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Reclassification of Acute Myeloid Leukemia According to the 2016 WHO Classification

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We reviewed our leukemia database to reclassify 610 patients previously diagnosed as having acute myeloid leukemia (AML) according to the updated 2016 WHO classification. Nine patients were categorized as having myelodysplastic syndrome and myeloid neoplasms with germline predisposition. AML with recurrent genetic abnormalities accounted for 57.4% (345/601) of the patients under the 2016 WHO classification. AML with mutated *NPM1* was the most common form (16.5%), with the majority associated with monocytic differentiation (63.6%). AML with double *CEBPA* mutations accounted for 8.3% of these cases, and the majority were previously diagnosed as AML with/without maturation (78.0%). These newly classified mutations were mutually exclusive without overlapping with other forms of AML with recurrent genetic abnormalities. AML with mutated *NPM1* and AML with myelodysplasia-related changes comprised the oldest patients, whereas AML with *RUNX1-RUNX1T1* included the youngest patients. The leukocyte count was highest in AML with mutated *NPM1*, and the percentage of peripheral blood blasts was the highest in AML with double *CEBPA* mutations. Our results indicate that implementation of the 2016 WHO classification of AML would not pose major difficulties in clinical practice. Hematopathologists should review and prepare genetic tests for the new classification, according to their clinical laboratory conditions.

Key Words: 2016 WHO classification, Acute myeloid leukemia, *NPM1*, *CEBPA*

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The diagnosis of acute myeloid leukemia (AML) has evolved over the past 15 years into a disease characterization technique that is largely based on cytogenetic and molecular analysis. Since the WHO classification was first proposed, it has been established as a formal tool for the diagnosis of hematologic malignancies [1]. The 2016 WHO classification incorporated new in-

formation that has emerged since the previous 2008 WHO classification, including acceptance of previous provisional entities such as AML with mutated *NPM1* and AML with double *CEBPA* mutations as definite ones [2]. However, this classification system must be contemporary and match with the rate of accumulating evidence; thus, there has been pressure to revise the AML classification after a certain period by collecting data and assessing their relevance. From a diagnostic perspective, application of the new criteria should be preceded by an understanding of the expected changes, accompanied by a revised classification. We reclassified our AML database that was created according to the 2008 WHO classification, and estimated the changes in data distribution and position on application of the 2016 WHO classification. Particularly, we focused on the newly introduced mutations, including *NPM1* and *CEBPA*.

We reviewed 610 patients who were diagnosed as having AML and were treated at Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea between January 2014 and June 2017. This study was approved by the Institutional Review Board (IRB) of St. Mary's Hospital affiliated with The Catholic University of Korea (IRB No: KC18RESI0227). Patient characteristics according to the 2016 WHO classification are summarized in Supplemental Data Table S1. The patients included 335 males and 275 females with a median age of 50 years (range, 1–88 years). Patient bone marrow aspirates and biopsy samples were reviewed independently by three hematopathologists. The patients' medical records, including history of chemotherapy, were reviewed. Cytogenetic abnormalities were classified according to the 2016 International System for Human Cytogenetic Nomenclature guidelines [3]. Multiplex reverse transcriptase-PCR was performed to detect the presence of *RUNX1/RUNX1T1*, *CBFB/MYH11*, *PML/RARA*, *MLLT3/KMT2A*, *DEK/NUP213*, and *BCR/ABL1* using a HemaVision kit (Bio-Rad Laboratories, Hercules, CA, USA). Mutations in the *CEBPA* and *NPM1* genes were analyzed by bidirectional Sanger sequencing using primers designed through Primer3 (http://bioinfo.ut.ee/primer3/) and described based on reference to GenBank sequences (*CEBPA*, NM_004364.4; *NPM1*, NM_002520.6).

Seven (1.1%) patients were categorized as having myelodysplastic syndrome (MDS) with excess blasts, because the definition of myeloid neoplasms with erythroid predominance was modified by shifting the main criteria for calculating blast percentage from non-erythroid cells to all nucleated marrow cells. Two patients were classified as having myeloid neoplasms with germline predisposition because they harbored a germline *CEBPA* mutation. AML with recurrent genetic abnormalities accounted for 57.4% (345/601) based on the 2016 WHO classification. AML with mutated *NPM1* was the most common form, followed by AML with *RUNX1-RUNX1T1*, AML with double *CEBPA* mutations, and AML with *CBFB-MYH11* (5.3%, N=32) (Fig. 1). Among AML with myelodysplasia-related changes (MRC) patients, seven (8.6%) had a history of MDS or myelodysplastic/myeloproliferative neoplasm, and 40 (49.4%) had MDS-related cytogenetic abnormalities. Because del(9q) was removed as a defining cytogenetic abnormality for AML-MRC, 10 patients with del(9q) were removed from the AML-MRC group; six of these patients were moved to AML with double *CEBPA* mutations and one diagnosis was changed to AML with mutated *NPM1*, while the others were reclassified as AML, not otherwise specified (NOS) (Table 1).

We investigated differences in hematologic variables according to the 2016 WHO classification using a one-way analysis of variance followed by the Bonferroni post hoc test. We used SPSS 12.0.1 for Windows (SPSS Inc., Chicago, IL, USA) and considerd $P \leq 0.05$ (two-sided) statistically significant. Patient age was the highest in the groups AML with mutated *NPM1* and AML-MRC, but lowest in the group AML with *RUNX1-RUNX1T1* ($P<0.001$). Leukocyte count was higher in AML with mutated *NPM1* than in AML with *RUNX1-RUNX1T1* and AML with *PML-*

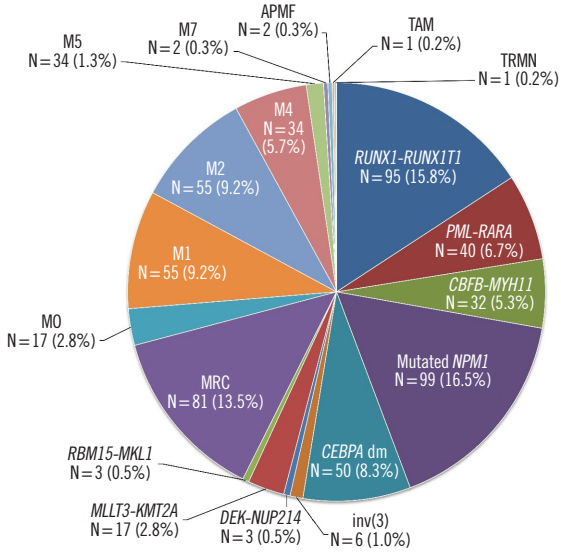

Fig. 1. Distribution of subtypes in AML patients classified according to 2016 WHO classification.
Abbreviations: AML, acute myeloid leukemia; N, number of patients (%); dm, double mutation; MRC, AML with myelodysplasia-related changes; M0, AML with minimal differentiation; M1, AML without maturation; M2, AML with maturation; M4, acute myelomonocytic leukemia; M5, acute monoblastic and monocytic leukemia; APMF, acute panmyelosis with myelofibrosis; TAM, transient abnormal myelopoiesis associated with Down syndrome; TRMN, therapy-related myeloid neoplasms.

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Table 1. Comparison of the distribution of AML patients based on the 2008 and 2016 WHO classifications

	2016 WHO	*RUNX1-RUNX1T1*	*PML-RARA*	*CBFB-MYH11*	*Mutated NPM1*	*CEBPA* dm	inv(3)	*DEK-NUP214*	*MLLT3-KMT2A*	*RBM15-MKL1*	MRC	M0	M1	M2	M4	M5	M7	APMF	TAM	TRMN	Total
2008 WHO	*RUNX1-RUNX1T1*	95																			95
	PML-RARA		40																		40
	CBFB-MYH11			32																	32
	RPN1-EVI1						6														6
	DEK-NUP214							3													3
	MLLT3-MLL								17												17
	RBM15-MKL1									3											3
	MRC				14	8					80		1	1	1						106
	M0											17									17
	M1				5	18							54								77
	M2				14	21								54							89
	M4				37	2									33						72
	M5				26											8					34
	M6A				1																1
	M6B				1	1					1										3
	M7																2				2
	APMF				1													2			3
	TAM																		1		1
	TRMN																			1	1
	Total	95	40	32	99	50	6	3	17	3	81	17	55	55	34	8	2	2	1	1	601

Abbreviations: AML, acute myeloid leukemia; dm, double mutation; MRC, AML with myelodysplasia-related changes; M0, AML with minimal differentiation; M1, AML without maturation; M2, AML with maturation; M4, acute myelomonocytic leukemia; M5, acute monoblastic and monocytic leukemia; M6A, erythroleukemia; M6B, pure erythroid leukemia; APMF, acute panmyelosis with myelofibrosis; TAM, transient abnormal myelopoiesis associated with Down syndrome; TRMN, therapy-related myeloid neoplasms.

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RARA (P=0.005). The blast percentage in peripheral blood was the highest in AML with double *CEBPA* mutations, which was greater than that in AML with *RUNX1-RUNX1T1* or *PML-RARA* (P<0.001). However, there was no significant difference in the Hb or platelet count (Supplemental Data Fig. S1).

The 2016 WHO classification newly defined *NPM1* and double *CEBPA* mutations for diagnostic classification. Therefore, we evaluated the previous diagnoses of these two new classifications. AML with mutated *NPM1* was the most common form of AML in our database (N=99, 16.5%). It was notable that all the patients in this group were over 18 years old. The majority of the patients in the AML with mutated *NPM1* group had previously been diagnosed as having AML-NOS, including acute myelomonocytic leukemia (N=37), acute monoblastic/monocytic leukemia (N=26), AML with maturation (N=14), AML without maturation (N=5), acute erythroid leukemia (N=2), and acute panmyelosis with myelofibrosis (N=1), according to the 2008 WHO classification. Others (N=14) had been diagnosed as having AML-MRC showing only dyspoiesis without MDS-related cytogenetic abnormalities. The association of monocytic differentiation and *NPM1* mutation was consistent with previous results [4, 5]. *NPM1* mutation in exon 12 was the most frequently detected mutation in cytogenetically normal AML patients. This is supported by our observation that 93 (93.9%) patients diagnosed as having AML with mutated *NPM1* showed a normal karyotype. Five of the six patients with an abnormal karyotype showed mosaicism with a normal karyotype, which can be explained as an acquired secondary event [6]. The characteristics of *NPM1* mutation (N=102) are summarized in Supplemental Data Fig. S2: type A mutation was predominant, followed by type B, type D, and type I, whereas type C, type J, type N, type R, and four individual mutations (c.863_864insTAAA, c.863_864insTTTG, c.864_876delinsCCAAGATCTCTGGCATT, c.869_873delinsCCTTGGCTC) were detected in one patient each.

Table 2. Distribution of *NPM1* and *CEBPA* mutations in 2016 WHO classified AML patients

2016 WHO classification	*NPM1* (+)			*NPM1* (-)			Total
	CEBPA dm	*CEBPA* sm	*CEBPA* (-)	*CEBPA* dm	*CEBPA* sm	*CEBPA* (-)	
RUNX1-RUNX1T1						95	95
PML-RARA						40	40
CBFB-MYH11						32	32
mutated *NPM1*		6	93				99
CEBPA dm				50			50
inv(3)						6	6
DEK-NUP214						3	3
MLLT3-KMT2A						17	17
RBM15-MKL1						3	3
MRC			3	2	6	70	81
M0						17	17
M1					5	50	55
M2					5	50	55
M4					1	33	34
M5					1	7	8
M7						2	2
APMF					1	1	2
TAM						1	1
TRMN						1	1
Total	0	6	96	52	19	428	601

Abbreviations: AML, acute myeloid leukemia; MRC, AML with myelodysplasia-related changes; M0, AML with minimal differentiation; M1, AML without maturation; M2, AML with maturation; M4, acute myelomonocytic leukemia; M5, acute monoblastic and monocytic leukemia; APMF, acute panmyelosis with myelofibrosis; TAM, transient abnormal myelopoiesis associated with Down syndrome; TRMN, therapy-related myeloid neoplasms; (+), mutation detected; dm, deouble mutation; sm, single mutation; (-), no mutation.

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There were 77 (12.8%) patients with *CEBPA* mutations in our database, including 46 (59.7%) with double *CEBPA* mutations. Four of these patients (5.2%) had one homozygous mutation, two patients had three mutations, and the other 25 (32.5%) had a monoallelic mutation. Thus, AML with double *CEBPA* mutations accounted for 8.3% of all forms of AML in the database. Previous classifications of AML-NOS (N=50), including AML with maturation (N=21), AML without maturation (N=18), acute myelomonocytic leukemia (N=2), acute erythroid leukemia (N=1), and AML-MRC (N=8) were reclassified as AML with double *CEBPA* mutations according to the 2016 WHO classification. Thirty-eight (76.0%) of these 50 patients had a normal karyotype, and six patients with del(9q) were also classified in this group, which is recognized as a cytogenetic abnormality for AML-MRC according to the 2008 WHO classification. Previous studies demonstrated that the majority of patients with del(9q) were diagnosed as having AML with or without maturation and a normal karyotype [7, 8]. In addition, these patients tended to have an earlier age of onset, higher Hb levels, and lower platelet counts than patients with other AML classifications, although the differences were not statistically significant [9]. Moreover, patients with double *CEBPA* mutations were reported to show a homogeneous gene expression profile and a favorable clinical outcome [10, 11].

Interestingly, the two new classifications of *NPM1* and double *CEBPA* mutations were mutually exclusive without overlapping with any other form of AML with recurrent genetic abnormalities (Table 2). There were five patients with *NPM1* mutations (N=3) or double *CEBPA* mutations (N=2) who were classified as having AML-MRC because of the diagnostic precedence of MDS-related cytogenetic abnormalities. AML with mutated *NPM1* became the most common AML in the 2016 WHO classification. Although approximately 80% of *NPM1* mutations identified were type A, other types cannot be ignored.

Next-generation sequencing (NGS) has recently replaced Sanger sequencing as the main method to identify common and significant mutations simultaneously, with excellent detection power [12]. The other newly included classification, AML with double *CEBPA* mutations, was the third most frequent form of AML with recurrent genetic abnormalities. Detection of *CEBPA* mutation is hampered mainly by the high GC content of this gene. Although more efficient and sensitive techniques such as NGS are emerging to address this challenge, Sanger sequencing still plays an important role in clinical *CEBPA* testing [13].

In summary, we found that implementation of the updated 2016 WHO classification of AML would not pose major difficulties in clinical practice and could help reduce the rate of ambiguous diagnoses for a more accurate prognosis. Hematopathologists should review and prepare genetic tests that are essential for the new classification, according to their clinical laboratory conditions. Future studies incorporating prognosis in the 2016 WHO classification would help provide baseline data for upgrading the classification to achieve better risk stratification and patient-oriented therapeutic strategies.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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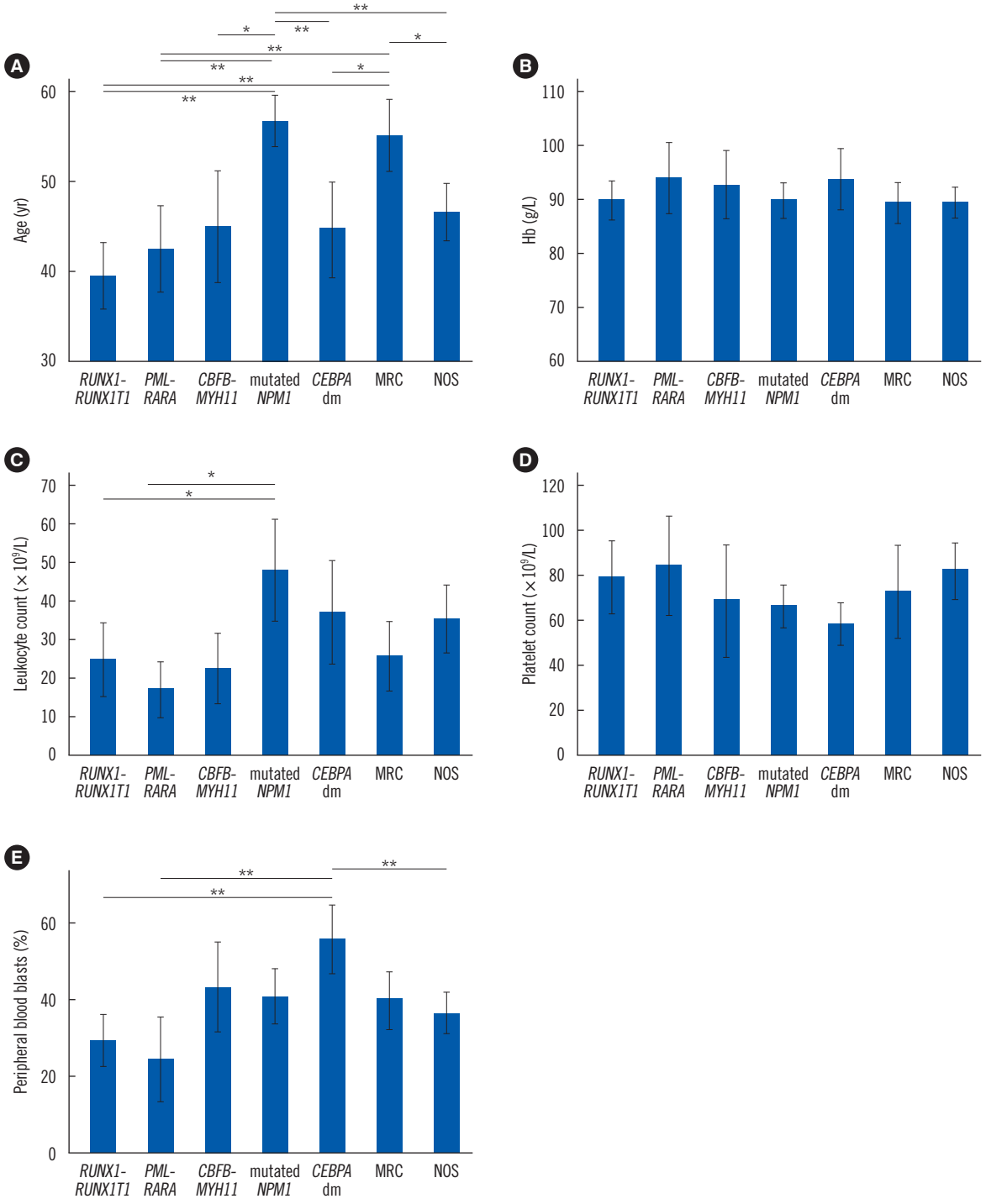
Supplemental Data Table S1. Characteristics of patients diagnosed as having AML according to the 2016 WHO classification

	N	Sex male (%)	Age, yr median (range)	Leukocytes, $\times 10^9$/L median (range)	Hb, g/L median (range)	Platelets, $\times 10^9$/L median (range)	PB blast, % median (range)
RUNX1-RUNX1T1	95	59 (62.1)	40 (5–82)	8.17 (0.58–258.80)	88 (60–143)	59 (5–546)	20 (0–98)
PML-RARA	40	19 (47.5)	45 (15–73)	4.22 (0.55–84.47)	96 (44–133)	71.5 (9–344)	1.5 (0–99)
CBFB-MYH11	32	22 (68.8)	46.5 (12–78)	16.54 (0.79–131.16)	905 (55–142)	50.5 (14–395)	45 (0–91)
Mutated *NPM1*	99	40 (40.4)	58 (18–83)	18.12 (0.71–368.68)	88 (31–135)	55 (11–281)	25 (0–98)
CEBPA dm	50	27 (54.0)	46 (4–82)	17.95 (0.84–174.92)	885 (62–152)	50.5 (5–146)	60.5 (0–99)
inv(3)	6	4 (66.7)	45 (34–71)	11.04 (2.50–72.15)	99 (68–122)	62 (42–197)	23.5 (2–71)
DEK-NUP214	3	3 (100)	48 (39–50)	18.34 (8.13–29.49)	107 (83–109)	77 (28–85)	86 (11–93)
MLLT3-KMT2A	17	6 (35.3)	54 (3–64)	23.41 (1.01–248.62)	98 (73–131)	50 (27–124)	23 (0–98)
RBM15-MKL1	3	1 (33.3)	2 (2–3)	2.22 (2.15–13.16)	86 (86–114)	50 (13–50)	0 (0–2)
MRC	81	53 (65.4)	58 (3–88)	10.43 (0.56–225.31)	88 (60–143)	53 (5–765)	37 (0–95)
M0	17	9 (52.9)	55 (3–88)	10.62 (0.89–175.27)	85 (54–96)	54 (20–127)	30 (0–98)
M1	55	33 (60.0)	48 (2–83)	10.56 (0.64–266.21)	91 (54–130)	62 (5–445)	26 (0–96)
M2	55	28 (50.9)	51 (4–81)	7.37 (0.90–161.40)	85 (47–135)	59 (6–643)	23 (0–98)
M4	34	18 (52.9)	48.5 (2–77)	18.51 (0.48–316.26)	85 (58–139)	61.5 (6–307)	31 (0–94)
M5	8	6 (75.0)	50 (13–62)	3.28 (0.78–57.13)	85 (69–109)	61 (38–212)	0 (0–34)
M7	2	0 (0)	2 (1–3)	52.41 (20.01–84.80)	108.5 (85–132)	71.5 (28–115)	15 (5–25)
APMF	2	2 (100)	46.5 (14–79)	66.11 (4.02–128.18)	112.5 (107–118)	247 (56–438)	47 (0–94)
TAM	1	0	2.00	12.73	80	85	2
TRMN	1	1	9.0	5.09	127	128	0
Total	601	331 (55.1)	50 (1–88)	11.61 (0.48–368.68)	89 (31–152)	56 (5–765)	27 (0–99)
P		0.023	<0.001	0.008	0.140	0.277	<0.001

Abbreviations: AML, acute myeloid leukemia; dm, double mutation; MRC, AML with myelodysplasia-related changes; M0, AML with minimal differentiation; M1, AML without maturation; M2, AML with maturation; M4, acute myelomonocytic leukemia; M5, acute monoblastic and monocytic leukemia; APMF, acute panmyelosis with myelofibrosis; TAM, transient abnormal myelopoiesis associated with Down syndrome; TRMN, therapy-related myeloid neoplasms; PB, peripheral blood.



Supplemental Data Figure S1. Comparison of age (A) and hematologic results, including Hb, platelet and leukocyte count (B-D), and blasts in peripheral blood (E) in AML patients, according to the 2016 WHO classification.
Data are presented as mean and SE.
*P<0.05; **P<0.01.
Abbreviations: AML, acute myeloid leukemia; dm, double mutation; MRC, AML with myelodysplasia-related changes; NOS, not otherwise specified.

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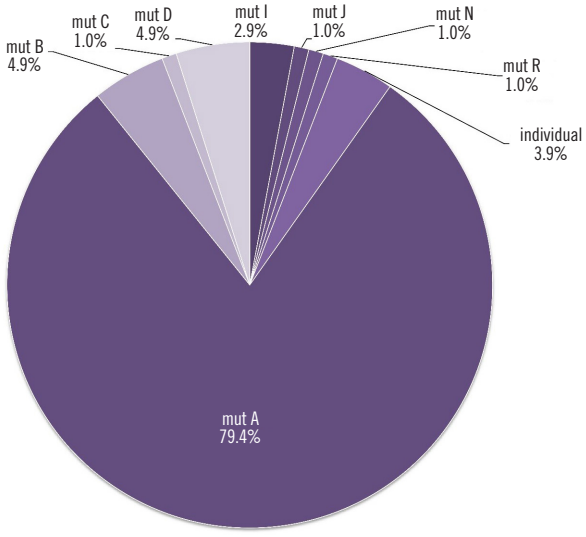

Supplemental Data Figure S2. Frequencies of *NPM1* mutation types.