Prognostic value of European LeukemiaNet 2022 criteria and genomic clusters using machine learning in older adults with acute myeloid leukemia

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Abstract

This study aimed to validate the new European Leukemia Net (ELN) 2022 criteria for genetic risk stratification in older adults with acute myeloid leukemia (AML) and to determine the most likely set of clusters of similar cytogenetic and mutation properties correlated with survival outcomes in three treatment groups: intensive chemotherapy (IC), hypomethylating agents (HMA) alone, and HMA plus venetoclax (HMA/VEN). The study included 279 patients (aged ≥60 years) who received IC (N=131), HMA (N=76), and HMA/VEN (N=72) between July 2017 and October 2021. No significant differences were observed in survival among the groups according to ELN 2022 risk stratification. Unsupervised hierarchical clustering analysis identified nine genomic clusters (C1-9) with varying survival outcomes depending on treatment type. For example, C4 (predominant for core binding factor-AML) displayed a favorable prognosis in the IC group, but not in the HMA or HMA/VEN groups. The HMA/VEN group had better outcomes than the HMA group in many clusters (C1, 2, 3, and 5); however, the addition of VEN to HMA or IC did not improve the survival outcomes compared with those of HMA alone in C7 and C9 (predominant for -5, del(5q), -7, -17/abn(17p), complex karyotypes, and mutated *TP53*). The study highlights the limitations of ELN genetic risk stratification in older adults with AML. It emphasizes the need for a more comprehensive approach that considers co-occurring somatic mutations to guide treatment selection in older adults with AML.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous group of diseases with variable prognoses,[1-3] resulting primarily from the complexity of the clonal architectures due to various chromosomal abnormalities, genetic mutations, or epigenetic changes.[1-4] In addition to this biological heterogeneity, age is an important prognostic indicator.[5,6] For instance, older adults with AML have been considered a distinct entity representing a vulnerable population characterized by inferior response and lower tolerance to conventional chemotherapy.[6,7] Therefore, treating older adults with AML is challenging. Classically, medical fitness is regarded as the major factor in determining candidacy for intensive chemotherapy (IC). Older adults with AML can receive IC if they meet the assessment criteria because IC is considered the best choice for obtaining a higher chance of remission and longer survival.[7-10] However, with the introduction of novel agents in recent years,[11] less intensive therapeutics, such as a combination of venetoclax (VEN) and hypomethylating agents (HMA),[12-14] have been used more frequently than IC, not only in patients with poor-risk cytogenetics, but also for those with *NPM1* and/or *IDH* mutations known to benefit from VEN-based regimens.[15,16] Consequently, fitness cannot be the sole basis for treatment decisions, and clinicians have to consider best-fit treatment options on an individualized basis according to the patient's disease-specific features, including cytogenetics and the mutational profile.[17] As more information about AML genomic landscapes becomes available, the prognostication system for AML has continued to evolve. Until recently, the European LeukemiaNet (ELN)

2017 genetic risk stratification[2] (now up-dated to the 2022 version[3]) was regarded as the gold standard for AML prognostication, and proved to be effective in predicting outcomes in independent validation cohorts of AML patients aged <65 years.[18,19] Although it is broadly accepted that the ELN risk model fits well and can serve as a basis for guiding treatment strategies in young patients with AML, the same risk strata are not as informative for older patients.[20,21] Therefore, the newly up-dated ELN 2022 guideline should be validated using multiple cohorts, and if outcomes are not predictable, there is an urgent need for appropriate tools to guide physicians in precisely tailoring a treatment strategy for this patient population.

Machine learning (ML) methods are effective for analyzing vast amounts of data and identifying complex relationships between variables. These advantages can promote better understanding of individual heterogeneities within a given disease. They can also delineate diagnostic and prognostic subgroups more precisely, and these can be used to develop individualized therapeutic strategies.[22-24] Some studies have classified the genomic landscapes of patients with AML using the unsupervised ML method and attempted to categorize survival outcomes of each cluster.[22,25-27] However, these studies focused on relatively young patients with AML. Given the need to validate the ELN 2022 classification in older patients with AML, and the scarcity of genomic classification data for this population, we aimed to investigate the effectiveness of ELN 2022 in this population and attempted to identify distinct genomic subtypes of older patients with AML via unsupervised clustering. In addition, we compared the patient outcomes of three treatment modalities, namely, IC, HMA alone, and HMA plus VEN, within the identified genomic subtypes and evaluated the potential impact of genomic clustering by ML on the care of elderly patients.

Methods

Patient selection

The patient selection criteria were: a) older ≥ 60 years patients with AML newly diagnosed at Seoul St. Mary's Hospital between July 2017 and October 2021; b) having available information on chromosome and gene mutations at diagnosis; and c) having received either IC, HMA alone, or HMA plus VEN (HMA/VEN) as their first-line treatment. Patient consent was not required because of the retrospective nature of the study. Patients who received only the best supportive care were not included in this study, which was approved by the Institutional Review Board and Ethics Committee of the Catholic Medical Center in South Korea (KC21RISI0572).

Molecular and cytogenetic studies

Bone marrow karyotyping was performed on G-banded metaphase chromosomes using conventional techniques. The karyotypes were interpreted using the International Standing Committee on Human Cytogenomic Nomenclature (ISCN) 2016.[28] For molecular analysis, next-generation sequencing was performed using a customized myeloid panel (SM panel), as described in our previous report.[29] The SM panel contains 67 genes that are frequently mutated in patients with AML. (See the *Online Supplementary Appendix* for details.)

For the detection of the FMS-like tyrosine kinase 3-internal tandem duplication (*FLT3*-ITD) mutation, polymerase chain reaction (PCR) for fragment analysis was performed using a previously published modified protocol, as detailed in the *Online Supplementary Appendix*.[30]

Definition of outcomes and statistical analyses

The baseline clinical and molecular characteristics of the older patients with AML were compared using the χ^2 test or Fisher exact test for categorical variables and a two-sample *t* test or Mann-Whitney U test for continuous variables. The *P* value was corrected using the Bonferroni method when multiple tests were indicated. Overall survival (OS) was defined as the time from the initiation of treatment to death from any cause. OS was estimated using the Kaplan-Meier method, and the groups were compared using the log-rank test. The Cox proportional hazards model was used for the univariate and multivariate analyses of OS. Variables with $P<0.10$ by univariate analysis were considered for entry into the multivariate analysis.

Unsupervised clustering and stratification

Unsupervised techniques were evaluated in our cohort and internally validated without testing external data. The abnormalities in karyotypes and genetic mutations that were used for risk stratification in ELN 2022 and additional mutations found in >3% of all patients in our cohort were used for the analysis. Detailed genomic variables are described in *Online Supplementary Table S1*. The number of clusters was determined using the parameter NbClust,[31] which explored a range of 3-12 clusters. The optimal number of clusters was chosen based on a voting process involving several measures, including the maximum value of the index, the maximum difference between the hierarchy levels of the index, and the minimum value of second differences between levels of the index. This process is further explained in detail in *Online Supplementary Table S2* and *Online Supplementary Figure S1*. The Ward1 algorithm with Euclidean distances were used for hierarchical agglomerative clustering and compared with k-means, clustering with partitioning around medoids (PAM), self-organizing maps (SOM), and the Gaussian mixture model (GMM). (See the *Online Supplementary Appendix* for details.) For internal validation, the clustering algorithms were compared by the clValid package.[32] The correlation network map demonstrated the weight if the correlation coefficient was >0.02. The analysis was performed using R software for statistical computing (v.0.2; R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics and their comparison between the treatment groups

A total of 279 patients who met the inclusion criteria were selected; median age at diagnosis was 68 years (range 60-84 years). As first-line treatment, 131 patients received IC (47.0%), 76 patients received HMA alone (27.2%), and 72 patients received HMA/VEN (25.8%). Median age of patients who received IC, HMA alone, and HMA/VEN was 64, 75, and 70 years, respectively (Table 1). Median age of patients in the HMA group was significantly higher than that of patients in the other groups. Most patients had *de novo* AML (N=238, 85.3%). There were more patients with secondary AML in the HMA/VEN group (30.6%) than in the other groups (IC: 9.2%; HMA: 9.2%). Among the genetic mutations at AML diagnosis, the distribution of *FLT3*-ITD and *PTPN11* mutations differed significantly among the three groups. The frequency of mutated *FLT3*-ITD was the lowest in patients receiving HMA/VEN (IC: 16%; HMA: 19.7%; HMA/VEN: 2.8%; *P*=0.018), whereas the *PTPN11* mutation was observed the least in the IC group (IC: 2.3%; HMA: 13.2%; HMA/VEN: 8.3%; *P*=0.03).

Survival outcomes after each treatment in older patients with acute myeloid leukemia according to European Leukemia Net 2022 criteria risk stratification

The clinical outcomes of each treatment are shown in Figure 1. After a median follow-up of 26.3 months, the median OS in the IC, HMA, and HMA/VEN groups was 20 months (95% CI: 14.9-32.5), 8.8 months (95% CI: 6.0-11.3), and 10 months

Table 1. Baseline demographic and clinical characteristics of the study cohort (N=279).

Variables	IC N=131	HMA N=76	HMA/VEN N=72	IC *vs.* HMA	HMA *vs.* HMA/VEN	HMA/VEN *vs.* IC	*P*
				P	*P*	*P*	
Age at diagnosis in years, median (range)	64.0 (62.0-67.0)	75.0 (71.0-77.0)	70.0 (66.5-74.0)	<0.001	<0.001	<0.001	<0.001
Sex, female, N (%)	54 (41.2)	25 (32.9)	40 (55.6)	0.298	0.009	0.019	0.057
Disease type, N (%)							
De novo	119 (90.8)	69 (90.8)	50 (69.4)	>0.999	0.002	<0.001	<0.001
Secondary	12 (9.2)	7 (9.2)	22 (30.6)				
Karyotype abnormality, N (%)							
Complex	8 (6.1)	9 (11.8)	6 (8.3)	0.236	0.664	0.757	>0.999
-5 or del(5q);-7;-17/abn(17p)	6 (4.6)	4 (5.3)	10 (13.9)	>0.999	0.131	0.037	0.111
Monosomal	5 (3.8)	5 (6.6)	10 (13.9)	0.577	0.23	0.019	0.084
RUNX1-RUNX1T1	10 (7.6)	4 (5.3)	3 (4.2)	0.713	>0.999	0.506	>0.999
CBFB-MYH11	6 (4.6)	1 (1.3)	4 (5.6)	0.393	0.331	>0.999	>0.999
Mutation, N (%)							
NPM1	31 (23.7)	20 (26.3)	10 (13.9)	0.795	0.094	0.14	0.447
*NPM1*mut without *FLT3-ITD*	14 (10.7)	10 (13.2)	8 (11.1)	0.757	0.897	>0.999	>0.999
DNMT3A	23 (17.6)	18 (23.7)	19 (26.4)	0.376	0.849	0.192	0.885
TET2	20 (15.3)	20 (26.3)	16 (22.2)	0.079	0.698	0.294	0.417
FLT3-ITD	21 (16.0)	15 (19.7)	2 (2.8)	0.626	0.003	0.009	0.018
FLT3-TKD	4 (3.1)	6 (7.9)	2 (2.8)	0.219	0.311	>0.999	0.579
IDH1	8 (6.1)	7 (9.2)	3 (4.2)	0.581	0.371	0.795	>0.999
IDH2	23 (17.6)	6 (7.9)	5 (6.9)	0.085	>0.999	0.059	0.105
RUNX1	12 (9.2)	12 (15.8)	8 (11.1)	0.226	0.554	0.841	>0.999
ASXL1	11 (8.4)	12 (15.8)	6 (8.3)	0.161	0.256	>0.999	0.585
NRAS	5 (3.8)	8 (10.5)	7 (9.7)	0.105	>0.999	0.163	0.366
PTPN11	3 (2.3)	10 (13.2)	6 (8.3)	0.005	0.497	0.1	0.03
SRSF2	9 (6.9)	5 (6.6)	5 (6.9)	>0.999	>0.999	>0.999	>0.999
BCOR	5 (3.8)	7 (9.2)	5 (6.9)	0.196	0.839	0.518	0.831
TP53	4 (3.1)	7 (9.2)	5 (6.9)	0.114	0.839	0.351	0.486
TP53 (VAF ≥10%)	3 (2.3)	7 (9.2)	1 (1.4)	0.057	0.082	>0.999	0.063
U2AF1	3 (2.3)	7 (9.2)	4 (5.6)	0.057	0.593	0.413	0.261
Non-bZIP *CEBPA*	4 (3.1)	4 (5.3)	3 (4.2)	0.674	>0.999	0.989	>0.999
bZIP in-frame *CEBPA*	3 (2.3)	3 (3.9)	0 (0.0)	0.798	0.263	0.493	0.756
JAK2	5 (3.8)	1 (1.3)	4 (5.6)	0.546	0.331	0.826	>0.999
SETBP1	2 (1.5)	5 (6.6)	2 (2.8)	0.124	0.483	0.932	0.408

IC: intensive chemotherapy; HMA: hypomethylating agent; HMA/VEN: HMA plus venetoclax; N: number; RUNX1-RUNX1T1: t(8;21)(q22;q22) rearrangement; CBFB-MYH11: inv(16)(p13.1q22) or t(16;16)(p13.1;q22) rearrangement; VAF: variant allele frequency.

(95% CI: 6.3-27.0), respectively (*P*<0.01). We applied the ELN 2022 risk stratification to the three treatment groups. For patients in the IC group, the median OS was estimated to be 44.1, 23.2, and 13.9 months according to the risk groups of favorable (FAV), intermediate (INT), and adverse (ADV) risk by ELN 2022, respectively. Moreover, long-term survival rate has a distinct trend that was not significantly different between the risk groups (*P*=0.069). As observed in the IC group, there was no significant difference in OS according to the ELN 2022 risk in the HMA (*P*=0.926) or HMA/VEN (*P*=0.498) groups. These data suggest that ELN 2022 genetic risk stratification does not impact survival outcome with respect to treatment modalities in older patients with AML.

Genetic factors that are associated with worse overall survival in older patients with acute myeloid leukemia by the treatment groups

To determine which ELN 2022 genetic risk stratification factors are associated the most with OS in each treatment group, we conducted univariate and multivariate analyses of these factors (Table 2). Mutated genes that were found in >3% of the patients and the cut-off age of 75 years were included in this analysis. Among the patients in the IC group, monosomal karyotype, mutated *TP53* with at least 10% variant allele frequency (VAF), *U2AF1*, and *SETBP1* result in inferior OS according to the multivariate analysis. In the HMA group, *CBFB-MYH11* fusion, mutated *SRSF2*, and non-biZIP *CEBPA* were independently associated with worse OS. In the HMA/VEN group, no variables significantly impacted OS in the multivariate analysis. These data suggest that certain genetic mutations are associated with OS in distinct treatment groups of older patients with AML.

Unsupervised clustering with respect to molecular aberrations

Hierarchical clustering with respect to the molecular and cytogenetic analysis results indicates that older patients with AML can be classified into one of the nine clusters (Figure 2A). The dominant cytogenetic features of each cluster were as follows. Cluster 1 (C1): no dominant genomic alterations; C2: non-bZIP *CEBPA* and *FLT3*-ITD mutations; C3: mutated *ASXL1* and *RUNX1*; C4: *RUNX1-RUNX1T1* or *CBFB-MYH11* fusion; C5: *BCOR* and *IDH2* mutations; C6: mutated *NPM1* with *FLT3*-ITD wild type, *IDH1*, *NRAS*, *PTPN11*, bZIP in-frame *CEBPA*, and *JAK2*; C7: abnormal karyotype (-5 or del(5q); -7;-17/abn(17p)); C8: mutated *SRSF2* and *TET2*; and C9: complex karyotype and *TP53* mutation at a VAF of at least 10%.

To determine which molecular aberrations are associated with each other in a paired fashion, we mapped their correlation network (Figure 2B). In this data analysis, the following pairs of molecular aberrations were highly correlated: monosomal karyotype and either -5, del(5q), -7 or -17/abn(17p), *RUNX1-RUNX1T1* and *KIT* mutations, and complex karyotype and *TP53* mutations with at least 10% VAF. The node with the most concomitant mutation or genetic events (also referred to as the highest betweenness centrality) was mutated *SETBP1*, followed by *NRAS* mutation. In contrast, the betweenness centrality was lowest in *CBFB-MYH11* fusion, followed by the bZIP in-frame *CEBPA* mutation, complex karyotype, and *SRSF2* mutations. Detailed proportions of the cytogenetic abnormalities and genetic mutations in each cluster are described in Figure 3. (See also *Online Supplementary Table S1*.) There were some overlaps across the clusters that may be due to the inherent limitations of the clustering method employed and the modest sample size, or they could reflect the characteristics of AML, where mutational overlapping across clusters is unavoidable. In addition, we also compared different cluster methods and found that the hierarchical agglomerative clustering used in this study showed the best score in connectivity and stability (average proportion of non-overlap and the average distance between means) (*Online Supplementary Table S3*). According to the Meila

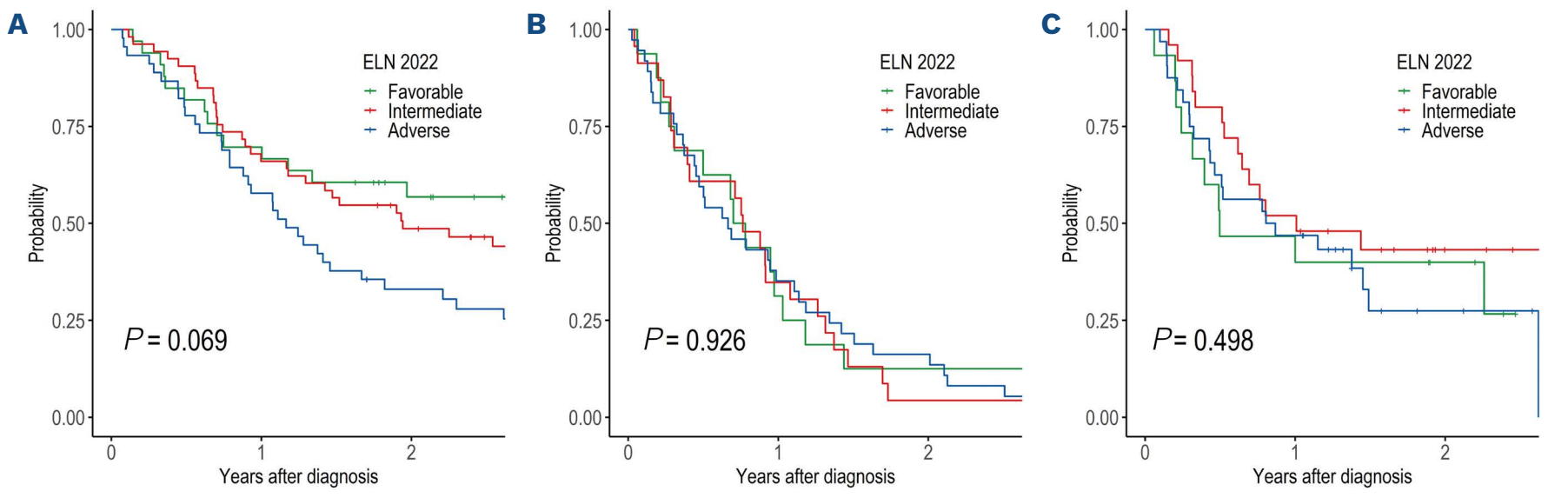


Figure 1. Prognostic significance of the European LeukemiaNet 2022 risk classification in predicting the overall survival of older patients with acute myeloid leukemia. Survival curves of patients who received (A) intensive chemotherapy, (B) hypomethylating agent, and (C) hypomethylating agent plus venetoclax.

Table 2. Univariate and multivariate analysis for survival outcomes related to genetic alteration.

Variable	IC				HMA				HMA/VEN	
	Univariate		Multivariate		Univariate		Multivariate		Univariate	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age >75 years	4.32 (1.04-18.0)	0.044	3.35 (0.58-19.4)	0.177	1.16 (0.73-1.85)	0.533	-	-	1.79 (0.94-3.41)	0.078
Complex	2.18 (1.00-4.77)	0.05	1.02 (0.38-2.73)	0.966	1.3 (0.64-2.63)	0.463	-	-	1.08 (0.39-3.03)	0.878
-5 or del(5q)-7;-17/ abn(17p)	1.56 (0.57-4.26)	0.389	-	-	0.8 (0.29-2.22)	0.674	-	-	1.58 (0.73-3.41)	0.241
Monosomal	7.24 (2.79-18.8)	<0.001	8.21 (2.85-23.7)	<0.001	0.7 (0.28-1.74)	0.437	-	-	1.57 (0.73-3.37)	0.248
RUNX1-RUNX1T1	0.82 (0.33-2.03)	0.667	-	-	0.93 (0.34-2.56)	0.887	-	-	0.94 (0.23-3.88)	0.929
CBFB-MYH11	0.6 (0.19-1.90)	0.383	-	-	37 (3.35-408)	0.003	47.2 (4.23-526)	0.002	0.81 (0.19-3.39)	0.774
*NPM1*mut without *FLT3-ITD DNMT3A*	1.03 (0.50-2.13)	0.94	-	-	0.81 (0.38-1.70)	0.575	-	-	1.48 (0.62-3.50)	0.375
TET2	1.37 (0.77-2.43)	0.29	-	-	1.16 (0.69-1.97)	0.579	-	-	1.39 (0.71-2.70)	0.335
FLT3-ITD	1.1 (0.62-1.95)	0.754	-	-	1.44 (0.81-2.55)	0.214	-	-	1 (0.14-7.26)	0.997
FLT3-TKD	2.45 (0.89-6.72)	0.082	2.11 (0.61-7.35)	0.239	0.55 (0.22-1.37)	0.202	-	-	1.79 (0.43-7.49)	0.425
IDH1	0.85 (0.34-2.10)	0.722	-	-	1.2 (0.55-2.63)	0.649	-	-	1.91 (0.59-6.19)	0.28
IDH2	0.7 (0.39-1.25)	0.231	-	-	0.94 (0.38-2.35)	0.901	-	-	0.47 (0.11-1.99)	0.305
RUNX1	0.68 (0.30-1.56)	0.364	-	-	0.69 (0.36-1.32)	0.262	-	-	0.89 (0.35-2.28)	0.804
ASXL1	0.7 (0.30-1.61)	0.397	-	-	0.85 (0.44-1.61)	0.608	-	-	0.79 (0.28-2.24)	0.658
NRAS	1.33 (0.42-4.21)	0.63	-	-	0.56 (0.25-1.24)	0.155	-	-	1.15 (0.45, 2.98)	0.768
PTPN11	1.24 (0.31-5.07)	0.761	-	-	1.23 (0.63-2.40)	0.547	-	-	1.63 (0.64-4.15)	0.308
SRSF2	1.1 (0.48-2.52)	0.83	-	-	2.36 (0.93-5.98)	0.07	2.65 (1.04-6.75)	0.041	1.82 (0.71-4.65)	0.209
BCOR	0.7 (0.22-2.22)	0.547	-	-	0.71 (0.32-1.56)	0.395	-	-	0.72 (0.17-3.00)	0.656
TP53 (VAF ≥10%)	7.1 (2.18-23.2)	0.001	7.1 (2.18-23.2)	0.004	1.2 (0.55-2.63)	0.647	-	-	1.02 (0.14-7.42)	0.985
KIT	0.62 (0.23-1.69)	0.347	-	-	1.89 (0.59-6.06)	0.286	-	-	1.2 (0.29-4.98)	0.802
U2AF1	6.7 (2.07-21.7)	0.002	6.7 (2.07-21.7)	<0.001	1.49 (0.68-3.27)	0.318	-	-	0.33 (0.05-2.39)	0.272
Non-bZIP *CEBPA*	0 (0.00-Inf)	0.995	-	-	3.54 (1.25-10.0)	0.018	4.03 (1.41-11.5)	0.009	0.7 (0.16-3.05)	0.634
bZIP in-frame *CEBPA*	0 (0.00-Inf)	0.996	-	-	1.46 (0.46-4.69)	0.521	-	-	-	-
JAK2	1.38 (0.56-3.42)	0.485	-	-	0.67 (0.09-4.86)	0.693	-	-	1.1 (0.34-3.56)	0.87
SETBP1	3.57 (0.87-14.6)	0.078	4.19 (1.01-17.4)	0.048	0.88 (0.35-2.20)	0.784	-	-	2.35 (0.56-9.90)	0.246

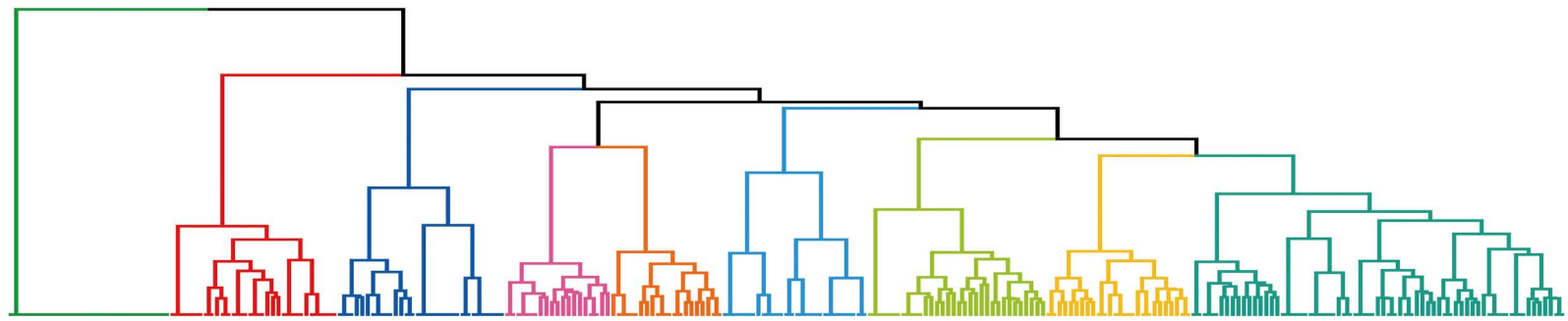
IC: intensive chemotherapy; HMA: hypomethylating agent; HMA/VEN: HMA plus venetoclax; HR: Hazard Ratio; CI: Confidence Interval; *RUNX1-RUNX1T1*: t(8;21)(q22;q22) rearrangement; *CBFB-MYH11*: inv(16)(p13.1q22) or t(16;16)(p13.1;q22) rearrangement; VAF: variant allele frequency.

variance information, clusters from PAM and SOM were the most similar, and hierarchical agglomerative clustering and GMM were the most different.

Genomic clustering by hierarchical agglomerative clustering and survival outcomes

To determine whether clusters formed with respect to molecular aberrations are associated with survival outcomes in older patients with AML, we compared and analyzed the survival of patients in clusters classified by molecular and cytogenetic aberrations. The median OS of all patients was 12.8 months, and those of C1, C3, C4, and C5 were >13 months. C7 showed the worst OS (median 6.7 months) among the clusters (*Online Supplementary Table S4*). The OS of each cluster according to the treatment type (*Online Supplementary Figure S2*) showed that survival could be significantly different across clusters when subject to the same treatment. Linking the clusters with survival outcomes of each treatment arm (Figure 4A, B, C, *Online Supplementary Figure S3*) enabled stratification (favorable, intermediate, and adverse) with significant differences in OS. Among the 131 patients in the IC group, the survival of C3 and C4 was superior to those of C2, C7, and C9 (Hazard Ratio [HR]: 2.79; 95% CI: 1.30-5.99; *P*=0.009). In contrast, C1, C3, and C7 achieved better survival (HR: 2.97; 95% CI: 1.28-6.88; *P*=0.011) than C2 and C4 when treated with HMA. In the HMA/VEN group, C1, C3, and C5 showed favorable survival outcomes, whereas C6 and C8 showed poorer outcomes (HR: 3.97; 95% CI: 1.60-9.84; *P*=0.001).

A



Cluster, N	C1, N=29	C2, N=30	C8, N=30	C7, N=19	C9, N=20	C4, N=26	C3, N=32	C5, N=26	C6, N=67
Dominant alteration	No dominant alteration	Non-bZIP *CEBPA* *FLT3-ITD*	*SRSF2* *TET2*	-5 or del(5q); .-7;-17/ abn(17p)	Complex Karyotype	*RUNX1-RUNX1T1*, *CBFB-MYH11* *KIT*	*ASXL1* *RUNX1*	*BCOR* *IDH2* *DNMT3A*	*NPM1*mut *FLT3-ITD* wild, bZIP in-frame *CEBPA* *NRAS* *PTPN11*

B

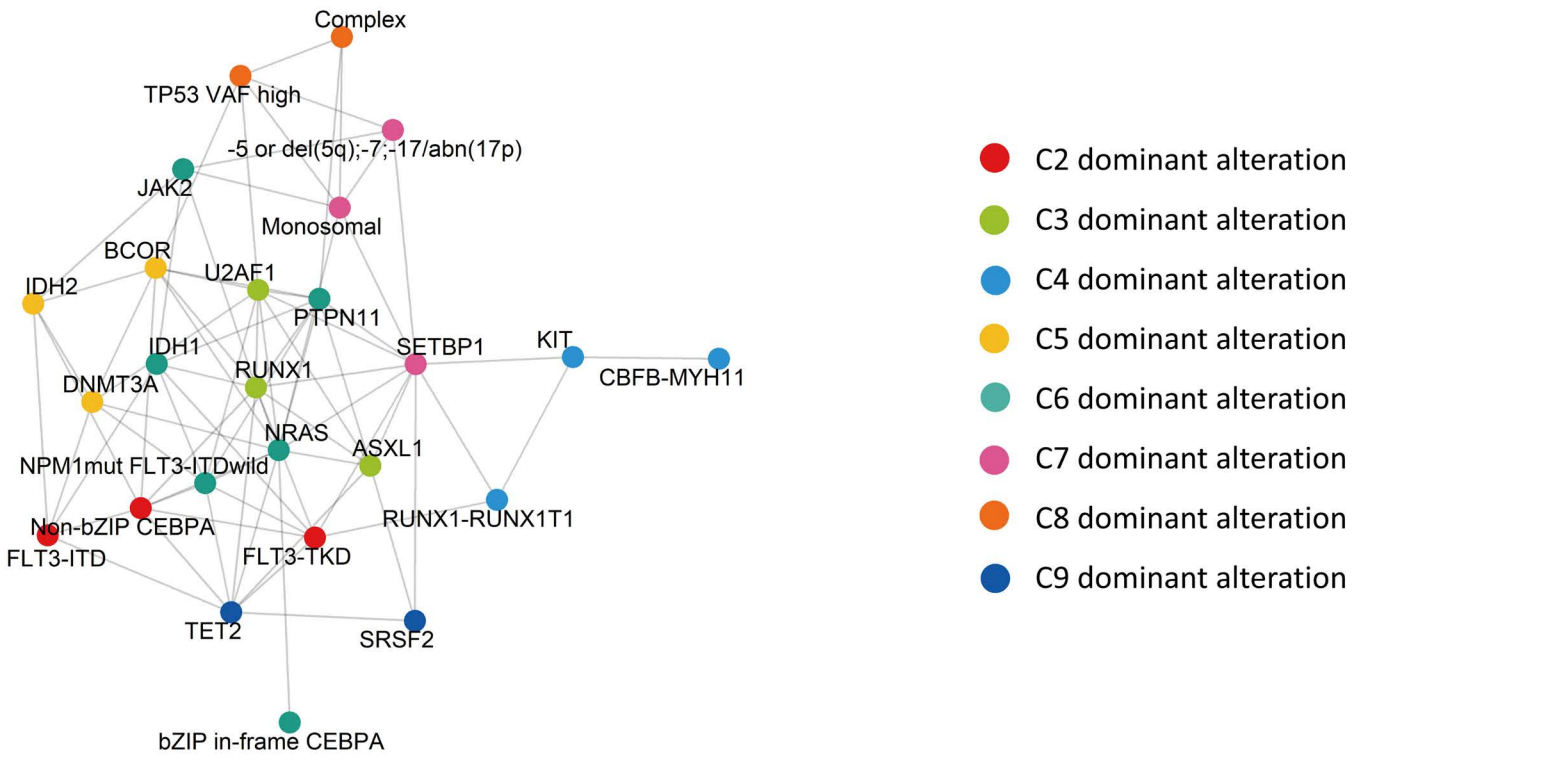


Figure 2. Classification of hierarchical clustering in older patients with acute myeloid leukemia. (A) Dendrogram showing patients classified into nine distinct subgroups based on the frequently assembled mutations and chromosomal abnormalities. (B) Nodes in the network map represent genomic alterations found in dominant individual clusters. The edges, arranged in relation to each gene, represent the correlations between different genes. Colors of the nodes represent the nine clusters, which include the dominant genes.

Stratification of the clusters differed depending on the treatment type, and survival outcomes of each cluster were substantially different according to different treatment arms (Figure 4D-F). Among C4, C6, and C8, IC showed better outcomes than HMA/VEN did (7 *vs.* 23.6 months; *P*=0.009). The HMA/VEN arm showed better outcomes than HMA only (HMA *vs.* HMA/VEN: 9.2 *vs.* 31.4 months; *P*=0.002) among C1, C2, C3, and C5. C7 and C9 showed no differences in OS among the HMA, HMA/VEN, and IC groups (8.6 *vs.* 9.5 *vs.* 9.4 months; *P*=0.904).

Discussion

With the recent release of the up-dated version of the ELN risk stratification, several groups have reported real-world prognostic validation results that proved that ELN 2022 performs well in stratifying patients with AML into prognostically different favorable, intermediate, and adverse risk groups.[33,34] However, these results were derived only from patients who received IC; consequently, information on the prognostic utility of ELN 2022 in older patients who may or may not be candidates for IC is lacking. In this study, we applied ELN 2022 to verify its prognostic predictability among patients with AML ≥ 60 years of age. We observed that it could not distinguish survival prognosis in these aged populations regardless of the treatments they received. The poor predictive ability of ELN 2022 was particularly noticeable in the patients receiving lower-intensity treatment compared with that of the IC group. Our results are consistent with previous findings that demonstrated the limited value of ELN 2017 in predicting prognosis in older adults with AML.[20,21] This highlights the need for a distinct prognostic system from that used for young patients with AML to guide therapeutic approaches in this older population.

Given the lack of prognostic models derived from disease features in older adults with AML, we examined the feasibility of prognostication based on clustering with respect

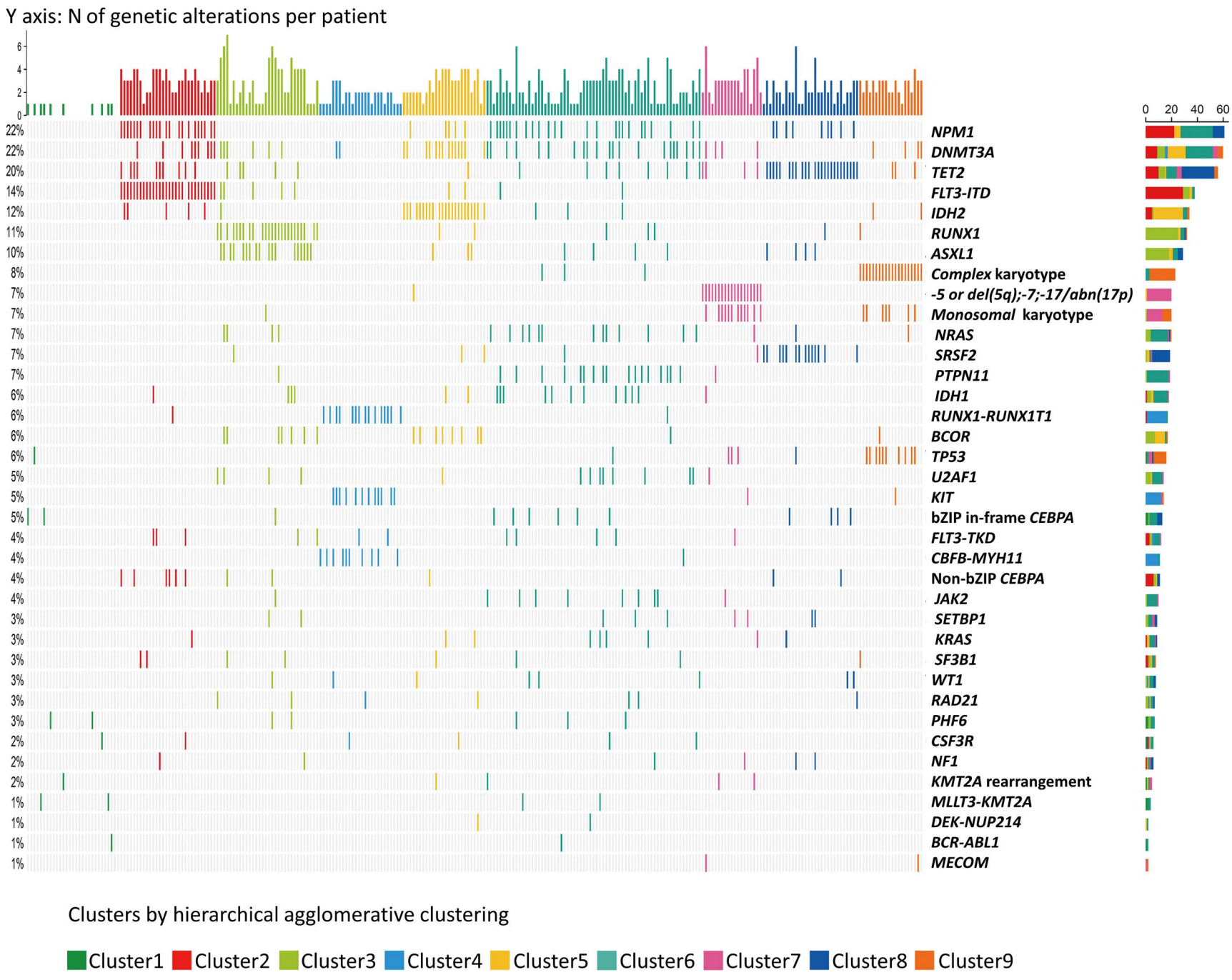


Figure 3. Heatmap of individual genomic lesions. Rows represent individual genomic lesions; columns represent patients included in the study. Vertical lines are used to indicate the presence of a specified driver mutation in each patient.

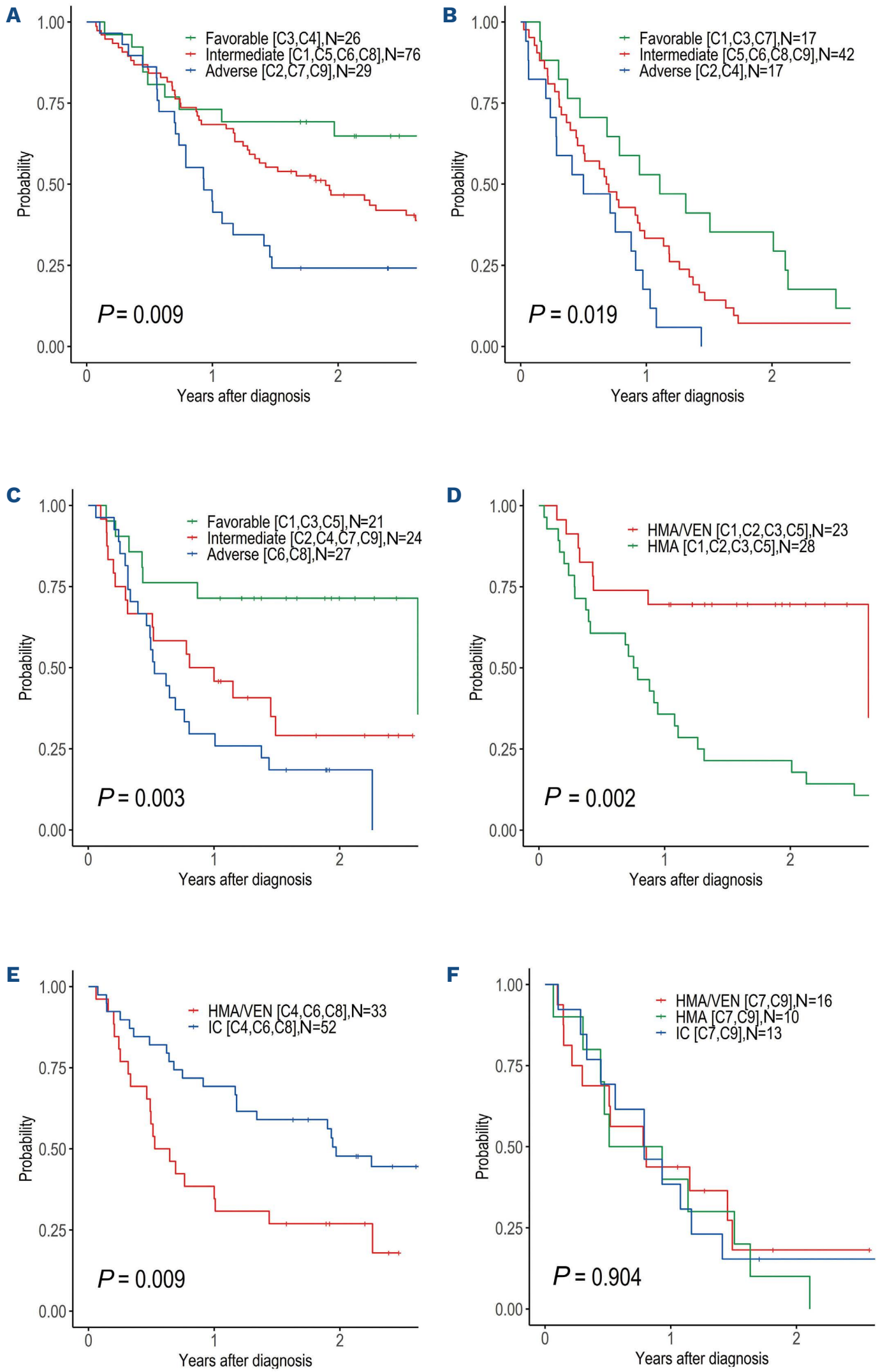


Figure 4. Association of overall survival and hierarchical clustering in older patients with acute myeloid leukemia. Risk stratification of individual genomic cluster according to the three treatment arms: (A) intensive chemotherapy (IC), (B) hypomethylating agent (HMA), and (C) hypomethylating agent plus venetoclax (HMA/VEN). Clusters showing better survival (D) in the HMA/VEN arm than in the HMA treatment arm and (E) in the IC treatment arm than in the HMA/VEN treatment arm. (F) Clusters showing similar survival between the three treatment arms. OS: overall survival.

to abnormalities in karyotypes and genetic mutations. Prior to our study, several scoring systems had been proposed to assist in determining which treatment may be beneficial to older adults with AML in terms of OS, early mortality, or comprehensive geriatric quality of life.[6-8,35,36] However, these were limited in that the development of such decision models was usually conducted regardless of the treatment type, with the result that they were unable to provide information regarding outcomes according to different treatment types. In addition, previous risk stratification was mainly focused on survival outcomes of exclusive genetic alterations that did not overlap.[26,27] As various genes were verified and characterized as affecting each other, subgrouping was needed to promote better interpretation.[37] Analyzing the interaction of each gene with several genes was possible via conventional statistical methods; however, because more genes were added, calculating the effect of the gene was impossible without applying an ML method. In particular, the ML method could validate whether these classifications were well-divided. In this study, we conducted an unsupervised hierarchical clustering analysis and classified older adults with AML into nine clusters with different genetic profiles at baseline. Although clustering is not straightforward because of the presence and complexity of co-occurring somatic mutations, each cluster has its own molecular signature based on predominantly mutated genes or karyotype abnormalities. Intriguingly, we observed that each genomic cluster was mapped to different prognostic positions by the changes in treatment modalities; a genomic cluster that showed favorable survival outcomes after IC would not necessarily show favorable outcomes after HMA or HMA/VEN, but could instead be the one that shows a poor prognosis.

In this context, one of the noticeable clusters was C4, in which core-binding factor-AML (CBF-AML) fusion dominance was observed. Among the AML subtypes, CBF-AML is a genetically distinct group of AML associated with chromosomal changes in t(8;21) and inv(16)(p13q22) or t(16;16)(p13;q22). It has several clinically distinctive characteristics compared with other forms of AML, such that it often begins in young adults. Patients aged >60 years with CBF-AML make up only about 5-15% of all adult CBF-AML.[38-41] It often presents a good prognosis given its favorable response to cytarabine-based IC. Moreover, it is generally accepted that older adults with CBF-AML should be offered IC if they are considered fit. Accordingly, patients with CBF-AML in our study were primarily offered IC unless deemed ineligible to receive such therapy. However, apart from data concerning IC, there are few data regarding the management of older adults with CBF-AML. Furthermore, phase III studies evaluating the effectiveness of lower-intensity treatments, such as HMA or HMA+VEN, in older adults with AML excluded CBF-AML for study inclusion.[13,42,43] In this study, we observed that C4, which mostly consisted of patients with CBF-AML (25/26, 96.2%), had a good prognosis in the IC group but not in the HMA or HMA/VEN groups. Moreover, the addition of VEN to HMA did not provide additional survival benefits compared with HMA alone for C4 patients. Our findings are in line with the results of a previous study in which the possibility of VEN resistance in CBF-AML was considered given the unpredictably poor event-free survival or OS in relapsed or refractory CBF-AML patients receiving the VEN-combined intensive regimen.[44] However, this conflicts with the findings of another study showing remarkable activity of HMA+VEN in favorable-risk AML, including cases of CBF-AML (10/46, 22%).[45] Given the limited data on the activity of HMA or HMA+VEN in patients with CBF alterations, further research with a larger cohort is needed.

Acute myeloid leukemia with higher-risk cytogenetics and mutated *TP53* have a dismal prognosis following conventional IC, making these patients candidates for innovative therapies that have the potential to improve prognosis. Our findings also showed that IC has no survival benefit over HMA or HMA+VEN in patients belonging to C7 and C9, where unfavorable cytogenetics, such as -5, del(5q), -7, -17/abn(17p), or complex karyotypes with mutated *TP53* were predominant. The median OS was similar between the IC, HMA, and HMA-VEN groups (9.4 *vs.* 8.6 *vs.* 9.5 months; P=0.904). Moreover, VEN combined with HMA did not improve the survival outcome compared with HMA alone, consistent with the results from the subgroup analysis of the phase III VIALE-A study.[13] Similarly, in a phase II study of 10-day decitabine plus VEN,[14] the median OS for adverse-risk cytogenetics was reported to be 8.0 months, whereas the median estimates were not reached for newly diagnosed *de novo* AML. In addition, multivariable analysis in this phase II study indicated that *TP53* mutation is associated with inferior survival and higher risk of relapse. Taken together, these data suggest an urgent need for novel therapeutic approaches targeting this group of patients. Based on the evidence that enhanced immune infiltrations are frequently observed in *TP53*-mutated AML, promising preliminary data with novel immune-based agents such as magrolimab,[46] flotetuzumab,[47] sabatolimab, and eprenetapopt[48] have been reported. In particular, given that *TP53* mutation is frequently accompanied by a complex karyotype (as shown in C9), the results of ongoing clinical trials for *TP53*-mutated AML are awaited with the hope of improving survival.[49]

With regard to treatment selection for older adults with AML, the current guideline[50] recommends azacitidine plus VEN as a preferred category 1 regimen for the majority of elderly patients unless they are suitable for IC. However, we have found that some clusters treated with HMA/VEN did not show any remarkable survival advantage over HMA alone. In addition, in real-world practice, we have observed a more prominent cytopenia or morphologic leukemia-free state with an uncertain response when using the VEN combination compared to HMA alone. This potentially leads to significant morbidity or mortality, highlighting the need for speedy identification of patients who are less likely to respond to the VEN combination. To date, however, beyond current risk factors such as genomics, we cannot be sure of the best way to identify the

best-fit population for specific treatments, including HMA/VEN. In this way, exploring a model that can reliably predict overall benefit through a summation of potential benefit and toxicity, which could perhaps be complemented with a physical function measure[51] or an *ex vivo* drug sensitivity test,[52] would be helpful for older adults with AML who usually consider HMA/VEN as their first choice.

The current study has several limitations. First, its retrospective nature and the relatively modest sample size in each treatment group, sometimes with a very small number of patients receiving specific treatments, make it challenging to draw definitive conclusions. Second, the lack of an independent validation cohort restricts the generalizability of our findings, and further validation of the clustering groups using external data or prospective cohorts is needed. Third, other possible factors affecting survival outcomes, such as fitness at diagnosis, quality of treatment response, and type of post-remission treatment, were not taken into account in the survival model. Fourth, we could not assess the impact of CPX-351, *FLT3*, or *IDH* inhibitors on survival outcomes because these agents were not available when the patients included in the study were treated. Fifth, the unsupervised clustering approach employed may have inherent weaknesses as it does not incorporate the physicians' clinical standpoints. For instance, C6 encompasses diverse genetic subgroups that are clustered together, where the co-assignment of various genetic mutations in the same cluster does not necessarily imply a strong relationship between them. Instead, it appears to reflect a tendency for C6 to possess less distinct genetic characteristics compared to the other clusters within the fixed cluster size (N=9) that was determined by the voting process involving multiple indices. Nevertheless, to the best of our knowledge, this is the first study to validate the new ELN 2022 risk stratification in older adults with AML. Furthermore, this is the first attempt to correlate genomic subtypes of AML revealed by unsupervised ML clustering with treatment-dependent prognostic prediction, which was confined to the setting of older adults with AML. This provides new insights into how therapeutic benefits vary by and depend on an individual genetic signature, which may aid clinicians in determining suitable treatments for older adults with AML. In conclusion, we demonstrate the limits of the new ELN 2022 for predicting outcomes in older adults with AML, highlighting the need for a different prognostic approach in this population. We also show that ML might be used to categorize genetic variables that influence prognosis depending on the type of treatment. When large-scale data analysis is possible, ML technology can allow genomic data to be classified more accurately based on how well different treatments work. This may improve our understanding of the unique clonal architecture of each patient, and enable a heterogeneous group of older adults with AML to receive the most precise and individualized treatment.

Disclosures

No conflicts of interest to disclose.

Contributions

TYK and SP collected and analyzed the data, and wrote the manuscript. DK, JML, MSK and YK provided patients and materials, and reviewed the manuscript. JK provided the materials and reviewed the manuscript. AR, TKK and HJK reviewed the manuscript and analyzed the data. BSC designed the study, provided patients and materials, analyzed the data, and supervised the writing of the manuscript. All authors read and agreed to the final version of the manuscript for publication.

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Data-sharing statement

The data presented in this study are available on request from the corresponding author. The data are not publicly available for privacy and ethical reasons.

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