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PROGRESS IN HEMATOLOGY

Recent progress in pediatric leukemia

Comprehensive molecular understanding of pediatric acute myeloid leukemia

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Abstract

Pediatric acute myeloid leukemia (AML) is a heterogeneous disease with various genetic abnormalities. Recent advances in genetic analysis have enabled the identification of causative genes in > 90% of pediatric AML cases. Fusion genes such as *RUNX1::RUNX1T1*, *CBFB::MYH11*, and *KMT2A::MLLT3* are frequently detected in > 70% of pediatric AML cases, whereas *FLT3*-internal tandem duplication, *CEBPA*-bZip, and *NPM1* mutations are detected in approximately 5–15% of cases, respectively. Conversely, mutations in *DNMT3A, TET2,* and *IDH*, which are common in adults, are extremely rare in pediatric AML. The genetic characteristics of pediatric AML are slightly different from those of adult AML. For accurate risk stratification and treatment intensity, genome analysis should be performed in a simple, fast, and inexpensive manner and the results should be returned to patients in real time. As with acute lymphoblastic leukemia, the presence or absence of minimal residual disease is an important factor in determining the success of treatment against AML, and it is important to predict prognosis and formulate treatment strategies considering the genetic abnormalities. For the development and clinical application of new molecularly targeted therapies based on identified genetic abnormalities, it is necessary to explore when and in which combinations drugs will be most effective.

Keywords Pediatric AML · Minimal residual disease · Risk stratification · DNA methylation

Introduction

Annually, approximately 150 new cases of pediatric acute myeloid leukemia (AML) occur in Japan. Acute promyelocytic leukemia (APL) accounts for 10–15% of these cases with t(15;17)(q24;q21), and most cases of APL are caused by translocations forming *PML::RARA* chimeric genes. All-trans retinoic acid-based differentiation induction therapy has been established as a standard treatment for APL [1], while attenuated chemotherapy has been established as the standard treatment for most cases of AML associated with Down syndrome (ML-DS) [2]. Therefore, in this article, I review the necessity for future reconstruction of risk stratification and the potential of new treatment strategies in addition to focusing on genes used for the risk stratification of de novo AML, excluding APL and ML-DS, in the AML-20 phase III clinical trial currently being conducted by the JCCG in Japan.

Characteristics of the molecular basis of pediatric AML

AML is a heterogeneous disease characterized by a variety of chromosomal and genetic abnormalities. Chromosomal abnormalities are more frequent in pediatric AML cases than in adult AML cases. Approximately, 40% of adult AML cases have a normal karyotype, but 70% of pediatric AML cases exhibit leukemia cell-specific translocations or structural abnormalities.

Chromosomal abnormalities and resulting fusion genes play a vital role in the development of pediatric AML. Many of these abnormalities in pediatric AML are known to correlate with treatment response and prognosis and are crucial markers for selecting treatment according to

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risk and for determining the indication for hematopoietic stem cell transplantation (HSCT) [3, 4]. The occurrence of genetic abnormalities varies depending on the patient's age; *KMT2A* rearrangements and *CBFA2T3::GLIS2* are observed in patients with AML aged < 3 years; *RUNX1::RUNX1T1*, *CBFB::MYH11*, *NUP98::NSD1*, and *DEK::NUP214* are observed in patients with AML aged 3–14 years; *RUNX1::RUNX1T1* and *CBFB::MYH11* are observed in the adolescent and young adult group (15–39 years), although the incidence is lower than that in patients aged < 14 years. The frequency of fusion genes clearly decreases in patients with AML aged at least 40 years, whereas the frequency of genetic mutations increases [3, 4].

Compared with Europe and the United States, *RUNX1::RUNX1T1* are highly detected in Japan, accounting for about 25–30% of all cases, and together with *CBFB::MYH11*, this most prevalent subgroup is known as core binding factor (CBF)-AML, accounting for one-third of all cases and exhibiting relatively low risk.

By contrast, mutations in *FLT3*-internal tandem duplication (ITD), *NPM1*, *CEBPA*, *KIT*, *RAS*, *WT1*, and *KMT2A*-partial tandem duplication (PTD) have been identified through previous genetic analysis studies, and the prognostic significance of *FLT3*-ITD, *KIT*, *NPM1*, and *CEBPA* mutations, which are frequently detected, has been examined. Numerous studies have been conducted on AML in both adult and pediatric populations (Table 1) [5, 6]. Since 2009, with the development of microarrays and next-generation sequencing, several genes including *DNMT3A*, *TET2*, and *IDH1/2*, as well as the gene mutations occurring in pediatric AML, have been identified in AML cases with a high prognostic value [7, 8]. However, these abnormalities are rare in pediatric AML, and the genetic background of pediatric AML development is somewhat different from that of adult AML [9].

Since it accounts for 5–10% of pediatric AML cases, acute megakaryoblastic leukemia (AMKL) has been regarded as a clinically and molecularly distinct disease subgroup. However, in ongoing international collaborative studies, *CBFA2T3::GLIS2* and *NUP98::KDM5A* have been identified in AMKL via comprehensive analysis using next-generation sequencing [10–12]. *CBFA2T3::GLIS2* was detected in 13–27% of pediatric AMKL cases, while *NUP98::KDM5A* was detected in 8–10% of cases, indicating that the former is a highly frequent abnormality. Other than AMKL, *NUP98::KDM5A* has been detected in various other AMLs [13]. The *GATA1* mutation, which is a frequent finding in ML-DS, was detected in some non-ML-DS de novo AML cases. In addition, the molecular basis of AMKL is now becoming more obvious, and *JAK2* and *MPL* mutations, which are frequently detected in myeloproliferative malignancies, have also been detected [14].

Table 1 Genetic aberrations related to prognosis

Good prognostic factors
t(8;21)(q22;q22)/*RUNX1::RUNX1T1*
inv(16)(p13.1;q22) or t(16;16)(p13.1;q22)/*CBFB::MYH11*
NPM1 mutation with or without *FLT3*-ITD
CEBPA-bZip mutation with or without *FLT3*-ITD
Poor prognostic factors
FLT3-ITD without *NPM1* or *CEBPA*-bZip mutation
t(16;21)(p11;q22)/*FUS::ERG*
t(5;11)(q35;p15.5)/*NUP98::NSD1*
inv(16)(p13.3q24.3)/*CBFA2T3::GLIS2*
t(6;11)(q27;q23)/*KMT2A*::*AFDN(AF6)*
t(10;11)(p12;q23)/*KMT2A::MLLT10*
t(10;11)(p11.2;q23)/*KMT2A::ABI1*
t(4;11)(q21;q23.3)/*KMT2A::AFF1(AF4)*
t(11;12)(p15;p13)/*NUP98::KDM5A*
t(7;11)(p15.4;p15)/*NUP98::HOXA9*
t(6;9)(p23;q34)/*DEK::NUP214*
t(8;16)(p11;p13)/*KAT6A::CREBBP*
t(7;12)(q36;p13)/*ETV6::HLXB*
t(3;21)(q26.2;q22)/*RUNX1::MECOM*
t(16;21)(q24;q22)/*RUNX1::CBFA2T3*
inv(3)(q21.3q26.2)/t(3;3)(q21.3q26.2)/*RPN1::MECOM*
t(3;5)(q25;q34)/*NPM1::MLF1*
t(10;11)(p12.3;q14.2)/*PICALM::MLLT10*
5q-, monosomy 5, monosomy 7

Recently, Yamato et al. have reported that genome-wide DNA methylation patterns are useful to predict prognosis. Four clusters linked to genetic alterations might be identified among pediatric patients with AML. Besides, combined with the gene expression status, the accuracy to predict relapse and survival rate was much improved (Fig. 1) [15]. Multiomics analysis might help to reveal the molecular basis of pediatric AML.

Treatment strategies for pediatric AML based on risk factors

Recent advances in treatment and supportive care have improved the overall survival of pediatric cancers. However, AML exhibits a relatively low survival rate (approximately 70%), and the prognosis of patients with relapsed/refractory AML is poor [3]. In clinical trials of pediatric AML in Japan so far (AML99 and AML-05 clinical trials), approximately 10% of patients failed to achieve complete remission (CR) and approximately 30% relapsed. These findings suggest that reconstructing risk stratification and improving survival rates are essential challenges.

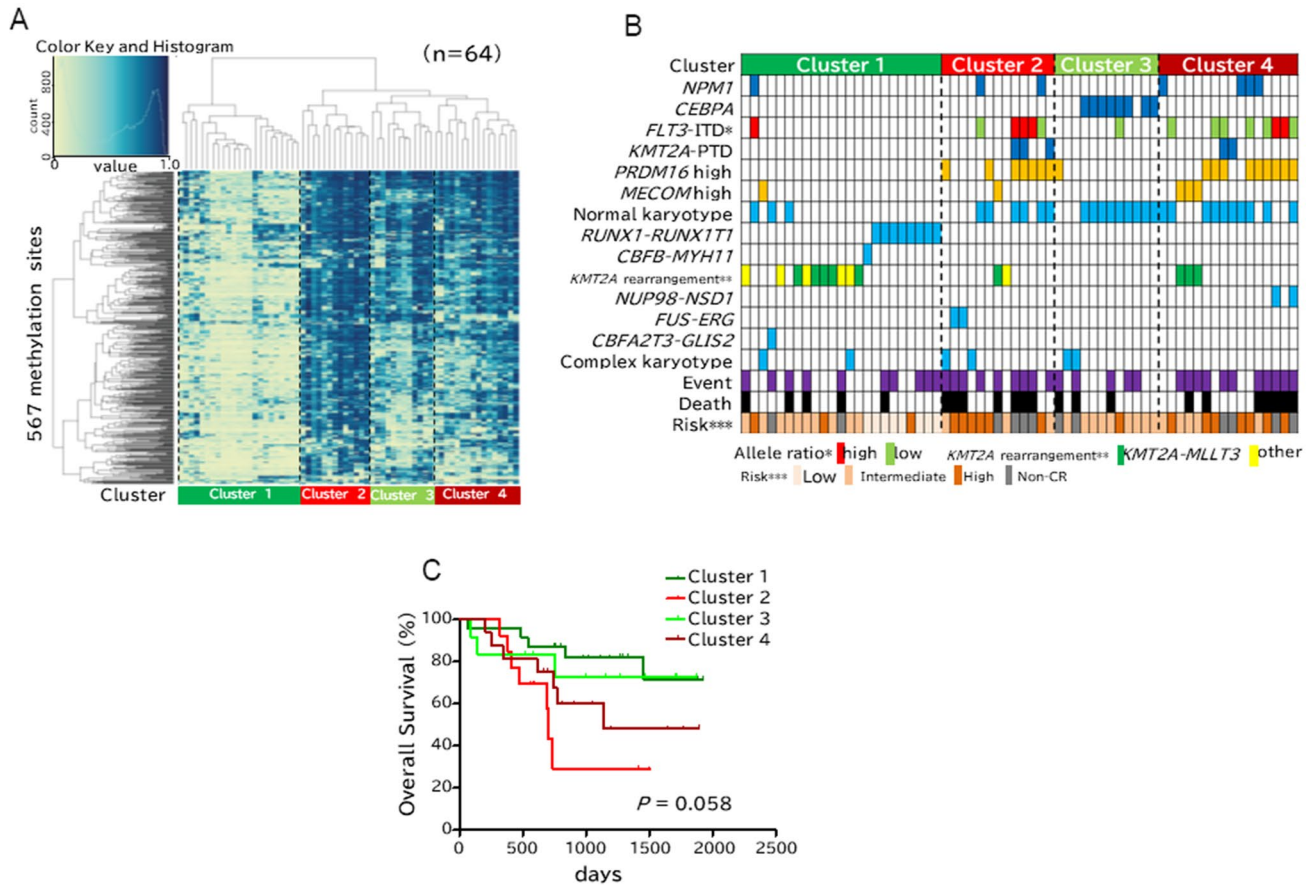

Fig. 1 Unsupervised hierarchical clustering of DNA methylation profiles and associations between DNA methylation clusters and additional parameters. (**A** and **B**) Heatmap of the DNA methylation profiles of 64 AMLs based on unsupervised hierarchical clustering. Clustering was based on the 567 CpG sites with the most variable methylation values in the 64 studied cases. Four clusters were generated: 1, 2, 3, and 4. DNA methylation levels were classified into 3 groups according to their β value: hypermethylation (> 0.67), intermediate methylation (0.34–0.66), and hypomethylation (< 0.33), respectively. Light blue, orange, and dark orange indicate the presence of the specified mutation, high gene expression, and chromosomal aberration, respectively. Brown indicates *KMT2A-MLLT3* fusion, and dark blue indicates *FLT3*-ITD with high allele ratio (> 0.7). Purple and black indicate non-complete remission (CR) and events and deaths, respectively. **C** Comparison of the Kaplan–Meier curves of OS among clusters 1–4. *PTD* partial tandem duplication

Usefulness of minimal residual disease

Similar to acute lymphoblastic leukemia (ALL), initial response to chemotherapy has been reported to be prognostically important in AML. In the St. Jude AML02 study, Inaba et al. evaluated initial response to treatment by morphology, minimal residual disease using flow cytometry (FCM-MRD), and quantitative PCR targeting fusion gene products, such as *RUNX1::RUNX1T1*, and concluded that FCM-MRD after induction 1 or 2 was the most sensitive marker to predict prognosis [16]. In this St Jude AML02 study, patients with FCM-MRD < 0.1% after induction 1 had a 3-year event-free survival (EFS) rate of 73.6% (95% CI 68.6–78.6%), whereas patients with FCM-MRD ≥ 0.1% had a 3-year EFS rate of 43.1% (95% CI 36.2–50.0%, $p < 0.0001$), indicating a significantly worse prognosis for FCM-MRD-positive patients after initial induction remission therapy [17]. In addition, in the Children's Oncology Group (COG) AAML03P1 study, patients with FCM-MRD < 0.1% had a relapse-free survival rate of 65.0% (95% CI 56.0–74.0%) following initial induction remission therapy, whereas patients with FCM-MRD ≥ 0.1% had a relapse-free survival rate of 30.0% (95% CI 15.0–45.0%, $p < 0.001$) [18]. Consequently, FCM-MRD may serve as a well-established marker in patients with AML, and the AML-20 clinical trial in Japan has just implemented FCM-MRD classification [19].

Cytogenetic characteristics of and treatment strategies for low-risk groups

In the AML-20 clinical trial, patients with CBF-AML were essentially placed in the low-risk group (cases positive for *FLT3*-ITD or having MRD ≥ 0.1% at the end of induction remission therapy-1 are at elevated risk in the

intermediate-risk group) (Table 2). In the AML-05 clinical trial, the 3-year OS was more than 90% in the CBF-AML group, showing a favorable prognosis. Although *RUNX1::RUNX1T1* and *CBFB::MYH11* are often analyzed together, patients with *RUNX1::RUNXIT1* showed a higher relapse rate than those with *CBFB::MYH11*. Tokumasu et al. detected *KIT* mutations in 47 of 107 patients with *RUNX1::RUNX1T1* and reported that patients with *KIT* mutations in exons 8 and 17 had considerably higher rates of relapse and cumulative events (*KIT* mutations in exons 10 and 11 had no impact on prognosis) [20]. Faber et al. also reported that *KIT* exon17 mutations were more frequently identified in *RUNX1::RUNX1T1* cases and had a considerably poorer prognosis [21]. The frequency of D816V mutations in *KIT* exon 17 was also examined using droplet digital PCR, and it was discovered that CBF-AML had a higher frequency of low allelic mutations than non-CBF-AML. In the present study, it was discovered that the presence of this *KIT* D816V-positive minor clone was associated with decreasing EFS (Fig. 2). This finding was consistent with the finding that AML cells with the *KIT* D816V mutation are more resistant to chemotherapy than AML cells without the mutation. However, many of the relapsed patients positive for *RUNX1::RUNX1T1* were rescued via subsequent hematopoietic stem cell transplantation (HSCT), and their 3-year OS rate was approximately 80% (Fig. 3A), indicating the effectiveness of HSCT. Thus, the transplantation source at the time of relapse should be considered from an early stage.

Despite the generally excellent prognosis of *CBFB::MYH11*, patients under the age of 3 years have a high risk of relapse. Hara et al. reported that the EFS was approximately 50% in 46 patients enrolled in the AML-05 clinical trial (Fig. 4) [22].

Table 2 Risk stratification of clinical trial of JCCG AML-20

Low risk	t(8;21)(q22;q22), inv(16)(p13.1;q22) or t(16;16)(p13.1;q22), and MRD < 0.1% after induction 1 and FLT3-ITD negative.
Mediate risk	1. t(8;21)(q22;q22), inv(16)(p13.1 q22) or t(16;16)(p13.1;q22) and MRD ≧ 0.1% after induction 1 and/or FLT3-ITD positive
	2. Negative for low and high-risk factors and MRD<0.1% after induction 1.
High risk	1. Positive for each of high-risk cytogenetic/genetic factor shown below. Monosomy 5/5q-, monosomy 7, inv(3)(q21.3q26.2)/t(3;3)(q21.3q26.2), FLT3-ITD(excluding patients with CBF), BCR-ABL1 Major/Minor, KMT2A-AFF1(AF4), KMT2A-AFDN(AF6), KMT2A-MLLT10(AF10), DEK-NUP214, NUP98-HOXA9, NUP98-NSD1, NUP98-KDM5A, CBFA2T3-GLIS2, FUS-ERG, MNX1-ETV6, PICALM-MLLT10, TBL1XR1-RARB
	2. Non complete remission after induction 1
	3. Negative for low risk factors and MRD ≧ 0.1% after induction 1

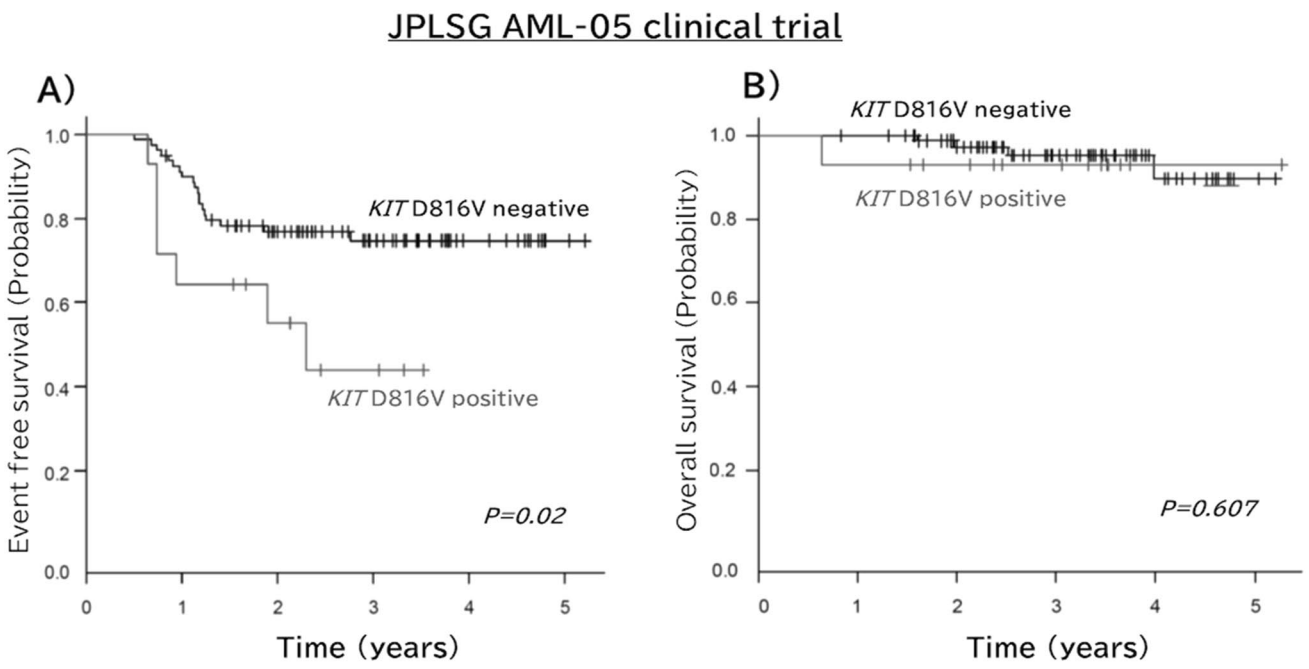

Fig. 2 Kaplan–Meier analysis of event-free survival and overall survival in the AML-05 clinical trial cohort. The 5-year event-free survival of the patients with the *KIT* D816V mutation was significantly inferior to that of those without *KIT* D816V mutation

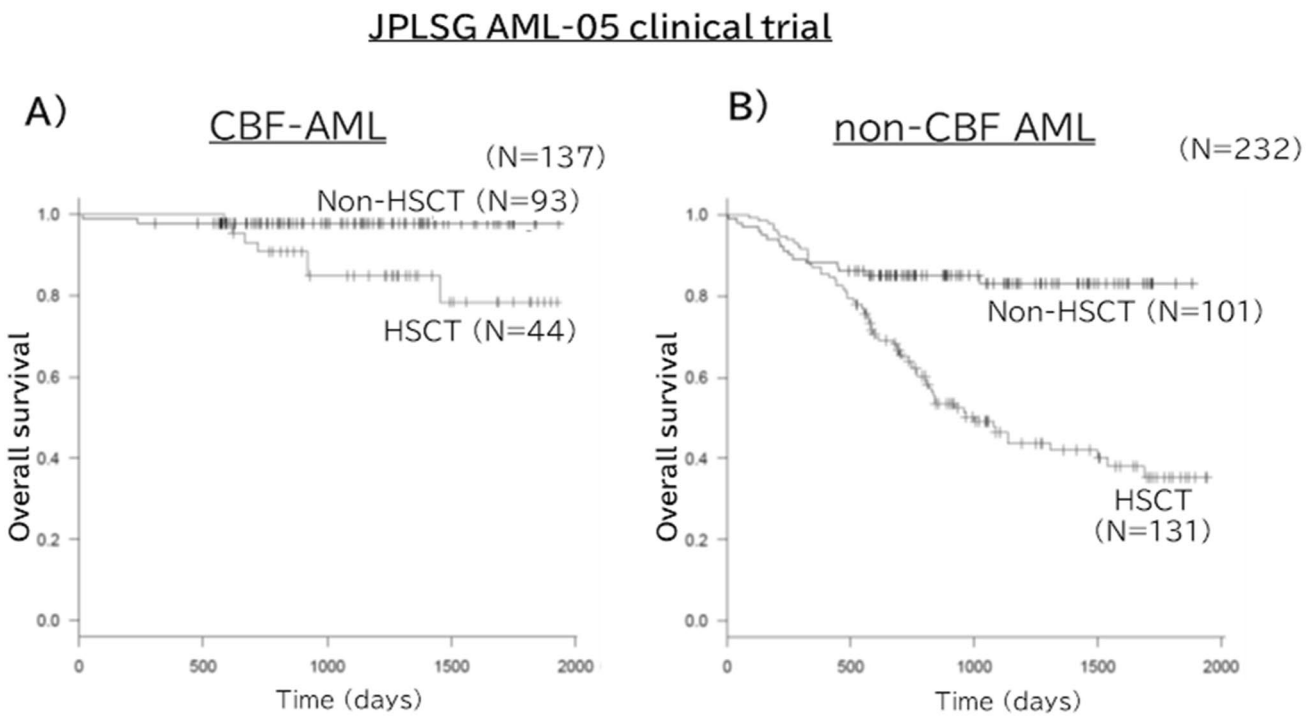

Fig. 3 Comparing efficacy of stem cell transplantation between AML patients with CBF (**A**) and non-CBF (**B**) who received stem cell transplantation enrolled in the AML-05 clinical trial. *CBF* core binding factor, *HSCT* hematopoietic stem cell transplantation

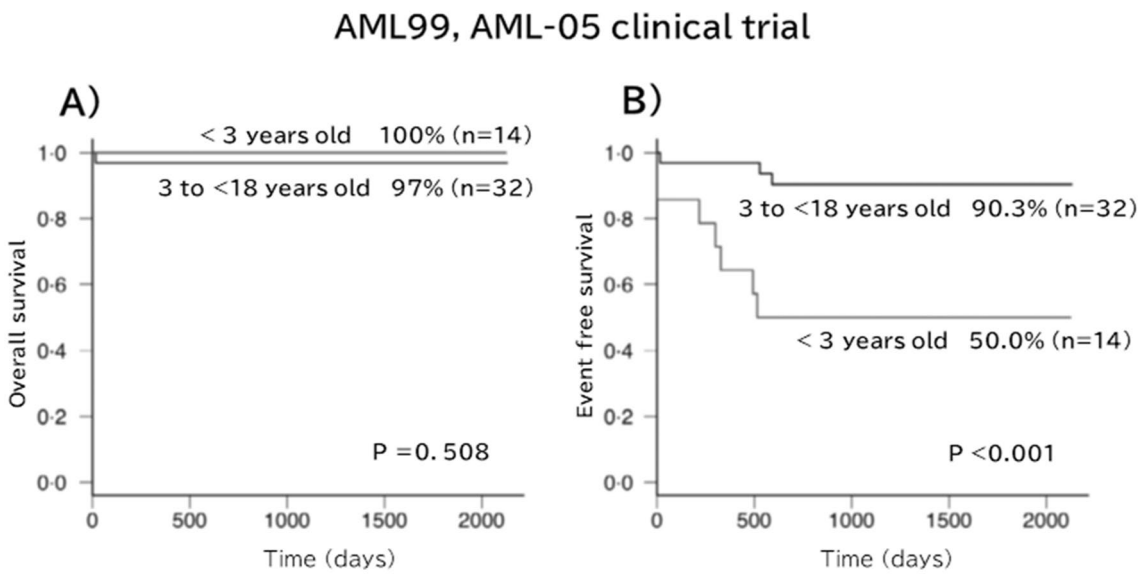

Fig. 4 Comparison of the prognosis between the younger (< 3 years old) and older (3– < 18 years old) groups among all patients with *CBFB-MYH11* enrolled in AML99 and AML-05 clinical trial. The younger group had significantly poorer EFS than the older group, although the OS was excellent in both groups

Cytogenetic characteristics of and treatment strategies for intermediate-risk groups

This intermediate-risk group (IR) group is composed of various genetic alterations. As a result, this group contains a mix of cases with a good prognosis and those with a poor prognosis. For example, the prognosis of patients with *KMT2A* rearrangements depends on partner genes

and/or *MECOM* expression. In particular, patients with a normal karyotype exhibit different genetic abnormalities. Patients with mutations in *NPM1* and *CEBPA*-bZip mutations showed a good prognosis, whereas patients positive for *KMT2A*-PTD showed a poor prognosis.

Therefore, it is necessary to consider adopting these abnormalities as risk factors in future clinical trials. Jo et al. and Matsuo et al. reported that patients with AML with *KMT2A::MLLT3* who exhibited high *MECOM* expression frequently relapsed and had a poor prognosis, while those who exhibited low *MECOM* expression had a fair prognosis [23, 24]. While patients with favorable prognosis can be cured with existing chemotherapies, CR cannot be maintained in most of patients positive for *KMT2A*-PTD or in patients with *KMT2A* rearrangement with high *MECOM* expression using conventional chemotherapies. Since chemotherapy significantly yields the refractory clones after relapse, a new therapeutic approach may be required. As an exploratory study, AML-20 will assess the prognostic significance of high *MECOM* expression.

Cytogenetic characteristics of and treatment strategies for high-risk groups

In the ongoing AML-20 clinical trial, *CBFA2T3::GLIS2*, *NUP98::KDM5A*, and other recently identified genetic abnormalities were added as high-risk factors in addition to monosomy 7, *FUS::ERG*, *KMT2A::AFDN (AF6)*, and *NUP98::NSD1*, which have already been adopted in the AML-12 clinical trial. Besides, AML-20 clinical trial also attempted to reduce the risk of CBF-AML to intermediate risk even in patients positive for *FLT3*-ITD (Table 2) [19].

In this review, a comprehensive gene expression analysis was performed on samples obtained from patients enrolled in the AML99 clinical trial using microarray. The results of this analysis revealed a characteristic gene expression pattern shared by patients with *NUP98::NSD1*. The characteristics of this signature were highly associated with high *PRDM16* expression, which is a homologous gene of *MECOM* and plays an important role in the activation of the *HOX* pathway. Many of these patients were refractory to treatment, did not experience remission, had a high rate of relapse, and did not fully recover even after HSCT. In case of non-CR, the protocol is discontinued, and second-line treatment is provided at each institution. Intriguingly, in approximately 20% of all pediatric AML cases, *NUP98::NSD1*-negative cases displayed the same gene expression pattern as *NUP98::NSD1*-positive cases, and the prognosis for these cases was poor [25]. In particular, the prognosis of patients with high *PRDM16* expression and *FLT3*-ITD positivity is extremely poor (Fig. 5). Recently, *UBTF*-ITD has been identified, and patients with this mutation have been reported to show poor prognosis and elevated *PRDM16* expression

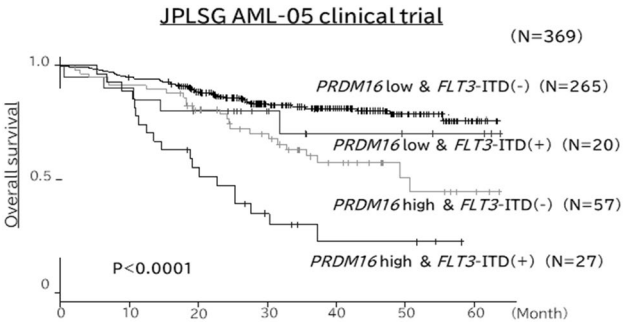

Fig. 5 Overall survival of AML patients based on *PRDM16* expression and *FLT3*-ITD status in AML-05 clinical trial

[26]. This significance will be assessed retrospectively in the AML-20 clinical trial.

Treatment strategies for patients with relapsed/refractory disease and non-complete remission for induction therapy

The standard chemotherapy regimen for relapsed/refractory pediatric AML has not been established, and the combination of daunorubicin (DNR), cytarabine (Ara-C), and etoposide (VP-16) used in the initial remission induction therapy should be often repeated. In particular, fludarabine, Ara-C, and granulocyte colony-stimulating factor (G-CSF) (FLAG) or FLAG with idarubicin (FALG-IDA) therapy are often used. Of the 369 AML-05 patients for whom genetic information was available, 232 were non-CBF-AML cases. Among them, the 3-year OS of 101 patients who did not receive HSCT was 80% and that of 131 patients who received HSCT was 30% (Fig. 3B). These findings suggested that conventional HSCT could not treat non-CBF-AML patients who experienced non-CR or relapse.

Exome analysis of four patients with relapsed disease showed that the clones that were initially minor and resistant to therapy were selected from multiple clones found at the time of initial relapse and that the clones with specific genetic mutations at the time of relapse proliferated. Leukemia cells exhibit a various type of clonal evolution. This suggests that leukemia cells are not a single population, but rather these cells develop resistance to anticancer drugs through a series of cytogenetic events that occur when these cells undergo repeated proliferation under the exposure of anticancer drugs. This may lead to treatment resistance, such as relapsed/refractory leukemia. These findings demonstrated the significance of initial treatment using drugs that cause total remission without the development of any refractory clones following initial induction therapy [9].

In the St. Jude AML02 study performed in the United States, patients with FCM-MRD $\geq$ 25% after the first course of induction therapy were treated with ADE

(small dose Ara-C + DNR + VP-16) + gemtuzumab ozogamicin (GO, 3 mg/m^2). Furthermore, patients with FCM-MRD ≥ 0.1% following the second course of induction therapy (Induction-2) were treated with GO alone (6 mg/m^2). After confirming the safety of ADE + GO, ADE + GO was administered to patients with MRD ≥ 1.0% after completion of Induction-1, and GO alone (6 mg/m^2) was discontinued. Among 29 patients with MRD ≥ 1.0% after Induction-1, 28 patients showed a decrease in MRD level and 13 patients turned negative for MRD, while 14 of 17 patients who received GO alone (6 mg/m^2) showed a decrease in MRD level [27]. In the COG-AAML0531 study, a subgroup analysis of IR and HR patients who had undergone HSCT revealed a trend toward improved disease-free survival and OS in the GO combination group compared with patients who received only chemotherapy [28]. GO administration before transplantation has been reported to improve prognosis in patients who relapsed [29]. The AML-20 clinical trial compared the poor prognosis of the IR and HR groups in each course of consolidation chemotherapy with or without GO to determine whether the addition of GO improved survival rates in the IR and HR groups (Fig. 6). Clinical trials using methylation inhibitors, BCL2 inhibitors, DOT1L inhibitors, HDAC inhibitors, JAK inhibitors, MENIN inhibitors, and other agents are mostly being conducted in the United States for pediatric AML [30]. Clinical trials of CAR-T cell therapy targeting CD123 are being conducted, and other targets are also being tested in clinical trials [30]. Various therapies are currently under development. Until a new standard strategy is established, these new molecularly targeted therapies should be tailored according to the cytogenetic characteristics of each patient, and it is necessary to investigate new treatment modalities in Japan.

Conclusion

Recent advances in molecular biology have been remarkable, and with the introduction of microarrays and next-generation sequencers, diagnostic methods for AML have evolved significantly and the cytogenetic background of AML has been clarified. Genetic analyses, as well as domestic and international analyses, have provided a better perspective on the molecular basis of AML (Fig. 7). The genomic profile of each clone at initial diagnosis should be evaluated to increase the cure rate of pediatric AML. Combination of various conventional or novel targetable drugs, cellular immunity, and conventional anticancer drugs with few side effects enable the reduction of relapse and therapy-related mortality.

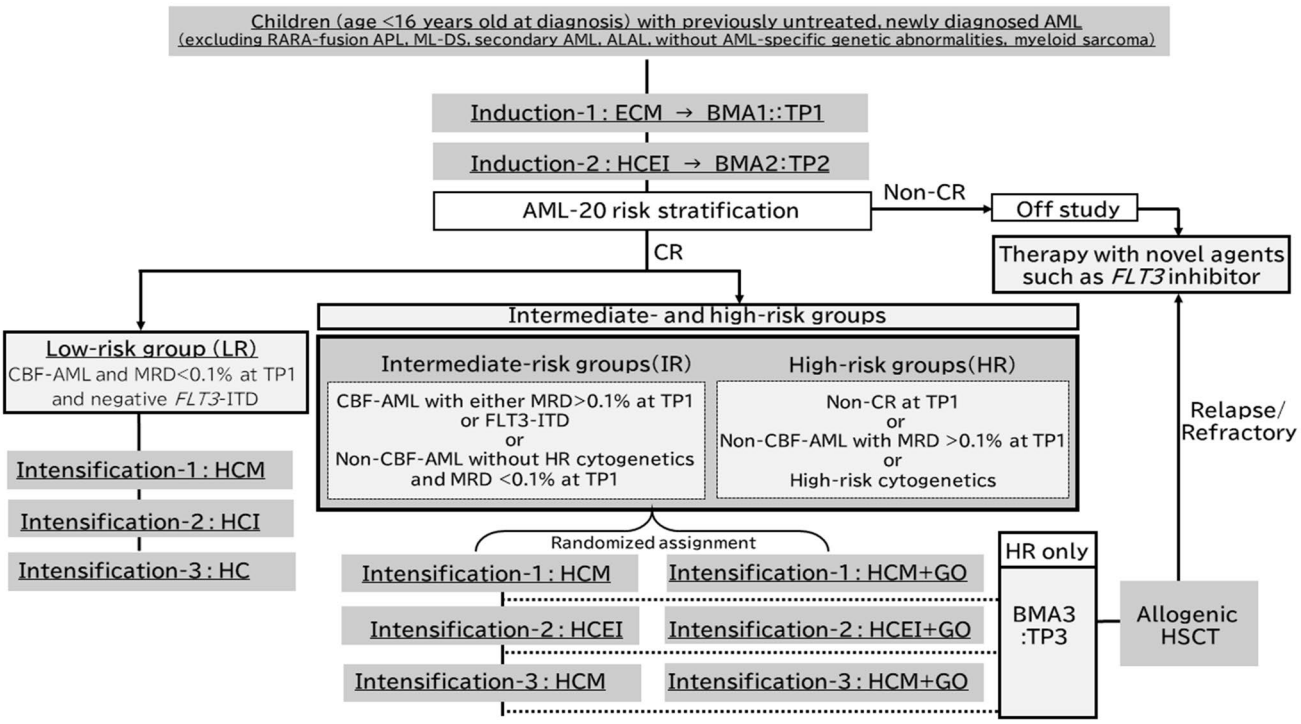

Fig. 6 Treatment scheme of the AML-20 clinical trial. *ALAL* acute leukemia of ambiguous lineage, *AML* acute myeloid leukemia, *APL* acute promyelocytic leukemia, *BMA* bone marrow aspiration, *CBF* core binding factor, *CR* complete remission, *HSCT* hematopoietic stem cell transplantation, *ML-DS* myeloid leukemia associated with Down syndrome, *MRD* minimal residual disease, *TP* timepoint

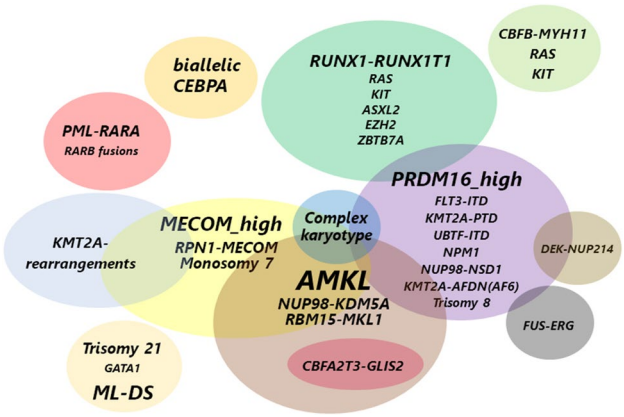

Fig. 7 Schematic diagram of AML subgroups classified by chromosomal aberrations, gene mutations, expressions, and fusion genes

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Declarations

Conflict of interest The author has nothing to declare in relation to the content of this paper's presentation.

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