

Significance of Gene Diagnosis in Acute Myeloid Leukemia with the Emergence of New Molecular Target Drug Treatment

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Acute myeloid leukemia (AML) is a heterogeneous hematopoietic malignancy accompanied by impaired differentiation and autonomous proliferation of hematopoietic stem cells. Standard induction therapy results in first complete remission among 70% of patients with AML; however, approximately half of these patients relapse and become refractory. Allogeneic hematopoietic cell transplantation is a useful treatment for relapsed and refractory cases. However, transplantation-related mortality is approximately 20%, which is not a low value, and quality of life after transplantation decreases. Therefore, there is a need to stratify the prognosis of each patient and implement this treatment appropriately. Owing to recent advances in genome analysis technology, many gene mutations involved in onset and recurrence of AML have been discovered. These abnormalities and mutations not only have clinical application as prognostic factors and minimal residual disease markers, but they may also contribute to novel molecular targeted drug development. Many new drugs such as first-generation FMS-like tyrosine kinase 3 (FLT3), isocitrate dehydrogenase 1 and 2 (IDH1/2), and B cell lymphoma 2 (BCL2) inhibitors have been developed in the West. In addition, the second-generation FLT3 inhibitors gilteritinib and quizartinib were developed in Japan, and treatment outcomes for patients with AML have improved. However, there is still a large disparity in drug availability between the West, and Japan. As a result, treatment guidelines in the West cannot be applied in the clinical setting in Japan. In this study, we assessed the molecular target drug treatment by gene diagnosis for treatment of AML patients.

(J Nippon Med Sch 2022; 89: 470-478)

Key words: acute myeloid leukemia, allogeneic hematopoietic cell transplantation, FLT3 inhibitor, gene diagnosis, molecular target drug treatment

Introduction

Acute myeloid leukemia (AML) is a heterogeneous hematopoietic malignancy characterized by the development of myeloblasts into the bone marrow, peripheral blood, and other tissues accompanied by impaired differentiation and autonomous proliferation of hematopoietic cells. Standard induction therapy with anthracyclines and cytarabine results in complete remission (CR) in approximately 70% of cases. However, over half of patients with CR experience recurrence, and their five-year survival is approximately 40%. For such relapsed and refractory AML, allogeneic hematopoietic cell transplantation (allo-HSCT) is a useful treatment with the aim of radical cure

of AML. However, allo-HSCT is associated with a high transplantation-related mortality (TRM) rate, and the quality of life (QOL) after transplantation also decreases. Therefore, allo-HSCT needs to be implemented with caution taking into account the predicted prognosis. Stratifying prognosis according to age, performance status (PS), onset type (de novo or secondary), and chromosomal/genetic mutations has been shown to be useful. In recent years, several molecular-targeted drugs, such as FLT3 inhibitors, have also been shown to be useful for AML, and new options are emerging in a field where chemotherapy and allo-HSCT used to be the only options for AML. However, due to the lag in availability of drugs in Japan

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https://doi.org/10.1272/jnms.JNMS.2022_89-313

Journal Website (<https://www.nms.ac.jp/sh/jnms/>)

Table 1 Prognostic classification of AML by ELN guidelines 2017

Risk category	Genetic abnormality
Favorable	t (8;21) (q22;q22.1): <i>RUNX1-RUNX1T1</i> Inv (16) (p13.1q22) or t (16;16) (p13.1;q22): <i>CBFB-MYH11</i> Mutated <i>NPM1</i> without <i>FLT3-ITD</i> or with <i>FLT3-ITD</i> ^{low*} Biallelic mutated <i>CEBPA</i>
Intermediate	Mutated <i>NPM1</i> and <i>FLT3-ITD</i> ^{high*} Wild type <i>NPM1</i> without <i>FLT3-ITD</i> or with <i>FLT3-ITD</i> ^{low*} (without adverse risk genetic lesions) t (9;11) (p21.3;q23.3); <i>KMT2A-MLLT3</i> [¶] Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t (6;9) (q23;q34.1): <i>DEK-NUP214</i> t (v;11) (v;q23): <i>KMT2A</i> rearranged t (9;22) (q34.1;q11.2); <i>BCR-ABL1</i> inv (3) (q21.3q26.2) or t (3;3) (q21.3;q26.2); <i>GATA2, MECOM (EVII)</i> -5 or del (5q), -7, -17 or abn (17p) Complex karyotype [§] , monosomal karyotype [†] Wild type <i>NPM1</i> and <i>FLT3-ITD</i> ^{high*} Mutated <i>RUNX1</i> [‡] Mutated <i>ASXL1</i> [‡] Mutated <i>TP53</i> [#]

* Low: low allelic ratio (<0.5), high: high allelic ratio (≥0.5)

¶ Takes precedence over rare, concurrent adverse-risk gene mutations.

§ Three or more unrelated cytogenetic abnormalities in the absence of the following translocations or inversions: t (8;21), inv (16) / t (16;16), t (9;11), t (v;11) (v;q23.3), t (6;9), inv (3) / t (3;3), BCR-ABL1

† Defined by the presence of 1 single monosomy (excluding loss of X or Y) in association with at least 1 additional monosomy or structural chromosome abnormality (excluding core-binding factor AML).

‡ These markers should not be used as an adverse prognostic marker if they co-occur with favorable-risk AML subtypes.

TP53 mutations are significantly associated with AML with complex and monosomal karyotype.

compared with the West, treatment guidelines in the West cannot be applied to actual clinical practice in Japan. Therefore, this paper outlines how to conceptualize prognosis and stratification of treatment in the current clinical practice of AML treatment in Japan.

Importance of Genetic Mutation as a Prognostic Factor

HSCT is one of the major factors in improvements in AML treatment outcomes. The Japan Adult Leukemia Study Group reported no significant differences in the change in CR rates from the AML87 to AML201 study, but the percentage of patients who underwent HSCT increased greatly from approximately 7% to approximately 45%, and there is no doubt that this was the reason for the improved treatment results¹. Advances in supportive care, non-myeloablative conditioning, cord blood transplantation, and haploidentical HSCT have continued to increase the number of transplantation patients². However, AML usually affects older individuals, with an average onset age of over 65 years. Moreover, allo-HSCT has high transplantation-related toxicity. Therefore, the importance of prognostic factors has become increasingly

important. To date, chromosomal analysis has been the most important prognostic factor for AML patients of age indicated for transplantation, but the issue with chromosomal analysis is that approximately 60% of cases fall into the intermediate prognosis group. Therefore, further stratification of prognosis using gene mutations was attempted for the intermediate chromosomal prognosis group. The European Leukemia Net (ELN) guidelines (2017 edition) incorporated various genetic abnormalities as prognostic factors (Table 1)³. In particular, *FLT3-ITD*, *NPM1*, and *CEBPA* mutations were significant prognostic factors. For example, *FLT3-ITD*-negative *NPM1* mutation-positive patients and *CEBPA* biallelic mutation-positive patients with normal karyotype have a favorable prognosis with an overall survival (OS) of approximately 75%⁴. In particular, *CEBPA* biallelic mutation-positive patients are highly responsive to chemotherapy, even with recurrence, and approximately 85% of them achieve a second CR (CR2)⁵. Therefore, allo-HSCT is not indicated for *CEBPA* biallelic mutation-positive patients during the first CR (CR1) when considering TRM and decreased QOL after transplantation⁴. We also recently reported that even

if *CEBPA* single allele mutations are present, the prognosis is favorable if mutations in the C-terminal bZIP domain are present⁶. In the future, it may be sufficient to analyze only this domain for analysis of prognosis in actual clinical practice.

Do Low Allelic Frequency *FLT3*-ITD-Positive *NPM1* Mutation-Positive Patients Truly Have a Favorable Prognosis?

FLT3-ITD is found in approximately 20% of AML patients, and it is the most significant factor in a poor prognosis. Furthermore, patients with *FLT3*-ITD with a higher mutant allelic ratio (AR) have a poorer prognosis⁷⁻⁹. However, the latest ELN guidelines (shown in **Table 1**) classify *FLT3*-ITD low-AR-positive *NPM1* mutation-positive patients as having a favorable prognosis, and therefore, they should not be indicated for allo-HSCT during CR1³. Many clinicians were taken by surprise by the sudden shift to a favorable prognosis for some *FLT3*-ITD-positive patients, as they were previously considered to have a poor prognosis. In reality, there are no references in the ELN guidelines that clearly state that *FLT3*-ITD low-AR-positive *NPM1* mutation-positive patients have a favorable prognosis³. Reports to date have indicated that *FLT3*-ITD low-AR-positive patients have a significantly favorable prognosis compared to high-AR-positive patients, but that OS was $\leq 50\%$ and did not necessarily indicate a favorable prognosis⁷⁻⁹. Furthermore, the ELN guidelines indicate that *FLT3*-ITD low-AR-positive *NPM1* mutation-positive patients should not be indicated for allo-HSCT during CR1. It has certainly been reported that allo-HSCT during CR1 does not improve treatment results compared to chemotherapy among *FLT3*-ITD low-AR-positive patients⁷. However, we published a contradictory report stating that not conducting allo-HSCT during CR1 for *FLT3*-ITD low-AR-positive *NPM1* mutation-positive patients results in an extremely poor prognosis of approximately 10%¹⁰. The CR1 rate of *FLT3*-ITD-positive AML is the same as that for *FLT3*-ITD-negative AML, but given the increase in patients with non-remission and allo-HSCT because of the low CR2 rate of chemotherapy for relapsed patients, not conducting allo-HSCT during CR1 will result in a poor prognosis^{5,10}. Given the above, it is thought that initial-onset *FLT3*-ITD-positive AML patients, whose treatment options in Japan were limited to conventional chemotherapy and allo-HSCT, have a poor prognosis regardless of the mutant AR of *FLT3*-ITD and should be indicated for allo-HSCT during CR1.

Usefulness of *FLT3* Inhibitors for *FLT3*-ITD-Positive AML

The results of clinical trials on several important *FLT3* inhibitors have continued to change the treatment strategies for *FLT3*-ITD-positive AML patients in the West. **Table 2** shows the results of representative clinical trials of the *FLT3* inhibitors published so far. First, the RATIFY trial results show that a combination of the conventional 3/7 therapy with the first-generation *FLT3* inhibitor midostaurin was becoming the standard therapy for initial-onset *FLT3*-ITD-positive AML¹¹. The RATIFY trial results of stratification analysis by gene mutation showed that the OS of *FLT3*-ITD low-AR-positive *NPM1* mutation-positive cases is approximately 75%¹². Furthermore, these patients were shown to have an OS of approximately 75% even without conducting allo-HSCT during CR1. In light of this result, it is possible that the ELN guidelines gave these patients a favorable prognosis and also did not indicate them for allo-HSCT during CR1. The legitimacy of this decision was finally demonstrated in the literature approximately 3 years after the ELN guidelines were published.

Next, gilteritinib, which is a second-generation Type-1 *FLT3* inhibitor (effective for *FLT3*-ITD and *FLT3*-tyrosine kinase domain), and quizartinib, a second-generation Type-2 *FLT3* inhibitor (effective for *FLT3*-ITD), were approved in Japan for relapsed and refractory *FLT3*-ITD-positive AML. The results of the ADMIRAL trial for gilteritinib and the QuANTUM-R trial for quizartinib^{13,14} showed that these second-generation *FLT3* inhibitors improve the CR2 rate, allo-HSCT implementation rate, and OS for relapsed and refractory *FLT3*-ITD-positive AML (**Table 2**). Furthermore, trials on the usefulness of 1) combining the conventional 3/7 therapy with quizartinib (NCT02668653) or gilteritinib (NCT02310321) for initial-onset *FLT3*-ITD-positive AML; 2) gilteritinib and azacitidine (AZA) for initial-onset *FLT3*-ITD-positive AML not indicated for standard intensive chemotherapy (NCT02752035); or 3) maintenance therapy with gilteritinib after allo-HSCT (NCT02997202) and others, have been conducted, and their results will likely be reported in the near future.

What Treatment Strategy Changes are Expected for *FLT3*-ITD-Positive AML Change in Japan?

In Japan, gilteritinib and quizartinib have been approved for relapsed and refractory *FLT3*-ITD-positive AML, but Midostaurin has not yet been approved. Given this fact, it is currently thought in Japan that allo-HSCT during CR

Table 2 Clinical trials of representative FLT3 inhibitor for AML

Study	Phase	Object	Treatment	Effect	Reference
RATIFY study	Phase III	18-60.9 years First lien <i>de novo</i> AML <i>FLT3</i> -ITD or TKD	Induction therapy (3/7) +consolidation therapy (HDAC) +Midosutaurin (n=360) vs Placebo (n=357)	CR rate: Mid 59% vs Pla 54% p=0.15 OS: Mid 74.7 mo vs Pla 25.6 mo, p=0.009 EFS: 8.2 mo vs 3.0 mo, p=0.002 DFS: 26.7 mo vs 15.5 mo, p=0.01	11
ADMIRAL study	Phase III	19-85 years Refractory or relapsed <i>de novo</i> AML <i>FLT3</i> -ITD or TKD	Gilteritinib (n=247) vs Salvage chemotherapy (n=124)	CR/CRi rate: Gil 54.3% vs Sal 21.3%, HR 32.5 OS: Gil 9.3 mo vs Sal 5.6 mo, p<0.001 EFS: Gil 2.3 mo vs Sal 0.7 mo HR 0.499	13
QuANTUM-R study	Phase III	44-66 years Refractory or relapsed <i>de novo</i> AML <i>FLT3</i> -ITD	Quizartinib (n=245) vs Salvage chemotherapy (n=122)	CR/CRi rate: Qui 48% vs Sal 27% OS: Qui 6.2 mo vs Sal 4.7 mo, p=0.02 EFS: Gil 1.4 mo vs Sal 0.9 mo HR 0.9	14

Mid: Midosutaurin, mo: month, Pla: placebo, Gil: gilteritinib, Sal: salvage chemotherapy, CR: complete remission, CRi: remission in which the recovery of some blood cells could not be obtained, OS: overall survival, DFS: disease-free survival, EFS: event-free survival, HR: hazard ratio, Qui: Quizartinib, TKD: tyrosine kinase domain

1 will be the first-line treatment for initial-onset *FLT3*-ITD-positive AML, regardless of AR, if a suitable donor can be secured. Meanwhile, given that second-generation FLT3 inhibitors have been approved for use against relapsed and refractory *FLT3*-ITD-positive AML, there is also a strategy whereby allo-HSCT during CR1 for *FLT3*-ITD low-AR-positive *NPM1* mutation-positive is not conducted, second-generation FLT3 inhibitors are administered when the first recurrence occurs, and then allo-HSCT is introduced during CR2. However, even though second-generation FLT3 inhibitors improve the CR2 rate, approximately half of patients do not achieve CR2. Furthermore, results of stratification analysis by AR for *FLT3*-ITD in the ADMIRAL trial did not show improved OS with gilteritinib relative to chemotherapy among low-AR patients¹³. When considering that over 70% of initial-onset patients achieve CR1 with conventional chemotherapy, CR1 is thought to be the optimal time for allo-HSCT from a probabilistic perspective in Japan.

Usefulness of the *NPM1* Mutation Minimal Residual Disease Marker (MRD) for *NPM1* Mutation-Positive AML

Being able to conduct an MRD search is extremely important in a clinical sense when determining the treatment policy, as well as prognostic factors of a disease. An

MRD search is conducted in clinical practice using chimeric gene mutations for AML with translocation-type chromosomal abnormalities in the favorable chromosomal prognosis group, such as t(15;17) (q22;q21) or t(8;21) (q22;q22). The ability to conduct an MRD search is also a factor for the favorable prognosis of AML with these translocation-type chromosomal abnormalities. Meanwhile, an MRD search cannot be conducted for the majority of AML patients, including those of the normal karyotype. Therefore, MRD search investigations have focused on several gene mutations. Among these, *NPM1* mutation search using the high-sensitivity quantitative PCR method has been reported to be a clinically useful MRD search method when predicting recurrence in *NPM1* mutation-positive AML^{15,16}. *FLT3*-ITD-negative *NPM1* mutation-positive AML has a favorable prognosis, and allo-HSCT is not the first-line treatment during CR1; therefore, monitoring with MRD markers for *NPM1* mutations is highly useful. Furthermore, *NPM1* mutations co-exist in approximately half of *FLT3*-ITD-positive AML patients. Therefore, it is also useful for judging the treatment effect in *NPM1* mutation-positive *FLT3*-ITD-positive AML. It is particularly useful in cases where allo-HSCT is not conducted during CR1 for *FLT3*-ITD low-AR-positive *NPM1* mutation-positive patients and for monitoring the introduction of FLT3 inhibitors after allo-

HSCT. However, it has been reported that driver mutations such as *NPM1* mutations disappear at the time of recurrence in approximately 20% of patients¹⁷. This is also the case when using the high-sensitivity quantitative PCR method; therefore, an MRD search using driver gene mutations will likely have been associated with false negatives, unlike with chimeric gene mutations.

Usefulness of *DNMT3A* Mutations in Stratifying AML Prognosis

DNMT3A mutations are additional genetic mutations, other than those shown in the ELN guidelines, that are useful for stratification of prognosis. *DNMT3A* mutations are observed in approximately 20% of AML patients, and it is the third most common genetic mutation after *FLT3* mutations and *NPM1* mutations¹⁸. There are many reports to date showing that *DNMT3A* mutations are a poor prognostic factor in AML^{18,19}. We have also shown that mutations in any of the epigenetic regulatory genes, including the *DNMT3A*, have a poor prognosis¹⁸. Furthermore, approximately half of *DNMT3A* mutations are those of the codon 882 mutation, and the *DNMT3A* R 882 mutation has been reported to have an even poorer prognosis even when compared to other *DNMT3A* mutations¹⁸. A revised edition of the ELN guidelines has been proposed in recent years based on these results, where it was proposed that the *DNMT3A* mutation was a poor prognosis factor²⁰. The abovementioned results suggest that even if mutation analysis cannot be conducted for the full length of the *DNMT3A* gene, analyzing even just the R882 mutation would be useful for stratifying approximately 10% of AML patients.

Potential of Therapeutic Stratification by *KIT* Gene

Mutation in Core Binding Factor (CBF)-AML

AML with chromosomal abnormalities of t(8;21)(q22;q22) or inv(16)(p13.1q22) is collectively called CBF-AML and is classified as having a favorable prognosis³. CBF-AML results in CR1 in over 90% of patients with standard remission induction therapy, and it has been shown that high-dose cytarabine for three or more cycles is effective as a post-remission therapy. Meanwhile, approximately 40% of CBF-AML patients experience recurrence, with some of these showing resistance to chemotherapy following recurrence, resulting in a poor prognosis. However, there are no clear research results regarding the stratification of CBF-AML with poor prognosis.

In CBF-AML, *KIT* mutation has often been reported as a poor prognosis factor for early recurrence and survival,

and in fact, previous National Comprehensive Cancer Network (NCCN) guidelines have treated CBF-AML patients with *KIT* gene mutations as an intermediate prognosis group. Meanwhile, some reports indicate that *KIT* mutations do not affect prognosis in CBF-AML; therefore, the 2016 NCCN guidelines did not set CBF-AML as an intermediate prognosis group. It was only mentioned in the margins of the guidelines that CBF-AML with *KIT* mutations may have a worse prognosis than usual, thereby diminishing their significance as a prognostic factor²¹⁻²³.

Therefore, we conducted a study to clarify the clinical significance of the *KIT* mutation in CBF-AML. First, we developed a high-sensitivity mutation detection method called the mutation-biased PCR (MB-PCR), where we discovered that there are minute *KIT* mutation clones that cannot be detected by the conventional direct sequencing using Sanger method at the onset of CBF-AML²⁴. The minute *KIT* mutation clones at the time of onset showed treatment resistance and had a high rate of recurrence. Furthermore, in order to verify this discovery, we conducted prognosis analysis of CBF-AML138 patients that achieved CR1, the results of which showed that only patients with the D816 mutation among those with *KIT* mutations had a poor prognosis²⁵. It was also confirmed in an *in vitro* study that the *KIT* 816 mutation had higher cell proliferation activity and anti-apoptotic ability than other *KIT* mutations²⁶. The results of studies suggest that the reason why the significance of *KIT* mutations as a prognostic factor in CBF-AML was not clarified was because of the low sensitivity of the detection method and because of the lack of analysis with consideration to the *KIT* mutation site.

Usefulness of Gemtuzumab Ozogamicin (GO)

Combined with Chemotherapy for CBF-AML

GO is an immunoconjugate in which a calicheamicin derivative, a cytotoxic compound, is bound to a humanized anti-CD33 antibody. When GO first appeared, it was initially anticipated that it would improve treatment results by combining its use with conventional chemotherapy for initial-onset CD33-positive AML (induction therapy: 3/7 therapy + 6 mg/m², post-remission therapy: chemotherapy + 5 mg/m²), but a subsequent verification by the Southwest Oncology Group (SWOG) indicated that not only did it not improve the treatment result, it also increased the mortality rate in induction therapy, and therefore, its approval in the U.S. was revoked²⁷. However, it was subsequently shown that administering GO

in small doses (induction therapy: 3/7 therapy + 3 mg/m² day 1, 4, 7; post-remission therapy: chemotherapy + 3 mg/m²) reduced therapeutic toxicity and improved the treatment results (two-year recurrence-free mortality rate: placebo group, 22.7% vs. GO group, 50.3%, $p = 0.0003$; two-year OS: placebo group, 41.9% vs. GO group, 53.2%, $p = 0.03768$)²⁸. Furthermore, meta-analysis results of clinical trials to date showed that the effect of combined GO and chemotherapy was most effective in the favorable chromosomal prognosis group, and no effects were observed in the poor prognosis group (OS improvement rate by combined GO use in chromosomal prognosis classification: favorable prognosis group, 20.7% ($p = 0.0006$); intermediate prognosis group: 5.7% ($p = 0.005$); poor prognosis group, 2.2% (no significant difference))²⁹. These results have led to combined GO and chemotherapy gradually becoming a standard therapy for initial-onset CBF-AML in the West. Furthermore, combined GO and chemotherapy has been shown to potentially reduce the recurrence rate and improve the prognosis of CBF-AML patients with *KIT* exon17 mutations, including D 816 mutations³⁰. The usefulness of combined GO and chemotherapy may have been achieved by improving the prognosis of *KIT* D816 mutation patients who responded poorly to conventional chemotherapy.

Effectiveness of CPX-351 for Secondary AML with Poor Prognosis or AML Accompanied by Myelodysplasia-Related Changes

Secondary AML or AML with myelodysplasia-related changes (AML-MRC) has a very poor prognosis due to the low effectiveness of conventional chemotherapy and the difficulty of allo-HSCT-based treatment strategies due to the high number of older patients³¹. CPX-351 contains Cytarabine and Daunorubicin in a double-lipid membrane, and one unit of CPX-351 contains 1 mg of Cytarabine and 0.44 mg of Daunorubicin. In the abovementioned study, the molar ratio of Cytarabine to Daunorubicin was 5:1, which was considered to be the molar ratio with the greatest synergistic effect and least amount of antagonistic activity. Furthermore, by wrapping both agents in a lipid membrane, this molar ratio was maintained for over 24 h. Phase II trials on first-recurrence patients with AML aged <65 years and the elderly showed that CPX-351 significantly improved the remission rate (including remission in which the recovery of some blood cells could not be obtained [CRi]). It also improved the event-free survival rate for AML with poor chromosomal prognosis or secondary AML^{32,33}. Phase III trials

that were conducted for secondary AML and AML with myelodysplasia-related changes in older adults based on these results showed that CPX-351 showed significant improvements when compared to 3/7 therapy for remission rate (CR + CRi) (47.7% vs. 33.3%, $p = 0.016$) and OS (median value, 9.56 vs. 5.95 months, $p = 0.005$)³⁴. In particular, the CPX-351 group had significantly more cases of allo-HSCT in remission (CPX-351 group, 76.9% vs. 3/7 therapy group, 61.5%) and a 12-month OS of over 60%, exhibiting favorable results. Based on these results, the first-line treatment for secondary AML or AML with myelodysplasia-related changes, for which conventional chemotherapy was indicated, is gradually shifting to CPX-351 in the West. However, stratification analysis of the Phase III trial showed that patients with a history of treatment with hypomethylating agents (HMAs), such as AZA, during pre-treatment had a CR + CRi that dropped to 36% for CPX-351, which was no longer significantly different from that of 3/7 therapy. Based on this result, new treatment strategies may be required for AML caused by myelodysplastic syndrome that has become refractory to HMAs.

Gene Mutation Analysis and Treatment Selection for Patients for Whom Standard Intensive Chemotherapy Is Not Indicated

Novel molecular-targeted drugs have continued to be proven useful for older adults and patients for whom standard intensive chemotherapy is not indicated due to complications. A Phase III trial that investigated the combined therapy of the BCL2 inhibitor venetoclax (VEN) and AZA in 431 patients with AML aged ≥ 75 years who were not indicated for standard intensive chemotherapy showed that the VEN+AZA group had a significantly higher CR + CRi rate (66.4% vs 28.3%, $p < 0.001$) and longer OS (median value of 14.7 vs. 9.6 months, $p < 0.001$) than the placebo group³⁵. In particular, *IDH2* mutation-positive patients had a CR + CRi rate of 85%, and *IDH1/2* mutation-positive patients had a CR + CRi rate of 75%; these patients had a longer OS and were more effective than positive patients with other gene mutations. Furthermore, a Phase III trial that investigated the combined therapy of VEN and low-dose cytarabine (LDAC) in 211 AML patients either aged ≥ 75 years, or who were not indicated for standard intensive chemotherapy due to complications, showed that the VEN+LDAC group did not have a significantly different OS (median value 7.2 vs. 4.1 months, $p = 0.11$) compared to the placebo group, but did have a significantly higher CR

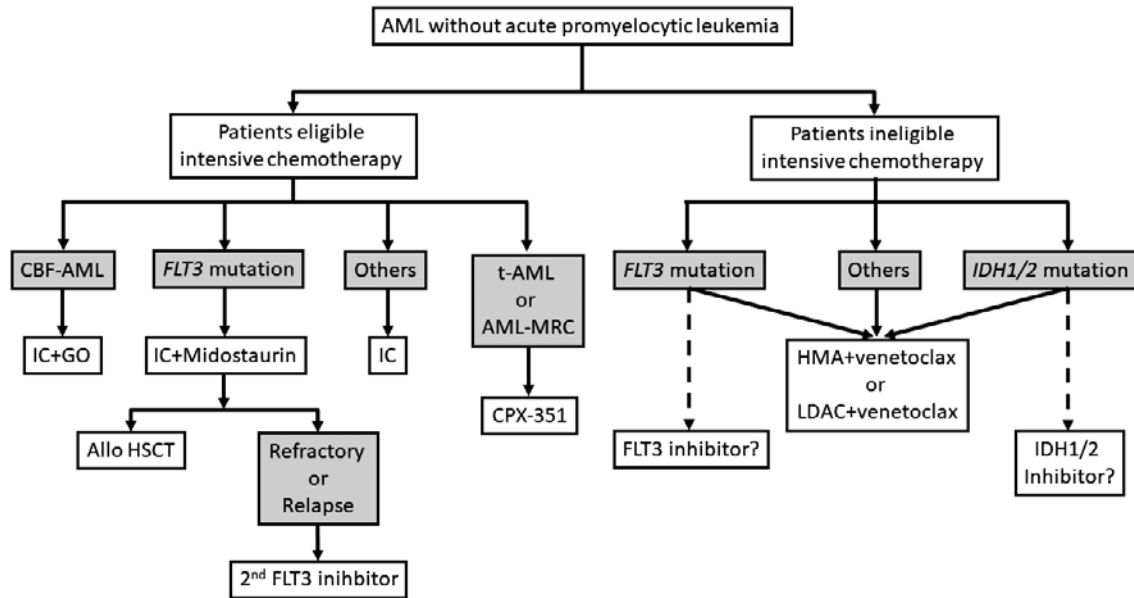


Fig. 1 The paradigm shift of gene diagnosis and treatment of AML in the future

CBF-AML: core binding factor acute myeloid leukemia, IC: intensive chemotherapy, GO: gemtuzumab ozogamicin, allo HSCT: allogeneic hematopoietic stem cell transplantation, t-AML: treatment related acute myeloid leukemia, AML-MRC: acute myeloid leukemia with myelodysplasia-related changes

+ CRi rate (48% vs. 13%, $p < 0.01$)³⁶. VEN + HMA is a standard therapy for older AML patients or those who are not indicated for standard intensive therapy due to complications. Furthermore, although it was a retrospective study, a trial reported that patients with poor prognostic factors for ELN such as *RUNX1* mutations and secondary AML had a higher CR + CRi and OS with VEN + HMA treatment than with standard chemotherapy. In the future, there is a possibility that VEN + HMA treatment may be indicated for some AML patients for whom standard chemotherapy is indicated³⁷.

The IDH1 inhibitor ivosidenib (IVO) and IDH2 inhibitor enasidenib have been shown to be useful for older AML patients with *IDH1/2* mutations^{38,39}. In one study, CR + CRi was achieved in 42.9% of older initial-onset *IDH1* mutation-positive AML patients treated with IVO alone³⁸. Additionally, a Phase III trial investigated the efficacy of the combined therapy of IVO and AZA in 146 patients with *IDH1* mutation-positive AML who were not indicated for standard chemotherapy, and they showed that the IVO + AZA group had significantly higher efficacy than the AZA only group (53% vs. 18%; $p < 0.001$). The IVO + AZA group also had significantly longer median EFS and median OS values than the AZA only group (EFS: hazard ratio, 0.33, 95% confidence interval, 0.16-0.69, $p = 0.002$; median OS: 24.0 vs. 7.9 months, $p = 0.001$)⁴⁰. Furthermore, among older initial-onset *IDH2* mutation-positive AML patients, as a result of enasidenib

alone, 26% had CR, or CRi, or achieved a state of the disappearance of leukemia but without blood cell recovery³⁹. One characteristic of IDH inhibitors is the differentiating effect of AML cells into neutrophils, and approximately 20% of patients treated with enasidenib showed the differentiating syndrome seen in ATRA treatment of acute promyelocytic leukemia⁴¹. Furthermore, the degree of myelosuppression is milder than in other drugs, and this is an oral drug with high tolerability. Thus, it is likely to become one of the treatment options for older AML patients with *IDH* mutations.

Conclusion

Figure 1 shows the stratification of AML treatment in the future based on the contents of this paper, but the field is undergoing a major shift from chemotherapy being the only initial-onset AML treatment. However, there is a large drug availability lag between the West and Japan. Many molecular-targeted drugs can be used in actual clinical practice in the West, while only second-generation FLT3 inhibitors and VEN are approved in Japan. Therefore, Western guidelines cannot be directly applied to actual clinical practice in Japan. Furthermore, in Japan, AML gene mutation analysis (*FLT3* mutation analysis) is approved only once for relapsed and refractory patients. Therefore, currently the gene mutation analysis described in this paper cannot be conducted in actual clinical practice for initial-onset AML cases in Ja-

pan, and this is a major obstacle in the medical care of AML, bigger than that of the drug lag.

The IDH1 inhibitor IVO and IDH2 inhibitor enasidenib has been shown to be useful for AML patients with IDH1/2 mutations. Therefore, in the future, some gene mutations will be able to be analyzed in actual clinical practice as companion diagnostics^{38,39}. Furthermore, as mentioned in this paper, even if an analysis of the full length of the gene, such as for *CEBPA* mutations or *DNMT3A* mutations, is not conducted, important value to actual clinical practice can still be provided via the analysis of only the domain of the gene that is necessary for AML prognosis analysis. It is our hope that the spread of gene mutation analysis in actual clinical practice and the elimination of the drug lag will facilitate progress in AML treatment, thereby improving patient outcomes in Japan.

Conflict of Interest: Hiroki Yamaguchi received honoraria from Novartis Co., Ltd., and received research support from Astellas Co., Ltd.

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(Received, March 3, 2022)

(Accepted, July 8, 2022)

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