Results of the APML3 trial incorporating all-*trans*-retinoic acid and idarubicin in both induction and consolidation as initial therapy for patients with acute promyelocytic leukemia

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ABSTRACT

Background

Initial therapy for patients with acute promyelocytic leukemia most often involves the combination of all-*trans*-retinoic acid with anthracycline-based chemotherapy. The role of non-anthracycline drugs in induction and consolidation is less well-established and varies widely between different cooperative group protocols.

Design and Methods

In an attempt to minimize relapse and maximize survival for patients with newly diagnosed acute promyelocytic leukemia, the Australasian Leukaemia and Lymphoma Group utilized all-*trans*-retinoic acid and idarubicin as anti-leukemic therapy for both induction and consolidation. The protocol (known as APML3) was subsequently amended to incorporate maintenance with all-*trans*-retinoic acid, methotrexate and 6-mercaptopurine.

Results

Eight (8%) of 101 patients died within 30 days, and 91 (90%) achieved complete remission. With a median estimated potential follow-up of 4.6 years, 4-year overall survival was 84%, and 71% of the patients remained in remission at 4 years. The cumulative incidence of all relapses was 28.1%, with 15 of the 25 relapses initially identified as an isolated molecular relapse. Both *FLT3* mutations (internal tandem duplications and codon 835/836 kinase domain mutations) and increased white cell count at diagnosis were associated with inferior overall survival, but in multivariate analyses only *FLT3* mutations remained significant (hazard ratio 6.647, P=0.005). Maintenance therapy was significantly associated with improved remission duration (hazard ratio 0.281, P<0.001) and disease-free survival (hazard ratio 0.290, P<0.001).

Conclusions

The combination of all-*trans*-retinoic acid and just two cycles of idarubicin followed by triple maintenance produced durable remissions in most patients, but patients with high-risk disease, especially those with *FLT3* mutations, require additional agents or alternative treatment approaches. The significant reduction in relapse seen after the addition of maintenance to the protocol supports a role for maintenance in the context of relatively low chemotherapy exposure during consolidation. *(actr.org.au identifier: ACTRN12607000410459)*

Key words: ATRA, idarubicin, induction, consolidation, acute promyelocytic leukemia.

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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-RARα).[1] This transcription factor disrupts normal myeloid differentiation programs, but at the same time imparts a unique sensitivity of APL cells to pharmacological doses of all-*trans*-retinoic acid (ATRA),[2] such that that this drug is now an indispensable component of anti-leukemic therapy in APL. The combination of ATRA with chemotherapy has achieved remarkable improvements in the long-term outcome of patients with APL,[3-6] but precisely what constitutes optimal chemotherapy remains contentious. While it has been argued that outcomes are not improved by the addition of multiple chemotherapy agents to a combination of idarubicin and mitoxantrone,[7] comparison of the European APL2000 and the PETHEMA LPA99 protocols suggests that patients with a white cell count (WCC) of 10×10^9/L or higher may benefit when cytarabine is added to daunorubicin during induction and consolidation.[8]

In 1997, the Australasian Leukaemia and Lymphoma Group (ALLG) initiated the APML3 protocol which used GIMEMA-style induction with ATRA and four doses of idarubicin (based on the AIDA0493 protocol[4]), but the protocols differed in their approach to consolidation. Whereas the AIDA protocol incorporated varying combinations of idarubicin, cytarabine, mitoxantrone, etoposide and 6-thioguanine in three cycles of consolidation, the ALLG exploited the perceived superiority of anthracyclines over other chemotherapeutic agents in APL by using another four doses of idarubicin in one chemotherapy consolidation cycle. In order to further reduce minimal residual disease[9] without inducing resistance,[10] additional ATRA was then administered on an intermittent basis in three 2-week cycles for all patients in hematologic remission. The initial protocol involved regular molecular monitoring without further therapy, but it was subsequently amended to include 2 years of triple maintenance with ATRA, methotrexate and 6-mercaptopurine following the demonstrated benefit of this approach in the European APL93 trial.[11] In this report we describe mature outcome data for the ALLG APML3 trial, with particular emphasis on: (i) the prognostic significance of pre-treatment variables, (ii) the anti-leukemic activity of only two cycles of idarubicin in combination with ATRA, and (iii) the impact of maintenance therapy on remission duration.

Design and Methods

Eligibility

This single arm trial was approved by human research ethics committees in all participating centers, and accrued patients between August 1997 and October 2002. Inclusion criteria were a morphological diagnosis of *de novo* APL according to French-American-British (FAB) criteria, demonstration of *PML-RARA* fusion transcripts by reverse transcriptase-polymerase chain reaction (RT-PCR) or cytogenetic demonstration of a t(15;17) translocation, age 18 years or over, left ventricular ejection fraction greater than 50%, a negative pregnancy test in females of childbearing age, written informed consent prior to the commencement of study drugs, and absence of serious cardiac, pulmonary, hepatic or renal disease. Patients with genetic variants of APL (*X-RARA* where X was not *PML*) were ineligible for the study.

Outline of therapy

Induction consisted of: (i) ATRA 45 mg/m^2/day in divided doses (capped at 80 mg/day) from day 1 until complete remission, (ii) idarubicin 12 mg/m^2 i.v. on days 2, 4, 6 and 8 (9 mg/m^2 for patients aged 61-70 years, and 6 mg/m^2 for patients aged ≥ 71 years), and (iii) oral prednisone[12] 50 mg/day if the WCC exceeded 10×10^9/L at diagnosis or following initiation of therapy, or if clinical features of APL differentiation syndrome appeared; prednisone was continued until the WCC fell below 10×10^9/L and/or differentiation syndrome resolved. Supportive care was according to local institutional practice. Following hematologic recovery, a second identical cycle of idarubicin without prednisone was administered unless contraindicated by cardiac dysfunction, in which case a non-anthracycline "7/7" protocol was used (7 days each of continuous intravenous infusion cytarabine 100 mg/m^2/day, and etoposide 75 mg/m^2/day i.v.). For patients whose marrow showed hematologic or cytogenetic evidence of incomplete remission prior to the second cycle of chemotherapy, ATRA was continued until hematologic recovery after cycle 2. Following the second cycle of chemotherapy, all patients received three cycles of intermittent ATRA (iATRA) 45 mg/m^2/day, given for the first 2 weeks in each 4-week cycle. Bone marrow morphology, cytogenetics, and RT-PCR for *PML-RARA* were performed after each chemotherapy cycle, and after completion of the third cycle of iATRA. Subsequent assessments were made every 3 months for 18 months, then every 6 months for 18 months. The protocol was amended in June 2000 to include 2 years of maintenance with (i) 2 weeks of ATRA 45 mg/m^2/day (maximum 80 mg/day) every 3 months, (ii) oral methotrexate 15 mg/m^2 once weekly, and (iii) oral 6-mercaptopurine 90 mg/m^2/day. Methotrexate and 6-mercaptopurine doses were adjusted for excessive myelosuppression and hepatotoxicity.

Molecular monitoring

The majority of bone marrow samples were submitted to a central laboratory (Royal Prince Alfred Hospital) either fresh, or processed in Trizol reagent (Life Technologies) following Ficoll-Hypaque density centrifugation for isolation of mononuclear cells. Peripheral blood was only used for diagnostic samples when bone marrow was not available. A minority of marrow samples underwent RNA extraction at the local center or were cryopreserved in dimethylsulfoxide prior to submission to the central laboratory. Samples from New Zealand were analyzed at Auckland Hospital, but when sufficient RNA was available, they were subsequently reanalyzed centrally. Overall, 97% of 958 informative samples were analyzed at the central laboratory. A semi-nested qualitative RT-PCR protocol with a sensitivity of at least 10^{-4} was employed for prospective molecular monitoring. Total RNA was heated at 65°C for 5 min prior to cDNA synthesis at 42°C for 2 h with a final RNA concentration of 50 ng/μL in 1x PCR buffer II (Applied Biosystems), 1 mM dNTP mix (Fisher Biotech), 5 mM $MgCl_2$, 2.5 μM random hexamer primers (Geneworks), 1 U/μL RNasin (Promega) and 2.5 U/μL RNase H$^-$ MMLV reverse transcriptase (Promega). PCR primers used were M2, M4, R5 and R8[13] and RARE33B (5'- ACA AAG CAA GGC TTG TAG ATG CGG-3'). All PCR amplifications were in 1x PCR buffer II, 1.5 mM $MgCl_2$, 200 μM dNTP mix, 140 nM of each primer and 0.03 U/μL AmpliTaq Gold DNA polymerase (Applied Biosystems). Cycling conditions for each reaction were 95°C for 5 min followed by 45 cycles of 30 s at 60°C, 45 s at 72°C and 30 s at 95°C. *PML-RARA* bcr1 and bcr2 transcripts were detected from 5 μL of cDNA by PCR with primers M2 and R5 in the first round followed by a second round

PCR from 1 μL of template with primers M2 and R8. *PML-RARA* bcr3 transcripts were similarly detected, with M4 and RARE33B primers in the first round and M4 and R8 in the second round. *PML-RARA* transcripts were also quantified retrospectively on archival diagnostic RNA samples using a one-step, RT-PCR DNAzyme-mediated reaction.[14]

FLT3 *mutational status*

Expression of mutant *FLT3* transcripts was assessed for both internal tandem duplications (ITD) and codon 835/836 kinase domain mutations. Published primers were used to amplify the region of the *FLT3* transcript coding for the juxtamembrane domain.[15] High resolution analysis of fluorescently labeled PCR products was performed by electrophoresis on denaturing polyacrylamide gels using an ABI 373 DNA sequencer and Genescan 672 software. Cleavage of 100 bp from the 5' end of both normal size (wild-type) and length mutant (ITD) amplicons by *DraI* digestion was used to confirm their origin from *FLT3* transcripts. Selected *FLT3* PCR products bearing ITD were cloned and sequenced to further confirm their identity (*data not shown*). To identify *FLT3* codon 835/836 mutations, cDNA encoding exon 20 was amplified and digested with *Eco*RV, whose recognition sequence (GATATC) encompasses codons 835 and 836. Digestion resistant bands were sequenced to identify the nature of the codon 835/836 mutation.

Definitions and study end-points

Hematologic complete remission required: (i) absence of symptoms and signs relating to leukemia; (b) peripheral blood absolute neutrophil count of 1.5×10^9/L or greater, and absence of abnormal cells in the peripheral blood differential; (iii) platelet count of at least 100×10^9/L; (iv) disappearance of abnormal promyelocytes and blast cells, and return of active normal hematopoiesis; and (v) absence of the t(15;17) cytogenetic abnormality in a bone marrow karyotype analysis, provided this abnormality had been demonstrable in the pre-treatment sample. Molecular complete remission was defined as absence of *PML-RARA* transcripts assessed by RT-PCR in a qualitative assay with a sensitivity of at least 10^{-4}. Relapse was defined as either: (i) reappearance of abnormal blast cells and/or promyelocytes as described above (hematologic relapse); (ii) return of t(15;17) cytogenetic abnormality (cytogenetic relapse); or (iii) reversion to *PML-RARA* positivity following previously documented RT-PCR negativity (molecular relapse). Endpoint definitions were as follows: overall survival, time from commencement of ATRA therapy to death from any cause; remission duration, time from documented complete remission to the first occurrence of hematologic, cytogenetic or molecular relapse; disease-free survival, time from documented complete remission to the earliest of relapse or death from any cause; failure-free survival, time from commencement of therapy to relapse, death from any cause, or off-protocol before completion of the third cycle of iATRA due to failure to achieve remission, excessive toxicity, or patient's refusal.

Statistical methods

Categorical factors at baseline which were investigated for their prognostic value included WCC, platelet count, Sanz risk stratification,[7] *PML* breakpoint (bcr1 *versus* bcr2 *versus* bcr3), and *FLT3* status (wild-type *versus* ITD and/or codon 835/836 mutations). Continuous-scale factors included age and log-transformed normalized *PML-RARA* transcripts. A maintenance-cohort factor which indexed patients according to whether they were registered before or after activation of the maintenance amendment enabled an intention-to-treat (ITT) analysis of post-remission maintenance as a prognostic factor. Given that some patients who were registered prior to the amendment did receive maintenance, and some patients registered after the amendment did not, analysis of the association of actual post-remission maintenance with survival was also conducted; in this analysis separate indicator variables for ATRA, 6-mercaptopurine and methotrexate maintenance administration in each 3-month period after consolidation were included as time-dependent covariates.

The association of baseline prognostic variables with both the incidence of early death and the achievement of complete remission were investigated using binary logistic regression. For time-to-event endpoints of survival and duration, unifactor associations of categorical prognostic factors were investigated with the log-rank test while unifactor associations of continuous-scale covariates and all multifactor analyses were investigated via Cox proportional hazards regression and the score test. Competing events, such as relapse and death in complete remission, were analyzed by estimating cumulative incidences using a competing risks analysis. Unless otherwise stated, all statistical tests were two-tailed tests without adjustment for multiple comparisons, and were carried out using SAS (version 9.2) and S-Plus software.

Results

Patients' characteristics

Of 107 registered patients, six were excluded because of failure to demonstrate either t(15;17) or *PML-RARA* transcripts at diagnosis, leaving 101 patients from 26 institutions in Australia and New Zealand eligible for analysis of both efficacy and toxicity (Table 1). With a study close-out (censor) date of 19 April 2005, the estimated median potential follow-up time using the reverse Kaplan-Meier method was 4.6 years (95% CI: 4.3 - 5.1 years). A strong association of *FLT3* mutation status with both WCC at diagnosis and *PML* breakpoint location was observed (Table 2). *FLT3* mutation-positive patients, especially those with ITD, were more likely to have a WCC greater than 10×10^9/L ($P<0.0001$) and a bcr3 *PML* breakpoint ($P<0.0001$).

Early deaths

Eight patients (8%) died within 30 days of study entry. Causes of death were usually multifactorial, and included hemorrhage (7 patients, with 4 intracerebral, 2 pulmonary, and 1 gastrointestinal), differentiation syndrome (4 patients), sepsis (3 patients), multi-organ failure (3 patients), and necrotizing enteritis (1 patient). Differentiation syndrome was a major contributing factor to the deaths of two patients. The WCC at diagnosis was strongly associated with an increased risk of early death, with a cut-off of 2.5×10^9/L being the most discriminatory count; no early deaths occurred among the 55 patients (0%) with a WCC of 2.5×10^9/L or lower, whereas 8 of 46 patients (17%) with a WCC above 2.5×10^9/L died within 30 days ($P<0.002$). The platelet count at diagnosis, age, and *FLT3* mutations were not associated with early death, nor was WCC at diagnosis when a cut-off of 10×10^9/L was used.

Remission

(i) Hematologic complete remission

Of 93 patients who survived more than 30 days, two were taken off study because of failure to achieve hematologic complete remission. One patient was withdrawn prematurely at day 18 because of persistent disseminated

intravascular coagulation; a progress marrow aspirate showed 11% blasts and 38% promyelocytes, with 100% abnormal metaphases. The other patient was assessed at day 36; a marrow aspirate produced only a blood tap, but contained numerous abnormal promyelocytes. Cytogenetics showed 60% abnormal metaphases, and interphase fluorescence *in situ* hybridization showed t(15;17) in 33% of cells. The remaining 91 patients (90%) achieved complete remission with protocol therapy (95% CI, 83-95%), including four who received 7/7 cytarabine/etoposide in the second cycle. Seventy-three patients (72%) achieved complete remission after the first cycle of idarubicin, and 18 (18%) after the second cycle of chemotherapy. Failure to achieve complete remission after cycle 1 was determined at the treating institution on the basis of morphology or by the persistence of t(15;17). The WCC at diagnosis was associated with the achievement of complete remission; 100% of the 55 patients with a WCC of 2.5×10^9/L or below achieved complete remission compared to 78% of the 46 patients with a WCC greater than 2.5×10^9/L (P=0.0002).

(ii) Molecular complete remission

After the first cycle of idarubicin, molecular results for *PML-RARA* were available for 79 patients (including the 2 who were withdrawn because of persistent leukemia). Only 24 were negative (molecular complete remission rate 30%, 95% CI±10.1%). After the second cycle of chemotherapy, 69 of 74 patients tested had achieved molecular compete remission (93%, 95% CI±5.7%). Of the 85 patients who completed three cycles of iATRA, all of the 81 patients tested were RT-PCR negative (molecular complete remission 100%); the four patients not tested were already in molecular remission before commencing iATRA. Thus there was no evidence of minimal residual disease in patients who completed induction and consolidation therapy.

(iii) Remission duration

Of the 91 patients who achieved complete remission, 25 (27%) relapsed. In 15 patients, a confirmed molecular relapse preceded cytogenetic or hematologic relapse, and enabled commencement of salvage therapy before frank leukemic recurrence. The cumulative incidence of any form of relapse (molecular, cytogenetic, or hematologic) at 4 years was 28.1% (SE±4.8%), and the Kaplan-Meier estimate of the proportion of patients remaining in remission at 4 years was 71.2% (95% CI: 60.4-79.6%). Both unifactor and multifactor analyses indicated a significant difference in remission duration between the ITT maintenance cohorts (HR=0.281, 95% CI: 0.128–0.619, P<0.001, 4-year remission duration: 48.7% *versus* 80.1%, Figure 1A). None of the remaining prognostic factors was statistically significant; however, an increase in the risk of relapse in patients with a bcr2 *PML* breakpoint relative to bcr1 was observed (Table 3).

Eighty-five of the 91 patients who achieved complete remission completed three cycles of iATRA within 145 to 230 days from the time treatment commenced. Accordingly, a landmark analysis of remission duration restricted to these 85 patients, and measured from day 231, was also performed. Both the ITT maintenance factor (HR=0.296, 95% CI: 0.132–0.665, P=0.003) and the treating hematologist's determination of whether complete remission was achieved after the first or second cycle of idarubicin (HR=2.581, 95% CI: 1.097–6.075, P=0.030) were statistically significantly associated with remission duration in multifactor analysis (Table 3 and Figure 1B).

Table 1. Baseline characteristics of eligible patients (n = 101 for each characteristic unless otherwise indicated).

Characteristic	Median (Range)	Number (%)
Age	40 years (19 – 73)	
Sex		
Male		53 (52)
Female		48 (48)
ECOG status		
0		52 (51)
1		32 (32)
2		13 (13)
3		3 (3)
4		1 (1)
FAB classification		
M3		81 (80)
M3v		20 (20)
White cell count	2.4×10^9/L (0.4-109)	
Platelet count	24×10^9/L (4-180)	
Sanz risk category		
Low		26 (26)
Intermediate		52 (51)
High		23 (23)
PML breakpoints (n = 97)		
bcr1		52 (54)
bcr2 (confirmed by sequencing)		11 (11)
bcr3		34 (35)
Karyotype (n = 97)		
t(15;17)		64 (66)
t(15;17) + additional abnormalities		30 (31)
Normal		3 (3)
FLT3 (n = 90)		
ITD		30 (33)
D835/I836 mutation		8 (9)
ITD + D835/I836 mutation		2 (2)
Wild type		50 (56)
PML-RARA:BCR (%) (n = 71)	258 (16-4320)	

Table 2. Relationship between *FLT3* mutation status, WCC at diagnosis, and *PML* breakpoint location.

FLT3 mutation status	Presenting WCC (x 10^9/L)			*PML* breakpoint		
	≤ 10	> 10	*P* value	bcr1 or bcr2	bcr3	*P* value
ITD						
Positive	18 (56%)	14 (44%)	< 0.0001	8 (25%)	24 (75%)	< 0.0001
Negative	54 (93%)	4 (7%)		50 (86%)	8 (14%)	
Codon 835/836						
Positive	6 (60%)	4 (40%)	0.11	4 (40%)	6 (60%)	0.16
Negative	66 (82%)	14 (18%)		54 (68%)	26 (32%)	
ITD and/or codon 835/836						
Positive	24 (60%)	16 (40%)	< 0.0001	12 (30%)	28 (70%)	< 0.0001
Negative	48 (96%)	2 (4%)		46 (92%)	4 (8%)	

Survival

(i) Disease free survival

In addition to the 25 relapses, two patients died in complete remission as a result of complications of the second cycle of idarubicin (one from suspected cardiac arrhythmia, one with intracerebral fungal infection and hemorrhage). The estimated 4-year disease-free survival was 69.7% (95% CI: 58.9–78.1%). Multifactor analysis indicated that ITT maintenance cohort was the only statistically significant prognostic factor associated with disease-free survival (HR=0.290, 95% CI: 0.136–0.620, $P<0.001$, 4-year disease-free survival: 46.9% *versus* 78.9%, Table 3). In addition, disease-free survival was reduced in patients with a bcr2 *PML* breakpoint relative to those with bcr1 (Table 3).

(ii) Overall survival

Prior to the close-out date there were a total of 16 deaths (8 early deaths, 2 deaths in complete remission, 5 patients died after relapsing, and 1 patient was withdrawn from the study after induction because of resistant leukemia and died during salvage therapy). The 4-year actuarial

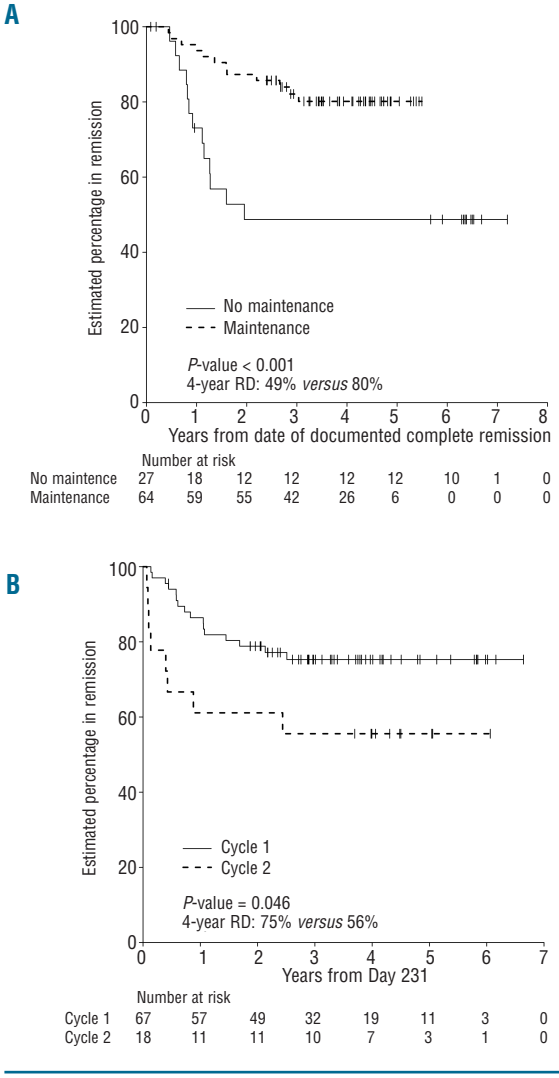

Figure 1. (A) Remission duration (RD) stratified by maintenance amendment. (B) RD from day 231 according to the cycle when complete remission was achieved.

Table 3. Multifactor Cox regression analyses for survival and remission duration end-points (the best fitting model for each end-point includes variables with *P* value < 0.05; for non-significant prognostic factors, results are those on omission from the best model).

	Hazard Ratio	95% CI	*P* value
Remission duration			
Maintenance cohort (No maintenance : Maintenance)	0.281	0.128, 0.619	<0.001
FLT3 (wildtype : 835/836 mutation or ITD)	1.681	0.711, 3.974	0.237
Risk stratification (Low : Intermediate)	1.302	0.462, 3.665	
Risk stratification (Low : High)	2.342	0.743, 7.383	0.295
Risk stratification (Intermediate : High)	1.799	0.715, 4.528	
PML breakpoint (bcr1 : bcr2)	2.967	1.084, 8.124	
PML breakpoint (bcr1 : bcr3)	1.013	0.403, 2.546	0.076
PML breakpoint (bcr2 : bcr3)	0.342	0.116, 1.009	
log[*PML-RARA*]	0.955	0.705, 1.293	0.766
Remission duration after day 231			
Maintenance cohort (No maintenance : Maintenance)	0.296	0.132, 0.665	0.003
CR achievement (Cycle 1 : Cycle 2)	2.581	1.097, 6.075	0.030
FLT3 (wildtype : 835/836 mutation or ITD)	2.404	0.918, 6.291	0.074
Risk stratification (Low : Intermediate)	1.618	0.524, 4.994	
Risk stratification (Low : High)	4.379	1.197, 16.02	0.057
Risk stratification (Intermediate : High)	2.706	0.991, 7.391	
PML breakpoint (bcr1 : bcr2)	2.139	0.716, 6.387	
PML breakpoint (bcr1 : bcr3)	1.162	0.449, 3.004	0.391
PML breakpoint (bcr2 : bcr3)	0.544	0.166, 1.780	
log[*PML-RARA*]	0.958	0.706, 1.301	0.785
Disease-free survival			
Maintenance cohort (No maintenance : Maintenance)	0.290	0.136, 0.620	<0.001
FLT3 (wildtype : 835/836 mutation or ITD)	1.970	0.860, 4.511	0.109
Risk stratification (Low : Intermediate)	1.293	0.459, 3.638	
Risk stratification (Low : High)	2.949	0.987, 8.807	0.081
Risk stratification (Intermediate: High)	2.281	0.971, 5.359	
PML breakpoint (bcr1 : bcr2)	2.834	1.037, 7.747	
PML breakpoint (bcr1 : bcr3)	1.283	0.540, 3.050	0.123
PML breakpoint (bcr2 : bcr3)	0.453	0.161, 1.270	
log[*PML-RARA*]	1.008	0.755, 1.345	0.959
Overall survival			
Maintenance cohort (No maintenance : Maintenance)	1.529	0.413, 5.667	0.525
FLT3 (wildtype : 835/836 mutation or ITD)	6.647	1.456, 30.34	0.005
Risk stratification (Low : Intermediate)	1.031	0.199, 5.346	
Risk stratification (Low : High)	1.410	0.254, 7.820	0.873
Risk stratification (Intermediate : High)	1.368	0.377, 4.971	
PML breakpoint (bcr1 : bcr2)	0.001	0.000, undefined*	
PML breakpoint (bcr1 : bcr3)	0.634	0.173, 2.327	0.790
PML breakpoint (bcr2 : bcr3)	undefined*	0.000, undefined*	
log[*PML-RARA*]	0.743	0.481, 1.148	0.181
Failure-free survival			
Maintenance cohort (No maintenance : Maintenance)	0.425	0.227, 0.797	0.008
FLT3 (wildtype : 835/836 mutation or ITD)	1.330	0.592, 2.986	0.490
Risk stratification (Low : Intermediate)	1.090	0.472, 2.516	
Risk stratification (Low : High)	2.552	1.067, 6.100	0.030
Risk stratification (Intermediate : High)	2.341	1.161, 4.724	

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PML breakpoint (bcr1 : bcr2)	1.757	0.674, 4.578	
PML breakpoint (bcr1 : bcr3)	0.890	0.394, 2.010	0.416
PML breakpoint (bcr2 : bcr3)	0.507	0.176, 1.456	
log[*PML-RARA*]	0.924	0.707, 1.207	0.560

** As there were no deaths in patients with bcr2 breakpoints, the hazard ratio and confidence intervals cannot be adequately defined.*

overall survival was 83.7% (95% CI: 74.8–89.7%). In unifactor analyses, both *FLT3* mutations and increasing WCC were associated with decreased overall survival, but only *FLT3* mutation status remained statistically significant in multifactor analysis (HR=6.647, 95% CI: 1.456–30.34, *P*=0.005, 4-year overall survival: 96.0% *versus* 74.4%, Table 3 and Figure 2). When considered separately in unifactor analysis, both *FLT3* ITD (HR=3.769, 95% CI: 1.135–12.52, *P*=0.020) and codon 835/836 mutations (HR=4.397, 95% CI: 1.322–14.63, *P*=0.008) were significantly correlated with overall survival.

(iii) Failure free survival

Among the 101 eligible patients, a total of 40 treatment failures occurred. In a competing risks analysis, the cumulative incidences of death, relapse, and off-protocol before either death or relapse were 8.9% (SE±2.8%), 24.4% (SE±4.4%), and 6.9% (SE±2.5%) respectively. Four-year failure-free survival was 59.7% (95% CI, 49.3% – 68.7%), and multifactor analyses indicated that ITT maintenance therapy was associated with a reduced risk of failure (HR=0.425, 95% CI: 0.227–0.797, *P*=0.008, 4-year failure-free survival 40.9% *versus* 67.7%) along with Sanz risk stratification (*P*=0.030) (Table 3).

Additional analysis with time-dependent covariates for actual post-remission maintenance

Additional exploratory analysis of remission duration, disease-free survival and failure-free survival with time-dependent covariates indicated that actual post-remission ATRA, 6-mercaptopurine and methotrexate maintenance were all statistically significant in unifactor analyses while only ATRA remained statistically significant in multifactor analyses (*P*<0.001). The best multifactor model for each of remission duration, disease-free survival and failure-free survival considering time-dependent maintenance covariates is shown in Table 4. In comparison with Table 3, *FLT3* mutation status and *PML* bcr2 breakpoint location are additional prognostic factors statistically significantly associated with remission duration and disease-free survival. As the number of patients with bcr2 breakpoints was small, the relevance of this latter observation is uncertain.

Discussion

The APML3 protocol accrued patients between 1997 and 2002. Our strategy of utilizing idarubicin as the only chemotherapy agent for both induction and consolidation most closely resembles the PETHEMA group's approach, as typified by LPA96 and LPA99.[16] These PETHEMA protocols were modeled on the GIMEMA AIDA0493 protocol,[4] but omitted non-anthracycline/anthraquinone drugs from all three cycles of consolidation. Although the total anthracycline/anthraquinone exposure in APML3 (idarubicin 96 mg/m^2) is considerably less than that used in AIDA0493 and LPA96 (idarubicin 80 mg/m^2 and mitoxantrone 50 mg/m^2), the complete remission, disease-free survival and overall survival rates are broadly comparable, provided the comparison is restricted to the cohort of APML3 patients who were registered after the maintenance amendment. For example, the cumulative incidence of relapse in the APML3 maintenance cohort was 19.6% at 4 years, compared with 17.2% at 3 years in the LPA96 trial[16] and 27.7% at 6 years in the AIDA0493 trial.[18] One possible explanation for the comparability of results despite the reduced chemotherapy in APML3 is our use of intermittent ATRA in consolidation. The subsequent PETHEMA LPA99 protocol adopted a similar approach for intermediate- and high-risk patients, together with increased anthracycline therapy (idarubicin 100 mg/m^2 and mitoxantrone 50 mg/m^2), and that strategy was associated with a significant improvement in overall cumulative incidence of relapse (11% at 4 years).[17]

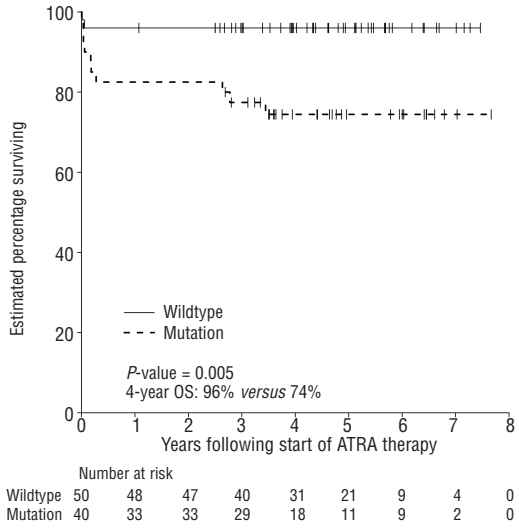

Figure 2. Overall survival (OS) by *FLT3* mutation status.

At the time APML3 was initiated, the benefits of maintenance therapy had not been demonstrated in a randomized fashion, and hence maintenance was not originally included. However the clear benefits of maintenance reported in the European APL93 study,[11] and in the North American Intergroup APL protocol,[19] persuaded the ALLG to amend APML3 in an attempt to reduce what was emerging as an unacceptably high relapse rate. The two approaches examining the impact of maintenance in APML3 that are reported here (an ITT analysis and an exploratory analysis in which actual post-remission maintenance components were treated as time-dependent covariates) both demonstrate statistically significant improvements in remission duration, disease-free survival and failure-free survival, in both univariate and multivariate analysis. In contrast to the European APL93 and the North American Intergroup randomized studies, the GIMEMA AIDA0493 study failed to show an improvement associated with maintenance;[20] this difference has been attributed to the more intensive chemotherapy employed in the AIDA0493 study. Since APML3 also utilized less total chemotherapy than AIDA0493, it is not sur-

Table 4. Best fitting multifactor Cox regression models considering time-dependent maintenance covariates for remission duration, disease-free survival and failure-free survival.

	Hazard Ratio	95% CI	*P* value
Remission duration			
Maintenance ATRA	0.050	0.011, 0.234	<0.001
FLT3 (wildtype : 835/836 mutation or ITD)	4.550	1.499, 13.81	0.007
PML breakpoint (bcr1 : bcr2)	2.579	0.833, 7.979	
PML breakpoint (bcr1 : bcr3)	0.350	0.100, 1.226	0.018
PML breakpoint (bcr2 : bcr3)	0.136	0.034, 0.544	
Disease-free survival			
Maintenance ATRA	0.054	0.012, 0.250	<0.001
FLT3 (wildtype : 835/836 mutation or ITD)	4.252	1.462, 12.37	0.008
PML breakpoint (bcr1 : bcr2)	2.448	0.795, 7.535	
PML breakpoint (bcr1 : bcr3)	0.482	0.149, 1.562	0.047
PML breakpoint (bcr2 : bcr3)	0.197	0.054, 0.721	
Failure-free survival			
Maintenance ATRA	0.079	0.023, 0.271	<0.001
Risk stratification (Low : Intermediate)	1.126	0.489, 2.593	
Risk stratification (Low : High)	2.589	1.084, 6.182	0.032
Risk stratification (Intermediate : High)	2.300	1.140, 4.641	

prising that maintenance had a significant effect on the APML3 relapse rate.

The adverse prognostic impact of *FLT3* mutations in non-APL acute myeloid leukemia is well documented,[21-24] especially in the context of normal karyotype acute myeloid leukemia. In APL, data supporting an adverse impact of *FLT3* mutations are less consistent,[25-30] despite the high prevalence of these mutations. In the present study, as in previous ones, *FLT3* mutations were closely correlated with bcr3 breakpoints and high WCC at diagnosis. We found a significant correlation with overall survival, and also observed that the association persisted when ITD and codon 835/836 mutations were assessed separately. *FLT3* mutations also emerged as important predictors of remission duration and disease-free survival in multivariate analyses when maintenance components were treated as time-dependent covariates. Our data are in close agreement with those of a large meta-analysis[29] of *FLT3* mutations in 1063 APL patients. In that analysis, *FLT3* ITD were significantly associated with both overall and disease-free survival, and a trend towards adverse outcomes was also observed for patients with codon 835 mutations (although only two studies reported codon 835 data). A subsequent report[30] of *FLT3* mutations in the PETHEMA group's LPA96 and LPA99 trials also showed inferior overall and relapse-free survival for patients with ITD in univariate analysis, but ITD were not significant in multivariate analysis because of their close relationship with high WCC. However, the PETHEMA report included ITD and codon 835 data for only 41% and 29% of the patients, respectively. Although the number of patients in the present report is relatively small, *FLT3* mutation data were available for 89% of the study population and, therefore, selection bias is unlikely to be a factor in the adverse impact of *FLT3* mutations that we observed. Whether the addition of *FLT3* inhibitors would influence outcomes is unknown, especially as the adverse impact of *FLT3* mutations is less likely to be apparent when protocols which employ more intensive chemotherapy or arsenic trioxide are used.

The 2009 European LeukemiaNet guidelines for APL[31] do not recommend cytogenetic or molecular analysis for determination of response after induction, and caution against morphological diagnosis of incomplete remission due to delayed maturation because virtually all patients treated with AIDA-style induction protocols eventually achieve complete remission. Two patients in the current study were withdrawn because of "failure to achieve complete remission", and with the benefit of hindsight those withdrawals were probably inappropriate. Similarly, those patients who were deemed not to have achieved morphological complete remission after the first cycle of idarubicin may have been assessed prematurely, since they all achieved molecular complete remission by the end of consolidation, consistent with the European LeukemiaNet recommendations. Nevertheless, our observation of a shorter remission duration in these patients based on a landmark analysis at the end of consolidation is of interest, since it highlights a potentially important difference in long-term outcome in patients with delayed maturation, and indirectly suggests that more effective induction regimens that achieve a greater degree of early leukemic cytoreduction might be associated with better relapse-free survival.

In summary, the APML3 protocol shows that an induction and consolidation strategy that combines ATRA with relatively low anthracycline exposure (delivered in only two chemotherapy cycles), followed by 2 years of triple maintenance, is capable of achieving prolonged disease-free survival in the majority of patients. However, high risk subgroups (e.g. patients with *FLT3* mutations and/or high WCC) require either more intensive therapy,[17] or therapy with alternative anti-leukemic agents such as arsenic trioxide.[32,33] The recently completed ALLG APML4 protocol has combined arsenic trioxide with ATRA and idarubicin for induction, and uses only ATRA and arsenic trioxide without further anthracycline for consolidation (total idarubicin exposure only 48 mg/m^2). Preliminary data indicate that this strategy has been highly successful in reducing relapses and improving long-term disease free survival[34] (and *unpublished data*).

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

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