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Poor Prognostic Implication of *ASXL1* Mutations in Korean Patients With Chronic Myelomonocytic Leukemia

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Background: Molecular genetic abnormalities are observed in over 90% of chronic myelomonocytic leukemia (CMML) cases. Recently, several studies have demonstrated the negative prognostic impact of *ASXL1* mutations in CMML patients. We evaluated the prognostic impact of *ASXL1* mutations and compared five CMML prognostic models in Korean patients with CMML.

Methods: We analyzed data from 36 of 57 patients diagnosed as having CMML from January 2000 to March 2016. *ASXL1* mutation analysis was performed by direct sequencing, and the clinical and laboratory features of patients were compared according to *ASXL1* mutation status.

Results: *ASXL1* mutations were detected in 18 patients (50%). There were no significant differences between the clinical and laboratory characteristics of *ASXL1*-mutated (*ASXL1*$^+$) CMML and *ASXL1*-nonmutated (*ASXL1*$^-$) CMML patients (all $P>0.05$). During the median follow-up of 14 months (range, 0–111 months), the overall survival (OS) of *ASXL1*$^+$ CMML patients was significantly inferior to that of *ASXL1*$^-$ CMML patients with a median survival of 11 months and 19 months, respectively (log-rank $P=0.049$). An evaluation of OS according to the prognostic models demonstrated inferior survival in patients with a higher risk category according to the Mayo molecular model (log-rank $P=0.001$); the other scoring systems did not demonstrate a significant association with survival.

Conclusions: We demonstrated that *ASXL1* mutations, occurring in half of the Korean CMML patients examined, were associated with inferior survival. *ASXL1* mutation status needs to be determined for risk stratification in CMML.

Key Words: Chronic myelomonocytic leukemia, *ASXL1*, Risk stratification, Prognostic models, Mayo molecular model, Korea

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INTRODUCTION

Chronic myelomonocytic leukemia (CMML) is a clonal hematologic neoplasm characterized by overlapping features of myelodysplastic and myeloproliferative syndrome with peripheral blood monocytosis [1, 2]. According to the 2016 WHO classification, CMML is further divided into three groups based on peripheral blood (PB) and bone marrow (BM) blast counts: CMML-0 (PB blasts <2%, BM blasts <5%), CMML-1 (PB blasts 2–4%, BM blasts 5–9%), and CMML-2 (PB blasts 5–19%, BM blasts 10–

19%) [2]. In addition, distinction according to the French-American-British (FAB) subtypes — proliferative type (white blood cell [WBC] count $\geq 13\times10^9$/L) and dysplastic type (WBC count $<13\times10^9$/L) — is also recommended [2].

Clonal cytogenetic abnormalities are seen in approximately 20–30% of CMML cases, and molecular genetic abnormalities are seen in over 90% of CMML cases [3-6]. The most commonly mutated genes in CMML involve epigenetic regulation (*TET2*, ~60%), spliceosome machinery (*SRSF2*, ~50%), chromatin/histone modulation (*ASXL1*, ~50%), and cell signaling (*RAS*, ~30%), and these genetic changes can be used as clonal evidence supporting the diagnosis of CMML, particularly in patients with a normal karyotype [2]. With regard to prognosis, to date, only frameshift or nonsense *ASXL1* mutations have been consistently reported as a poor prognostic factor, which has led to the incorporation of *ASXL1* mutations into prognostic models of CMML [7-9]. The most common *ASXL1* mutation is c.1934dupG (p.Gly646Trpfs*12), which accounts for 40–50% of *ASXL1* mutations.

Several prognostic models have been proposed for CMML: the Spanish cytogenetic risk stratification [3], the MD Anderson prognostic scoring system (MDAPS) [10], the CMML-specific prognostic scoring system (CPSS) [11], the Groupe Français des Myélodysplasies (GFM) model [8], the Mayo prognostic model [12], and the Mayo molecular model (MMM) [9]. These models never been validated for Korean patients. The objectives of this study were to evaluate the prognostic impact of *ASXL1* mutations and to compare previously reported CMML prognostic models in Korean patients with CMML.

METHODS

1. Study patients

A total of 57 patients were diagnosed as having CMML from January 2000 to March 2016 at Samsung Medical Center, Seoul, Korea. Of them, 21 patients whose samples were unavailable for genetic analysis were excluded, and the remaining 36 were included in this retrospective study. CMML was diagnosed and divided into subtypes according to the 2008 and 2016 WHO classifications [2, 13]. The clinical and laboratory information of patients, including age, sex, complete blood counts, BM blasts (%), cytogenetic study results, and treatment history was collected from electronic medical records (Table 1 and Supplemental Data Table S1). The patients were stratified into different risk categories according to the five prognostic scoring systems: the Spanish cytogenetic risk stratification, MDAPS, the GFM model, the Mayo model, and MMM. The Spanish cytogenetic risk stratification divides patients into low risk (normal karyotype or loss of Y chromosome), high risk (presence of trisomy 8, or abnormalities of chromosome 7, or a complex karyotype), and intermediate risk (all others) [3]. MDAPS stratifies patients into four risk groups based on the following risk factors: hemoglobin <12 g/dL, presence of circulating immature myeloid cells (IMC), absolute lymphocyte count $>2.5\times10^9$/L, and BM blasts >10% [10]. The GFM model stratifies patients into three risk groups based on age >65 years, WBC count $>15\times10^9$/L, anemia (<11 g/dL for males and <10 g/dL for females), platelet count $<100\times10^9$/L, and *ASXL1* mutation [8]. The Mayo model stratifies patients into three groups based on the following risk factors: absolute monocyte count (AMC) $>10\times10^9$/L, presence of circulating IMC, hemoglobin <10 g/dL, and platelet count $<100\times10^9$/L. MMM has added *ASXL1* mutation to the Mayo prognostic model components as a risk factor and stratifies patients into four groups: low (no risk factor), intermediate-1 (one risk factor), intermediate-2 (two risk factors), and high risk (≥3 risk factors) [9, 12]. The study protocol was approved by the Institutional Review Board of Samsung Medical Center, and written informed consent was obtained from all patients.

2. *ASXL1* mutation analysis

Genomic DNA was isolated from BM aspirate samples at the time of initial diagnosis using the Wizard genomic DNA purification kit (Promega, Madison, WI, USA), according to the manufacturer's instructions, and *ASXL1* mutation analysis was performed on the archived samples. Exon 14 of *ASXL1* and its flanking intronic regions were amplified by PCR using primers designed by the authors (See Supplemental Data Table S2) on a Thermal Cycler 9700 (Applied Biosystems, Foster City, CA, USA), with the following cycling conditions: 94°C for 5 minutes, followed by 32 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, and a final extension at 72°C for 7 minutes. Direct sequencing was performed using the ABI Prism 3130xl genetic analyzer (Applied Biosystems) with the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems).

3. Statistical analysis

Patient characteristics according to *ASXL1* mutation status were compared using the chi-square test or Fisher's exact test for categorical variables and the Mann-Whitney test or two sample t-test for continuous variables. Overall survival (OS) was deter-

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Table 1. Clinical and laboratory characteristics of 36 patients with CMML

Characteristics	All (N=36)	*ASXL1* mutation status		
		*ASXL1*mut$^-$ (N=18)	*ASXL1*mut$^+$ (N=18)	*P*
Age (yr)	71 (38–83)	72 (50–83)	70 (38–79)	0.776
≥65 yr, N (%)	24 (66.7)	12 (66.7)	12 (66.7)	1
Male, N (%)	26 (72.2)	12 (66.7)	14 (77.8)	0.457
Hb (g/dL)	9.0 (5.3–13.5)	9.3 (5.7–13.5)	8.9 (5.3–13.3)	0.364
WBC ($\times 10^9$/L)	16.8 (3.8–231.0)	15.2 (5.4–81.9)	37.3 (3.8–231.0)	0.129
ANC ($\times 10^9$/L)	10.0 (0.4–134.0)	8.2 (0.9–50.0)	11.0 (0.4–134.0)	0.146
AMC ($\times 10^9$/L)	3.6 (1.4–48.5)	2.6 (1.5–28.2)	5.8 (1.4–48.5)	0.411
ALC ($\times 10^9$/L)	2.8 (0.8–26.3)	2.6 (0.8–11.9)	3.0 (1.2–26.3)	0.327
Platelets ($\times 10^9$/L)	76 (23–709)	61 (23–396)	117 (34–709)	0.094
PB blast (%)	1 (0–16)	1 (0–15)	1 (0–16)	1
BM blast (%)	5 (1–19)	4 (1–19)	9 (2–18)	0.252
Presence of circulating IMC, N (%)	28 (77.8)	14 (77.8)	14 (77.8)	1
Cytogenetic study				
Normal karyotype	26 (72.2)	12 (66.7)	14 (77.8)	0.457
Abnormal karyotype	10 (27.8)	6 (33.3)	4 (22.2)	
-7 or del(7q)	2 (20.0)	0 (0)	2 (50.0)	
+8	2 (20.0)	1 (16.7)	1 (25.0)	
-Y	2 (20.0)	2 (33.3)	0 (0)	
+21	1 (10.0)	0 (0)	1 (25.0)	
Others	3 (30.0)	3 (50.0)	0 (0)	
Treatment, N/total (%)				
Decitabine or hydroxyurea	27/33 (81.8)	13/16 (81.3)	14/17 (82.4)	0.935
Supportive care	6/33 (18.2)	3/16 (18.8)	3/17 (17.6)	
Leukemic transformation, N/total (%)	12/33 (33.3)	4/17 (23.5)	8/16 (50.0)	0.114
Death, N (%)	26 (72.2)	11 (61.1)	15 (83.3)	0.137
Proliferative and dysplastic subtypes, N (%)				
Proliferative type	26 (72.2)	11 (61.1)	15 (83.3)	0.137
Dysplastic type	10 (27.8)	7 (38.9)	3 (16.7)	
2008 WHO morphological subtypes, N (%)				
CMML-1	21 (58.3)	11 (61.1)	10 (55.6)	0.735
CMML-2	15 (41.7)	7 (38.9)	8 (44.4)	
2016 WHO morphological subtypes, N (%)				
CMML-0	12 (33.3)	9 (50.0)	3 (16.7)	0.06
CMML-1	9 (25.0)	2 (11.1)	7 (38.9)	
CMML-2	15 (41.7)	7 (38.9)	8 (44.4)	

Values are reported as median (range) or number (percentage).
Abbreviations: WBC, white blood cell; ANC, absolute neutrophil count; AMC, absolute monocyte count; ALC, absolute lymphocyte count; PB, peripheral blood; BM, bone marrow; IMC, immature myeloid cell; CMML, chronic myelomonocytic leukemia.

mined from the time of initial diagnosis to death from any cause or last follow-up. Leukemic transformation-free survival (LFS) was determined from the time of initial diagnosis to leukemic transformation, death, or last follow-up. Survival analyses were performed using Kaplan-Meier plots, and differences in survival were compared using the log-rank test. Univariate and multivar-

iate analyses were performed using Cox proportional hazards regression. $P<0.05$ was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY, USA).

RESULTS

1. *ASXL1* mutations and cytogenetic abnormalities

An abnormal karyotype was found in ten (27.8%) patients. Trisomy 8 (+8), abnormalities of chromosome 7 (monosomy 7 [-7] and deletion 7q [del7q]), and loss of Y chromosome were each observed in two patients (5.6%) and a complex karyotype including abnormalities of chromosome 3, 5, 6, and 15 was found in one patient (2.8%). *ASXL1* mutations were detected in 18 patients (50%). All identified *ASXL1* mutations were either frameshift or nonsense mutations, of which four were listed in the Catalogue of Somatic Mutations in Cancer (COSMIC) database (http://cancer.sanger.ac.uk/cosmic) (Table 2). The most common mutation was c.1934dupG (p.Gly646Trpfs*12), which was found in 12 patients (66.7%). Six unique mutations were detected in the other six patients, of which three were novel: c.1766_1767insAGTA (p.Thr590Valfs*30), c.2973_2985del (p.Leu992Valfs*28), and c.3001dupA (p.Thr1001Asnfs*4). *ASXL1* mutations were detected in 14 (54%) patients with a normal karyotype and in four (40%) patients with cytogenetic abnormalities.

2. Patient characteristics and risk stratification according to *ASXL1* mutation status

Clinical and laboratory characteristics did not significantly differ between *ASXL1*-mutated (*ASXL1*$^+$) CMML and *ASXL1*-non-mutated (*ASXL1*$^-$) CMML patients (Table 1). Of the different risk stratification classifications, only the GFM prognostic risk categories were significantly different between *ASXL1*$^+$ CMML and *ASXL1*$^-$ CMML ($P=0.006$; Table 3).

Table 2. Spectrum of *ASXL1* mutations in 18 patients with chronic myelomonocytic leukemia

cDNA change	Amino acid change	Patients, N (%)	Patients with normal karyotype (N)	Mutation ID†
c.1766_1767insAGTA	p.Thr590Valfs*30	1 (5.6)	1	-
c.1773C>G	p.Tyr591*	1 (5.6)	1	COSM53200
c.1900_1922del	p.Glu635Argfs*15	1 (5.6)	0	COSM36165
c.1934dupG	p.Gly646Trpfs*12	12 (66.7)	9	COSM34210
c.2254dupG	p.Ala752Glyfs*22	1 (5.6)	1	COSM96391
c.2973_2985del	p.Leu992Valfs*28	1 (5.6)	1	-
c.3001dupA	p.Thr1001Asnfs*4	1 (5.6)	1	-

†Mutation ID was assigned based on the Catalogue of Somatic Mutations in Cancer (COSMIC) database (http://cancer.sanger.ac.uk/cosmic).

3. Survival analyses

At the median follow-up of 14 months (range, 0–111 months), 33% of the patients had developed leukemic transformation and 72.2% of the patients had expired (median survival 15 months and median LFS 23 months). Leukemic transformation and death occurred more frequently in *ASXL1*$^+$ CMML than in *ASXL1*$^-$ CMML, but this finding was not statistically significant (50% vs 23.5% and 83.3% vs 61.1%, respectively; Table 1). The OS of *ASXL1*$^+$ CMML was significantly lower than *ASXL1*$^-$

Table 3. Risk stratification of 36 patients with chronic myelomonocytic leukemia according to five different risk stratification models

Risk model	All patients (N=36)	*ASXL1* mutation status		*P*
		*ASXL1*mut$^-$ (N=18)	*ASXL1*mut$^+$ (N=18)	
Spanish cytogenetic risk stratification, N (%)				1.000
Low	28 (77.8)	14 (77.8)	14 (77.8)	
Intermediate	3 (8.3)	2 (11.1)	1 (5.6)	
High	5 (13.9)	2 (11.1)	3 (16.7)	
MDAPS, N (%)				0.172
Low	8 (22.2)	4 (22.2)	4 (22.2)	
Intermediate-1	9 (25.0)	7 (38.9)	2 (11.1)	
Intermediate-2	11 (30.6)	3 (16.7)	8 (44.4)	
High	8 (22.2)	4. (22.2)	4 (22.2)	
GFM model, N (%)				0.006
Low	5 (13.9)	4 (22.2)	1 (5.6)	
Intermediate	21 (58.3)	13 (72.2)	8 (44.4)	
High	10 (27.8)	1 (5.6)	9 (50.0)	
Mayo model, N (%)				0.443
Low	-	-	-	
Intermediate	9 (25.0)	3 (16.7)	6 (33.3)	
High	27 (75.0)	15 (83.3)	12 (66.7)	
Mayo molecular model, N (%)				0.131
Low	-	-	-	
Intermediate-1	3 (8.3)	3 (16.7)	-	
Intermediate-2	14 (38.9)	8 (44.4)	6 (33.3)	
High	19 (52.8)	7 (38.9)	12 (66.7)	

Abbreviations: MDAPS, MD Anderson prognostic scoring system; GFM, Groupe Français des Myélodysplasies.

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CMML with a median survival of 11 months and 19 months, respectively (log-rank $P=0.049$; Fig. 1A). Moreover, the LFS was shorter in *ASXL1*$^+$ CMML, albeit without statistical significance (log-rank $P=0.132$; Fig. 1B). Univariate analysis showed that the presence of *ASXL1* mutation tended towards inferior survival (Hazard ratio [HR] 2.260, 95% confidence interval [CI] 0.972–5.255; $P=0.058$) and that BM blasts >10% was associated with inferior LFS (HR 3.566, 95% CI 1.039–12.245; $P=0.043$; Table 4). However, BM blasts >10% did not retain their prognostic significance in multivariate analysis.

When OS was evaluated according to the prognostic models, survival was significantly inferior in patients with a higher risk category according to MMM, with a median survival of 11 months, 28 months, and 46 months in the high, intermediate-2, and intermediate-1 risk groups, respectively (log-rank $P=0.001$); however, the other scoring systems did not demonstrate a significant association with survival (all log-rank $P>0.05$; Fig. 2). According to the Spanish cytogenetic risk stratification, although the survival of the low, intermediate, and high groups was not significantly different (log-rank $P=0.064$), the survival of the high-risk group was significantly inferior to that of the combined low and intermediate risk groups (log-rank $P=0.019$).

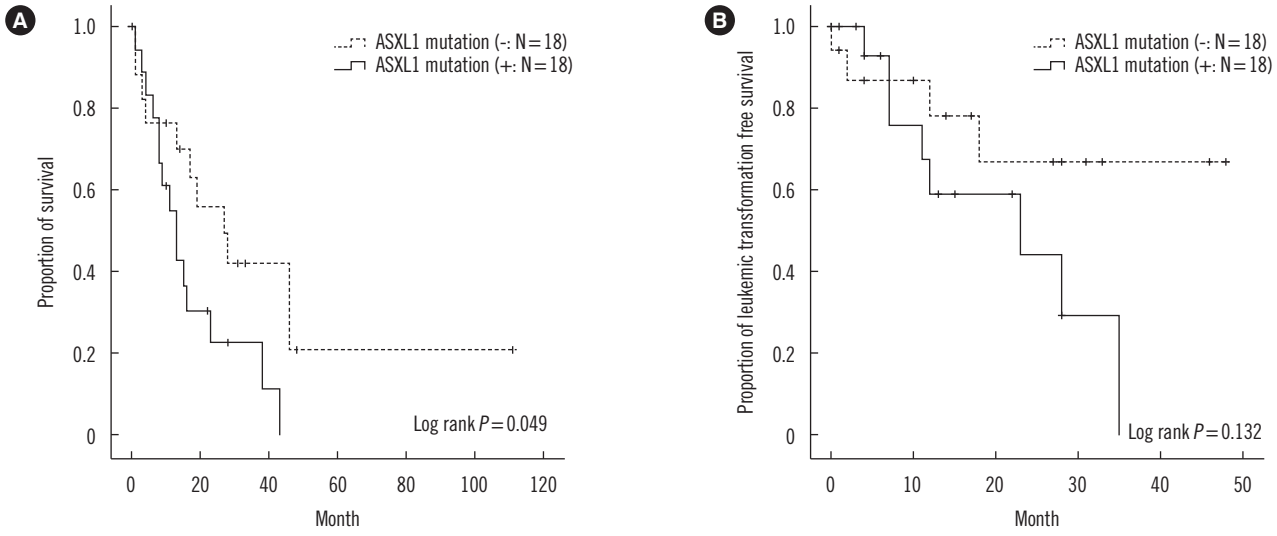

Fig. 1. Kaplan-Meier plots of the (A) overall survival and (B) leukemic transformation-free survival in patients with chronic myelomonocytic leukemia according to *ASXL1* mutation status.

Table 4. Univariate analysis for overall survival and leukemic transformation-free survival in 36 patients with chronic myelomonocytic leukemia

	Overall survival		Leukemic transformation-free survival	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age >65 yr	0.772 (0.343–1.739)	0.533	0.543 (0.170–1.741)	0.304
Hb <10 g/dL	1.690 (0.669–4.269)	0.267	1.477 (0.383–5.698)	0.571
WBC $>13\times10^9$/L	1.820 (0.679–4.874)	0.234	3.746 (0.482–29.092)	0.207
AMC $>10\times10^9$/L	1.680 (0.741–3.810)	0.214	1.752 (0.545–5.631)	0.347
Platelets $<100\times10^9$/L	1.570 (0.710–3.473)	0.265	1,559 (0.466–5.214)	0.471
Presence of circulating IMC	2.238 (0.761–6.581)	0.143	3.677 (0.471–28.713)	0.214
PB blasts >5%	0.722 (0.248–2.101)	0.550	2.260 (0.582–8.781)	0.239
BM blasts >10%	0.808 (0.350–1.864)	0.617	3.566 (1.039–12.245)	0.043
Abnormal karyotype	1.380 (0.607–3.136)	0.442	1.052 (0.282–3.922)	0.939
Presence of *ASXL1* mutation	2.260 (0.972–5.255)	0.058	2.439 (0.728–8.169)	0.148

Abbreviations: HR, hazard ratio; CI, confidence interval; WBC, white blood cell; AMC, absolute monocyte count; IMC, immature myeloid cell; PB, peripheral blood; BM, bone marrow.

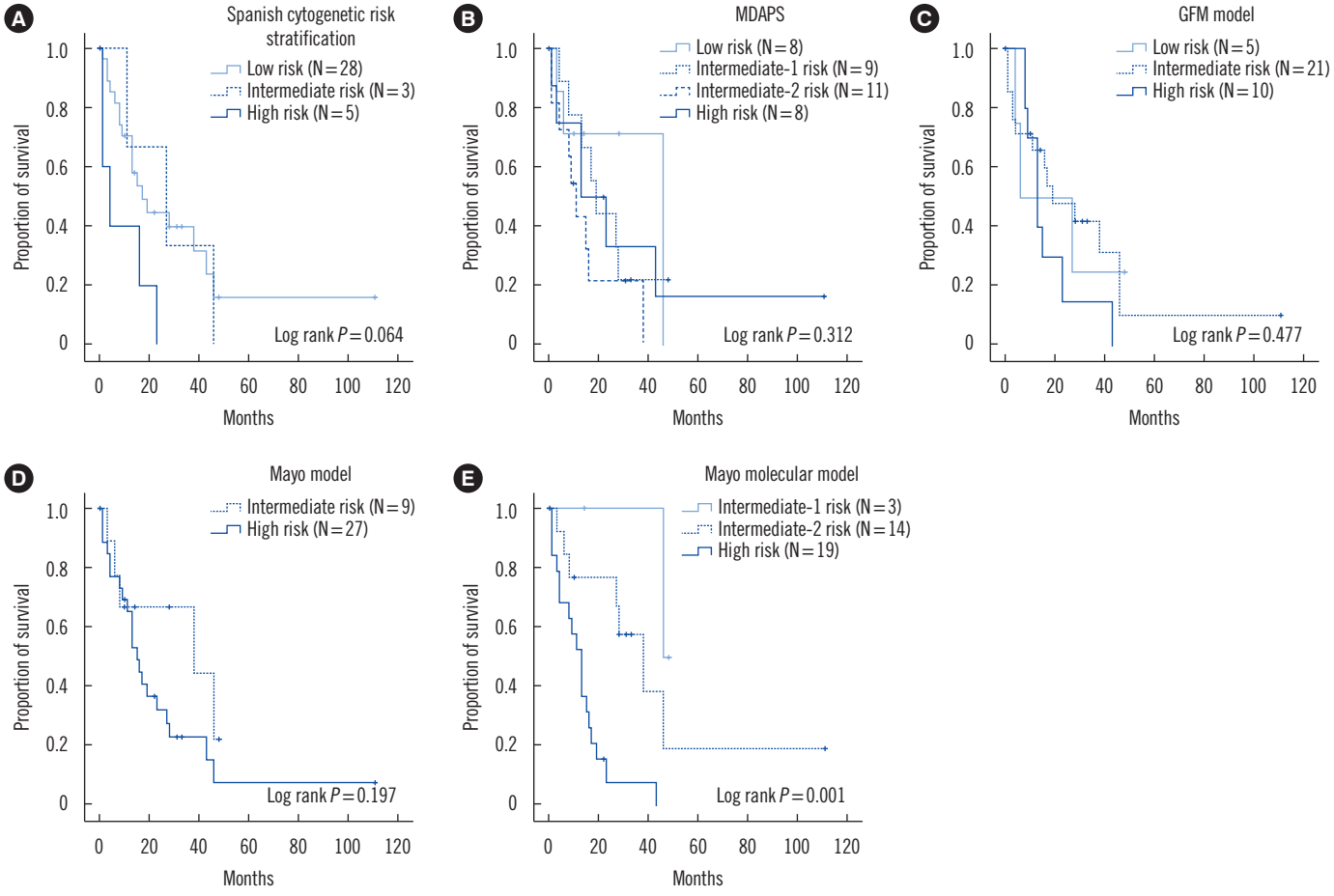

Fig. 2. Kaplan-Meier plots of the overall survival in patients with chronic myelomonocytic leukemia according to the (A) Spanish cytogenetic risk stratification, (B) MDAPS, (C) GFM model, (D) Mayo model, and (E) Mayo molecular model.
Abbreviations: MDAPS, MD Anderson prognostic scoring system; GFM, Groupe Français des Myélodysplasies.

DISCUSSION

In our study, *ASXL1* mutations were detected in half of the CMML patients, all of which, including three novel mutations, were frameshift or nonsense mutations (Table 2). Approximately 67% of *ASXL1* mutations were c.1934dup, and no other mutation hotspots were found. With regard to genotype-phenotype correlations, previous studies have shown an association between *ASXL1* mutations and proliferative phenotypes including higher WBC, higher AMC, and the presence of circulating IMC [5, 9]. However, we found no significant difference between the clinical or laboratory characteristics of *ASXL1*$^+$ and *ASXL*$^-$ CMML patients, which might be due to the limited number of patients. Of note, a higher tendency of WBC and AMC median values was observed in *ASXL1*$^+$ CMML than in *ASXL1*$^-$ CMML patients. Cytogenetic abnormalities have been reported in approximately 20–30% of CMML patients, and +8, loss of Y, -7/del7q, and trisomy 21 (+21) have been established as common abnormalities [3-5, 14]. Our study showed similar findings; approximately 28% of our patients had cytogenetic abnormalities, and 70% of the cytogenetic abnormalities were +8 (20%), loss of Y (20%), -7/del7q (20%), and +21 (10%). Regarding the frequency of *ASXL1* mutations, a previous study has demonstrated that *ASXL1* mutations cluster with an abnormal karyotype (45% vs 34%, P=0.04) [4]; however, in our series, the difference was not statistically significant (40% vs 54%, P=0.457).

The natural course of CMML is highly variable and the OS ranges from 12 months to 35 months [3, 8-11]. Furthermore, in a recent study involving young adult (≤65 years) CMML patients, the median survival was 55 months [15]. These variable clinical courses led to the development of various risk stratification models based on either complete blood counts or cytogenetic or molecular abnormalities. The Spanish cytogenetic risk stratification categorizes patients into three groups; an initial

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study reported the 5-year OS in the low, intermediate, and high-risk groups to be 35%, 26%, and 4%, respectively [3]. A study on 417 CMML patients demonstrated a significantly different OS for each risk group, with a median survival of 33 months, 24 months, and 14 months for the low, intermediate, and high-risk groups, respectively [14]. In our study, only the high-risk group with +8, -7/del7q, or a complex karyotype showed significantly inferior survival compared with the low/intermediate risk groups; the median survival was 17 months and 1 month for the low/intermediate and high-risk groups, respectively, which was lower than in a previous study [14].

The inferior survival in *ASXL1*$^+$ CMML patients was first demonstrated in a study by Gelsi-Boyer *et al* [7], which included 53 CMML patients. Subsequently, GFM has demonstrated the independent poor prognostic value of *ASXL1* mutation in 312 CMML patients and thus incorporated *ASXL1* mutation into the GFM prognostic model [8]. In contrast, the initial Mayo Clinic study found no prognostic impact for *ASXL1* mutation in 226 CMML patients, which led to the development of the Mayo prognostic model without incorporation of *ASXL1* mutation. While only *ASXL1* nonsense and frameshift mutations were included in the GFM study, all nucleotide variations, including missense variation, were included as *ASXL1* mutations in the Mayo Clinic study, which is thought to account for the conflicting prognostic impact of the *ASXL1* mutation between the two studies [9]. To further clarify the prognostic relevance of *ASXL1* mutation, 466 CMML patients from the Mayo Clinic and the GFM study were analyzed; only frameshift and nonsense *ASXL1* mutations were demonstrated to constitute as an independent prognostic factor for OS, but not for leukemic transformation [9]. Therefore, the new MMM was developed by adding *ASXL1* mutation to the Mayo model. We also demonstrated the inferior prognostic impact of *ASXL1* mutation in Korean patients with CMML (Fig. 1A). When previous prognostic scoring systems were applied to our patients, the survival rates of the different risk groups were not significantly different according to the risk stratification of MDAPS, the GFM prognostic model, or the Mayo model (Fig. 2B-D). Only MMM stratification showed a significant difference between the risk groups (Fig. 2E), and median survival rates were similar to that of a previous MMM study (the median survival of the low, intermediate-1, intermediate-2, and high-risk groups was 97, 59, 31, and 16 months, respectively) [9]. There were no low risk group patients in our study, presumably because of the limited number of patients. Previously, the MDAPS and MMM were also compared for 146 CMML patients in Spain [16]. In contrast to our results, the OS was significantly different in each of the MDAPS risk groups, and in the Mayo model, the low/intermediate-risk groups exhibited better OS than the high-risk groups, although there were no differences between the low and intermediate-risk groups.

Leukemic transformation is one of the main causes of death in CMML, and its incidence ranges from 15% to 30% [9-12]. While Gelsi-Boyer *et al* [7] showed that *ASXL1* mutation is also associated with leukemic transformation, Patnaik *et al* [17] reported that the risk factors for leukemic transformation are the presence of circulating blasts and female sex, but not the karyotype or mutational status of *ASXL1*. In our study, leukemic transformation occurred in 33.3% of patients, more frequently in *ASXL1*$^+$ CMML patients (50%) than in *ASXL1*$^-$ patients (23.5%), but this finding was not statistically significant. Based on univariate analysis, only BM blasts >10% were associated with the risk of leukemic transformation.

This study had some limitations. Our study did not enroll a sufficiently large number of CMML patients. Moreover, we did not evaluate any other genetic abnormalities (i.e., *TET2*, *DNMT3A*). Therefore, further large-scale studies using targeted next-generation sequencing are needed.

In conclusion, *ASXL1* mutations found in half of the Korean CMML patients in this study were associated with inferior survival. Of the risk classification models, the MMM best stratified the patient risk groups despite the limited number of patients. *ASXL1* mutation status needs to be determined for risk stratification in Korean patients with CMML.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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Supplemental Data Table S1. Detailed clinical and laboratory features of 36 patients with CMML

No. Case	Age	Sex	*ASXL1* mutation	Karyotype	2008 WHO subtypes	2016 WHO subtypes	FAB subtype	Spanish cytogenetic risk stratification	MDAPS	GFM model	Mayo prognostic model	Mayo molecular model	Outcome
1	73	M	c.1934dupG (p.Gly646Trpfs*12)	46,XY[20]	CMML-1	CMML-0	Proliferative type	Low	Intermediate-1	High	Intermediate	Intermediate-2	Dead
2	71	F	c.1934dupG (p.Gly646Trpfs*12)	46,XX[4]	CMML-1	CMML-1	Proliferative type	Low	Intermediate-1	High	High	High	Dead
3	66	M	c.1934dupG (p.Gly646Trpfs*12)	46,XY[2]	CMML-1	CMML-1	Proliferative type	Low	Intermediate-2	High	High	High	Dead
4	55	F	c.2973_2985del (p.Leu992Valfs*28)	46,XX[20]	CMML-1	CMML-1	Proliferative type	Low	Intermediate-2	Intermediate	Intermediate	Intermediate-2	Dead
5	50	F	-	46,XX,del(9)(q13q22)[2]/46,XX[3]	CMML-2	CMML-2	Dysplastic type	High	Intermediate-1	Low	High	Intermediate-2	Dead
6	83	M	-	46,X,der(Y)t(Y;1)(q12;q21)[20]	CMML-1	CMML-0	Proliferative type	Intermediate	Low	Intermediate	Intermediate	Intermediate-1	Dead
7	58	M	c.1934dupG (p.Gly646Trpfs*12)	46,XY,del(7)(q22)[9]	CMML-1	CMML-1	Proliferative type	High	Intermediate-2	Intermediate	High	High	Dead
8	73	M	-	46,XY[20]	CMML-1	CMML-0	Dysplastic type	Low	Intermediate-1	Intermediate	High	High	Dead
9	53	M	-	47,XY,+8[2]/46,XY[28]	CMML-1	CMML-0	Proliferative type	High	Intermediate-2	Intermediate	High	High	Dead
10	38	F	c.1934dupG (p.Gly646Trpfs*12)	46,XX[20]	CMML-1	CMML-1	Dysplastic type	Low	Low	Low	Intermediate	Intermediate-2	Dead
11	71	M	-	45,X,-Y[50]	CMML-1	CMML-0	Dysplastic type	Low	Low	Intermediate	High	Intermediate-2	Dead
12	74	M	-	46,XY[20]	CMML-1	CMML-1	Proliferative type	Low	Intermediate-1	Intermediate	High	Intermediate-2	Dead
13	72	M	-	46,XY[20]	CMML-1	CMML-0	Dysplastic type	Low	Intermediate-1	Low	Intermediate	Intermediate-1	Alive
14	74	M	c.1934dupG (p.Gly646Trpfs*12)	46,XY[20]	CMML-1	CMML-1	Proliferative type	Low	Intermediate-2	Intermediate	High	High	Dead
15	58	M	-	46,XY[20]	CMML-1	CMML-0	Proliferative type	Low	Intermediate-1	Low	High	High	Dead
16	78	M	-	46,XY[20]	CMML-1	CMML-0	Proliferative type	Low	Intermediate-1	Intermediate	High	Intermediate-2	Alive
17	68	M	c.3001dupA (p.Thr1001Asnfs*4)	46,XY[20]	CMML-1	CMML-0	Proliferative type	Low	Low	Intermediate	Intermediate	Intermediate-2	Dead
18	56	F	-	46,XX[20]	CMML-1	CMML-0	Proliferative type	Low	Low	Intermediate	Intermediate	Intermediate-1	Alive
19	70	M	-	46,XY[20]	CMML-1	CMML-1	Dysplastic type	Low	Intermediate-2	Intermediate	High	Intermediate-2	Alive
20	55	M	c.1900_1922del (p.Glu635Argfs*15)	47,XY,+21[19]/46,XY[1]	CMML-1	CMML-1	Proliferative type	High	Intermediate-2	Intermediate	High	High	Dead
21	59	F	-	46,XX[20]	CMML-2	CMML-2	Proliferative type	Low	High	Intermediate	High	Intermediate-2	Alive

(*Continued to the next page*)

ANNALS OF LABORATORY MEDICINE

Supplemental Data Table S1. Continued

No. Case	Age	Sex	*ASXL1* mutation	Karyotype	2008 WHO subtypes	2016 WHO subtypes	FAB subtype	Spanish cytogenetic risk stratification	MDAPS	GFM model	Mayo prognostic model	Mayo molecular model	Outcome
22	83	M	-	45,X,-Y[12]	CMML-2	CMML-2	Dysplastic type	Low	Low	Low	High	Intermediate-2	Alive
23	72	M	c.1773C>G (p.Tyr591*)	46,XY[20]	CMML-2	CMML-2	Proliferative type	Low	High	High	High	High	Dead
24	60	F	-	46,XX[20]	CMML-2	CMML-2	Proliferative type	Low	High	Intermediate	High	High	Dead
25	76	M	c.1934dupG (p.Gly646Trpfs*12)	45,XY,-7[10]/46,XY[10]	CMML-2	CMML-2	Proliferative type	High	High	High	High	High	Dead
26	75	F	c.1934dupG (p.Gly646Trpfs*12)	46,XX[20]	CMML-2	CMML-2	Proliferative type	Low	High	High	High	High	Dead
27	72	F	-	46,XX[20]	CMML-2	CMML-2	Proliferative type	Low	Intermediate-2	Intermediate	High	Intermediate-2	Alive
28	75	M	c.1766_1767insAGTA (p.Thr590Valfs*30)	46,XY[20]	CMML-2	CMML-2	Dysplastic type	Low	Low	Intermediate	Intermediate	Intermediate-2	Alive
29	79	M	c.1934dupG (p.Gly646Trpfs*12)	46,XY[20]	CMML-2	CMML-2	Proliferative type	Low	Intermediate-2	High	High	High	Dead
30	59	M	c.1934dupG (p.Gly646Trpfs*12)	46,XY[20]	CMML-2	CMML-2	Proliferative type	Low	High	High	High	High	Alive
31	75	M	-	45,XY,del(3)(p21),der(5;6)(p10;p10),add(15)(p11.2)[20]	CMML-2	CMML-2	Dysplastic type	High	High	Intermediate	High	High	Dead
32	70	M	c.2254dupG (p.Ala752Glyfs*22)	46,XY[20]	CMML-2	CMML-2	Dysplastic type	Low	Low	Intermediate	Intermediate	Intermediate-2	Alive
33	75	M	c.1934dupG (p.Gly646Trpfs*12)	46,XY[18]	CMML-2	CMML-2	Proliferative type	Low	Intermediate-2	High	High	High	Dead
34	65	M	c.1934dupG (p.Gly646Trpfs*12)	47,XY,+8[20]	CMML-1	CMML-0	Proliferative type	High	Intermediate-2	Intermediate	High	High	Dead
35	74	M	-	46,XY[20]	CMML-1	CMML-0	Proliferative type	Low	Intermediate-1	Intermediate	High	High	Dead
36	72	F	-	46,XX[10]	CMML-2	CMML-2	Proliferative type	Low	High	High	High	High	Dead

Abbreviations: FAB, French-American-British; MDAPS, MD Anderson prognostic scoring system; GFM, Groupe Français des Myélodysplasies; M, male; F, female; CMML, chronic myelomonocytic leukemia.

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Supplemental Data Table S2. PCR and sequencing primers for the *ASXL1* gene

Primer name	Forward sequence (5′→3′)	Reverse sequence (3′→5′)	Product size (bp)
ASXL1_E14-1	tgccatgacccttaagctact	AAGGCGGCAGTAGTTGTGTT	479
ASXL1_E14-2	GATGAGGGAGGTGGCAGAG	TCCCACTAGAGACGGAATGG	492
ASXL1_E14-3	TCCTCCCAAACCTCAGTAGC	CTGGTTTGGGCTGTTTCACT	445
ASXL1_E14-4	GCCTGCAGAACAGAGCATTT	GTGTCAGCCTCACTGCTGTC	487
ASXL1_E14-5	TGTCAACAGGTGGACATTGAA	TGGTACTTGTGGGGATTCTGA	489
ASXL1_E14-6	CTCAGTGGAGGCCACTAACC	GATCTCCTGGGCTCTTTCCT	500
ASXL1_E14-7	AGAGCAGTTCTCTTCCTTTAGTTG	AGTGACCCACCAGTTCCAG	500
ASXL1_E14-8	GTTGGGACCAAGCACAAACT	ACACTGGAGCGAGATGCTTT	508
ASXL1_E14-9	CTTCTCTCCCCTCCCAACTC	gcaagagtgctcctgcctaa	462

Uppercase letters and lowercase letters represent exonic and intronic sequences, respectively.