Ph.D. Dissertation of Medicine

DNMT3A mutation pattern and clinical features in Korean acute myeloid leukemia patients

한국인 급성골수성백혈병에서 *DNMT3A* 유전자 변이와 임상 양상 분석

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Abstract

Background

Acute myeloid leukemia (AML) is a complicated disease characterized by heterogeneous and simultaneous variant genetic alterations. Epigenetic dysregulation is one of the most important carcinogenesis mechanisms and frequently discovered aberration in AML. The correlation of DNA methylation with carcinogenesis and progression has been investigated for years. However, genetic alteration status, including locus and allele frequency of genes associated with DNA methylation, especially *DNMT3A*, the most prevalent genetic alteration in adult AML, has not been sufficiently investigated. Thus, the objective of this study was to determine clinical significance of *DNMT3A* mutation and the efficacy of hypomethylating agents (HMA) based on detailed mutation patterns.

Materials and Methods

For the genetic study part, 96 samples from AML patients were analyzed by target sequencing to investigate the *DNMT3A* mutation status. For the clinical cohort study part, clinical medical records of newly diagnosed AML patients (n = 216) were collected, and *DNMT3A* mutated Korean AML patients (n = 62, 30.5%) were reviewed, compared, and combined with Western datasets to analyze detailed alteration profiles.

Results

In the genetic study, 191 variants were detected from 70 analyzable samples after variant filtering. The mutation prevalence of *DNMT3A* was 34.3%: Point mutation

at R882 of the *DNMT3A* gene was observed at 14.3% in total and 41.7% in *DNMT3A* mutation. Mutations at non-R882 were observed in 58.3% of *DNMT3A* mutations. The median variant allele frequency (VAF) of *DNMT3A* mutation was 43.1%. In the clinical cohort study part, among 216 patients, the median age at diagnosis was 61.3 years old (range, 18-88 years), 47.7% of patients (n = 103) were adverse prognosis group, and 33.8% (n = 73) had more than 4 mutations.

DNMT3A mutant group was sorted out to study detailed alteration profiles. The median age was 64.9 years old (range, 37-87 years) and 49.3% (n = 34) was the adverse risk group. The prevalence of *DNMT3A* mutation was 30.5% (n = 69) and presented less frequent favorable cytogenetics (P = 0.037). *DNMT3A* WT group was more responsive to intensive chemotherapy (P = 0.014) than the *DNMT3A* mutant group, while the response to the first line HMA was not different (P = 0.244). The presence of *DNMT3A* mutation was associated with shorter overall survival (OS, P = 0.0001). Among *DNMT3A* mutant group, the prevalence of R882 mutation was 47.8%. The mutation prevalence of *DNMT3A* methyltransferase domain where R882 residue was located was 76.8%. *DNMT3A* mutated patients were classified into *DNMT3A* mutation low (VAF $\leq$ 47.6%, n = 54) and *DNMT3A* mutation high (VAF > 47.6%, n = 12) except 3 cases with unknown locations of mutations. Low *DNMT3A* VAF did not present survival benefit. Low VAF of R882 point mutation (P = 0.0015) and methyltransferase domain mutation (P = 0.0284) presented longer OS. Patients with low VAF of total *DNMT3A* mutation (P = 0.0047) and methyltransferase domain mutation lived longer after initial HMA therapy (P = 0.0268).

To validate the clinical impact of *DNMT3A* mutation in a larger dataset, Korean dataset was combined with Western datasets, and the tendency of better

survival in the low *DNMT3A* VAF group was maintained for methyltransferase domain mutation (P = 0.0068), including R882 (P = 0.0027) and total *DNMT3A* mutant cases (P = 0.0138). Methyltransferase domain mutant patients treated by HMA had more prolonged survival when their *DNMT3A* mutation VAF was low (P = 0.0079).

Conclusion

This study presented different *DNMT3A* mutation patterns between Korean and Western AML patients. There were no significant survival differences or frequency of methyltransferase domain mutations between Korean and Western patients. Results of this study suggest that higher allele frequency of *DNMT3A* methyltransferase domain mutations might be suggested as an adverse prognostic factor for survival and a predictable factor for initial HMA therapy in elderly AML patients.

Keyword : Acute myeloid leukemia; Next-generation sequencing; Mutation profiling; DNA methylation; Epigenetics

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List of Abbreviations

AML; acute myeloid leukemia

DNMT3A; deoxynucleic acid methyltransferase 3A

R882; arginine 882

MTD; methyltransferase domain

VAF; variant allele frequency

OS; overall survival

EFS; event-free survival

HMA; hypomethylating agent

NGS; next-generation sequencing

Introduction

Since the expected survival of the population is increasing, the number of newly diagnosed cancer patients is also increasing, particularly in those with hematologic malignancies. The most common hematologic malignancy in adults is non-Hodgkin's lymphoma. Acute myeloid leukemia (AML) is the second most common blood cancer and the most common disease among myeloid neoplasms. The treatment paradigm of AML has not been changed for decades; the achievement of complete remission with anthracycline and cytarabine combined induction chemotherapy followed by allogeneic hematopoietic stem cell transplantation (HSCT) [1]. Because conventional induction chemotherapy is highly intensive with toxicities, only young and fit patients could be candidates for the standard treatment. AML is a well-known age-related disease, with approximately half of AML patients being older than 70 years when they are diagnosed. Hypomethylating agents developed in early 2000 have slightly improved clinical outcomes and survival rates of elderly AML patients [2].

Traditionally, treatment decisions to conduct consolidation chemotherapy and to perform HSCT were made based on patients' physical conditions and conventional risk groups with cytogenetic profiles, including promyelocytic leukemia/retinoic acid receptor alpha (*PML/RARA*), core-binding factor subunit beta/myosin heavy chain 11 (*CBFb/MYH11*), and runt-related transcription factor1/runt-related transcription factor1 translocation partner 1 (*RUNX1/RUNX1T1*) translocation [1]. Chromosomal abnormalities categorized AML before the molecular genomics era. However, recent advance in the genomics of hematopoietic stem cells and progenitors has made it possible to discover

pathogenic heterogeneity and simultaneous alterations in multiple genes in AML.

Since 2010, with the development of genomics and sequencing technology, molecular alterations have been frequently discovered in normal karyotypes of AML including fms related receptor tyrosine kinase 3 (*FLT3*), nucleophosmin 1 (*NPM1*), *RAS,* deoxyribonucleic acid methyltransferase 3A (*DNMT3A*), CCAAT enhancer binding protein alpha (*CEBPA*), isocitrate dehydrogenase 1/2 (*IDH1/2*), and tet methylcytosine dioxygenase 2 (*TET2).* Novel molecular agents targeting FLT3 mutation or IDH1/2 mutation have been developed and introduced to clinical practice based on genomic information [3]. The treatment strategy for AML is changing from limited cytotoxic chemotherapy followed by HSCT or hypomethylating agent to the availability of novel target therapies [1]. However, FLT3 inhibitor acting on a tyrosine kinase receptor and IDH1/2 inhibitors interrupting deoxyribonucleic acid (DNA) epigenetic changes could be applied to only a small portion of patients who have those types of genetic alterations.

With the recent development of genomics, various mutations of AML have been discovered and categorized into transcription factors, kinases, cell cycle regulators, spliceosomal genes, and epigenetic regulators as the most frequent mutations [3, 4]. Alterations of epigenetic modifications essential for normal cell biology including DNA methylation and histone acetylation have been identified. Epigenetic dysregulation is one of the most important carcinogenesis mechanisms implying that it is a potential critical event in AML pathogenesis [5]. Some epigenetic changes could be reversed. These epigenetic alterations have become targets of novel drug development. Administration of hypomethylating agent for AML patients who are not feasible for intensive chemotherapy is one major

treatment strategy.

The genetic alteration status including locus and allele frequency of genes associated with DNA methylation, especially *DNMT3A*, has not been sufficiently investigated. *DNMT3A* mutations have been reported as the most prevalent genetic alterations in adult AML, occurring in up to 20-25% of cases [6, 7]. The correlations of DNA methylation with carcinogenesis and progression have been investigated for years. Many new drug developments for AML are focused on epigenetic modifications [1, 8]. DNA methylation process is the formation of 5-methylcytosine (5mC) by the addition of a methyl group to the C5 of the pyrimidine ring of cytosines at CpG islands [9, 10]. Aberrant DNA hypermethylation of CpG islands in the promotor region is associated with downstream tumor suppressor gene silencing [11]. *DNMT3A* interacts with DNA in a regulatory manner through target recognizing domain loop by a structural change to form catalytic DNA-binding surface [12].

The most common *DNMT3A* mutation occurs in arginine 882 (R882) residue with around 60-70% prevalence. R882 mutant AML cells presented focal methylation loss compared to normal CD34 cells, while wild-type *DNMT3A* AML cells displayed CpG island hypermethylation, suggesting *DNMT3A* R882 mutation could cause hypomethylation [13]. However, several studies reported the presence of *DNMT3A* mutation was associated with a better response rate to hypomethylating agents in AML and myelodysplastic syndrome (MDS) [14-16]. More research is needed because the most common *DNMT3A* mutation is associated with methylation loss, while the presence of *DNMT3A* mutation is associated with a better response to hypomethylating agents.

In a previous report, mutations at the methyltransferase domain (MTD) of

DNMT3A could decrease the catalytic activity. Mutations at MTD were observed at C710, V716, P718, E756, R790, R792, T834, and R836. Those mutations decreased methyltransferase catalytic activity by altering methylation specificity and substrate binding site mutation [12]. DNMT3A methylation acts through regulatory and autoinhibitory ways, decreased activity might silence tumor suppressor genes by promotor CpG islands hypermethylation. Hypomethylation of tumor suppressor genes and induction of DNA damage response pathway is one of the mechanisms of action of hypomethylating agents [17]. In that, the hypothesis could be suggested that not only the most common *DNMT3A* R882 mutation but also mutations at MTD are associated with leukemia.

Recently, variant allele freuquency (VAF) of mutated genes has been studied to clarify its clinical meaning in hematologic malignancies. Including *TP53* and *DNMT3A*, impact of various genetic mutations on the outcomes of AML were studied [18]. AML patients with more than 40% of mutant *TP53* VAF had poor OS in a previous report [19]. For *DNMT3A*, high *DNMT3A* VAF more than 44% was reported as an adverse prognostic factor in de novo AML [20].

Since 2017, the NGS test for newly diagnosed and relapsed AML and MDS have been reimbursed by National health insurance in Korea. The objective of this study was to perform an integrative analysis using NGS test results of Korean patients in comparison with Western patients to address the clinical implication of alterations of *DNMT3A* and the efficacy of hypomethylating agents based on detailed mutation profiles.

Materials and methods

Genetic study part

Patient samples

From 2015 to 2017, bone marrow (BM) or blood samples at diagnosis were obtained from 252 AML and MDS patients who were diagnosed at Seoul National University Hospital. Patients had to be adults older than 19 years. Diagnosis was made by the 2008 World Health Organization criteria in terms of cytomorphological evaluation and cytogenetic or molecular abnormalities [21]. At the time of this research, 96 samples available for genetic tests were selected from AML patients with the verifiable clinical course by electronic medical record (EMR). Three samples from benign idiopathic thrombocytopenic purpura patients were included in the sequencing study as controls.

Targeted sequencing

Genomic DNAs were extracted from patients' samples using QIAamp DNA Mini kit (QIAGEN, Germany) following the manufacturer's instruction. The quality of DNA was assessed using a Qubit fluorometer. Target genes for sequencing were selected based on clinical significance and literature review. Included genes were *FLT3, NPM1, CEBPA, IDH1, IDH2, DNMT3A, TET2, TP53, RUNX1, KIT, KMT2A, ASXL1, ETV6, EZH2, GATA2, KRAS, NRAS, PHF6, PTPN11, SF3B1, SRSF2, WT1, CARM1, BRAF,* and *ASH1L*. Targeted sequencing was performed with TWIST custom panel (TWIST Bioscience, CA, USA) using an Illumina HiSeq® 2500 platform (Illumina, CA, USA). Library construction was

performed using the xGEN™ DNA Library Kit (Integrated DNA Technologies, Iowa, USA) following the manufacturer's instructions. The average coverage depth was more than 1579X. Sequencing reads trimming, mapping, elimination of duplicates and recalibration were performed adequately. To call somatic variants, Mutect2 was used.

Clinical cohort study part

Patient data collection

The NGS test for newly diagnosed and relapsed AML and MDS began to get reimbursed by National health insurance in the fourth quarter of 2017 in Korea. AML is known to have a more unified disease severity, clinical manifestation, and prognosis than MDS. Thus, I focused on AML and excluded MDS. Medical records of newly diagnosed AML patients who underwent the NGS test between 2017 and 2020 were retrospectively collected from three institutions: Seoul National University Hospital (SNUH), Seoul National University Bundang Hospital (SNUBH) and National Cancer Center (NCC). Inclusion criteria were 1) a trackable clinical course through EMR, 2) verifiable NGS test results from clinical practice, and 3) in case of patients in *DNMT3A* mutant subgroup, the presence of *DNMT3A* mutation available to figure out mutation profile details, including mutation locus and variant allele frequency (VAF). Patients' clinical data from the Genetic study part who turned out to have *DNMT3A* mutations were added to the *DNMT3A* mutant subgroup analysis.

The following clinical variables were collected: 1) patient-related factors, including age, gender, comorbidities, and body mass index, 2) disease-related factors, including percent of BM blast, karyotype, BM cellularity, NGS result,

laboratory findings, and extramedullary disease, and 3) therapy-related factors including the type of treatment, treatment response, hematopoietic stem cell transplantation, donor type, and adverse event during treatment. According to European Leukemia Network (ELN) 2017 risk stratification criteria [22], patients were classified into favorable, intermediate, and adverse risk groups. Treatment was categorized as conventional intensive chemotherapy consisting of an anthracycline with cytarabine and hypomethylating agent (HMA), including azacitidine and decitabine. Treatment response was evaluated as complete remission (CR) if it had less than 5% of bone marrow blast without detecting molecular abnormalities versus non-CR. CR versus non-CR evaluation criteria could underestimate treatment response of HMA-treated patients. Thus, patients' response was also evaluated as 'responsive' versus 'refractory'. 'Responsive' included CR, partial remission, and stable disease. Target genes of NGS panel sequencing of each hospital were similar for clinically important genes. In SNUBH, NGS test was performed using Ion S5XL® system and Torrent Suit pipeline. The mean target coverage was ≥ 875X, % target base coverage was 98% for ≥ 500X. In NCC, NGS test was performed using hybridization with oligonucleotide probes and Illumina MiSeq® DX platform, with a mean coverage of depth 1935X. Variants were detected with VarSCAN2. A similar test method was used in SNUH, certified and controlled by laboratory quality management. Illumina MiSeq® DX platform was used until January 2019 and Illumina NextSeq® 550DX was used since January 2019. The minimum coverage depth was 280X. However, minor differences were found for less significant genes. Common genes included *CEBPA*, *NPM1*, *FLT3-ITD*, *FLT3-TKD*, *IDH1*, *IDH2*, *DNMT3A*, *TET2*, *TP53*, *RUNX1*, *KIT*, *ASXL1*, *ETV6*, *EZH2*, *GATA2*, *NRAS*, *PHF6*, *SF3B1*, *SRSF2*, and *WT1*.

Public data source

To compare clinical impact of *DNMT3A* mutation in terms of locus and VAF, pre-published data sources were searched. Data sources were included if they met the following criteria: 1) available for analyses of clinical detail and survival and 2) analyzable detailed mutation profile of *DNMT3A*. Three public datasets [23-25] satisfied these criteria. Datasets were in the form of Microsoft Excel spreadsheets. They were downloaded from journal websites and cBioportal [26-28].

Statistical analysis

Survival analysis was performed with the Kaplan-Meier method and log-rank test. Multivariate analyses were performed using the Cox proportional hazard regression model. Overall survival (OS) duration was defined as the time between the date of diagnosis and the date of death or last visit. Events were defined as followings: Relapse after CR or persistent disease without treatment response in patients received first line intensive chemotherapy; progressive disease after achieving treatment response including CR, partial remission, and stable disease in first line HMA-treated patients. Event-free survival (EFS) duration was defined as the time between the date of diagnosis and the date of first event or death. When patients did not experience any event or death, EFS was defined as the time from diagnosis to the last visit. Categorical variables were analyzed using Pearson's χ^2 test and Fisher's exact test. Continuous variables were analyzed with Student's t-test and one-way analysis of variance (ANOVA). Two-sided P-values of less than 0.05 were considered statistically significant. All statistical analyses were performed using STATA, version 16 (StataCorp LP, TX, USA).

Ethics

For prospective sample collection, written informed consent was obtained from every patient. For retrospective data analysis, a waiver of consent was applied. This study was approved by the Institutional Review Board (IRB) of Seoul National University Hospital (IRB approval number: 1103-004-353). It was conducted in accordance with Declaration of Helsinki provisions.

Result

Genetic study part

Distinct mutation pattern

Among matched patients with 96 samples, 57.3% (n = 55) were younger than 65. Males (n = 63) accounted for 65.6%, and females (n = 33) accounted for 34.4%. After somatic variant calls were detected with MuTect2, a filtering process was applied with gnomAD allele frequency ≤1%, variant allele frequency ≥20%, read depth ≥10, and putative strand-specific bias. Variant calls were annotated with ANNOVAR and checked with ClinVAR, and known as benign/likely benign variants were excluded. Finally, 191 variants were detected from 70 samples. The list of genes and details are summarized in Table 1.

Table 1. List of genes in target sequencing

Gene	Chromosome	start	end	length	Detected mutation type
FLT3	13	28,577,411	28,674,729	97,318	Missense, frameshift
ASXL1	20	30,946,155	31,027,122	80,967	Missense, nonsense, frameshift
SRSF2	17	74,730,197	74,733,456	3,259	Missense
KIT	4	55,524,085	55,606,881	82,796	Missense
DNMT3A	2	25,455,845	25,565,459	109,614	Missense, frameshift
GATA2	3	128,198,270	128,212,028	13,758	Missense
NRAS	1	115,248,090	115,259,515	11,425	Missense
TET2	4	106,067,032	106,200,973	133,941	Missense, nonsense, frameshift
ETV6	12	11,802,788	12,048,336	245,548	Missense, frameshift
KRAS	12	25,357,723	25,403,870	46,147	
NPM1	5	170,814,120	170,838,141	24,021	Frameshift
PHF6	X	133,507,283	133,562,820	55,537	Missense, nonsense, frameshift
PTPN11	12	112,856,155	112,947,717	91,562	
CEBPA	19	33,790,840	33,793,470	2,630	Missense, nonsense, frameshift
SF3B1	2	198,254,508	198,299,815	45,307	Missense, frameshift
IDH1	2	209,100,951	209,130,798	29,847	Missense
WT1	11	32,409,321	32,457,176	47,855	Missense, frameshift
TP53	17	7,565,097	7,590,856	25,759	Missense,

					nonsense, frameshift
IDH2	15	90,626,277	90,645,736	19,459	Missense
KMT2A	11	118,307,205	118,397,539	90,334	Missense
CARM1	19	10,982,189	11,033,453	51,264	
BRAF	7	140,419,127	140,624,564	205,437	
RUNX1	21	36,160,098	37,376,965	1,216,867	Missense, frameshift
EZH2	7	148,504,475	148,581,413	76,938	Missense, frameshift
ASH1L	1	155,305,059	155,532,598	227,539	Missense

DNMT3A mutation status was the main interesting topic. In Western studies, the prevalence of *DNMT3A* mutations has been reported to be around 20-25%, with residue R882 in the methyltransferase domain being the most common mutation or hotspot with a frequency of around 60% [6, 29]. Sequencing results of specimens from Korean patients showed that the mutation prevalence of *DNMT3A* was only 34.3% (24 out of 70). Point mutation at R882 of the *DNMT3A* gene was observed in 10 cases (14.3% in total, 41.7% in *DNMT3A* mutation). Mutations at other locations of the *DNMT3A* gene (non-R882 mutations) were found in 14 cases (20% in total, 58.3% in *DNMT3A* mutation). The median variant allele frequency of *DNMT3A* mutation was 43.1%. Three had a double *DNMT3A* mutation. Mutation details with distribution and allele frequency are described in Table 2, Figure 1, and Figure 2. Because the mutation prevalence and hotspot were different from those in Western studies, Korean data were compared with Western data.

Table 2. Mutation distribution and allele frequency in *DNMT3A* mutated cases.

Pt No	location	Functional domain	AA change	VAF (%)	type
1	25470581	PWWP	G298V	38.9	Missense
1	25458604	MTD	D857N	38.0	Missense
2	25457243	MTD	R882S	37.0	Missense
3	25457242	MTD	R882H	40.6	Missense
4	25469488	-	-	-	Splice site mutation
5	25471057	Splicing zone	E235GX	50.0	Frameshift
6	25457242	MTD	R882H	42.8	Missense
7	25464508	MTD	S669P	24.8	Missense
7	25464505	MTD	-669-690X	24.5	Frameshift
8	25469948	PWWP	Y365C	44.3	Missense
9	25457242	MTD	R882H	40.9	Missense
10	25457182	MTD	F902X	41.9	Frameshift
11	25457243	MTD	R882C	48.1	Missense
12	25457164	MTD	Y908C	33.6	Missense
13	25457242	MTD	R882H	45.9	Missense
14	25457242	MTD	R882H	48.9	Missense
15	25457243	MTD	R882C	42.7	Missense
16	25464437	MTD	Q692HX	43.3	Frameshift
17	25462023	MTD	W795S	45.6	Missense
18	25463232	MTD	L754P	36.0	Missense
19	25457243	MTD	R882C	47.6	Missense
20	25457242	MTD	R882H	37.7	Missense
21	25467430	ADD	C549Y	46.2	Missense
21	25463287	MTD	R736S	43.9	Missense

Abbreviation: AA, amino acids; ADD, ATRX-DNMT3-DNMT3L domain; MTD, methyltransferase domain; PWWP, Pro-Trp-Trp-Pro domain; VAF, variant allele frequency.

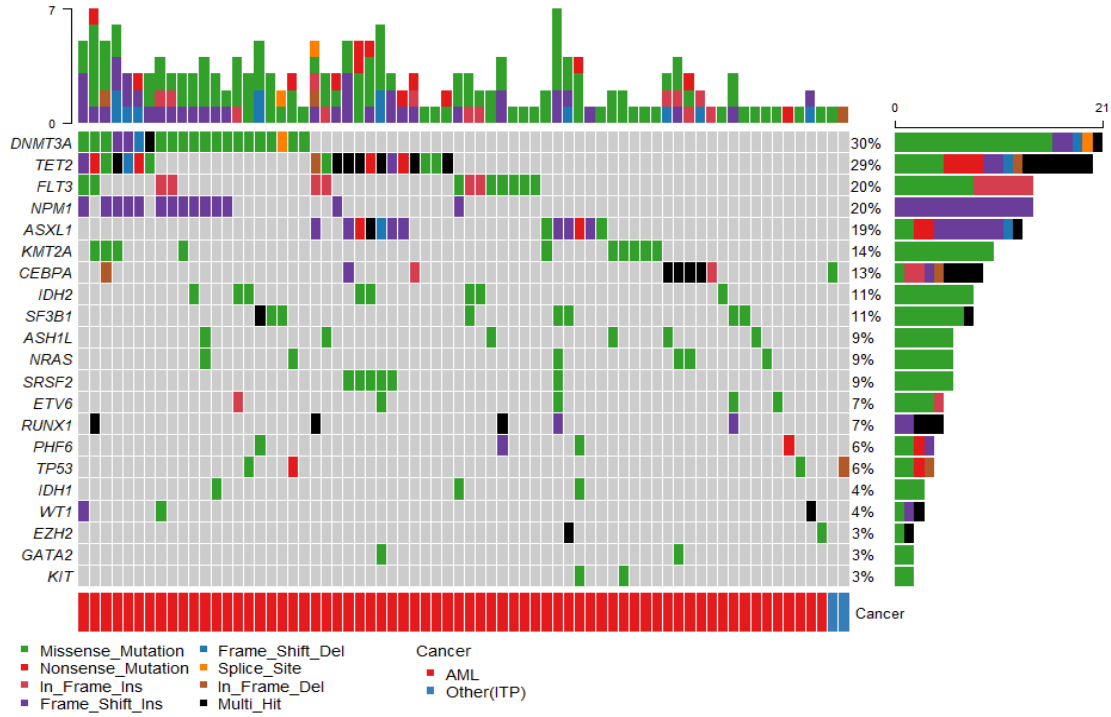


Figure 1. Distribution of mutations. From 70 samples, including 2 benign ITP samples as control, 191 variants were detected. One column matches with a single patient. Abbreviation: AML, acute myeloid leukemia; ITP, immune thrombocytopenic purpura.

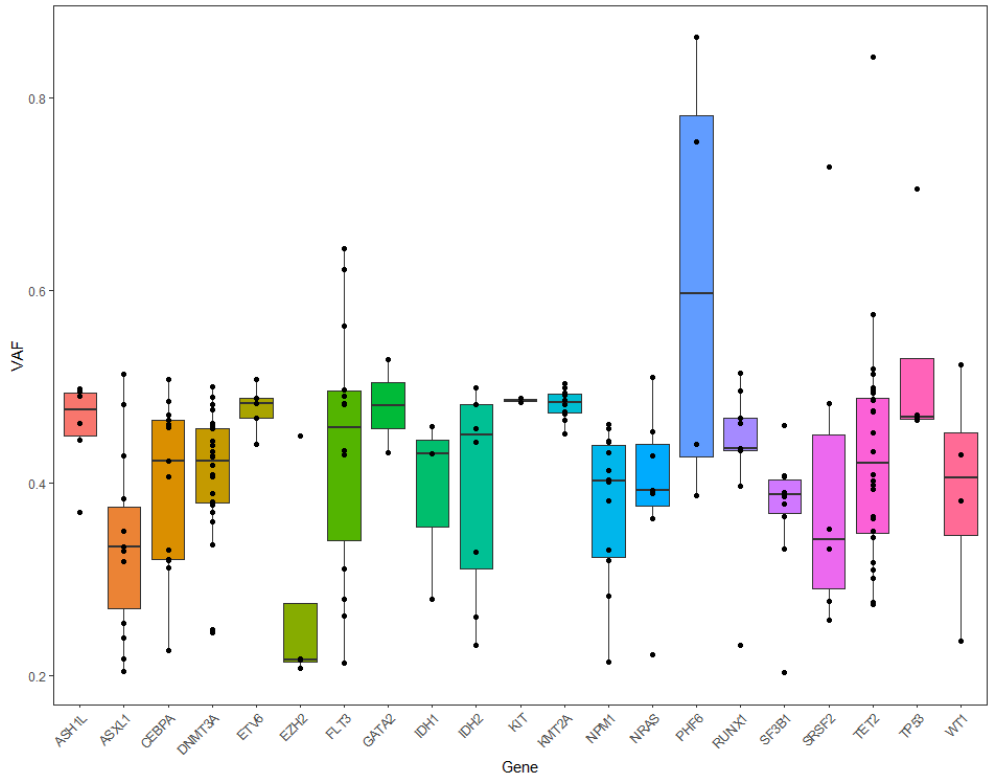


Figure 2. Variant allele frequency of detected mutation. *DNMT3A* mutation VAF range was 24.5%-48.9%. Median VAF was 43.1%. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; VAF, variant allele frequency.

Clinical cohort study part

Baseline clinical parameters

Of 226 newly diagnosed AML patients from three hospitals (125 from SNUH, 50 from SNUBH, and 51 from NCC), 10 patients were excluded for not fulfilling inclusion criteria. The median age at diagnosis was 61.3 years old (range 18-88 years). About 50% of patients (n = 103, 47.7%) were classified into the adverse prognosis group according to ELN2017 risk criteria. The median number of genetic mutations was 3 (range, 0-11). Patients who had more than 4 mutations were 33.8% (n = 73). The hypomethylating agent was used as the first line treatment in 63 patients (29.2%) and 42.6% of patients (n = 92) who received hematopoietic stem cell transplantation. The baseline characteristics of total Korean patients are summarized in Table 3.

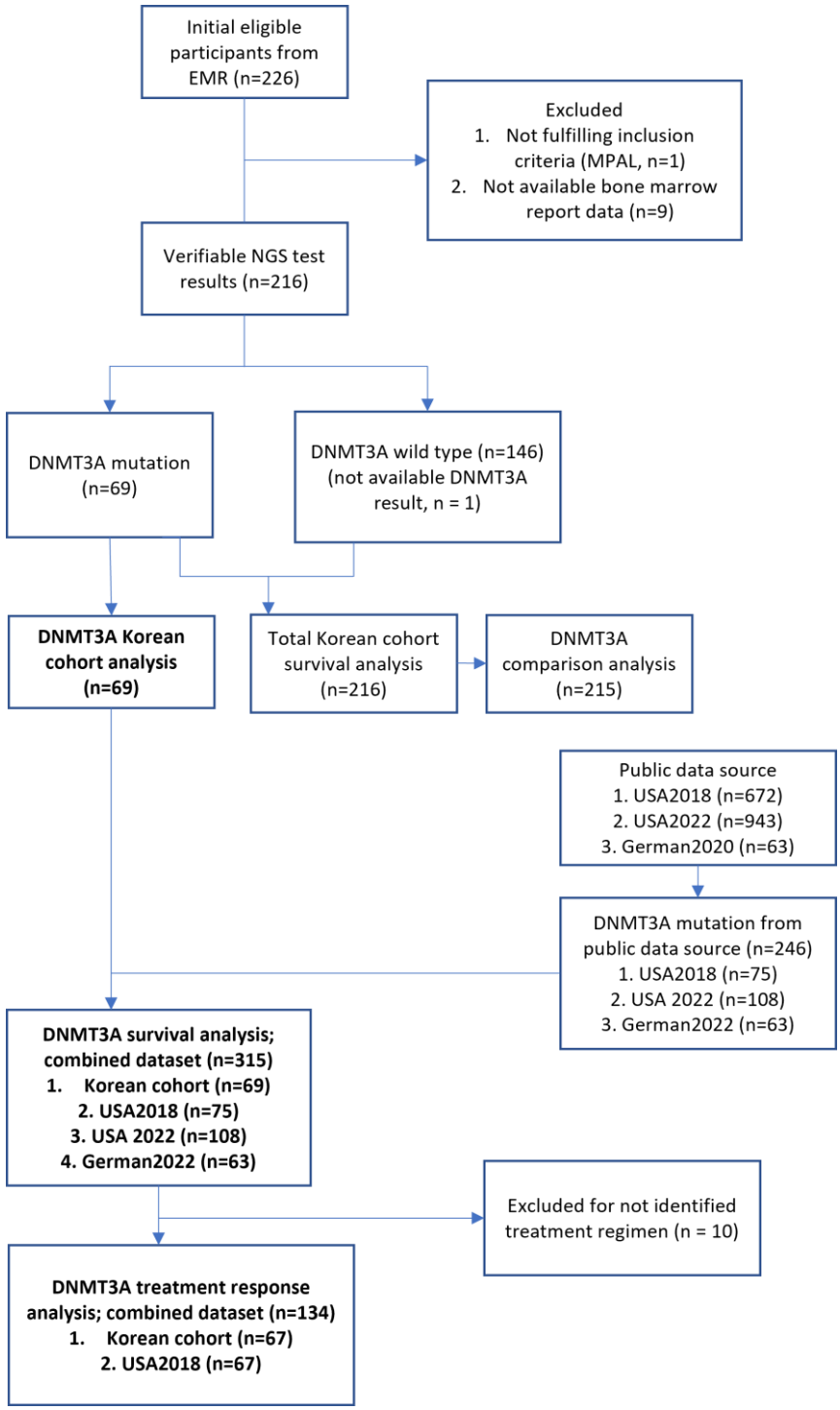


Figure 3. Consort diagram. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; EMR, electric medical records; NGS, next generation sequencing; MPAL, mixed phenotype acute leukemia; VAF, variant allele

frequency. Public datasets were referred from the United States for the year 2018 (USA2018) and year 2022 (USA2022) and Germany for the year 2020 (German2020) [23-25].

Table 3. Baseline characteristics of Korean patients (n=216).

	Patient number (n=216)	**%**	**Median (Range)**
Age at diagnosis (years)			61.3 (18-88)
< 65	129	59.7	
≥ 65	87	40.3	
Gender			
Male	115	53.2	
Female	101	46.8	
Comorbidities			
< 2	184	85.2	
≥ 2	32	14.8	
Other malignancy history			
Yes[1]	10	16.1	
No	52	83.9	
Bone marrow blast[2]			63 (19-98.4)
Karyotype[3]			
Favorable	32	14.8	
Intermediate	138	63.9	
Adverse	42	19.4	
Not evaluable[4]	4	1.9	
ELN2017 risk			
Favorable	65	30.1	
Intermediate	48	22.2	
Adverse	103	47.7	
Extramedullary disease			
Yes	20	9.3	
No	196	90.7	

1st line treatment			
HMA	63	29.2	
Intensive chemotherapy	147	68.0	
Unknown	6	2.8	
Hematopoietic stem cell transplantation			
Yes	92	42.6	
No	124	57.4	
Mutations prevalence		%	
FLT3-ITD	43	19.9	
FLT3-TKD	14	6.5	
NPM1	48	22.2	
CEBPA	24	11.1	
IDH1	13	6.0	
IDH2	30	13.9	
RUNX1	36	16.7	
KIT	23	10.6	
TP53	17	7.9	
DNMT3A	69	31.9	
ASXL1	35	16.2	
ETV6	7	3.2	
EZH2	9	4.2	
GATA2	4	1.8	
NRAS	31	14.3	
PHF6	6	2.8	
SF3B1	9	4.2	
SRSF2	15	6.9	
WT1	6	4.2	
TET2	42	19.4	
Number of mutations			3 (0-11)
< 4	143	66.2	
≥ 4	73	33.8	

Abbreviation: ELN2017, European Leukemia Net 2017; HMA, hypomethylating agent.

[1] Stomach cancer and prostate cancer in a single patient, colon cancer in 3, melanoma in 1, thyroid cancer in 3, primary myelofibrosis in 1, cutaneous T-cell

lymphoma in 1, diffuse large B-cell lymphoma in 1, multiple myeloma in 2, chronic myelomonocytic leukemia in 1, non-small cell lung cancer in 3, hepatocellular carcinoma in 1, breast cancer in 5.

[2] Bone marrow blast count data was not evaluable in 15 patients and excluded from the result.

[3] Favorable cytogenetics includes t(8;21), in(16) or t(16;16), and t(15;17). The complex cytogenetics includes 3 or more unrelated chromosomal abnormalities without WHO-designated recurring alterations.

[4] Four patients that karyotype data were not evaluable had adverse risk genetic mutations: 1 had *FLT3-ITD* (VAF 91.7%) mutation with wild-type *NPM1*, 1 had *FLT3-ITD* mutation (VAF 66.8%) and *RUNX1* mutation, 1 had *ASXL1* mutation, and 1 had *FLT3-ITD* (VAF 64.63%) mutation, *NPM1* mutation, and *ASXL1* mutation.

Among 216 Korean patients with verifiable NGS test results, 147 had wild-type (WT) *DNMT3A*, and 69 (30.5%) presented *DNMT3A* mutations. *DNMT3A* result was not available in a single patient who was excluded from the *DNMT3A* comparison analysis. Among 69 *DNMT3A* mutant patients, the median age at diagnosis was 64.9 years old (range 37-87 years). Half (49.3%, n = 34) were classified into the adverse prognosis group according to ELN2017 risk criteria. Compared to the *DNMT3A* wild-type WT patients, *DNMT3A* mutant patients presented less frequent favorable cytogenetics ($P = 0.037$) and more mutations ($P = 0.000$). *NPM1* ($P = 0.000$), and *IDH2* ($P = 0.007$) mutations were more frequent co-mutations in *DNMT3A* mutant patients, while *CEBPA* ($P = 0.008$) and *KIT* ($P = 0.038$) mutations were more frequent in *DNMT3A* WT patients. A comparison of baseline characteristics according to the presence of DNMT3A mutation is summarized in Table 4.

Table 4. Comparison of baseline characteristics of Korean patients according to *DNMT3A* mutation.

	DNMT3A WT (n=146)	DNMT3A mut (n=69)	*p*
Age at diagnosis (years)			0.070
< 65	93 (63.7%)	35 (50.7%)	
≥ 65	53 (36.3%)	34 (49.3%)	
Gender			0.395
Male	81 (55.5%)	34 (49.3%)	
Female	65 (44.5%)	35 (50.7%)	
Comorbidities			0.478
< 2	20 (13.7%)	12 (17.4%)	
≥ 2	126 (86.3%)	57 (82.6%)	
Other malignancy history			0.046
Yes	12 (8.2%)	12 (17.4%)	
No	134 (91.8%)	57 (82.6%)	
Bone marrow blast[1]			0.494
< 63%	72 (51.1%)	27 (45.8%)	
≥ 63%	69 (48.9%)	32 (54.2%)	
Karyotype[1]			0.037
Favorable	27 (19.0%)	5 (7.3%)	
Intermediate	86 (50.6%)	53 (76.8%)	
Adverse	29 (20.4%)	11 (15.9%)	
ELN2017 risk			0.839
Favorable	46 (31.5%)	19 (27.5%)	
Intermediate	32 (21.9%)	16 (23.2%)	
Adverse	68 (46.6%)	34 (49.3%)	
Extramedullary disease			0.224
Yes	130 (89.0%)	65 (94.2%)	
No	16 (11.0%)	4 (5.8%)	
1st line treatment			0.300
HMA	38 (26.0%)	25 (36.2%)	
Intensive chemotherapy	104 (71.2%)	42 (60.9%)	

Unknown	4 (2.7%)	2 (2.9%)	
Hematopoietic stem cell transplantation			0.214
Yes	80 (54.8%)	44 (63.8%)	
No	66 (45.2%)	25 (36.2%)	
Mutations prevalence			
FLT3-ITD	24 (16.4%)	18 (26.1%)	0.096
FLT3-TKD	8 (7.2%)	6 (8.7%)	0.372
NPM1	19 (13.0%)	29 (42.0%)	0.000
CEBPA	22 (15.1%)	2 (2.9%)	0.008
IDH1	6 (4.1%)	7 (10.1%)	0.083
IDH2	14 (9.6%)	16 (23.2%)	0.007
RUNX1	25 (17.1%)	10 (14.5%)	0.626
KIT	20 (13.7%)	3 (4.4%)	0.038
TP53	12 (8.2%)	5 (7.3%)	0.805
ASXL1	26 (17.8%)	8 (11.6%)	0.244
ETV6	6 (4.1%)	1 (1.5%)	0.305
EZH2	8 (5.5%)	1 (1.5%)	0.168
GATA2	4 (2.7%)	0 (0.0%)	0.165
NRAS	22 (15.1%)	9 (13.0%)	0.693
PHF6	3 (2.1%)	2 (2.9%)	0.702
SF3B1	5 (3.4%)	4 (5.8%)	0.417
SRSF2	12 (8.2%)	3 (4.4%)	0.298
WT1	4 (2.7%)	2 (2.9%)	0.947
TET2	25 (17.1%)	17 (24.6%)	0.195
Number of mutations			0.000
< 4	106 (74.1%)	33 (48.6%)	
≥ 4	37 (25.9%)	35 (51.5%)	

Abbreviation: ELN2017, European Leukemia Net 2017; HMA, hypomethylating agent; WT, wild-type.

[1] Missing data was excluded from the analysis.

To access Western data, public data sets were used from pre-published literature searches, including cBioPortal and BEATAML projects that were available to analyze detailed *DNMT3A* mutation profiles and survival. Three data sets were available, two from the United States for the year 2018 (USA2018) and year 2022 (USA2022) and one from Germany for the year 2020 (German2020) [23-25]. The positive rate of *DNMT3A* mutation was 11.1% (75 out of 672) in USA2018, 11.5% (108 out of 943) in USA2022, and 100% (63 out of 63) in Germany2020.

Compared to Western data, the percentage of patients older than 65 tended to be higher in Korean data, although the difference was not statistically significant. The prognosis group, according to ELN2017 risk stratification, showed no difference among USA2018, USA 2022, and Korean data (P = 0.304). Fewer adverse risk group patients were included in the German2020 data.

Locations of *DNMT3A* mutations were significantly different between groups. Compared to Western patients (51.8%-71.4%), Korean patients had less frequent R882 mutations (47.8%, P = 0.039). However, there was no significant difference in the prevalence of mutation of MTD where R882 residue was located (P = 0.145). Patients who had mutations in MTD accounted for 76.8% (n = 53) in Korean data, 77.3% in USA2018 data, 74.1% in USA2022 data, and 88.9% in German2020 data. *NPM1* co-mutation was significantly more frequent in Western data (P = 0.004). Treatment and response evaluation was possible from Korean data and USA2018 data. More patients received conventional intensive chemotherapy in the Western than in Korean data (P = 0.001). CR rate after the first treatment was not significantly different (P = 0.119). A comparison of four datasets is described in Table 5. Detailed amino acid changes and mutation types

are listed in Table 6.

To validate the clinical impact of *DNMT3A* mutation in a larger dataset, the Korean dataset was combined with pre-published Western data. OS analysis was available for all three public datasets, and treatment and response data were available in USA2018. Type of amino acid change and locus information of *DNMT3A* mutation data were available in all three datasets, while variant allele frequency (VAF) data were available in USA2018 and USA2022.

Table 5. Comparison of baseline characteristics of each dataset.

	Total Korean (n=216)	**Korean DNMT3A (n=69)**	**USA2018 (n=75)**	**USA2022 (n=108)**	**German2020 (n=63)**
Median age (range)	61.3 (18-88)	64.9 (37-87)	61 (33-86)	62 (33-86)	NA
Age ≥ 65	129 (59.7%)	34 (49.3%)	28 (37.3%)	44 (40.7%)	NA
Age < 65	87 (40.3%)	35 (50.7%)	47 (62.7%)	64 (59.3%)	NA
Gender					
Male	115 (53.2%)	34 (49.3%)	35 (46.7%)	47 (43.5%)	28 (44.4%)
Female	101 (46.8%)	35 (50.7%)	40 (53.3%)	61 (56.5%)	35 (55.6%)
ELN2017 risk					
Favorable	65 (30.1%)	19 (27.5%)	20 (26.7%)	23 (21.3%)	23 (36.5%)
Intermediate	48 (22.2%)	16 (23.2%)	18 (24.0%)	17 (15.7%)	29 (46.0%)
Adverse	103 (47.7%)	34 (49.3%)	37 (49.3%)	68 (63.0%)	11 (17.5%)
1st line therapy					
HMA	63 (29.2%)	25 (36.2%)	9 (12.0%)	NA	NA
Intensive chemotherapy[1]	147 (68.0%)	42 (60.9%)	58 (77.3%)	NA	NA
Unknown	6 (2.8%)	2 (2.9%)	8 (10.7%)	NA	NA
1st line					

therapy CR					
CR	121 (56.0%)	32 (46.4%)	40 (53.3%)	NA	NA
Non-CR	71 (32.9%)	27 (39.1%)	18 (24.0%)	NA	NA
unknown	25 (11.6%)	10 (14.5%)	17 (22.7%)	NA	NA
1st line therapy response[2]					
Responsive	134 (62.0%)	37 (53.6%)	40 (53.3%)	NA	NA
Refractory	58 (26.9%)	22 (31.9%)	18 (24.0%)	NA	NA
unknown	25 (11.6%)	10 (14.5%)	17 (22.7%)	NA	NA
***DNMT3A* mutation type**					
R882	33 (47.8%)	33 (47.8%)	45 (60.0%)	56 (51.8%)	45 (71.4%)
Non-R882	33 (47.8%)	33 (47.8%)	29 (38.7%)	45 (41.7%)	18 (28.6%)
unknown	3 (4.4%)	3 (4.4%)	1 (1.3%)	7 (6.5%)	0 (0.0%)
***DNMT3A* mutation locus**					
MTD	53 (76.8%)	53 (76.8%)	58 (77.3%)	80 (74.1%)	56 (88.9%)
Non-MTD	13 (18.8%)	13 (18.8%)	16 (21.4%)	21 (19.4%)	7 (11.1%)
unknown	3 (4.4%)	3 (4.4%)	1 (1.3%)	7 (6.5%)	0 (0.0%)
Co-mutation prevalence					
FLT3-ITD	43 (19.9%)	18	30	42	28 (44.4%)

		(26.1%)	(40.0%)	(38.9%)	
FLT3-TKD	14 (6.5%)	6 (8.7%)	NA	8 (7.4%)	10 (15.9%)
NPM1	48 (22.2%)	29 (42.0%)	39 (52.0%)	58 (53.7%)	46 (73.0%)
CEBPA	24 (11.1%)	2 (2.9%)	2 (2.7%)	7 (6.5%)	NA
IDH1	13 (6.0%)	7 (10.1%)	15 (20.0%)	18 (16.7%)	NA
IDH2	30 (13.9%)	16 (23.2%)	NA	18 (16.7%)	NA
RUNX1	36 (16.7%)	10 (14.5%)	NA	11 (10.2%)	NA
TP53	17 (7.9%)	5 (7.3%)	6 (8.0%)	6 (5.6%)	

Abbreviation: CR, complete remission; *DNMT3A*, deoxynucleic acid methyltransferase 3A; ELN, European Leukemia Network; HMA, hypomethylating agent; MTD, methyltransferase domain; R882, arginine 882.

[1] Conventional intensive chemotherapy: anthracycline and cytarabine.

[2] 'Responsive' includes complete remission, partial remission, and stable disease. 'Refractory' includes progressive and persistent disease.

Table 6. *DNMT3A* mutation profiles in Korean dataset.

Pt No	DNA change	AA change	type	VAF (%)
1	c.1646G>A	p.Cys549Tyr	Missense	51.4
2	c.2644C>T	p.Arg882Cys	Missense	32.63
3	c.2254T>A	p.Phe752Ile	Missense	17.39
4	c.2645G>A	p.Arg882His	Missense	48.32
5	-	-	Splice site mutation	57.1
6	c.2309C>T	p.Ser770Leu	Missense	46.45
7	c.2644C>T	p.Arg882Cys	Missense	44.88
8	c.2645G>A	p.Arg882His	Missense	37.2
9	c.2261T>G	p.Leu754Pro	Missense	39.0
10	c.1250C>T	p.Ser417fs	Missense	43.07
11	c.2209_2213delGTACGTCCTC	p.736Leu_740Alaf	Frameshift	58.4
12	c.1627G>T	p.Gly543Cys	Missense	46.37
13	c.2645G>A	p.Arg882His	Missense	40.7
14	-	-	-	52.5
15	c.2645G>A	p.Arg882His	Missense	57.1
16	c.2645G>A	p.Arg882His	Missense	42.65
17	c.2644C>T	p.Arg882Cys	Missense	42.23
18	c.2645G>C	p.Arg882Pro	Missense	46.37
19	c.2204A>G	p.Tyr735Cys	Missense	4.49
20	c.2558dupA	p.Asn853fs	Frameshift	41.2
21	c.2645G>A	p.Arg882His	Missense	30.84
22	c.2645G>A	p.Arg882His	Missense	47.38
23	c.2645G>A	p.Arg882His	Missense	47.53
24	c.2645G>A	p.Arg882His	Missense	52.9
25	c.2645G>A	p.Arg882His	Missense	37.2
26	c.2645G>A	p.Arg882His	Missense	41.3
27	c.893	p.Gly298Val	Missense	38.9
28	c.2644C>A	p.Arg882Ser	Missense	37.0
29	c.2645G>A	p.Arg882His	Missense	40.6
30	-	-	Splice site mutation	38.9

31	-	p.Glu235	Frameshift	50.0
32	c.2645G>A	p.Arg882His	Missense	42.8
33	c.2005T>C	p.Ser669Pro	Missense	24.8
34	c.1094>G	p.Tyr365Cys	Missense	44.3
35	c.2645G>A	p.Arg882His	Missense	40.9
36	c.2704T>?	p.Phe902	Frameshift	41.9
37	c.2644C>T	p.Arg882Cys	Missense	48.1
38	c.2723A>G	p.Tyr908Cys	Missense	33.6
39	-	p.Gln692	Frameshift	43.3
40	c.2385G>	p.Trp795Ser	Missense	45.6
41	c.2644C>T	p.Arg882Cys	Missense	47.6
42	c.2644C>T	p.Arg882Cys	Missense	43.6
43	c.2150A>G	p.Asn717Ser	Missense	48.3
44	c.2645G>A	p.Arg882His	Missense	37.9
45	c.2645G>A	p.Arg882His	Missense	9.9
46	c.2193_2195delCTT	p.Phe732del	Deletion (in frame)	42.3
47	c.2645G>A	p.Arg882His	Missense	46.3
48	c.2017_2023del	p.Gly673Cysfs*30	Missense	35.3
49	c.1914dup	p.Leu639fs*3	Missense	43.9
50	c.2210T>G	p.Leu737Arg	Missense	27.0
51	c.1084C>T	p.Gln362*	Nonsense	47.0
52	c.2222C>A	p.Ala741Glu	Missense	42.0
53	c.2357C>A	p.Ser786*	Nonsense	48.0
54	c.2645G>A	p.Arg882His	Missense	43.0
55	c.1416_1417insCAATA	p.Glu473Glnfs*2	Frameshift	33.0
56	c.1010C>T	p.Ser337Leu	Missense	46.3
57	c.2645G>A	p.Arg882His	Missense	43.0
58	c.1138G>C	p.Ala380Pro	Missense	32.0
59	c.1773delC	p.Tyr592Thrfs*59	Frameshift	26.0
60	c.2384G>A	p.Trp795Ter	Nonsense	31.0
61	c.2159G>A	p.Arg720His	Missense	27.0
62	c.2098C>G	p.Pro700Ala	Missense	3.6
63	c.2645G>A	p.Arg882His	Missense	48.9
64	c.2645G>A	p.Arg882His	Missense	40.7
65	c.2644C>G	p.Arg882Gly	Missense	48.96

66	c.1903C>T	p.Arg635Trp	Missense	40.22
67	c.2645G>A	p.Arg882His	Missense	43.87
68	c.939G>A	p.Trp313*	Missense	2.47
69	c.1715C>A	p.Ala572Asp	Missense	38.0

Abbreviation: AA, amino acid; VAF, variant allele frequency.

Treatment response

Korean cohort

In choosing the first-line treatment for the Korean patients between HMA and intensive induction chemotherapy, no significant difference was observed between the *DNMT3A* WT group and *DNMT3A* mutant group (P = 0.300). Thirty-eight patients (26.0%) in the *DNMT3A* WT group and 25 patients (36.2%) in *DNMT3A* mutant group received HMA as the first line treatment. In HSCT, significant difference was not observed. Eighty patients (54.8%) in the *DNMT3A* WT group and 44 (63.8%) in the *DNMT3A* mutant group underwent transplantation.

Regarding response, patients who received intensive chemotherapy as the first line treatment presented more response than those treated with HMA as expected (P = 0.000). In comparing the efficacy of treatment according to *DNMT3A* mutation, the first-line HMA treatment did not show a difference between the *DNMT3A* WT group and *DNMT3A* mutant group (P = 0.244). *DNMT3A* WT group was more responsive to intensive chemotherapy (P = 0.014, Table 8A).

Treatment response was analyzed according to VAF and mutation locus in the *DNMT3A* mutant group. Previous studies have reported that the high allele

frequency of the R882 mutation is associated with an adverse prognosis [20, 30]. In that, the mutated allele frequency was focused. *DNMT3A* VAF ranged from 2.47% to 58.4%, with a median of 42.3%. To set an adequate cut-off point, receiver operating characteristic (ROC) curve analysis was performed, and 47.6% of VAF was selected. Three cases with unknown locations of mutations were excluded, and 66 patients were partitioned into two groups: *DNMT3A* mutation low (VAF $\leq$ 47.6%, n = 54) and *DNMT3A* mutation high (VAF > 47.6%, n = 12). Clinical variables are compared in Table 7. Responses to intensive chemotherapy were not different according to VAF. Responses to HMA was better in lower VAF of total DNMT3A mutation (P = 0.020) and VAF of R882 (P = 0.028), however, the number of patients in each group was small (Table 8A, 8B).

Table 7. Baseline clinical parameters according to *DNMT3A* mutation allele frequency in Korean patients.

	DNMT3A mut low (n=54)	**DNMT3A mut high (n=12)**	***P***
Age ≥ 65	28 (51.9%)	7 (58.3%)	0.684
Gender			0.164
Male	24 (44.4%)	8 (66.7%)	
Female	30 (55.6%)	4 (33.3%)	
BM blast ≥ 67%	21 (45.7%)	8 (72.7%)	0.107
ELN2017 risk			0.805
Favorable	14 (25.9%)	4 (33.3%)	
Intermediate	13 (24.1%)	2 (16.7%)	
Adverse	27 (50.0%)	6 (50.0%)	
***DNMT3A* mutation type**			0.523
R882	46 (48.2%)	7 (58.3%)	
Non-R882	28 (51.9%)	5 (41.7%)	
***DNMT3A* mutation locus**			0.274
MTD	42 (77.8%)	11 (91.7%)	
Non-MTD	12 (22.2%)	1 (8.3%)	
Median VAF	40.7% (range, 2.47-47.6)	49.0% (range, 48-58.4)	
1st line therapy			0.321
HMA	18 (34.6%)	6 (50.0%)	
Intensive chemotherapy	34 (65.4%)	6 (50.0%)	
1st line CR[1]			0.342
CR	26 (56.5%)	4 (40.0%)	
Non-CR	20 (43.5%)	6 (60.0%)	
1st line response[1,2]			0.349
Responsive	31 (66.0%)	4 (40.0%)	
Refractory	16 (34.0%)	6 (60.0%)	

Abbreviation: BM, bone marrow; CR, complete remission; *DNMT3A*, deoxynucleic

acid methyltransferase 3A; ELN, European Leukemia Network; HMA, hypomethylating agent; MTD, methyltransferase domain; R882, arginine 882; VAF, variant allele frequency.

[1] Missing data was excluded from the analysis.

[2] 'Responsive' includes complete remission, partial remission, and stable disease. 'Refractory' includes progressive and persistent disease.

Table 8A. Treatment response to first line intensive chemotherapy according to various *DNMT3A* mutation status in Korean patients.

Intensive chemotherapy response[1]	***DNMT3A* WT**	***DNMT3A* mutation**	***P***
CR	80 (80.0%)	24 (60.0%)	0.014
Non-CR	20 (20.0%)	16 (40.0%)	
Intensive chemotherapy response[1]	***DNMT3A* mut low**	***DNMT3A* mut high**	[2]
CR	20 (62.5%)	3 (60.0%)	
Non-CR	12 (37.5%)	2 (40.0%)	
Intensive chemotherapy response[1]	***DNMT3A* R882 low**	***DNMT3A* R882 high**	[2]
CR	13 (76.5%)	2 (100.0%)	
Non-CR	4 (23.5%)	0 (0.0%)	
Intensive chemotherapy response[1]	***DNMT3A* MTD low**	***DNMT3A* MTD high**	[2]
CR	16 (66.7%)	3 (60.0%)	
Non-CR	8 (33.3%)	2 (40.0%)	

Abbreviation: CR, complete remission; *DNMT3A*, deoxynucleic acid methyltransferase 3A; MTD, methyltransferase domain; R882, arginine 882; WT, wild-type.

[1] Missing data was excluded from analysis.

[2] P-values were not shown in the table because the numbers of patients were too small. P-value for *DNMT3A* mut low vs. *DNMT3A* mut high was 0.915, *DNMT3A* R882 low vs. *DMT3A* R882 high was 0.440, and *DNMT3A* MTD low vs. *DNMT3A* MTD high was 0.775, respectively.

Table 8B. Treatment response to first-line HMA treatment according to various *DNMT3A* mutation statuses in Korean patients.

HMA response[1]	***DNMT3A* WT**	***DNMT3A* mutation**	***P***
Responsive	15 (48.4%)	13 (65.0%)	0.244
Refractory	16 (51.6%)	7 (35.0%)	
HMA response[1]	***DNMT3A* mut low**	***DNMT3A* mut high**	***P***
Responsive	11 (78.6%)	1 (20.0%)	0.020
Refractory	3 (21.4%)	4 (80.0%)	
HMA response[1]	***DNMT3A* R882 low**	***DNMT3A* R882 high**	[3]
Responsive	4 (80.0%)	0 (0.0%)	
Refractory	1 (20.0%)	3 (100.0%)	
HMA response[1]	***DNMT3A* MTD low**	***DNMT3A* MTD high**	[3]
Responsive	7 (70.0%)	1 (25.0%)	
Refractory	3 (30.0%)	3 (75.0%)	

Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; HMA, hypomethylating agent; MTD, methyltransferase domain; R882, arginine 882; WT, wild-type.

[1] Missing data was excluded from analysis.

[2] 'Responsive' includes complete remission, partial remission, and stable disease. 'Refractory' includes progressive and persistent disease.

[3] P-values were not shown in the table because the numbers of patients were too small. P-value for *DNMT3A* R882 low vs. *DNMT3A* R882 high was 0.028, and *DNMT3A* MTD low vs. *DNMT3A* MTD high was 0.124, respectively.

Combined dataset

In treatment response analyzable combined data (n = 134), first-line intensive chemotherapy was more effective in achieving response as expected (*P* = 0.003 in CR rate, *P* = 0.006 in response rate). In comparison, according to VAF, 132 patients were included, except 2 patients whose VAF results were not available. Response to intensive chemotherapy according to VAF of total *DNMT3A* mutation, R882, and MTD were not different from the VAF in the Korean cohort. First-line HMA was more effective in the *DNMT3A* mutation low group (n = 102) than in the *DNMT3A* mutation high group (n = 30, *P* = 0.027). Though the tendency of better response to HMA in lower VAF in terms of total DNMT3A mutation (*P* = 0.025), similar to the results of the Korean cohort, the number of response evaluable patients in HMA-treated group in combined data was too small (Table 9A, 9B).

Table 9A. Treatment response to first-line intensive chemotherapy according to various *DNMT3A* mutation statuses in the combined dataset.

Intensive chemotherapy response[1]	***DNMT3A* mut low**	***DNMT3A* mut high**	***P***
CR	45 (65.2%)	12 (63.2%)	0.868
Non-CR	24 (34.8%)	7 (36.8%)	
Intensive chemotherapy response[1]	***DNMT3A* R882 low**	***DNMT3A* R882 high**	***P***
CR	31 (72.1%)	7 (7.78%)	0.727
Non-CR	12 (27.9%)	2 (22.2%)	
Intensive chemotherapy response[1]	***DNMT3A* MTD low**	***DNMT3A* MTD high**	***P***
CR	40 (70.2%)	11 (68.8%)	0.913
Non-CR	17 (29.8%)	5 (31.3%)	

Abbreviation: CR, complete remission; *DNMT3A*, deoxynucleic acid methyltransferase 3A; HMA, hypomethylating agent; MTD, methyltransferase domain; R882, arginine 882; WT, wild-type.

[1] Missing data was excluded from the analysis.

Table 9B. Treatment response to first-line HMA treatment according to various *DNMT3A* mutation statuses in a combined dataset.

HMA response[1]	***DNMT3A* mut low**	***DNMT3A* mut high**	***P***
Responsive	14 (82.4%)	2 (33.3%)	0.025
Refractory	3 (17.7%)	4 (66.7%)	
HMA response[1]	***DNMT3A* R882 low**	***DNMT3A* R882 high**	[3]
Responsive	6 (85.7%)	1 (25.0%)	
Refractory	1 (14.3%)	3 (75.0%)	
HMA response[1]	***DNMT3A* MTD low**	***DNMT3A* MTD high**	[3]
Responsive	9 (75.0%)	2 (40.0%)	
Refractory	3 (25.0%)	3 (60.0%)	

Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; HMA, hypomethylating agent; MTD, methyltransferase domain; R882, arginine 882; WT, wild-type.

[1] Missing data was excluded from the analysis.

[2] 'Responsive' includes complete remission, partial remission, and stable disease. 'Refractory' includes progressive and persistent disease.

[3] P-values were not shown in the table because the numbers of patients were too small. P-value for *DNMT3A* R882 low vs. *DNMT3A* R882 high was 0.044, and *DNMT3A* MTD low vs. *DNMT3A* MTD high was 0.169, respectively.

Survival analysis

Korean cohort

Inhibition of DNMT activity by targeting the catalytic MTD that results in interruption and competition of DNA and enzyme binding, structural allosteric inhibition, and inhibition of protein-protein interactions have been suggested as mechanisms of novel *DNMT3A* target molecules in recent research [31]. Even though the number was small, higher VAF in total DNMT3A mutation and R882 mutation was associated with poor response of first-line HMA treatment in response analysis. The frequency of R882 mutation in Korean patients was significantly different from that in Western patients, while the prevalence of mutations in MTD where R882 residue was located had no significant difference. In that, the impact of VAF on survival in terms of R882 and MTD mutation was analyzed.

The survival result of the Korean dataset was analyzed first. In the total Korean AML dataset (n = 216), the median OS was 20.62 months (95% confidence interval [CI], 0.4117-0.5679), and the median EFS was 8.26 months (95% CI, 0.4270-0.5676, Figure 4A, 4B).

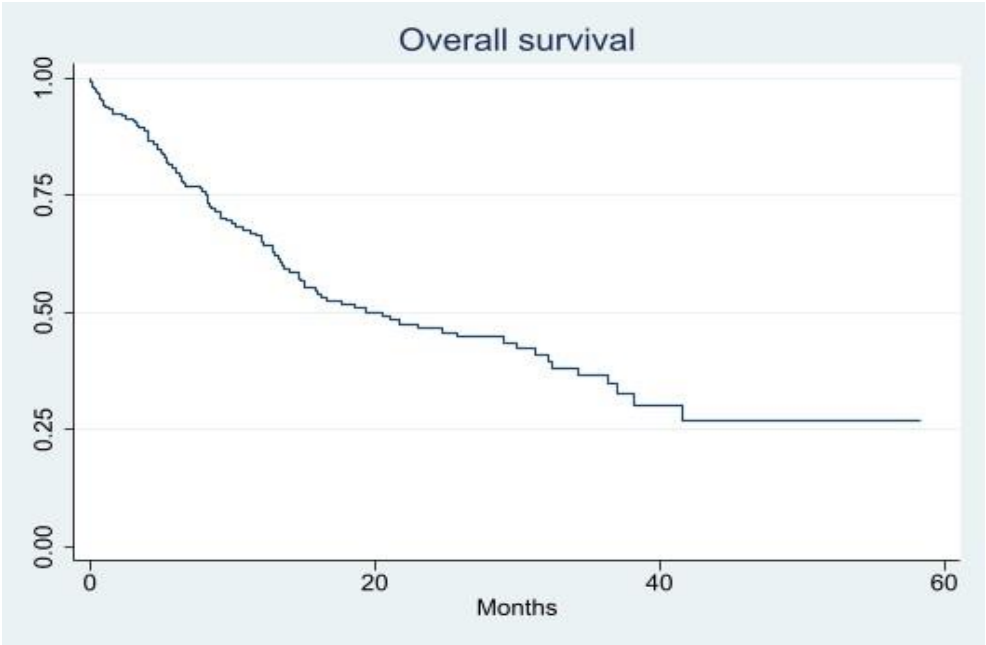


Figure 4A. OS in total Korean patients (n=216). The median OS was 20.62 months (95% CI, 0.4117-0.5679). Abbreviation: OS, overall survival

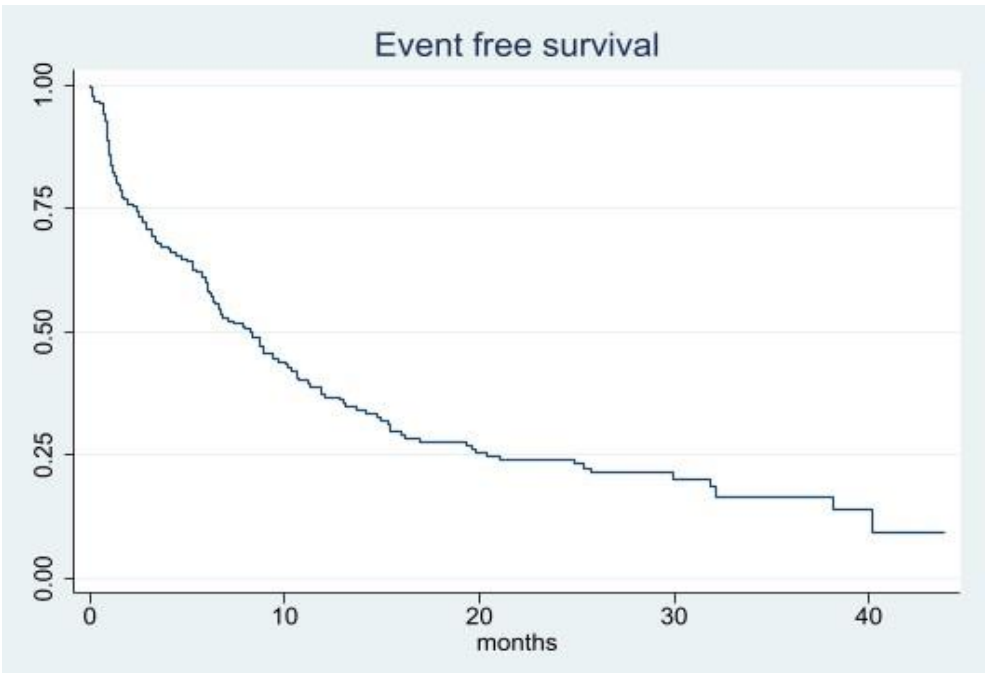
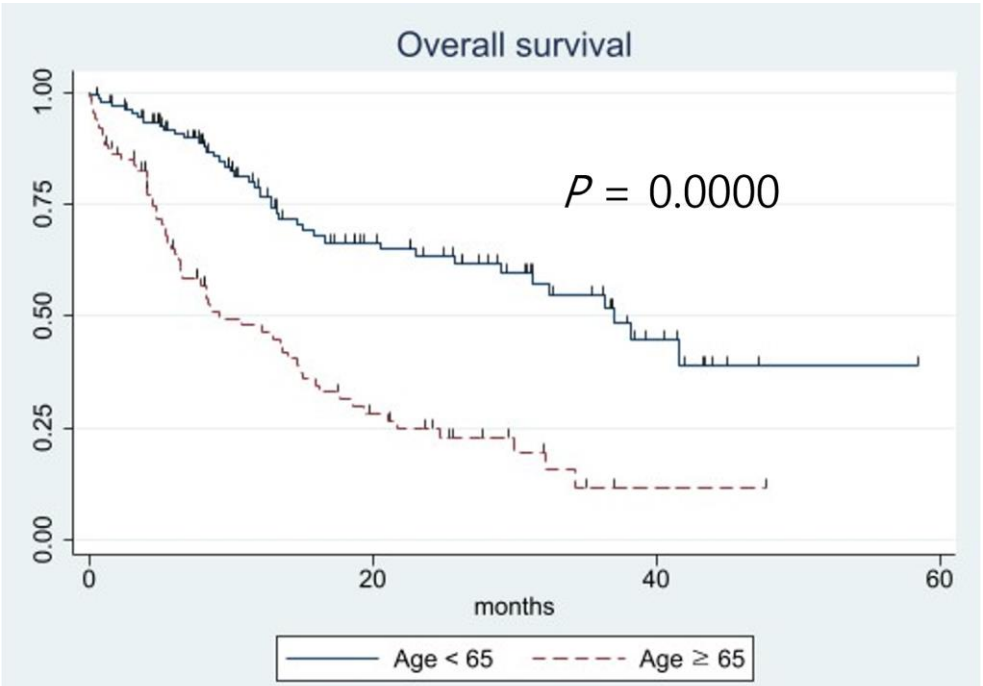


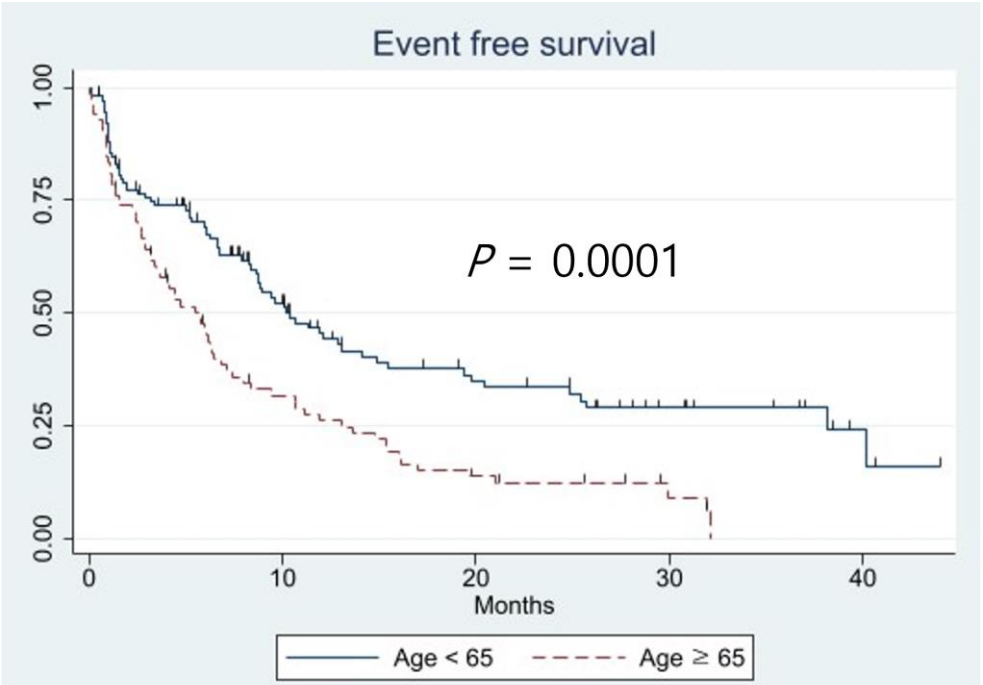
Figure 4B. EFS in total Korean patients (n=216). The median EFS was 8.26 months (95% CI, 0.4270-0.5676). Abbreviation: EFS, event-free survival

Patients who were younger than 65 years (P = 0.0000) or who had favorable risk by ELN2017 criteria (P = 0.0048), or who had less than 4 mutations (P = 0.0045) presented longer OS. Similarly, younger than 65 years old (P = 0.0001), favorable risk group by ELN2017 criteria (P = 0.0001), and less than 4 mutations (P = 0.0413) were associated with better EFS (Figure 5A-5F). The presence of DNMT3A mutation in the total Korean cohort was also associated with shorter OS with statistical significance (P = 0.0001, Figure 6A, 6B).



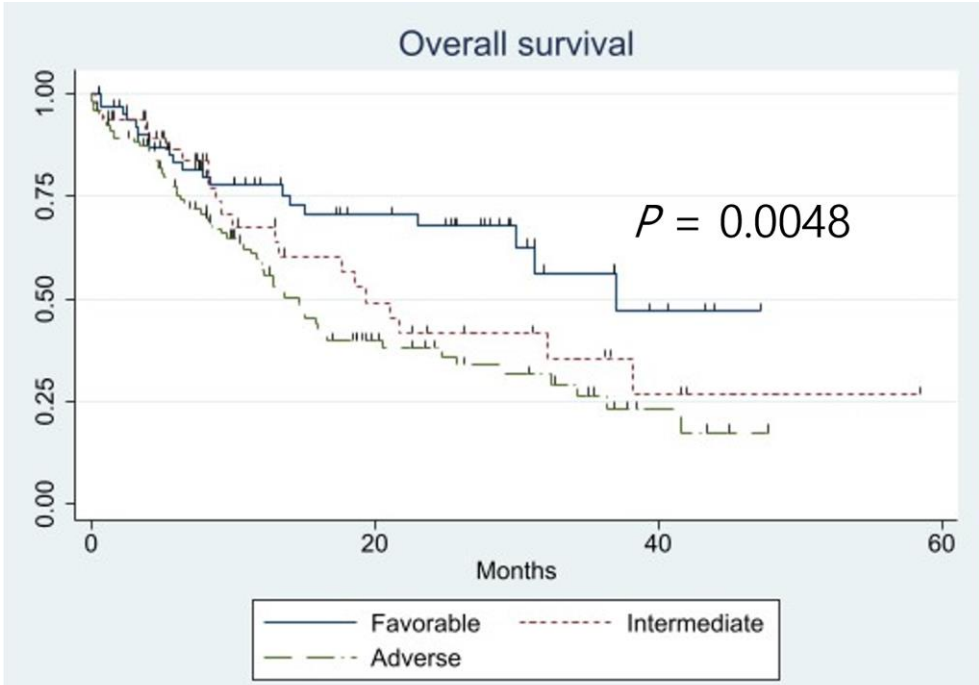
	Age < 65	Age ≥ 65
N	129	87
Median OS	36.98	8.492
95% CI	0.3442-06085	0.3909-0.6154

Figure 5A. Overall survival according to age in the total Korean cohort. Abbreviation: OS, overall survival.



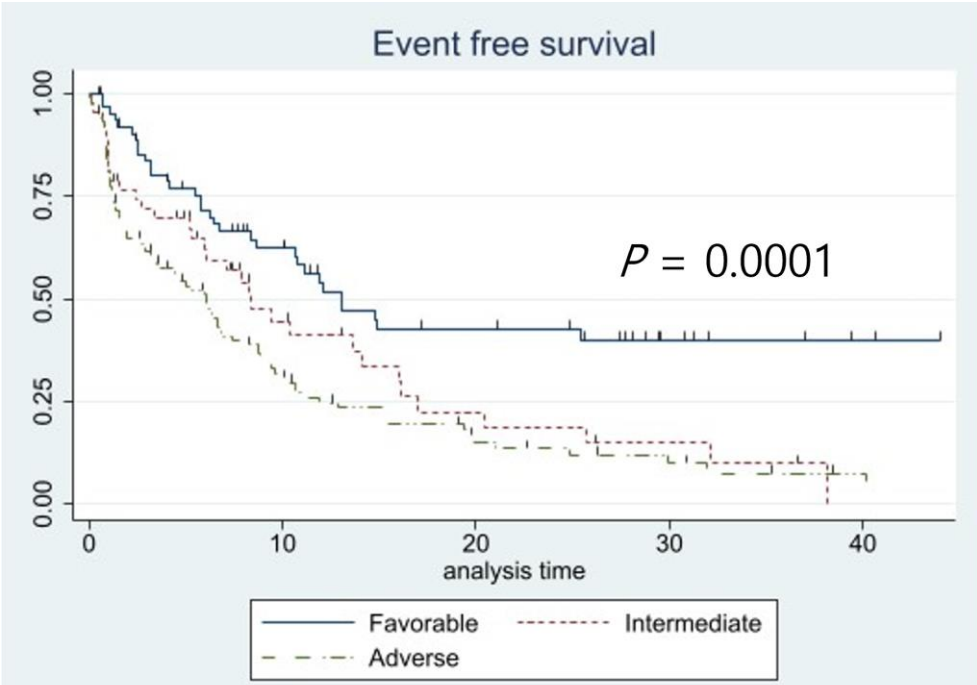
	Age < 65	Age ≥ 65
N	129	87
Median EFS	10.36	5.77
95% CI	0.3913-0.5808	0.3649-0.5800

Figure 5B. Event-free survival according to age in the total Korean cohort. Abbreviation: EFS, event-free survival.



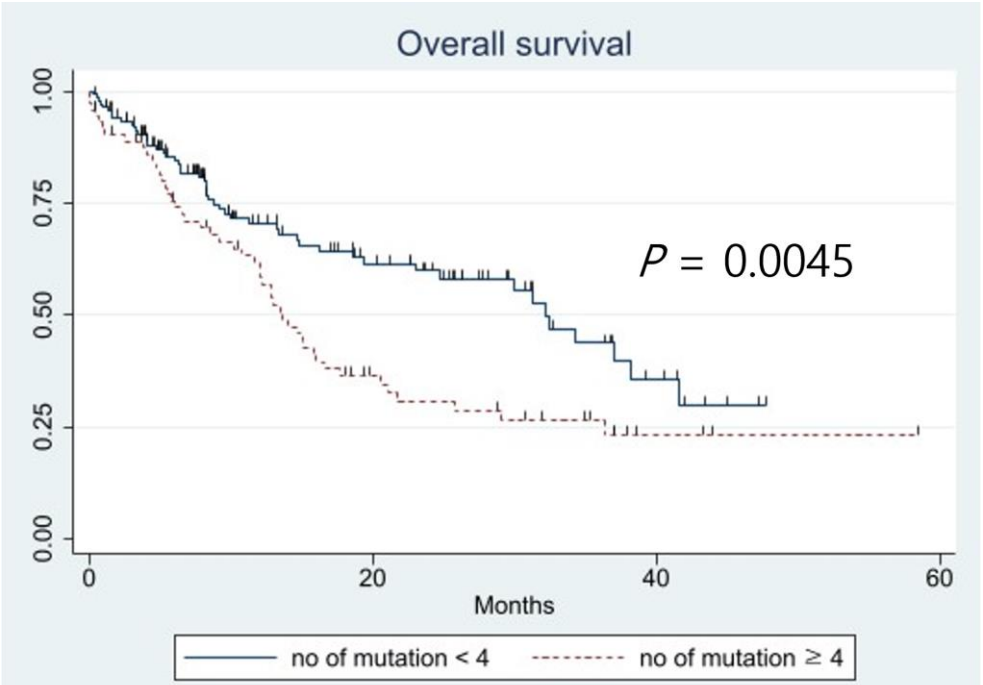
	Favorable	Intermediate	Adverse
N	65	48	103
Median OS	36.98	19.44	14.66
95% CI	0.2390-0.6703	0.3052-0.6527	0.3808-0.5929

Figure 5C. Overall survival according to ELN2017 risk criteria in the total Korean cohort. Abbreviation: ELN, European Leukemia Network; OS, overall survival.



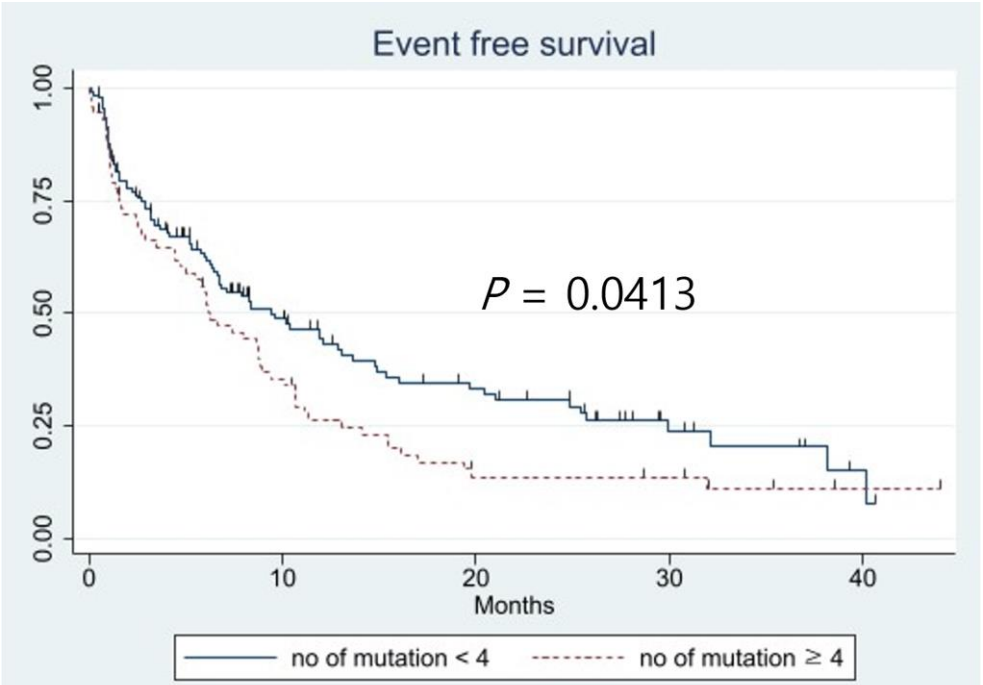
	Favorable	Intermediate	Adverse
N	65	48	103
Median EFS	13.02	8.33	6.09
95% CI	0.3541-0.6212	0.3141-0.6218	0.3857-0.5821

Figure 5D. Event-free survival according to ELN2017 risk criteria in the total Korean cohort. Abbreviation: ELN, European Leukemia Network; EFS, event-free survival.



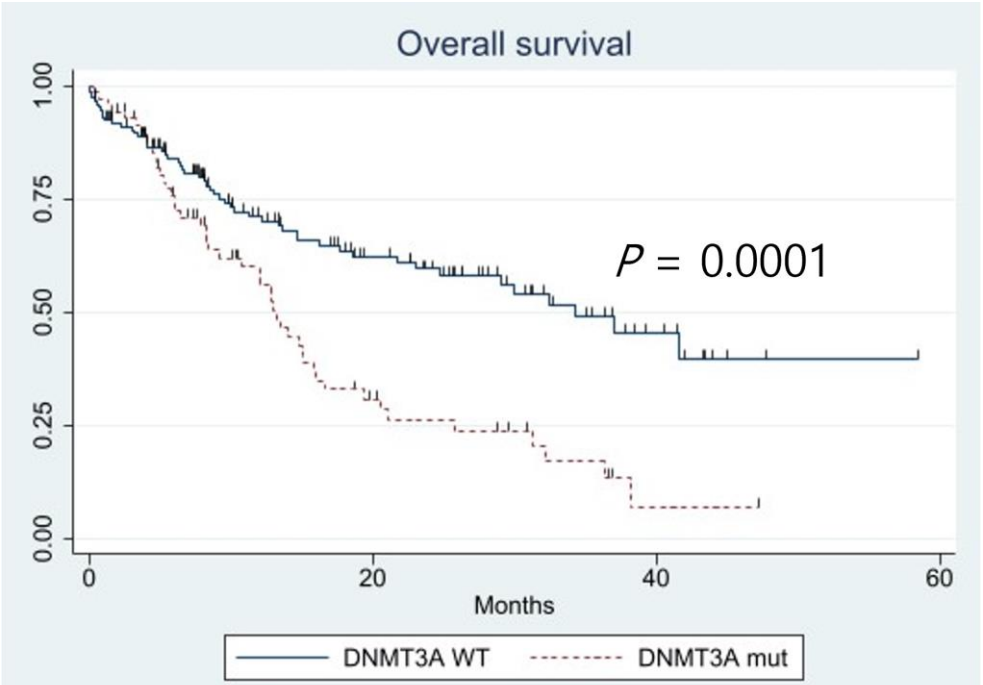
	No of mutation < 4	No of mutation ≥ 4
N	139	73
Median OS	32.16	13.61
95% CI	0.3690-0.6142	0.3648-0.6040

Figure 5E. Overall survival according to the number of mutations in the total Korean cohort. Abbreviation: OS, overall survival.



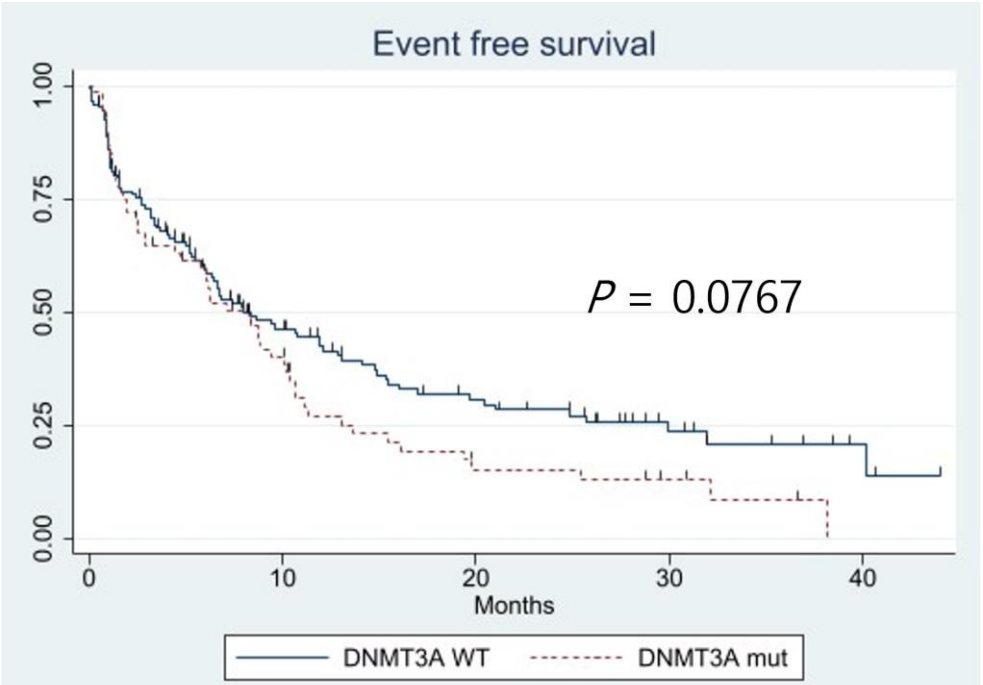
	No of mutation < 4	No of mutation ≥ 4
N	139	73
Median EFS	32.16	13.61
95% CI	0.3690-0.6142	0.3648-0.6040

Figure 5F. Event-free survival according to the number of mutations in the total Korean cohort. Abbreviation: EFS, event-free survival.



	DNMT3A WT	DNMT3A mut
N	146	69
Median OS	34.33	13.25
95% CI	0.3694-0.5994	0.3500-0.6056

Figure 6A. Overall survival according to the presence of *DNMT3A* mutation in the total Korean cohort. Abbreviation: OS, overall survival.



	DNMT3A WT	DNMT3A mut
N	146	69
Median EFS	8.328	8.262
95% CI	0.4028-0.5741	0.3597-0.5999

Figure 6B. Event-free survival according to the presence of *DNMT3A* mutation in the total Korean cohort. Abbreviation: EFS, event-free survival.

In multivariate analysis, age older than 65 years (HR 4.06, 95% CI, 2.6275-6.2747, P = 0.000), adverse risk group (HR 1.34, 95% CI, 1.0331-1.7386, P = 0.027), 1st line HMA treatment (HR 1.53, 95% CI, 1.3153-1.7976, P = 0.000) and the presence of *DNMT3A* mutation (HR 1.82, 95% CI, 1.1990-2.7651, P = 0.005) were associated with poor OS (Table 10B). Male was associated with longer OS (HR 0.52, 95%CI, 0.3431-0.8060, P = 0.003). Age older than 65 years (HR 2.23, 95% CI, 1.5861-3.1633, P = 0.000), adverse risk group (HR 1.51, 95% CI, 1.2391-1.8638, P = 0.000), and 1st line HMA treatment (HR 1.32, 95% CI, 1.1650-1.5158, P = 0.000) were poor risk factors in EFS also. (Table 11B).

Table 10A. Univariate analysis for OS in the total Korean cohort.

	HR	*P*	95% CI
Male	0.61	0.017	0.4126-0.9149
Age > 65	3.13	0.000	2.095-4.699
Adverse risk group	1.47	0.002	1.1591-1.8839
Extramedullary disease	0.78	0.519	0.3822-1.6254
No previous history of cancer	0.85	0.617	0.4545-1.5969
1st line HMA	1.31	0.0001	1.1156-1.5480
***DNMT3A* mutation**	2.12	0.000	1.4325-3.1651
Non-R882 mutation	1.20	0.050	0.9996-1.4471
Non-MTD mutation	0.84	0.272	0.6169-1.1456
More than 4 mutations	1.77	0.005	1.194-2.629

Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; HMA, hypomethylating agent;; HR, hazard ratio, MTD, methyltransferase domain; R882, arginine 882.

Table 10B. Multivariate analysis for OS in the total Korean cohort.

	HR	*P*	95% CI
Male	0.52	0.003	0.3431-0.8060
Age > 65	4.06	0.000	2.6275-6.2747
Adverse risk group	1.34	0.027	1.0331-1.7386
1st line HMA	1.53	0.000	1.3153-1.7976
***DNMT3A* mutation**	1.82	0.005	1.1990-2.7651

Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; HMA, hypomethylating agent; HR, hazard ratio, R882, arginine 882.

Table 11A. Univariate analysis for EFS in the total Korean cohort.

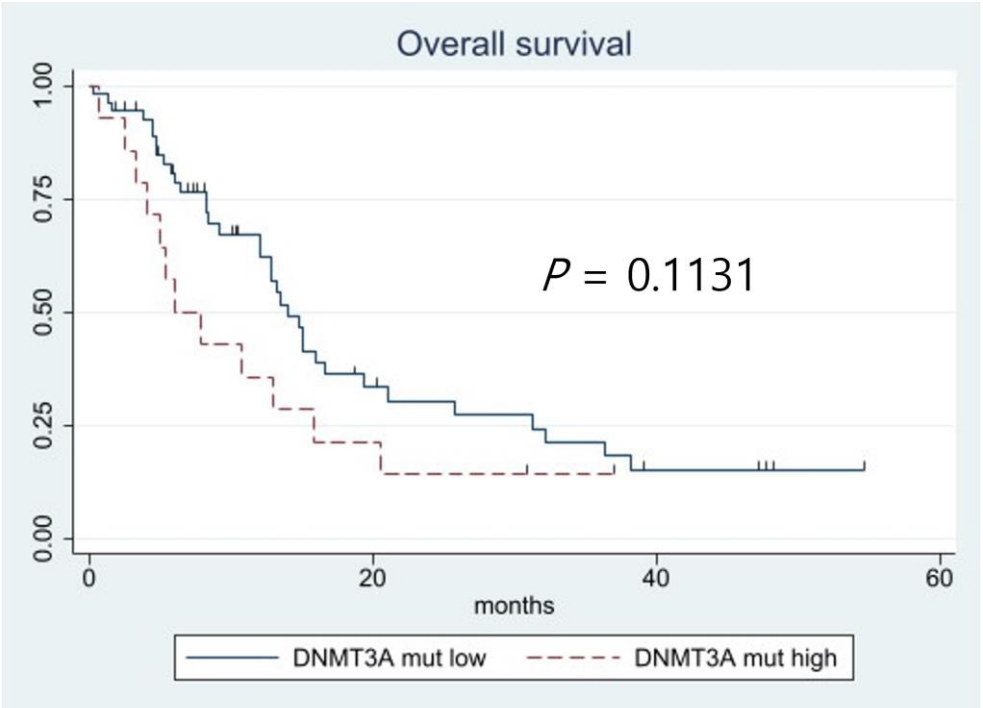
	HR	*P*	95% CI
Male	0.78	0.147	0.5659-1.0886
Age > 65	1.89	0.000	1.3625-2.689
Adverse risk group	1.55	0.000	1.2736-1.8948
Extramedullary disease	1.11	0.702	0.6406-1.9376
No previous history of cancer	1.25	0.388	0.7529-2.0753
1st line HMA	1.18	0.027	1.0194-1.3856
***DNMT3A* mutation**	1.37	0.064	0.9815-1.9271
Non-R882 mutation	1.06	0.497	0.8928-1.2630
Non-MTD mutation	0.66	0.034	0.4556-0.9693
More than 4 mutations	1.40	0.047	1.0041-1.9526

Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; HMA, hypomethylating agent; HSCT, hematopoietic stem cell transplantation; HR, hazard ratio, MTD, methyltransferase domain; R882, arginine 882.

Table 11B. Multivariate analysis for EFS in the total Korean cohort.

	HR	*P*	95% CI
Male	0.73	0.073	0.5218-1.0290
Age > 65	2.23	0.000	1.5861-3.1633
Adverse risk group	1.51	0.000	1.2391-1.8638
1st line HMA	1.32	0.000	1.1650-1.5158
***DNMT3A* mutation**	1.25	0.185	0.8959-1.7644

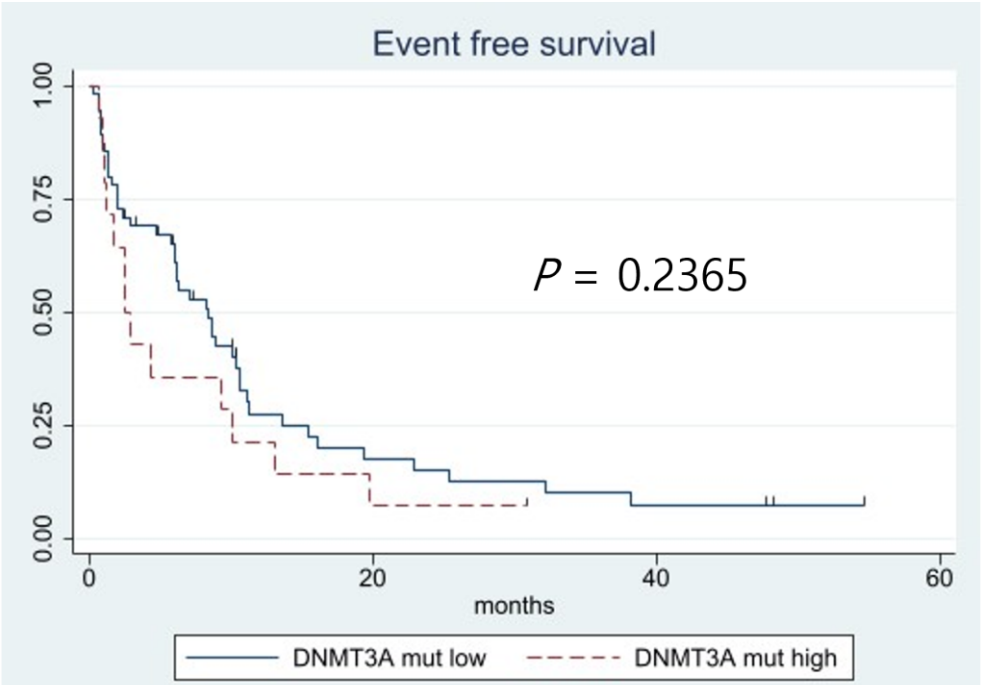
Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; HMA, hypomethylating agent; HR, hazard ratio; R882, arginine 882.



	DNMT3A mut low	DNMT3A mut high
N	54	12
Median OS	14.03	7.934
95% CI	0.3352-0.6302	0.1773-0.6604

Figure 7A. Overall survival according to VAF of *DNMT3A* mutation in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; OS, overall survival.

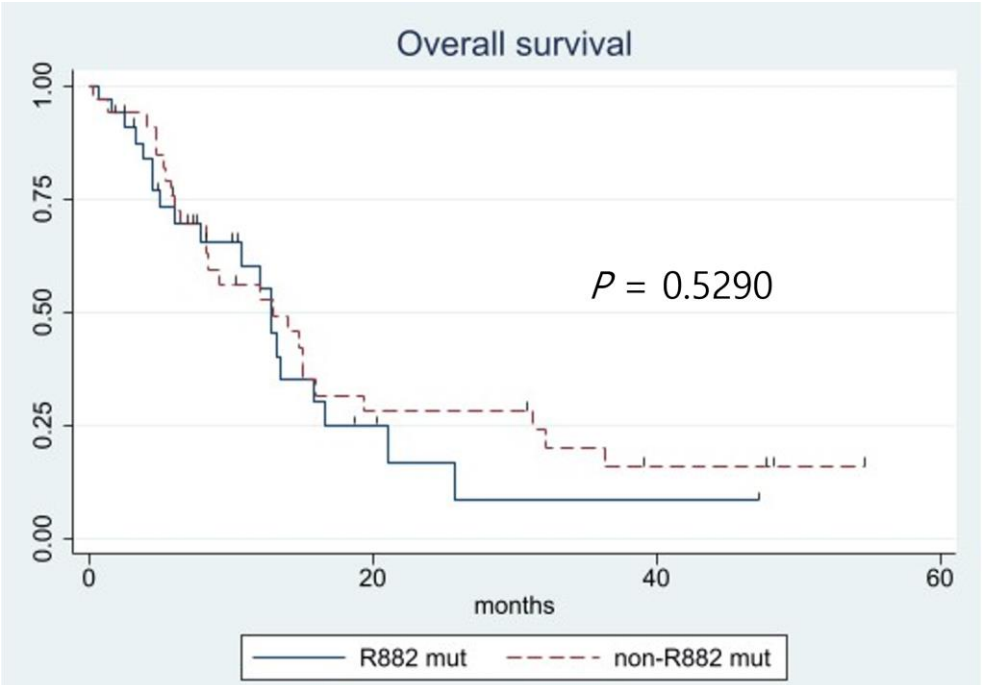
As presence of *DNMT3A* mutation was associated with poor OS, the further analysis among *DNMT3A* mutant group to find out more deeply associated factors. First, survival analysis between *DNMT3A* mutation low (VAF ≤ 47.6%) and *DNMT3A* mutation high (VAF > 47.6%) according to VAF was undergone. Significant differences in OS (P = 0.1131) and EFS (P = 0.2365) between the *DNMT3A* mutation low group and *DNMT3A* mutation high group were not observed. (Figure 7A, 7B)



	DNMT3A mut low	DNMT3A mut high
N	54	12
Median EFS	8.361	2.525
95% CI	0.3454-0.6145	0.2286-0.7221

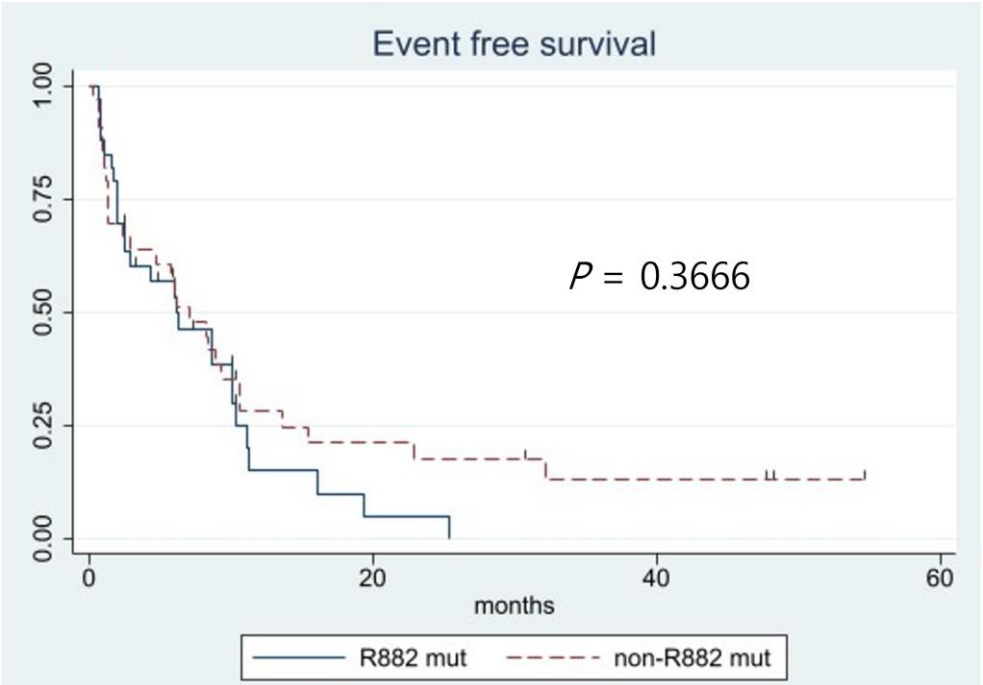
Figure 7B. Event-free survival according to VAF of *DNMT3A* mutation in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; EFS, event-free survival.

In the next step, the impact on survival of R882 mutations versus non-R882 mutations and MTD mutations versus non-MTD mutations was analyzed. Statistically significant survival differences according to mutation point or domain were not observed (Figure 8A, 8B and Figure 9A, 9B).



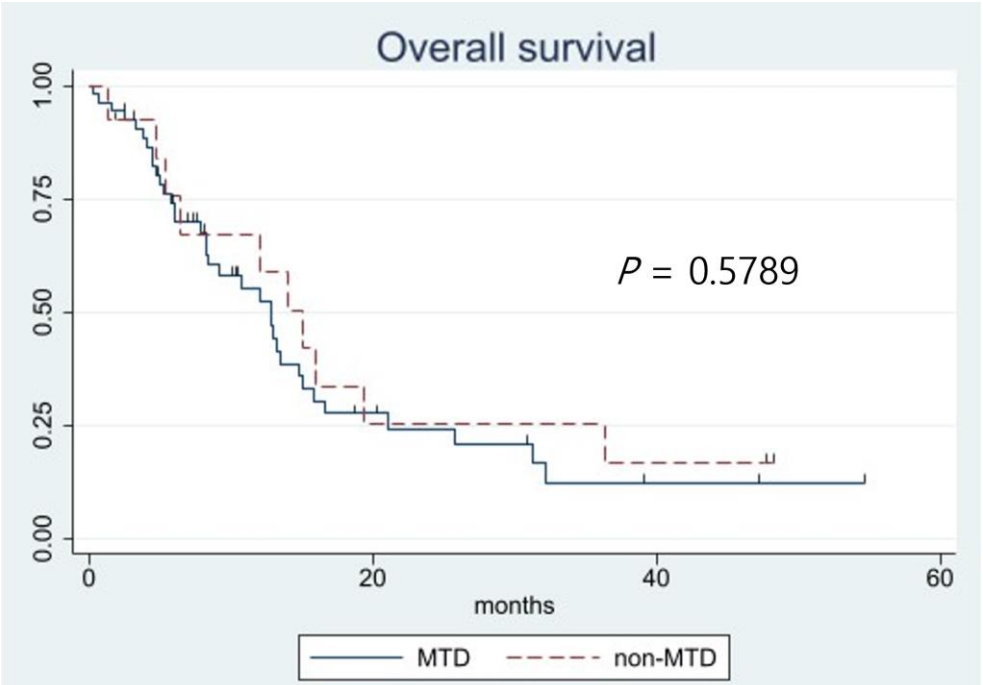
	R882	Non-R882
N	33	33
Median OS	12.89	12.95
95% CI	0.2463-0.6384	0.3081-0.6521

Figure 8A. Overall survival according to the presence of R882 mutation in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; OS, overall survival; R882, arginine 882.



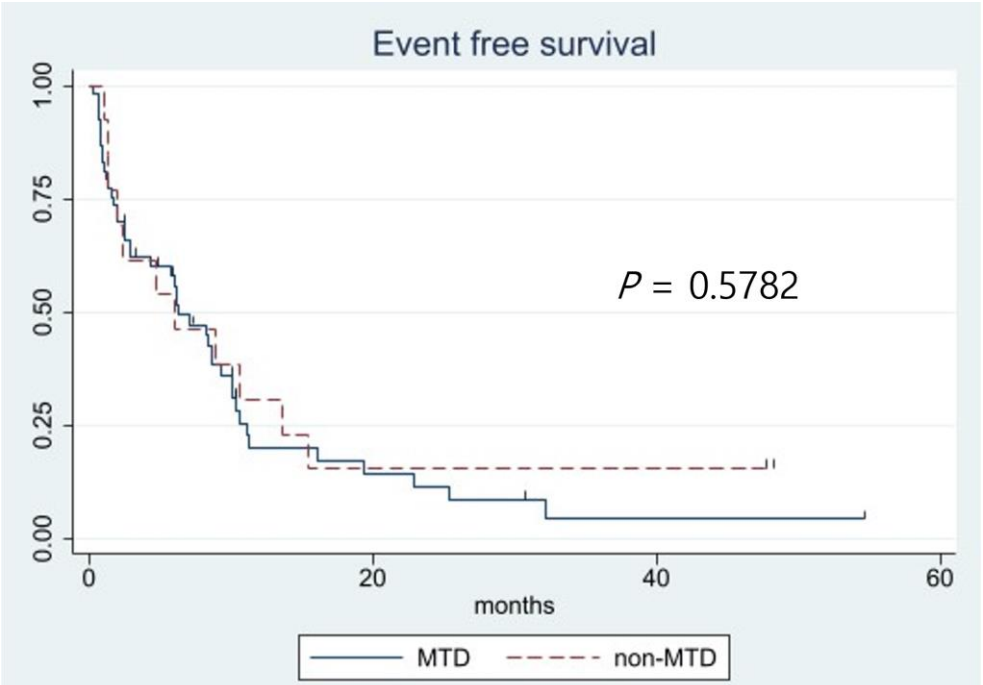
	R882	Non-R882
N	33	33
Median EFS	6.164	7.148
95% CI	0.3139-0.6565	0.3023-0.6371

Figure 8B. Event-free survival according to the presence of R882 mutation in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; EFS, event free survival; R882, arginine 882.



	MTD	Non-MTD
N	53	13
Median OS	12.89	14.03
95% CI	0.3140-0.6098	0.2110-0.7389

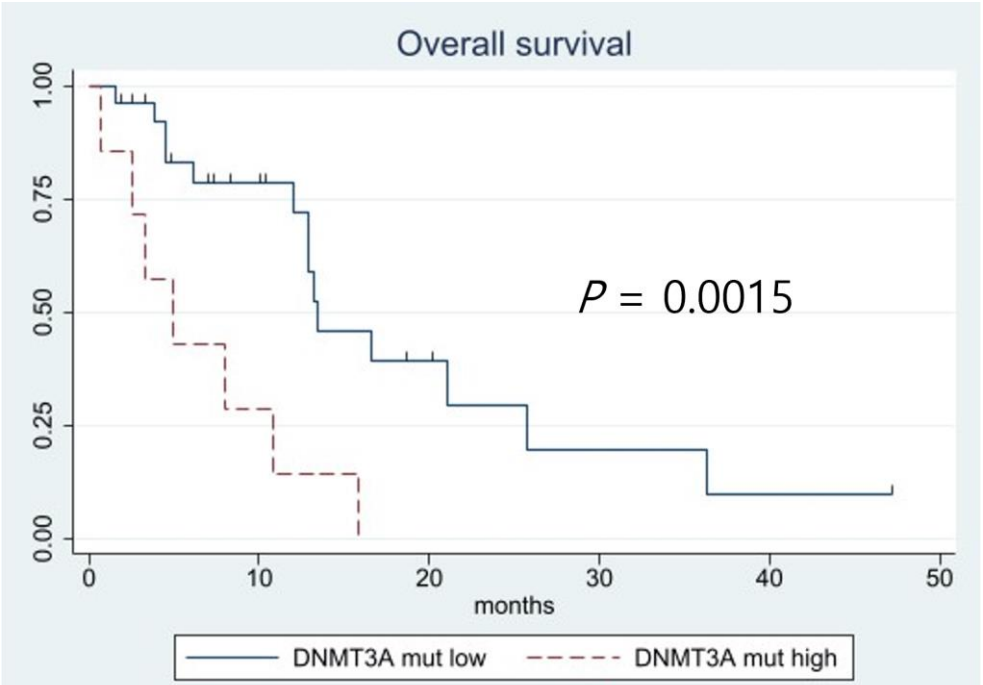
Figure 9A. Overall survival according to the presence of MTD mutation in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; MTD, methyltransferase domain; OS, overall survival.



	MTD	Non-MTD
N	53	13
Median EFS	6.295	6.033
95% CI	0.3504-0.6223	0.1916-0.6964

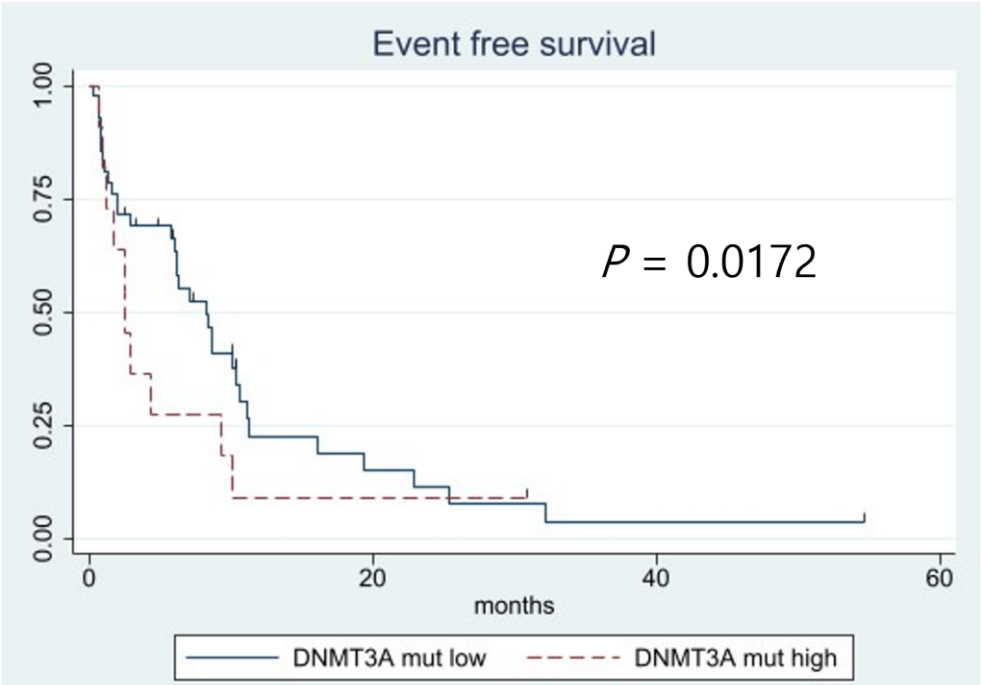
Figure 9B. Event-free survival according to the presence of MTD mutation in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; EFS, event-free survival; MTD, methyltransferase domain.

Next, survival was analyzed according to VAF in MTD mutation, R882 point mutation, and non-R882 mutation. High VAF at R882 point mutation (P = 0.0015) and MTD (P = 0.0284) presented longer OS. High VAF at non-R882 mutation did not show a positive impact on OS (P = 0.3377, Figure 10A, 11A, 12A).



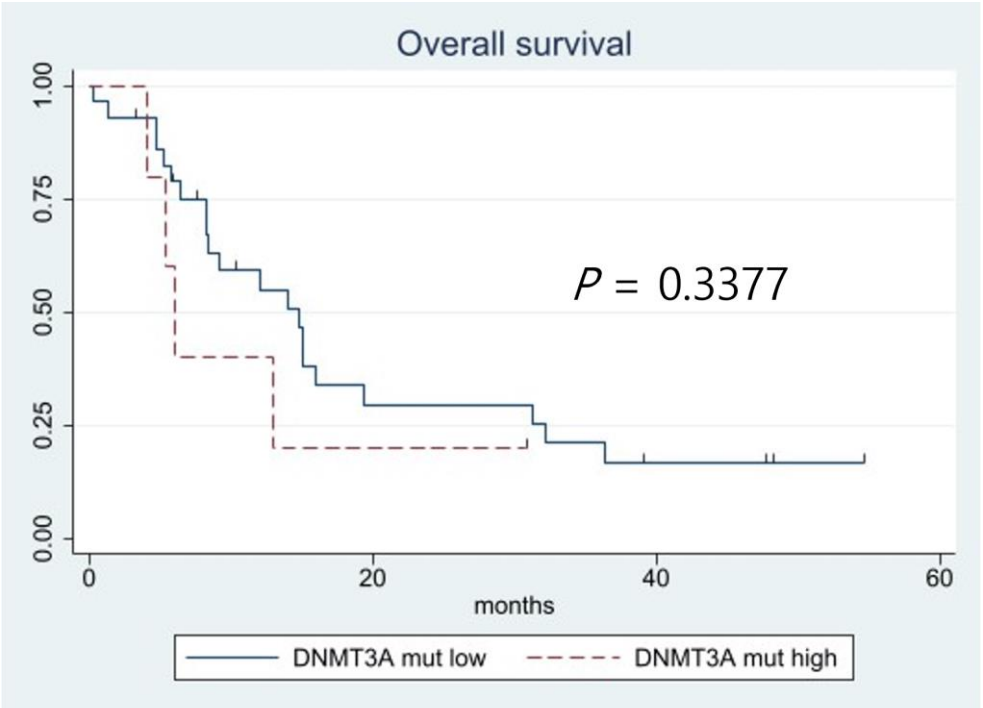
	DNMT3A mut low	DNMT3A mut high
N	26	7
Median OS	13.48	4.951
95% CI	0.2172-0.6710	0.0978-0.7344

Figure 10A. Overall survival according to VAF of R882 mutation in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; OS, overall survival; R882, arginine 882.



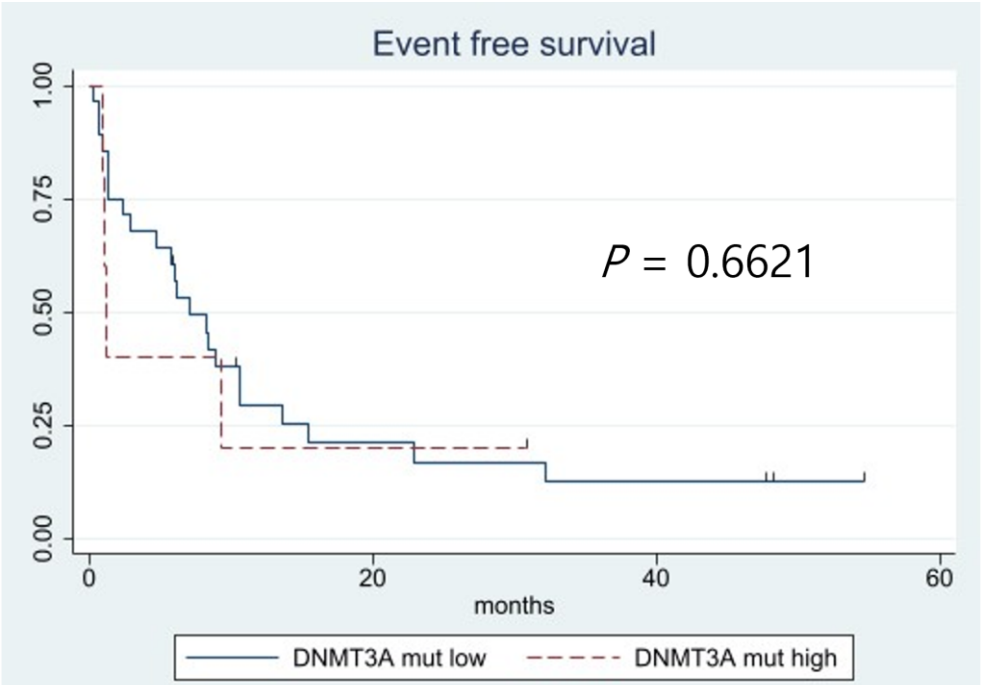
	DNMT3A mut low	DNMT3A mut high
N	26	7
Median EFS	8.721	2.525
95% CI	0.2449-0.6406	0.0978-0.7344

Figure 10B. Event-free survival according to VAF of R882 mutation in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; EFS, event free survival; R882, arginine 882.



	DNMT3A mut low	DNMT3A mut high
N	28	5
Median OS	14.85	6.033
95% CI	0.2669-0.6422	0.0520-0.7528

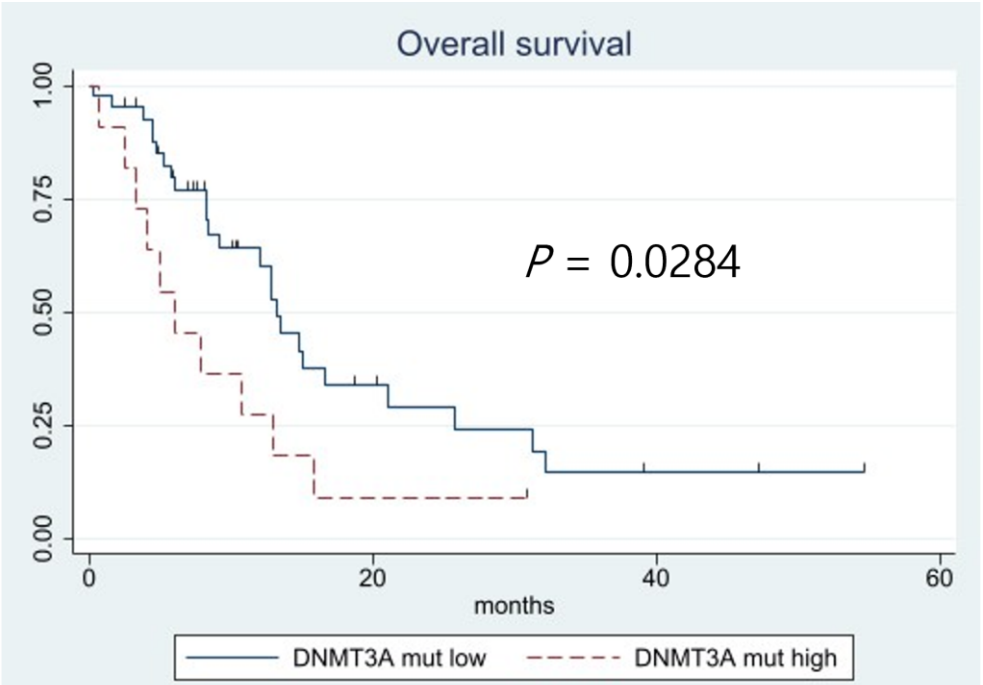
Figure 11A. Overall survival according to VAF of non-R882 mutation in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; OS, overall survival; R882, arginine 882.



	DNMT3A mut low	DNMT3A mut high
N	28	5
Median EFS	7.148	1.18
95% CI	0.2984-0.6617	0.0520-0.7528

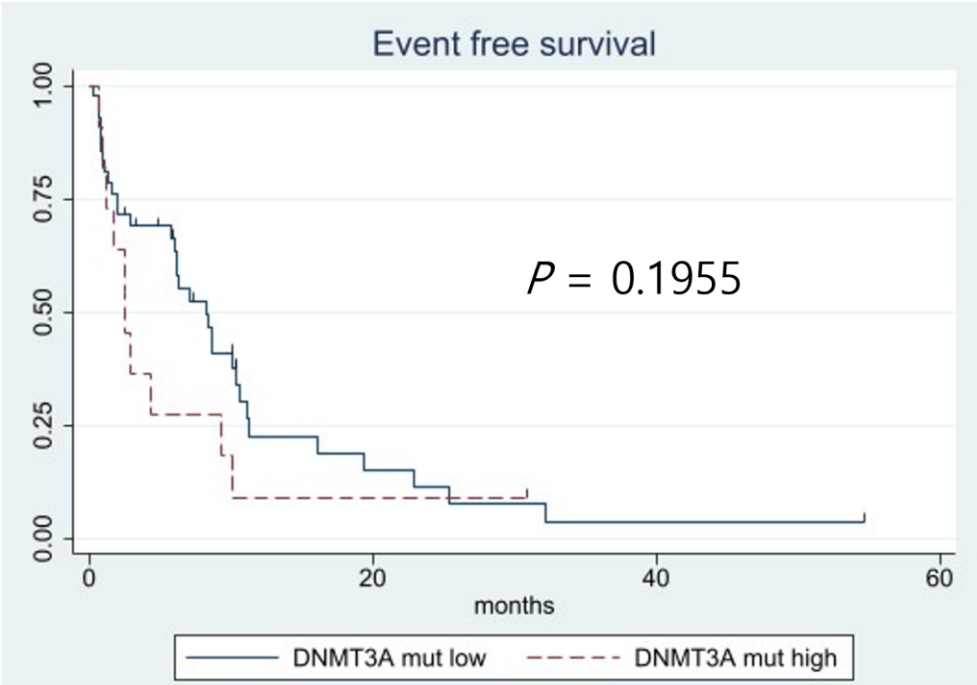
Figure 11B. Event-free survival according to VAF of non-R882 mutation in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; EFS, event free survival; R882, arginine 882.

Higher VAF in R882 point mutation was associated with poor EFS (P = 0.0172). However, significant differences were not observed according to VAF in MTD mutation (P = 0.1955) or non-R882 point mutation (P = 0.6621, Figure 10B, 11B, 12B).



	DNMT3A mut low	DNMT3A mut high
N	42	11
Median OS	13.25	6.033
95% CI	0.3055-0.6510	0.1666-0.7069

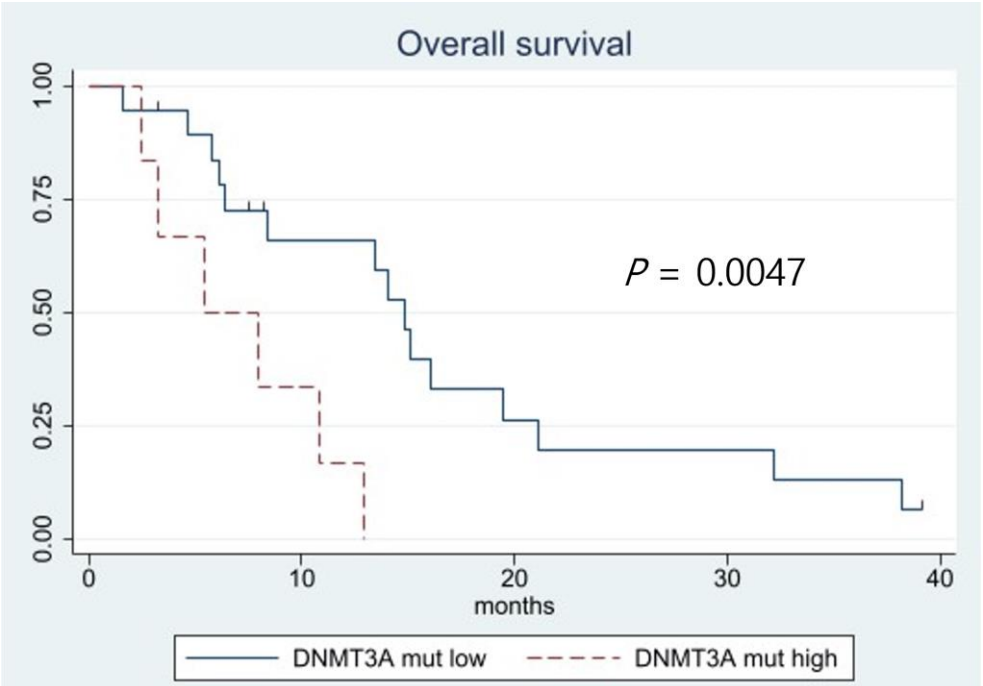
Figure 12A. Overall survival according to VAF of MTD mutation in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; MTD, methyltransferase domain; OS, overall survival.



	DNMT3A mut low	DNMT3A mut high
N	42	11
Median EFS	8.262	2.525
95% CI	0.3309-0.6408	0.1666-0.7069

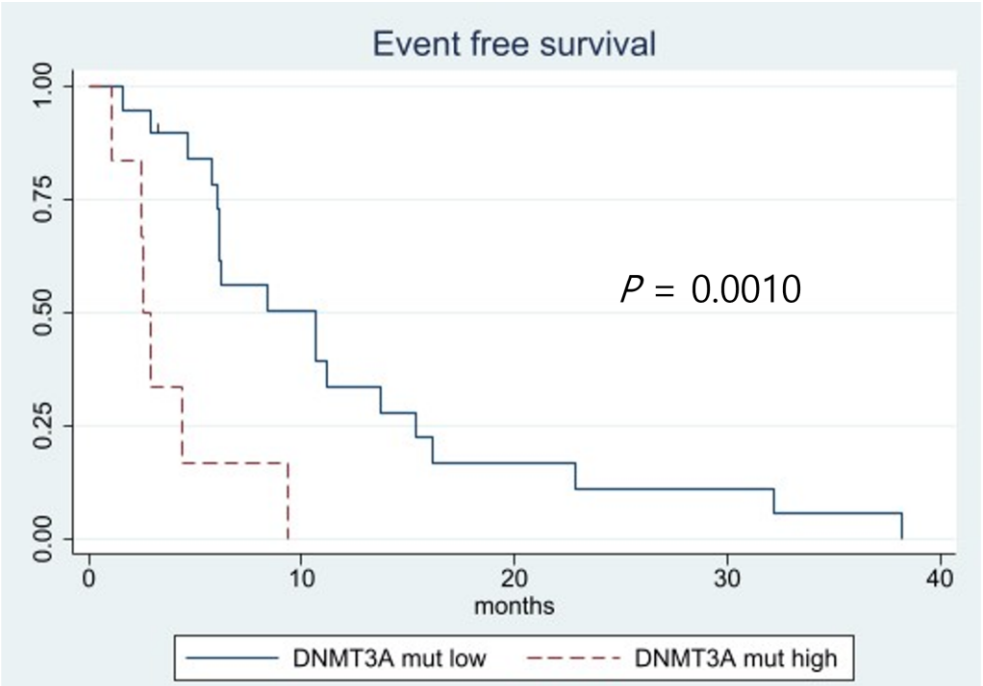
Figure 12B. Event-free survival according to VAF of MTD mutation in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; EFS, event-free survival; MTD, methyltransferase domain.

DNMT3A mediates DNA methylation to regulate epigenetic modification of gene expression [20, 29]. Aberration of DNA methylation can result in DNA hypermethylation and inhibition of tumor suppressor genes [1]. Thus, the impact of HMA on survival according to *DNMT3A* mutation status was evaluated. Twenty-five patients received initial HMA treatment. One patients with an unknown mutation spot was not included in the survival analysis. Patients who received initial intensive chemotherapy (n = 42) were excluded because their young and fit condition could be a bias. OS (P = 0.0047) and EFS (P = 0.0010) were longer in the *DNMT3A* mutation low group than the *DNMT3A* mutation high group treated with initial HMA therapy (Figure 13A, 13B).



	DNMT3A mut low	DNMT3A mut high
N	18	6
Median OS	14.85	5.443
95% CI	0.21569-0.6765	0.1109-0.8037

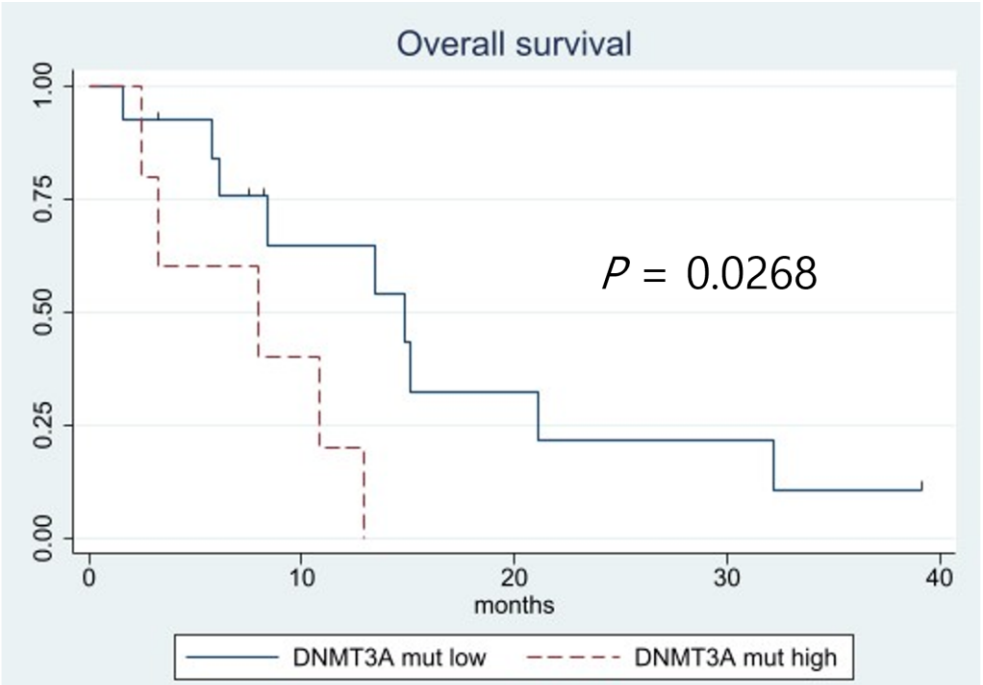
Figure 13A. Overall survival according to VAF of *DNMT3A* mutation in patients who received initial HMA treatment in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; HMA, hypomethylating agent; OS, overall survival.



	DNMT3A mut low	DNMT3A mut high
N	18	6
Median EFS	10.66	2.525
95% CI	0.2180-0.6539	0.1109-0.8037

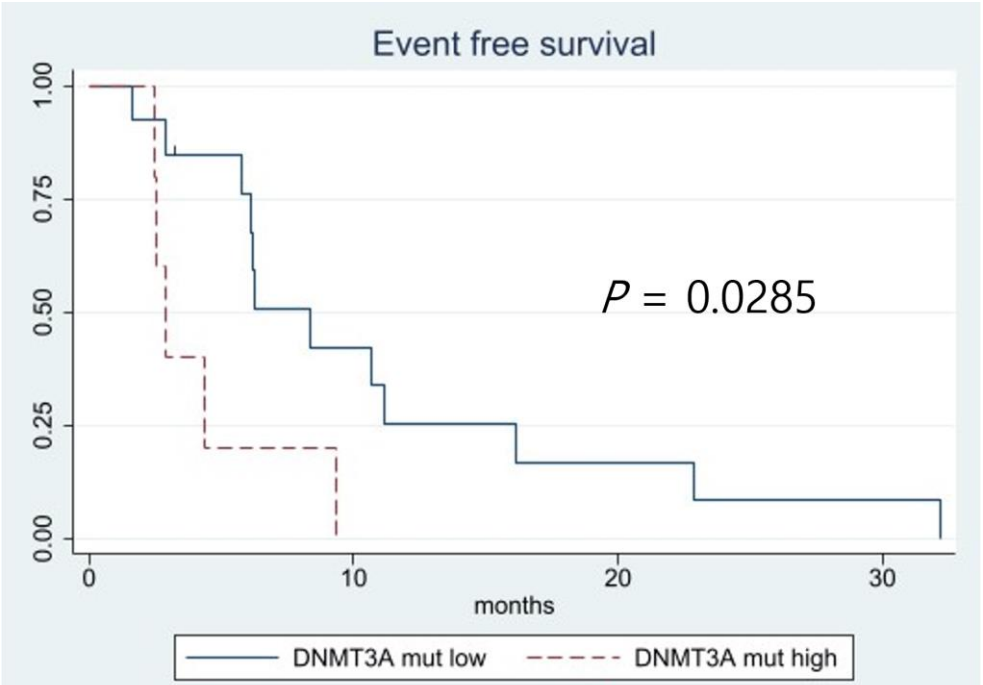
Figure 13B. Event-free survival according to VAF of *DNMT3A* mutation in patients who received initial HMA treatment in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; EFS, event-free survival; HMA, hypomethylating agent.

Among initial HMA-treated patients, OS by VAF in MTD mutation presented longer in the *DNMT3A* mutation low group (P = 0.0268, Figure 14A). R882 point mutation with high VAF treated with HMA did not significantly impact survival. (P = 0.1630, Figure 15A). However, in EFS analysis, both MTD (P = 0.0285, Figure 14B) or R882 (P = 0.0276, Figure 15B) *DNMT3A* mutation low group treated with initial HMA therapy presented superior results.



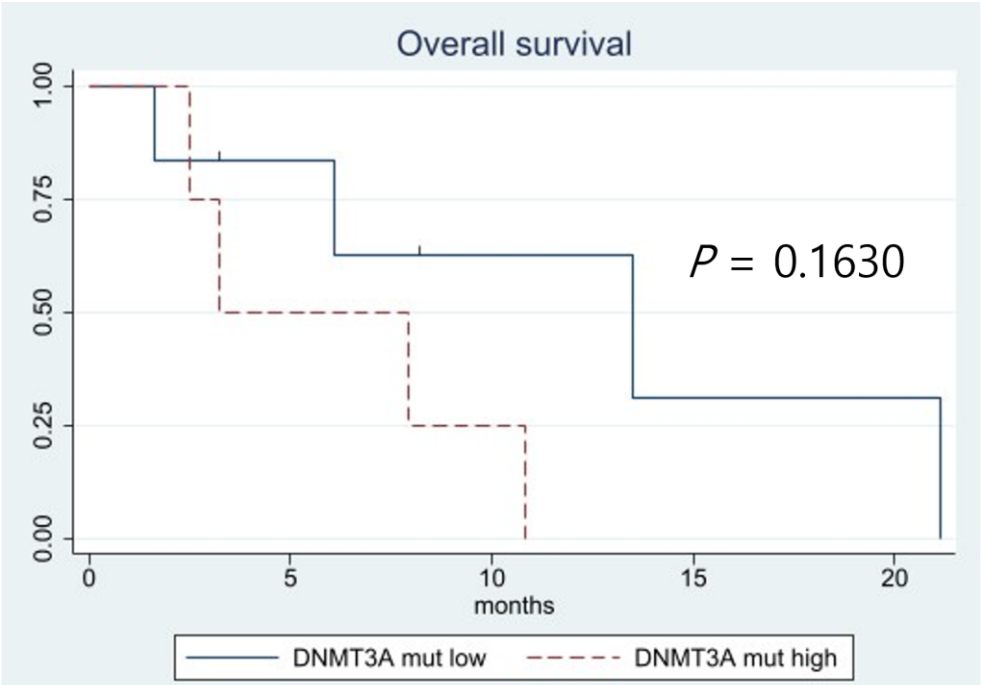
	DNMT3A mut low	DNMT3A mut high
N	13	5
Median OS	14.85	7.934
95% CI	0.1392-0.6990	0.0520-0.7528

Figure 14A. Overall survival according to VAF of MTD mutation in patients who received initial HMA treatment in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; HMA, hypomethylating agent; MTD, methyltransferase domain; OS, overall survival.



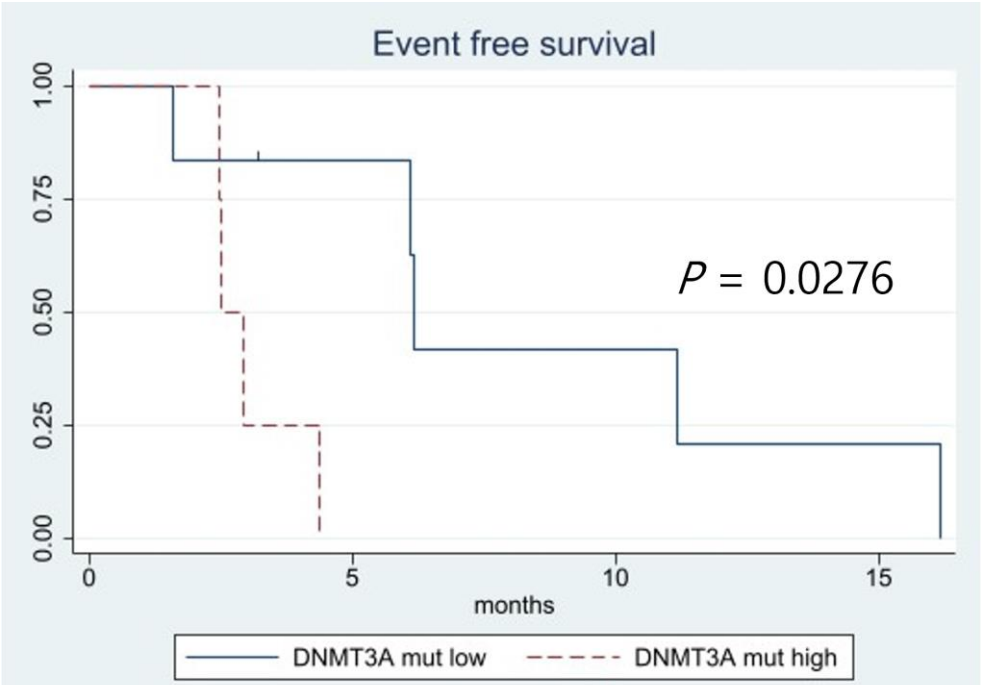
	DNMT3A mut low	DNMT3A mut high
N	13	5
Median EFS	8.361	2.918
95% CI	0.1562-0.6713	0.0520-0.7528

Figure 14B. Event-free survival according to VAF of MTD mutation in patients who received initial HMA treatment in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; EFS, event-free survival; HMA, hypomethylating agent; MTD, methyltransferase domain.



	DNMT3A mut low	DNMT3A mut high
N	6	4
Median EFS	1.348	3.246
95% CI	0.0127-0.7337	0.0578-0.8449

Figure 15A. Overall survival according to VAF of R882 mutation in patients who received initial HMA treatment in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; HMA, hypomethylating agent; OS, overall survival; R882, arginine 882.



	DNMT3A mut low	DNMT3A mut high
N	6	4
Median EFS	6.164	2.525
95% CI	0.0560-0.7665	0.0578-0.8449

Figure 15B. Event-free survival according to VAF of R882 mutation in patients who received initial HMA treatment in *DNMT3A* mutant Korean patients. Cases with unknown mutation loci were excluded. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; EFS, event-free survival; HMA, hypomethylating agent; R882, arginine 882.

In multivariate analysis, *DNMT3A* mutation high (HR 2.28, 95% CI, 1.1094-4.6950, $P = 0.025$), and the non-R882 mutation (HR 0.62, 95% CI, 0.3987-0.9715, $P = 0.037$) were associated with OS. (Table 12A, 12B)

Table 12A. Univariate analysis for OS in *DNMT3A* mutant Korean cohort.

	HR	*P*	95% CI
Male	0.73	0.304	0.4151-1.3150
Age > 65	2.00	0.019	1.1189-3.5981
Adverse risk group	1.19	0.329	0.8377-1.6959
Extramedullary disease	3.09	0.070	0.9124-10.4941
No previous history of cancer	0.85	0.711	0.3843-1.9199
1st line HMA	0.62	0.117	0.3470-1.1252
***DNMT3A* mut VAF > 47.6%**	1.69	0.118	0.8751-3.2744
Non-R882 mutation	0.72	0.123	0.4891-1.0890
Non-MTD mutation	0.73	0.139	0.4903-1.1044

Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; HMA, hypomethylating agent; HSCT, hematopoietic stem cell transplantation; HR, hazard ratio, MTD, methyltransferase domain; R882, arginine 882; VAF, variant allele frequency.

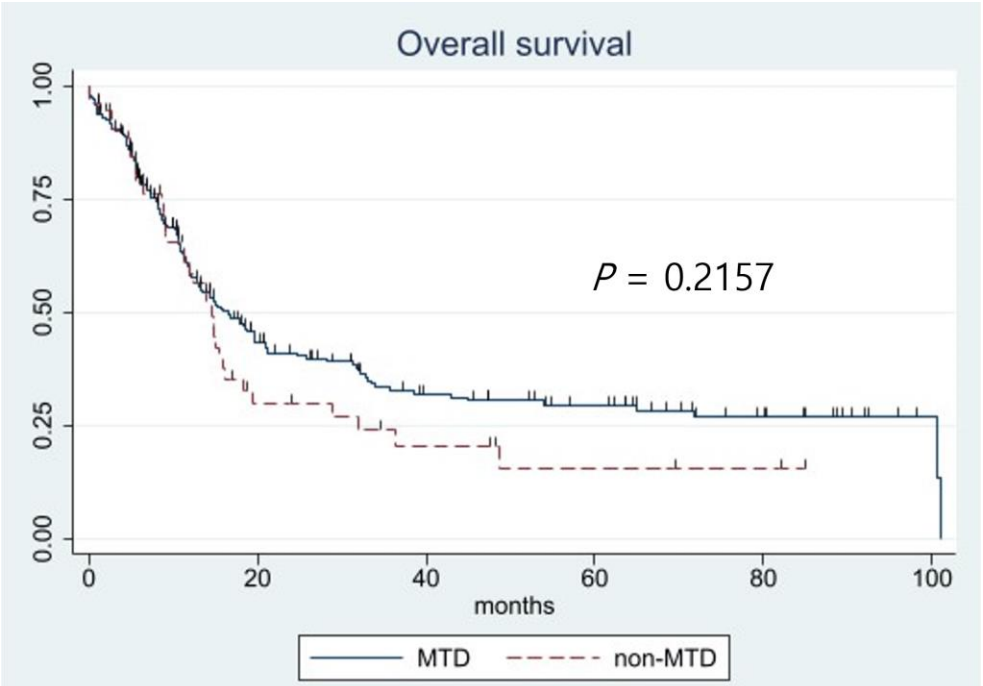
Table 12B. Multivariate analysis for OS in *DNMT3A* mutant Korean cohort.

	HR	*P*	95% CI
Age > 65	1.88	0.129	0.8323-4.2582
***DNMT3A* mut VAF > 47.6%**	2.28	0.025	1.1094-4.6950
Non-R882 mutation	0.62	0.037	0.3987-0.9715

Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; HR, hazard ratio; R882, arginine 882; VAF, variant allele frequency.

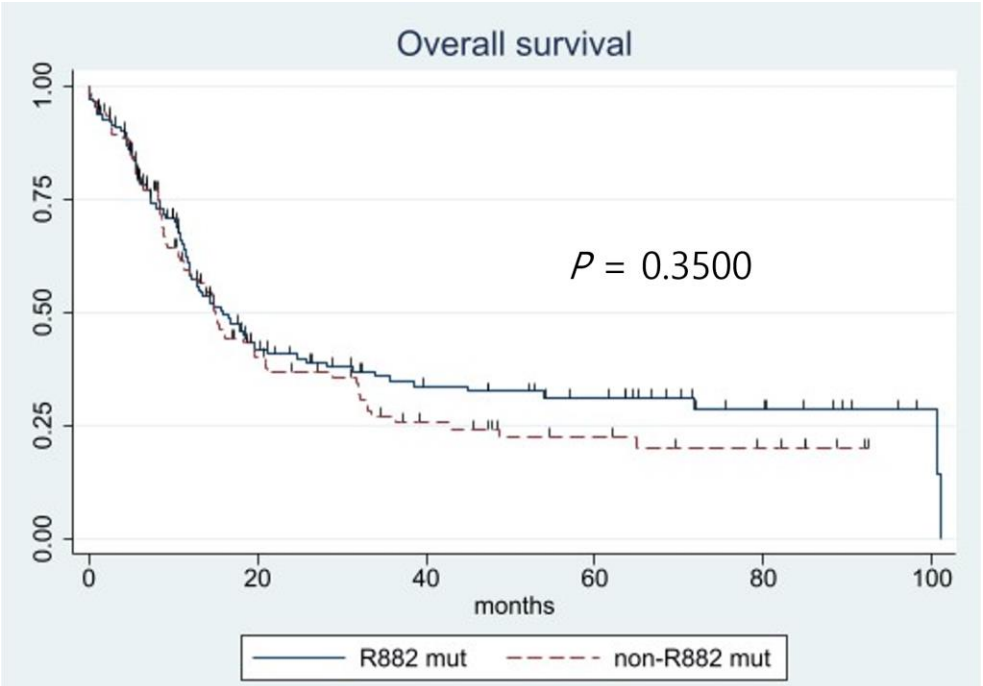
Combined dataset

In combined data, the presence of MTD mutation or R882 mutation was not enough to show survival differences (Figure 16A, 16B).



	MTD	Non-MTD
N	247	57
Median OS	16.62	14.09
95% CI	0.4260-0.5617	0.3423-0.6265

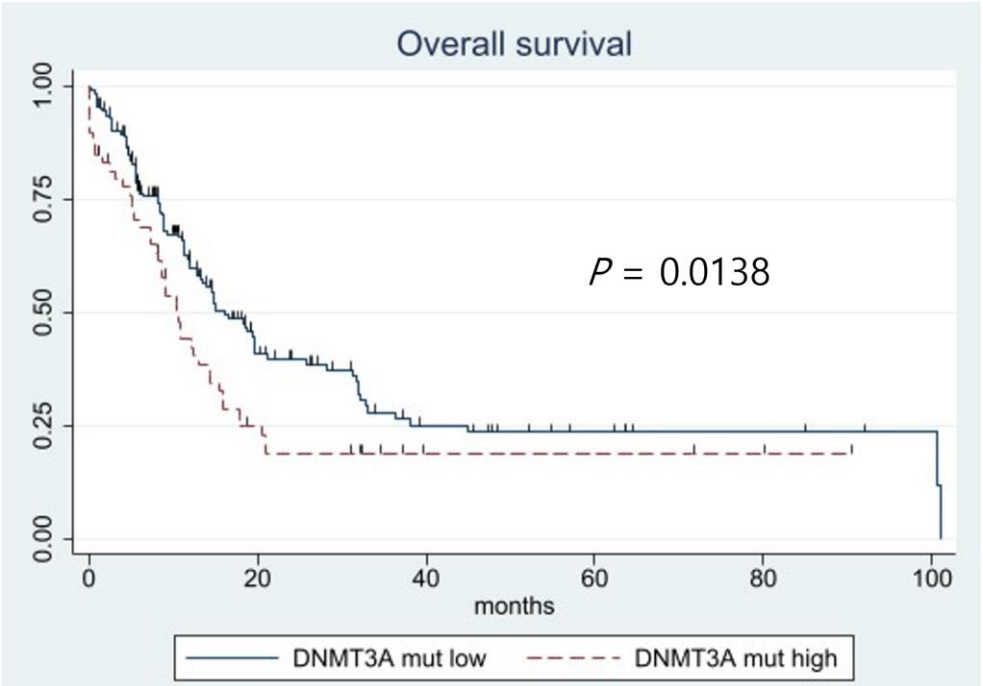
Figure 16A. OS in combined datasets according to the presence of MTD mutation. Unanalyzable survival (n=9) and unknown mutation location (n=11) cases were excluded. The combined datasets consisted of 69 patients from Korean, 75 from USA2018, 108 from USA2022, and 63 from German2020. Abbreviation: MTD, methyltransferase domain; OS, overall survival.



	R882 mut	Non-R882 mut
N	179	125
Median OS	15.87	15.08
95% CI	0.4131-0.5746	0.3956-0.5835

Figure 16B. OS in combined datasets according to the presence of R882 mutation. Unanalyzable survival (n=9) and unknown mutation location (n=11) cases were excluded. The combined datasets consisted of 69 patients from Korean, 75 from USA2018, 108 from USA2022, and 63 from German2020. Abbreviation: R882, arginine 882; OS, overall survival.

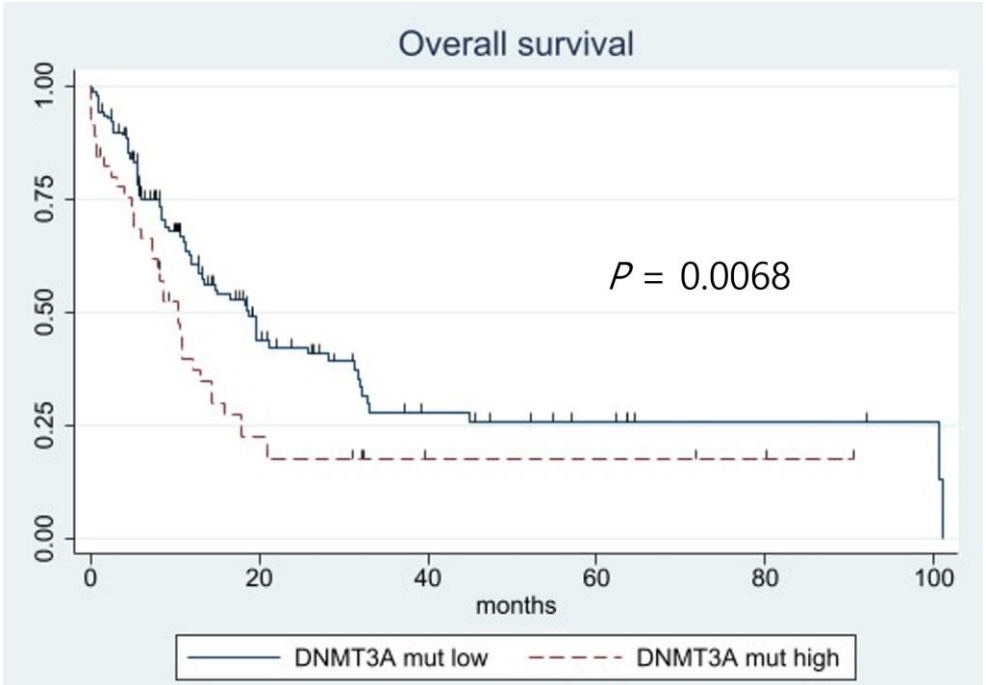
However, in the analysis of VAF, the *DNMT3A* mutation low group showed better survival (P = 0.0138, Figure 17).



	DNMT3A mut low	DNMT3A mut high
N	183	61
Median OS	16.03	10.49
95% CI	0.4078-0.5739	0.3613-0.6220

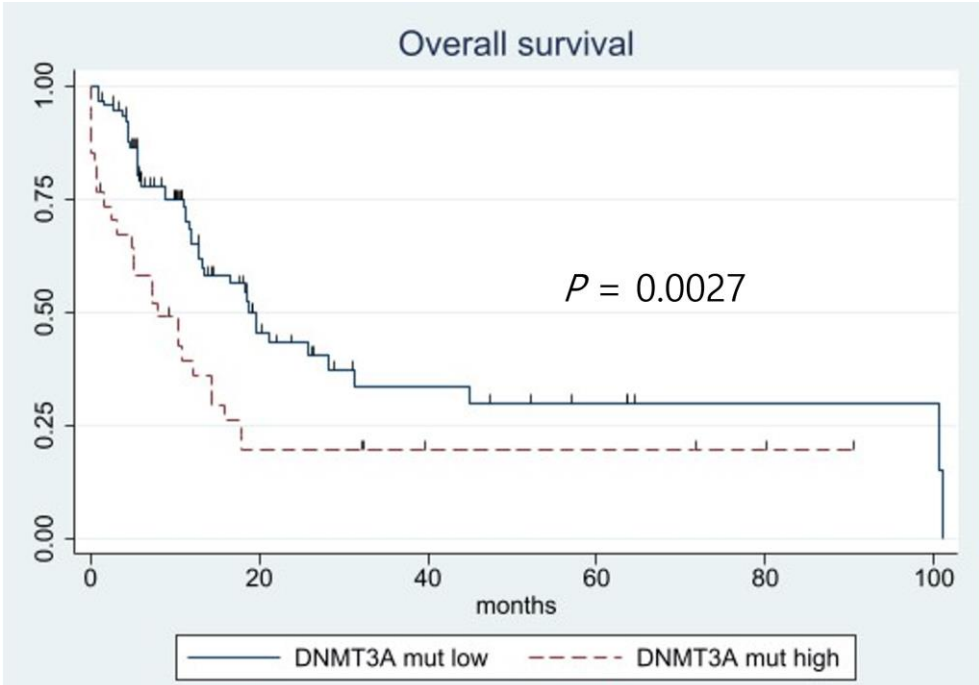
Figure 17. OS in combined datasets according to VAF of *DNMT3A* mutation. Unanalyzable survival (n=9) and unknown mutation location (n=11) cases were excluded. The combined datasets consisted of 69 patients from Korean, 75 from USA2018, 108 from USA2022, and 63 from German2020. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; OS, overall survival; VAF, variant allele frequency.

In patients with MTD mutation (P = 0.0068) or R882 point mutation (P = 0.0027), the *DNMT3A* mutation low group also lived longer than the *DNMT3A* mutation high group (Figure 18A, 18B).



	DNMT3A mut low	DNMT3A mut high
N	140	45
Median OS	18.68	10.46
95% CI	0.3900-0.5822	0.3421-0.6345

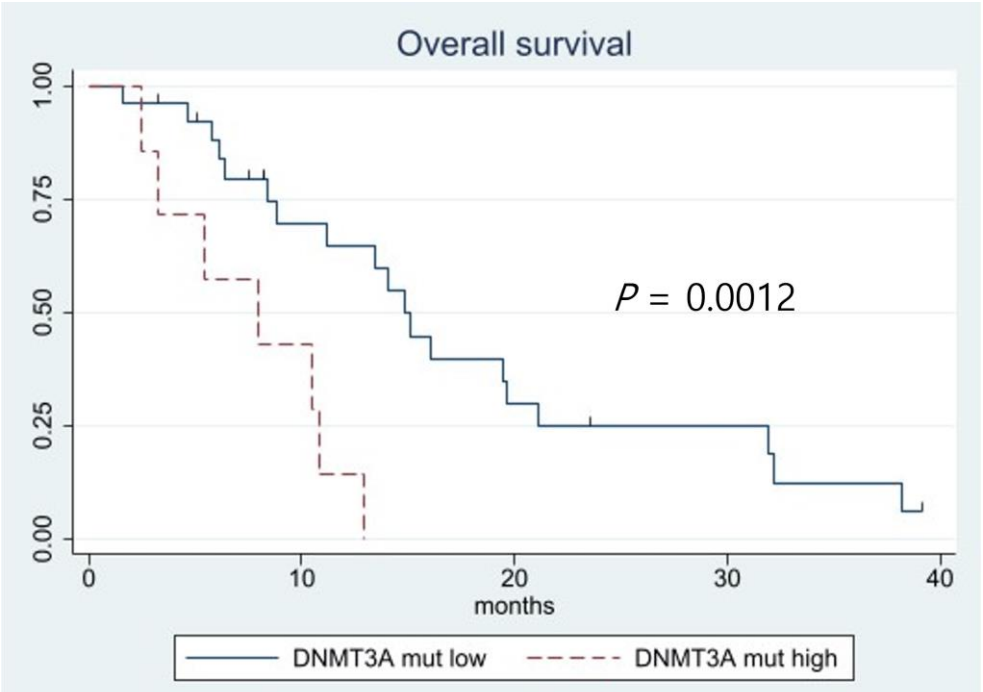
Figure 18A. OS in combined datasets according to VAF of MTD mutation. Unanalyzable survival (n=9) and unknown mutation location (n=11) cases were excluded. The combined datasets consisted of 69 patients from Korean, 75 from USA2018, 108 from USA2022, and 63 from German2020. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; MTD, methyltransferase domain; OS, overall survival; VAF, variant allele frequency.



	DNMT3A mut low	DNMT3A mut high
N	94	34
Median OS	19.57	7.934
95% CI	0.3245-0.5773	0.3125-0.6447

Figure 18B. OS in combined datasets according to VAF of R882 mutation. Unanalyzable survival (n=9) and unknown mutation location (n=11) cases were excluded. The combined datasets consisted of 69 patients from Korean, 75 from USA2018, 108 from USA2022, and 63 from German2020. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; OS, overall survival; R882, arginine 882; VAF, variant allele frequency.

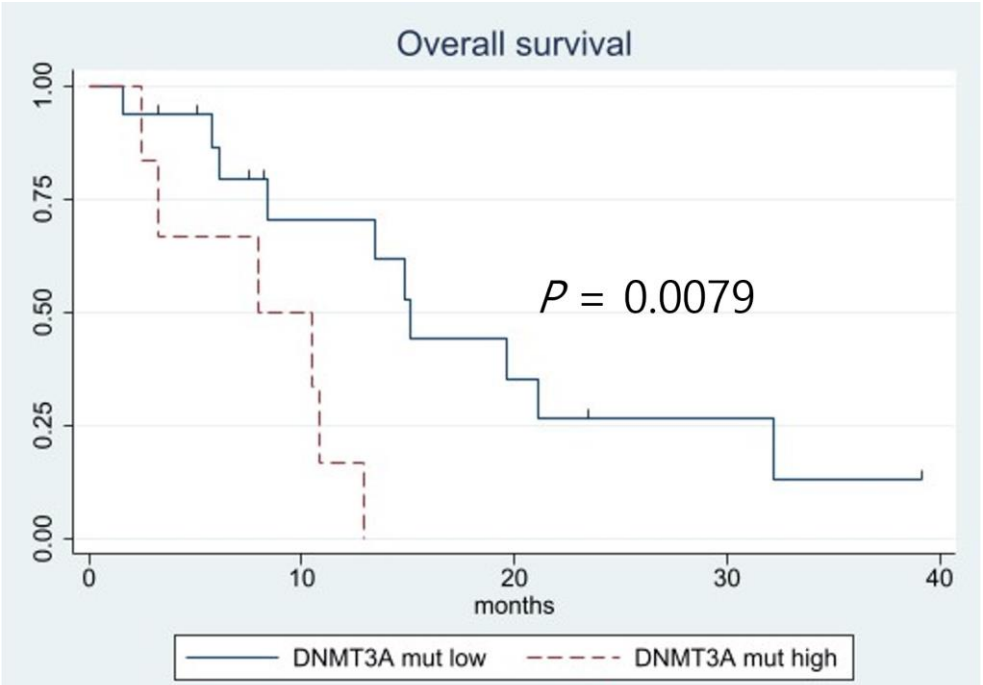
Initial HMA therapy resulted in better survival in the *DNMT3A* mutation low group in combined data, similar to that in Korean data (P = 0.0012, Figure 19).



	DNMT3A mut low	DNMT3A mut high
N	26	7
Median OS	14.85	7.934
95% CI	0.2748-0.6855	0.0978-0.7344

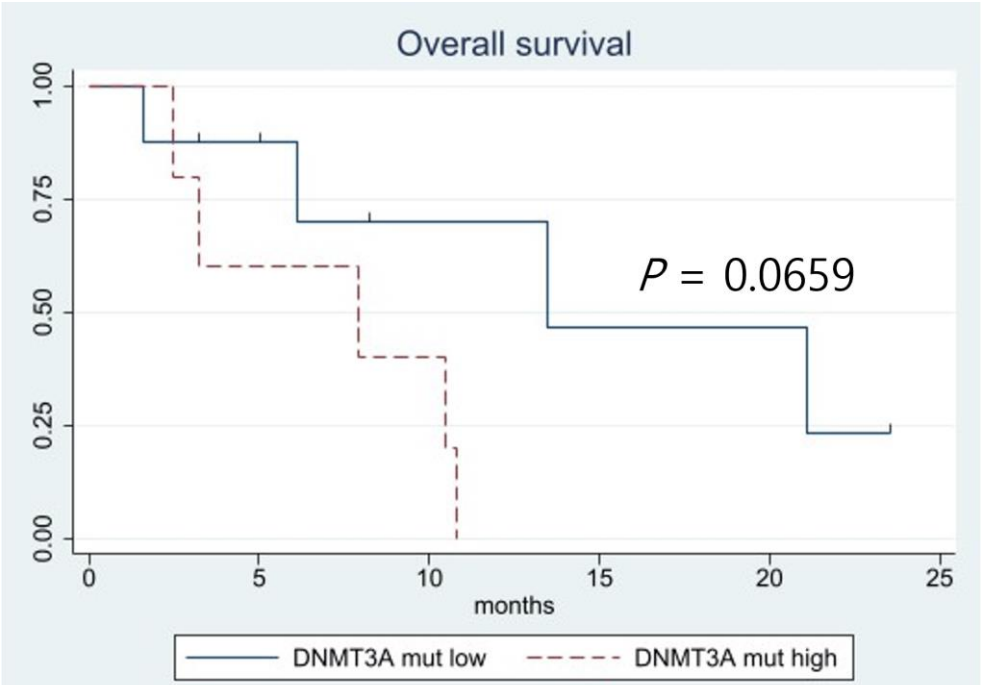
Figure 19. Impact of initial HMA on OS according to VAF of *DNMT3A* mutation in combined dataset (n=33). Treatment analysis was available in Korean and USA2018. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; HMA, hypomethylating agent; OS, overall survival; VAF, variant allele frequency.

Although the number of cases was small, patients who had mutations in MTD treated by HMA had longer survival when their *DNMT3A* mutation VAFs were low (P = 0.0079). The impact of HMA in R882 point mutation with high VAF treated was not statistically significant (P = 0.0659, Figure 20A, 20B).



	DNMT3A mut low	DNMT3A mut high
N	17	6
Median OS	15.08	7.934
95% CI	0.1666-0.6875	0.1109-0.8037

Figure 20A. Impact of initial HMA on OS according to VAF of MTD mutation in combined dataset (n=23). Treatment analysis was available in Korean and USA2018. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; HMA, hypomethylating agent; MTD, methyltransferase domain; OS, overall survival; VAF, variant allele frequency.



	DNMT3A mut low	DNMT3A mut high
N	9	5
Median EFS	1.348	7.934
95% CI	0.0708-0.8030	0.0520-00.7528

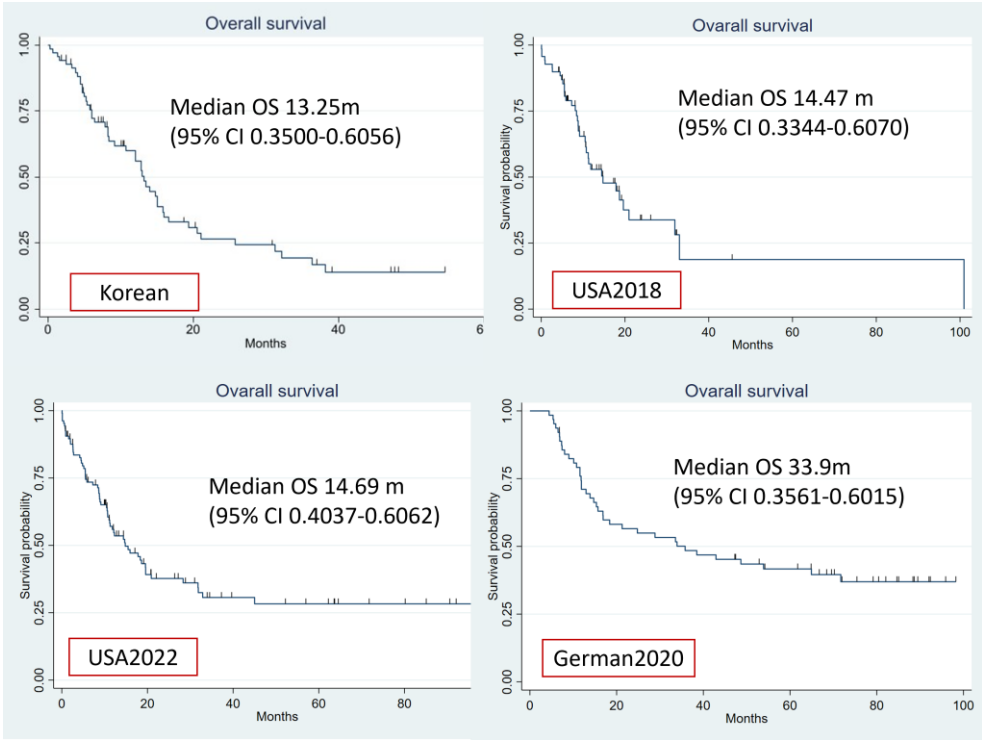
Figure 20B. Impact of initial HMA on OS according to VAF of R882 mutation in combined dataset (n=14). Treatment analysis was available in Korean and USA2018. Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; HMA, hypomethylating agent; OS, overall survival; R882, arginine 882; VAF, variant allele frequency.

In multivariate analysis, age older than 65 years (HR 1.62, 95% CI, 1.1570-2.2699, P = 0.005), the adverse risk group by ELN2017 criteria (HR 1.38, 95% CI, 1.1263-1.7049, P = 0.002) and DNMT3A mutation high (HR 1.61, 95% CI, 1.1245-2.3238, P = 0.009) was associated with inferior survival after stratified by first-line treatment and response. (Table 13)

Table 13. Multivariate analysis for OS in *DNMT3A* mutant combined cohort.

	HR	***P***	**95% CI**
Age > 65	1.62	0.005	1.1570-2.2699
Adverse risk group	1.38	0.002	1.1263-1.7049
***DNMT3A* mut VAF > 47.6%**	1.61	0.009	1.1245-2.3238

Abbreviation: *DNMT3A*, deoxynucleic acid methyltransferase 3A; VAF, variant allele frequency.



Supplementary figure. Median OS in *DNMT3A* mutation patients of each dataset. Korean n=69, USA2018 n=75, USA2022 n=108, German2020 n=63.

Discussion

In this study, I investigated *DNMT3A* mutation patterns in Korean AML patients and their clinical features. Mutation of *DNMT3A* is one of the common genetic alterations in cancer. Changes from arginine 882 to other amino acids are the most common mutations in hematologic malignancies, with a prevalence of around 60%-70% [6, 7, 20]. In this study, the prevalence of *DNMT3A* R882 mutation in Korean was not as high as in Western (P = 0.039). In panel sequencing, a point mutation at R882 of *DNMT3A* gene was observed in 41.7%, which is similar to the frequency of R882 cases in the clinical database (47.8%).

The presence of R882 mutation has been reported to be an adverse prognostic factor that could imply a lower prevalence of R882 mutation in Korean patients could act as a favorable prognostic factor for survival [29, 30, 32]. However, the median OS in Korean patients was similar to that in Western patients (Supplementary Figure). In that, the mutation composition of AML that could affect prognosis might be different between Korea and other countries. R882 residue is contained in the methyltransferase domain (MTD) [33]. Because the mutation prevalence at MTD was not different between Korean and Western data (P = 0.145), a hypothesis was suggested that mutation occurring at *DNMT3A* MTD might be a factor associated with prognosis in AML. This hypothesis was tested with the Korean dataset and expanded to combined datasets. First, treatment response and survival were analyzed in the total Korean AML cohort, including *DNMT3A* WT patients and *DNMT3A* mutant patients. As well as conventional prognostic factors including age older than 65 years, poor risk group by ELN2017

criteria, and first line HMA treatment rather than intensive chemotherapy, the presence of *DNMT3A* mutation also was an adverse prognostic factor for OS. To investigate the clinical meaning of *DNMT3A* in a more detailed way, mutated allele frequency and location were the topics of interest. For this reason, *DNMT3A* mutant group was sorted out from the total Korean AML cohort. In OS analysis, high VAF at R882 point mutation and MTD mutation had an adverse impact on survival. For patients who received HMA as the first-line treatment, high allele frequency of total *DNMT3A* mutation and MTD mutation were adverse factors for OS and EFS. High VAF of R882 mutation presented significantly shorter EFS in initial HMA-treated patients. Treatment responses to HMA were inferior in groups with high VAF of DNMT3A mutation and R882 mutation, however, the evaluable number of patients were small. HMA response in MTD mutation high group was not statistically significant even though the number of patients was very small.

Because limitations existed due to the small number of each group, Korean dataset was combined with the Western public dataset to validate the role of *DNMT3A* in a larger dataset. In the combined dataset, the negative impact of high allele frequency of total *DNMT3A* mutation, MTD mutation, and R882 mutation in survival was observed as in the Korean dataset. The negative impact of high VAF of total *DNMT3A* mutation and MTD mutation in patients who received HMA as initial therapy was also maintained. Tendency of inferior treatment response patterns to HMA in high VAF of *DNMT3A* mutation and R882 mutation were similar to those in the Korean dataset.

Genes associated with epigenetic mechanisms are more altered in elderly patients than younger patients. The prevalence of mutations of *DNMT3A*, *TET2*, and *IDH* is 3-5 times more in elderly AML and MDS patients than in younger

patients, according to The Cancer Genome Atlas (TCGA) data [1]. Epigenetic changes are inherited alterations in DNA without changing its sequence. Epigenetic alterations are DNA methylation, histone modification, and chromatin remodeling. Altered gene expression caused by epigenetic dysregulations is a well-known carcinogenesis mechanism [34, 35].

DNMT regulates gene expression by adding a methyl group to the carbon-5 position of the cytosine ring from S-adenosyl methionine (SAM) to make S-adenosyl-l-homocysteine (SAH) [8, 34, 36]. *DNMT1* maintains a DNA methylation pattern on cell division, while *DNMT3A* and *DNMT3B* mediates de novo DNA methylation [10]. Altered DNA methylation at CpG islands of tumor suppressor genes can contribute to leukemogenesis and lead to poor prognosis in intermediate-risk cytogenetics patients [2, 29].

The most common epigenetic alterations in AML occur at *DNMT3A.* They affect DNA methylation. They are correlated with carcinogenesis and progression [8, 29]. 23 exons encode DNMT3A protein on chromosome 2p23. The structure of *DNMT3A* consists of an N-terminal domain, a Pro-Trp-Trp-Pro (PWWP) domain, an ATRX-DNMT3-DNMT3L (ADD) domain, and a catalytic methyltransferase (MTD) domain [6, 33]. DNA binds to the N-terminal domain and interacts with the PWWP and ADD domain to present auto-inhibitory regulation by binding trimethylated histone motifs. DNMT3A exists in a homodimeric form or a hetero-dimeric form with DNMT3L. At the MTD domain, DNMT3A dimer can make a heterotetramer structure and creates a DNA binding site at the catalytic pocket [36]. A previous study has reported the catalytic activity of DNMTs could be under allosteric control of modulation of various domains [37]. The majority of *DNMT3A* mutations occur in the MTD domain. The mutational hot

spot is arginine 882. More than 50% of *DNMT3A* mutations have been observed at the R882 residue in the MTD domain, which makes premature truncation by a nonsense or frameshift mutation. Many studies have reported that the R882 mutation can act as a dominant-negative mutation with catalytic activity decreased by 80%. R882 mutated *DNMT3A* AML patients tend to present resistance to anthracycline [38]. Thus, they show inferior survival outcome than wild-type patients [30]. However, response to HMA in R882 or MTD mutation has not been reported. Currently, it is unclear how R882 mutations affect leukemogenesis [39]. R882 mutated *DNMT3A* AML cells show impaired apoptosis, contributing to leukemogenesis *in vivo* [40]. On the other hand, differences of global 5mC level and gene expression level were not observed between R882 mutated AML and wild type AML despite decreased catalytic activity [13].

Non-R882 mutations in MTD having 20-25% of portion of *DNMT3A* mutations also show changes of activity and specificity of DNA methylation [12]. Interestingly, the hazard ratio of AML occurrence was higher for those with non-R882 mutations than those with R882 mutations [41]. Several research studies have reported that DNMT3A mutated AML has higher response rates to hypomethylating agents than wild-type AML, which was not reproduced in this study [14]. Previously, it was expected that *DNMT3A* R882 mutation AML presented extensive hypermethylation at CpG island. However, constant global methylation levels and hypomethylation at a specific region in CpG island have been recently reported [13, 42]. This finding could not explain the higher response rate of *DNMT3A* mutated AML to hypomethylating agents.

DNMT3A is the most frequently mutated gene in AML and clonal hematopoiesis (CH) [43]. In AML, *DNMT3A* mutation is considered as an early

event in leukemogenesis. The prevalence of R882 variants is higher in AML than in CH [44]. However, not all R882 mutated healthy people cases proceed to AML or MDS [45]. Rather than focusing on the presence of the R882 mutation itself, it has to be considered the common feature of MTD mutations that could alter catalytic activity. In addition, methylation specificity can result in substrate binding site mutation and decreased DNA binding activity [12]. Thus, the degree of interruption of *DNMT3A* action might be important. Taken together, mutations at a specific domain, the MTD for *DNMT3A*, that could interrupt the structural action and the allosteric effect might act as a leukemogenesis associated genetic change. Higher VAF of *DNMT3A* mutation might mean higher proportion and the predominant activity of corresponding leukemic myeloid cells.

The clinical significance of VAF has been reported in variable genes [18, 19, 46]. The prognostic impact of *DNMT3A* VAF in AML has been reported in a previous research [20]. The inferior survival correlated with high VAF was reproduced in this study for R882 and MTD mutations. Taking one step forward, the predictive significance of high VAF of *DNMT3A* mutations in HMA therapy was presented. This study also found survival disadvantages in high VAF of MTD mutations in initial HMA therapy.

DNMT3A mutant group had more number of mutations. *IDH2* and *NPM1* were more frequent in the *DNMT3A* mutant group, while *CEBPA* and *KIT* mutation were more frequent in *DNMT3A* WT group. Compared to Western data, the Korean DNMT3A mutant group presented less frequent *NPM1* co-occurrence. The clinical meaning of co-mutations needs more investigation.

DNMT inhibition is a major treatment strategy in elderly AML and MDS patients. HMA treatment, including azacitidine and decitabine, are the most

researched DNMT inhibitors. Azacitidine incorporates into RNA and decitabine into DNA and causes degradation of DNMT and DNA hypomethylation [4, 47]. DNMT inhibition can restore normal molecular function and bring mutated DNA damage. It can lead to an anti-leukemic immune reaction by expressing tumor-associated antigens [5, 48, 49]. Both HMAs presented clinical efficacy in AML and MDS patients who were not indicated for intensive induction chemotherapy or HSCT in international phase III studies [50-53]. Response of HMA in real-world practice is various, suggesting that the high allele frequency of *DNMT3A* mutation might be a predictable factor.

In conclusion, this study presented different *DNMT3A* mutation patterns between Korean and Western AML patients. Observing not significant survival differences between Korean and Western patients and similar frequency of MTD mutations, higher allele frequency of *DNMT3A* MTD mutations might be suggested as an adverse prognostic factor for survival and a predictable response factor for initial HMA therapy in elderly AML patients.

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초 록

연구 배경

급성골수성백혈병은 다양한 유전체와 염색체 변이가 발생하는 복잡한 질환이다. DNA 메틸화를 비롯한 후성유전체조절의 변이는 성인 급성골수성백혈병에서 흔하게 발견되는 돌연변이의 하나로 암발생의 주요 기전 중 하나로 알려져 있다. DNA 메틸화가 암발생과 암 진행에 미치는 영향에 대해서는 이전부터 연구되어 온 바 있다. 그러나 성인 급성골수성백혈병에서 가장 흔히 발견되는 DNA 메틸화 관련 유전자 돌연변이인 *DNMT3A* 유전자의 돌연변이 발생 위치나 대립형질 발생 빈도가 질병에 미치는 영향에 관해서는 아직 연구 결과가 부족하다. 본 연구에서는 *DNMT3A* 유전자 돌연변이 패턴에 따른 임상 양상과 저메틸화제제(hypomethylating agent, HMA)의 효과에 관해 알아보고자 한다.

연구 방법

유전자 분석 파트에서는 DNMT3A 유전자 돌연변이를 확인하기 위해 96명의 검체를 수집하여 차세대염기서열분석을 시행하였다. 임상 코호트 연구 파트에서는 새로 진단된 216명의 한국인 급성골수성백혈병 환자의 임상 양상을 수집하고 그 중 DNMT3A 유전자 돌연변이가 있는 급성골수성백혈병 환자 62명의 임상 검사 결과와 경과를 취합하여 공개 데이터 소스에서 수집한 서양인 데이터와 비교 및 통합 분석을 시행하였다.

연구 결과

유전자 분석 파트에서는 96개의 검체 중 70개 검체가 분석에 적합하였고, 191개의 유전체 변이가 확인되었다. 이 중 DNMT3A 돌연변이 유병율은 34.3%였다. DNMT3A R882 점돌연변이는 14.3%에서 확인되었으며, DNMT3A 유전자 돌연변이가 있는 환자들만 대상으로 했을 때에는

R882 돌연변이는 41.7%, 비-R882 돌연변이는 58.3%였다. 돌연변이 대립형질 빈도(VAF)의 중앙값은 43.1%였다. 임상 코호트 연구 파트에서, 새로 진단된 216명의 급성골수성백혈병 환자의 연령 중앙값은 61.3세(범위, 18-88세)였고 47.7%(n = 103)가 불량예후그룹에 속했으며 33.8%(n = 73)에서 유전자 돌연변이가 4개 이상 확인되었다. *DNMT3A* 유전자 돌연변이는 30.5%(n = 69)에서 관찰되었고, 연령 중앙값은 64.9세 (범위, 37-87세)였으며 49.3%(n = 34)가 불량예후그룹에 속했고, *DNMT3A* 돌연변이가 없는 그룹에 비해 양호한 염색체 이상 발생 빈도가 낮았다(P = 0.037). *DNMT3A* 돌연변이가 없는 그룹은 세포독성 관해유도 항암치료에 대한 반응 (P = 0.014)이 *DNMT3A* 유전자 돌연변이 그룹보다 높았으나 HMA 치료 반응의 차이는 유의하지 않았다(P = 0.244). *DNMT3A* 돌연변이의 존재는 짧은 전체 생존기간과 관련있었다(P = 0.0001). *DNMT3A* 유전자 돌연변이 그룹에서 R882 돌연변이는 47.8%로 확인되었고, R882 아미노산이 위치하는 메틸전이효소영역의 돌연변이 발생 빈도는 76.8%였다. R882(P = 0.0015)와 메틸전이효소영역(P = 0.0284)에서 *DNMT3A* VAF가 낮은 환자들은 (VAF 기준 47.6%) 높은 환자들에 비해 생존기간이 길었으며, HMA 치료를 받은 경우 VAF가 낮은 전체 *DNMT3A* 돌연변이 그룹(P = 0.0047)과 메틸전이효소영역 돌연변이 그룹(P = 0.0268)이 장기 생존하였다.

DNMT3A 돌연변이의 임상적 의의를 대규모 데이터에서 검증하기 위하여 한국인 데이터와 서양 데이터를 통합 분석한 결과 VAF가 낮은 그룹의 장기 생존 경향은 메틸전이효소영역 돌연변이(P = 0.0068), R882 점돌연변이(P = 0.0027) 뿐만 아니라 전체 DNMT3A 돌연변이 그룹에서 유지되었다(P = 0.0138). HMA 치료를 받은 경우 VAF가 낮은 메틸전이효소영역 돌연변이 그룹이 VAF가 높은 메틸전이효소영역 돌연변이 그룹보다 장기 생존하였다(P = 0.0079).

결론

본 연구에서는 한국인과 서양인 급성골수성백혈병 환자의 DNMT3A 유전자 돌연변이 양상의 차이점과 유사점을 밝혔다. R882 점돌연변이의 발생율은 한국인에서 낮았으나 R882가 위치하는 메틸전이효소영역의 돌연변이 발생율에는 차이가 없었다. 메틸전이효소영역 돌연변이의 높은 VAF는 성인 급성골수성백혈병 환자에서 좋지 않은 예후와 관련되어 있으며 저메틸화제제치료를 받은 경우의 생존 예측인자로 고려해 볼 수 있을 것이다.

주요어 : 급성골수성백혈병; 차세대염기서열분석; 유전자변이 발현양상; 유전체 메틸화; 후성유전체학

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