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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 $\chi^2$ 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