High event-free survival rate with minimum-dose-anthracycline treatment in childhood acute promyelocytic leukaemia: a nationwide prospective study by the Japanese Paediatric Leukaemia/Lymphoma Study Group

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We evaluated the efficacy of treatment using reduced cumulative doses of anthracyclines in children with acute promyelocytic leukaemia (APL) in the Japanese Paediatric Leukaemia/Lymphoma Study Group AML-P05 study. All patients received two and three subsequent courses of induction and consolidation chemotherapy respectively, consisting of all-trans retinoic acid (ATRA), cytarabine and anthracyclines, followed by maintenance therapy with ATRA. Notably, a single administration of anthracyclines was introduced in the second induction and all consolidation therapies to minimize total doses of anthracycline. The 3-year event-free (EFS) and overall survival rates for 43 eligible children were 83.6% [95% confidence interval (CI): 68.1–91.8%] and 90.7% (95% CI: 77.1–96.4%), respectively. Although two patients died of intracranial haemorrhage or infection during induction phases, no cardiac adverse events or treatment-related deaths were observed during subsequent phases. Patients not displaying M1 marrow after the first induction therapy, or those under 5 years of age at diagnosis, showed inferior outcomes (3-year EFS rate; 33.3% (95% CI: 19.3–67.6%) and 54.6% (95% CI: 22.9–78.0%), respectively). In conclusion, a single administration of anthracycline during each consolidation phase was sufficient for treating childhood APL. In younger children, however, conventional ATRA and chemotherapy may be insufficient so that alternative therapies should be considered.

Keywords: acute promyelocytic leukaemia, anthracyclines, childhood leukaemia, clinical trial, recombinant human soluble thrombomodulin.

© 2016 John Wiley & Sons Ltd
British Journal of Haematology, 2016, 174, 437–443
First published online 31 March 2016
doi: 10.1111/bjh.14068
The prognosis of patients with acute promyelocytic leukaemia (APL), a rare subtype of acute myeloid leukaemia, has improved with the introduction of all-trans retinoic acid (ATRA) for both adults and children. In paediatric APL, the event-free (EFS) and overall survival (OS) rates were reported as around 80% and 90% respectively, in recent clinical trials consisting of ATRA and anthracycline-based chemotherapy [de Botton et al, 2004; Ortega et al, 2005; Testi et al, 2005; Sanz et al, 2009; Creuzig et al, 2010; Imaizumi et al, 2011]. However, anthracycline-induced long-term cardiac toxicity could be problematic, especially in paediatric patients. Therefore, reduction of the cumulative doses of anthracyclines without compromising treatment outcome is one of the major issues in the treatment of childhood APL.

In a previous Japanese study, designated AML99-M3, combinations of ATRA, cytarabine (Ara-C) and anthracyclines were used in a total of seven courses of remission induction and consolidation therapies [Imaizumi et al, 2011]. Among the 58 patients enrolled in this study, 7-year OS and EFS rates of 93.1% and 91.4% respectively, were achieved. However, severe bacterial infections occurred in about 5–10% of cases in each consolidation course, requiring the modification of treatment for some patients. Accordingly, on the basis of high survival rates obtained from AML99-M3 study, the Japanese Paediatric Leukaemia/Lymphoma Study Group (JPLSG) conducted a nationwide, non-randomized prospective study, AML-P05, to evaluate the efficacy of treatment using reduced intensity consolidation therapy, as well as minimizing cumulative doses of anthracyclines, for children with APL.

Materials & methods

Patients

Between April 2006 and March 2011, 46 children with newly diagnosed APL were enrolled in the AML-P05 study. The eligibility criteria of this study were as follows: (i) A diagnosis of APL and t(15;17)(q22;q12)/PML-RARA or other RARA variants confirmed by either karyotyping or by fluorescence in situ hybridization (FISH), (ii) age less than 18 years, (iii) an Eastern Cooperative Oncology Group (ECOG) performance status score of 0–2, or of 3 when leukaemia-related, (iv) no history of previous chemotherapy or radiation therapy, (v) sufficient hepatic, renal, and cardiac function, and (vi) written informed consent obtained from guardians. Patients with central nervous system haemorrhage, which is likely to interfere with protocol therapy, or patients with t (11;17)(q23;q21)/PLZF-RARA were excluded from the study. This trial is registered with the UMIN Clinical Trials Registry (UMIN-CTR, URL: http://www.umin.ac.jp/ctr/index.htm), number UMIN000000645.

Treatment

Treatment details of this study are shown in Table SI. Briefly, all patients received at least 3 d of ATRA monotherapy (45 mg/m² per day for 35 d in total), followed by chemotherapeutic agents, consisting of Ara-C (200 mg/m² per day for 7 d) and daunorubicin (DNR, 45 mg/m² per day for 3 d), as the first induction therapy. The starting day for Ara-C and DNR administration varied according to the daily
white blood cell (WBC) count to minimize the development of disseminated intravascular coagulation (DIC). Briefly, for patients with a WBC count higher than $20 \times 10^9/\text{l}$, either at diagnosis or after the initiation of 3-day ATRA monotherapy, Ara-C and DNR therapy was commenced on day 4. For patients whose WBC counts exceeded $20 \times 10^9/\text{l}$ from days 4 to 7, Ara-C and DNR were commenced on the day the high WBC count was identified. For patients whose WBC counts did not exceed $20 \times 10^9/\text{l}$ during the initial 7 d of ATRA monotherapy, Ara-C and DNR was commenced on day 8. To prevent APL differentiation syndrome (APL-DS), dexamethasone ($0.2 \text{ mg/kg, bid}$) was administered to patients whose WBC count exceeded $5 \times 10^9/\text{l}$. For young children who could not swallow ATRA capsules, ATRA was administered by dissolving capsules in warm milk.

The second induction therapy and subsequent three courses of consolidation therapy consisted of ATRA, either a high- or intermediate-dose of Ara-C, triple intrathecal injection (TIT), and a single dose of anthracycline; 10 mg/m$^2$ of mitoxantrone (MIT) in the first two courses and 45 mg/m$^2$ of pirarubicin (tetraydropryanyl-doxorubicin, THP) in the last two courses. THP is a doxorubicin (DXR) derivative manufactured in Japan and is considered to be less cardiotoxic [Shimomura et al, 2011]. Finally, patients received 1-year intermittent maintenance therapy with ATRA for 15 d every 3 months. Consequently, the cumulative anthracycline dose in this study was converted to 246 mg/m$^2$ of DXR; 1:0:83 for DNR, 1:4 for MIT and 1:0:6 for THP according to the JPLSG criteria of anthracycline equivalents, drafted on the basis of previous reports [Ewer et al, 2003; Sakata-Yanagimoto et al, 2004] with slight modifications.

The decision to use recombinant human soluble thrombomodulin-alpha (rTM) during induction therapy, which was approved in Japan for the treatment of DIC in 2008, was at the discretion of each institutional physician.

Definitions and statistics

The initial diagnosis of APL was evaluated by central review (morphology, flow cytometry and karyotypes). The morphological treatment response was also evaluated by central review, and was defined as follows: M1 marrow, $<5\%$ blasts; M2 marrow, $\geq 5\%$ and $<25\%$ blasts; M3 marrow, $\geq 25\%$ blasts; complete remission (CR), M1 marrow with regeneration of normal haematoopoiesis and no leukaemia-related symptoms or extramedullary leukaemic invasion observed. CR was estimated after the completion of two courses of induction therapy. DIC was diagnosed according to Japanese Ministry of Health, Labour and Welfare Diagnostic Criteria [Kobayashi et al, 1983]. APL-DS was defined as the presence of at least four of the following seven signs: unexplained fever, respiratory distress, body weight gain ($>$10\%), pulmonary infiltration, pleural or pericardial effusion, hypotension, or renal failure.

The OS rate was defined as the length of time from the diagnosis of APL to death from any cause. The EFS rate was defined as the length of time from diagnosis to the last follow-up or first event: failure to achieve CR, relapse, secondary malignancy, or death from any cause. OS and EFS rates were analysed using the Kaplan–Meier method.

All data analyses were performed using STATA version 13.0 (StataCorp, College Station, Texas, USA), estimated by the log-rank test and considered to be significant when a $P$ value was $<0.05$. Follow-up data were actualized as of 31 March 2014.

Results

Patient characteristics

Of the 46 patients registered in the AML-P05 study, three were excluded because of PML-RARA negativity. The remaining 43 patients, including two with molecular variants of $t(11;17)(q13;q12)/\text{NUMA-RARA}$ and $t(4;17)(q12;q21)/\text{FIP1L1-RARA}$, were evaluated. The relevant initial clinical and haematological data of the 43 patients are shown in Table SI. The median follow-up period was 4:47 years (0:00–7:45 years). The median age at diagnosis was 9 years (range, 11 months–16 years old); 11 patients (25\%) were less than 5 years of age at diagnosis. No patients were diagnosed as having the microgranular variant (M3v) according to the French American British (FAB) classification. Fms-like tyrosine kinase 3 ($\text{FLT3}$)-like internal tandem duplication (ITD) and -kinase domain mutations (D835) were detected in 15\% (6 out of 40) and 15\% (5 out of 34) of patients examined, respectively. No patients exhibited concurrent ITD and D835 mutations.

Treatment outcomes

Among the 43 patients enrolled in the study, three patients exhibited DIC [7-0\%, 95\% confidence interval (CI): 1-5–19-1\%] during the first course of induction therapy, and one died due to intracranial and pulmonary haemorrhages. Two patients developed APL-DS (4-7\%, 95\% CI: 0-6–15-8\%) but this resolved with the temporary cessation of ATRA and supportive therapies. Six out of 42 patients (14-3\%) did not achieve M1 marrow after the completion of the first course of induction therapy; three of these successfully achieved CR after the second course. One patient died of sepsis in the second induction therapy. Consequently, two patients died during induction phases and three patients did not achieve CR, even after two courses of induction therapy. After excluding one patient whose bone marrow specimen was not sent to the central laboratory, the overall CR rate was 85-7\% (36/42, 95\% CI: 71-5–94-6\%). One patient, although having achieved CR, ceased the protocol study in accordance with their physician’s decision after contracting severe bacterial infections during the induction phases.
In every patient, grade 4 leucopenia and neutropenia, as evaluated by the Common Terminology Criteria of Adverse Events, version 3.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf), as well as grade 3–4 anaemia and thrombocytopenia were observed during almost all induction and consolidation phases. Febrile neutropenia and grade 4 infection were not documented during each consolidation course. Neither adverse cardiac events, nor treatment-related deaths, were observed during remission.

Three patients relapsed in the bone marrow during or after maintenance therapy, and one patient developed secondary acute myeloid leukaemia. Consequently, the 3-year EFS and OS rates were 83.6% (95% CI: 68.6–91.8%) and 90.7% (95% CI: 77.1–96.4%), respectively (Fig 1). The OS rate was equivalent to the preceding Japanese childhood APL study, AML99-M3, although the EFS rate in this study was slightly lower than that in AML99-M3 (83.6% vs. 91.4%; P = 0.027). Two patients with molecular variants are alive in continuous CR.

Recombinant thrombomodulin treatment

Recombinant TM was used to treat DIC in six patients (rTM+ group). In these patients, the length of time required for a normalization of the DIC score was shortened by about 3 d compared to patients who did not receive rTM (13.0 vs. 16.3 d for rTM+ and rTM- groups respectively), although this difference was not significant. Adverse haemorrhagic events were not documented for the rTM+ group.

Prognostic factors

Table I displays clinical and haematological subgroups and their outcomes in this study. A high WBC count (>10 × 10⁹/l), low platelet count (<40 × 10⁹/l), additional chromosomal abnormality and type of PML-RARA fusion did not influence the risk of events. Regarding the mutational status of the FLT3 gene, neither ITD nor D835 correlated with outcome.

In contrast, patients who did not achieve M1 marrow after the first induction therapy experienced a significantly inferior outcome [3-year EFS rate; 33.3% (95% CI: 19.3–67.6%) for M2/M3 marrow, 94.3% (95% CI: 79.0–98.5%) for M1 marrow, P < 0.001]. Patients less than 5 years of age at diagnosis also had a significantly lower 3-year EFS rate compared to that of older children [54.6% (95% CI: 22.9–78.0%) and 93.8% (95% CI: 77.3–96.8%) respectively, P = 0.005; Fig 2A]. Figure 2B shows the age distribution and type of events in this cohort. The cohort could be divided into two age groups at 4 years of age, with most haematological events (induction failure and relapse) occurring in the younger age group. Table SIII lists the characteristics and outcomes of five patients less than 5 years of age and haematological events. The presence of known prognostic factors, such as platelet count, obesity, type of PML-RARA, additional chromosomal abnormalities, and FLT3 mutations, seemed unlikely in these cases, except for a trend of hyperleucocytosis. Regarding salvage treatment, four out of five patients received arsenic trioxide (ATO), followed by stem cell transplantation in two patients. One patient died due to a transplantation-associated adverse event, and the other three patients are alive in continuous CR. Eventually, the OS rate did not differ significantly between the two age groups [3-year OS; 81.8% (95% CI: 44.7–95.1%) and 93.8% (95% CI: 77.3–98.4%), P = 0.271], indicating the efficacy of salvage treatment consisting of ATO.

Outcome by minimal residual disease

The significance of minimal residual disease (MRD) was evaluated by measuring PML-RARA fusion transcripts using
real-time quantitative polymerase chain reaction. However, because MRD was analysed as an add-on study, therefore, at most, only 20 samples were available for each time point. MRD was positive in 13 of 20 patients at the end of the first induction therapy and did not influence outcomes. At the end of the second induction therapy, two of 19 patients were MRD-positive; one had not obtained morphological CR at this time point and the other was in continuous CR. No patients exhibited MRD-positivity, either at the end of consolidation or maintenance therapy, including one patient who eventually relapsed in bone marrow 5 years after the completion of treatment. Unfortunately, MRD could not be monitored in another two patients who experienced relapse.

Discussion

The prognosis of patients with APL has improved with the introduction of ATRA combined with anthracyclines. However, a dose-dependent risk of long-term cardiac toxicity is sometimes problematic, especially in paediatric patients. In
one study, children who received more than 300 mg/m² of cumulative doses of anthracyclines had more than a 10-fold increased risk of chronic heart failure compared to those who received less than 300 mg/m² [Kremer et al, 2009]. According to the JPLSG criteria of anthracycline equivalents, the cumulative anthracycline dose in this study was converted to 246 mg/m² of DXR. The dose was much lower than that used in recent US or European studies, especially those designed primarily for adult APL patients for whom more than 600 mg/m² of anthracyclines were used (Table SIV). We were able to achieve similar results to these studies with minimal doses of anthracyclines (3-year EFS and OS rates were 83-6% and 90-7%, respectively). Therefore, a single administration of anthracyline in each consolidation phase seemed sufficient in the treatment of childhood APL.

DIC and APL-DS are the major life-threatening complications that develop during remission induction therapy in APL [Sanz et al, 2009]. In this study, we introduced at least 3 d of ATRA monotherapy to minimize the development of DIC. Grade 4 DIC was observed in three cases [7-0% (95% CI: 1-5–19-1%)] and was fatal in only one, which seems a lower rate than those reported in previous studies [Testi et al, 2005; Creuzig et al, 2010; Imaizumi et al, 2011] although the differences were not significant. ATRA mono-therapy may induce hyperleucocytosis leading to APL-DS. Prophylactic use of dexamethasone successfully prevented this condition; only two cases developed APL-DS, and this resolved immediately with the temporary cessation of ATRA and supportive therapies.

The control of DIC is still an issue for the initial management of APL even in the ATRA era. Recombinant TM ameliorates DIC by inhibiting the coagulation pathway through the activation of protein C. However, because activated protein C also inhibits plasminogen activator inhibitor-1 [Neyrick et al, 2009], rTM may facilitate the fibrinolytic pathway and cause severe haemorrhagic complications in cases of DIC triggered by APL. In a retrospective study of adult patients with APL [Matsushita et al, 2014], early rTM treatment resulted in a reduction of haemorrhagic complications, with the authors concluding that rTM had a beneficial effect. In our study of childhood APL, the duration of DIC in six patients who received rTM was shortened by about 3 d compared to that in patients who did not receive rTM, although the difference was not significant because of the limited number of cases in the rTM+ group. However, it is notable that there was no severe haemorrhage or death in the rTM+ group. This suggests that rTM may be safely used for the management of childhood APL-related DIC.

In this study, we found that young children less than 5 years of age showed a significantly higher incidence of haematological events than older children: three patients showed induction failure and two out of 11 cases showed relapse, resulting in inferior outcomes compared to older children (54-6% and 93-8% respectively, \(P = 0.005\)). A similar finding has also been reported by the European APL group, which found the 5-year cumulative incidence of relapse rate in children aged less than 5 years was 52%, compared to 17-6% in children aged 5–12 years [Bally et al, 2012]. Hence, APL arising in younger children may possess distinct biological or genetic characteristics compared to that in older children and adults. Conventional therapy consisting of ATRA and chemotherapy may not be sufficient and alternative therapy that includes ATO should be considered in the treatment of these patients. In the ongoing AML-P13 trial for childhood APL in Japan, all three consolidation phases have been replaced by ATO to further minimize the cumulative dose of anthracyline and to maximize the effect of treatment, especially in younger children with APL.

In conclusion, reduction of the cumulative dose of anthracyline to \(<300\,\text{mg/m}^2\) seems sufficient in the treatment of childhood APL. However, children aged \(<5\) years have a higher risk of haematological events so that alternative treatment modalities including ATO should be considered for treating these patients.

Acknowledgments

The authors deeply appreciate the invaluable cooperation of the large number of physicians working in the institutions, central diagnostic laboratories, and in the office and data centre of JPLSG. We thank Dr Masue Imaizumi for supporting this study. This work was supported by a Grant for Clinical Cancer Research and a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan.

Author contributions

HT (principal investigator), AK, Y Yuza, H Moritake, KT, SI, HN, AS, K Kudo, DT, T Taka, AT and SA designed the research study. HT, DT, T Taka and SA designed the research study. HT, DT, T Taka and SA reviewed the data analysis and interpretation and were the main authors of the manuscript. TW and AMS conducted the statistical analysis. Y Yuza was responsible for the rTM study. T Taki contributed the cytogentic diagnostics. MY, H Matsushita and H Miyachi performed morphological diagnosis. Y Yamashita and KH performed molecular diagnosis. HT and AK were responsible for integrating central diagnostics. K Koike, AO and YK recruited patients. KH, TN and SA contributed to the financial and administrative support of the study.

Conflict of interest

The authors declare that they have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table SI. Treatment schedule for the AML-P05 study.

Table SII. Patients’ characteristics.
Table SIII. Characteristics, haematological events and outcomes for patients aged less than 5 years.

Table SIV. Cumulative doses of anthracyclines and outcomes in recent childhood APL trials.

References


