in the absence of mutated *CEBPA* and with concurrent positive *FLT3*-ITD, with a high WBC count (>75 × 10^9/L), >80% PB and BM blasts, and anemia and thrombocytopenia, similar to the presentation of AML-*NPM1*^{mut} cases in this study.

4. Discussion

An enormous amount of information is available regarding genetic mutations and pathology of de novo and secondary AML at the level of the genome, which formed the basis of the current WHO classification. Limited information is available, however, about the epidemiology and distribution of the WHO-defined categories of AML. By carefully applying the principles and criteria of the WHO classification to a cohort of consecutively diagnosed cases of AML, this study provided a distribution of AML cases classified by WHO criteria at a single institution and, furthermore, identified cases of the new molecularly defined entities of AML with gene mutations in *NPM1* and biallelic *CEBPA*, thereby validating the principles of the WHO classification for identifying cases of AML-*NPM1*^{mut} and AML-bi*CEBPA*^{mut} entities.

In the studies that described and established the prognostic significance of *NPM1* mutations in conjunction with mutations in *FLT3* and other genes, the AML cases were described using FAB criteria. A critical analysis of prior publications [13,17-23], in Table 4**,** shows that similar to previous studies, this entire cohort was comprised of a greater proportion of de novo (91%) cases, but age was ≤89 years, with the median at 62 years, in contrast with earlier studies with median age in the fourth decade [17,21,22] or with age inclusion primarily ≤60 years [13,18,22]. Men predominated, similar to cohorts that included ≥60 years [21,23] but in contrast with those with a younger age range or younger median age, which showed overall female predominance [17,18,20,23]. Secondary AML cases were also included previously [13,18,20,22], with 12.45% complex karyotypes reported in 1 large study [20], virtually identical in percentage (12.58%) for complex karyotypes in this study.

This small AML-*NPM1*^{mut} cohort showed, in similarity to previous studies, female predominance [17,18,20,22], high WBC counts [13,17-23], high percentages of PB and BM blasts [18-20,23], the presence of myelomonocytic or monocytic features in leukemic cells [13,17-23], extramedullary involvement in at least 27% including gingival hyperplasia and lymphadenopathy [18,23], CD34-negative myeloblasts [13,18], cytogenetics predominantly normal with abnormalities including trisomy 8 similar to most prior studies [13,17,18,20-23], and *FLT3*-ITD positivity in 41% very similar in percentage to previous studies [18,20,22,23], but thrombocytopenia was present in similarity [23], and in contrast with thrombocytosis [18,20], and symptoms of anemia were common presenting features. Presentation with a rapidly spreading cutaneous rash is a novel feature for AML-*NPM1*^{mut}. This study suggested that presenting features for AML-*NPM1*^{mut} can be varied, and although some clinicopathologic profiles among AML-NOS might suggest the possibility of AML-*NPM1*^{mut}, definitive classification required molecular analysis for gene mutations.

As shown in Table 5, similarities with prior studies according to *FLT3*-ITD status included female predominance in both *FLT3*-ITD^{pos} and *FLT3*-ITD^{neg} (when also *FLT3*-TKD^{neg}), elevated WBC counts and predominantly normal cytogenetics with low or absent complex karyotypes in both groups, higher WBC and percentages of PB and BM blasts,

Table 5 Characteristics of AMLs with mutant *NPM1* according to *FLT3*-ITD status in prior studies compared with this study

Characteristics	*NPM1* mutated *FLT3*-ITD^{pos}			*NPM1* mutated *FLT3*-ITD^{neg}		
	Döhner [18]	Thiede [20]	This study	Döhner [18]	Thiede [20]	This study
n	59	164	9	86	244	13
Median age, y (range)	47 (18-60)	57.5 (19-81)	64 (34-84)	49 (25-60)	60 (18-83)	54 (23-73)
Female, %	71.2%	62.2%	66.6%	59.3%	55.3%	62% if also *FLT3*-TKD^{neg} [a]
Median hemoglobin value, g/dL (range)	9.3 (5.8-14.0)	NA	7.9 (5.8-11.9)	9.0 (5.2-14.9)	NA	8.7 (7.1-12.9)
Median platelet count, $\times 10^9$/L (range)	61 (16-248)	56 (3-514)	29 (11-171)	68 (12-746)	68 (7-302)	64 (17-320)
Median WBC, $\times 10^9$/L, (range)	43.5 (0.7-345)	50 (1.1-372)	32.4 (7.9-137.8)	19.8 (0.2-328)	26.3 (0.5-380)	12.46 (2.58-53.41)
Median PB blasts, % (range)	50 (0-100)	NA	66 (9.4-98)	40 (0-99)	NA	27 (2-77); 1 NA
Median BM blasts, % (range)	85 (0-100)	75.5 (29.5-100)	77.5 (44-90)	80 (0-100)	67.3 (6-95)	53 (17-97)
Cytogenetics normal, n (%)	100%	142 (93.4)	8 (89)	100%	182 (79.1)	12 (92)
Cytogenetics abn, n (%)	0 (0)	10 (6.6)	1 (11)	0 (0)	48 (20.9)	1 (8)
Complex karyotype [b] n (%)	0 (0)	0 (0)	0 (0)	0 (0)	4	0 (0)
FLT3-TKD^{pos}, n (%)	NA	8/133 (6.0)	0 (0)	NA	31/204 (15.2)	5/22 (22.7)
De novo AML, n (%)	53/59 (89.8%)	150 (91.5)	100%	78/86 (90.6)	223 (91.4)	100%

Abbreviations: *FLT3*-ITD^{pos}, positive for *FLT3* internal tandem duplication mutation; *FLT3*-ITD^{neg}, negative for *FLT3*-ITD; *FLT3*-TKD^{neg}, negative for *FLT3* tyrosine kinase domain mutation; *FLT3*-TKD^{pos}, positive for *FLT3*-TKD.

[a] Seven of 13 *FLT3*-ITD^{neg} cases were also negative for *FLT3*-TKD; women comprised 46% of all 13 *FLT3*-ITD^{neg} cases in this study.

[b] Complex karyotype indicates ≥3 clonal abnormalities, as per cytogenetic nomenclature.

and lower platelet counts in *FLT3*-ITD^{pos} than in the *FLT3*-ITD^{neg} group [18,20]. The differences included a tendency for higher median age in *FLT3*-ITD^{pos}, lower hemoglobin values in *FLT3*-ITD^{pos}, and less elevated WBC counts in both *FLT3*-ITD^{pos} and *FLT3*-ITD^{neg} groups in this study with only de novo AML-*NPM1*^{mut} in contrast with previously included secondary cases [18,20].

Next, a review of literature indicates that the incidence and features of patients with mutations of *CEBPA* vary with the composition of the AML cohorts and whether *CEBPA* mutations were monoallelic or biallelic. Mutations in *CEBPA* were initially found only in ages <61 years, with the highest frequency in AML with FAB M2 morphology, with such features likely because the function of *CEBPA* is crucial for granulocytic differentiation [15,24,25]. Table 6 is provided after a critical analysis of prior publications for biallelic *CEBPA* mutations in AML, for comparison with this study [25-31]. The incidence of AML-bi*CEBPA*^{mut} among all cytogenetically normal AMLs was 3.8%, 4.3%, 5.8%, and 5.97% among 4 cohorts (3 from Germany, 1 from the United Kingdom) [25-27,30], 7.7% in a combined European cohort [29], and 11% in an Italian cohort [31], in comparison with 3% in this study; percentages among de novo AMLs included 4.4% in the UK study [27], 4.68% (28/598) in a Dutch cohort [28], 7% in the Italian cohort [31], and 5.3% of all (12/224) AMLs in a Swiss cohort [24], in contrast with 1.5% of de novo or 1.4% of all AMLs in this study. Women comprised 45% [26], 49.45% [29], 50% [25], 50.8% [27], and 60.57% [30] among prior AML-bi*CEBPA*^{mut}, in comparison with 50% in this study.

Interestingly, in the cohort with the youngest median age (34 years) among AML-bi*CEBPA*^{mut} [27], the 15-29–year age group comprised 36% of AML-bi*CEBPA*^{mut} cases, with incidence decreasing progressively with age, such that the ≥60-<67–year group included only 2 (3.38%)/59 cases [27]. The 2 cases in this study included both limits of the age range, including the seventh decade that was included in prior studies [25,26,30,31], with AML-bi*CEBPA*^{mut} in age 74 included in at least 2 studies [26,30]. Finally, as given in the last row in Table 6, familial leukemia was reported in only the study with combined cohorts [29] and in 1 of the 2 AML-bi*CEBPA*^{mut} cases in this study.

Furthermore, features of familial AML-bi*CEBPA*^{mut} were previously reviewed [32]. The age range for prior reported 37 familial AML cases with *CEBPA* mutations among 14 pedigrees with a positive family history was 1.75-62 years, with median age of 25 years at AML diagnosis (from Table 2 of that publication) [32]. Except for age of 74 years, all features in the familial AML-bi*CEBPA*^{mut} case in this study were consistent with familial disease, with morphology similar to prior reported FAB M2 morphology [33] and with the occurrence of relapse as is the natural course of this familial disease [34], indicating that familial AML-bi*CEBPA*^{mut} can present at a higher age than currently recognized, further suggesting that additional studies would be valuable for sporadic and familial AML with *CEBPA* mutations. A greater emphasis on family history is suggested, with genetic counseling offered for all AML-bi*CEBPA*^{mut} cases to identify familial disease.

Whether or not there was any selection bias among the AML cases in this study is difficult to ascertain; however,

Table 6 Characteristics of total AML cohorts and of AMLs with bi*CEBPA*^{mut} from prior studies in comparison with this study

Characteristics	Frohling et al [25],	Dufour et al, [26]	Green et al [27],	Taskesen et al [29],	Fasan et al [30],	Manelli et al [31],	This study
Year of publication, cohorts	2004, German	2009, German	2010, UK	2011, Dutch, Belgian, German Austrian	2014, German	2009, Italian	2019, USA.
Pathologic classification	FAB	FAB	FAB	FAB	FAB and WHO	WHO	WHO 2008 & 2016/2017
Total AML cohorts							
Total AML, n	236 AML^{NK}	467 AML^{NK}	1427	1182^{NK}	2296	251	143
Total AML, age, median (range), y	NA (16-60)	NA	43 (15-68)	NA (16-60)	68.4 (15.7-100.4)	57 (16-81)	62 (18-89)
Total M/F, n	NA	NA	699: 728	NA	1244: 1052	NA	79: 64
AML de novo, n (%)	NA	NA	1317 (92)	NA	NA	228 (91)	130 (91)
AML^{sec}, n (%)	Included; n(%) NA	NA	110 (8)	NA	NA	23 (9)	13 (9)
Total AML^{NK}, n (%)	236 (100)	467	583 (40.8)	1182 (100)	1742 (76)	117 (47)	66 (46)
AML, complex karyotype, n (%)	0 (0)	0 (0)	Adverse risk 141 (9.8)	0 (0)	Adverse risk 122 (5.3)	Adverse risk 38 (15)	18 (12.58) of 143
AML with biallelic *CEBPA* mutations							
Total number, n (% of total AML)	9 (3.8) biallelic	20 (4.3)	59 (3.85)	91 (7.7)	104 (4.5)	16 (6.4)	2/143 (1.4)
n (% of all AML^{NK})	9/236 (3.8)	20 (4.3)	34/583 (5.8)	91 (7.7) in combined cohorts	104 (5.97)	13/117 (11.1)	2/66 (3)
n (% of all de novo AMLs)	NA	19/NA (NA); 1 from MDS	58/1317 (4.4)	NA	101/NA (NA)	16/228 (7)	2/130 (1.5)
Median age, y, (range)	47 (18-80) [a]	62 (28-75)	35 (16-67)	44 (16-60)	56.3 (15.7-87.6)	48.5 (23-72)	29, 74
Total M/F, n	50% each [a]	11: 9	29: 30	46: 45	41: 63	NA	1:1
Median Hgb, g/dL (range)	9.8 (5.4-14.6) [a]	10.3 (8.6-12.5)	NA	NA	9.6 (4.0-14)	10.6 (4.1-13.4)	9.0, 10.2
Median platelet count, $\times 10^9$/L (range)	45 (8-746) [a]	40 (18-176)	NA	38 (4-265)	34.0 (5.8-183)	24 (10-193)	77, 152
Median WBC, $\times 10^9$/L, (range)	28.9 (1.1-345) [a]	10.5 (0.9-103.5)	19 (3.6-480)	28 (1.5-262)	17.1 (0.9-182)	8.1 (1.2-166.0)	2.6, 109
Median PB blasts, % (range)	62 (13-100) [a]	NA	NA	NA	NA	NA	33, 93
Median BM blasts, % (range)	80 (2-99) [a]	80 (30-95)	NA	78 (2-100)	65 (8.5-95)	80 (40-100)	25, 81
CD34^{pos} blasts	NA	NA	NA	NA	Yes	Yes	Yes
Cytogenetics normal, n (%)	100%	100%	34/59 (57.6)	1172 (100)	78 (75)	13/16 (81)	2 (100)
Cytogenetics abnormal, n (%)	0 (0)	0 (0)	13 (22); 12 unknown	0 (0)	26 (25)	1/16 (6); 2 NA	0 (0)
Other features	FAB M1, M2 most common	12/20 (60%) FAB M2	FAB M1, M2: 47/59 (80%)	Combined cohorts [c]	FAB M1, M2 95/104 (91%)	Dysplasia [b] in 5/16 (31%)	1 consistent with FAB M2
Family history AML or leukemia, n	NA	NA	NA	2 [c]	NA	NA	1 of 2 with family history leukemia

Abbreviations: bi*CEBPA*^{mut}, biallelic mutated *CEBPA*; AML^{sec}, secondary AML; AML^{NK}, AML with normal karyotype; CD34^{pos}, positive for CD34.

[a] These values were for total 36 AML cases that included both single and biallelic mutations of *CEBPA* [25].

[b] Dysplasia multilineage by WHO criteria (≥2 lineages with 50% or more dysplastic cells) [31].

[c] Taskesen et al [29] combined cohorts including a Dutch cohort possibly included in an earlier publication [28]; germline mutations of *CEBPA* were present in 5 (7%) of 71, including 4 with biallelic mutations of *CEBPA*, with 2 of those 4 (ages 25 and 28 years) positive for a family history of AML, and in the other 2/4 (ages 51, 59 years), there was no family history of AML [29].

the vast majority of patients treated at the medical center for AML were from the surrounding geographical region, in contrast with larger referral centers in the United States wherein, typically, the entire country could be included in the referral region for hematologic malignancies. The author does not have knowledge of numbers of AML patients who may have been diagnosed at other institutions in the area and who may have been referred or treated elsewhere. Nevertheless, regardless of any selection bias that may have been present, or even if there might have been minimal selection bias, certainly the true prevalence or proportion comprised by familial cases among all AML *cannot* be determined from this study. Much larger studies would be needed for that purpose, possibly encompassing all institutions within a geographical region wherever a new diagnosis of AML may have been made.

Significantly, a genetic predisposition for, or familial AML-*NPM1*^{mut} has not been reported thus far, to the best of the author's review of literature. The presence of a family history of leukemia in at least 18% (4/22) of AML-*NPM1*^{mut} in this study, including 3(75%) familial molecularly confirmed cases with nonsmoking history, requires further studies to determine if indeed there are heritable or familial or other yet unknown predisposing factors for the development of AML-*NPM1*^{mut}. Given that smoking increases the risk of AML [35] and has also been shown to increase the risk of somatic mutations including in *NPM1* [36], whether the molecular pathogenesis of familial AML-*NPM1*^{mut} might differ from that of sporadic AML-*NPM1*^{mut} and whether there may be a predominant component of genetics (intrinsic factors that may also be heritable) in familial disease versus in conjunction also with a significant contribution of external factors (smoking) in sporadic AML is intriguing and requires further studies.

Furthermore, this study raises questions related to the demographics of AML entities with gene mutations in *NPM1* and biallelic *CEBPA*. Because AML occurs predominantly in elderly age groups and in men, what are the factors that lead to predominance of these AML entities in women, including whether that predominance is present only for mutations in *NPM1* or also for *CEBPA*, and whether younger women indeed have a higher incidence of these AML entities with gene mutations?

From a practical diagnostic aspect, the WHO classification is meant to be applied worldwide in situations with very varied available resources. Consequently, an understanding of what can be achieved with various resources available worldwide is necessary, especially for a uniformly fatal disease, if untreated, such as AML. Toward that goal, this study provides proof of principle that, with careful application of the WHO classification, the principles of which are based on enormous genomic advances, even limited single-gene molecular analyses can, indeed, precisely identify AML-*NPM1*^{mut} and AML-bi*CEBPA*^{mut} cases.

The other end of the spectrum, however, includes the continuously rapid pace of genomic advances, including with prognostic and progressively increasing therapeutic significance with the advent of new therapeutic agents, including for AML, which lead to the challenging task of accurate recognition of molecularly defined entities with clinical relevance accomplished routinely by pathologists with easily understood and reproducible criteria that can be applied with cost-effective tools. Towards that goal, Table 7 shows the AML cases in this cohort as classified by WHO$^{2016/2017}$ in comparison with the subgroups identified by genomic analyses in the landmark study with clinicopathologic correlation [37]. As expected, all cases in the respective AML-RGA categories were identified in this study because those genetic abnormalities were detected by cytogenetics and, notably, with percentages for each of those entities in this small cohort less than 5%, similar to the corresponding percentages by the genomic classification [37]. Percentages for AML-*NPM1*^{mut} and AML-bi*CEBPA*^{mut} were likely lower in this study because molecular analyses were not performed in all retrospectively studied cases.

Conversely, the limitations of applying only limited molecular analyses for the classification of AML subtypes were also identified, which in this study essentially precluded further characterization of the remaining AML-NOS cases despite careful integration of all clinical, pathologic, immunophenotypic, and cytogenetic findings; importantly, even if a diagnostic classification could be suspected based on all of the above traditionally available tools, definitive confirmation required molecular genetic analysis. Consequently, as depicted in the lower part of Table 7, the AML-NOS, t-AML, and the AML-MRC categories in this study would most likely include 2 genomic subgroups in the table [37], with no factors identified to further separate prognostic categories in AML-NOS except with molecular analyses. Therefore, this study indicates that future correlative studies including with multigene molecular profiling will be required for further integration of genomic analyses into diagnostic criteria that can be applied to precisely identify all clinically relevant subtypes of AML.

Based on this study, the following insights and recommendations are provided for further refinement of the WHO$^{2016/2017}$ classification for the AML-NOS category: (1) Although morphologic evaluation skills are necessary for accurate morphology-based AML diagnosis, precise FAB subclassification within AML-NOS is not necessary for diagnostic classification or clinical management; the absence of precise FAB categorization did not affect any aspect of patient care in this study by the author who was also classically trained in morphologic including cytochemical evaluation. (2) In fact, classifying AML-NOS by morphology-based criteria leads to confusion because the features described for the FAB categories within AML-NOS overlap, as expected, with the other (specific) clinically relevant AML entities by the WHO classification [11]. Furthermore, any pathologist, particularly in a situation with limited access to molecular tests, could easily erroneously conclude that those AML-NOS subclasses are true AML entities (which they are not) and diagnose as such per current criteria. (3) Instead, emphasis needs to shift toward other integrated

Table 7 The AML cases in this study as classified by the 2016/2017 WHO criteria compared with the corresponding subgroups in the genomic classification of AML

The genomic classification of AML [37], 2016 (n = 1540)			AML cases in this study classified by the 2016/2017 WHO criteria [11]	
AML genomic subgroup [37] [a]	Class-defining mutated genes & cytogenetic abnormalities (% within subgroup)[37] [a]	% of total n [37] [a]	2016/2017 WHO classification category of AML	n (%) of cases in this study
AML with mutated *NPM1*	*NPM1* (100%)	27%	AML with mutated *NPM1*	22 (15%)
AML with inv(16) or t(16;16)	inv(16) (100%)	5%	AML with inv(16) or t(16;16)	5 (~4%)
AML with mutated biallelic *CEBPA*	*CEBPA* biallelic (100%)	4%	AML with mutated biallelic *CEBPA*	2 (1%-2%)
AML with t(15;17)	t(15;17) (100%)	4%	AML with t(15;17)	6 (~4%)
AML with t(8;21)	t(8;21) (100%)	4%	AML with t(8;21)	4 (~3%)
AML with *MLL* fusion genes	t(x;11q23) (100%)	3%	AML with *MLL* fusion genes	4 (~3%)
AML with inv(3) or t(3;3)	inv(3) (100%)	1%	AML with inv(3) or t(3;3)	1 (0.69%)
AML with t(6;9)	t(6;9) (100%)	1%	AML with t(6;9)	1 (0.69%)
AML with mutations of RNA spliceosome genes or chromatin modifier genes or transcription factor *RUNX1*	*RUNX1* (39%), MLL^{PTD} (25%), *SRSF2* (22%), *ASXL1* (17%), *STAG2* (16%)	18%	AML, not otherwise specified = 42 (29%), and AML with myelodysplasia-related changes = 53 (37%), and therapy-related AML = 3 (2%)	
AML with *TP53* mutations, chromosomal aneuploidy or both	Complex karyotype (68%), −5/5q (47%), −7/7q (44%), *TP53* (44%), −17/17p (31%), −12/12p (17%), −8/8q (16%)	13%	Total n of these 3 WHO categories in this study = 98 (68.5%)	
AML with $IDH2^{R172}$ mutations and no other class defining lesion	$IDH2^{R172}$ (100%)	1%	Unclear from this study; *IDH1/IDH2* mutations not examined in this study	

[a] Data for genomic subgroups in the left 3 columns from the referenced publication [37].

features, most importantly for clinical features, to diagnose as a pathologist the real clinicopathogenetic entities, the "drivers" for which ultimately determine the clinical features, natural disease course, prognosis, and clinical management. This study showed that careful integration of presenting clinical features with hematologic indices and pathologic examination alone with cytogenetics *can* guide toward the possible diagnosis of the molecularly defined entity of AML-$NPM1^{mut}$. (4) The high cost of AML workup including with increasing molecular genetic tests that often would *not* have contributed toward patient care in this study, even if the tests had been performed, was a constant and justifiable concern among the treating clinicians for AML. Similar to AML-$NPM1^{mut}$ and AML-bi$CEBPA^{mut}$ categories in this study that were reclassified from WHO^{2008} AML-NOS using single-gene molecular analysis, careful evaluation of integrated clinical, pathologic, and genetic features for $WHO^{2016/2017}$ AML-NOS cases in further studies could lead to eventual development of criteria (from integrated clinicopathologic profiles) for identification of clinically relevant AML entities (other than AML-$NPM1^{mut}$) using cost-effective tools. (5) For situations when only limited molecular testing may be possible, although all AML-NOS cases with morphologic monocytic features do *not* represent AML-$NPM1^{mut}$, any AML-NOS with even mild absolute monocytosis, if with morphologic monocytic features in leukemic cells or $CD34^{neg}$ leukemic myeloblasts, particularly if in a young previously healthy patient, should *not* be classified as AML-NOS until at least the presence of *NPM1* mutations is definitely excluded, especially because AML-$NPM1^{mut}$ comprised the largest single AML entity with prognostic significance [37] (6) Any AML with pathologic features suggestive of AML with maturation or FAB M2, if negative for the t(8;21) translocation, should not be classified as AML-NOS until *CEBPA* mutations are ruled out by a reliable molecular analytic method. (7) Lastly, for future direction, because AML-NOS is *not* a specific entity and is meant to include those cases which have been adequately examined, including for mutations in *NPM1* and *CEBPA*, and then found to be unclassifiable into any of the specific AML entities, it would be valuable to distinguish the true $WHO^{2016/2017}$ AML-NOS cases as defined from those AML-NOS cases wherein, for any reason, molecular or cytogenetic analysis might not have been either possible or was inadequate or inconclusive. The latter cases would currently also be classified as AML-NOS, although in reality these might represent a true specific entity that simply could not be diagnosed because of incomplete testing or incompletely

fulfilled current criteria. A different terminology for such cases (as an example, "not otherwise classified") to distinguish from the true "not otherwise specified" AML-NOS cases would flag the former "not otherwise classified" cases among AML-NOS to indicate that some required evaluation or tests were still needed or were incomplete (could be any required information or tests, including nongenetic) and thus facilitate further study and precision among the heterogenous cases in the AML-NOS category for further refinement in the WHO$^{2016/2017}$ classification.

Indeed, this study can be criticized for not providing new molecular genetic information; however, that was not the objective for this study. Accurate diagnosis and precise classification using universally available tools for criteria that can be easily understood and reproducibly applied by pathologists everywhere, as in the WHO classification, are by themselves, the major first steps necessary for translation of the tremendous progress in our understanding of AML into similar improvements in routine clinical care, which was a purpose behind this study. In that respect, the study underscores the value of integrating pathologic, immunophenotypic, and genetic findings with clinical features not only for precise diagnostic classification but also as an invaluable tool for discovery of yet unidentified aspects, or possible new entities, with the first report of familial AML-*NPM1*^{mut} disease as an example of discovery in this study. The importance of incorporating all findings into an integrated pathology report for AML and the resources that may be required for that purpose must be emphasized given the inevitable time lag from diagnosis of AML to the time when multiple genetic findings are reported.

In conclusion, this study confirms that applying the principles and criteria of the revised fourth edition of the WHO classification indeed identifies both new entities of AML with gene mutations in *NPM1* and biallelic *CEBPA* and thus validates the current principles and the immense value of the WHO classification in identifying cases comprising both of those 2 categories. The study provided a distribution of AML subtypes by the WHO classification in consecutive cases at a single institution, characterized the features of the WHO-defined AML-*NPM1*^{mut} entity, which included at least 4 (18%) previously unreported familial leukemia cases including in 3 nonsmokers in a small cohort, and provided future direction for further refinement of the WHO$^{2016/2017}$ AML-NOS category. Larger studies would be valuable to characterize the complete spectrum of the AML-bi*CEBPA*^{mut} category as defined by the WHO classification and are necessary for familial AML including for the WHO-defined entity of AML with mutations in *NPM1*.

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Author contributions

R. K. designed the study, wrote the IRB proposal as the Principal Investigator that was approved by the IRB at PSHMC, reviewed and analyzed data, and wrote the paper.

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