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Comparison of mutational profiles and clinical outcomes in patients with acute myeloid leukemia with mutated *RUNX1* versus acute myeloid leukemia with myelodysplasia-related changes with mutated *RUNX1*

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Abstract

Studies comparing the prognostic role of *RUNX1* mutations (*RUNX1^{mut}*) in acute myeloid leukemia (AML) and acute myeloid leukemia-with myelodysplasia-related changes (AML-MRC) are limited. Our study examines the genetic profile of 118 *RUNX1^{mut}* AML patients including 57 AML with *RUNX1^{mut}* and 61 AML-MRC with *RUNX1^{mut}* and 100 AML, NOS patients with wild type *RUNX1* (*RUNX1^{wt}*). Results revealed that AML-MRC patients with *RUNX1^{mut}* had shorter median overall survival (OS) (11 ± 3.3 months) when compared to AML with *RUNX1^{mut}* (19 ± 7.1 months) and AML, NOS with *RUNX1^{wt}* (not reached) ($p = .001$). The most common concurrent mutations observed in AML-MRC with *RUNX1^{mut}* patients were *DNMT3A*, *SRSF2*, *ASXL1*, and *IDH2* while in AML with *RUNX1^{mut}* patients were *ASXL1*, *SRSF2*, *TET2*, *IDH2*, and *DNMT3A*. *ASXL1* and *TET2* mutations appeared to adversely affect OS in AML-MRC, but not in AML with *RUNX1^{mut}*. Concurrent *RUNX1/DNMT3A* mutations, in contrast had negative impact on OS in AML with *RUNX1^{mut}*, but not in AML-MRC with *RUNX1^{mut}*.

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Keywords

RUNX1; AML; AML-MRC; NGS

Introduction

Acute myeloid leukemia (AML) is a clonal proliferation of immature myeloid precursors with a variety of clinical and immunophenotypic presentations depending on the inherited and acquired genetic aberrations [1,2]. AML is an aggressive disease with a 5-year overall survival (OS) rate of 27.4% according to the National Cancer Institute, Surveillance, Epidemiology, and End Results Program (SEER) [3]. Emerging evidence shows that the clinical prognosis of AML largely depends on several key factors including the patient's age, coexisting comorbidities, and the precise disease sub-classification based on morphology, cytogenetics, and molecular signatures as identified by the World Health Organization (WHO) [1,4,5]. In addition to recurrent chromosomal aberrations, somatic mutations in *RUNX1*, *NPM1*, *FLT3*, *TP53*, *KMT2A/MLL*, and *CEBPA* genes are critical in risk stratification of AML patients [2,6].

As one of the key transcriptional factors, *RUNX1* plays an important role in the regulation of normal hematopoietic stem cells differentiation [7]. Therefore, translocation or intragenic mutation of *RUNX1* leads to impaired hematopoiesis and ultimately leukemia [8–11]. Two large cohort studies have shown that somatic *RUNX1* mutations (*RUNX1^{mut}*) are frequently identified in myelodysplastic syndromes (MDS) and therapy-related AML (t-AML) [12,13]. Additionally, approximately 10% of patients with *de novo* AML, not otherwise specified (AML-NOS), were shown to have *RUNX1^{mut}*, particularly patients with intermediate-risk and/or non-complex cytogenetics [11,14,15]. Furthermore, AML patients with *RUNX1^{mut}* are shown to be more resistant to induction chemotherapies and associated with inferior survival outcomes when compared to AML, NOS with *RUNX1^{wt}* [1,11,14].

AML with *RUNX1^{mut}* is a new provisional entity in the 2017 WHO classification of Tumors of Hematopoietic and Lymphoid Tissue [5,16], distinguishing it from AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes (AML-MRC) and t-AML [4,16]. However, studies comparing the clinical impact of *RUNX1^{mut}* and its associated genetic landscape in AML-MRC and AML with *RUNX1^{mut}* are very limited. This is clinically important for accurate risk stratification and personalized treatment approach for AML patients. Therefore, our study aimed to investigate the clinical outcomes in AML with *RUNX1^{mut}* and AML-MRC with *RUNX1^{mut}* and identify potential cooperative mutations and cytogenetic abnormalities that may have prognostic significance in these specific subgroups of AML.

Methods and material

Patient population

This study was approved by the Institutional Review Board (IRB) and Scientific Review Committee of H. Lee Moffitt Cancer Center and Research Institute (Moffitt). Between 2012

and 2017, a total of 118 AML patients harboring *RUNX1*^{mut} were detected by next-generation sequencing (NGS) using the Illumina Trusight myeloid sequencing panel (54 genes). The patients with *RUNX1*^{mut} were further categorized as AML with *RUNX1*^{mut} (*n* = 57) or AML-MRC with *RUNX1*^{mut} (*n* = 61) (Table 1). A total of 100 AML, NOS patients with *RUNX1*^{wt} were included as a control group after matching for age, sex, bone marrow (BM) biopsy, and NGS test period. Clinical parameters including diagnosis, complete blood count (CBC), molecular data, and treatment regimen were collected. t-AML and AML with recurrent genetic aberrations beyond *RUNX1* (*PML-RARA*, *RUNX1/RUNX1T1*, *CBFB-MYH11*, *BCR-ABL1* etc.) were excluded. Patients with known congenital hematological malignancies were also excluded. Germline studies for *RUNX1* mutation were not performed in this study.

Conventional karyotyping/fluorescence *in situ* hybridization (FISH)

Routine cytogenetic analysis using standard trypsin-Giemsa banding technique by Laboratory Corporation of America (Burlington, NC, USA) was performed on all patients with sufficient BM aspirate specimen and reported in accordance with an International System for Human Cytogenetic Nomenclature, 2016 (ISCH 2016, Karger). FISH probe sets designed for MDS [del(5q)/-5, del(7q)/-7, 8, del(17p)/-17, and del(20q)/-20] and AML [t(8;21)/p*RUNX1*-*RUNX1T1*, t(15;17)/*PML-RARA*, and inv(16)/*CBFB-MYH11*] were conducted based on the manufacturer's instructions (Vysis, Downers Grove, IL, USA) at Moffitt Cancer Center.

NGS and mutation analysis

NGS was performed as previously described [17]. The panel used in our study encompasses a total of 54 genes including *ABL1*, *ASXL1*, *ATRX*, *BCOR*, *BCORL1*, *BRAF*, *CALR*, *CBL*, *CBLB*, *CBLC*, *CDKN2A*, *CEBPA*, *CSF3R*, *CUX1*, *DNMT3A*, *ETV6/TEL*, *EZH2*, *FBXW7*, *FLT3*, *GATA1*, *GATA2*, *GNAS*, *HRAS*, *IDH1*, *IDH2*, *IKZF1*, *JAK2*, *JAK3*, *KDM6A*, *KIT*, *KRAS*, *MLL*, *MPL*, *MYD88*, *NOTCH1*, *NPM1*, *NRAS*, *PDGRA*, *PHF6*, *PTEN*, *PTPN11*, *RAD21*, *RUNX1*, *SETBP1*, *SF3B1*, *SMC1A*, *SMC3*, *SRSF2*, *STAG2*, *TET2*, *TP53*, *U2AF1*, *WT1*, and *ZRSR2*. The filter setting for variant detection was set to identify any somatic, non-synonymous DNA mutations of single nucleotide variants (SNVs), insertions, and deletions. Fifty nanograms of DNA were extracted using the Qiagen AllPrep DNA extraction kit (Qiagen, Germantown, MD, USA) and quantitated by NanoDrop spectrophotometry (Agilent, Santa Clara, CA, USA). The samples were sequenced using an Illumina NextSeq 500 sequencer (Illumina, San Diego, CA, USA), with variant detection by the PierianDx bioinformatics pipeline. The variant filters were set at 5% variant allele frequency (VAF) for SNV and at 10% for insertions/deletions (indels). Any variants that met the threshold of the filters were automatically placed into a high confidence group. A second low-confidence group was established for specimens that did not meet the desired filter thresholds. In these cases, individual variants were manually interrogated by an integrated genomic viewer (IGV). If a mutation was clinically significant, it was then manually added to the final molecular pathology report. All *RUNX1* mutations described were categorized as levels 1–4 variants excluding known germline polymorphisms or level 5 variants. Given that all the patients developed adult-onset AML, no additional tissue was harvested to test for *RUNX1* germline mutation.

Statistical analysis

The Chi-square or Fisher exact test was used for continuous variables and Chi-squared test for categorical variables. Both tests encompassed baseline demographic data, laboratory, and BM biopsy findings and disease features. Kaplan–Meier method was used for estimated overall survival (OS) analysis and log-rank test was used for comparison of median OS. The OS was calculated from the time of AML diagnosis.

Results

Clinical characteristics of AML with *RUNX1^{mut}* and AML-MRC with *RUNX1^{mut}*

Of the 118 patients harboring *RUNX1^{mut}*, 57 patients (48.3%) were classified as AML with *RUNX1^{mut}* and the remaining 61 patients (51.7%) were categorized as AML-MRC with *RUNX1^{mut}* according to 2017 WHO classification. Patients with AML with *RUNX1^{mut}* had a median age of 67 years (range, 21–86) and were predominantly male (M:F ratio 1.85) while patients with AML-MRC with *RUNX1^{mut}* had a median age of 72 years (range, 31–92) also showing male predominance (M:F ratio 2.21). Patients with AML, NOS with *RUNX1^{wt}* had a median age of 60.9 years (range, 19–85) with a M:F ratio of 1.16. Table 1 summarizes the clinicopathologic features of these three cohorts. Among 57 patients with AML with *RUNX1^{mut}*, missense mutations constituted 38.6% *RUNX1^{mut}* followed by frameshift (36.8%) and nonsense (24.6%) mutations. Among 61 AML-MRC patients with *RUNX1^{mut}*, 45.9% had missense mutations followed by frameshift (41.0%) and nonsense (13.1%) mutations. These numbers were not statistically different between these two groups ($p > .05$). In this study, patients with AML with *RUNX1^{mut}* or AML, NOS with *RUNX1^{wt}* were more likely to receive induction chemotherapy than their AML-MRC with *RUNX1^{mut}* counterparts. Approximately 20–30% of AML patients from each cohort subsequently received allogeneic stem cell transplantation (Allo-SCT) (Table 1).

Concurrent mutations in AML with *RUNX1^{mut}* and AML-MRC with *RUNX1^{mut}*

The most frequent mutations in AML with *RUNX1^{mut}* were *DNMT3A* (33.3%), *ASXL1* (31.6%), *SRSF2* (28.1%), and *IDH2* (17.5%); while in AML-MRC with *RUNX1^{mut}* the most common mutations were *ASXL1* (37.7%), *SRSF2* (29.5%), *TET2* (26.2%), *IDH2* (18%), and *DNMT3A* (18%). Other mutations were also identified in these cohorts with VAF ranging from less than 1 to 10% (Table 1). Of note, *FLT3-ITD* were more commonly seen in the patients with AML with *RUNX1^{mut}* as compared to AML, NOS with *RUNX1^{wt}* (7/62 versus 1/100, $p = .003$).

Clinical outcomes in AML with *RUNX1^{mut}* and AML-MRC with *RUNX1^{mut}*

AML-MRC patients with *RUNX1^{mut}* demonstrated shorter median OS (18.9 ± 1.9 months) when compared to patients with AML with *RUNX1^{mut}* (25.1 ± 2.8 months) or *RUNX1^{wt}* AML (39.5 ± 4.0 months) ($p = .001$) (Figure 1). When *RUNX1^{mut}* patients were stratified by concurrent gene mutations, *TET2* appeared to adversely affect OS in AML-MRC with *RUNX1^{mut}* (*RUNX1*, 21.9 ± 3.0 months versus *RUNX1/TET2*, 9.7 ± 2.2 months, $p = .009$) (Figure 2(A)), but not in AML with *RUNX1^{mut}* (*RUNX1*, 26.3 ± 3.1 months versus *RUNX1/TET2*, 12.4 ± 3.2 months, $p = .123$) (Figure 2(B)). Concurrent *DNMT3A* mutation

also had a negative impact on OS in AML with *RUNX1^{mut}* (*RUNX1*, 29.8 ± 3.5 months versus *RUNX1/DNMT3A*, 14.7 ± 3.5 months, $p = .002$), but not in AML-MRC with *RUNX1^{mut}* (*RUNX1*, 20.7 ± 2.9 months versus *RUNX1/DNMT3A*, 12.3 ± 2.1 months, $p = .099$) (Figure 3(A,B)). Concurrent *IDH1* mutation was associated with worse clinical outcome in AML-MRC with *RUNX1^{mut}* patients (*RUNX1*, 19.5 ± 2.5 months versus *RUNX1/IDH1*, 2.6 ± 1.4 months, $p = .008$), but it did not affect OS in patients with AML with *RUNX1^{mut}* (*RUNX1*, 26.4 ± 3.1 months versus *RUNX1/IDH1*, 15.6 ± 7.8 months, $p = .168$); however, there were only seven patients with *IDH1* in AML-MRC and seven patients in AML with *RUNX1^{mut}* cohorts. The combination of *RUNX1/ASXL1* mutations did show a slightly worse statistically significant clinical outcome in AML patients with *RUNX1^{mut}* (*RUNX1*, 27.8 ± 3.6 months versus *RUNX1/ASXL1*, 19.4 ± 4.2 months, $p = .203$) (Figure 4(A)). There was a difference in clinical outcome in AML-MRC patients with *RUNX1^{mut}* (*RUNX1*, 20.3 ± 3.3 months versus *RUNX1/ASXL1*, 16.8 ± 3.5 months, $p = .51$) (Figure 4(B)), but this did not reach statistical significance. Other concurrent gene mutations (*ETV6*, *EZH2*, *FLT3*, *IDH2*, *NRAS*, *SRSF2*, *TP53*, and *U2AF1*, etc.) showed no effect on OS in AML with *RUNX1^{mut}* or AML-MRC with *RUNX1^{mut}* cohorts. Additionally, the presence of three or more gene mutations (including *RUNX1*) significantly decreased OS in patients with AML with *RUNX1^{mut}* (19.5 ± 2.9 months, versus 36.3 ± 4.2 months $p = .004$) (Figure 5(A)); while having no significant impact on OS in patients with AML-MRC with *RUNX1^{mut}* (17.8 ± 2.4 months versus 24.9 ± 7.5 months, $p = .508$) (Figure 5(B)).

Clinical impact of cytogenetic alterations in AML with *RUNX1^{mut}* and AML-MRC with *RUNX1^{mut}*

Conventional karyotyping data was retrieved in 51 of 57 patients with AML with *RUNX1^{mut}*, 60 of 61 patients with AML-MRC with *RUNX1^{mut}*, and 98 of 100 AML, NOS with *RUNX1^{wt}* patients. Patients with normal karyotype were more commonly observed in the AML regardless of *RUNX1* mutation status (AML with *RUNX1^{mut}* and AML, NOS with *RUNX1^{wt}*) when compared to AML-MRC with *RUNX1^{mut}* ($p < .01$) (Table 1). Monosomy 7 or del(7q) was more commonly identified in AML-MRC with *RUNX1^{mut}* (13%) compared to the other two groups (0.0% in AML with *RUNX1^{mut}* and 5.1% in AML, NOS with *RUNX1^{wt}*, $p = .0072$). The prevalence of trisomy 8 was similar in AML with *RUNX1^{mut}* (11.8%) and AML-MRC with *RUNX1^{mut}* (11.7%), while trisomy 13 appeared in approximately 6% of patients with AML with *RUNX1^{mut}* and in 3.3% of AML-MRC with *RUNX1^{mut}* patients.

Discussion

Studies have shown that *RUNX1^{mut}* are associated with poor prognosis in AML [5,18,19]. However, whether clinical outcome of AML-MRC with *RUNX1^{mut}* is worse than AML with *RUNX1^{mut}* is yet unknown and there has been debate regarding prognostic impact of multilineage dysplasia in AML patients with *RUNX1^{mut}*. Similar to a previous study reported by Haferlach's group [20], mild morphologic dysplasia in hematopoietic precursor did not adversely affect the clinical outcome in our patient with AML with *RUNX1^{mut}* or AML-MRC with *RUNX1^{mut}* (data not shown). However, we observed that AML-MRC with

RUNX1^{mut} is associated with inferior OS compared to AML with *RUNX1^{mut}*. This observation is clinically important since poor treatment response and inferior survival outcome associated with *RUNX1^{mut}* could be further compromised in AML-MRC patients [21,22]. Although CPX-351 was recently approved as a front-line therapy in AML-MRC patients and hypomethylating agents are commonly used in AML patients who are not eligible for the intensive chemotherapy [23–27], there is still lack of data regarding the optimal treatment in this unique group of AML-MRC with *RUNX1^{mut}* patients. Future studies are warranted to further improve survival outcomes in this poor prognostic group.

Additionally, recent studies have shown that *ASXL1*, *RAS*, *FLT3-ITD*, and *IDH1/2* mutations frequently concur in patients with AML with *RUNX1^{mut}* [21]. Also, the incidence of *ASXL1* mutation was higher in *RUNX1^{mut}* versus AML, NOS with *RUNX1^{wt}* patients with normal karyotype (36% versus 8%) [22] and Gaidzik et al. reported that in addition to aforementioned genes, *EZH2*, *SRSF2*, *SF3B1*, *STAG2*, *PHF6*, and *BCOR* mutations frequently concur in AML with *RUNX1^{mut}* patients [14]. Similar to these studies, we observed a relatively high incidence of mutations in *SRSF2*, *TET2*, *DNMT3A*, *ASXL1*, *IDH1/2*, *EZH2*, *BCOR*, *PHF6*, *STAG*, and *FLT3-ITD* genes in *RUNX1* mutant patients, either AML with *RUNX1^{mut}* or AML-MRC with *RUNX1^{mut}* group. In our study, some of these co-mutations appear prognostically important; *RUNX1/TET2* co-mutations were associated with worse OS compared to *RUNX1^{mut}* alone in AML-MRC while concurrent *RUNX1/DNMT3A* mutations were associated with worse OS in patients with AML with *RUNX1^{mut}* than AML-MRC with *RUNX1^{mut}*. Concurrent *RUNX1/ASXL1* mutations also showed negative clinical impact on OS in patients with AML with *RUNX1^{mut}* and *RUNX1/IDH2* co-mutations were associated with worse clinical outcome than *RUNX1^{mut}* alone in AML-MRC patients, but not in AML with *RUNX1^{mut}*. However, these observations require cautious interpretation because of the limited number of patients included from a single institution, requiring further validation with independent patient cohorts.

Recent studies have demonstrated that AML with *RUNX1^{mut}* has a unique cytogenetic signature [20,28]. Trisomy 13 is reportedly strongly associated with *RUNX1* mutation in AML (9%, 13/152) [20,29]. However, the presence of trisomy 13 was extremely low in our study appearing only in 5.9% and 3.3% of patients with AML with *RUNX1^{mut}* and AML-MRC with *RUNX1^{mut}*, respectively. Following normal karyotype, trisomy 8 was the most common aberration making up 11.8% of AML with *RUNX1^{mut}* and 11.7% of AML-MRC with *RUNX1^{mut}*.

Although *RUNX1* mutations have been identified in 10–33% of MDS and AML patients [18,19,30,31], the proportion of germ-line versus somatic mutations is largely unknown. Patients with germ-line *RUNX1* mutations develop early onset MDS/AML with a median age of 33 years at diagnosis whereas sporadic MDS/AML patients typically present later time in life with median age of 65–68 years [32,33]. A recent study showed that the carriers of *RUNX1* germ-line mutations are typically asymptomatic, but have a high cumulative risk of developing a clonal myeloid neoplasm by age 50, suggesting that secondary mutations are necessary to trigger MDS/AML [34,35]. The median age of patients in the groups in our study was 60–72 years old and only six patients were diagnosed before 35 years. This

suggests that the majority of patients in our cohort likely have *RUNX1* somatic mutations, but this is inconclusive given the lack of germ-line mutation analysis.

Despite the need for studies to further elucidate the association between AML-MRC and *RUNX1^{mut}* to predict poor clinical outcome, our study provides some insight to potential points of clinical interest. We have demonstrated that patients with AML-MRC with *RUNX1^{mut}* are a unique poor prognostic group and certain co-mutations and/or cytogenetics could further affect their clinical outcomes. This warrants routine investigation by a comprehensive myeloid mutation panel to have better risk stratification and identify those that may benefit from future targeted therapies.

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Figure 1. Overall survival curves for cases of AML with *RUNX1*^{mut}, AML-MRC with *RUNX1*^{mut} and AML, NOS with *RUNX1*^{wt}.

Figure 2. (A) Overall survival curves for cases of AML-MRC with concurrent *RUNX1/TET2* mutation compared to AML-MRC cases with *RUNX1* mutation alone. (B) Overall survival curves for cases of AML with *RUNX1*^{mut} with or without concurrent *TET2* mutation.

Figure 3.

(A) Overall survival curves for cases of AML with *RUNX1*^{mut} with or without concurrent *DNMT3A* mutation. (B) Overall survival curves for cases of AML-MRC with concurrent *RUNX1/DNMT3A* mutation compared to cases with *RUNX1* mutation alone.

Figure 4.

(A) Overall survival curves for cases of AML with *RUNX1*^{mut} with or without concurrent *ASXL1* mutation. (B) Overall survival curves for cases of AML-MRC with concurrent *RUNX1/ASXL1* mutation compared to cases with *RUNX1* mutation alone.

Figure 5. (A) Overall survival curves for cases of AML with *RUNX1*^{mut} with three or more mutations versus cases with fewer than three mutations. (B) Overall survival curves for cases of AML-MRC with three or more mutations versus fewer than three mutations (including *RUNX1*).

Patient demographics and frequency of genetic aberrations in patients with AML with *RUNX1^{mut}*, AML-MRC with *RUNX1^{mut}*, and AML, NOS with *RUNX1^{wt}*.

Characteristics	AML with <i>RUNX1^{mut}</i> (n = 57)	AML-MRC with <i>RUNX1^{mut}</i> (n = 61)	AML, NOS with <i>RUNX1^{wt}</i> (n = 100)	p-Value
Median age (range, years)	67 (21–86)	72 (31–92)	60.9 (19–85)	>.05
Gender (Male: Female)	1.85	2.21	1.16	>.05
CBC data (median)				
WBC, × 10 ⁹ /L (range)	4.5 (0.6–103.2)	3.8 (0.7–134.1)	7.1 (0.9–248)	N/A
ANC, × 10 ⁹ /L (range)	0.9 (0–20.7)	2.1 (0–23.8)	1.0 (0–24.9)	
Hemoglobin, g/dL (range)	8.5 (6.6–12.2)	8.7 (7.4–12.1)	9.1 (4.5–13.2)	
Platelets, × 10 ⁹ /L (range)	41 (0.5–136)	43 (2–597)	56 (8–351)	
Prior MDS, MDS/MPN, MPN	20.8 (18–95.5)	22 (18–59)	50.5 (13.2–94)	
Prior t-MDS	0 (0%)	42 (68.8%)	0 (0%)	
Cytogenetics				
Normal karyotype	(n = 51)	(n = 60)	(n = 98)	.05639
<3 abnormalities	28 (54.9%)	25 (41.7%)	60 (61.2%)	.03496*
3 abnormalities	NA	11 (18.3%)	NA	NA
–5/del(5q)	0 (0%)	4 (6.7%)	0 (0%)	N/A
–7/del(7q)	0 (0%)	8 (13.3%)	5 (5.1%)	.0072*
+8	6 (11.8%)	7 (11.7%)	3 (3.1%)	.063618
+13	3 (5.9%)	2 (3.3%)	1 (1.0%)	.676687
–17/del(17p)	0 (0%)	1 (1.7%)	1 (1.0%)	N/A
–20/del(20q)	0 (0%)	5 (8.3%)	4 (4.1%)	N/A
Somatic mutations	(n = 57)	(n = 61)	(n = 100)	
<i>ASXL1</i>	18 (31.6%)	23 (37.7%)	18 (18%)	.016166*
<i>DNMT3A</i>	19 (33.3%)	11 (18.0%)	29 (29%)	.146216
<i>IDH2</i>	10 (17.5%)	11 (18.0%)	12 (12%)	.49133
<i>SRSF2</i>	16 (28.1%)	18 (29.5%)	4 (4%)	<.00001*
<i>TET2</i>	5 (8.8%)	16 (26.2%)	20 (20%)	.048484*
<i>BCOR</i>	5 (8.8%)	2 (3.3%)	5 (5%)	.406604

Characteristics	AML with <i>RUNX1^{mut}</i> (n = 57)	AML-MRC with <i>RUNX1^{mut}</i> (n = 61)	AML, NOS with <i>RUNX1^{wt}</i> (n = 100)	p-Value
<i>BCORL</i>	4 (7.0%)	3 (4.9%)	3 (3%)	.506597
<i>CBL</i>	0 (0%)	4 (6.6%)	2 (2%)	N/A
<i>IDH1</i>	7 (12.3%)	6 (9.8%)	9 (9%)	.803816
<i>ETV6</i>	6 (10.5%)	4 (6.6%)	0 (0%)	N/A
<i>EZH2</i>	3 (5.3%)	3 (4.9%)	0 (0%)	N/A
<i>FLT3</i>	7 (12.3%)	0 (%)	1 (1%)	N/A
<i>GATA2</i>	3 (5.3%)	1 (1.6%)	1 (1%)	.21159
<i>KMT2A</i>	2 (3.5%)	1 (1.6%)	2 (2%)	.692851
<i>NRAS</i>	7 (12.3%)	4 (6.6%)	8 (8%)	.555879
<i>PHF6</i>	3 (5.3%)	4 (6.6%)	3 (3%)	.555413
<i>SF3B1</i>	0 (0%)	4 (6.6%)	0 (%)	N/A
<i>SETBP</i>	2 (3.5%)	0 (0%)	2 (2%)	N/A
<i>STAG2</i>	2 (3.5%)	2 (2.3%)	0 (0%)	N/A
<i>TP53</i>	3 (5.35)	4 (6.6%)	1 (1%)	.144823
<i>U2AF1</i>	6 (10.5%)	6 (9.83%)	9 (9%)	.950672
<i>WT1</i>	3 (5.3%)	0 (0%)	2 (2%)	N/A
<i>ZRSR2</i>	1 (1.76%)	3 (4.9%)	1 (1%)	.474143
Others ^a	1 (1.8%)	1 (1.6%) or less	1 (1%) or less	.90689
Number of mutations				
<3 mutations	17 (29.8%)	12 (19.6%)	50 (59.5%)	.000267*
3 mutations	40 (70.2%)	49 (80.3%)	34 (40.5%)	<.00001 *
No mutations	—	—	16	N/A
Treatment				
Induction chemotherapy	25 (43.9%)	10 (16.3%)	44 (44%)	.000733*
Hypomethylation agents ^b	7 (12.2%)	23 (37.7%)	11 (11%)	.000049*
Allo-SCT	15 (26.3%)	13 (21.3%)	29 (29%)	.559621
Other	10 (17.5%)	15 (24.5%)	16 (16%)	.384509

Allo-SCT: allogeneic hematopoietic stem cell transplant; ANC: absolute neutrophil count; CBC: complete blood count; MDS: myelodysplastic syndromes; MPN: myeloproliferative neoplasms; t-MDS: therapy-related MDS; WBC: white blood cells. AML: acute myeloid leukemia; AML-MRC: acute myeloid leukemia with myelodysplasia-related changes; NOS: not otherwise specified.

^aOther somatic mutations include *CSF3R*, *CEBPA*, *JAK2*, *KIT*, *ZRSR2*, *KDM6A*, *PDGFR*, *NOTCH1*, *ABL1*, *TRX*, *FBXW7*, and *RAD21*.

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Hypomethylation agents include azacitidine and decitabine.
* Indicates statistical significance.