ORIGINAL ARTICLE

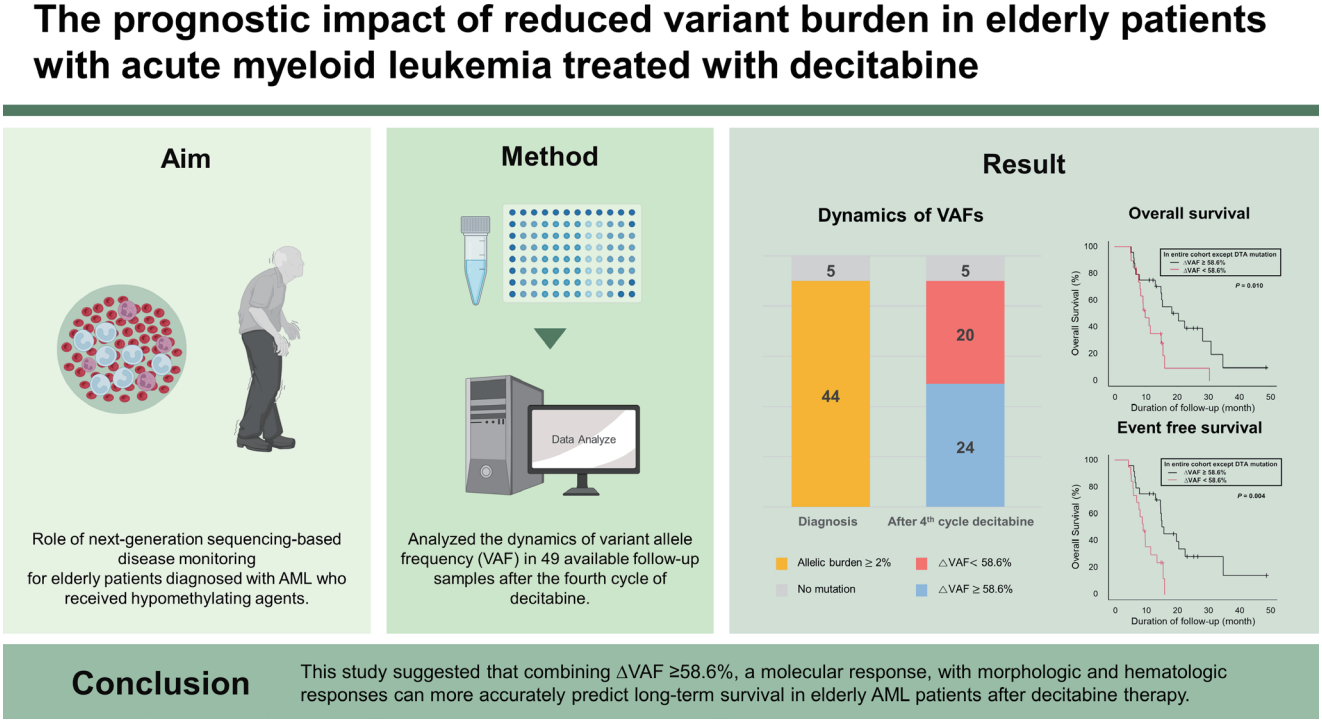
Korean J Intern Med 2023;38:534-545
https://doi.org/10.3904/kjim.2022.396

The prognostic impact of reduced variant burden in elderly patients with acute myeloid leukemia treated with decitabine

Mihee Kim[1,*], TaeHyung Kim[2,3,*], Seo-Yeon Ahn[1], Jun Hyung Lee[4], Ju Heon Park[5], Myung-Geun Shin[5], Sung-Hoon Jung[1], Ga-Young Song[1], Deok-Hwan Yang[1], Je-Jung Lee[1], Seung Hyun Choi[6], Mi Yeon Kim[6], Jae-Sook Ahn[1,6], Hyeoung-Joon Kim[1,6], and Dennis Dong Hwan Kim[7]

[1]Department of Hematology-Oncology, Chonnam National University Hwasun Hospital, Chonnam National University Medical School, Hwasun, Korea; [2]Department of Computer Science, University of Toronto, Toronto; [3]Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Canada; [4]GC Labs, Yongin; [5]Department of Laboratory Medicine, Chonnam National University Hwasun Hospital, Chonnam National University Medical School, Hwasun; [6]Genomic Research Center for Hematopoietic Diseases, Chonnam National University Hwasun Hospital, Hwasun, Korea; [7]Department of Medical Oncology and Hematology, Princess Margaret Cancer Centre, University of Toronto, Toronto, Canada

*These authors contributed equally to this manuscript.



Background/Aims: We evaluated the role of next-generation sequencing (NGS)-based disease monitoring for elderly patients diagnosed with acute myeloid leukemia (AML) who received decitabine therapy.

Methods: A total of 123 patients aged > 65 years with AML who received decitabine were eligible. We analyzed the dynamics of variant allele frequency (VAF) in 49 available follow-up samples after the fourth cycle of decitabine. The 58.6%

pISSN 1226-3303
eISSN 2005-6648
http://www.kjim.org

VAF clearance (Δ, [VAF at diagnosis – VAF at follow-up] × 100 / VAF at diagnosis) was the optimal cut-off for predicting overall survival (OS).

Results: The overall response rate was 34.1% (eight patients with complete remission [CR], six of CR with incomplete hematologic recovery, 22 with partial responses, and six with morphologic leukemia-free status). Responders (n = 42) had significantly better OS compared with non-responders (n = 42) (median, 15.3 months vs. 6.5 months; $p < 0.001$). Of the 49 patients available for follow-up targeted NGS analysis, 44 had trackable gene mutations. The median OS of patients with ΔVAF ≥ 58.6% (n=24) was significantly better than that of patients with ΔVAF < 58.6% (n = 19) (20.5 months vs. 9.8 months, $p = 0.010$). Moreover, responders with ΔVAF ≥ 58.6% (n = 20) had a significantly longer median OS compared with responders with VAF < 58.6% (n = 11) (22.5 months vs. 9.8 months, $p = 0.004$).

Conclusions: This study suggested that combining ΔVAF ≥ 58.6%, a molecular response, with morphologic and hematologic responses can more accurately predict OS in elderly AML patients after decitabine therapy.

Keywords: Acute myeloid leukemia; Elderly patients; Decitabine; Allele frequency; Residual neoplasm

INTRODUCTION

The incidence of acute myeloid leukemia (AML) increases with age, with a median age of approximately 70 years at diagnosis [1,2]. The prognosis of elderly patients with AML is poor for various reasons, including patients' medical comorbidities, performance status, and disease biology [3-5]. Compared with younger patients, elderly patients with AML have different genetic characteristics, and the treatment of elderly patients with AML remains challenging due to intolerance and resistance to intensive chemotherapy [4,6,7]. These factors lead physicians to favor less-intensive treatment rather than standard intensive chemotherapy [8,9]. Hypomethylating agents (HMAs) are widely used in clinical practice because they are relatively well tolerated with low treatment-related toxicity [10]. HMAs are particularly appropriate in the treatment of AML in elderly patients with comorbidities, poor performance status, and intolerance to combination therapy with venetoclax [10].

The genomic approach in clinical medicine has improved by developing next-generation sequencing (NGS). Thus, with NGS testing, AML can be categorized according to genetic risk [9,11]. However, the risk stratification of 2017 European LeukemiaNet (ELN) is generally focused on younger patients who are fit on standard induction treatment [11,12]. In addition, the therapeutic responses of AML patients were defined by morphologic complete remission (CR) [11]. Most patients with AML have molecular mutations at diagnosis, and NGS analysis has been widely used to detect and trace gene mutations. Therefore, NGS-based measurable residual disease (MRD) monitoring has been used to predict relapse in patients who have undergone intensive treatment [13,14]. Recent studies have been conducted to detect MRD to predict relapse risk [13-17]. However, the genetic mutations associated with prognosis among older HMA-treated AML patients have not been well delineated, and an appropriate MRD marker for elderly AML patients has yet to be established [18,19].

In this retrospective study, we analyzed the prognostic impact of genetic mutations at AML diagnosis in elderly patients treated with decitabine. Additionally, we aimed to determine the prognostic significance of NGS-based disease monitoring in our cohort.

METHODS

Patients

Patients diagnosed with AML between 2013 and 2020 were enrolled from a single institution. Eligible patients were those aged 65 years or older, with a confirmed diagnosis of AML according to the 2016 World Health Organization criteria, receiving decitabine for first-line therapy, and available for NGS using bone marrow (BM) samples at diagnosis. Patients diagnosed with acute promyelocytic leukemia were excluded. All patients received decitabine in standard doses (20 mg/m^2 by intravenous infusion for 5 consecutive days) every 4 weeks. Among 64 patients who received at least four cycles of decitabine, 55 underwent follow-up BM biopsy after the fourth decitabine cycle, and two patients

underwent BM biopsy after the second and third decitabine cycles, respectively, due to disease progression. Forty-nine patients' follow-up BM samples underwent sequential targeted NGS sequencing. The study was conducted according to the principles of the Declaration of Helsinki. This study was approved by the Institutional Review Board of the Chonnam National University Hwasun Hospital, Korea (IRB No. CNUHH-2020-147). The details of the patients who underwent treatment are summarized in Supplementary Fig. 1.

Molecular analysis: NGS

NGS was performed on 123 samples collected from BM at the time of initial diagnosis and 49 follow-up BM samples. Deep sequencing was performed by targeting the coding regions of 51 genes with recurrent driver mutations based on data extracted from large cohort studies investigating AML and other myeloid malignancies (Supplementary Table 1) [20,21]. The targeted panel was constructed using a custom Agilent probe set (Agilent Technologies, Santa Clara, CA, USA). We defined the threshold of MRD positivity as variant allelic frequency (VAF)≥ 0.2% for follow-up BM biopsies based on the mutation locus background error rates, given that the mutations were present at diagnosis at VAF ≥ 2% [13]. The detailed sample preparation, NGS, and variant calling procedures are provided in the Supplementary Materials.

Statistical analysis

Clinical characteristics were analyzed using chi-square tests for categorical variables, and two-sided Student's *t* tests were used to analyze the quantitative variables. The statistical significance of VAF changes was determined using paired *t* tests. VAF clearance (ΔVAF) was calculated using the following formula: $(VAF_{dx} – VAF_{fu}) / VAF_{dx} \times 100$. Using the R package 'rpart' for recursive partitioning to define a significant clearance rate (Δ) of VAF, we defined Δ58.6% as the optimal cut-off value (Supplementary Fig. 2). For patients with multiple genetic alterations, the maximum VAF clearance rate was considered the ΔVAF. The genetic risk stratification, along with definitions of CR, CR with incomplete hematologic recovery (CRi), morphologic leukemia-free state (MLFS), partial remission (PR), and no response, followed the 2017 ELN recommendations [11]. The overall response rate was defined as the proportion of patients who achieved CR, CRi, PR, and MLFS. Overall survival (OS) was calculated from the diagnosis of AML to

Table 1. Clinical characteristics of AML patients (n = 123)

Characteristic	Value
Age, yr	75 (67–89)
Gender (male)	62 (50.4)
Type of AML	
De novo	112 (91.1)
Secondary	11 (8.9)
ELN risk stratification	
Favorable	22 (17.9)
Intermediate	62 (50.4)
Adverse	39 (31.7)
Karyotype	
Favorable risk	
t(8;21)	2 (1.6)
inv(16)	1 (0.8)
Intermediate risk	
Normal karyotype	71 (57.7)
t(9;11)	1 (0.8)
del(13q)	2 (1.6)
Other numerical	5 (4.0)
Other structural	6 (4.8)
Adverse risk	
Complex karyotype	20 (16.3)
-5 or del(5q)	2 (1.6)
-17	1 (0.8)
No mitotic cells	3 (2.4)
Blast percentage	
Bone marrow, %	57.5 (10–95.5)
Peripheral blood, %	32.8 (0–99)
Bone marrow cellularity, %	65 (10–90)
White blood cells, /μL	32,190 (400–244,500)
Hemoglobin, g/dL	8.5 (3.6–15.0)
Platelet count, /μL	103,000 (2,000–1,809,000)
Median cycle of decitabine	6 (1–41)
Follow-up duration of surviving patients, months	15 (9.5–48.6)
Cause of death	109 (88.6)
Infection	53 (43.1)
Disease progression	51 (41.5)
Bleeding	3 (2.4)
Myocardial infraction	1 (0.8)
Combined other malignancy	1 (0.8)

Values are presented as median (range) or number (%).
AML, acute myeloid leukemia; ELN, European LeukemiaNet.

https://doi.org/10.3904/kjim.2022.396

the date of death or last follow-up. Event-free survival (EFS) was calculated as the interval from the date of the first administration of decitabine to the date of disease progression or date of death from any cause, whichever occurred first. The Kaplan-Meier method was used to analyze OS and EFS. The log-rank test was used to analyze the survival outcomes. Cox regression models were used for the multivariate analysis of various factors. To clarify the immortal time bias, landmark analyses were performed with patients (n = 84) who survived at least 3.6 months (because the median time from treatment initiation to follow-up BM examination after four decitabine cycles was 3.6 months). A *p* value less than 0.05 was considered statistically significant. Data analysis was performed using SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA) and EZR, version 1.54 (Jichi Medical University, Saitama, Japan) [22].

RESULTS

Characteristics of enrolled patients

A total of 123 patients diagnosed with AML were eligible. With a median age of 75 years (range, 67 to 89 years), 112 patients (91.1%) were diagnosed with *de novo* AML and 11 (8.9%) with secondary AML. According to the 2017 ELN risk stratification, 22 patients (17.9%) were classified as favorable, 62 (50.4%) as intermediate, and 39 (31.7%) as adverse risk. Favorable cytogenetic abnormalities were detected in three patients (2.4%), and most patients (n = 71, 57.7%) had normal karyotypes. Twenty patients (16.3%) had complex aberrant karyotypes. The median number of decitabine cycles was 6 (range, 1 to 41), and the median follow-up duration was 15 months (range, 9.5 to 48.6 months) among survivors. There were 109 deaths (88.6%), and the most common causes of death were infection (43.1%) and disease progression (41.5%). The median OS was 6.3 months (95% confidence interval [CI], 5.1 to 8.1; Supplementary Fig. 3A), and the median EFS was 6.3 months (95% CI, 4.5 to 7.7; Supplementary Fig. 3B). Other details of the patients' characteristics and clinical courses are summarized in Table 1 and Supplementary Fig. 1.

Genetic mutation status at diagnosis

In the diagnostic samples, 108 patients (87.8%) had detectable genetic mutations, with a median of two mutations per patient (range, 1 to 6; mean, 2.3 per patient) and a me-

Table 2. Clinical correlation of overall response after four cycles of decitabine (n = 64)

	Overall response		*p* value
	Responder (n = 41)	Non-responder (n = 23)	
ELN risk stratification			
Favorable risk (n = 11)	7 (17.1)	4 (17.4)	0.557
Intermediate risk (n = 33)	23 (56.1)	10 (43.5)	
Adverse risk (n = 20)	11 (26.8)	9 (39.1)	
Cytogenetics			
Normal karyotype (n = 35)	22 (53.7)	13 (56.5)	0.517
Complex karyotype (n = 11)	6 (14.6)	5 (21.7)	0.347
Categories of related genes			
Activated signaling genes (n = 28)	17 (41.5)	11 (47.8)	0.408
DNA-methylation-related genes (n = 24)	15 (36.6)	9 (39.1)	0.524
Chromatin-modifying genes (n = 3)	2 (4.9)	1 (4.3)	0.709
Transcription-factor fusion genes (n = 13)	8 (19.5)	5 (21.7)	0.537
Tumor-suppressor genes (n = 11)	5 (12.2)	6 (26.1)	0.143
NPM1 (n = 11)	8 (19.5)	3 (13.0)	0.386
Spliceosome-complex genes (n = 11)	5 (12.2)	5 (26.1)	0.143
Cohesin-complex genes (n = 4)	1 (2.4)	2 (8.7)	0.291

Values are presented as number (%).
ELN, European LeukemiaNet; *NPM1*, nucleophosmin 1.

dian VAF of 32.5% (range, 2.0% to 95.0%). The patients' mutational profile is shown in Supplementary Fig. 4. The most frequently detected mutations (in terms of categories of related genes) were those of activated signaling genes (n = 58; 47.2%), followed by DNA-methylation–related genes (n = 55, 44.7%), the gene encoding nucleophos-

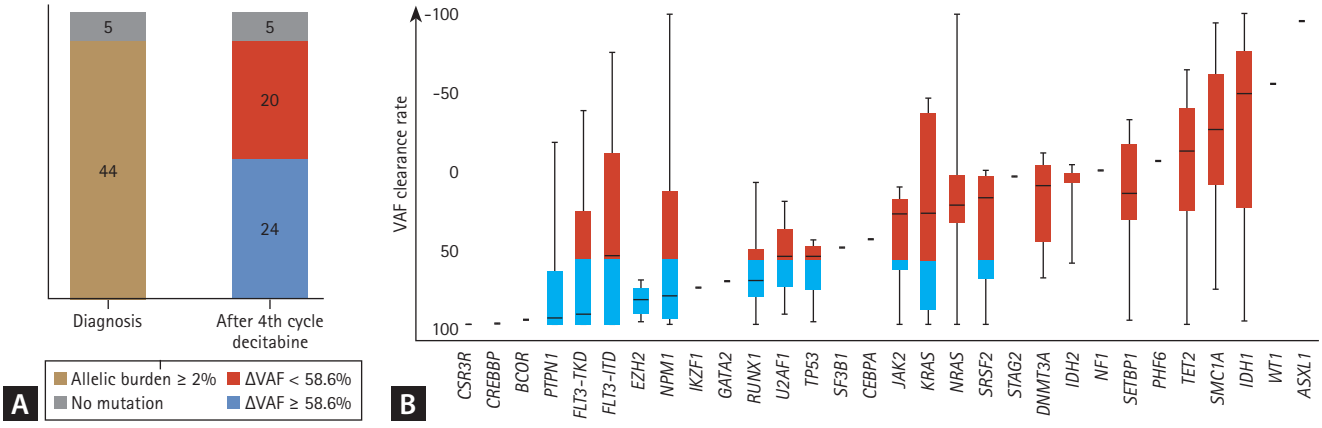


Figure 1. Variant allele frequency clearance rate (ΔVAF) at follow-up; ΔVAFs measured from paired samples at diagnosis and the follow-up bone marrow samples after four cycles of decitabine. (A) ΔVAFs at follow-up, (B) ΔVAF at follow-up in association with each genetic mutation.

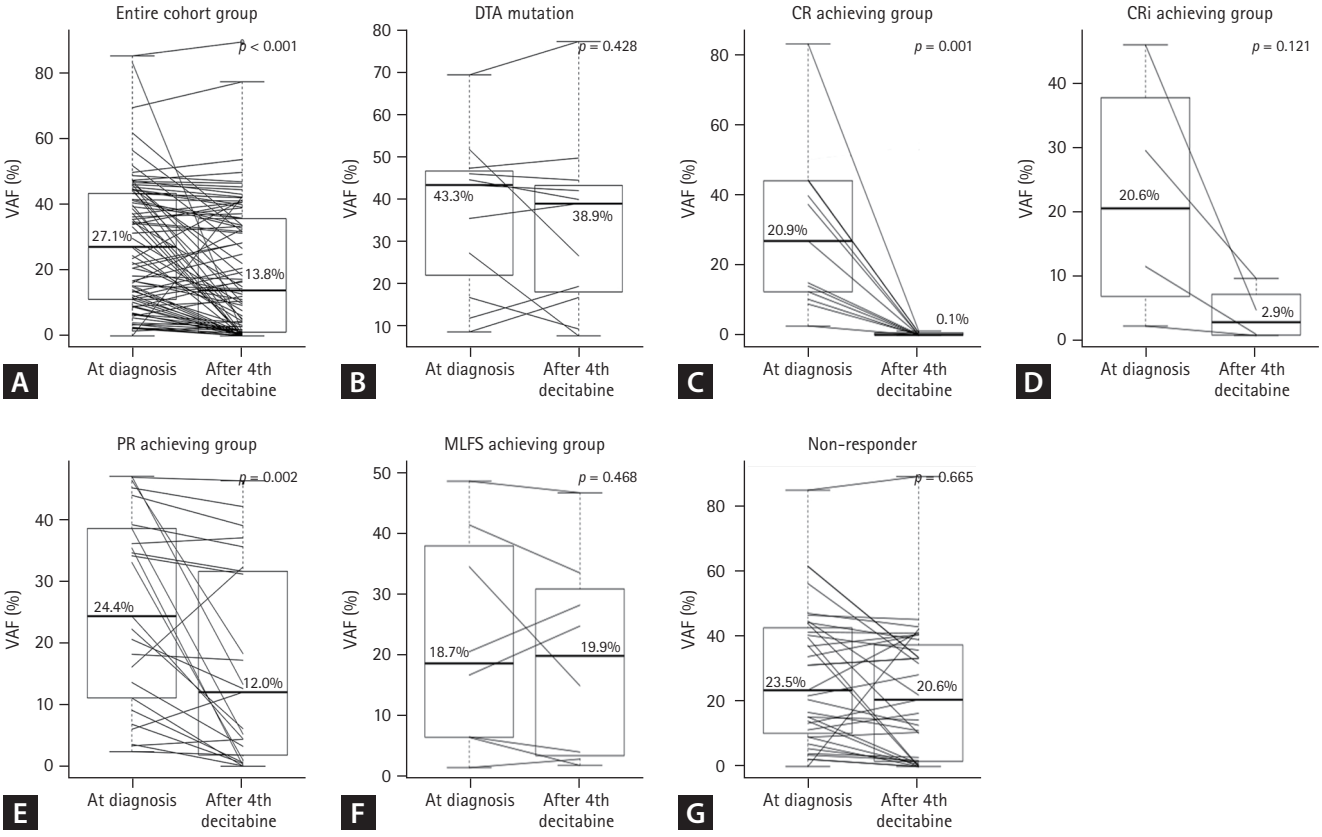


Figure 2. Dynamics of variant allele frequencies (VAFs) of mutations at two time points (at diagnosis and after the fourth cycle of decitabine) in elderly acute myeloid leukemia HR patients. (A) Entire cohort; (B) group with *DNMT3A*, *TET2*, and *ASXL1* (DTA) mutations; (C) complete remission (CR) group; (D) complete remission with incomplete hematologic recovery (CRi) group; (E) partial remission (PR) group; (F) morphological leukemia-free state (MLFS) group; and (G) non-responders.

https://doi.org/10.3904/kjim.2022.396

min 1 (n = 26, 21.1%), spliceosome-complex genes (n = 26, 21.1%), transcription-factor fusions (n = 21, 17.1%), and tumor-suppressor genes (n = 18, 14.6%). The most frequently detected mutations at diagnosis were mutations of *FLT3* (n = 34, 27.6%; *FLT3*-ITD, n = 24 [high ratio, n = 10; low ratio, n = 14)]; *FLT3*-TKD, n = 10), followed by *IDH1/IDH2* (24.3%; *IDH1*, n = 12; *IDH2*, n = 19), *NRAS/KRAS* (n = 18, 14.6%), *DNMT3A* (n = 18, 14.5%), *TP53* (n = 15, 12.1%), and *TET2* (n = 14, 11.0%) (Supplementary Fig. 4C). DNA methylation-related genes were more commonly detected in the intermediate ELN risk group than other risk groups (p = 0.019). Among 15 patients who carried no detectable mutations of known AML-associated driver genes, nine patients had normal karyotypes, and one patient had a complex karyotype.

Prognostic impact of cytogenetics, mutation status at diagnosis, and treatment response

Patients with complex karyotypes (n = 20; hazard ratio [HR], 1.52; 95% CI, 0.93 to 2.48; p = 0.095) showed a trend of poor survival compared with the other patients (Supplementary Fig. 5A). Patients with *TET2* (n = 14; HR, 2.05; 95% CI, 1.15 to 3.63; p = 0.014) and *TP53* (n = 15; HR, 1.64; 95% CI, 0.95 to 2.56; p = 0.097) mutations had poor OS. Patients with mutations of tumor-suppressor genes trended toward poor survival compared with the other groups (HR, 1.54; 95% CI, 0.92 to 2.56; p = 0.097). The survival analysis, conducted according to genetic mutations at diagnosis, is depicted in Supplementary Fig. 5B.

The overall response rate to decitabine treatment in this cohort was 34.1% (42/123; CR, n = 8; CRi, n = 6; PR, n = 22; MLFS, n = 6; Supplementary Fig. 6A). There were no significant differences in overall response rates according to 2017 ELN risk stratification, cytogenetic abnormality, genetic mutation, or category of related genes (Supplementary Fig. 6B, Table 2). Responders (n = 42; those who achieved CR, CRi, PR, or MLFS) had significantly better survival outcomes compared with non-responders (n = 42) in the land-

Table 3. Clinical correlation between ΔVAF ≥ 58.6% and ΔVAF < 58.6% after the fourth cycle chemotherapy (n = 44)[a)]

	ΔVAF		
	ΔVAF ≥ 58.6% (n = 24)	ΔVAF < 58.6% (n = 20)	*p* value
ELN risk stratification			0.726
Favorable risk (n = 6)	4 (16.7)	2 (10.0)	
Intermediate risk (n = 26)	13 (54.2)	13 (65.0)	
Poor risk (n = 12)	7 (29.2)	5 (25.0)	
Cytogenetics			
Normal karyotype (n = 26)	13 (54.2)	13 (65.0)	0.338
Complex karyotype (n = 6)	3 (12.5)	3 (15.0)	0.575
Categories of related genes			
Activated signaling gene (n = 22)	13 (54.2)	9 (45.0)	0.381
DNA-methylation-related genes (n = 18)	6 (25.0)	12 (60.0)	0.020
Chromatin modifying gene (n = 3)	2 (8.3)	1 (5.0)	0.570
NPM1 (n = 7)	5 (20.8)	2 (10.0)	0.290
Transcription-factor fusion genes (n = 12)	7 (29.2)	5 (25.0)	0.514
Tumor-suppressor genes (n = 5)	1 (4.2)	4 (20.0)	0.121
Spliceosome-complex genes (n = 10)	4 (16.7)	6 (30.0)	0.245
Cohesin-complex genes (n = 3)	0	3 (15.0)	0.086
Overall response			0.043
Responder (n = 31)	20 (83.3)	11 (55.0)	
Non-responder (n = 13)	4 (16.7)	9 (45.0)	

Values are presented as number (%).
ΔVAF, variant allele frequency clearance rate; ELN, European LeukemiaNet; *NPM1*, nucleophosmin 1.
[a)]Five of 49 patients who underwent follow-up bone marrow biopsy did not have traceable gene mutation.

mark analysis (median OS, 15.3 months vs. 6.5 months; $p < 0.001$; median EFS, 14.7 months vs. 6.5 months; $p < 0.001$) (Supplementary Fig. 3C, D). Among responders, the achievement of CR/CRi (n = 14) or PR (n = 22) was associated with better OS, but MLFS (n = 6) was associated with inferior OS (median OS, 19.3 months vs. 16.0 months vs. 8.1 months; $p = 0.023$) and inferior EFS (median EFS, 15.3 months vs. 15.1 months vs. 7.2 months; $p = 0.036$) (Supplementary Fig. 3E, F).

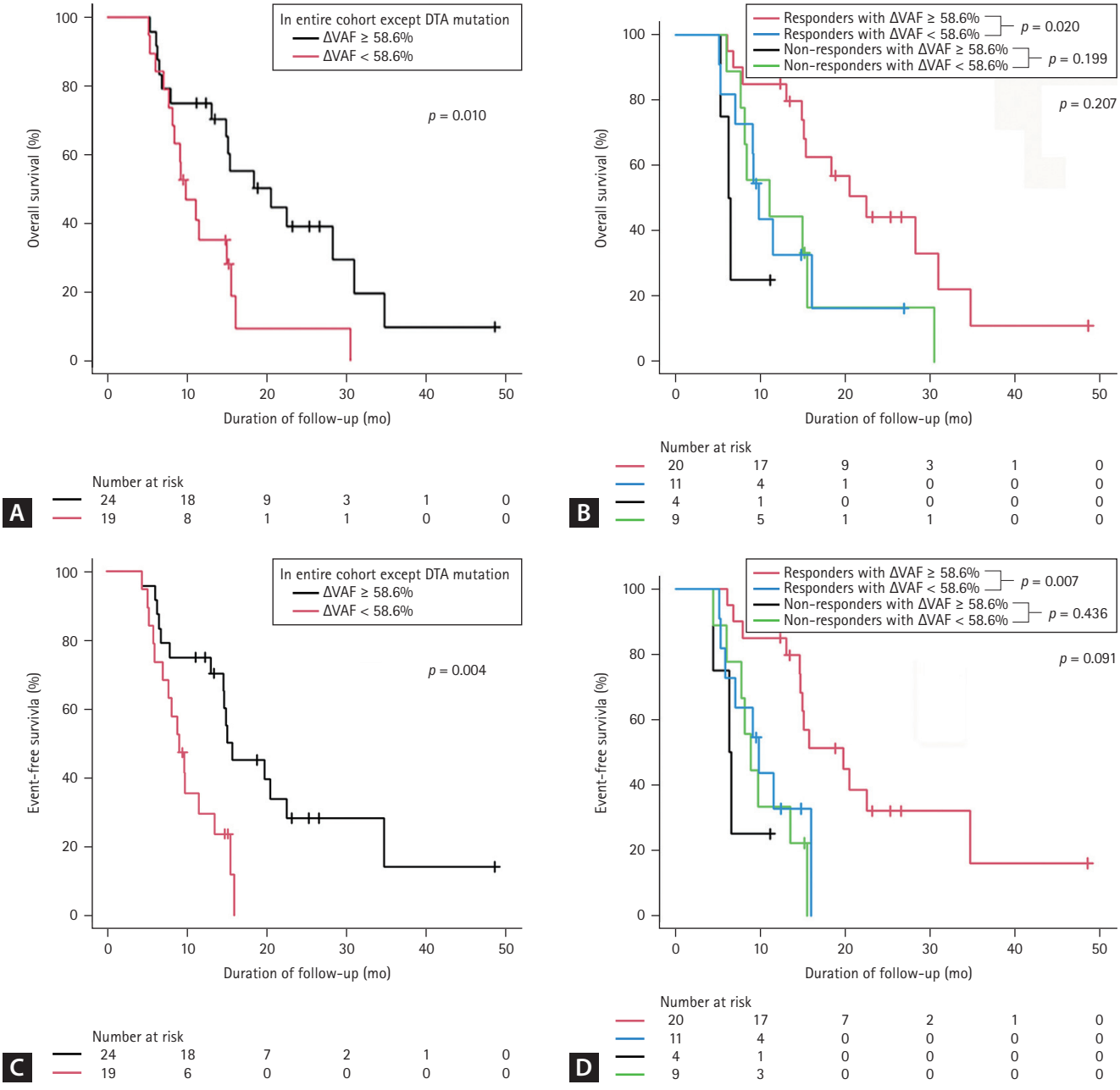


Figure 3. Kaplan-Meier curves using landmark analysis (3.6 months) for elderly acute myeloid leukemia patients according to variant allele frequency VAF clearance (ΔVAF). (A) Overall survival (OS) in the entire cohort excluding those with *DNMT3A*, *TET2*, and *ASXL1* (DTA) mutations; (B) OS in the non complete remission (non-CR)/CR with incomplete hematologic recovery (CRi) group; (C) event-free survival (EFS) in the entire cohort excluding those with DTA mutations; (D) EFS in the non-CR/CRi group. After landmark analysis, the 39 patients who died before 3.6 months were excluded from the survival analysis. Others included the stable VAF group (ΔVAF < 58.6%), the group of patients with no mutations, and the group that did not have available follow-up BM samples in each cohort.

https://doi.org/10.3904/kjim.2022.396

NGS-based MRD status

Of the 49 patients available for targeted NGS analysis after the fourth decitabine cycle, 44 had trackable gene mutations at diagnosis (Fig. 1). Eight of 44 patients (18.2%) achieved MRD negativity after four cycles of decitabine treatment. Among patients with each mutation, the median clearance rate of VAF was Δ43.7%. Figure 2 shows the clearance rates of VAF after the fourth decitabine cycle. Mutations of *DNMT3A, TET2,* and *ASXL1* (DTA genes) were detected in samples from 29 patients at diagnosis. The DTA mutations, which are associated with age-related clonal hematopoiesis, were also persistently highly detected after the fourth cycle of decitabine ($p = 0.428$, Fig. 2B). Therefore, DTA mutations were excluded from the VAF clearance analysis. In the CR and CRi groups, the median VAFs at diagnosis decreased significantly after the fourth decitabine cycle (from 20.9% to 0.1% in the CR group, $p = 0.001$, Fig. 2C; from 20.6% to 2.9% in the CRi group, $p = 0.121$, Fig. 2D). In the PR group, the median VAF also decreased from 24.4% to 12.0% ($p = 0.002$, Fig. 2E). However, in the MLFS and non-responder groups, the median VAF was persistently maintained (from 18.7% to 19.9% in the MLFS group, $p = 0.468$, Fig. 2F; from 23.5% to 20.6% in the non-responder group; $p = 0.665$, Fig. 2G).

In the survival analysis, a 10% increase in mutation clearance improved OS by 11.7% (HR, 0.883; 95% CI, 0.837 to 0.930; $p = 3.00 \times 10^{-6}$). Based on a clearance rate of VAF Δ58.6%, which was the most informative cut-off point, 24 patients (54%) had VAF reductions ≥ 58.6% (Fig. 1A). There were no associations of 2017 ELN risk stratification, cytogenetics, and categories of related gene abnormalities between responders and non-responders (Table 2). However, there was a significantly higher proportion of responders than non-responders ($p = 0.043$, Table 3) with reduced VAF (ΔVAF ≥ 58.6%).

PTPN1, *FLT3-TKD*, *EZH2*, *RUNX1*, and *TP53* mutations were associated with a median ΔVAF ≥ 58.6%. DNA-methylation gene (*DMNT3A*, *TET2*, *IDH1*, and *IDH2*) mutations were associated with a median ΔVAF < 58.6% (Fig. 1B).

Correlation between survival and ΔVAF

The median OS of patients with ΔVAF ≥ 58.6% (n = 24) was significantly longer than that of patients with ΔVAF < 58.6% (n = 20 [median OS, 20.5 months vs. 9.8 months; $p = 0.033$]). MRD-negative patients had a good prognosis, with a median OS and EFS of 20.5 months, respectively. After DTA mutations were excluded, the survival outcomes remained similar to those derived from the analysis that in-

Table 4. Univariate and multivariate Cox proportional hazards regression analyses predicting OS and EFS in elderly AML patients

Variable	Overall survival				Event free survival			
	Univariate HR (95% CI)	*p* value	Multivariate HR[a] (95% CI)	*p* value	Univariate HR (95% CI)	*p* value	Multivariate HR[a] (95% CI)	*p* value
Age[b]	1.06 (1.01–1.11)	0.009	1.09 (0.97–1.23)	0.149	1.05 (1.01–1.10)	0.020	1.06 (0.95–1.19)	0.309
TET2	2.05 (1.15–3.63)	0.014	4.54 (1.27–16.25)	0.020	2.00 (1.13–3.55)	0.018	4.67 (1.29–16.85)	0.019
TP53	1.65 (0.95–2.86)	0.077	3.97 (1.12–14.04)	0.032	1.77 (1.02–3.07)	0.044	1.72 (0.32–9.29)	0.530
Complex karyotype	1.52 (0.93–2.48)	0.095	0.98 (0.26–3.69)	0.978	1.76 (1.07–2.89)	0.026	3.79 (1.43–10.07)	0.008
ΔVAF[c]								
≥ 58.6%	0.47 (0.22–0.98)	0.049	0.46 (0.21–0.99)	0.047	0.36 (0.17–0.80)	0.012	0.39 (0.17–0.86)	0.019
< 58.6%	Reference				Reference			

Univariate analysis and multivariate Cox proportional hazards regression analysis was conducted using the log-rank method. Multivariate analysis was performed using the covariates that yielded *p* values < 0.1 in the univariate analysis.

OS, overall survival; EFS, event-free survival; AML, acute myeloid leukemia; HR, hazard ratio; CI, confidence interval; VAF, variant allele frequency; ΔVAF, VAF clearance rate.

[a]Multivariate Cox regression analysis was conducted on 43 patients for whom the VAF clearance rate was measurable.

[b]Continuous variable.

[c]Forty-three patients who could be classified as VAF ≥ 58.6% and ΔVAF < 58.6% in entire cohort, except for those with *DNMT3A*, *TET2*, and *ASXL1* mutations, were included in the univariate and multivariate analysis.

cluded DTA mutations (median OS of ΔVAF ≥ 58.6% [n = 24], 20.5 months; ΔVAF < 58.6% [n = 19], 9.8 months; $p = 0.010$; Fig. 3A). In addition, the median EFS of patients with reduced VAF was significantly longer than that of patients with ΔVAF < 58.6% (median EFS, 15.7 months vs. 9.1 months, $p = 0.007$). In the analysis excluding the DTA mutations, the survival outcomes remained similar to those derived from the analysis that included the DTA mutations (median EFS of ΔVAF ≥ 58.6% [n = 24], 15.7 months; ΔVAF < 58.6% [n = 19], 9.1 months; $p = 0.004$, Fig. 3C).

ΔVAF ≥ 58.6% was more frequently observed in responders ($p = 0.043$, Table 3). Therefore, we further analyzed the prognostic significance of ΔVAF ≥ 58.6% in responders. Responders with ΔVAF ≥ 58.6% had significantly longer OS and EFS than responders with ΔVAF < 58.6% (Fig. 3B, D). The median OS in the responders with ΔVAF ≥ 58.6% (n = 20) was 22.5 months, which was longer than the median 12.7 months in responders with ΔVAF < 58.6% (n = 11, $p = 0.020$, Fig. 3B).

Table 4 summarizes the results of the univariate and multivariate Cox regression analyses to evaluate the variables associated with OS and EFS. Univariate analysis revealed ΔVAF ≥ 58.6%, *TET2* mutation, and *TP53* mutation to be significant predictors of OS and EFS (all, $p < 0.05$). Multivariate Cox regression analysis revealed ΔVAF ≥ 58.6% (OS [$p = 0.047$; HR, 0.46; 95% CI, 0.21 to 0.99], EFS [$p = 0.019$; HR, 0.39; 95% CI, 0.17 to 0.86]), *TET2* (OS [$p = 0.020$; HR, 4.54; 95% CI, 1.27 to 16.25], and EFS [$p = 0.019$; HR, 4.67; 95% CI, 1.29 to 16.85]) to be independent predictors of OS and EFS (Table 4).

DISCUSSION

This study aimed to identify the prognostic significance of VAF reductions in elderly AML patients after decitabine treatment. The group with reduced VAF (ΔVAF ≥ 58.6%) had significantly longer OS and EFS than the group without ΔVAF ≥ 58.6%. Additionally, responders with ΔVAF ≥ 58.6% had significantly longer OS and EFS. For elderly AML patients after decitabine treatment, long-term responses can be more accurately predicted when the ΔVAF ≥ 58.6%, with additional evidence from morphologic and hematologic HMA treatment responses.

MRD assessments are widely studied as prognostic factors after induction chemotherapy and after transplantation [13,14,23]. However, NGS-based MRD assessment in patients receiving low-intensity treatment has not been fully evaluated. The goal of intensive treatment is to achieve CR, and MRD clearance is an important predictable molecular marker of relapse. Recently, Boddu et al. [24] demonstrated the clinical relevance of MRD negativity in older patients with AML treated with HMAs. Using multicolor flow cytometry, they showed a significant advantage of MRD negativity in terms of the cumulative incidence of relapse [24]. However, this result did not translate to improved survival [24]. In that study, 13 patients were MRD-negative among 24 patients in the reduced-VAF group. The expectation of molecular clearance with only HMA treatment in older AML patients is questionable because somatic mutations with relatively high VAF remain after HMA administration [25,26]. Therefore, MRD negativity is not a conclusive prognostic marker for elderly AML patients treated with HMAs. In our study, reduced VAF (ΔVAF ≥ 58.6%) was shown to be a feasible prognostic marker for predicting OS and EFS for elderly patients. These results emphasize the importance of VAF reduction, along with MRD negativity, in elderly AML patients treated with HMAs. The goal of HMA treatment in this patient population is to prolong survival rather than achieve cure.

Our study demonstrated that decitabine-treated elderly AML patients who achieved CR/CRi or PR had prolonged survival. Similar to our study, Molica et al. [27] found that the achievement of CR or PR after HMA treatment was significantly associated with better outcomes; they also observed no differences between CR and PR in elderly AML patients. However, that study did not clearly define MLFS. Our study found that such favorable outcomes were not associated with an MLFS in elderly AML patients. One of the reasons for the non-survival benefit in the MLFS group could be the accompanying infection and bleeding due to the cytopenia associated with an MLFS. Although limited in number, four out of six patients died from complications of cytopenia in our cohort. The other reason might be that an MLFS may make it difficult to predict a morphologic response due to the associated BM aplastic state. In our study, the MLFS patients had relatively stable VAFs compared with other responders (CR/CRi or PR)—the median ΔVAFs were 11.5% vs. 88.0%, respectively. The MLFS can be interpreted as a depletion of leukemic cells but not a true decrease in leukemic burden compared with normal hematopoietic cells. Our study findings suggested that combining the MLFS

https://doi.org/10.3904/kjim.2022.396

evidence with ΔVAF ≥ 58.6% may address the limitations associated with the aplastic state in the MLFS.

Our data also demonstrate the prognostic significance of genetic mutations in the diagnosis of elderly AML patients receiving decitabine treatment. Metzeler et al. [28] found that the mutation spectrum of elderly AML patients differed from that of younger patients. The high rates of *SRSF2*, *DNMT3A*, *TET2*, and *TP53* mutations in our elderly AML patients were concordant with other study findings [28,29]. These genes are age-associated and mediate clonal hematopoiesis. They are frequently mutated in elderly patients, and these results represent biological differences in the AML afflicting younger and elderly patients [30,31]. Unlike other studies, in our study, *DNMT3A* and *RUNX1* mutations at diagnosis were not associated with inferior survival outcomes [32,33]. Differences in treatment modalities (intensive vs. non-intensive treatment) between studies could partially explain these conflicting findings. Since our study focused on the group receiving decitabine monotherapy, it was meaningful in that it demonstrated the significance of the genetic mutations in the HMA-treated group. Consistent with previous studies, we identified that *TET2* and *TP53* mutations at diagnosis were genetic risk predictors of poor survival [32,33]. It can be concluded that it is difficult to improve the survival of elderly AML patients with *TP53* or *TET2* mutations using HMA monotherapy. Elderly AML patients who carry these mutations could be recommended to register for clinical trials for early access to novel treatments. In our study, *IDH1/IDH2* mutations were potentially associated with shorter survival in this patient population. A recent study found that patients with *IDH1/IDH2*-mutated AML had favorable outcomes when treated with HMAs and venetoclax [34]. Our data may provide additional evidence to recommend HMAs combined with venetoclax or *IDH1/IDH2* inhibitors because it is difficult to expect improved survival with HMAs alone in elderly AML patients with *IDH1/IDH2* mutations [35,36].

There were several limitations to our study. First, the use of ΔVAF is associated with selection bias because only patients who received at least four cycles of decitabine were included in the ΔVAF groups. For this reason, it is difficult to predict early mortality or early treatment failure at diagnosis. Second, this retrospective cohort included a small number of patients. However, our study provides appropriate evidence for applying NGS-based disease monitoring to elderly AML patients treated with HMAs. This study also suggests that a different approach to MRD monitoring is required for elderly AML patients.

Recently, HMAs with venetoclax has become a new standard treatment for older patients with AML [9,35,37]. However, the estimated median OS for patients with reduced VAF (ΔVAF ≥ 58.6%) was 20.5 months, which was higher than that associated with the combination of HMA therapy and venetoclax (17.5 months) [38]. Some elderly AML patients are not suitable candidates for venetoclax plus HMAs because of treatment-related myelosuppression, comorbidities, and economic issues. ΔVAF is an early predictor of long-term survival associated with decitabine monotherapy, and such prediction can help determine whether to continue treatment or consider other treatments at an early stage before the loss of the treatment response. Based on our data, VAF clearance could be an additional prognostic indicator of survival outcomes for elderly AML patients, particularly those who achieved PR or better responses after HMA treatment. Despite achieving overall responses to HMAs, patients with stable VAF may be unlikely to achieve long-term survival, and an early regimen change should be considered.

This study investigated the clinical significance of genetic mutations at the time of diagnosis in elderly AML patients receiving HMAs. Moreover, VAF clearance could provide additional information for predicting long-term survival in elderly AML patients responding to decitabine. ΔVAF ≥ 58.6% combined with evidence from morphologic and hematologic treatment responses could be proposed as a marker for determining whether to maintain decitabine treatment or combine novel agents to improve survival prognosis.

KEY MESSAGE

1. Variant allele frequency (VAF) clearance could provide additional information for predicting long-term survival in elderly acute myeloid leukemia patients responding to decitabine
2. VAF clearance with morphologic and hematologic responses could be proposed as a marker for determining whether to maintain decitabine treatment or combine novel agents to improve survival prognosis.

REFERENCES

1. Prassek VV, Rothenberg-Thurley M, Sauerland MC, et al. Genetics of acute myeloid leukemia in the elderly: mutation spectrum and clinical impact in intensively treated patients aged 75 years or older. Haematologica 2018;103:1853-1861.
2. Thein MS, Ershler WB, Jemal A, Yates JW, Baer MR. Outcome of older patients with acute myeloid leukemia: an analysis of SEER data over 3 decades. Cancer 2013;119:2720-2727.
3. Hourigan CS, Karp JE. Development of therapeutic agents for older patients with acute myelogenous leukemia. Curr Opin Investig Drugs 2010;11:669-677.
4. Ossenkoppele G, Löwenberg B. How I treat the older patient with acute myeloid leukemia. Blood 2015;125:767-774.
5. Wang ES. Treating acute myeloid leukemia in older adults. Hematology 2014;2014:14-20.
6. Klepin HD. Elderly acute myeloid leukemia: assessing risk. Curr Hematol Malig Rep 2015;10:118-125.
7. Rao AV, Valk PJ, Metzeler KH, et al. Age-specific differences in oncogenic pathway dysregulation and anthracycline sensitivity in patients with acute myeloid leukemia. J Clin Oncol 2009;27:5580-5586.
8. Rao AV. Fitness in the elderly: how to make decisions regarding acute myeloid leukemia induction. Hematology 2016;2016:339-347.
9. Hwang SM. Classification of acute myeloid leukemia. Blood Res 2020;55:S1-S4.
10. Dombret H, Seymour JF, Butrym A, et al. International phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with >30% blasts. Blood 2015;126:291-299.
11. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017;129:424-447.
12. Wang B, Liu Y, Hou G, et al. Mutational spectrum and risk stratification of intermediate-risk acute myeloid leukemia patients based on next-generation sequencing. Oncotarget 2016;7:32065-32078.
13. Ahn JS, Kim T, Jung SH, et al. Allogeneic transplant can abrogate the risk of relapse in the patients of first remission acute myeloid leukemia with detectable measurable residual disease by next-generation sequencing. Bone Marrow Transplant 2021;56:1159-1170.
14. Kim T, Moon JH, Ahn JS, et al. RNA sequencing as an alternative tool for detecting measurable residual disease in core-binding factor acute myeloid leukemia. Sci Rep 2020;10:20119.
15. Thol F, Gabdoulline R, Liebich A, et al. Measurable residual disease monitoring by NGS before allogeneic hematopoietic cell transplantation in AML. Blood 2018;132:1703-1713.
16. Ivey A, Hills RK, Simpson MA, et al. Assessment of minimal residual disease in standard-risk AML. N Engl J Med 2016;374:422-433.
17. Thol F, Kölking B, Damm F, et al. Next-generation sequencing for minimal residual disease monitoring in acute myeloid leukemia patients with FLT3-ITD or NPM1 mutations. Genes Chromosomes Cancer 2012;51:689-695.
18. Krug U, Gale RP, Berdel WE, et al. Therapy of older persons with acute myeloid leukaemia. Leuk Res 2017;60:1-10.
19. Hilberink JR, Morsink LM, van der Velden WJFM, et al. Pretransplantation MRD in older patients with AML after treatment with decitabine or conventional chemotherapy. Transplant Cell Ther 2021;27:246-252.
20. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med 2016;374:2209-2221.
21. Ley TJ, Miller C, Ding L, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med 2013;368:2059-2074.
22. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 2013;48:452-458.
23. Jongen-Lavrencic M, Grob T, Hanekamp D, et al. Molecular minimal residual disease in acute myeloid leukemia. N Engl J Med 2018;378:1189-1199.
24. Boddu P, Jorgensen J, Kantarjian H, et al. Achievement of a negative minimal residual disease state after hypomethylating agent therapy in older patients with AML reduces the risk of relapse. Leukemia 2018;32:241-244.
25. Falconi G, Fabiani E, Piciocchi A, et al. Somatic mutations as markers of outcome after azacitidine and allogeneic stem cell transplantation in higher-risk myelodysplastic syndromes. Leukemia 2019;33:785-790.
26. Buccisano F, Dillon R, Freeman SD, Venditti A. Role of minimal (measurable) residual disease assessment in older patients with acute myeloid leukemia. Cancers (Basel) 2018;10:215.
27. Molica M, Mazzone C, Niscola P, et al. Identification of predictive factors for overall survival and response during hypomethylating treatment in very elderly (≥75 years) acute myeloid leukemia patients: a multicenter real-life experience. Cancers (Basel) 2022;14:4897.
28. Metzeler KH, Herold T, Rothenberg-Thurley M, et al. Spectrum

https://doi.org/10.3904/kjim.2022.396

and prognostic relevance of driver gene mutations in acute myeloid leukemia. Blood 2016;128:686-698.
29. Tsai CH, Hou HA, Tang JL, et al. Genetic alterations and their clinical implications in older patients with acute myeloid leukemia. Leukemia 2016;30:1485-1492.
30. Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med 2014;371:2477-2487.
31. Park DJ, Kwon A, Cho BS, et al. Characteristics of DNMT3A mutations in acute myeloid leukemia. Blood Res 2020;55:17-26.
32. Tsai CH, Hou HA, Tang JL, et al. Genetic alterations and their clinical implications in older patients with acute myeloid leukemia. Leukemia 2016;30:1485-1492.
33. Wang SY, Cheng WY, Mao YF, et al. Genetic alteration patterns and clinical outcomes of elderly and secondary acute myeloid leukemia. Hematol Oncol 2019;37:456-463.
34. Pollyea DA, Dinardo CD, Arellano ML, et al. Results of venetoclax and azacitidine combination in chemotherapy ineligible untreated patients with acute myeloid leukemia with IDH 1/2 mutations. Blood 2020;136:5-7.
35. Park S, Cho BS, Kim HJ. New agents in acute myeloid leukemia (AML). Blood Res 2020;55:S14-S18.
36. Byun JM, Yoo SJ, Kim HJ, et al. IDH1/2 mutations in acute myeloid leukemia. Blood Res 2022;57:13-19.
37. Maiti A, Qiao W, Sasaki K, et al. Venetoclax with decitabine vs intensive chemotherapy in acute myeloid leukemia: a propensity score matched analysis stratified by risk of treatment-related mortality. Am J Hematol 2021;96:282-291.
38. DiNardo CD, Pratz K, Pullarkat V, et al. Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia. Blood 2019;133:7-17.

Received : December 17, 2022
Revised : February 3, 2023
Accepted : February 20, 2023

Correspondence to
Jae-Sook Ahn, M.D., Ph.D.
Department of Internal Medicine, Chonnam National University Hwasun Hospital, Chonnam National University, 322 Seoyang-ro, Hwasun-eup, Hwasun-gun 58128, Korea
Tel: +82-61-379-7635, Fax: +82-61-379-8097
E-mail: f0115@chonnam.ac.kr
https://orcid.org/0000-0003-3078-5024

Acknowledgments
The biospecimens used in this study were provided by the Biobank of Chonnam National University Hwasun Hospital, a member of the Korea Biobank. The whole-exome data used in this study have been deposited in the Clinical & Omics Data Archive (CODA, http://coda.nih.go.kr) under accession # R000007.

CRedit authorship contributions
Mihee Kim: conceptualization, data curation, formal analysis, methodology, visualization, writing - original draft; TaeHyung Kim: data curation, methodology, visualization, writing - original draft; Seo-Yeon Ahn: data curation, formal analysis; Jun Hyung Lee: data curation, formal analysis; Ju Heon Park: methodology; Myung-Geun Shin: methodology; Sung-Hoon Jung: data curation, formal analysis; Ga-Young Song: data curation, formal analysis; Deok-Hwan Yang: formal analysis; Je-Jung Lee: formal analysis; Seung Hyun Choi: data curation, methodology; Mi Yeon Kim: data curation, methodology; Jae-Sook Ahn: conceptualization, formal analysis, funding acquisition, project administration, visualization, writing - review & editing; Hyeoung-Joon Kim: conceptualization, project administration; Dennis Dong Hwan Kim: conceptualization, project administration

Conflicts of interest
The authors disclose no conflicts.

Funding
This research was supported by the Basic Science Research Program, through the National Research Foundation of Korea (NRF), funded by the Ministry of Science, ICT, and Future Planning (NRF-2015R1A2A1A10054579) and the National R&D Program for Cancer Control, Ministry of Health & Welfare, Republic of Korea (1720160). This study was supported by a grant (HCRI21006) from Chonnam National University Hwasun Hospital Institute for Biomedical Science. This work was also supported by a National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2018R1A2A1A05078480, 2022R1F1A1063836) and by Foundation of cutting-edge supporting infrastructure for precision medicine industry (P0017144).

KJIM

SUPPLEMENTARY MATERIALS

Sample preparation and next-generation sequencing

One hundred and twenty-three samples taken at initial diagnosis and follow-up samples from bone marrow after treatment (n = 49) were sequenced. Deep-sequencing was performed by targeting the coding regions of 51 genes known to have recurrent driver mutations, as observed in large cohort studies of acute myeloid leukemia (AML) and other myeloid malignancies [1,2]. Genomic DNA was extracted using QIAamp DNA blood mini-kits (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. After library preparation, all samples were subjected to targeted sequencing per the manufacturer's instructions. Using the Agilent (SureSelect Target Enrichment, CITY, STATE, COUNTRY) custom probe set targeting the coding region of 51 genes (COMPANY, Santa Clara, CA, USA), all samples were sequenced using an Illumina Hiseq 2500 (COMPANY, San Diego, CA, USA) with a 101-bp pair-end mode.

Next-generation sequencing read processing and procedure for variant calling

The read processing and variant calling procedure were followed as in our previous studies [3,4]. For samples without corresponding T-cell samples, the same read processing was used. For variant calling of those samples, we also searched for variants from samples with corresponding T-cell samples in addition to variants in the list compiled from two other AML and MDS studies [1,2], and ran the DeepSNV R package, which implements the Shearwater algorithm using its default parameters [5]. Once lists of candidate somatic mutations were compiled, we filtered out germline mutations and rare SNPs as in our previous studies [3,4] and based on population frequency (over 0.1% according to NHLBI GO Exome Sequencing Project [ESP]) [6,7].

All sequencing data have been deposited at the European Nucleotide Archive (Accession number: PRJEB48636).

SUPPLEMENTARY REFERENCES

1. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood 2013;122:3616-3627; quiz 3699.
2. Cancer Genome Atlas Research Network; Ley TJ, Miller C, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med 2013;368:2059-2074.
3. Kim T, Tyndel MS, Kim HJ, et al. The clonal origins of leukemic progression of myelodysplasia. Leukemia 2017;31:1928-1935.
4. Kim T, Yoshida K, Kim YK, et al. Clonal dynamics in a single AML case tracked for 9 years reveals the complexity of leukemia progression. Leukemia 2016;30:295-302.
5. Gerstung M, Papaemmanuil E, Campbell PJ. Subclonal variant calling with multiple samples and prior knowledge. Bioinformatics 2014;30:1198-1204.
6. Fu W, O'Connor TD, Jun G, et al. Analysis of 6,515 exomes reveals the recent origin of most human protein-coding variants. Nature 2013;493:216-220.
7. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 2010;38:e164.

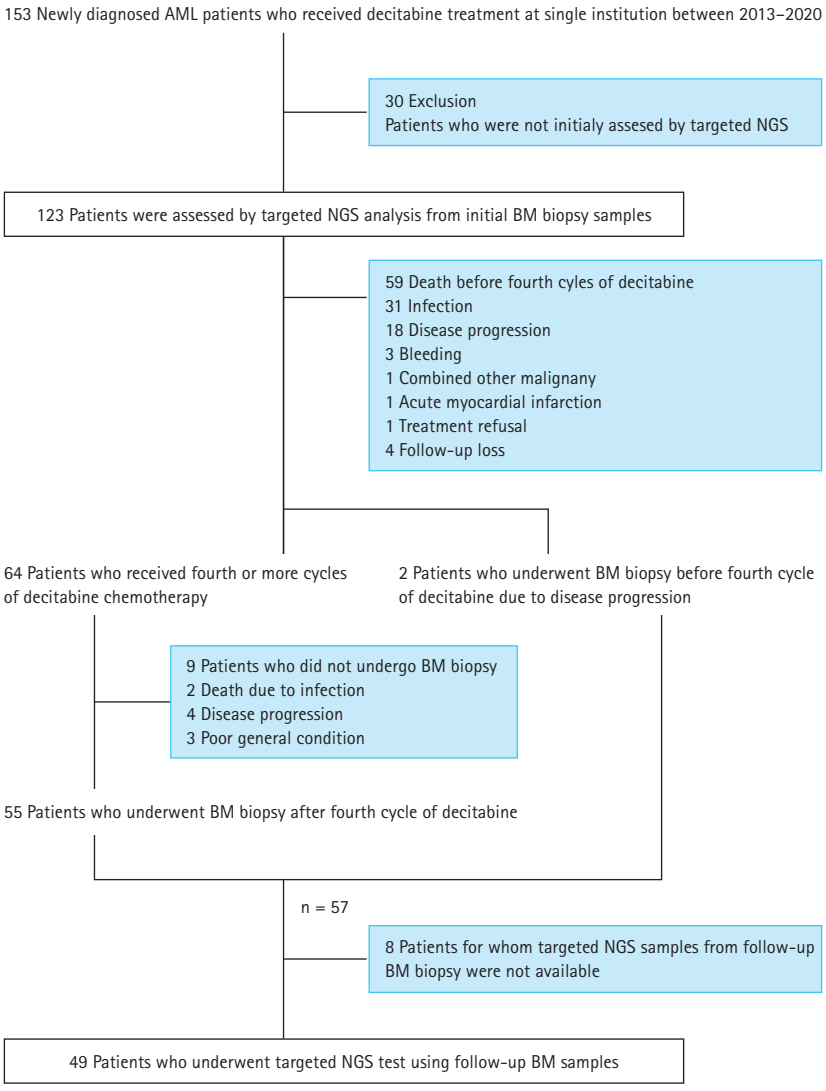
Supplementary Table 1. Gene list

ABL1
ASXL1
ATM
BCOR
BRAF
CALR
CBL
CDKN2A
CEBPA
CREBBP
CSF3R
DDX41
DNMT3A
ETV6
EZH2
FBXW7
FLT3
GATA2
IDH1
IDH2
IKZF1
JAK1
JAK2
JAK3
KDM6A
KIT
KMT2A
KRAS
MECOM
MPL
MYD88
NF1
NOTCH1
NPM1
NRAS
PDGFRA
PDGFRB
PHF6
PTPN11
RB1
RUNX1
SETBP1

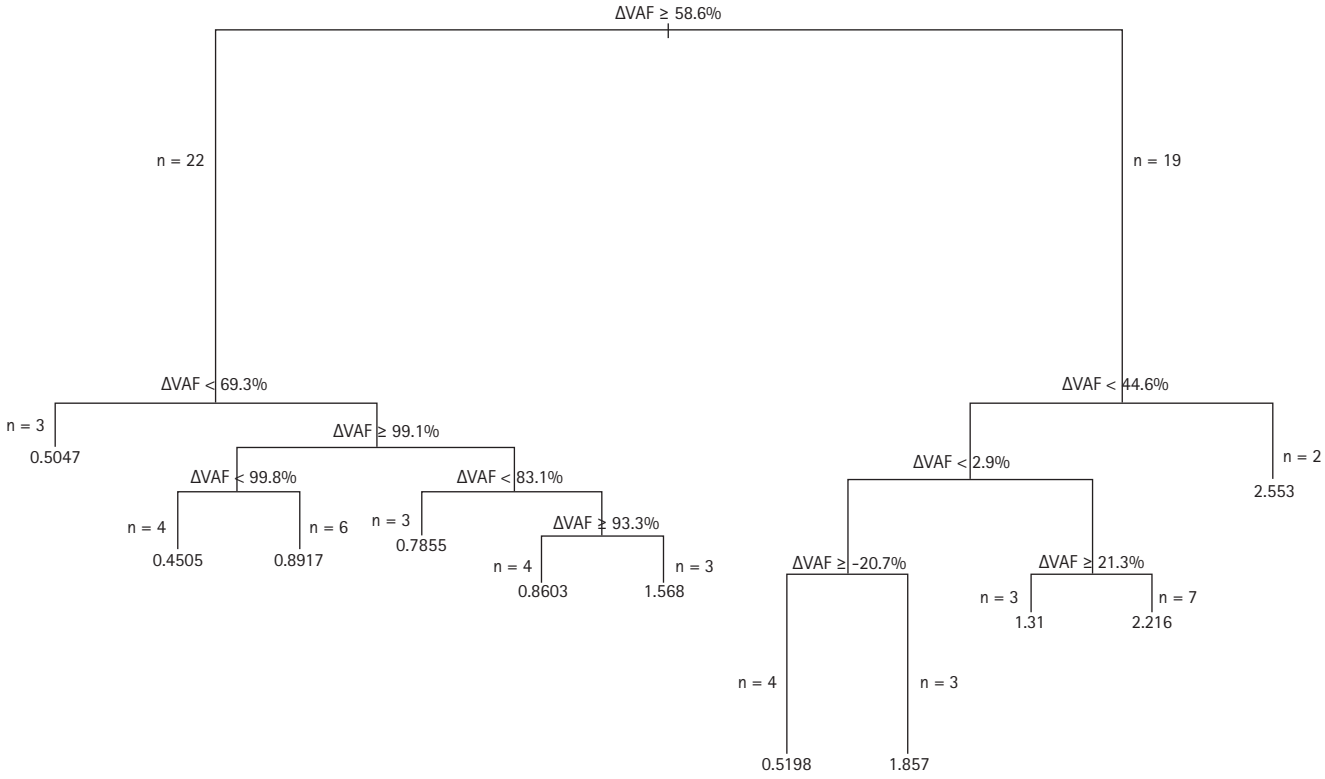
Supplementary Table 1. Continued

SF3B1
SMC1A
SRSF2
STAG2
TET2
TP53
U2AF1
WT1
ZRSR2

https://doi.org/10.3904/kjim.2022.396

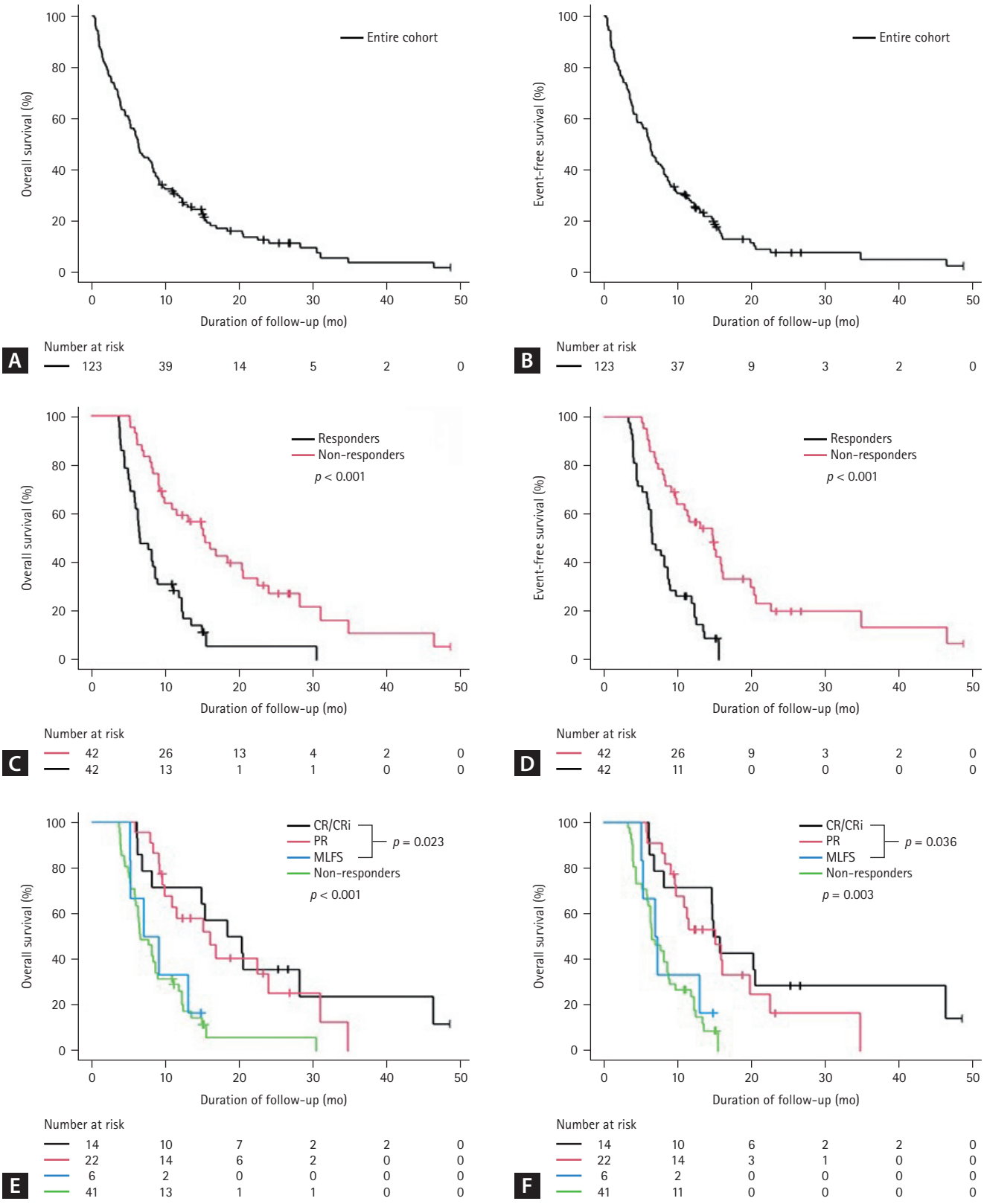


Supplementary Figure 1. Case distribution of genetic mutations assessed by next-generation sequencing (NGS) analysis. AML, acute myeloid leukemia; BM, bone marrow.

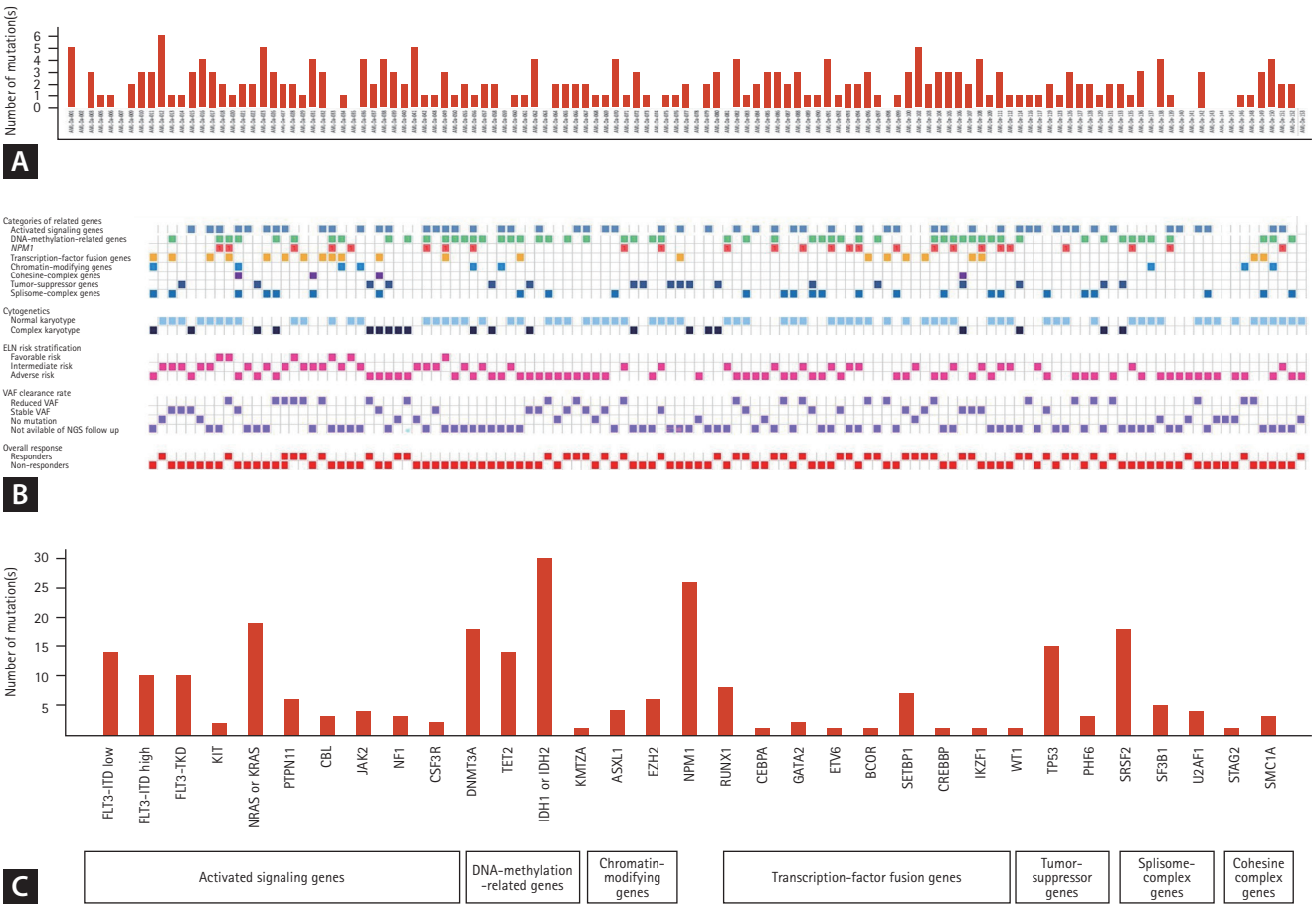


Supplementary Figure 2. Recursive partitioning for defining significant clearance rate (Δ) of variant allele frequency (VAF).

https://doi.org/10.3904/kjim.2022.396

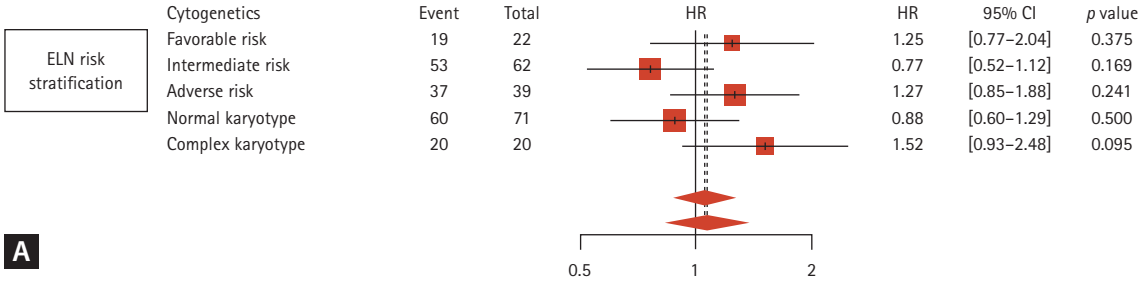


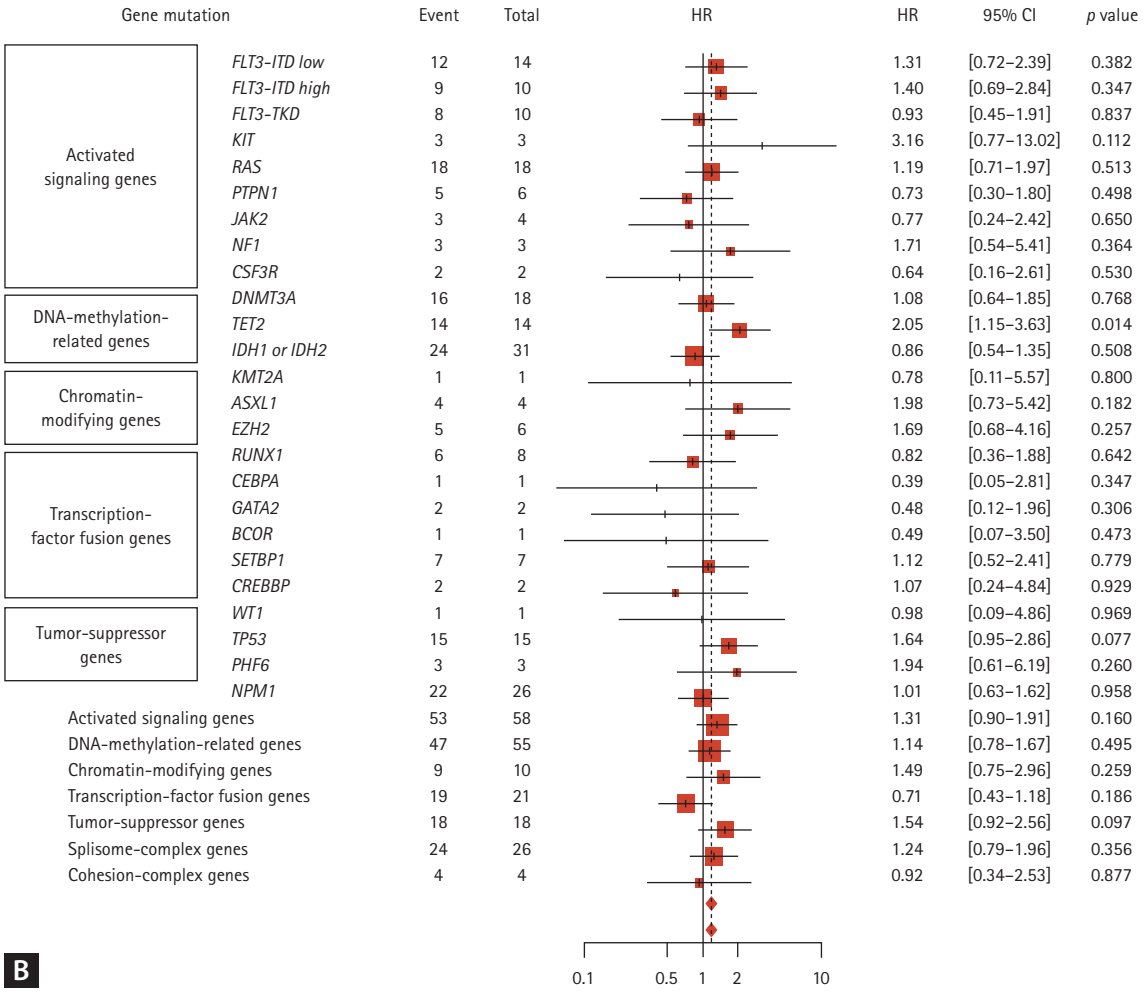
Supplementary Figure 3. (A) Overall survival (OS) curve of the entire cohort, (B) event-free survival (EFS) curves of the entire cohort, (C) OS and (D) EFS according to response and non-response, (E) OS and (F) EFS according to treatment response. CR, complete remission; CRi, CR with incomplete hematologic recovery; PR, partial remission; MLFS, morphologic leukemia-free state.



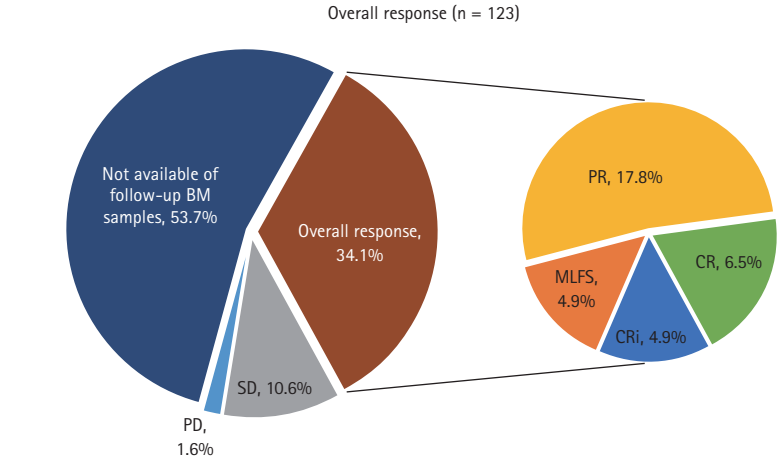
Supplementary Figure 4. Genetic mutations in elderly AML patients (n = 123) treated with decitabine. (A) Number of mutations detected in elderly AML patients at diagnosis. (B) Heat map clustered by functional gene pathway, cytogenetics, ELN stratification, VAF clearance rate, and overall response. (C) Frequency of mutations by functional gene pathway. *NPM1*, nucleophosmin 1; VAF, variant allele frequency; AML, acute myeloid leukemia; ELN, European LeukemiaNet.

https://doi.org/10.3904/kjim.2022.396

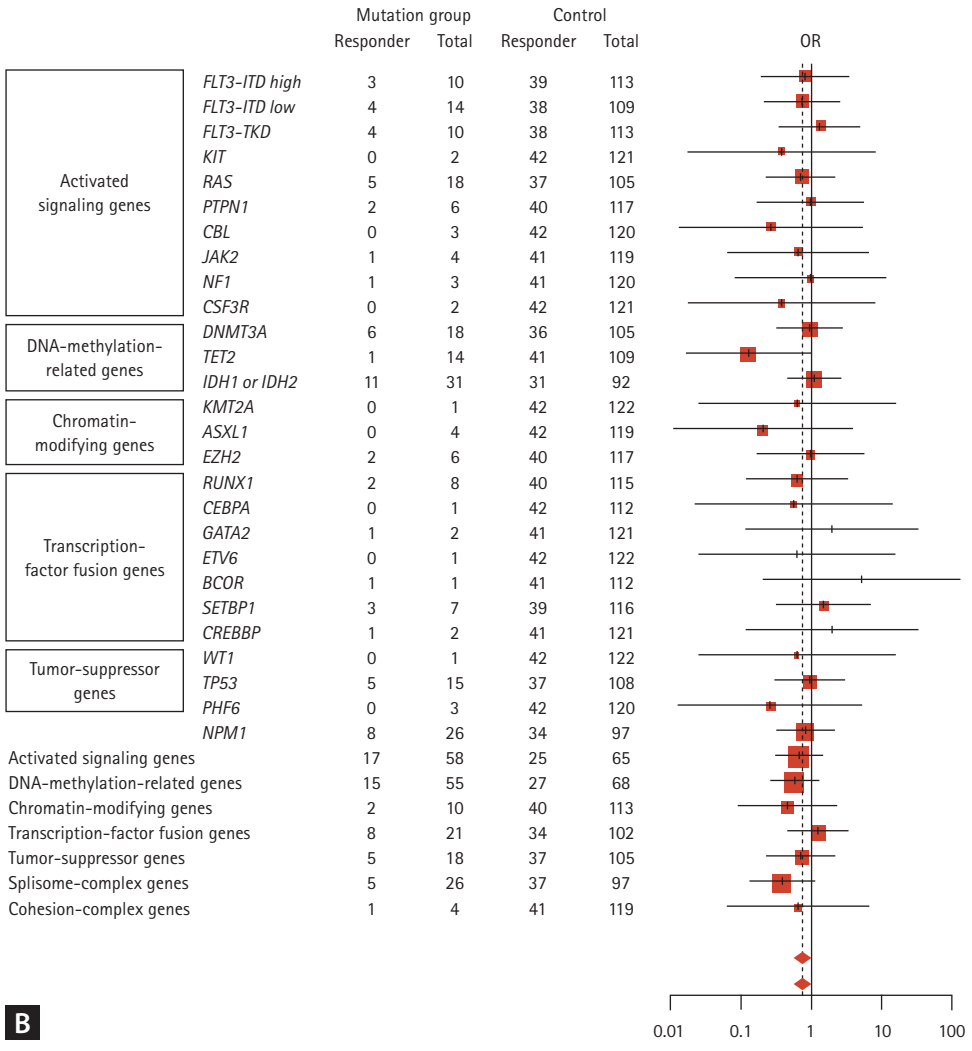




Supplementary Figure 5. Forest plot for overall survival (OS) by (A) cytogenetic abnormality and (B) gene mutations at diagnosis. Forest plot shows the hazard ratios (HRs, the solid squares) with 95% confidence intervals (Cis) for OS in elderly acute myeloid leukemia patients. ELN, European LeukemiaNet.



		Mutation group		Control				
		Responder	Total	Responder	Total	OR	OR	95% CI
Activated signaling genes	*FLT3-ITD high*	3	10	39	113		0.81	[0.20; 3.32]
	FLT3-ITD low	4	14	38	109		0.75	[0.22; 2.54]
	FLT3-TKD	4	10	38	113		1.32	[0.35; 4.95]
	KIT	0	2	42	121		0.37	[0.02; 7.97]
	RAS	5	18	37	105		0.71	[0.23; 2.14]
	PTPN1	2	6	40	117		0.96	[0.17; 5.48]
	CBL	0	3	42	120		0.26	[0.01; 5.23]
	JAK2	1	4	41	119		0.63	[0.06; 6.29]
	NF1	1	3	41	120		0.96	[0.08; 10.94]
	CSF3R	0	2	42	121		0.37	[0.02; 7.97]
DNA-methylation-related genes	*DNMT3A*	6	18	36	105		0.96	[0.33; 2.76]
	TET2	1	14	41	109		0.13	[0.02; 1.01]
	IDH1 or IDH2	11	31	31	92		1.08	[0.46; 2.54]
Chromatin-modifying genes	*KMT2A*	0	1	42	122		0.63	[0.03; 15.84]
	ASXL1	0	4	42	119		0.20	[0.01; 3.85]
	EZH2	2	6	40	117		0.96	[0.17; 5.48]
Transcription-factor fusion genes	*RUNX1*	2	8	40	115		0.62	[0.12; 3.24]
	CEBPA	0	1	42	112		0.55	[0.02; 13.88]
	GATA2	1	2	41	121		1.95	[0.12; 32.00]
	ETV6	0	1	42	122		0.63	[0.03; 15.84]
	BCOR	1	1	41	112		5.17	[0.21; 129.80]
	SETBP1	3	7	39	116		1.48	[0.32; 6.95]
	CREBBP	1	2	41	121		1.95	[0.12; 32.00]
Tumor-suppressor genes	*WT1*	0	1	42	122		0.63	[0.03; 15.84]
	TP53	5	15	37	108		0.96	[0.31; 3.01]
	PHF6	0	3	42	120		0.26	[0.01; 5.23]
	NPM1	8	26	34	97		0.82	[0.32; 2.09]
Activated signaling genes		17	58	25	65		0.66	[0.31; 1.41]
DNA-methylation-related genes		15	55	27	68		0.57	[0.26; 1.23]
Chromatin-modifying genes		2	10	40	113		0.46	[0.09; 2.25]
Transcription-factor fusion genes		8	21	34	102		1.23	[0.47; 3.25]
Tumor-suppressor genes		5	18	37	105		0.71	[0.23; 2.14]
Splisome-complex genes		5	26	37	97		0.39	[0.13; 1.11]
Cohesion-complex genes		1	4	41	119		0.63	[0.06; 6.29]



Supplementary Figure 6. Overall response in entire cohort (n = 123), (A) pie graph of overall response rates. (B) Gene mutations at diagnosis in elderly AML patients. Forest plots show the odds ratios (ORs, the solid squaress) of overall response with 95% confidence intervals (CIs) for each gene mutation. BM, bone marrow; PD, progressive disease; SD, stable disease; PR, partial remission; MLFS, morphological leukemia-free state; CR, complete remission; CRi, CR with incomplete hematologic recovery.

https://doi.org/10.3904/kjim.2022.396