Acute Megakaryoblastic Leukaemia (AMKL) in Children: A Comparison of AMKL with and without Down Syndrome

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Summary

To characterize childhood acute megakaryoblastic leukaemia (AMKL), we reviewed 45 children with AMKL diagnosed between 1986 and 2005 at Nagoya University Hospital and Japanese Red Cross Nagoya First Hospital. Twenty-four patients (53%) had AMKL associated with Down syndrome (DS-AMKL) and 21 (47%) had non-DS-AMKL. The median age of DS-AMKL patient was 21 months (range, 8-38 months) and that of non-DS-AMKL was 15 months (range, 2-185 months). The morphology of blast cells was categorized into three groups according to the stage of megakaryocyte maturation. The blast cells were more immature in DS-AMKL than in non-DS-AMKL in terms of morphology and immunophenotyping. Cytogenetic abnormalities of leukaemic cells were classified into 7 categories: normal karyotype including constitutional trisomy 21 in DS-AMKL; numerical abnormalities only; t(1;22)(p13;q13); 3q21q26 abnormalities; t(16;21)(p11;q22); −5/del(5q) and/or −7/del(7q); and other structural changes. The outcome of both children with DS-AMKL and non-DS-AMKL is excellent. The estimate of 10-year overall survival was 79% (95% CI: 54-90) with DS-AMKL and 76% (95% CI: 58-91) with non-DS-AMKL (P = 0.81) with a median follow-up of 78 months (range, 20-243 months). Our study shows the diverse heterogeneity of childhood AMKL and the need for subclassification according to cytogenetic and morphological features.

Keywords: acute megakaryoblastic leukaemia; Down syndrome; children; cytogenetics; morphology

Introduction

Acute megakaryoblastic leukaemia (AMKL), M7 according to the French-American-British (FAB) classification, is a subtype of acute myeloid leukaemia (AML) (Bennett *et al,* 1985). Until now, only a few series of AMKL have been reported in a consecutive cohort of children (Ribeiro *et al,* 1993; Athale *et al,* 2001; Paredes-Aguilera *et al,* 2003; Reinhardt *et al,* 2005). Large collaborative studies have shown that AMKL occurs in 4.1% to 15.3% of patients with childhood AML (Ravindranath *et al,* 1992; Gamis *et al,* 2003; Creutzig *et al,* 2005; Zeller *et al,* 2005; Rao *et al,* 2006). These previous studies revealed a diverse heterogeneity of the disease including morphology and cytogenetic studies. In children, 2 major subgroups have been described: AMKL developing in patients with Down syndrome (DS-AMKL) and AMKL in patients without Down syndrome (non-DS-AMKL). AMKL is the most frequent type of AML in children with DS (Zipursky *et al,* 1987). Somatic mutations of *GATA1* gene are found in almost all children with DS-AMKL (Wechsler *et al,* 2002; Hirose *et al,* 2003; Rainis *et al,* 2003). It is well known that patients with DS-AMKL have excellent outcome with less intensive chemotherapy. In general, the remission rate is approximately 90% with event-free survival (EFS) of 70% to 80% (Gamis 2005). The favorable outcome in patients with DS-AMKL has been explained by the increased sensitivity of leukaemic cells to anticancer drugs (Taub *et al,* 1999, 2000). On the other hand, patients with non-DS-AMKL appear to be more heterogeneous and several cytogenetic groups have been identified (Lu, *et al* 1993; Dastugue, *et al* 2002; Duchayne, *et al* 2003). Among these groups, the occurrence of t(1;22)(p13;q13) is restricted to infants with AMKL (Carroll, *et al* 1991; Lion, *et al* 1992; Bernstein, *et al* 2000). The outcome of children with non-DS-AMKL is generally poor (Ribeiro *et al,* 1993; Athale *et al,* 2001), but a recent study reported long-term survivors after intensive chemotherapy (Reinhardt *et al,* 2005).

We reviewed 45 children with AMKL (DS-AMKL, 24; non-DS-AMKL, 21) diagnosed between 1986 and 2005 at Nagoya University Hospital and Japanese Red Cross Nagoya First Hospital. The main purpose of this study was to compare clinical and biological characteristics of patients with DS-AMKL and non-DS-AMKL.

Patients and methods

Patients and diagnostic criteria of AMKL

Forty-five children with newly diagnosed AMKL (DS-AMKL, 24; non-DS-AMKL, 21) at Nagoya University Hospital and Japanese Red Cross Nagoya First Hospital between 1986 and 2005 were retrospectively reviewed. Acute leukaemia was diagnosed by the presence of at least 20% blasts in the bone marrow (BM) according to the World Health Organization classification (Harris *et al,* 1999). In patients with poor quality BM aspiration smears, the presence of more than 20% blasts in the BM core biopsy or 20% or more circulating blasts were used to support the diagnosis of acute leukaemia. The diagnosis of AMKL was established on the basis of FAB classification (Bennett *et al,* 1985) by studies of cell morphology and cytochemistry and was confirmed by immunophenotyping.

For immunophenotyping of leukaemic blasts, mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation from the BM. In patients who did not have an adequate BM aspirate, the immunologic studies were performed on their peripheral blood (PB) mononuclear cells. The cells were analyzed by flow cytometry with a panel of monoclonal antibodies. For the assessment of megakaryocytic lineage, at least 10% of the blast cells needed to be positive for one or more of the platelet-associated antigens (CD36, CD41, CD42 or CD61) (San Miguel*, et al* 1988). For the scoring of the other immunologic markers, the samples were defined as positive if more than 20% of the cells were stained. In the absence of immunophenotyping, the diagnosis was confirmed by electron microscopic identification of platelet peroxidase (PPO) activity or CD41 expression in the histopathologic examination in malignant cells.

Cytogenetic studies were performed on BM or peripheral blood samples taken at the time of diagnosis; samples were processed and analyzed by standard methods.

Analysis of GATA1 mutation

After obtaining informed consent from the parents of the children for the purpose of sample banking and molecular analysis, BM or PB samples were obtained from all of the patients with AMKL at the time of diagnosis. High-molecular weight DNA was extracted from the samples using standard methods. For screening of *GATA1* mutations, we amplified the genomic DNA that corresponded to exon 2 of *GATA1* by using polymerase chain reaction that employed 1 primer pair as previously reported (Hirose *et al,* 2003).

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Amplified products were cloned into the pGEM-T Easy vector (Promega, Madison, WI, USA) and sequenced on a DNA sequencer (310; Applied Biosystems, Foster City, CA, USA) using a BigDye terminator cycle sequencing kit (Applied Biosystems).

Treatment

In patients with DS-AMKL, 6 patients received chemotherapy consisting of cytosine arabinoside (AraC) and dounorubicin (Kojima *et al*, 1990, 1993) and 16 patients received chemotherapy consisting of AraC, etoposide, and dounorubicin or pirarubicin (Kojima *et al*, 2000). One patient with mosaic DS received intensive chemotherapy according to non-DS-AML protocol containing high-dose AraC (AML99) (Shimada *et al*, 2006) and 1 patient received chemotherapy according to acute lymphoblastic leukaemia (ALL) oriented protocol (Kojima *et al*, 1990). One patient who failed to achieve complete remission (CR) by induction therapy received bone marrow transplant (BMT) from an HLA-matched unrelated donor.

In the patients with non-DS-AMKL, 18 patients were treated in 1 of 2 national cooperative studies for AML (12 on ANLL91, 6 on AML99). Two patients received less intensive chemotherapy according to DS-AMKL protocol (Kojima *et al*, 1993). Twelve patients received BMT (autologous, 7; allogeneic, 5) in the first CR. Three patients who failed to achieve CR after induction therapy received allogeneic stem cell transplant (SCT: BM, 2; cord blood, 1). Two patients who relapsed after allogeneic BMT in the first CR (one from a matched sibling, the other from a matched unrelated donor) received a second allogeneic BMT (one from a matched unrelated donor, the other from a haploidentical related donor). The indication for SCT varied over the study period and was defined by individual protocol. In the ANLL91 study, allogeneic BMT was indicated in the first CR for patients who had a matched sibling donor; patients without a matched sibling donor were eligible for autologous BMT. AML99 study did not indicate SCT for patients with AMKL in the first CR without patients with poor prognostic chromosome abnormalities such as monosomy 7 or t(16;21). Conditioning regimens included busulfan and melphalan; fludarabine was added in the unrelated donor settings. Prophylaxis against graft-versus-host disease consisted of cyclosporine and short-term methotrexate therapy in BMT from a matched related donor and tacrolimus and short-term methotrxate in SCT from an unrelated donor or a mismatched related donor.

Statistical analysis

Non-parametric Mann-Whitney test was used to analyze statistical differences in the distribution of

continuous variables. χ^2 test or Fisher's exact test was used for differences in frequencies. Survival distributions were estimated by using the method of Kaplan and Meier (Kaplan *et al*, 1958) and were compared using the log-rank test. All estimates of outcomes are reported with 95% confidence intervals (CI). The duration of overall survival (OS) was defined as the period between the date of diagnosis and the date of death from any cause or the date of the last follow-up examination. The duration of EFS was defined as the period between the date of diagnosis and the date of an adverse event (relapse, death from any cause) or the most recent follow-up examination. Early death or remission induction failure was recorded as an event at zero time, with an EFS value of zero. SAS release 6.12 software (SAS Institute, Cary, NC) was used to perform the statistical analysis.

Results

Patients

During the study, 194 children with AML were diagnosed in two hospitals; 45 (23.2%) of these had AMKL. Twenty-four patients (53.3%) had DS-AMKL and 21 (46.7%) had non-DS-AMKL. Among them, 11 patients with DS-AMKL were previously reported (Kojima *et al,* 1990, 1993, 2000). Twenty-seven children with DS-AML/myelodysplastic syndrome (MDS) were diagnosed during the same period of current study. Among them, 1 patient was diagnosed as AML (M0) and 2 were diagnosed as MDS. One of the patients with DS-AMKL was trisomy 21 mosaicism. In the patients with DS-AMKL, 8 patients (33.3%) had the history of transient myeloproliferative disorder and 12 (50.0%) had prior MDS. Congenital heart anomalies were present in 11 patients (45.8%) with DS-AMKL. None of the patients with AMKL had secondary leukaemia or mediastinal germ cell tumor. The median age of DS-AMKL patient was 21 months (range, 8-38 months) and that of non-DS-AMKL was 15 months (range, 2-185 months). Patients younger than 4 years at diagnosis accounted for 95.6% (43 of 45) in both groups.

Clinical and laboratory features

Table I shows the clinical and laboratory findings at the time of diagnosis. There were no statistically significant differences in these findings between DS-AMKL and non-DS-AMKL patients. The initial leukocyte count, BM blast cell count, and lactic dehydrogenase activity tended to be higher in patients with non-DS-AMKL than in those with DS-AMKL, but differences were not significant. Nine patients

(DS-AMKL, 8; non-DS-AMKL, 1) had less than 20% blasts in the BM; the BM of all nine patients was difficult to aspirate, contributing to the low estimates of percentage of blasts. Six patients (DS-AMKL, 5; non-DS-AMKL, 1) underwent BM biopsy, which confirmed the presence of more than 20% blast cells and the diagnosis of acute leukaemia. Three DS-AMKL patients had more than 20% blasts in the peripheral blood; which supported the diagnosis of acute leukaemia.

Morphological features

Forty-two BM smears were studied, as 3 smears of patients with non-DS-AMKL were unevaluable. The leukaemic cells of all 42 patients were negative for myeloperoxidase, chloroacetate esterase, and alpha naphthyl butyrate esterase activity. The morphology of blast cells was extremely varied and we tried to categorize it into three groups according to the stage of megakaryocyte maturation: type 1, completely undifferentiated blasts with nucleolus or vacuoles in the cytoplasm (Figs 1A,B); type 2, intermediately differentiated blasts with cytoplasmic blebs, sometimes a large cytoplasm and azurophilic granules (Figs 1C,D); and type 3, blasts with dysmegakaryocytopoiesis (Fig 1E) including the presence of micromegakaryocytes (Fig 1F). The blast cells with deep blue cytoplasm (type 1b, 2b) (Fig 1B,D) were distinguished from type 1a or 2a blasts (Fig 1A,C). The morphology of blast cells in DS-AMKL and non-DS-AMKL were distributed as follows: type 1 (63%, 39%), type 2 (25%, 39%), and type 3 (12%, 22%) (Tables Ⅱ,Ⅲ). Type 1b and 2b blasts were detected in 8 of 24 patients (33%) with DS-AMKL. The blast cells tended to be less mature in DS-AMKL than in non-DS-AMKL in terms of morphology. Seven patients presented with type 3 morphology: 4 patients (DS-AMKL, 1; non-DS-AMKL, 3) had increased numbers of micromegakaryocytes with type 1 blasts, 2 patients with DS-AMKL had increased numbers of dysmegakaryocytopoiesis, and 1 non-DS-AMKL patient with t(16;21) showed type 3 blasts with emperipolesis and cytophagocytosis, which are characteristic in patients with t(16;21) (Imashuku *et al*, 2000). Dysplasia in trilineage blood cells was seen in 2 patients (DS-AMKL, 1; non-DS-AMKL, 1). Prominent dyserythropoiesis without involvement of the myeloid cell lineage was found in 5 patients (DS-AMKL, 3; non-DS-AMKL, 2). Emperipolesis was observed in 3 patients (DS-AMKL, 2; non-DS-AMKL, 1).

Immunophenotyping

All but one patient had immunophenotyping studies (Tables Ⅱ,Ⅲ); the BM sample of 1 patient with

non-DS-AMKL who showed CD41 expression in blast cells by the histopathologic examination and translocation t(1;22) was insufficient and could not evaluated. The leukaemic cells of 44 patients expressed at least one platelet-associated antigen (CD36, CD41, CD42, or CD61). The blast cells with low expression of platelet-associated antigens in 2 patients (DS-AMKL, 1; non-DS-AMKL, 1) were positive for PPO. Among the myeloid antigens, CD13/CD33 was expressed by leukaemic blast cells in 78%/53% and 60%/78% of patients with DS-AMKL and non-DS-AMKL, respectively. Atypical expression of lymphoid-associated antigens CD7 was detected in 88% and 53% of patients with DS-AMKL and non-DS-AMKL, respectively ($P = 0.003$). Glycophorin A was detected only on the leukemic cells of 47% of patients with DS-AMKL ($P = 0.009$). Interestingly, 86% of the type 1b and 2b blasts were positive for glycophorin A.

Cytogenetic findings

Cytogenetic studies were performed for 21 patients with DS-AMKL and 21 patients with non-DS-AMKL (Tables Ⅱ,Ⅲ). Three patients with DS-AMKL had insufficient BM samples. Cytogenetic abnormalities of leukemic cells were classified into 7 categories: normal karyotype including constitutional trisomy 21 in DS-AMKL; numerical abnormalities only; t(1;22)(p13;q13); 3q21q26 abnormalities; t(16;21)(p11;q22); −5/del(5q) and/or −7/del(7q); and other structural changes. Normal karyotype including constitutional trisomy 21 was found in 5 patients with DS-AMKL and in 2 patients with non-DS-AMKL. Numerical chromosomal abnormalities were common in non-DS-AMKL. Patients with non-DS-AMKL had trisomy 8 (6 patients), trisomy 19 (5 patients), trisomy 21 (7 patients), and monosomy 7 (2 patients). Six patients with DS-AMKL had −7/del(7q) and 1 of them had both monosomy 5 and 7. The translocation t(1:22) was found in 2 patients with non-DS-AMKL and 3q21q26 abnormalities, which are common in adult AMKL (Lu *et al*, 1993; Tallman *et al*, 2000; Dastugue *et al*, 2002; Duchayne *et al*, 2003), was found in 1 patient with non-DS-AMKL. The translocation t(16;21) was found in 1 patient with non-DS-AMKL. These recurrent structural changes were not observed in patients with DS-AMKL. The 11q23 abnormalities and the Philadelphia chromosomes were not detected in either group. Other structural changes, such as t(5;12)(p15;q21) was found in DS-AMKL and t(2;7)(p12;p22), t(2;11;19)(q31;q13;q13) were found in non-DS-AMKL.

GATA1 gene mutations

We performed the analysis of *GATA1* mutation in 17 of 24 patients with DS-AMKL and 11 of 21 patients

with non-DS-AMKL. *GATA1* mutations were observed in all patients with DS-AMKL and 1 patient with non-DS-AMKL. Ten of 17 patients with *GATA1* mutations in DS-AMKL were previously reported (Hirose *et al,* 2003).

Outcome

In patients with DS-AMKL, 23 of 24 (96%) achieved CR. Three patients relapsed and died, and 2 other patients with congenital heart anomalies died of congestive heart failure. Of the 24 patients with DS-AMKL, 19 (79%) are currently alive. Of the 21 patients with non-DS-AMKL, 16 (76%) achieved CR. One patient with t(1;22) did not receive induction therapy because of multiple organ failure at the day of admission. Three of 4 non-responders to induction therapy underwent successful allogeneic SCT and 1 patient died of pneumonia. Twelve patients received BMT in the first CR, 4 of whom relapsed and 3 of whom died. Five patients received chemotherapy only, 4 of whom have remained in CR. Overall, 16 of 21 patients (76%) are currently alive.

The estimate of 10-year OS was 79% (95% CI: 54-90) for patients with DS-AMKL and 76% (95% CI: 58-91) for patients with non-DS-AMKL with a median follow-up of 78 months (range, 20-243 months) (P = 0.81, Fig 2). The estimate of 10-year EFS was 79% (95% CI: 58-91) for patients with DS-AMKL and 57% (95% CI: 36-77) for patients with non-DS-AMKL (P = 0.09, Fig 3). The outcome of DS-AMKL and non-DS-AMKL was comparable. In non-DS-AMKL, the estimated OS of 15 children who received SCT (79%, 95% CI: 51-93) did not differ from 5 children treated with chemotherapy alone (80%, 95% CI: 30-97) (P = 0.95).

Discussion

In the current series, 23.2% of patients with AML were identified as AMKL, which is higher than findings of several collaborative group studies for childhood AML (Ravindranath *et al,* 1992; Gamis *et al,* 2003; Creutzig *et al,* 2005; Zeller *et al,* 2005; Rao *et al,* 2006). The relative frequency of AMKL has varied markedly, ranging from 4.1% to 15.3%. There are possible explanations for the high proportion of AMKL in our series. One is the difference in prevalence of DS-AMKL. The proportion of DS-AMKL in this study was 53.3%, which was much higher than those of other institutes. For example, the ratio of DS-AMKL to non-DS-AMKL was 6 to 35 in the report from St. Jude Children's Research Hospital

(Athale *et al,* 2001). In the 1980s and early 1990s, the outcome of patients with DS-AMKL was generally poor (Levitt *et al,* 1990). Most DS-AMKL patients were not enrolled in the clinical studies. In German Berlin-Frankfurt-Munster (BFM) cooperative group studies, the percentage of patients with DS has gradually increased since study 78 (1.9%), study 83 (5.6%), study 87 (8.1%), study 93 (9.7%) and study 98 (12.9%) (Creutzig *et al,* 2005). On the other hand, among patients registered in the population-based Nordic study between 1984 and 2001, 72 of 515 (14.0%) children with AML had DS (Zeller *et al,* 2005), which is similar to the percentage in our study, in which 25 of 194 patients (12.9%) with AML had DS. Because we started the clinical trial for DS-AMKL using a less intensive regimen that was specifically designed for DS-AMKL since mid the 1980s (Kojima *et al,* 1990, 1993, 2000), patients with DS-AMKL were not excluded from the data file, which may account for the higher proportion of DS-AMKL in our series. In a report from Mexico, 29 of 152 (19.1%) children with AML were diagnosed as AMKL among whom only one patient had DS (Paredes-Aguilera *et al,* 2003).

The incidence of non-DS-AMKL was 12.4% in our non-DS-AML series, which might also be higher than those of other reports. Immunophenotyping with platelet-associated antigens and electron microscopic identification of PPO were introduced relatively long ago in our hospitals (Kojima *et al,* 1990). The incidence of AMKL might have been underestimated because of its diverse clinical presentation and requirement of specific laboratory methods in early reports. As previously described, 19.1% of children with AML had AMKL in a report from Mexico. The incidence of AMKL might be different between western countries and non-western countries. We speculate that AMKL represents approximately 10% of all cases of non-DS-AML in children.

In the current study, the morphology of blast cells was categorized into three groups (type 1, type 2, or type 3) according to the stage of megakaryocyte maturation, modeling after FAB classification of myeloid leukemia (Fig 1). The blast cells with deep blue cytoplasm (type 1b, 2b) were only detected in patients with DS-AMKL and positive for glycophorin A in all but 1 patient. Erythroid specific mRNAs encoding γ-globin and erythroid δ-amino levulinate synthase were expressed in blasts from all patients with DS-AMKL (Ito *et al,* 1995). These findings suggest that type 1b and 2b blasts arise from bipotent megakaryocyte/erythrocyte progenitors. A high incidence of the coexpression of the T cell-associated marker CD7 in patients with DS-AMKL compared with patients with de novo AMKL was also observed. In addition to mature T cells, the CD7 antigen is expressed on immature haematopoietic cells (Kita *et al,* 1993; Creutzig *et al,* 1995). In terms of morphology and immunophenotyping, the blast cells were more

immature in patients with DS-AMKL than in those with non-DS-AMKL.

Cytogenetic profile of AMKL is complex, which reflects the heterogeneity of the disease. In the current study, seven cytogenetic groups were identified. The translocation t(1;22)(p13;q13) which produces OTT-MAL (RBM15-MKL1) fusion gene (Ma *et al,* 2001; Mercher *et al,* 2001, 2002), was detected in 2 patients (4.3%) with non-DS-AMKL. This frequency was lower than in the another study (Dastugue *et al*, 2002; Duchayne, *et al* 2003). Dastugue et al (Dastugue *et al,* 2002) reported that OTT-MAL transcript was detected in 1 patient with a normal karyotype, suggesting that the molecular determination method may increase the detection of t(1;22) translocation. The 3q21q26 abnormalities were observed in 1 patient with non-DS-AMKL. The 3q21q26 abnormalities are rare in childhood AMKL (6.7% in the Dastugue's report) (Dastugue *et al,* 2002), while they are seen in 17% to 20% of adult AMKL patients (Lu *et al,* 1993; Tallman *et al,* 2000; Dastugue *et al,* 2002; Duchayne *et al,* 2003). The translocation t(16;21)(p11;q22) generating a TLS/FUS-ERG transcript (Kong *et al,* 1997) was found in 1 patient with non-DS-AMKL. This translocation has been found in all subtypes of AML except M3, including several cases with AMKL. The patient with t(16;21) in our series showed morphologically type 3 blasts with emperipolesis and cytophagocytosis. These morphological findings of BM are characteristic of patients with t(16;21), regardless of FAB classification (Imashuku *et al,* 2000). These recurrent structural changes were not observed in patients with DS-AMKL. On the other hand, monosomy 7/del(7q) was more frequent in patients with DS-AMKL (29%) than non-DS-AMKL (9.5%). Monosomy 5/del(5q) was found in only 1 patient with DS-AMKL who had also monosomy 7. In non-DS-AMKL, trisomies (+8, +19, +21) were more frequent than DS-AMKL, as previously reported (Dastugue *et al,* 2002, Duchayne *et al,* 2003, Lu *et al,* 1993, Reinhardt *et al,* 2005). An acquired trisomy 21 is a popular chromosome gain of childhood AMKL, reported in 23% to 43% of patients with non-DS-AMKL (Ribeiro *et al,* 1993; Athale *et al,* 2001,). In our study, an acquired trisomy 21 was found in 33% of patients with non-DS-AMKL. Coupled with other reports, an acquired trisomy 21 seems to have a higher incidence in non-DS-AMKL in children than in other childhood non-DS-AML. The 11q23 abnormalities are often detected in childhood AMKL (Athale *et al,* 2001; Reinhardt *et al,* 2005), although not in the current study. The Philadelphia chromosomes or i(12)(p10) with mediastinal germ cell tumor, which were mainly found in adult AMKL (Dastugue *et al,* 2002, Duchayne *et al,* 2003), were also not found in the current study.

We performed the analysis of *GATA1* mutation in 17 of 24 patients with DS-AMKL and 11 of 21 patients with non-DS-AMKL. *GATA1* mutations were observed in all patients with DS-AMKL as

previously reported (Wechsler *et al,* 2002; Hirose *et al,* 2003). In contrast to DS-AMKL, *GATA1* mutations were rarely found in patients with non-DS-AMKL. Until now, only 4 children with *GATA1* mutations in non-DS-AMKL were reported (Rainis *et al,* 2003; Bourquin *et al,* 2006). Interestingly, all of them had acquired trisomy 21 in their leukaemic cells. Our patient with *GATA1* mutation in non-DS-AMKL did not have acquired trisomy 21 in his leukaemic cells.

Before the 1990s, most patients with DS-AML were treated outside of clinical studies and received suboptimal therapies, resulting in poor outcomes (Levitt *et al,* 1990). Following the recognition of the favorable outcome when treated with protocols of the collaborative study group for AML (Ravindranath *et al,* 1992), there has been an increase in recruitment into protocol studies. However, it has become apparent that resistant disease is rare but treatment-related deaths are frequent in most series (Creutzig *et al,* 1996, Lange *et al,* 1998). Since then, several collaborative groups adapted their AML protocols for DS-AML by reducing the dose of chemotherapeutic agents (Creutzig *et al,* 2005; Zeller *et al,* 2005; Rao *et al,* 2006). In recent reports, 5-year survival rate have been in excess of 80%, largely because of reductions in treatment-related deaths with a decrease from 30% to 40% in the early 1990s to around 10% in recent studies(Creutzig *et al,* 2005; Zeller *et al,* 2005; Rao *et al,* 2006). Since mid 1980s, we have used a less intensive regimen specifically designed for DS-AML (Kojima *et al,* 1990, 1993, 2000). The excellent outcome of DS-AMKL may originate from early use of a regimen specified for DS-AML.

The 10-year OS in our series was 76% (95% CI: 58-91) for patients with non-DS-AMKL, which was superior to those of other reports (Ribeiro *et al,* 1993; Athale *et al,* 2001; Reinhardt *et al,* 2005). The prognosis for children with non-DS-AMKL was poor in previous reports. According to the report from St Jude Children's Research Hospital, 2-year OS was only 14%, which was significantly higher after allogeneic SCT (30%) than after chemotherapy alone (0%) (Athale *et al,* 2001). The result of a recently published report on AMKL from the European Group for Blood and Marrow Transplantation (EBMT) was excellent (Garderet *et al,* 2005). Three-year OS was 82% in 19 children after allogeneic SCT and 61% in 38 children after autologous SCT. The authors recommend allogeneic SCT when an HLA-matched sibling is available and otherwise, autologous SCT for children with AMKL in the first CR. However, this report included 11 children with DS and analyzed the outcome of children with DS and without DS together, which confused the interpretation of the results. In the current study, 5-year OS was 79% in patients who received SCT, which did not differ from patients achieving CR and being treated with chemotherapy alone (80%, P = 0.98). In the recent report from BFM collaborative group, the 5-year

OS was 43% in the SCT group and 54% in the chemotherapy group (P = 0.37) (Reinhardt *et al*, 2005). The recent use of intensified chemotherapy may abrogate the indication of allogeneic SCT for children with non-DS-AMKL.

In conclusion, our study shows the diverse heterogeneity of childhood AMKL and the differences in the clinical and biological presentation between DS-AMKL and non-DS-AMKL. Subclassification according to megakaryocyte maturation and cytogenetic abnormalities in childhood AMKL is warranted.

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Titles and legends to figures

Fig 1. **Morphological categories of the blasts.** May-Giemsa staining of the bone marrow smears. (**A**) Type 1a: complete undifferentiated blasts. (**B**) Type 1b: complete undifferentiated blasts with deep blue cytoplasm. (**C**) Type 2a: intermediately differentiated blasts with cytoplasmic blebs. (**D**) Type 2b: intermediately differentiated blasts with cytoplasmic blebs and deep blue cytoplasm. (**E**) Type 3: blasts with dysmegakaryocytopoiesis. (**F**) Type 3: blasts with micromegakaryocytes. Original magnification×1000 for all panels.

Fig 2. **Estimated probability of overall survival for 24 patients with DS-AMKL and 21 patients with non-DS-AMKL.** The estimate of 10-year OS was 79% (95% CI: 54-90) for patients with DS-AMKL and 76% (95% CI: 58-91) for patients with non-DS-AMKL (P = 0.81). DS-AMKL indicates

Down syndrome-associated acute megakaryoblastic leukaemia.

Fig 3.   Estimated probability of event-free survival for 24 patients with DS-AMKL and 21 patients with non-DS-AMKL. The estimate of 10-year EFS was 79% (95% CI: 58-91) for patients with DS-AMKL and 57% (95% CI: 36-77) for patients with non-DS-AMKL (P = 0.09). DS-AMKL indicates Down syndrome-associated acute megakaryoblastic leukaemia.