All-trans-retinoic acid, idarubicin, and intravenous arsenic trioxide as initial therapy in acute promyelocytic leukemia (APML4)

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Running title: ATRA, idarubicin and arsenic therapy for APL
ABSTRACT

The treatment of acute promyelocytic leukemia has improved considerably following recognition of the effectiveness of all-trans-retinoic acid (ATRA), anthracycline-based chemotherapy, and arsenic trioxide (ATO). Here, we report the use of all 3 agents in combination (APML4 phase-II protocol). For induction, ATO was superimposed upon an ATRA and idarubicin backbone, with scheduling designed to exploit anti-leukemic synergy whilst minimizing cardiotoxicity and the severity of differentiation syndrome. Consolidation comprised 2 cycles of ATRA and ATO without chemotherapy, followed by 2 years of maintenance with ATRA, oral methotrexate and 6-mercaptopurine. Of 124 evaluable patients, there were 4 (3.2%) early deaths, 118 (95%) achieved hematological complete remission, and all 112 patients who commenced consolidation attained molecular complete remission. The 2-year rate for freedom from relapse is 97.5%, failure-free survival 88.1%, and overall survival 93.2%. These outcomes were not influenced by FLT3 mutation status, whereas failure-free survival was correlated with Sanz risk stratification ($P_{[\text{trend}]}=0.03$). Compared with our previously reported ATRA/idarubicin-based protocol (APML3), APML4 patients had statistically significantly improved freedom from relapse ($P=0.006$) and failure-free survival ($P=0.01$). In conclusion, the use of ATO in both induction and consolidation achieved excellent outcomes despite a substantial reduction in anthracycline exposure. This trial was registered at www.anzctr.org.au (#ACTRN1260500070639).
INTRODUCTION

Acute promyelocytic leukemia (APL) is a discrete subtype of acute myeloid leukemia characterized by a t(15;17) translocation, rearrangement of the PML and RARA genes, and formation of an abnormal chimeric retinoic acid receptor transcription factor (PML-RARA). The latter disrupts normal myeloid differentiation programs, but simultaneously imparts a unique sensitivity on APL cells to pharmacological doses of all-trans-retinoic acid (ATRA). The combination of ATRA and anthracycline-based chemotherapy for induction and consolidation has achieved dramatic improvements in outcome for patients with APL, with long term disease-free survivals now exceeding 80%.

Arsenic trioxide (ATO) is also a highly effective anti-leukemic agent in APL. ATRA and ATO synergistically degrade PML-RARA, resulting in eradication of APL leukemia-initiating cells, and ATO is currently the agent of choice for treating relapse after initial ATRA-anthracycline therapy. Several studies have now reported its use, either as a single agent or in combination with ATRA, as initial induction therapy for APL, with relapse rates approximating those that have been achieved with ATRA and multiple cycles of chemotherapy. The benefit of adding ATO in consolidation has also been demonstrated in a randomised North American Leukemia Intergroup trial.

In an attempt to build upon the proven benefits of ATRA and idarubicin, and exploit the potent anti-leukemic efficacy of ATO, the Australasian Leukaemia and Lymphoma Group (ALLG) initiated a single arm phase-II study employing triple induction with ATRA, idarubicin and ATO for patients with newly diagnosed APL.
Consolidation consisted of two additional cycles of ATRA and ATO without chemotherapy, and was followed by maintenance with ATRA, oral methotrexate (MTX) and 6-mercaptopurine (6MP). This manuscript reports the results of a protocol-specified interim analysis showing outstanding anti-leukemic efficacy combined with acceptable tolerability when delivered in a multi-institutional setting. The ALLG’s previous APML3 study, which used ATRA and idarubicin in both induction and consolidation without ATO, provides an appropriate historical control to illustrate the benefits of incorporating ATO in the initial therapy for APL.

PATIENTS AND METHODS

Patients

This trial was approved by human research ethics committees in all participating ALLG and Australian & New Zealand Children’s Haematology/Oncology Group (ANZCHOG) centers, and was registered at the Australian New Zealand Clinical Trials Registry (www.anzctr.org.au, #ACTRN12605000070639). Patients were accrued between November 2004 and September 2009. Eligibility criteria included a morphological diagnosis of de novo APL according to French-American-British criteria, demonstration of PML-RARA fusion transcripts by reverse transcriptase-polymerase chain reaction (RT-PCR) or cytogenetic demonstration of t(15;17), ECOG performance status 0-3, age > 1 year with no upper limit, normal left ventricular ejection fraction and Q-Tc interval < 500 msec, a negative pregnancy test in females of child-bearing potential, no prior treatment for APL, no history of grand mal seizures or cancer (other than basal cell skin cancer or carcinoma of the cervix in situ), absence of serious cardiac, pulmonary, hepatic, or renal disease, and written informed consent. Patients with genetic variants of APL (X-RARA where X was not
PML) were ineligible. Patient registration, data collection and validation, and statistical analyses were performed at the Centre for Biostatistics and Clinical Trials (Peter MacCallum Cancer Centre, East Melbourne, Australia).

**APML4 protocol (Table 1)**

Induction comprised ATRA 45 mg/m²/day plus age-adjusted intravenous idarubicin, given on days 2, 4, 6 and 8, and followed on day 9 by intravenous ATO 0.15 mg/kg/day (supplied by Phebra, Lane Cove, Australia). Blood product support was administered to achieve protocol-specified platelet and coagulation targets. Since both ATRA and ATO can induce APL differentiation syndrome (DS), prednisone 1 mg/kg/day was administered prophylactically to all patients for at least 10 days, regardless of white blood cell (WBC) count at presentation. The use of heparin, antifibrinolytics and G-CSF was discouraged. Decisions regarding when to use antibiotics, antivirals, and antifungals, and the choice of specific agents, were left to individual investigators. Twice weekly electrocardiographic surveillance was combined with three times per week electrolyte assessment to minimise the risk of arrhythmias due to arsenic-associated Q-Tc prolongation. For grade 3-4 non-hematologic toxicity, treatment with ATRA and/or ATO was either temporarily suspended, or reduced to doses still known to be active (25 mg/m²/day and 0.08 mg/kg/day respectively). When ATRA or ATO were omitted for 3 or more days, the treatment duration was extended beyond day 36 to compensate for the omitted doses.

Induction was followed by 2 consolidation cycles with ATRA and ATO (Table 1); both agents were given continuously in cycle 1, and intermittently in cycle 2 (to facilitate
outpatient administration of ATO and to minimise the risk of developing ATRA resistance). Maintenance therapy continued for 2 years, and consisted of eight 3-monthly cycles. ATRA was administered alone for the first 2 weeks of each cycle; oral MTX and 6MP were taken for the remainder of each cycle, targeting a neutrophil count of 1-2 x 10^9/L, with dose adjustments for excessive myelosuppression or hepatotoxicity. Bone marrow morphology, cytogenetics, and quantitative RT-PCR for PML-RARA were performed after induction and each consolidation cycle. Subsequent bone marrow assessments occurred every 3 months for 36 months, ie. until 12 months after completion of maintenance.

**Molecular monitoring**

Either peripheral blood or bone marrow was acceptable for demonstration of PML-RARA transcripts at diagnosis, but only marrow was acceptable for molecular monitoring after treatment commenced. Of 910 informative samples collected prior to the study close-out date, 906 (99.6%) were analyzed at a central laboratory (Royal Prince Alfred Hospital). RNA was extracted from mononuclear cells isolated by Ficoll-Hypaque density centrifugation, or if necessary from bone marrow simultaneously collected into RNA later (Invitrogen). The majority of samples (84%) were assayed by quantitative RT-PCR for PML-RARA fusion transcripts using FusionQuant kits (Ipsogen). The remainder were assayed with a semi-nested qualitative RT-PCR protocol with a sensitivity of at least 10^-4, especially those with more proximal bcr2 breakpoints not amenable to FusionQuant analysis. Identification of mutant FLT3 transcripts, both internal tandem duplications (ITD) and codon 835/836 mutations, was performed as previously described.
Definitions and study end-points

Hematologic complete remission (hCR) was assessed according to criteria described by the International Working Group\textsuperscript{17}. Molecular complete remission (mCR) required absence of detectable \textit{PML-RARA} transcripts. Relapse was defined as either (a) reappearance of abnormal blast cells and/or promyelocytes, or the development of extramedullary disease (hematologic relapse), or (b) reversion to \textit{PML-RARA} positivity, confirmed on serial samples, following previously documented negativity (molecular relapse), whichever occurred first. The primary endpoint of the study, freedom from relapse (FFR), was calculated as the time from documented hCR to hematological or molecular relapse. Secondary end-points were measured as follows: overall survival (OS), time from commencement of ATRA therapy to death from any cause; disease-free survival (DFS), time from documented hCR to the earliest of relapse or death; failure-free survival (FFS), time from commencement of ATRA therapy to the earliest of treatment failure, relapse, or death, where treatment failure included failure to achieve mCR by the end of consolidation, or withdrawal from protocol therapy due to patient refusal to continue or excessive toxicity. Early death was defined as death during induction (ie. within 36 days from the commencement of ATRA therapy). Adverse events (AEs) were reported using the Common Terminology Criteria for Adverse Events v3.0 (CTCAE, National Cancer Institute, Bethesda, MD).

Statistical methods

This interim analysis was planned to take place approximately one year after accrual had ceased. At the time of analysis a study close-out (censor) date was set as the earliest of the dates of last contact of patients who were still alive and being followed
up. Therefore, with the exception of patients who had been lost to follow-up, the status of all patients in the trial for the time-to-event endpoints was known at this date. FFR, OS, DFS and FFS curves were estimated using the Kaplan-Meier product limit method; 2-year survival estimates and 95% confidence intervals (95% CI) were also calculated. The associations of \textit{FLT3} status (wild type versus ITD and/or codon 835/836 mutations) and Sanz risk stratification\textsuperscript{18} with time-to-event outcomes were assessed using Cox proportional hazards regression models. The relationship between WBC count at baseline and early death was investigated using binary logistic regression. AEs were summarised descriptively using number of events and percentages for induction and consolidation separately; McNemar’s test was used to compare the incidence of specific grade 3-4 AEs between induction and the first consolidation cycle, and also between the first and second consolidation cycles. All statistical analyses were performed in R version 2.10 (R Development Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, 2011. http://www.R-project.org).

\textbf{APML3 historical control data}

To better assess the impact of (i) adding ATO in induction and consolidation, and (ii) eliminating chemotherapy from consolidation, data from the ALLG’s previous APML3 trial\textsuperscript{16} were used as a historical control. APML3 involved ATRA and idarubicin induction, and consolidation utilised a second cycle of idarubicin followed by three 14-day blocks of ATRA. APML3 was subsequently amended during the study to incorporate 2 years of maintenance with ATRA, oral MTX and 6MP. A total of 101 eligible patients were registered on the APML3 trial, but the comparator group used here has been restricted to the 70 patients registered after the maintenance
amendment was activated in order to eliminate differing post-consolidation therapy as a confounding variable. Their median potential follow-up time determined by the reverse Kaplan-Meier method was 48 (range 30-68) months. The time interval between the first patient registered in the APML3 comparator cohort and the first patient registered in the APML4 cohort was 63 months. Comparisons between these cohorts were performed for early death using Fisher’s exact test, and for time-to-event outcomes using the logrank test and Cox proportional hazards regression.

RESULTS

Induction

One hundred and twenty-nine patients from 27 Australian centers were registered. Five patients were excluded from the analysis; three patients were negative for both t(15;17) and PML-RARA, and one of these was subsequently found to have a genetic variant APL (PRKAR1A-RARA)\(^{19}\), one patient withdrew consent prior to commencing therapy, and one patient with an eligibility infringement (prior malignancy) also withdrew consent. The characteristics of the 124 evaluable patients are summarised in Table 2. With a study close-out date of 16 November 2009, the median potential follow-up time was 24 (range 2-61) months.

Four of 124 patients died during induction (early death rate 3.2%). The causes of death were myocardial ischemia and cardiac arrest (day 1), intracerebral hemorrhage (day 3 and day 7), and cerebral edema (day 30). Early deaths were associated with age > 70 years (\(P=0.02\)), but not with WBC count > 10 x 10\(^9\)/L at diagnosis (\(P=0.17\)). Two patients withdrew from study during induction without subsequent evaluation of remission status; one withdrew on day 29 because he no
longer wanted to attend for daily ATO infusions, and the other withdrew on day 16 because of grade 4 DS which had commenced prior to day 9. The latter patient did not receive any ATO before withdrawal, but was subsequently treated with ATO off-protocol. The remaining 118 patients (95%) had hCR documented a median of 53 days (range 34-83) from the start of ATRA therapy. **Table 3** lists grade 3-4 non-hematologic AEs observed during induction. Grade 3-4 DS (CTCAE v3.0 retinoic acid syndrome) was reported in 14%, but there were no deaths attributable to DS. Q-Tc prolongation to > 500 msec occurred during ATO therapy in 14% of patients, but there were no instances of *torsades de pointes* or other severe arrhythmias. One patient developed marked T-wave inversion following the first dose of ATO. Despite cessation of ATO, T-wave inversion persisted and the patient was withdrawn from the study. Whether the preceding 4 doses of idarubicin (total dose 35 mg/m²) or the single dose of ATO (0.15 mg/kg) was responsible is unclear. Biochemical hepatotoxicity was common, but responded to protocol-specified ATO dose reduction, and resolved after ATO withdrawal. Other potential contributors to the observed hepatotoxicity include ATRA, idarubicin, allopurinol, and anti-fungal prophylaxis. **Table 4** lists the spectrum of infections that were reported, and the organisms that were identified. Six patients withdrew consent before commencing consolidation due to unacceptable toxicity during induction (severe rash [2], infection [2], persistent deep T-wave inversion [1], and peripheral neuropathy [1]). All 6 were in hCR after induction, and 4 were also in mCR.

**Consolidation**

Of 118 patients in hCR, 112 (95% of hCR patients, and 90% of the total study population) commenced consolidation at a median of 6.5 days (range 0-28) after
documentation of hCR, and all 112 (100%) were in mCR by the end of consolidation cycle 2. There were no instances of DS, and no deaths were attributed to either of the two cycles of consolidation. Grade 3-4 non-hematologic AEs experienced in each consolidation cycle are shown in Table 3, and the spectrum of infections are listed in Table 4. In general, consolidation was associated with considerably less toxicity than induction, and this was especially evident for hepatic, gastrointestinal, infective and metabolic AEs. Compared with the first cycle of consolidation, biochemical hepatotoxicity and infections were also statistically significantly less frequent in the second cycle when ATO and ATRA were given on an intermittent schedule (weekdays only for ATO, alternate weeks for ATRA). Myelotoxicity associated with ATO was also schedule-dependent, since any grade 3-4 cytopenia occurred in 52% of patients during the first cycle of consolidation compared with 24% during the second cycle (P<0.0001). This myelotoxicity was predominantly manifest as neutropenia (51% in cycle 1 and 23% in cycle 2). Q-Tc prolongation was less frequent during consolidation than induction, but the differences were not statistically significant. One episode of ventricular tachycardia occurred in a single patient during the first cycle of consolidation, but this was transient and was not associated with serious sequelae. Toxicities experienced by the four pediatric and adolescent patients (aged 3, 15, 16 and 17 years) were comparable with those seen in adult patients.

**Outcomes**

At the close-out date, two patients were known to have relapsed following completion of consolidation. One patient (intermediate risk) had a molecular marrow relapse 166 days after consolidation cycle 2, followed soon after by overt central
nervous system relapse, and died of progressive disease. The other patient (high risk) had an isolated molecular relapse 189 days after consolidation cycle 2, and died of infective complications related to salvage chemotherapy with intermediate-dose cytarabine and etoposide. The 2-year FFR rate is 97.5% (95% CI: 90.4%-99.4%, Figure 1A), and was unaffected by Sanz risk stratification ($P_{\text{trend}}=0.17$, Figure 1B). Since there have been no protocol-associated deaths in remission, the estimates for DFS and FFR at 2-years are identical.

The actuarial 2-year rate for FFS is 88.1% (95% CI: 80.7%-92.8%, Figure 2A), and for OS is 93.2% (95% CI, 85.8%-96.8%, Figure 2B). Sanz risk stratification did not impact on OS ($P_{\text{trend}}=0.16$), but was statistically significantly correlated with FFS ($P_{\text{trend}}=0.03$, Figure 2C). However, the relevance of this association is tempered by the fact that FFS, as defined in this study, included withdrawal due to patient refusal or excessive toxicity in addition to relapse, death, or failure to achieve mCR. Accordingly, FFS is a less precise endpoint than FFR, DFS and OS in identifying patient subgroups with a poorer prognosis. When considered as a continuous covariate, age was not statistically significantly correlated with FFR/DFS ($P=0.92$), OS ($P=0.10$) or FFS ($P=0.07$).

**Dose delivery**

The vast majority of patients received total doses of idarubicin, ATRA and ATO above the minimum specified for each cycle of the protocol (Table 5), and most received at least 80% of the maximum specified doses. These data demonstrate the feasibility of combining all 3 drugs, and delivering APML4 in a multi-institutional setting.
Comparison with APML3 historical control data

There were no statistically significant differences in age, sex distribution, median WBC count, median platelet count, Sanz risk classification or FLT3 mutation status between the APML3 and APML4 cohorts (Table 2). The difference in early death rate between the two studies (7.1% in APML3, 3.2% in APML4) was not statistically significant ($P=0.29$), nor was the difference in OS (hazard ratio $= 0.47$, 95% CI: 0.18-1.23, $P=0.12$; APML4 93% versus APML3 90% at 2 years). In contrast, we observed statistically significant improvements in FFR, DFS and FFS associated with the use of front-line ATO (APML4) compared with APML3 (Figure 3A-C).

In APML3, FLT3 mutation status was the most important predictor of OS ($P=0.005$), and this association with inferior OS was true for both ITDs and codon 835/836 mutations$^{16}$. In APML4, however, OS did not differ by FLT3 mutation status ($P=0.93$, Figure 4A), nor was there any impact on FFR ($P=0.83$, Figure 4B) or FFS ($P=0.98$).

DISCUSSION

The potential mechanisms of the striking anti-leukemic activity of ATO in APL have been extensively studied. ATO has the ability to induce both differentiation and apoptosis of APL cells in a dose-dependent manner$^{20}$. Furthermore, ATO has potent and relatively selective activity against APL leukemia-initiating cells as a result of its ability to induce PML-RARA fusion protein degradation$^{5,21}$. ATO is universally recognized as the treatment of choice for patients who relapse after initial therapy with ATRA and chemotherapy$^{6-8}$, and in most instances it is active even in patients whose leukemic cells exhibit ATRA and chemotherapy resistance. Several reports have established its activity in the management of previously untreated patients,
including its use as a single agent\textsuperscript{9,10} in countries where access to both ATRA and chemotherapy is limited, and tetra-arsenic tetra-sulfide (As\textsubscript{4}S\textsubscript{4})\textsuperscript{22} has also been successfully employed as a single agent for the treatment of newly diagnosed and relapsed APL. However, while single agent arsenic is undoubtedly effective, outcomes with this approach do not appear superior to those achieved by protocols employing the combination of ATRA with anthracycline-based chemotherapy (\textbf{Table 6}). Synergism of ATO and ATRA has been demonstrated in a mouse model of human APL\textsuperscript{23}, and has been clinically confirmed in a small randomized trial\textsuperscript{24}. As the benefit of ATO is now well established, identification of the optimal way in which it can be incorporated into front-line therapy remains one of the major challenges in the treatment of APL.

The North American Leukemia Intergroup Study C9710\textsuperscript{13} demonstrated that the addition of two cycles of ATO to ATRA and chemotherapy during consolidation significantly improved event-free survival. In a similar ATRA and chemotherapy protocol, post-induction ATO has been used as a substitute for the second cycle of consolidation chemotherapy\textsuperscript{25}. The Shanghai\textsuperscript{11} and Changsha\textsuperscript{26} groups have also demonstrated excellent outcomes with ATRA- and ATO-based induction therapy, although both studies variably employed additional cytotoxic agents during induction for high-risk patients. In addition, both studies used substantial post-remission chemotherapy (daunorubicin, cytarabine, and homoharringtonine) in multiple cycles of consolidation (\textbf{Table 6}). In contrast, we attempted to maximize anti-leukemic activity during induction by adding ATO to ATRA and idarubicin in the APML4 protocol, and also omitted all other chemotherapeutic agents from consolidation. This approach has proven extremely successful, with DFS and OS in the APML4
study reported here that compare favorably with other published data (Table 6). Furthermore, the APML4 results have been achieved at relatively low cumulative ATO and anthracycline exposures, and without the use of other chemotherapeutic agents that have recently been advocated for high-risk patients\textsuperscript{3,27,28}, such as intermediate- or high-dose cytarabine. Although the median followup in this interim analysis is relatively short, it is comparable with several other studies whose median followup was less than 3 years at the time of reporting\textsuperscript{3,12,13,22,25,26}. Nevertheless, we recognize additional relapses are likely to be seen with a longer period of observation, and a final APML4 analysis will be performed when the last registered patient has been followed for a minimum of 2 years after consolidation.

The strategy that most closely resembles APML4 was reported by investigators at the MD Anderson Cancer Center\textsuperscript{12}. They omitted anthracycline chemotherapy entirely, utilizing only ATRA and ATO in both induction and consolidation, although gemtuzumab ozogamicin was added for patients with high-risk disease. The OS and DFS data reported here with APML4 are at least as good as the MD Anderson data (Table 6), despite the use of less ATO and a similar median duration of follow-up, suggesting that some anthracycline in induction is beneficial in maximizing long-term outcomes. Since gemtuzumab ozogamicin is no longer readily accessible following its voluntary withdrawal from the market, the combination of idarubicin with ATO and ATRA in induction represents a highly effective and readily available therapeutic approach, especially for patients with high-risk disease.

The potent anti-leukemic activity of ATO-based combination therapy is particularly evident when FLT3 mutation data are taken into account. Although the prognostic
impact of FLT3 mutations in APL has been more controversial than in non-APL acute myeloid leukemia, a large meta-analysis encompassing 1063 patients with APL identified FLT3 ITD as significantly associated with inferior OS and DFS, and a trend towards adverse outcomes was also observed for patients with codon 835 mutations. An association between WBC count and FLT3 ITDs was noted in that report, and it is not entirely clear whether the adverse impact of FLT3 mutations was independent of WBC count. However, it is interesting to note that 10 of the 11 reports that were included in that meta-analysis utilized ATRA plus chemotherapy. The final study, which showed no association between FLT3 mutations and adverse outcomes, was a study of single agent ATO. In our previous study of ATRA and idarubicin (APML3), our FLT3 mutation data were in close agreement with the meta-analysis, since FLT3 mutation status was the single most important predictor of OS in multivariate analysis, and the association with inferior survival persisted when ITD and codon 835/836 mutations were assessed separately. Furthermore, FLT3 mutations also emerged as important predictors of remission duration and DFS in multivariate analyses when maintenance components were treated as time-dependent covariates. In contrast, the OS and FFR curves that we have observed in the current APML4 study for FLT3 wild type and mutant subgroups are superimposable (Figure 4A-B). Thus, any adverse effect of FLT3 mutations that is evident when APL is treated with ATRA plus chemotherapy appears to be abrogated by inclusion of ATO during induction and consolidation. Accordingly, despite the frequent occurrence of FLT3 mutations in APL, it is highly unlikely that FLT3 inhibitor therapy will have any role in the future management of APL.
The early death rate is typically identified as 5-10% in most APL series, although it is undoubtedly higher in population-based studies\textsuperscript{31,32}, indicating that selection bias is inevitable in APL trials. While the same criticism can be directed at the current report, the low early death rate (3.2%) likely also reflects better supportive care during induction. In contrast to APML3, where hemostatic targets were not protocol-specified and hemostatic support was administered according to local practice, APML4 employed clear recommendations to maintain adequate hemostasis (Table 1). In addition, all patients in APML4 received prophylactic prednisone\textsuperscript{33} to minimise the frequency and severity of DS regardless of pre-treatment WBC count, whereas in APML3 prednisone was only employed if the WBC count exceeded 10 x 10\textsuperscript{9}/L during induction or once clinical features of DS were present. DS was a contributory factor in the early deaths of 2 patients in the APML3 study, whereas there were no fatalities due to DS in APML4. Thus despite the fact that the induction protocol in APML4 was more intensive than in APML3, a trend towards lower early deaths was evident, and most likely reflects improved supportive care.

Since both idarubicin and ATO are potentially cardiotoxic, we delayed initiation of ATO until day 9, following the fourth and final dose of idarubicin. Strict attention to electrolyte levels was specified in the protocol, and no instances of torsades de pointes or other life-threatening arrhythmias occurred during induction, despite 14% of patients experiencing Q-Tc prolongation to > 500 msec on at least one occasion. It is likely that the use of oral ATO\textsuperscript{34,35} will reduce the risk of significant arrhythmias, especially when administered during consolidation when outpatient therapy is desirable and feasible.
A second reason for delaying commencement of ATO until day 9 was to reduce the risk and severity of DS. Whilst DS is typically seen with ATRA therapy, ATO can also cause an identical syndrome\textsuperscript{36}, and we therefore delayed ATO until the protective cytoreductive effect of idarubicin chemotherapy was manifest. We also utilized prednisone prophylaxis in all patients, regardless of initial WBC count, to further reduce DS complications. Although the routine use of prophylactic corticosteroids has not conclusively been shown to prevent DS\textsuperscript{8}, there is some evidence\textsuperscript{33} suggesting they may be beneficial in patients receiving ATRA-based induction. Since our patients received both ATRA and ATO, we considered the potential benefits of short-term prophylactic prednisone outweighed any potential risks, and the absence of early deaths attributable to either DS or corticosteroid toxicity appears to have vindicated that decision. Furthermore, it is worth noting that of the 4 early deaths in this study, 3 occurred before day 9 when ATO therapy was initiated, and ATO was not implicated in any of the other 3 deaths that occurred beyond day 36. Accordingly, only 1 out of 7 deaths in our series of 124 patients was potentially associated with ATO toxicity, and we believe this highlights the lack of safety concern associated with the incorporation of ATO in the regimen described here.

The optimal number of consolidation cycles required to maximise FFR is unknown, and the number incorporated into currently used regimens is quite variable amongst the protocols listed in Table 6, ranging from 2 to 9. In APML4, only 2 cycles were used, further emphasising the value of arsenic-based consolidation, since our results for DFS are not inferior to protocols incorporating 3 or more cycles of chemotherapy-based consolidation. While our dose delivery data demonstrate the feasibility of the APML4 regimen, logistic issues associated with repeated intravenous infusions of
ATO constitute a relative disadvantage. Adopting an intermittent ATO schedule (5 days per week for 5 weeks) for both APML4 consolidation cycles would reduce toxicity, improve resource utilisation, and be more palatable for patients. Whether this would compromise outcomes is unknown, but any impact on efficacy is likely to be small. Alternatively, provided pharmacological and therapeutic equivalence were established, oral rather than intravenous ATO would significantly facilitate administration, but assuring protocol adherence would be more difficult, as seen with other long-term oral anti-cancer therapies.

Based on the benefits of maintenance that were observed in our previous APML3 study, maintenance with ATRA, oral MTX and 6MP was administered to all patients in APML4. The role of maintenance, however, remains controversial, since there are conflicting data from randomized studies. Maintenance treatment was associated with significantly better outcomes in both the European APL93 and North American Intergroup APL studies, whereas the AIDA 0493 report from GIMEMA showed no advantage for maintenance. All these studies were based on an ATRA/chemotherapy backbone, and it would be appropriate to re-examine the requirement for maintenance in the era of ATO-based therapy for newly diagnosed patients with APL.

In summary, APML4 induction therapy, which employs the three most active agents in APL (ATRA, idarubicin and ATO), combined with consolidation restricted to ATRA and ATO, is capable of achieving excellent DFS and OS. The total doses of anthracycline and ATO are at the low end of the spectrum of studies that have been associated with DFS in excess of 90% (Table 6), and APML4 is further characterized
by the omission of any other cytotoxic agents during consolidation. The toxicity profile is manageable, and the lack of deaths associated with consolidation therapy is a major advantage when compared with protocols that utilise intensive post-remission chemotherapy. Furthermore, the toxicity associated with the APML4 protocol is theoretically capable of further attenuation by the use of a risk-adapted reduction in idarubicin dose during induction, the incorporation of oral ATO during consolidation, and possibly reduction or elimination of maintenance. Our experience with APML4 adds further support to the use of ATRA and ATO as initial therapy for APL in both induction and consolidation, with minimal requirement for additional chemotherapy.

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AUTHORSHIP CONTRIBUTIONS

HJI was the principal investigator and takes primary responsibility for the paper. HJI, KB, JFS, MH, PB, FF, and JR designed the study. HJI, KB, MH, AG, FF, CT, KT, RF, MS, JT, JS, JM, JB and JFS recruited patients. SGS, AC and AH performed laboratory work. MC, JDI, HJI and JR performed data validation and interpretation. MC performed statistical analysis. HJI, JFS, and MC wrote the paper. All authors reviewed and approved the final manuscript.

DISCLOSURE OF CONFLICTS OF INTEREST

HJI, FF and JFS report a consultancy for Phebra. Apart from the provision of ATO, Phebra had no involvement in APML4 trial design, conduct of the trial, data analysis, or preparation of the manuscript. JR was a statistician for the ALLG during the APML4 study, but has since joined Novartis. However ATRA, idarubicin and ATO are not Novartis products. The remaining authors declare no competing financial interests.
REFERENCES


16. Iland HJ, Bradstock K, Seymour J, et al. Results of the APML3 trial incorporating all-trans-retinoic acid and idarubicin in both induction and consolidation


Table 1. Components of the APML4 protocol

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<tr>
<td>ATRA*</td>
<td>45 mg/m²/day PO†</td>
<td>days 1-36 in divided doses</td>
</tr>
</tbody>
</table>
| Idarubicin| • 12 mg/m²/day IV‡ (ages 1-60)  
• 9 mg/m²/day IV (ages 61-70)  
• 6 mg/m²/day IV (ages >70) | days 2, 4, 6 & 8 |
| ATO§      | 0.15 mg/kg/day IV | • days 9-36 as a 2-hour IV infusion  
• supplemental potassium and magnesium as required to maintain serum levels in the upper half of the respective normal ranges |
| Prednisone| 1 mg/kg/day PO | days 1-10, or until WBC count fell below 1 x 10⁹/L, or until resolution of differentiation syndrome (whichever occurred last) |
| Hemostatic support | Products administered once or twice daily as required to achieve specified targets | • platelets > 30 x 10⁹/L  
• normal prothrombin time  
• normal activated partial thromboplastin time  
• fibrinogen > 1.5 g/L |

Consolidation cycle 1 (3-4 weeks after the end of induction)

| ATRA | 45 mg/m²/day PO | days 1-28 |
| ATO  | 0.15 mg/kg/day IV | days 1-28 |

Consolidation cycle 2 (3-4 weeks after the end of consolidation cycle 1)

| ATRA | 45 mg/m²/day PO | days 1-7, 15-21, 29-35 |
| ATO  | 0.15 mg/kg/day IV | days 1-5, 8-12, 15-19, 22-26, 29-33 |

Maintenance - 8 cycles (3-4 weeks after the end of consolidation cycle 2)

| ATRA | 45 mg/m²/day PO | days 1-14 |
| MTX§ | 5-15 mg/m²/week PO | days 15-90 |
| 6MP¶ | 50-90 mg/m²/day PO | days 15-90 |
* all-trans-retinoic acid

† oral

‡ intravenous

§ arsenic trioxide

|| methotrexate

¶ 6-mercaptopurine
Table 2. Pre-treatment characteristics of evaluable patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>APML4</th>
<th>APML3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (%)</td>
<td>Median (Range)</td>
<td>Number (%)</td>
</tr>
<tr>
<td>Number of patients</td>
<td>124 (100)</td>
<td></td>
<td>70 (100)</td>
</tr>
<tr>
<td>Age, years</td>
<td>108 (87)</td>
<td>44 (3 - 78)</td>
<td>63 (90)</td>
</tr>
<tr>
<td>Age subgroup, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 60</td>
<td>108 (87)</td>
<td>61-70</td>
<td>6 (9)</td>
</tr>
<tr>
<td>&gt; 70</td>
<td>7 (6)</td>
<td></td>
<td>6 (9)</td>
</tr>
<tr>
<td>Sex</td>
<td>62 (50)</td>
<td></td>
<td>37 (53)</td>
</tr>
<tr>
<td>Male</td>
<td>62 (50)</td>
<td></td>
<td>33 (47)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECOG status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>64 (52)</td>
<td>37 (53)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>43 (35)</td>
<td>23 (33)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>11 (9)</td>
<td>9 (13)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6 (5)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>FAB* classification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>99 (80)</td>
<td>57 (81)</td>
<td></td>
</tr>
<tr>
<td>M3v</td>
<td>25 (20)</td>
<td>13 (19)</td>
<td></td>
</tr>
<tr>
<td>PML breakpoint†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bcr1</td>
<td>66 (53)</td>
<td>38 (57)</td>
<td></td>
</tr>
<tr>
<td>bcr2</td>
<td>8 (6)</td>
<td>6 (9)</td>
<td></td>
</tr>
<tr>
<td>bcr3</td>
<td>50 (40)</td>
<td>23 (34)</td>
<td></td>
</tr>
<tr>
<td>WBC count, x10⁹/L</td>
<td>2.4 (0.1 - 85.8)</td>
<td>2.4 (0.4 - 109.0)</td>
<td></td>
</tr>
<tr>
<td>Platelet count, x10⁹/L‡</td>
<td>22 (1 - 173)</td>
<td>22 (4 - 180)</td>
<td></td>
</tr>
<tr>
<td>Sanz risk category‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>32 (26)</td>
<td>20 (29)</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>67 (54)</td>
<td>35 (50)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>24 (20)</td>
<td>15 (21)</td>
<td></td>
</tr>
<tr>
<td>FLT3 status§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>66 (56)</td>
<td>37 (59)</td>
<td></td>
</tr>
<tr>
<td>Mutation</td>
<td>52 (44)</td>
<td>26 (41)</td>
<td></td>
</tr>
</tbody>
</table>
*French-American-British

†PML breakpoint was not available in 3 APML3 patients; therefore n=67.

‡Pre-transfusion platelet count was not available in one APML4 patient; therefore n=123.

§FLT3 status available for 118 APML4 patients (95%), and 63 APML3 patients (90%); FLT3 mutations include internal tandem duplications and/or codon 835/836 mutations.
Table 3. The number (%) of patients experiencing grade 3-4 non-hematologic adverse events during induction and consolidation

<table>
<thead>
<tr>
<th></th>
<th>Induction</th>
<th>Con 1*</th>
<th>Con 2†</th>
<th><em>P</em> (Induction versus Con 1)</th>
<th>†P (Con 1 versus Con 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients for whom adverse event data are available</td>
<td>120 (97%)</td>
<td>112 (100%)</td>
<td>110 (98%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac‡</td>
<td>1 (1%)</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Prolonged Q-Tc interval</td>
<td>17 (14%)</td>
<td>10 (9%)</td>
<td>4 (4%)</td>
<td>0.17</td>
<td>0.11</td>
</tr>
<tr>
<td>Hepatic§</td>
<td>53 (44%)</td>
<td>13 (12%)</td>
<td>2 (2%)</td>
<td>&lt; 0.0001</td>
<td>0.01</td>
</tr>
<tr>
<td>Gastrointestinal‖</td>
<td>33 (28%)</td>
<td>3 (3%)</td>
<td>1 (1%)</td>
<td>&lt; 0.0001</td>
<td>0.62</td>
</tr>
<tr>
<td>Infection¶</td>
<td>91 (76%)</td>
<td>21 (19%)</td>
<td>3 (3%)</td>
<td>&lt; 0.0001</td>
<td>0.0005</td>
</tr>
<tr>
<td>Differentiation syndrome</td>
<td>17 (14%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0.0005</td>
<td></td>
</tr>
<tr>
<td>Neurological#</td>
<td>7 (6%)</td>
<td>2 (2%)</td>
<td>0 (0%)</td>
<td>0.29</td>
<td>0.48</td>
</tr>
<tr>
<td>Headache</td>
<td>4 (3%)</td>
<td>2 (2%)</td>
<td>0 (0%)</td>
<td>0.68</td>
<td>0.48</td>
</tr>
<tr>
<td>Dermatological</td>
<td>5 (4%)</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Respiratory**</td>
<td>2 (2%)</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Metabolic‖</td>
<td>19 (16%)</td>
<td>4 (4%)</td>
<td>4 (4%)</td>
<td>0.002</td>
<td>1.0</td>
</tr>
<tr>
<td>Second malignancy</td>
<td>0 (0%)</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>
* consolidation cycle 1
† consolidation cycle 2
‡ conduction abnormalities other than Q-Tc prolongation or left ventricular systolic dysfunction
§ clinical liver failure, or elevation of bilirubin, ALT, AST or GGT
ǁ nausea, vomiting, diarrhea, mucositis or enterocolitis
¶ documented infection or febrile neutropenia
# dizziness, mood alteration, musculoskeletal pain or seizure
** dyspnea or hypoxia not attributed to differentiation syndrome
†† hyperglycemia, hypertriglyceridemia, hypoalbuminemia, hypokalemia, hypophosphatemia or renal failure
‡‡ squamous cell carcinoma (SCC) of skin; since the latency of skin cancer related to arsenic exposure is usually measured in
years or decades (Levine T, Marcus W, Chen C. US Environmental Protection Agency Risk Assessment Forum: Special Report
unlikely that this SCC was a consequence of the therapeutic ATO used in this protocol
Table 4. Spectrum and number of infections observed during induction and consolidation

<table>
<thead>
<tr>
<th></th>
<th>Induction</th>
<th>Consolidation cycle 1</th>
<th>Consolidation cycle 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood stream and/or catheter-related*</td>
<td>42</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Respiratory tract†</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Urinary tract‡</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Gastrointestinal§</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Skin / wound</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Viral¶</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Fungal#</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>


† includes *E. coli, coagulase negative Staphylococcus, L. bozemanii, S. maltophilia, H. influenzae*

‡ includes *E. coli, E. cloacae, K. pneumoniae*

§ includes *Clostridium species, E. faecium, E. faecalis*

|| includes *Serratia species, S. aureus*

¶ includes Herpes simplex, Herpes zoster

# includes *Aspergillus species*
Table 5: Summary of idarubicin, ATRA and ATO doses delivered according to protocol specifications and treatment cycle

<table>
<thead>
<tr>
<th></th>
<th>Number of patients with data available</th>
<th>Protocol-specified maximum / minimum* total dose</th>
<th>Median total dose delivered (range†)</th>
<th>Number that received at least 80% of the maximum total dose (%)</th>
<th>Number that received at least 100% of the minimum* total dose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induction (n=121‡)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idarubicin, mg/m²</td>
<td>121</td>
<td>24-48§ / N/A‖</td>
<td>47.5 (22.2-52.4)</td>
<td>118 (97)</td>
<td>N/A</td>
</tr>
<tr>
<td>ATRA, mg/m²</td>
<td>118</td>
<td>1620 / 850</td>
<td>1584.2 (136.1-1988.1)</td>
<td>106 (90)</td>
<td>112 (95)</td>
</tr>
<tr>
<td>ATO, mg/kg</td>
<td>117</td>
<td>4.2 / 2.08</td>
<td>4.03 (0-4.94)</td>
<td>84 (72)</td>
<td>109 (93)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consolidation cycle 1 (n=112)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATRA, mg/m²</td>
<td>110</td>
<td>1260 / 650</td>
<td>1269.8 (515.9-2655.0)</td>
<td>108 (98)</td>
<td>108 (98)</td>
</tr>
<tr>
<td>ATO, mg/kg</td>
<td>109</td>
<td>4.2 / 2.08</td>
<td>4.2 (1.97-4.72)</td>
<td>100 (92)</td>
<td>108 (99)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consolidation cycle 2 (n=112)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATRA, mg/m²</td>
<td>109</td>
<td>945 / 475</td>
<td>948.4 (522.6-1260.0)</td>
<td>106 (97)</td>
<td>109 (100)</td>
</tr>
<tr>
<td>ATO, mg/kg</td>
<td>111</td>
<td>3.75 / 1.84</td>
<td>3.75 (1.66-4.48)</td>
<td>100 (90)</td>
<td>110 (99)</td>
</tr>
</tbody>
</table>

* Protocol-specified dose reductions for ATRA (25mg/m²/day) and ATO (0.08mg/kg/day), and omission of up to 2 days per cycle for both drugs, were permitted according to clinical circumstances

† In each cycle, 1-3% of patients received more than 110% of the maximum specified doses due to protocol deviations

‡ Induction dose delivery data excludes the 3 patients who died prior to commencement of ATO therapy (day 9)

§ Idarubicin dosing was age-adjusted (Table 1)

‖ N/A (not applicable); there was no protocol-specified minimum idarubicin dose (apart from dose reduction for age > 60)
<table>
<thead>
<tr>
<th>Series (reference)</th>
<th>Number (age restriction)</th>
<th>Median followup, yrs</th>
<th>Number of arsenic treatment daysb</th>
<th>Idarubicin equivalentb,c, mg/m²</th>
<th>Cytarabineb, g/m²</th>
<th>Other cytotoxic agents in induction and/or consolidation</th>
<th>OSd</th>
<th>DFSd</th>
<th>EFSd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ades²⁸ APL2000</td>
<td>178 (less than 65)</td>
<td>5.2</td>
<td>-</td>
<td>99</td>
<td>10.8 - 22.8°</td>
<td></td>
<td>94% (3 yrs)</td>
<td>-</td>
<td>86% (3 yrs)</td>
</tr>
</tbody>
</table>
| Sanz³ LPA2005      | 402                      | 2.3                  | -                                 | 105 - 125°                     | 0 - 5.8°         | • etoposide⁷  
• 6-thioguanine⁸                                      | 88% (4 yrs) | 90% (4 yrs) | - |
| Lo Coco⁴ AIDA 2000 | 445 (less than 62)       | 4.9                  | -                                 | 121.7                          | 0 - 6.3°         | • hydroxyurea⁹  
• low dose anthracycline⁹                               | 87% (6 yrs) | 86% (6 yrs) | - |
| Matthews⁹ Vellore   | 72                       | 5.0                  | 112 - 158                         | -                              | -                | • hydroxyurea⁹                                      | 74% (5 yrs) | 80% (5 yrs) | 69% (5 yrs) |
| Ghavamzadeh¹⁰ Tehran| 197 (patients in CR)     | 3.2                  | 58 - 142°                         | -                              | -                | • hydroxyurea⁹                                      | 64% (5 yrs) | 67% (5 yrs) | - |
| Lu²² Beijing¹       | 19                       | 1.1                  | 546 - 597                         | -                              | -                |                                                      | -              | 77% (3 yrs) | - |
| Powell¹³ North American Intergroup C9710 | 243                       | 2.4                  | 50                                | 100                            | 1.4              | • hydroxyurea⁹                                      | 86% (3 yrs) | 90% (3 yrs) | 80% (3 yrs) |
| Gore²⁰ Baltimore    | 45                       | 2.7                  | 30                                | 72                             | 2.0              | • hydroxyurea⁹                                      | 88% (3 yrs) | 89% (3 yrs) | 76% (3 yrs) |
| Hu¹¹ Shanghai       | 85                       | 5.8                  | 175                               | 81                             | 17.7 - 26.7°     | • hydroxyurea⁹  
• idarubicin + cytarabine⁹  
• homoharringtonine¹   | 92% (5 yrs) | 95% (5 yrs) | 89% (5 yrs) |
| Dai²⁶ Changsha¹     | 90                       | 2.7                  | 68 - 84                           | 135                            | 2.6 - 3.6°       | • hydroxyurea⁹  
• homoharringtonine⁹                                                               | -              | 93% (2.5 yrs) | - |
| Ravandi¹² Houston   | 82                       | 1.9                  | 100 - 110                         | -                              | -                | • gemtuzumab ozogamicin⁹                             | 85% (3 yrs) | 81% (3 yrs) | - |
| Iland (current study) APML4 | 124                  | 2.0                  | 81                                | 48                             | -                |                                                      | 93% (2 yrs) | 98% (2 yrs) | 88% (2 yrs) |
References (3),(4),(28) are ATRA/chemotherapy protocols; references (9),(10),(22) are single agent arsenic protocols (ATO or As$_4$S$_4$); references (13),(25) are ATRA/chemotherapy protocols with ATO in consolidation; references (11),(26) are ATRA/ATO/chemotherapy protocols; reference (12) is an ATRA/ATO protocol with gemtuzumab ozogamicin for high risk patients.

Data for arsenic exposure (ATO or As$_4$S$_4$) include amounts used in induction, consolidation and (where appropriate) maintenance; data for idarubicin equivalent and cytarabine doses include amounts used in induction and consolidation.

Idarubicin equivalent calculated as follows$^{41}$: 10 mg idarubicin = 12 mg mitoxantrone = 50 mg daunorubicin

OS: overall survival; DFS: disease-free survival; EFS: event-free survival; survival data are single time-point figures at the times shown in parentheses.

Higher dose for high risk patients, except LPA2005 where intermediate risk group received highest idarubicin equivalent dose of anthracycline (idarubicin) plus anthraquinone (mitoxantrone).

Additional chemotherapy during consolidation for high risk patients.

Additional chemotherapy during induction for high risk patients.

Based on median number of days to CR = 30.

This study utilised single agent As$_4$S$_4$ rather than ATO.

Additional chemotherapy during consolidation for all patients.

Data reported as RFS with no deaths in CR; therefore included as DFS here.

Results shown are for the group B1 patients who received ATRA and ATO during induction; the total cytarabine doses ranged from 4.5-6.3 g, and have been adjusted for an average body surface area of 1.73 m$^2$.

EFS in this series was measured from the date of CR until relapse or death, and therefore listed here as DFS.
FIGURE LEGENDS

Figure 1. Freedom from relapse.
(A) All patients who commenced consolidation (n=112). (B). Stratification by Sanz risk category.

Figure 2. Kaplan-Meier survival curves.
(A) FFS. (B) OS. (C) FFS stratified by Sanz risk category.

Figure 3. Comparison of APML4 with APML3 (historical control).
(A) FFR. (B) DFS. (C) FFS.

Figure 4. Impact of FLT3 mutations on APML4 outcomes.
(A) OS. (B) FFR.
Figure 1

A

% relapse-free

100
80
60
40
20
0

Years from achievement of HCR

112
73
48
24
11
0

Number at risk

APML4

2 relapses in 112 patients

2-year relapse-free rate: 97.5%, 95% CI: 90.4% - 99.4%

B

% relapse-free

100
80
60
40
20
0

Years from achievement of HCR

Low

Intermediate

High

P-value (trend) = 0.17, 2-year relapse-free rate: 100%, 98%, 92%

Number at risk

Low 31 19 10 6 2 0
Inter 60 43 29 13 7 0
High 20 10 8 5 2 0
Figure 2

A

% alive and failure-free

APML4

14 failures in 124 patients
2-year FFS: 88.1%, 95% CI: 80.7% - 92.8%

Number at risk

Years from start of ATRA therapy

124 77 51 26 11 1

B

% surviving

APML4

7 deaths in 124 patients
2-year OS: 93.2%, 95% CI: 85.8% - 96.8%

Number at risk

Years from start of ATRA therapy

124 86 57 28 12 1

C

% alive and failure-free

Low
Intermediate
High

P-value (trend) = 0.03; 2-year FFS: 97%, 88%, 76%

Number at risk

Years from start of ATRA therapy

Low 32 26 10 7 2 0
Intermediate 45 31 13 7
High 24 11 9 6 2 1
Figure 3

A

% relapse-free

APML4

APML3

HR = 0.16, 95% CI: 0.03 - 0.70, P-value = 0.006
2-year relapse-free rate: 98% vs 87%

Number at risk

Years from achievement of HCR

APML4

APML3

112 73

64 59

48 24

55 42

26 11

0 0

0 6

0 0

B

% alive and relapse-free

APML4

APML3

HR = 0.14, 95% CI: 0.03 - 0.62, P-value = 0.003
2-year DFS: 98% vs 86%

Number at risk

Years from achievement of HCR

APML4

APML3

112 73

64 59

48 24

55 42

26 11

0 0

0 6

0 0

C

% alive and failure-free

APML4

APML3

HR = 0.43, 95% CI: 0.22 - 0.84, P-value = 0.01
2-year FFS: 88% vs 74%

Number at risk

Years from start of ATRA therapy

APML4

APML3

124 77

70 57

51 26

52 41

25 11

1 0

0 7
Figure 4

A

![Graph A](image)

No FLT3 mutation

FLT3 mutation

HR = 0.94, 95% CI: 0.21 - 4.19, P-value = 0.93

2-year OS: 93% vs 93%

B

![Graph B](image)

No FLT3 mutation

FLT3 mutation

HR = 1.35, 95% CI: 0.08 - 21.6, P-value = 0.83

2-year relapse-free rate: 98% vs 97%
All-trans-retinoic acid, idarubicin, and intravenous arsenic trioxide as initial therapy in acute promyelocytic leukemia (APML4)

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