**Table III.** Characteristics of patients with early death or relapse.

| Patients | Age (years) | Sex | WBC | PLT | PT | APTT | Fibrinogen | D-dimer | FAB | Breakpoint of *PML-RARA* | Clinical course | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 15 | M | 1·2 | 55 | 1·25 | 28·6 | 0·65 | 65 300 | M3 | bcr1 | ICH at 15 d in induction | Death at 24 d |
| 2 | 4 | F | 171·0 | 28 | 1·47 | 25·9 | 1·02 | 6200 | M3v | bcr3 | BM relapse at 15 months in maintenance and then BMT in 2CR | Alive at 83 months |
| 3 | 14 | M | 62·4 | 4 | 1·46 | 30·4 | 0·79 | 17 400 | M3v | n.e. | ICH at 2 d | Death at 16 d |
| 4 | 11 | F | 2·1 | 39 | 1·10 | 27·1 | 1·00 | 35 000 | M3 | n.e. | Pseudomonas sepsis and meningitis after four courses of consolidation | Death at 5 months |
| 5 | 12 | M | 1·8 | 16 | 1·45 | 26·9 | 1·10 | 6500 | M3 | n.e. | BM relapse at 19 months and then ICH during subsequent treatment | Death at 24 months |

WBC, white blood cell count ($\times 10^9$/l); PLT, platelet count ($\times 10^9$/l); PT, prothrombin time (s); APTT, activated partial thromboplastin time (s) Fibrinogen, mg/dl; D-dimer, μg/ml. FAB, French-American-British classification; ICH, intracranial haemorrhage; BM, bone marrow; BMT, bone marrow transplantation; 2CR, 2nd complete remission; n.e., not evaluated.

became PCR-negative after 6 months of therapy. No patient that was monitored for MRD exhibited re-conversion to PCR-positivity.

## Discussion

APL with the *PML-RARA* chimaeric gene is more homogenous than other types of AML and, for infrequent childhood APL, therapy has been often considered together with that of adult patients. However, for paediatric patients, who typically have physiological differences from adults, it has not been thoroughly understood whether the combination of cytarabine with ATRA and anthracyclines would be effective in terms of long-term prognosis.

As shown in Table IV, recent clinical studies of childhood APL, in which patients were enrolled from the mid-1990s to the early 2000s and followed up for median periods of 36 months or longer, were compared to our study (de Botton *et al*, 2004; Ortega *et al*, 2005; Testi *et al*, 2005). In all of these studies, induction therapy with administration of ATRA and anthracyclines with or without cytarabine achieved CR rates at >90% and incidence of early death at <10% respectively. In the state-of-the-art treatment guidelines (Sanz *et al*, 2009), anthracyclines should start together with ATRA (or as soon as possible) in high-risk patients. Regarding drug dosages and clinical parameters, the adjusted cumulative dosage of anthracyclines of our study (375–415 mg/m$^2$) was lower than other studies (390–750 mg/m$^2$), while that of cytarabine varied to a large extent among studies. Regarding long-term survival, other three studies presented EFS rates of 71–82% despite OS rates at around 90%, whereas our study achieved a 7-year EFS of 91·4% (Table IV). Accordingly, the 7-year CIR of our study (3·6%) was lower than reported by other studies (15·6–27%) (Table IV). Moreover, none of our patients suffered EM relapse, whereas the other studies reported five patients with EM relapse (skin, middle ear or CNS; Table IV). In our study, one patient exhibited asymptomatic prolongation of QTc interval, which may be associated with late effects of anthracyclines. One other study (Testi *et al*, 2005) reported that two patients developed t-MDS after 36 and 80 months from diagnosis.

In post-remission therapy studies including chemotherapy-based consolidation without ATRA, recurrent disease might develop late in the course, such as seven clinical relapses that occurred over 4–36 months in the APL93 study (de Botton *et al*, 2004) or 14 haematological and five molecular relapses at the median of 26 and 31 months respectively, in the AIDA (ATRA and idarubicin) study (Testi *et al*, 2005). The PETHEMA (Programa para el Estudio y Tratamiento de las Hemopatias Maligna) group reinforced the consolidation therapy of LPA96 study with single anthracycline agent by adding ATRA and increased dosage of idarubicin for intermediate and high-risk patients (LAP99 study). (Ortega *et al*, 2005) These reports indicated that addition of ATRA to anthracycline-based consolidation therapy improved the prognosis of APL patients, especially those with risk factors,


 

Table IV. Comparison of AML99-M3 with recent studies on childhood APL.

| Reports | de Botton *et al* | Testi *et al* | Ortega *et al* | Imaizumi *et al* |
|---|---|---|---|---|
| Protocol | APL93 | AIDA | LPA96/LPA99 | AML99-M3 |
| Year | 2004 | 2005 | 2005 | This study |
| Period of enrollment | 1993–1998 | 1993–2000 | 1996–2004 | 1997–2004 |
| Median follow-up time | 67 months | 79 months | 38 months | 86 months |
| No. of patients | 31 | 110 | 66 | 58 |
| Proportion of patients with WBC $\geq 10 \times 10^9$/l | 48% | 35% | 39% | 38% |
| Therapy | | | | |
| Induction | 1) ATRA → DNR + CA*<br>2) ATRA+DNR + CA* | ATRA + IDA | ATRA + IDA | ATRA + DNR + CA |
| Consolidation | 1) DNR + CA<br>2) DNR + HCA | 1) IDA + HCA<br>2) MIT + VP-16<br>3) IDA + CA + 6TG | 1) IDA + ATRA†<br>2) MIT + ATRA†<br>3) IDA + ATRA† | 1) ATR + MIT + HCA‡<br>2) ATRA + THP + CA‡<br>3) ATRA + ACM + HCA‡ |
| Maintenance | (−) or ATRA ± MP/MTX§ | ATRA or MP/MTX§ | ATRA + MP/MTX | ATRA alone |
| Dosage of anthracyclines (mg/m$^2$) | DNR (495) | IDA (80), MIT (50) | IDA (80–100), MIT (50) | DNR(135), MIT(20), THP(90), ACM(180) |
| Anthracycline dosage converted to DNR (mg/m$^2$)¶ | 495 | 390–650 | 390–750 | 375–415 |
| Cumulative dosage of cytarabine (mg/m$^2$) | 10800 | 6250 | 0 | 68000 |
| Cumulative dosage of ATRA (mg/m$^2$) | 1350–6750 | 750–6150 | 3750–4875 | 5940 |
| Incidence of headache/ pseudotumour cerebri (%) | 39/16 | 13/9 | 30/6 | 24/5 |
| Clinical outcome | | | | |
| Early death (%) | 3 | 3·6 | 7·5 | 3·4 |
| CR rate (%) | 97 | 96 | 92 | 96·6 |
| CIR (%) | 27 (5 years) | NA | 17 (5 years) | 3·6 (7 years) |
| Extramedullary relapse (sites) | 1 (skin) | 2 (middle ear) | 2 (CNS) | 0 |
| Overall survival rate (%) | 90 (5 years) | 89 (10 years) | 87 (5 years) | 93·1 (7 years) |
| Event-free survival rate (%) | 71 (5 years) | 76 (10 years) | 82 (5 years) | 91·4 (7 years) |
| Late cardiotoxicity | No | No | No | 1** |
| Secondary malignancy | No | 2 (tMDS) | No | No |

DNR, daunorubicin; IDA, idarubicin; MIT, mitoxantrone; THP, pirarubicin; ACM, aclarubicin; CA, cytarabine; HCA, high-dose CA; MP, mercaptopurine; MTX, methotrexate; CR, complete remission; CIR, cumulative incidence of relapse; CNS, central nervous sytem; tMDS, therapy-related myelodsysplastic syndrome; NA, not available.

*Patients with WBC $\leq 0{\cdot}5 \times 10^9$/l were randomized to 1) or 2), and those with WBC $>0{\cdot}5 \times 10^9$/l assigned to 2).

†In LPA96 anthracyclines alone; in LPA99 ATRA was combined and IDA dose was increased for intermediate and high-risk patients.

‡Each course was repeated twice.

§Patients were randomized.

¶Equivalent DNR doses were converted using ratios in1:3–1:5 for IDA/MIT, 1:1·6 for THP and 1:0·2 for ACM.

**One patient with asymptomatic prolongation of QTc interval in the examination with electrocardiogram.

although the trial to add cytarabine to ATRA and anthracycline-based consolidation remains undetermined.

In our study, which combined cytarabine with ATRA and anthracyclines both in induction and consolidation, the long-term outcome was improved and showed a low CIR level. Moreover, by adopting prarubicin (Lenk *et al*, 1990) and aclarubicin (Warrell, 1986), two agents of anthracyclines with relatively low acute cardiotocicity, the cumulative doses of anthracycllines were lowered to levels that did not exceed moderate dosages (approximately 300–550 mg/m$^2$). Late abnormalities of left ventricular performance were uncommon with cumulative anthracycline doses <300 mg/m$^2$, but late cardiotoxicity might be an important concern in patients with moderate or higher dosages.( Sorensen *et al*, 1997; Nysom

*et al*, 1998) However, our study included one patient who showed asymptomatic electrocardiographic changes of QTc prolongation which may be associated with late effects of anthracyclines (Bagnes *et al*, 2010) and, therefore, cautious observation might be important for children with a long prospect of survival.

It is to be noted, however, that our regimen with six reinforced courses of consolidation led to increased risks of infectious complications attributable to the prolonged duration of neutropenia. The incidence of sepsis in our study (5·6–10·9% in each consolidation block) was higher than that (3·3–6·6% of incidence) reported by the PETHEMA study (Ortega *et al*, 2005). Although all but one of patients in remission recovered from sepsis with treatment, the compliance of the regimen was decreased in five patients with inevitable omission or dose-reduction of Block 3 consolidation because of chemotherapy-related toxicities. On the basis of the decreased MRD shown during this combined consolidation therapy, the intensity of consolidation therapy should be adjusted to ensure safety. In the ongoing trial in Japan that succeeded AML99-M3, the intensity of consolidation therapy has been reduced from six to four courses, and the effects of this will be compared to AML99-M3.

Recently, the European APL Group suggested the possibility of additional cytarabine to reduce the chance of relapse for patients with APL.(Adès *et al*, 2006) More recently, in the comparative analysis between APL2000 trial with additional cytarabine and LPA99 trial without cytarabine, the 3-year OS and CIR of high-risk patients were respectively, 91·5% vs. 80·0% and 9·9% vs. 18·5%.(Adès *et al*, 2008) Furthermore, the PETHEMA group also demonstrated that the risk-adapted treatment with ATRA, idarubicin and cytarabine for high-risk patients significantly improved the 3-year CIR (11%) when compared to that (26%) of their previous study.(Sanz *et al*, 2010) These findings suggest an importance of risk-adapted treatment and additional cytarabine for high-risk patients.

While EM relapse involving mostly CNS occurs at an incidence of 1–5% (Liso *et al*, 1998; Ko *et al*, 1999; Specchia *et al*, 2001; Breccia *et al*, 2003), at least one in 10 relapses of APL have a CNS component (Sanz *et al*, 2009). For 81 children with relapse reported in the literature, six patients (7·4%) had CNS involvement and the incidence of isolated CNS of good risk patients was as low as 2/218 (0·92%).(Chow & Feusner, 2009) In a European study, which reported 169 relapses (23%) in 740 patients (de Botton *et al*, 2006), the 3-year cumulative incidence (5·0%) of EM relapse was more frequent in patients with WBC count $> 10 \times 10^9$/l, suggesting that high-risk patients may benefit from IT therapy for CNS prophylaxis. Accordingly, IT therapy was performed for high-risk patients (Sanz *et al*, 2005; Adès *et al*, 2008), whereas IT therapy for CNS prophylaxis is not currently recommended for low-risk patients (Chow & Feusner, 2009). As high-dose cytarabine could have contributed to the prevention of CNS relapse because of a high penetration property into the CNS, TI therapy for low-risk patients would be omitted in our regimen while holding CIR at low levels.

Secondary malignancy is another emerging problem, even if at low levels, for APL patients as their survival is prolonged. The PETHEMA LPA99 study, with 560 subjects, identified nine patients with second malignancies, including six t-MDS/AML, at a median interval of 41 months.(Sanz *et al*, 2008) More recently, the European APL group reported the very long-term outcome of 578 patients with a median follow-up of 10 years, in which the cumulative incidence of secondary tumours and t-MDS was 1·4% and 0·2% at 5 years respectively, and 2·7% and 1·1% at 10 years respectively.(Adès *et al*, 2010) It is of note that the risk of t-MDS/AML may be increased by exposure to moderate or high cumulative doses of anthracyclines, which act by inhibiting DNA topoisomerase II, for children with malignant tumours.( Zunino & Capranico, 1990; Le Deley *et al*, 2003) Although the risk of secondary malignancy may not be thoroughly understood with regard to the use of anthracyclines for APL, the cumulative dosage of anthracyclines may be an important perspective of the long-term outcomes and adverse effects for childhood APL.

Recently, therapy with arsenic trioxide, which induces differentiation as well as apoptosis of APL cells, has been shown to be effective for patients not only with relapsed but also with newly diagnosed APL (Ferrara, 2010). With accumulating evidence for the efficacy and safety of therapy with arsenic trioxide alone or in combination with other agents, it would be a promising approach for treatment of childhood APL in the near future (Zhang *et al*, 2008) (Zhou *et al*, 2010).

In conclusion, although this study, without risk-adjusted stratifications or randomized approaches, is insufficient to make definite conclusions, the improved outcome of paediatric APL patients in this study may provide useful implications in the perspective of long-term prognosis and late adverse effects of childhood APL. Further investigations are needed.

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Patient Reports

# Infantile acute promyelocytic leukemia without an RARα rearrangement

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**Key words** acute promyelocytic leukemia, infant, normal karyotype, RARα.

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia (AML), with a characteristic clinical course, distinct morphological features and gene rearrangement, and is assigned to M3 in the French–American–British (FAB) classification. APL accounts for less than 10% of pediatric AML, and is extremely rare in early childhood.[1] Almost all APL is typified by PML/RARα rearrangement and responds positively to all-*trans*-retinoic acid (ATRA). However, about 2% of APL patients lack the PML/RARα rearrangement and display features that differ somewhat from typical APL,[2] though detailed reports of such patients are rare. We describe the laboratory findings and clinical course of a female infant with APL lacking an RARα rearrangement.

## Case Report

An 11-month-old female infant presented with a 10-day history of high fever. On physical examination, she was pale and had hepatomegaly without a bleeding tendency. Her hemoglobin was 8.7 g/dL, with a normal platelet count of $193 \times 10^9$/L, an elevated leukocyte count of $35.4 \times 10^9$/L with 25% abnormal promyelocytes and dysplastic neutrophils with abnormal nuclei, such as hypersegmentation, ring-shape, and the pseudo-Pelger–Huet anomaly. Her serum lactate dehydrogenase level was elevated to 929 IU/L and coagulation studies were normal.

Bone marrow examination showed a hypercellular marrow with 81% neoplastic promyelocytes with cytoplasmic hypergranulation (Fig. 1). No Auer rods were seen in their cytoplasm. The shape of the nuclei was basically round, and bilobed or folded nuclei were sparse. These cells were strongly positive for myeloperoxidase (Fig. 2) and specific esterase staining (Fig. 3a). Some stained positive for non-specific esterase (Fig. 3a), and this staining was inhibited by sodium fluoride (Fig. 3b).

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Immunophenotyping analyses of these cells with flow cytometry revealed positive expression of CD13, CD15, CD33 and MPO; and negative expression of CD11b, CD14, CD34, CD56 and HLA-DR. These findings also supported the conclusion that the leukemic cells were at the promyelocyte stage of myeloid development. Cytogenetic analysis revealed a normal female karyotype. No chromosomal abnormalities were detected with spectral karyotyping-fluorescence *in situ* hybridization analysis.

Reverse transcriptase-polymerase chain reaction for PML-RARα was negative. Furthermore, no RARα gene rearrangements were detected with fluorescence *in situ* hybridization analysis. She was diagnosed as having APL without RARα gene rearrangement, based on the morphological features, cytochemical staining, and immunophenotyping of her leukemic cells.

Patients with APL, lacking rearrangement of PML/RARα, NPM/RARα or NuMA/RARα, are unlikely to benefit from ATRA. Thus, our patient received five cycles of a multi-agent chemotherapy regimen consisting of etoposide, cytarabine, mitoxantrone and idarubicine without ATRA. Coagulation studies remained normal during induction chemotherapy. A subsequent bone marrow examination revealed complete remission. She has remained in continuous first remission for over 2 years after completion of chemotherapy.

## Discussion

APL is characterized by FAB M3 blast cell morphology. The leukemic cells of our patient were not morphologically consistent with typical APL, as Auer rods were not present in their cytoplasm and blast cells with bilobed or folded nuclei were in a minority. However, they were classified as promyelocytes, because they had abundant azurophilic granules. Blasts with round nuclei may be predominant in atypical APL, in contrast to typical APL carrying the PML/RARα rearrangement.[3] In the present case, some of the blast cells were positive for non-specific esterase staining. APL is reported to have two types of cellular differentiation: pure neutrophilic or neutrophilic/monocytoid type.[4] The latter can express positively for non-specific esterase staining as in our case. APL immunophenotype is characterized

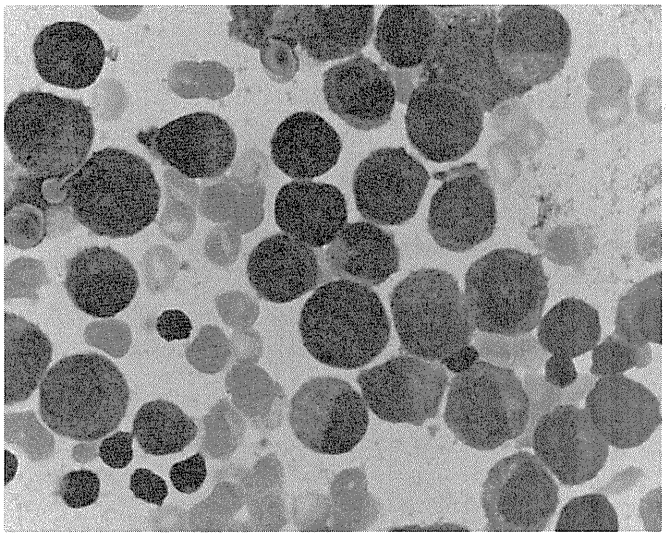

**Fig. 1** Bone marrow examination at onset. Most of the blast cells have regular nuclei and hypergranular cytoplasm without Auer or Faggot bodies (May–Giemsa staining, original magnification ×1000).

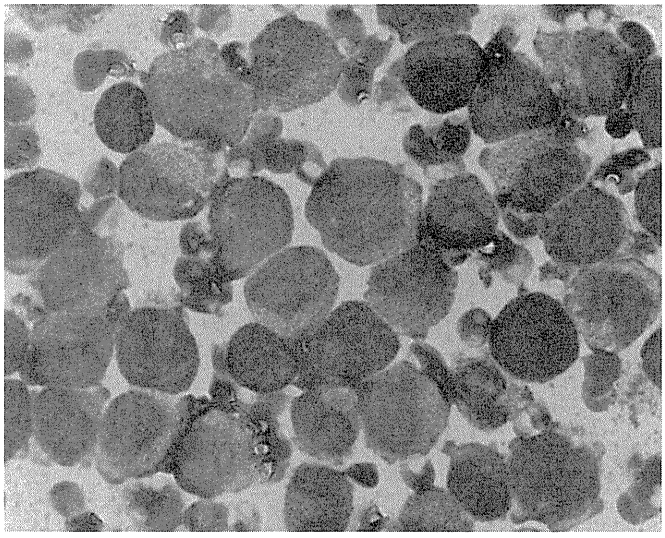

**Fig. 2** Blast cells showing strong positivity for myeloperoxidase (original magnification ×1000).

by frequent expression of CD13 and CD33, and rare expression of HLA-DR, CD34, CD11b and CD14.[5,6] The immunophenotypic features of our patient's blast cells were consistent with typical APL.

Another major characteristic of APL is a gene rearrangement, and 98% of patients with APL have a typical form of PML/RARα rearrangement, while the remaining 2% are atypical.[6] About half of the atypical forms involve other RARα rearrangements, including PLZF/RARα, NPM/RARα, NuMA/RARα or STAT5b/RARα; and the rest lack an RARα rearrangement.[6] Recently, a new variant APL with rearrangement of PRKAR1A/RARα was reported.[7] Our patient was negative for NuMA1-RARα, PLZF-RARα, STAT5B-RARα, NPM-RARα, and PRKAR1A-RARα transcripts by reverse transcriptase-polymerase chain reaction.

One of the most important distinctions between typical and atypical APL is the sensitivity to ATRA. The combination of ATRA and anthracycline-based chemotherapy is currently considered to be the gold standard first-line treatment for patients with APL. Like PML/RARα rearrangement-associated APL, patients with rearrangements of NPM/RARα and NuMA/RARα are sensitive to ATRA.[8,9] In contrast, APL patients carrying PLZF/RARα and STAT5b/RARα rearrangements are resistant to ATRA.[10,11] Sensitivity to ATRA of APL patients with PRKAR1A/RARα may also exist but is not yet confirmed.[7] The ATRA responsiveness of patients lacking an RARα rearrangement has not been formally confirmed, although failure to respond to ATRA treatment has been previously documented in such a patient.[3] Considering the mechanism of ATRA, which resolves the differentiation block induced by RARα fusion proteins, it is

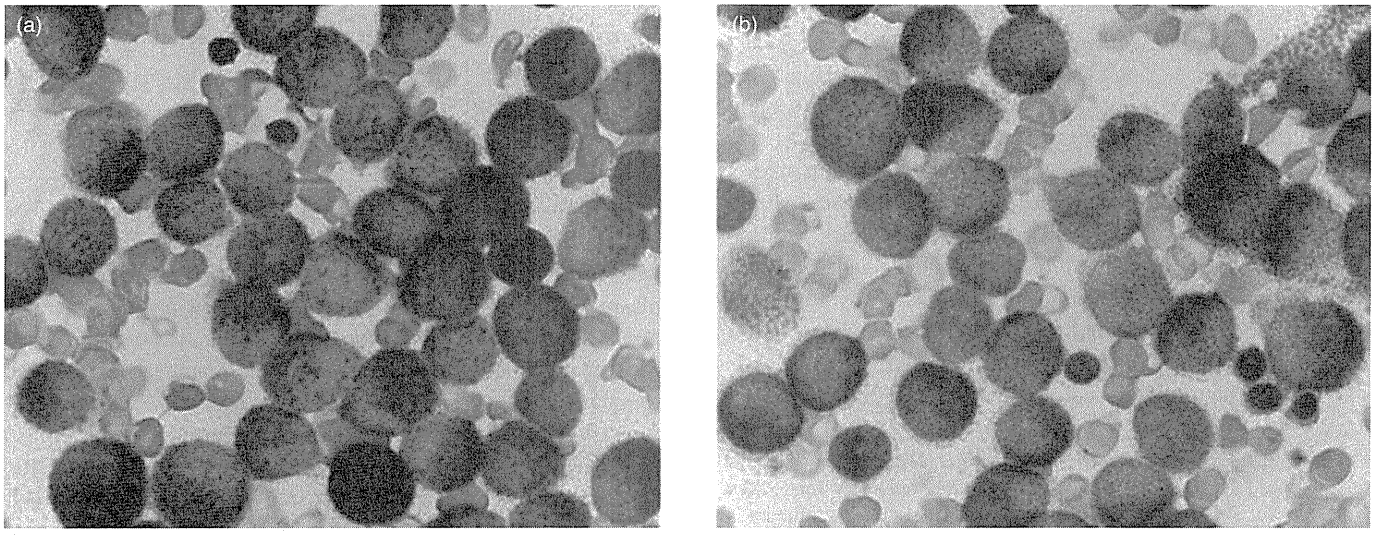


**Fig. 3** (a) Blast cells showing positivity for specific and non-specific esterase (original magnification ×1000). (b) The non-specific esterase positivity is totally inhibited by sodium fluoride (original magnification ×1000).

**Table 1** Acute promyelocytic leukemia cases lacking evidence for RARα rearrangements

| Patient no. | Reference no. | Age (years) | Sex | Initial WBC ($\times 10^9$/L) | Initial PLT ($\times 10^9$/L) | Hyper-fibrinolysis | Morphology | | Auer rods | Type of cellular differentiation | Chromosome | Immunophenotype | | Treatment with ATRA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Nucleus | Granularity | | | | Positive | Negative | |
| 1 | Our patient | 0.9 | F | 35.4 | 193 | No | Regular | Hypergranular | – | Neurotrophic/ monocytoid | 46, XX | CD13, 15, 33 | CD14, 34, 56, HLA-DR | NE |
| 2 | 12 | 20 | F | 112.85 | 34 | Yes | Irregular | Hypergranular | + | Pure neurotrophic | 47, XX, 2q+, +17[3]/47, idem, del(5)(p15)[11]/46, XX[11] | CD13, 15, 33 | CD14, 34, 56, HLA-DR | Effective |
| 3 | 13 | 49 | F | 38.9 | 28 | NA | Regular | Hypergranular | – | Pure neurotrophic | 46, X, t(X; 17)(q28; q12) | CD33, 56, 117 | CD14, 15, 34, HLA-DR | Not effective |
| 4 | 3,4 | NA | M | 3.3–84 (Median 11.3) | NA | NA | Regular | Hypergranular | ++ | NA | 45, X, –Y, der(7)t(7; 11)(q34; p15)ins(7; 12)(q34.3), der(11)t(7; 11)/46, XY | CD13(3/4), 33(3/4) | CD34(3/4), 56(2/2), HLA-DR(4/4) | NE |
| 5 | | | F | | | | Regular | Hypergranular | – | NA | 46, XX | | | NE |
| 6 | | | M | | | | Regular | Hypergranular | NA | NA | 46, XY | | | Not effective |
| 7 | | | M | | | | Irregular/regular | Hypergranular | ++ | NA | 46, XY | | | NE |
| 8 | | | F | | | | Irregular/regular | Hypergranular | ++ | NA | 45, XX, –2, –12, +13add(17)(q2?) | | | NE |

ATRA, all-*trans*-retinoic acid; F, female; M, male; NA, not available from the references; NE, not evaluable; PLT, platelet count; WBC, white blood cell count.

likely that ATRA therapy will be ineffective in treating APL without an RARα rearrangement. Recently, a patient with APL lacking RARα rearrangement who responded to ATRA was reported.[12] The mechanism of ATRA effectiveness was not discussed in the report, however an extra RARα gene (the trisome 17) in the APL cells might have been involved.

We have presented not only a very rare but also an evocative case report that would suggest important differences between clinical or biological features in typical APL and atypical APL lacking an RARα rearrangement. Table 1 summarizes the biological and clinical characteristics of the few patients lacking an RARα rearrangement previously reported in the literature. APL lacking an RARα rearrangement may possess an alternative mechanism mediating the differentiation block that causes APL. However, it remains possible that such cases could be heterogeneous and some of them could still involve the RARα gene via unknown pathways. Further understanding of this small subgroup of APL patients may suggest new therapeutic strategies.

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# *IDH1* and *IDH2* mutations are rare in pediatric myeloid malignancies

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Recently, recurrent somatic missense mutations in $NADP^+$-dependent isocitrate dehydrogenase gene (*IDH1*) at codon R132, as well as *IDH2* at codon R172, have been identified in low-grade gliomas/secondary glioblastoma by high-throughput sequencing.[1] Subsequent studies also revealed that acquired somatic mutations in *IDH1* frequently occurred in adult hematological malignancies, such as acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS).[2,3] More recently, Paschka *et al.*[4]reported that not only *IDH1* but also *IDH2* mutations occurred relatively frequently in adult AML, and that these mutations were associated with older age, poor prognosis, cytogenetically normal AML (CN-AML) and the genotype of mutated *NPM1* without *FLT3*-internal tandem duplication (ITD). Exon 4 of both *IDH1* and *IDH2*, which was previously identified as a hot spot for mutations in these genes, encodes three arginine residues (R100, R109 and R132 in *IDH1* and R140, R149, and R172 in *IDH2*) that are important for protein activities.[5] Tumor-derived *IDH1* and *IDH2* mutations impair the affinity of enzymes for substrates, and dominantly inhibit wild-type *IDH1* and *IDH2* activities through the formation of catalytically inactive heterodimers.[5] Ho *et al.*[6] previously reported that *IDH1* mutations are not detected in pediatric AML; however, little is known about the incidence and prognostic values of *IDH1* and *IDH2* mutations in pediatric myeloid malignancies. Here, we analyzed mutations that involve the activation sites of *IDH1* and *IDH2* (exon 4 and exon 7 in both *IDH1* and *IDH2*) using genomic DNA-polymerase chain reaction amplification/sequencing in a total of 199 samples of pediatric myeloid malignancies, including 17 AML-derived cell lines, 115 primary cases of AML, 28 primary cases of MDS, 15 primary cases of juvenile myelomonocytic leukemia (JMML), 6 chronic myeloid leukemia (CML)-derived cell lines and 18 primary cases of CML. Moreover, to assess whether *IDH1* and *IDH2* mutations overlap with known gene abnormalities, such as *FLT3*, *c-KIT* and *NPM1* mutations, mutational analyses of *FLT3*, *c-KIT* and *NPM1* were also performed in AML samples. This study was approved by the ethics committee of the University of Tokyo (Approval Number 3043).

The common *IDH2* R140Q mutation was detected in a single AML case, whereas no *IDH1* mutation including G123E, as well as no other *IDH2* mutations, such as R172K, were detected in our study (Figure 1). The *IDH2* R140Q mutation detected in the AML case was a heterozygous substitution. No *IDH1* and *IDH2* mutations were detected in the JMML, MDS or CML samples examined. As the additional activation sites of both *IDH1* and *IDH2* are located in exon 7 of these genes, direct sequencing of exon 7 of *IDH1* and *IDH2* was also performed, but no mutations were detected in our series. Six AML samples including one cell line had *c-KIT* mutations (D816V, N822K and D419fs), and 12 AML samples had *FLT3-ITD*. The *NPM1* mutation was detected in 2 of 132 AML samples. The AML case harboring the *IDH2* mutation, case 39, showed no abnormalities of *NPM1*, *c-KIT* and *FLT3*. Case 39 was a 12-year-old boy diagnosed as AML-M2 according to the French–American–British cooperative group classification system. Bone marrow blasts obtained at initial diagnosis showed t(8;21)(q22;q22). After complete remission was achieved by the ACMP (adriamycin, cytarabine, 6-mercaptopurine, prednisolone) two-step induction therapy, the patient underwent consolidation therapy every 5 weeks, but hematological relapse occurred 11 months after the initial diagnosis. He was treated with low-dose cytarabine, but died 5 months after relapse with progressive disease. To assess the genetic mechanisms involved in the pathogenesis of the disease of this case, we further performed genome-wide copy number analysis of bone marrow blasts obtained at initial diagnosis of this case, using single-nucleotide polymorphism (SNP)-genotype microarrays (Affymetrix GeneChip Mapping 250 K *Styl* arrays,

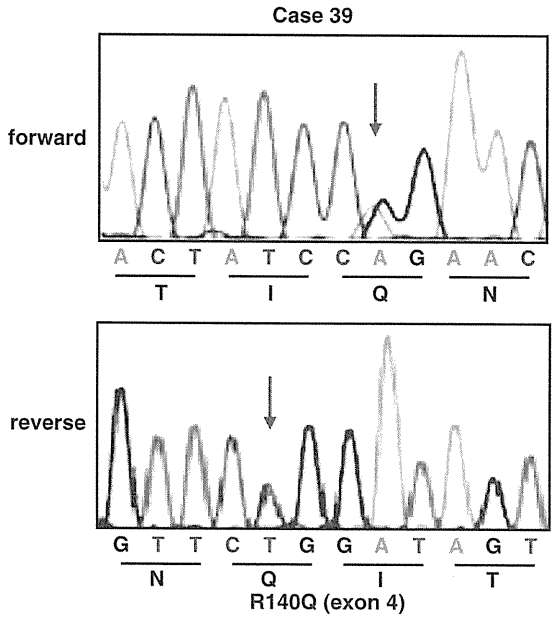


**Figure 1** Sequence chromatogram of the *IDH2* mutation detected in a pediatric AML patient. A heterozygous mutation at R140 in exon 4 of *IDH2* is shown (top and bottom: forward and reverse sequencing results, respectively). Mutated nucleotides are indicated by arrows.

Affymetrix, Inc., Santa Clara, CA, USA). As shown in Figure 2, complex chromosomal abnormalities, such as heterozygous deletions at chromosomes 7q11.2, 7q34-qter, 9q13-q21.33, 9q22.33, 16q23.1-q24.3 and 17q12qter, as well as gains of 4q24.3, 17q12-qter and 22q12.3-q13.33 were detected in leukemic cells of this patient (Figure 2).

To our knowledge, this is the first report to describe the *IDH2* mutation in a pediatric AML patient. In the present study, we detected the *IDH2* R140Q mutation in a single AML case out of 199 samples of pediatric myeloid malignancies, which suggests that the involvement of *IDH1* and *IDH2* mutations in the pathogenesis of pediatric AML is extremely rare compared with those in adult AML cases. Likewise, although *IDH* mutations are frequently observed in adult brain tumors, they are not observed in pediatric cases.[1] Therefore, somatically acquired *IDH1* and *IDH2* mutations may be related to an acquired neoplastic pathway exclusive to adult patients. Several groups have reported that *IDH1* and *IDH2* mutations are significantly associated with a normal karyotype in adult AML.[4,6] However, our patient with an *IDH2* mutation had t(8;21) together with complex chromosomal changes. Furthermore, a previously reported genome-wide study of pediatric AML revealed that, in contrast to our AML patients with *IDH2* mutation, pediatric *de novo* AML was characterized by a very low burden of genomic alterations.[7] These clinical and cytogenetic data suggest that pediatric AML with t(8;21) and *IDH2* mutation might be a specific subtype of AML with complex chromosomal abnormalities and poor prognosis. Thus, our result has important clinical and pathological implications regarding the role of *IDH2* mutations in the development of AML. t(8;21) is considered as a distinct AML subtype associated with characteristic morphology and a favorable prognosis.[8] Although approximately 90% of AML patients with t(8;21) achieve remission, relapse is frequent.[8] Once the disease relapses, the prognosis is poor, with an overall survival of 50% at 5 years.[8] Although the *c-KIT* mutation and *FLT3-ITD* are considered as poor prognostic factors in AML patients with t(8;21), these abnormalities occur in approximately 10% of AML patients with t(8;21).[9] Notably, *IDH1* and *IDH2* mutations constitute a poor prognostic factor in CN-AML with mutated *NPM1* without *FLT3-ITD*, which allows refined risk stratification of this AML subset.[4] Although treatment contents as well as clinical and genetic backgrounds were some of the parameters influencing the patient's outcome, our findings suggest that the *IDH2* mutation may also be related to an inferior outcome in pediatric AML patients with t(8;21) even if they lack the *c-KIT* mutation and *FLT3-ITD*. As *IDH2* mutation with t(8;21) is an extremely rare event and the prognostic values of *IDH2* mutations in AML

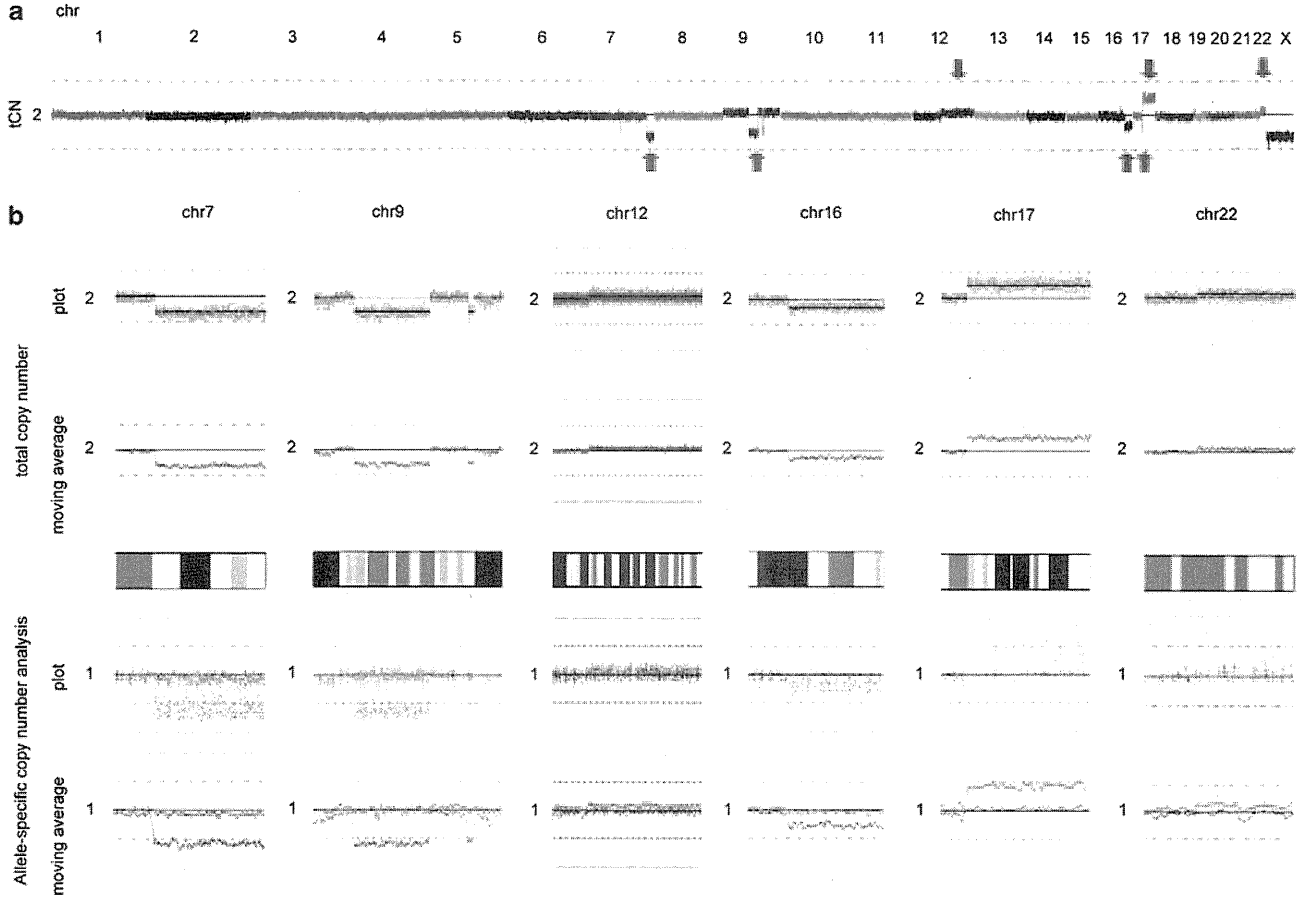


**Figure 2** The result of copy number analysis using SNP-genotyping microarrays. (**a**) The moving average of the total copy number plot is presented. Each chromosome is indicated by different colors. Deletions in the regions at 7q, 9q, 16q and 17q, and gains in the region at 12q, 17q and 22q are indicated by the red arrows. (**b**) Deletions of 7q, 9q, 16q and 17q, and gains of 12q, 17q and 22q. The total copy number plot from each probe (red points) and the moving average (blue line) are shown above the cytobands. The results of the allele-specific analysis with CNAG/AsCNAR are shown below the cytobands. The larger allele is presented in red, and the smaller allele is presented in green. The numbers located at the left edge of each lane indicate a normal copy number (2 for total copy number analysis and 1 for allele-specific copy number analysis).

with t(8;21) are still unclear, further data accumulation is necessary. Although uncommon in pediatric myeloid malignancies, *IDH1* and *IDH2* mutations, particularly *IDH2* mutations, could contribute to the advanced phenotype of AML. Our findings provide additional impetus for investigating the role of *IDH1* and *IDH2* in the pathophysiology of errors of metabolism and in neoplastic disorders.

## Conflict of interest

The authors declare no conflict of interest.

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# *CBL* mutation in childhood therapy-related leukemia

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Therapy-related leukemia and myelodysplastic syndrome (t-Leuk/MDS) are mainly caused by topoisomerase II inhibitors that cause acute myeloid leukemia (AML) with an 11q23 translocation or by alkylating agents that induce MDS/AML with an *AML1* mutation and monosomy 7.[1,2] Two types of t-Leuk/MDS can be distinguished, one of which has a long latency (⩾5–7 years) and is seen following alkylating agents, frequently with an preleukemic phase.[1] The other has a short latency period (1–3 years), no preleukemic phase, and is strongly associated with the administration of topoisomerase II inhibitors and chromosomal abnormalities involving 11q23 translocation/*MLL* rearrangement (*MLL*-R).[2] Repair of etoposide (VP-16)-stabilized DNA topoisomerase II covalent complexes may initiate *MLL*-R observed in patients.[3]

In this regard, recent reports of somatic mutations of the *CBL* proto-oncogene in myeloid neoplasms are intriguing, because

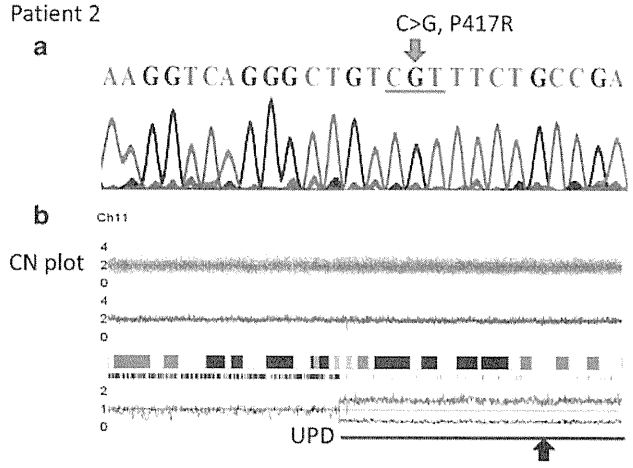


**Figure 1** Identification of acquired isodisomy of 11q and *CBL* mutation in therapy-related leukemia. (**a**) Homozygous mutation of the *CBL* gene was identified in patient 2. (**b**) Copy number (CN) analysis for the gene chip output for therapy-related leukemia in patient 2. Total CNs (red plot) are shown above the cytoband, and the result of allele-specific CN analysis with anonymous references plots are shown below the cytoband. Larger allele is presented in red line, and smaller allele is presented in green line. Allele-specific analysis showed 11q-aUPD (blue line), which contained *CBL* region (black arrow).

these *CBL* mutations were shown to result in aberrant tyrosine kinase signaling, which would lead also to activation of RAS signaling pathways. We and others reported that *CBL* mutations occurred in a variety of myeloid neoplasms, including *de novo* AML,[4] MDS[4] and myeloproliferative neoplasm,[4,5] especially in chronic myelomonocytic leukemia[5] and juvenile myelomonocytic leukemia.[6] The importance of *CBL* mutations concerning about leukemogenesis is substantially increased. This prompted us to search for possible *CBL* mutations in pediatric t-Leuk/MDS.

Analysis of *CBL* gene was carried out in 20 pediatric t-Leuk/MDSs, including 15 AMLs (range: 1 year and 10 months to 17 years; 8 males and 7 females), 4 MDSs (range: 7 years to 14 years; 4 males) and 1 acute lymphoblastic leukemia (4 years and 2 months; 1 male). Median age at diagnosis was 8 years and 1 months (range: 1 year and 10 months to 17 years; 13 males and 7 females). Rearrangements of *MLL* gene were found in 17 patients (85%), including 15 of 16 who received VP-16 (Sugita *et al.*[7]), and 2 of 4 who did not receive it. An initial diagnosis was made as non-Hodgkin's lymphoma in seven patients, neuroblastoma in five, acute lymphoblastic leukemia in five, AML in two and juvenile myelomonocytic leukemia in one.

Because *CBL* mutations thus far reported almost exclusively involved exons 8–9 that encode linker/RING finger domains,[4–6] we confined our mutation analysis to these exons, in which PCR-amplified exons 8–9 were subjected to direct sequencing using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Branchburg, NJ, USA). The study adhered to the principles of the Helsinki declaration, and was conducted under the regulations enacted by the Ethics Board of Gunma Children's Medical Center.

Homozygous mutation of the *CBL* gene was identified in 1 out of the 20 t-Leuk/MDS cases (5%), which were located in the RING finger domain (P417R in patient 2). As the frequency of 11q-acquired uniparental disomy (11q-aUPD) was reported ~85–90% in *CBL* mutations,[4–6] we analyzed his sample using Affymetrix GeneChip 250K *Nsp*I array (Affymetrix, Santa Clara, CA, USA), and found the presence of 11q-aUPD, which was the sole abnormality seen by single-nucleotide polymorphism array (Figure 1), confirming a strong association of *CBL* mutations with 11q-aUPD as previously described.[4–6] Furthermore, we examined *NRAS* and *KRAS* mutations in these patients whose samples were available and found *KRAS* mutation in one patient with t-Leuk (acute monocytic leukemia having t(9;11)(p21;q23) after B-cell precursor acute lymphoblastic leukemia having 6p−, 7q+, 9q+ and 12q−).

*CBL* mutation was detected in MDS cells from the patient with t-MDS after malignant lymphoma. The patient was initially diagnosed as having diffuse large T-cell type malignant lymphoma, whose biopsied specimen of the buccal lymph node showed MT1(+), MB1(−) and UCHL1(+), when he was 5 years old. He subsequently was treated with chemotherapy according to T-8801 protocol including VP-16 (200 mg/m$^2$) given twice weekly,[7] and obtained a complete remission. However, at 7 months after diagnosis, tumor appeared in the right maxilla, and was diagnosed as the relapsed lymphoma, then, he received local irradiation (30 Gy) and chemotherapy including ifosfamide, vincristine, THP-adriamycin and L-asparaginase. At 4 months later, enlarged spleen was resected, and the infiltrated tumor cells were microscopically seen in the tumor sections. At 6 months later, 19 months after initial diagnosis, blast cells appeared in peripheral blood. His laboratory data revealed leukocytosis (14 700/μl with 18% blast cells) and an elevated serum lactate dehydrogenase level (1458 U/l). Bone marrow aspiration revealed 9.8% blasts, which were positive for cytoplasmic myeloperoxidase, suggesting MDS. Surface marker analysis showed that the leukemic blasts in the bone marrow were positive for CD33. Chromosomal analysis of bone marrow cells revealed t(5;11)(q21;q23) in 11 of 20 cells. Rearrangement of *MLL* gene of these cells was identified by Southern blotting, however, no known chimeric mRNA with *MLL*, such as *MLL-AF5q31* and *MLL-GRAF* in t(5;11)(q31;q23), could be detected. These suggested that the gene at 5q21 was a novel partner gene of *MLL*. Although another chemotherapy for AML was performed, his blast cells increased >30% blasts in bone marrow at 25 months after initial diagnosis. Therefore, he was diagnosed as having t-Leuk resembling acute monoblastic leukemia due to VP-16. He died of mycotic infection at 35 months after initial diagnosis.

No *CBL* mutations were found in his lymphoma sample at diagnosis and in tumor cells in the enlarged spleen. We also performed tissue-fluorescence *in situ* hybridization analysis with *MLL* probe on paraffin-embedded tissue sections of the tumor cells in the enlarged spleen, however, no evaluable results could be detected because of poor quality of samples. No initial samples for tissue-fluorescence *in situ* hybridization analysis could be obtained.

The 11q23 translocation/*MLL*-R in t-Leuk/MDS was considered to be induced by VP-16,[3] however, gene alterations in addition to *MLL*-R have rarely reported. Recently, *CBL* mutations were found in a variety of myeloid neoplasms.[4–6] Among 2000 samples from the patients with myeloid neoplasms, *CBL* mutations have been found in ~5% samples, including AML transformed from MDS, but not *de novo* or therapy-related acute leukemia with 11q23 translocation/*MLL*-R. To our knowledge, this is the first t-Leuk/MDS patient with 11q23 translocation/*MLL*-R and *CBL* mutation. Interestingly, a *de novo* AML case with *MLL–CBL* fusion gene has also been reported.[8] These findings suggest that alterations of *CBL* gene and 11q23 translocation/*MLL*-R may cooperate in the pathogenesis of a subtype of t-Leuk/MDS and *de novo* leukemia.

## Conflict of interest

The authors declare no conflict of interest.

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# Total body irradiation and melphalan as a conditioning regimen for children with hematological malignancies undergoing transplantation with stem cells from HLA-identical related donors

Watanabe N, Takahashi Y, Matsumoto K, Horikoshi Y, Hama A, Muramatsu H, Yoshida N, Yagasaki H, Kudo K, Horibe K, Kato K, Kojima S. Total body irradiation and melphalan as a conditioning regimen for children with hematological malignancies undergoing transplantation with stem cells from HLA-identical related donors. Pediatr Transplantation 2011: 15: 642–649. © 2011 John Wiley & Sons A/S.

Abstract: Although some studies have reported that TBI and MEL offer an effective conditioning regimen for autologous SCT in acute leukemia, little has been reported regarding outcomes of allogeneic SCT. We retrospectively evaluated outcomes for 50 pediatric patients who underwent allo-SCT conditioned with intravenous MEL (180–210 mg/m$^2$) and fractionated TBI (12–13.2 Gy) from HLA-identical related donors. Nineteen patients were in CR1, 18 were in CR2, and 13 showed advanced-stage disease (≥CR3). Patients had received allo-SCT from HLA-identical siblings (n = 45) or phenotypically HLA-identical family donors (n = 5). Median duration of follow-up for all disease-free patients was 61 months (range, 8.8–177 months). At the time of analysis, 12 patients had died. Eleven of those died of relapse, and one died of TRM. DFS rates for all patients, patients with AML (n = 12), and patients with lymphoid malignancy (n = 38) were 61.4% and 82.1%, respectively. DFS rates for CR1, CR2, and ≥CR3 cases were 89.2%, 88.1%, and 23.1%, respectively ($p < 0.05$). MEL/TBI for pediatric patients with hematological malignancies was associated with lower relapse rates and no increase in toxicity, resulting in better survival.

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Key words: pediatrics – allogeneic stem cell transplantation – melphalan – hematological malignancies – preconditioning regimen – total body irradiation

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Abbreviations: ABL, acute biphenotypic leukemia; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CMV, cytomegalovirus; CNS, central nervous system; CR, complete remission; CR1, first complete remission; CR2, second remission; CsA, cyclosporine A; CY, cyclophosphamide; DFS, disease-free survival; G-CSF, granulocyte colony-stimulating factor; GVHD, graft-versus-host disease; ICU, intensive care unit; MEL, melphalan; MTX, methotrexate; NCI-CTC, National Cancer Institute Common Toxicity Criteria; SCT, stem cell transplantation; TAM, transplantation-associated microangiopathy; TBI, total body irradiation; TRM, transplant-related mortality; VOD, veno-occlusive disease.

Although various combinations of cytotoxic drugs have been proposed as conditioning regimens for various hematological malignancies (1–5), none of those proposals have been based on randomized studies. Furthermore, documented superiority over standard regimens such as high-dose CY (60 mg/kg/day for two consecutive days) and TBI has yet to be shown. MEL is an alkylating agent with non-cell cycle–specific activity that can further increase the killing of leukemic stem cells (6). Although MEL is not currently included as a part of first- or second-line chemotherapy protocols for childhood

leukemias, adding this alkylating agent to a TBI-based conditioning regimen could overcome previously acquired drug resistances of leukemic stem cells. Indeed, the relapse rate after marrow transplantation is reduced in patients with AML administered TBI and MEL compared with those treated using TBI and CY, but the benefit was offset by the probable toxicity of MEL (7). However, that report included results from pediatric and adult patients together, causing difficulties with defining outcomes in pediatric patients. Conversely, other studies have favored high-dose MEL for autologous bone marrow transplantation in children with acute leukemia (8–10). Little information has been reported regarding the outcomes of allogeneic transplantation for pediatric patients with hematological malignancies using MEL/TBI as a conditioning regimen. We evaluated the effect and safety of a MEL/TBI regimen for 50 children who received allogeneic SCT for hematological malignancies from HLA-identical related donors.

## Patients and methods

### Patient characteristics

The 50 pediatric patients included in this analysis received treatment between September 1988 and December 2009 at four transplant units and were followed up until September 1, 2010. Patients included 32, 12, 3, and three patients with ALL, AML, ABL, and malignant lymphoma, respectively. All patients received HLA-matched related donor grafts, as determined by serological HLA-A, HLA-B, or HLA-DR typing. Before transplantation, 19 patients (ALL, n = 9; AML, n = 8; ABL, n = 2) were in CR1, 18 (ALL, n = 14; AML, n = 2; lymphoma, n = 2) were in CR2, and 13 (ALL, n = 9; AML, n = 2; ABL, n = 1; lymphoma, n = 1) showed advanced-stage disease (≥CR3). All patients with CR1 in acute leukemia had either unfavorable chromosomal abnormalities, that is, t(9;22) translocation, corticosteroid resistance ($>1 \times 10^9$/L blasts in peripheral blood after seven days of prednisone administration in patients with ALL), or lack of remission by the end of the first induction phase. Median duration between diagnosis and transplant was 16.4 months (range, 4.4–157.5 months), and median duration of CR1 was 31 months (range, 1.1–80.6 months) for the 18 patients receiving SCT with CR2. Seventeen of the 18 patients with CR2 experienced an isolated or combined medullary relapse, and one patient experienced isolated extramedullary relapse. Informed consent to undergo the transplant procedure was obtained according to the requirements of the ethics committees of the four transplant units. Table 1 shows the clinical characteristics of the 50 patients.

### Conditioning regimen

All patients received MEL/TBI. This regimen consisted of MEL administered to 37 patients at a dose of 60 mg/m$^2$ or to 13 patients at a dose of 70 mg/m$^2$ once intravenously on days −6 and −4 (total dose, 180–210 mg/m$^2$) combined with fractionated TBI at 2–2.2 Gy twice daily on days −3 to −1 (total dose, 12–13.2 Gy). The variable doses of MEL and TBI were because of the decisions of the individual physicians. Half-value lung shields were used throughout irradiation. In seven patients who had developed CNS infiltration at some stage before transplantation, CNS boosts (2 Gy) were administered as part of the irradiation regimen, and all but six of the 35 male patients received a booster dose (4 Gy) to the testicle in one or two fractions. GVHD prophylaxis consisted of MTX only (15 mg/m$^2$ on day +1, 10 mg/m$^2$ on days +3, +6, and +11, and weekly thereafter until day +60) in 29 of 45 patients who received stem cells from HLA-identical sibling donors. Fifteen patients with HLA-identical sibling donors and three patients with a phenotypically HLA-identical family donor received CsA combined with a short course of MTX (15 mg/m$^2$ on day +1 and 10 mg/m$^2$ on days +3, +6, and +11). CsA was started on day −1 before transplant and administered intravenously at 0.5–1.5 mg/kg every 12 h. One patient with an HLA-identical sibling donor and two patients with a phenotypically HLA-identical family donor were continuously infused with tacrolimus (0.03 mg/kg/day) from day −1 and received a short course of MTX.

Table 1. Patient characteristics

| | |
|---|---|
| Total number of patients | 50 |
| Sex (male/female) | 35/15 |
| Median, yr (range) | 8 (2–18) |
| Date of SCT | |
| 1988–1999 | 23 |
| 2000–2008 | 27 |
| Diagnosis and remission status | |
| ALL | 32 |
| 1CR | 9 |
| 2CR | 14 |
| ≥3CR | 9 |
| AML | 12 |
| 1CR | 8 |
| 2CR | 2 |
| ≥CR3 | 2 |
| ABL | 3 |
| CR1 | 2 |
| ≥CR3 | 1 |
| Lymphoma | 3 |
| CR2 | 2 |
| ≥CR3 | 1 |
| Median duration between diagnosis and transplant (months) | 16.4 (4.4–157.5) |
| Stem cell source | |
| BM | 46 |
| PB | 4 |
| Nucleated cell dose (×10$^8$/kg) | 3.67 (0.92–35.2) |
| MEL dose | |
| 180 mg/m$^2$ | 37 |
| 210 mg/m$^2$ | 13 |
| TBI | |
| 12 Gy | 44 |
| 13.2 Gy | 6 |
| Donor type | |
| HLA identical sibling | 45 |
| Phenotypically HLA identical family donor | 5 |
| GVHD prophylaxis | |
| Cyclosporine + MTX | 18 |
| Tacrolimus + MTX | 3 |
| MTX alone | 29 |

BM, bone marrow; PB, peripheral blood.

### Supportive care

Transplants were performed in laminar airflow rooms. In 38 patients with ALL, ABL, and lymphoma, G-CSF was administered at 5 μg/kg/day from day +5 until engraftment and was not routinely applied to patients with AML. A standard regimen of antibiotic prophylaxis was administered to prevent bacterial, viral, fungal, and *Pneumocystis jiroveci* infections. Supportive treatments including total parenteral nutrition, blood product infusion, and broad-spectrum antibiotics for febrile neutropenia were administered according to the standard care protocols at each institution. Practices for CMV diagnosis were based on testing for CMV antigen in the blood twice weekly between engraftment and day 100 post-SCT, and preemptive treatment with ganciclovir was administered.

### Definitions and statistical analysis

Neutrophil and platelet engraftments were defined as the first of three consecutive days with a neutrophil count $>0.5 \times 10^9$/L and a platelet count $>20 \times 10^9$/L, respectively, regardless of platelet transfusion.

Acute and chronic GVHDs were classified according to standard criteria. Children with sustained donor engraftment who survived >14 and >100 days after transplantation were evaluated for the occurrence and severity of acute and chronic GVHD, respectively.

Toxicity related to the transplantation procedure was graded according to the NCI-CTC version 3.0.

DFS was calculated from the first day of transplant to the time of analysis or the first event. Kaplan–Meier product-limit estimates with a 95% confidence interval calculated from standard errors were used to estimate the probabilities of relapse and DFS. The log-rank procedure was used to assess the statistical significance of differences between subgroups of children with respect to DFS and probability of relapse. All statistical tests were two sided, and differences were considered statistically significant for values of $p < 0.05$. All data were statistically analyzed using SPSS software (SPSS, Chicago, IL, USA).

## Results

### Engraftment

All patients achieved durable engraftment. The median time to achieve neutrophil recovery was 15 days (range, 10–50 days), whereas the median time for a self-sustained platelet count $>20 \times 10^9$/L was 26 days (range, 12–115 days). Neutrophil counts recovered faster among patients who received G-CSF than among those who did not receive hematopoietic support (data not shown). None of the recipients developed secondary graft failure.

### Regimen-related toxicity

Table 2 shows a summary of organ toxicity and GVHD. All patients experienced profound granulocytopenia and thrombocytopenia immediately after transplantation, and febrile episodes were also common during the neutropenic period. Most patients responded to appropriate broad-spectrum antibiotic therapy and became afebrile, usually after granulocyte recovery. NCI grade ≥3 non-hematological organ toxicity developed in 35 (70%) patients. Although mucositis and diarrhea were frequent, occurring in 23 (46%) and 14 (28%) patients, respectively, these toxicities improved spontaneously. Two (4%) patients developed VOD, while TAM occurred in one (2%) patient. Reversible hemorrhagic cystitis (NCI grade ≥3) developed in two patients. Five patients showed idiopathic interstitial pneumonitis, resolving with steroid therapy in three patients, but developing to bronchiolitis obliterans thereafter in the other two patients. One patient was admitted to the ICU for six days because of VOD. The condition of this patient improved with intensive care, and he was alive as of the last follow-up. Although one boy developed osteosarcoma four yr after transplantation, he remained alive and disease-free as of the last follow-up, after being treated with multiagent chemotherapy and aggressive surgery.

Table 2. Organ toxicities and GVHD

| | n (%) |
|---|---|
| Organ toxicity* | |
| Any grade 3 toxicity | 35 (70) |
| Mucositis | 23 (46) |
| Diarrhea | 14 (28) |
| Bladder | 2 (4) |
| Pulmonary | 5 (10) |
| Second solid cancer | 1 (2) |
| VOD | 2 (4) |
| TAM | 1 (2) |
| Death because of organ toxicity | 1 (2) |
| GVHD | |
| Acute GVHD total | 20 (40) |
| Acute GVHD grade II–IV | 12 (24) |
| Chronic GVHD total | 15 (30) |
| Chronic GVHD extensive | 11 (22) |
| Death because of GVHD | 1 (2) |

*Toxicity graded using NCI-CTC. Only patients with grade 3 toxicity are presented. Some patients had toxicity in more than one organ system.

### GVHD

Table 2 shows details regarding acute and chronic GVHD incidence. Total and grade II–III acute GVHD occurred in 20 and 12 patients, with cumulative incidences of 40% and 24%, respectively. No patients developed grade IV acute GVHD. Fifteen (30%) patients developed chronic GVHD, and eight developed both acute and chronic GVHD. Four patients developed limited skin chronic GVHD, and 11 developed the extensive form of the disease. Seven of the 15 patients with chronic GVHD received MTX alone as GVHD prophylaxis, five patients

received low-dose CsA (1.0 mg/kg/day), and the remaining two patients received peripheral blood SCT. One death was related to chronic GVHD because of bronchiolitis obliterans, and the cumulative incidence of TRM was 2% as of the last follow-up.

### Survival

Thirty-eight of the 50 patients remained alive and were in CR at a median follow-up of 88 months (range, 12–177 months). The actuarial DFS rate in all patients was 69.7% (range, 60.8–78.8%) (Fig. 1). The actuarial DFS rate in patients with AML, with a median follow-up period of 65.6 months (range, 36.8–94.6 months), was 61.4% (range, 46.1–76.7%) (Fig. 2). The actuarial DFS rate in patients with lymphoid malignancies (including ALL, ABL, and lymphoma), with a median follow-up period of 143.9 months (range, 123.5–164.0 months), was 82.1% (range, 76.0–88.2%) (Fig. 2). Actuarial DFS rates for patients in CR1 and CR2 were 89.2% (range, 82.2–95.4%) and 88.1% (range, 80.2–96.0%), respectively, and the DFS rate in patients at the advanced stage (≥CR3) was 23.1% (range, 5.4–140.8%) (Fig. 3). This difference was statistically significant ($p < 0.05$). Eleven patients experienced relapse between 1.8 and 100.2 months after transplantation. Only 4 (10.8%) of 37 patients in the CR1 and CR2 groups experienced relapse. However, 7 (53.8%) of the 13 patients in the advanced group relapsed. Cause of death was relapse in all non-CR patients.

## Discussion

Although the MEL/TBI regimen has been confirmed as an effective antileukemia treatment for pediatric patients with hematological malignancies who undergo autologous bone marrow transplantation (8–10), few reports have described outcomes after allogeneic SCT using a preconditioning MEL/TBI regimen. This study retrospectively analyzed the safety and efficacy of a MEL/TBI regimen for preconditioning in patients with pediatric hematological malignancies who underwent allo-SCT. As MEL elicits a dose–response effect in high-dose regimens for relapsed AML and ALL (11), our patients received a higher dose (180–210 mg/m$^2$) of MEL than that previously described (4, 12, 13). Although grade 3 mucositis, diarrhea, and febrile episodes were common in the neutropenic period, frequencies of severe GVHD and TRM were not increased compared with those in previous studies that adopted other regimens (14, 15). This reason is that all patients in our study received transplantation from HLA-identical related donors. As Vettenranta et al. (16) reported, allograft from an unrelated donor might accentuate the gastrointestinal toxicity associated with MEL and TBI.

Various studies have found that the DFS after HLA-identical related donor transplantation in

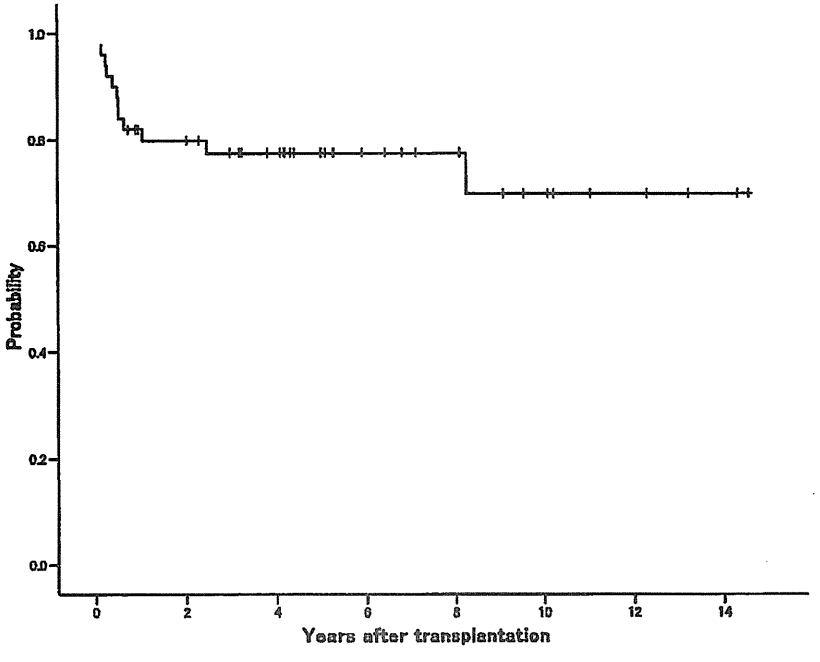


*Fig. 1.* Actuarial DFS rates for all patients. Median duration of follow-up for all patients was 88 months (range, 12–177 months).

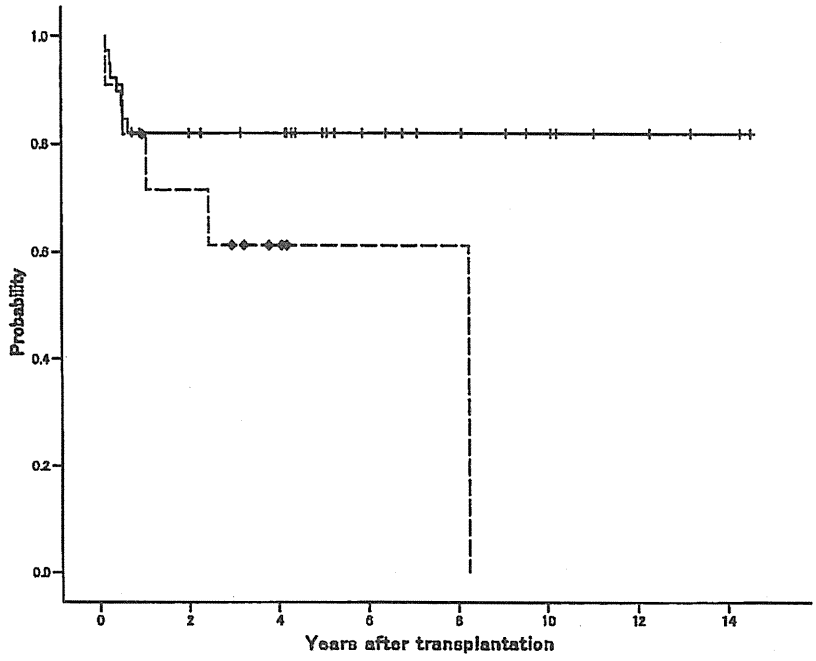


*Fig. 2.* Actuarial DFS rates for patients with lymphoid malignancies (—) vs. AML (- - - -).

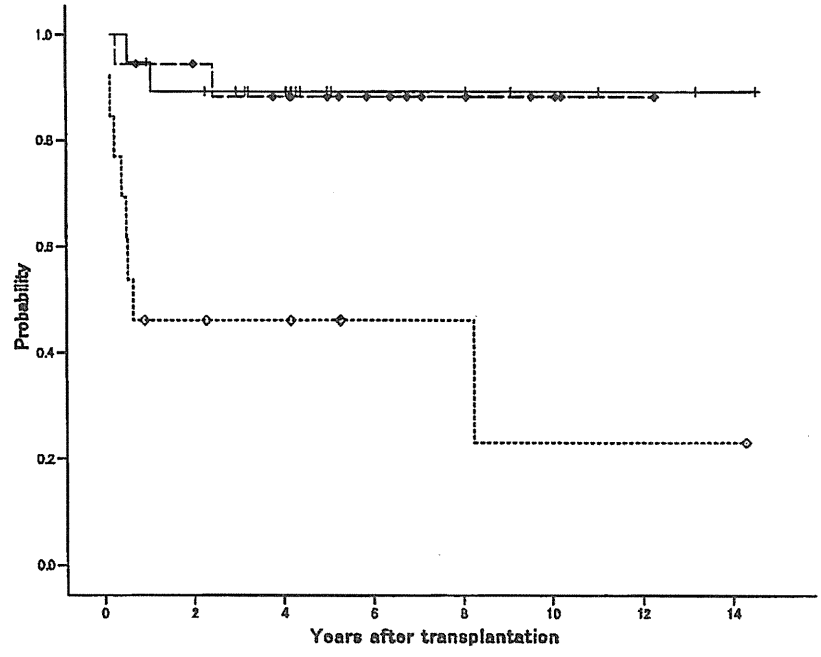


*Fig. 3.* Actuarial DFS rates for patients in CR1 (—) vs. CR2 (- - - -) vs. advanced disease (≥CR3) (.....).

children with acute leukemia in CR1 or CR2 ranges from 30% to 70% (17–21). Despite the apparent lack of randomized studies looking at different preparative regimens in children, some studies of adult patients have been described (22–24). Table 3 shows the outcomes of various preparative regimens. In our study, actuarial DFS was favorable for patients who received a transplant at CR1 and CR2. DFS rates for patients at CR1 and CR2 were 89.2% and 88.1%, respectively. Relapse rates in CR1 and CR2 were thus much lower than in previous reports (14, 15), suggesting that this conditioning regimen exerts powerful antileukemic effects.