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Research paper

Prognostic analysis according to the 2017 ELN risk stratification by genetics in adult acute myeloid leukemia patients treated in the Japan Adult Leukemia Study Group (JALSG) AML201 study

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ABSTRACT

Many genetic alterations that are associated with the prognosis of acute myeloid leukemia (AML) have been identified, and several risk stratification systems based on the genetic status have been recommended. The European LeukemiaNet (ELN) first proposed the risk stratification system for AML in 2010 (ELN-2010), and recently published the revised system (ELN-2017). We validated the long-term prognosis and clinical characteristics of each ELN-2017 risk category in Japanese adult AML patients who were treated in the Japan Adult Leukemia Study Group (JALSG) AML-201 study. We demonstrated that the 3-risk category system of the ELN-2017 successfully discriminated the overall survival and complete remission rates in our cohort in comparison with the 4-risk category of the ELN-2010. However, there were still genetic categories in which stratification of patients into favorable or intermediate risk categories was controversial; the low allelic ratio of *FLT3*-ITD was not necessarily associated with a better prognosis in patients with *FLT3*-ITD, and cytogenetic abnormalities may affect the prognosis in patients with favorable genetic lesions such as *NPM1* and *CEBPA* mutations. As many

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molecular targeting agents, such as FLT3 inhibitors, have been developed, we must continue to modify the genetic risk stratification system to match the progression of therapeutic strategies.

1. Introduction

Acute myeloid leukemia (AML) is a clinically and genetically heterogeneous disease [1,2]. Therefore, the evaluation of the prognostic risk is clinically important for AML patients to determine the appropriate therapeutic strategy. The Medical Research Council (MRC) developed the cytogenetic classification system in 1998, and it was refined by considering the clinical characteristics and prognostic relevance of rare cytogenetic abnormalities [3,4]. The refined MRC system, in which three cytogenetic risk groups are distinguished, is widely used for cytogenetic risk stratification of younger adults with AML. However, as there are limitations for patients in the intermediate-risk group, particularly those with cytogenetically normal (CN)-AML [4], more precise risk stratification systems based on genetic status have been proposed [5–20]. The European LeukemiaNet (ELN) first recommended the risk classification system based on the cytogenetic and genetic status in 2010 (ELN-2010) [2]. In this system, risk categories were divided into four groups; favorable-risk (FR), intermediate-I-risk (IR-I), intermediate-II-risk (IR-II) and adverse-risk (AR). It was a landmark in the genetic risk stratification of CN-AML that patients were able to be divided into two groups according to the mutation status of *NPM1*, *FLT3*-ITD and *CEBPA*. Although retrospective analysis demonstrated that the ELN-2010 was useful for further risk stratification of younger adult patients with CN-AML [21,22], the accumulation of information on the prognostic relevance of recurrent genetic alterations has required further modification to include genetic status [5,23].

Recently, the ELN published the revised risk stratification system for AML (ELN-2017), in which AML is divided into three risk categories (favorable, intermediate and adverse) rather than the previous 4-category system [24]. In the ELN-2017 system, several modifications have been made; biallelic mutated *CEBPA* is considered as favorable risk, the allelic ratio of *FLT3*-ITD is considered for the risk stratification, cytogenetic abnormality is excluded for stratification into favorable risk in patients with *NPM1* or biallelic *CEBPA* mutations, and *RUNX1, ASXL1* and *TP53* mutations, and monosomal karyotype are additionally included in the adverse risk category. In this study, we evaluated the usefulness of the ELN-2017 risk stratification system in comparison with the ELN-2010 and refined MRC systems for Japanese AML patients who were registered in the Japan Adult Leukemia Study Group (JALSG) AML201 study.

2. Patients and methods

2.1. Patients and treatment

The JALSG AML201 study was a multi-center phase 3 randomized study for newly diagnosed *de novo* adult AML patients, except for those with acute promyelocytic leukemia (UMIN Clinical Trials Registry C000000157, http://www.umin.ac.jp/ctrj/) [25,26]. The detailed protocol is presented in Supplemental information.

Morphological diagnosis, the French-American-British (FAB) classification and karyotypes were reviewed and confirmed by the central review committees of the JALSG using the bone marrow (BM) samples obtained at diagnosis The diagnosis of AML was based on the classification [27]. The AML201 study included 1057 patients, 197 of whom were available for comprehensive genetic analysis, and their clinical and genetic data were used for this study.

We obtained informed consent from all patients to use their clinical data and samples for banking and molecular analysis, and approval was obtained from the ethics committees of the participating institutes.

2.2. Cytogenetic and molecular analysis

Cytogenetic G-banding analysis was performed using standard methods. We also examined 11 chimeric gene transcripts (Major *BCR-ABL1*, Minor *BCR-ABL1*, *PML-RARA*, *RUNX1-RUNX1T1*, *CBFB-MYH11*, *DEK-NUP214*, *NUP98-HOXA9*, *MLLT1-KMT2A*, *MLLT2-KMT2A, MLLT3-KMT2A, MLLT4-KMT2A*) by reverse transcriptase-mediated quantitative PCR (RQ-PCR) as previously reported [28].

Mutation analysis and results were reported previously [29]. To measure the allelic ratio of *FLT3*-ITD, exons 14 and 15 of the *FLT3* gene were amplified from DNA by PCR using a fluorescently labeled primer, and the products were analyzed by fragment analysis on the Genetic Analyzer 3500 (Applied Biosystems, Foster City, CA).

3. Statistical analysis

Differences in continuous variables were analyzed by the Kruskal-Wallis test. Frequencies were analyzed with Pearson's χ^2 test. Survival probabilities were estimated by the Kaplan-Meier method, and differences in the survival distributions were evaluated using the log-rank test. OS was defined as the time from the date of entry into the AML201 study to death due to any cause or last follow-up. The prognostic significance of the clinical variables was assessed using the Cox proportional hazards model. These statistical analyses were performed with Stata version 13.1 (StataCorp, College Station, TX). For all analyses, the *P*-values were two-tailed, and a *P*-value of less than 0.05 was considered significant.

4. Results

4.1. Risk stratification according to the 2017 ELN recommendation

According to the ELN-2017 criteria, favorable, intermediate and adverse categories comprised 108 (54.8%), 43 (21.8%) and 46 (23.4%) patients, respectively (Table 1). In the ELN-2010 criteria, FR, IR-I, IR-II and AR consisted of 92 (47%), 35 (18%), 42 (21%) and 28 (14%) patients, respectively (Table 2), indicating that many patients were re-categorized into favorable or adverse risk groups with the ELN-2017 criteria. Based on the G-banding karyotype and chimeric transcript analyses, patients were assigned to favorable- (n = 55, 28%), intermediate- (n = 119, 60%) or adverse-risk (n = 23, 12%) groups according to the refined MRC criteria (Supplemental Table 1) [4]. Patient distributions according to the refined MRC, ELN-2010 and ELN-2017 criteria are shown in Fig. 1. Patient distribution of cytogenetic abnormalities according to the original MRC risk stratification system are shown in Supplemental Table 2.

The ELN-2017 favorable group consisted of 90 FR, 6 IR-I and 12 IR-II patients according to the ELN-2010 criteria (Fig. 2). All IR-I patients who were re-categorized into the favorable group in the ELN-2017 had mutated *NPM1* with *FLT3*-ITD^{low}. Of 12 IR-II patients who were re-categorized into the favorable group, seven patients had mutated *NPM1* without *FLT3*-ITD, two had mutated *NPM1* with *FLT3*-ITD^{low} and three had biallelic mutated *CEBPA*; however, all patients had cytogenetic abnormalities. The intermediate group consisted of 2 FR, 19 IR-I and 22 IR-II patients according to the ELN-2010 system (Fig. 2). All FR patients who were re-categorized into the intermediate group in the ELN-2017 system had monoallelic mutated *CEBPA*. Of 19 IR-I patients who were re-categorized into the intermediate group, 10 patients had wild-type *NPM1* without *FLT3*-ITD, seven had wild-type *NPM1* with *FLT3*-ITD^{low} and two had mutated *NPM1* with *FLT3*-ITD^{high}. Of 22 IR-II patients who

Table 1
Distribution of genetic abnormalities according to the ELN-2017 risk stratification system.

Risk category	Genetic abnormality	Number (%)
Favorable	t(8;21)(q22;q22): *RUNX1-RUNX1T1*	41 (20.8)
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22): *CBFB-MYH11*	14 (7.1)
	Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}	36 (18.3)
	Biallelic mutated *CEBPA*	17 (8.6)
	Total	108 (54.8)
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high}	2 (1.0)
	Wild type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}	28 (14.2)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*	1 (0.5)
	Cytogenetic abnormalities not classified as favorable or adverse	12 (6.1)
	Total	43 (21.8)
Adverse	t(6;9)(p23;q34): *DEK-NUP214*	3 (1.5)
	t(v;11)(v;q23): *KMT2A* rearranged	6 (3.0)
	t(9;22)(q34.1;q11.2); *BCR-ABL1*	2 (1.0)
	−7	1 (0.5)
	Complex karyotype	16 (8.1)
	monosormal karyotype	2 (1.0)
	Wild type *NPM1* and *FLT3*-ITD^{high}	3 (1.5)
	Mutated *RUNX1*	10 (5.1)
	Mutated *ASXL1*	3 (1.5)
	Total	46 (23.4)

Table 2
Distribution of genetic abnormalities according to the ELN-2010 risk stratification system.

Risk category	Genetic abnormality	Number (%)
Favorable	t(8;21)(q22;q22); *RUNX1-RUNX1T1*	41 (20.8)
	Inv(16)(p13.1q22) or t(16;16)(8p13.1;q22); *CBFB-MYH11*	14 (7.1)
	Mutated *NPM1*/Wild type *FLT3* (normal karyotype)	21 (10.7)
	Mutated *CEBPA* (normal karyotype)	16 (8.1)
	Total	92 (46.7)
Intermediate-I	Mutated *NPM1/FLT3*-ITD (normal karyotype)	8 (4.1)
	Wild type *NPM1/FLT3*-ITD (normal karyotype)	13 (6.6)
	Wild type *NPM1*/Wild type *FLT3* (normal karyotype)	14 (7.1)
	Total	35 (17.8)
Intermediate-II	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*	1 (0.5)
	Cytogenetic abnormalities not classified as favorable or adverse	41 (20.8)
	Total	42 (21.3)
Adverse	t(6;9)(q23;q34); *DEK-NUP214*	3 (1.5)
	t(v;11)(v;q23); *MLL* rearranged	6 (3.0)
	−7	1 (0.5)
	Complex karyotype	18 (9.1)
	Total	28 (14.2)

were re-categorized into the intermediate group, 12 patients had cytogenetic abnormalities not classified as favorable or adverse, nine had wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low} and one had *MLLT3-KMT2A*. The adverse group consisted of 10 IR-I, 8 IR-II and 28 AR patients according to the ELN-2010 system (Fig. 2). Of 10 IR-I patients who were re-categorized into the adverse group, three patients had wild-type *NPM1* with *FLT3*-ITD^{high}, six patients had mutated *RUNX1* and one patient had mutated *ASXL1*. Of eight IR-II patients who were re-categorized into the adverse group, four patients had mutated *RUNX1*, two had monosormal karyotypes and two had mutated *ASXL1*.

4.2. Patient characteristics according to the ELN-2017 categories

Patient characteristics according to the ELN-2017 system are listed in Table 3. There were no significant differences in age distribution or

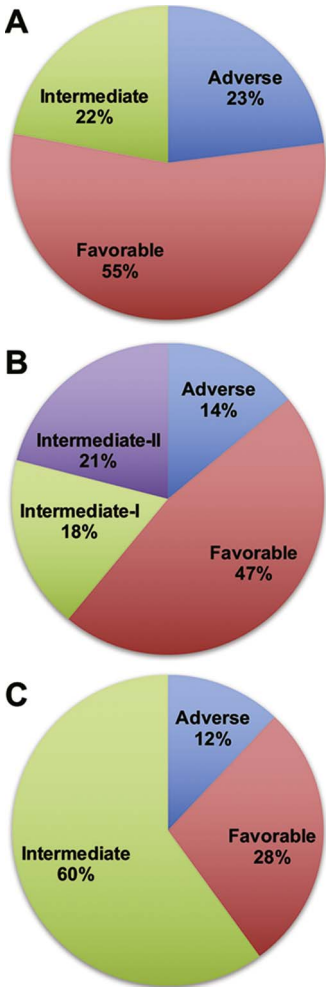

Fig. 1. Distribution of patients according to the refined MRC, ELN-2010 and ELN-2017 systems.
Distribution of patients according to the refined MRC (A), ELN-2010 (B) and ELN-2017 (C). The numbers of patients categorized into the favorable and adverse groups increased with the ELN-2017 system.

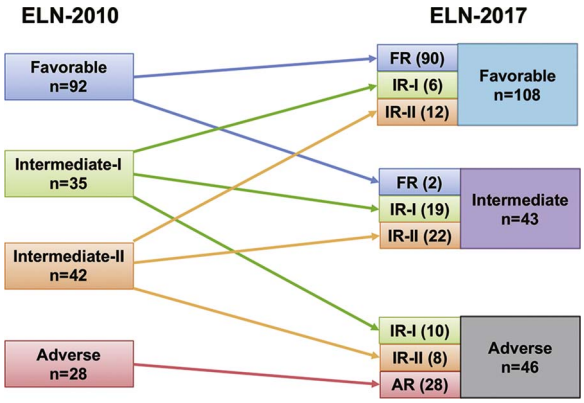

Fig. 2. Changes in the risk categories between the ELN-2010 and the ELN-2017.
With the ELN-2017 system, the numbers of patients in the favorable and adverse risk group increased due to changes in the risk categories based on genetic status.

WBC counts among the three categories. As the favorable risk category includes CBF-AML, the FAB M2 and M4 subtypes were frequently observed in this category. Of the 197 patients, 98 and 99 patients were assigned to IDR or HiDNR arms for induction therapy, respectively. Allo-SCT was conducted for 105 patients; 23 patients at the first CR and 82 after the first relapse.

Table 3
Characteristics of the 197 patients according to the ELN-2017 risk stratification system.

	Total (n = 197) Number (%)	Favorable (n = 108) Number (%)	Intermediate (n = 43) Number (%)	Adverse (n = 46) Number (%)	*P*-value
Age (yr)					0.222
15–19	6 (3.0%)	4 (3.7%)	1 (2.3%)	1 (2.2%)	
20–29	32 (16.2%)	14 (13.0%)	12 (27.9%)	6 (13.0%)	
30–39	35 (17.8%)	20 (18.5%)	7 (16.3%)	8 (17.4%)	
40–49	33 (16.8%)	22 (20.4%)	3 (7.0%)	8 (17.4%)	
50–59	69 (35.0%)	40 (37.0%)	15 (34.9%)	14 (30.4%)	
60–64	22 (11.2%)	8 (7.4%)	5 (11.6%)	9 (19.6%)	
FAB-type					0.008
M0	7 (3.6%)	0 (0%)	4 (9.3%)	3 (6.5%)	
M1	36 (18.3%)	13 (12.0%)	12 (27.9%)	11 (23.9%)	
M2	89 (45.2%)	59 (54.6%)	15 (34.9%)	15 (32.6%)	
M4	43 (21.8%)	26 (24.1%)	8 (18.6%)	9 (19.6%)	
M5	21 (10.7%)	10 (9.3%)	4 (9.3%)	7 (15.2%)	
M6	1 (0.5%)	0 (0%)	0 (0%)	1 (2.2%)	
Induction therapy					0.102
IDR + AraC	98 (49.7%)	61 (56.5%)	19 (44.2%)	18 (39.1%)	
DNR + AraC	99 (50.3%)	47 (43.5%)	24 (55.8%)	28 (60.9%)	
Consolidation therapy					0.797
High-dose Ara-C	80 (40.6%)	51 (47.2%)	15 (34.9%)	14 (30.4%)	
Multiagent CT	77 (39.1%)	50 (46.3%)	11 (25.6%)	16 (34.8%)	
WBC count ($x10^9$/L)					0.797
median	17.2	16.6	19.7	13.0	
range	0.05–367	0.05–367	0.50–323	0.08–200	

4.3. Prognostic analysis according to the ELN-2017 system

CR was achieved in 161 of 197 (81.7%) patients, and 80 and 77 patients were assigned to HiDAC or conventional consolidation therapies, respectively. The CR rate was significantly higher in the favorable risk group (102/108; 94.4%) than in the intermediate (28/43; 65.1%) and adverse (31/46; 67.4%) groups (Table 4). Notably, 91 of the 102 (89.2%) patients in the favorable risk group achieved CR by one course of induction therapy, whereas 10 of the 28 (35.7%) in the intermediate risk group and 9 of the 31 (29.0%) in the adverse risk group required two courses of induction therapy. With the ELN-2010 criteria, the CR rates in the FR, IR-I, IR-II and AR groups were 93.5%, 77.1%, 69.0% and 67.9%, respectively. With the refined MRC criteria, the CR rates in the favorable, intermediate and adverse groups were 90.9%, 79.8% and 69.6%, respectively. These results suggest that the ELN-2017 system more clearly distinguishes the risk groups for achieving CR.

The median follow-up time was 32.5 months for the 197 analyzed patients. Kaplan-Meier analyses for OS according to the refined MRC, ELN-2010 and ELN-2017 categories are shown in Fig. 3. OS according to the original MRC is shown in Supplemental Fig. 1. OSs at 5 years in the favorable, intermediate and adverse groups with the ELN-2017 were 59.1% (95% CI, 47.7–68.8%), 32.6% (95% CI, 14.9–51.7%) and 22.6% (95% CI, 11.6–35.8%), respectively (Fig. 3A). On the other hand, OSs at 5 years in the FR, IR-I, IR-II and AR groups with the ELN-2010 were 64.8% (95% CI, 52.3–74.8%), 17.8% (95% CI, 6.1–34.5%), 38.1% (95% CI, 20.7–55.4%) and 24.1% (95% CI, 10.2–41.2%), respectively (Fig. 3B). Therefore, the 3-risk category system of the ELN-2017 successfully discriminated OS in our cohort in comparison with the 4-risk category of the ELN-2010. However, the OS in the favorable group with the ELN-2017 was lower than that with the ELN-2010. We, therefore, compared the prognosis according to the ELN-2010 categories with each ELN-2017 category. In the favorable group with the ELN-2017, OS was significantly different among the FR, IR-I and IR-II groups with the ELN-2010 ($P < 0.0001$) (Fig. 4A). In particular, there was a significant difference between the FR and IR-I groups ($P < 0.0001$). All IR-I patients had mutated *NPM1* with *FLT3*-ITD^{low}, and all IR-II patients were cytogenetically abnormal and had mutated *NPM1* without *FLT3*-ITD, mutated *NPM1* with *FLT3*-ITD^{low} or biallelic mutated *CEBPA*. These results indicated that cytogenetic abnormalities may affect the prognosis of patients with mutated *NPM1* without *FLT3*-ITD, mutated *NPM1* with *FLT3*-ITD^{low} or biallelic mutated *CEBPA*. However, further analysis is required to confirm this difference because of the low number of patients in the IR-I and IR-II groups.

Furthermore, from these results, we questioned whether the *FLT3*-ITD allelic ratio affected the prognosis in our cohort. We compared the prognosis of patients with *FLT3*-ITD according to the allelic ratio, but did not find any significant differences between *FLT3*-ITD^{high} and *FLT3*-ITD^{low} patients (Fig. 5A). In addition, there were no significant differences between patients with CN-AML and those with wild-type or

Table 4
Comparison of the CR rates in each risk category among the refined MRC, ELN-2010 and ELN-2017.

		Favorable	Intermediate		Adverse
Refined MRC	number	55	119		23
	CR	50 (90.9%)	95 (79.8%)		16 (69.6%)
	No. of induction courses				
	1	46 (83.6%)	72 (60.5%)		13 (56.5%)
	2	4 (7.3%)	23 (19.3%)		3 (13.0%)
	non-CR	5 (9.1%)	24 (20.2%)		7 (30.4%)
ELN-2010			Intermediate-I	Intermediate-II	
	number	92	35	42	28
	CR	86 (93.5%)	27 (77.1%)	29 (69.0%)	19 (67.9%)
	No. of induction courses				
	1	76 (82.6%)	17 (48.6%)	24 (57.1%)	14 (50.0%)
	2	10 (10.9%)	10 (28.6%)	5 (11.9%)	5 (17.9%)
	non-CR	6 (6.5%)	8 (22.9%)	13 (31.0%)	9 (32.1%)
ELN-2017	number	108	43		46
	CR	102 (94.4%)	28 (65.1%)		31 (67.4%)
	No. of induction courses				
	1	91 (84.3%)	18 (41.9%)		22 (47.8%)
	2	11 (10.2%)	10 (23.3%)		9 (19.6%)
	non-CR	6 (5.6%)	15 (34.9%)		15 (32.6%)

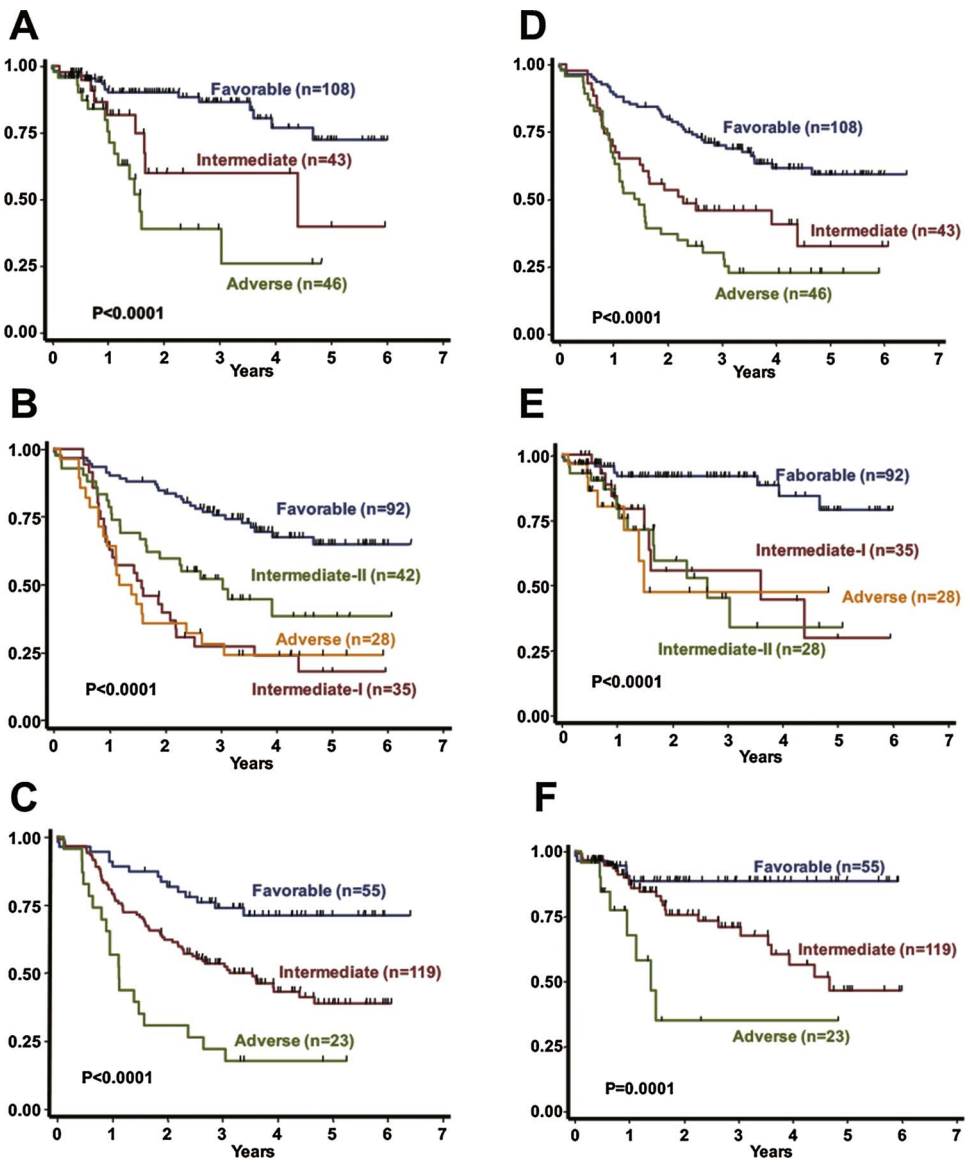

Fig. 3. Overall survivals according to the ELN-2017, ELN-2010 and refined MRC risk categories.
Overall survivals according to the ELN-2017 (A,D), ELN-2010 (B,E) and refined MRC (C,F) risk categories are shown. D, E and F are the results when allo-SCT was censored.

mutated *NPM1* (Fig. 5B–D).

In the intermediate risk group with the ELN-2017, the patients categorized in the FR of the ELN-2010, all of whom were CN-AML with single mutated *CEBPA,* exhibited a better prognosis than those in the IR-I and IR-II groups, but a significant difference was not observed because of the small number of patients (Fig. 4B). In the adverse risk group with the ELN-2017, there was no significant difference in OS among the IR-I, IR-II and AR groups with the ELN-2010 (Fig. 4C).

OSs at 5-yrs in the favorable, intermediate and adverse groups according to the refined MRC system were 71.1% (95% CI, 56.4–81.6%), 38.7% (95% CI, 28.3–49.0%) and 17.4% (95% CI, 5.4–35.0%), respectively (Fig. 3C). These results suggest that favorable and adverse risk cytogenetics should be considered for risk stratification of adult AML.

In addition to the ELN and MRC systems, the Dutch–Belgian Cooperative Trial Group for Hematology/Oncology (HOVON) and the Southwest Oncology Group (SWOG) have also recommended risk stratification systems [30,31]. Unfortunately, we were unable to evaluate the prognostic impact of the HOVON system on our cohort because we did not examine all of the genetic factors included in the HOVON system. OSs at 5-yrs in the favorable, intermediate and adverse groups according to the SWOG system were 67.2% (95% CI, 51.7–78.6%), 37.8% (95% CI, 26.1–49.4%) and 35.9% (95% CI, 21.1–51.0%), respectively (Supplemental Fig. 2 and Supplemental Table 3). Therefore, the SWOG system did not distinguish between the intermediate and adverse groups in our cohort.

We finally compared prognostic impacts of each risk category based on the ELN-2017, ELN-2010, refined MRC and SWOG systems. As shown in Table 5, the ELN-2017 system was able to more clearly discriminate the prognosis of intermediate and adverse risk groups than the ELN-2010 system in our cohort. However, the refined MRC system was also useful for risk stratification.

4.4. Association of mutations with therapeutic regimens

In the JALSG AML201 study, patients were randomized to receive either the standard dose of IDR + Ara-C or HiDNR + Ara-C induction therapy, and the CR patients were again randomized to receive either three courses of HiDAC or four courses of conventional standard-dose multiagent consolidation therapy. Therefore, we analyzed whether the therapeutic regimens affected the CR rate, OS or DFS according to the ELN-2017 system, but no significant differences were observed.

In our cohort, 105 patients underwent allo-SCT during the treatments. We additionally evaluated the prognosis of each risk category when allo-SCT was censored. Although the ELN-2017 and the refined MRC systems still discriminated OSs even if allo-SCT was censored, the

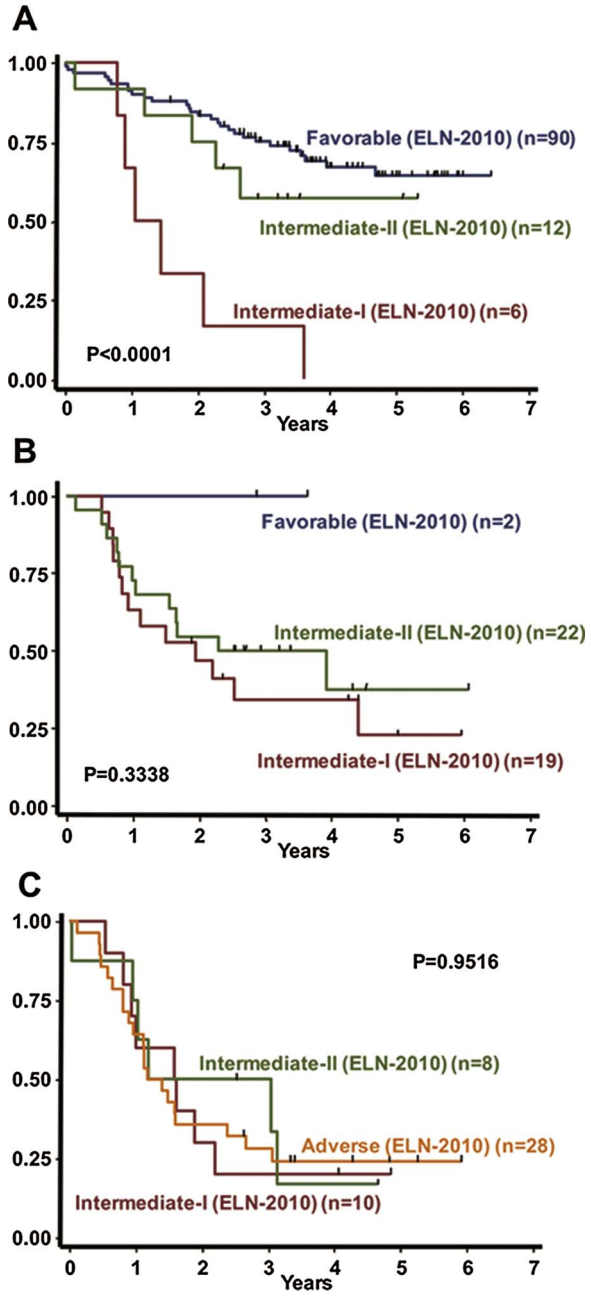

Fig. 4. Overall survivals according to the 2010 ELN risk categories with the 2017 ELN categories.
(A) In the favorable risk groups, the prognosis of patients categorized into IR-I and IR-II by the ELN-2010 was relatively poor. In particular, there was a significant difference between the FR and IR-I groups ($P < 0.0001$). (B) In the intermediate group, the prognosis of patients categorized into FR by the ELN-2010 was relatively better. (C) In the adverse group, there was no prognostic difference among the risk categories with the ELN-2010.

ELN-2010 system was unable to distinguish the prognosis among the IR-I, IR-II and AR groups (Fig. 3).

5. Discussion

Prognostic risk assessment is the most important step in providing AML patients with appropriate therapy. Although many risk factors for prognosis have been identified in AML patients, genetic alterations greatly affect the therapeutic strategies for patients who are eligible for intensive chemotherapy. In this study, we stratified the AML patients into each category according to the refined MRC, ELN-2010 and ELN-2017 risk categories, and validated each system for clinical application.

As CBF-AML is frequently found in Japanese patients, particularly in younger adults, our cohort included a higher number of favorable risk patients according to the refined MRC system. To more precisely stratify the intermediate risk groups, particularly the CN-patients, the ELN-2010 included the mutation status of *FLT3, NPM1* and *CEBPA* genes, and stratified the patients into four risk groups. The ELN-2010 system separated the favorable risk group from the CN-AML patients; however, it has been reported that the long-term prognosis in the IR-I group was not distinguishable from that in the IR-II and/or the AR groups [22]. We previously reported that the long-term prognosis in the IR-I group was almost the same as that in the AR group in our cohort [29]. Furthermore, *DNMT3A* and *RUNX1* mutations, and partial tandem duplication of the *MLL* gene (*MLL*-PTD) were identified as poor prognostic factors for OS in our cohort [29]. In the 35 IR-I group patients, 11 and six patients harbored *DNMT3A* mutation and *MLL*-PTD, respectively, and three patients harbored both mutations. In particular, *MLL*-PTD was a poor prognostic factor in the IR-I group [29]. Therefore, this mutation status reduced the OS in the IR-I group. However, *DNMT3A* mutation and *MLL*-PTD are not included in the ELN-2017 system. Multivariate analysis including these mutations and the adverse risk with the ELN-2017 demonstrated that these were independent poor prognostic factors for OS (Table 6). Further analysis with a large-scale cohort is necessary to confirm the prognostic effects of these mutations in Japanese AML patients.

Although the ELN-2010 system was able to select favorable risk patients from the cytogenetically intermediate risk group, the AR groups did not increase because genetic status was not considered for the AR category. The adverse risk group with the ELN-2017 system additionally includes *RUNX1, ASXL1* and *TP53* mutations, and monosomal karyotype, resulting in the increase of this group from 14.2% to 23.4% in our cohort. As we previously confirmed the poor prognosis of patients with these genetic abnormalities in our cohort, the adverse risk group with the ELN-2017 was more clearly distinguished from the intermediate risk group than with the ELN-2010.

In the intermediate risk group with the ELN-2017, the patients categorized into the FR group with the ELN-2010 had a better prognosis than those in the IR-I and IR-II groups (Fig. 4B). All patients categorized in the FR group with the ELN-2010 had CN-AML with single mutated *CEBPA*. It has been reported that single mutated *CEBPA* patients frequently acquire other genetic mutations [32]; however, those in our cohort did not have other mutations associated with poor prognosis. Further studies are required to evaluate the prognostic relevance of single *CEBPA* mutation in patients with CN-AML.

The most controversial issue in our cohort was the genetic category for stratification into the favorable risk group with the ELN-2017. As shown in Fig. 4A, the OS of the patients in the favorable risk group with the ELN-2017 was significantly different among the patients categorized into the FR, IR-I and IR-II groups with the ELN-2010. The IR-II patients were re-categorized into the favorable group with the ELN-2017 because the ELN-2017 does not consider cytogenetic abnormalities. As indicated above, the prognosis of CN-AML patients with single *CEBPA* mutation seemed better in our cohort. These results suggested that the prognostic implications of the normal karyotype are more precisely evaluated; however, there is a limitation in that cytogenetic abnormalities cannot be completely avoided using the conventional G-banding method, indicating that novel methods, such as next generation sequencing, may be necessary for evaluating the cytogenetic effects on the prognosis of AML patients. Of note is the poor prognosis of the IR-I patients who were categorized into the favorable risk group with the ELN-2017. These patients were categorized into the favorable risk group because of the low *FLT3*-ITD allelic ratio. As shown in Fig. 5, we were unable to distinguish the prognosis of patients with *FLT3*-ITD based on the allelic ratio. Although we analyzed the prognostic effects of *FLT3*-ITD ratio in other patients who were treated in the JALSG AML-87, -89 and -92 studies, we found no prognostic relevance for the *FLT3*-

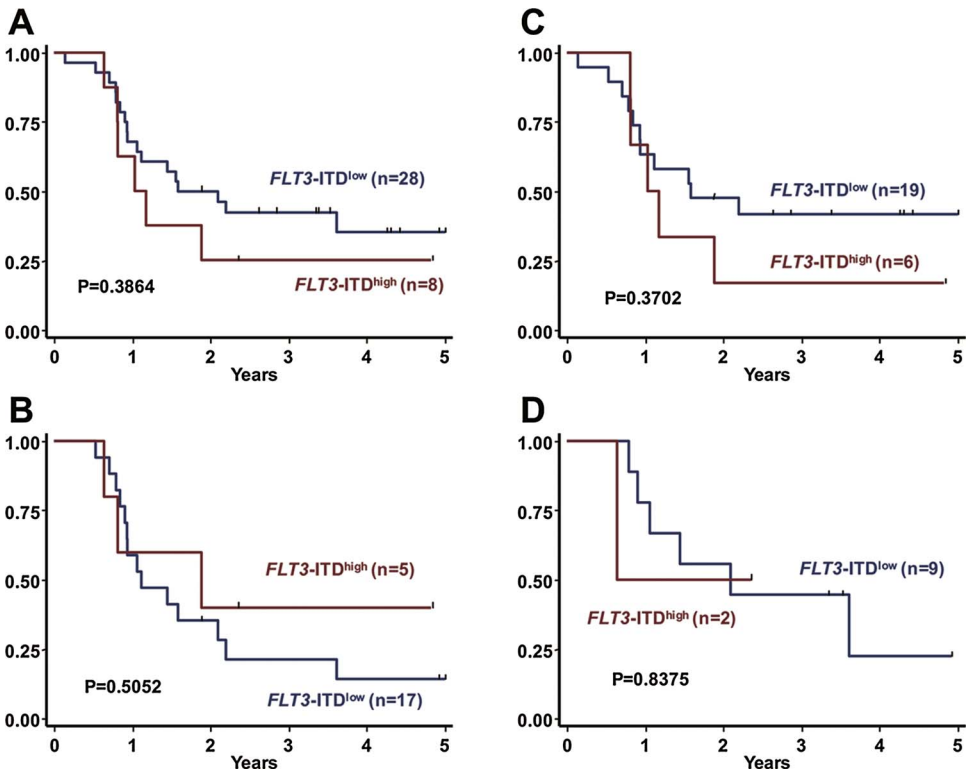

Fig. 5. Overall survivals according to the FLT3-ITD allelic ratio.
The allelic ratio of *FLT3*-ITD did not affect the prognosis in patients with *FLT3*-ITD (A), those with CN-AML (B), those with wild-type *NPM1* (C) nor those with mutated *NPM1* (D).

Table 5
Prognostic impact of each category.

	HR	95% CI	*P*-value
ELN2017			
Favorable	0.362	0.241–0.543	< 0.001
Intermediate	1.466	0.931–2.310	0.099
Adverse	2.632	1.736–3.988	< 0.001
ELN2010			
Favorable	0.295	0.190–0.459	< 0.001
Intermediate-I	2.404	1.539–3.755	< 0.001
Intermediate-II	1.247	0.781–1.990	0.356
Adverse	2.371	1.461–3.850	< 0.001
Refined MRC			
Favorable	0.359	0.207–0.622	< 0.001
Intermediate	1.311	0.866–1.985	0.201
Adverse	2.947	1.779–4.881	< 0.001
SWOG			
Favorable	0.438	0.257–0.749	0.003
Intermediate	1.271	0.856–1.887	0.235
Unfavorable	1.578	1.002–2.485	0.049
Unknown	1.186	0.482–2.920	0.710

Table 6
Multivariate analysis for overall survival.

Risk	HR	95% CI	*P*-value
ELN2017-Adverse	2.772	1.735–4.804	< 0.001
MLL-PTD	2.450	1.188–5.050	0.015
DNMT3A	2.187	1.322–3.619	0.002

ITD allelic ratio (data not shown) [33]. At present, it is unclear why the *FLT3*-ITD allelic ratio did not affect the prognosis in Japanese adult patients; however, this should be re-evaluated in patients treated with FLT3 inhibitors because the combination of chemotherapy and a FLT3 inhibitor, midostaurin, reportedly improved the prognosis of AML patients with *FLT3* mutation [34].

In conclusion, we demonstrated that the ELN-2017 risk stratification system for AML clearly distinguished long-term prognosis in Japanese adult patients with *de novo* AML. However, there are still controversial genetic categories in the favorable and intermediate risk groups. Further studies are required to confirm their prognostic relevance in Japanese AML patients.

Contributors

Y.H., Y.I., H. Kiyoi, S. Ogawa, I.M., Y.M. and T.N. designed the study and interpreted the data; Y.H., Y.I. and H. Kiyoi wrote the manuscript; Y.H., Y.I., R.K. and Y.N. performed molecular analysis and interpreted the data; N.A., S. Ohtake, S.M., Y.M., T.S., Y.O., N.U., H. Kanamori, Y.I, K.I., Y.S., S.K., K.K., E.S., M.O., A.T., F.I., H.S., Y.K. and I.M. collected samples and clinical data, contributed to the interpretation of the data, and critically reviewed the manuscript; all authors approved the final version submitted for publication.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.leukres.2018.01.008.

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