The significance of minimal residual disease kinetics in adults with newly diagnosed PML-RARα-positive acute promyelocytic leukemia: results of a prospective trial

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Seventy adults with acute promyelocytic leukemia were studied to clarify the significance of the level and kinetics of minimal residual disease (MRD) over their entire treatment course by real-time quantitative polymerase chain reaction. At a median follow-up of 44 months, nine relapses had occurred. The 5-year probabilities of relapse and disease-free survival were 17.3±5.4% and 81.5±5.4%, respectively. A MRD level of $>10^{-3}$ after first consolidation was the most powerful predictor of relapse (85.7±13.2% versus 7.3±4.1%, $p<0.001$) and disease-free survival (14.3±13.2% versus 91.2±4.3%, $p<0.001$). Prospective MRD monitoring may allow us to identify subgroups of patients at high risk of relapse earlier during treatment.

Key words: acute promyelocytic leukemia, minimal residual disease, real-time quantitative polymerase chain reaction.

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Recent improvements in the management of acute promyelocytic leukemia (APL) have increased long-lasting complete remissions and apparent cure rates to approximately 70%.[1-5] However, a proportion of patients continue to die eventually as a result of disease recurrence. Although a number of pretreatment characteristics have been identified that are correlated with an increased risk of relapse,[3,6,7] there is great expectation that minimal residual disease (MRD) monitoring will enable more precise identification of patients who will undergo relapse. Using conventional reverse transcription-polymerase chain reaction (RT-PCR) assays, it was evident that PCR positivity after consolidation completion is a strong predictor of subsequent hematologic relapse[7-13] However, the attainment of PCR negativity does not represent a guaranteed cure because 90% to 95% of patients are PCR-negative at this stage.[2,3,6,12,13] In addition, a number of different approaches (eg, modified RT-PCR, amplification of the reciprocal RARα-PML transcript) did not improve the predictive value of PCR positivity detected at a single point of time after consolidation completion.[3,14] These considerations indicate the need for prospective MRD monitoring during the entire treatment course. According to some reports,[15,16] real-time quantitative PCR (RQ-PCR) assays allow efficient, accurate MRD quantification in APL. We undertook a prospective study to clarify the significance of the level and kinetics of MRD over the entire treatment course by RQ-PCR in adults with newly diagnosed PML-RARα-positive APL.

Design and Methods

Between January 2000 and December 2003, 73 of 76 consecutive adults with APL were considered for this study. Of these, 70 who completed induction therapy,[2] achieved hematologic complete remission and intended to undergo consolidation and maintenance schedules were enrolled in this study. Six patients were excluded because of old age (n=1), refusal (n=2), or death during induction (n=3). All patients provided written informed consent, and the study protocol was approved by the institutional review board of The Catholic University of Korea. Patients received three consolidation courses consisting of the following: course 1, idarubicin (5 mg/m^2, days 1-4); course 2, mitoxantrone (10 mg/m^2, days 1-5); and course 3, idarubicin (12 mg/m^2, day 1). After completion of consolidation, patients received maintenance therapy for 2 years with 6-mercaptopurine (50 mg/m^2/day), methotrexate (15 mg/m^2/week), and all-trans retinoic acid (ATRA; 45 mg/m^2/day for 15 days every 3 months).[4] For MRD monitoring, 762 bone marrow samples were analyzed by RQ-PCR. Samples were collected at diagnosis, after induction, after each course of consolidation, and then at 3, 6, 9, 12, 18, 24, and 36 months after starting maintenance therapy. Total RNA was extracted using an RNAqueous kit (Ambion, Austin, TX, USA), and reverse transcription was performed using 1 μg RNA. Plasmid standard titrations with the defined copy numbers for *PML-RARα* and reference *ABL* were performed at the same time as patients' bone marrow samples were tested. Based on our previous experience,[17,18] we designed one set of primers for each type of *PML-RARα* transcript, forward (5'-GTCTTCCTGCCCAACAGCAACC-3') and reverse (5'-CTCACAGGCGCTGACCCCATAGT-3') for *bcr1* (190 bp), as well as forward (5'-AGCTCTTGCATCACCCAGGGGA-3') and reverse (5'-CTCACAGGCGCTGACCCCATAGT-3') for *bcr3* (165 bp). One set of primers was also designed for *ABL* (forward: 5'-GCCTCAGGGTCTGAGTGAAG-3', reverse: 5'-ACACCATTCCCCATTGTGAT-3'). The TaqMan probes for *PML-RARα* (5'-

Table 1. Presenting characteristics and potential variables in relation to relapse and survival.

Variables		No. (%)	Relapse (% at 5 y)	p Univ.	p Multiv.	DFS (% at 5 y)	p Univ.	p Multiv.	OS (% at 5 y)	p Univ.	p Multiv.
Follow-up, mo., median (range)	44 (20-68+)	70	17.3±5.4			81.5±5.4			88.0±4.8		
Age, y, median (range)	37 (18-59)										
	≤40	46 (65.7)	18.5±6.9	0.845	NT	81.5±6.9	0.804	NT	93.6±4.4	0.109	0.053
	>40	24 (34.3)	15.6±8.5			80.9±8.8			77.6±10.6		
Sex	Male	27 (38.6)	20.8±9.5	0.654	NT	76.2±9.6	0.408	NT	80.1±9.3	0.167	0.828
	Female	43 (61.4)	15.0±6.3			85.0±6.3			94.1±4.1		
Hb, g/dL,	7.5 (3.3-13.0)										
median (range)	≤80	41 (58.6)	22.0±7.5	0.295	NT	78.0±7.5	0.540	NT	85.3±7.0	0.744	NT
	>8.0	29 (41.1)	9.6±6.7			87.3±7.2			93.1±4.7		
WBC,×10^9/L,	2.1 (0.3-51.3)										
median (range)	≤10	63 (90.0)	13.4±5.2	0.002	0.238	86.6±5.2	<0.001	0.068	94.2±4.1	<0.001	0.031
	>10	7 (10.0)	55.6±22.2			38.1±19.9			38.1±19.9		
Platelet, ×10^9/L,	27.5 (4.0-149.0)										
median (range)	≤40	46 (65.7)	17.6±6.7	0.959	NT	82.4±6.7	0.696	NT	94.1±4.2	0.096	0.051
	>40	24 (34.3)	16.7±9.0			79.8±9.3			77.6±10.3		
Fibrinogen, g/L,	1.4 (0.2-7.2)										
median (range)	≤17	40 (57.1)	21.6±8.2	0.331	NT	76.4±8.2	0.232	NT	77.1±9.0	0.017	0.280
	>1.7	30 (42.9)	12.3±6.7			87.7±6.7			100		
Karyotype	t(15;17)/normal	50/2 (74.3)	14.2±6.0	0.211	NT	84.2±6.1	0.317	NT	89.2±5.4	0.697	NT
	Additional	18 (25.7)	26.0±11.3			74.0±11.3			85.6±9.7		
PML-RARα isoform	bcr1/bcr2	44/1 (64.3)	9.4±5.2	0.023	0.305	88.6±5.5	0.057	0.465	94.6±3.8	0.063	0.214
	bcr3	25 (35.7)	33.8±12.3			66.2±12.3			67.1±16.6		
MRD level at diagnosis,	2.83×10^{1}										
median (range)	(3.96×10^{-1}-8.30×10^{2})										
	<10^{1}	24 (34.3)	5.9±5.7	0.162	0.932	94.1±5.7	0.120	0.651	100	0.075	0.392
	≥10^{1}	46 (65.7)	22.7±7.2			75.6±7.3			82.0±7.2		
MRD level after induction*	Undetectable	34 (49.3)	9.2±6.2			90.8±6.2			100		
	<10^{-2}	47 (68.1)	5.8±4.0	0.001	0.628	94.2±4.0	<0.001	0.177	100	<0.001	0.115
	≥10^{-2}	22 (31.9)	44.1±13.2			53.4±12.8			60.0±14.4		
MRD level after C1*	Undetectable	57 (82.6)	7.9±4.4			90.5±4.6			98.2±1.7		
	<10^{-3}	62 (89.9)	7.3±4.1	<0.001	<0.001	91.2±4.3	<0.001	<0.001	98.4±1.6	<0.001	0.003
	≥10^{-3}	7 (10.1)	85.7±13.2			14.3±13.2			0		

Mo: months; y: year; Hb: hemoglobin; WBC: white blood cell count; MRD: minimal residual disease; C1: first consolidation; univ.: univariate; multiv.: multivariate; NT: not tested; DFS: disease-free survival; OS: overall survival. MRD could not be assessed in one patient who had bcr2 transcripts because of poor quality RNA.

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TATCTCTTCAGAACTGCTGCTCTGGGTCTC-3') and *ABL* (5'-AGAGTGTTATCTCCACTGGCCACAAAAT-CA-3') transcripts were designed to hybridize to the reverse strands of each sequence. RQ-PCR was performed in triplicate using iCycler software 2.1 (Bio-Rad, Hercules, CA, USA) with standard conditions (95°C for 10 min, 60 cycles at 95°C for 15 sec, and 60°C for 1 min). The 50-µL PCR reaction mix contained 5 µL 10×PCR buffer (4.5 mM $MgCl_2$, 0.2 mM deoxyribonucleoside triphosphate, 0.4 µM primer, 140 nM *Taq*Man probe, 1.25 U Ampli*Taq* gold DNA polymerase, and 4 µL target cDNA). The quantity of *PML-RARα* transcript was normalized for *ABL* expression (sensitivity, 10^{-4}).

The hematologic and molecular responses were defined using standard criteria.[7,19] Survival duration was calculated from the date of starting induction until death or the last date the patient was known to be alive. When calculating disease-free survival, both hematologic relapses and deaths in complete remission were counted as adverse events. The cumulative relapse rate was calculated using the same type of analysis as used for disease-free survival, except for patients who died in complete remission, who were censored at the time of death. Survival curves were plotted using the Kaplan-Meier method and compared using the log-rank test. The prognostic significances of variables a *p* value less than 0.20 in univariate analysis were determined using the Cox proportional hazard model.[20]

Results and Discussion

The main characteristics of the 70 patients are summarized in Table 1. After induction, MRD assessment was performed in 69 patients; one patient who had *bcr2* transcripts was not evaluable because of poor quality RNA. Of these 69 patients, 34 patients (49.3%) achieved molecular complete remission. No correlation was found between PCR positivity after induction and outcome. Our results are similar to those obtained by the GIMEMA[2] and PETHEMA[5] groups. In these studies, molecular complete remission rates after induction were 60.5% and 51.3%, respectively. They observed no influence of PCR positivity after induction on treatment outcome. Sixty-nine of the 70 patients completed their three courses of consolidation and proceeded to maintenance therapy as scheduled. One patient who was PCR-negative died of a fungal infection during second consolidation. After first consolidation, 23 of the 35 patients who were PCR-positive after induction became PCR-negative (Figure 1A). In the remaining 12 patients, the median *PML-RARα/ABL* ratio was 1.13×10^{-3} (range, 1.35×10^{-4}-7.86×10^{-2}); and the median *PML-RARα/ABL* ratio decreased by 1.28 (0.32-2.63) log, compared with levels after induction (Figure 1B). After second consolidation, 10 of the 12 patients who were PCR-positive after first consolidation showed a further reduction in MRD to an undetectable level. After third consolidation,

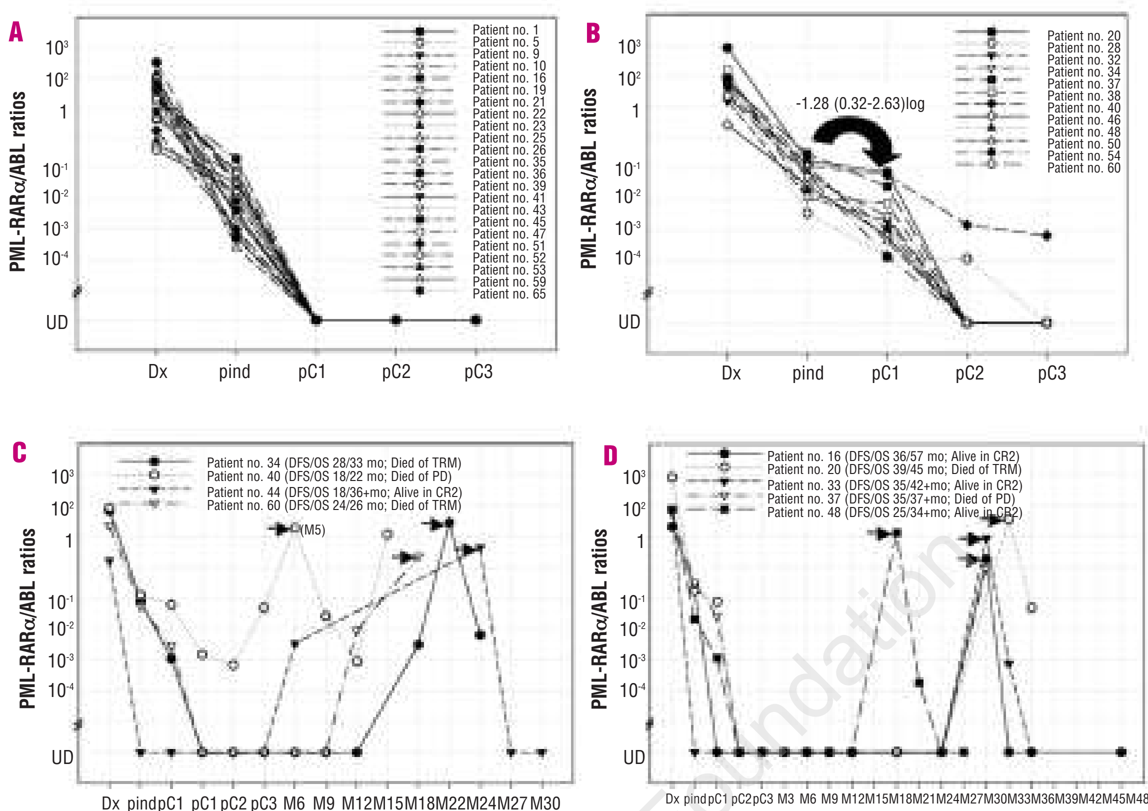

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Figure 1. **Kinetics of PML-RARα transcripts and their clinical correlations. A. Plot showing quantitative MRD levels for 23 patients who became PCR-negative after first consolidation. B. Plot showing quantitative MRD levels for 12 patients who had detectable MRD after first consolidation. C. Plot showing quantitative MRD levels for four relapsed patients who showed molecular relapse or an increase in MRD followed by hematologic relapse. D. Plot showing quantitative MRD levels for five relapsed patients who failed to show any evidence of molecular events preceding hematologic relapse. Arrow indicates starting point of salvage treatment (chemotherapy±stem cell transplantation). p: post (ie, after); Ind: induction; M: maintenance; UD: undetectable; TRM: treatment-related mortality; PD: disease progression.**

one patient still had detectable MRD. To date, nine patients (12.9%) relapsed at a median interval of 29 months (range, 11-39 months). Of these, four patients (nos. 34, 40, 44, and 60) showed molecular relapse or an increase in MRD followed by hematologic relapse at 2 to 18 months from first molecular events (Figure 1C), whereas five (nos. 16, 20, 33, 37, and 48) who tested persistently PCR-negative failed to show molecular events preceding hematologic relapse (Figure 1D). This may be due to our pre-established MRD assessment schedule, which involved testing at 6 or 12-month intervals from 1 year after the initiation of maintenance. Indeed, the latter five relapses occurred after durable first complete remission (range, 25-39 months). According to the GIMEMA group,[13] 20 of 21 patients who converted from PCR-negative to PCR-positive after consolidation completion underwent hematologic relapse at a median of 3 months (range, 1-14 months) after their first PCR-positive result, whereas eight (5.6%) of 142 patients who tested persistently PCR-negative relapsed after a median follow-up of 18 months (range, 6-38 months). The Memorial Sloan-Kettering group[6] also reported that three (7%) of 41 patients who

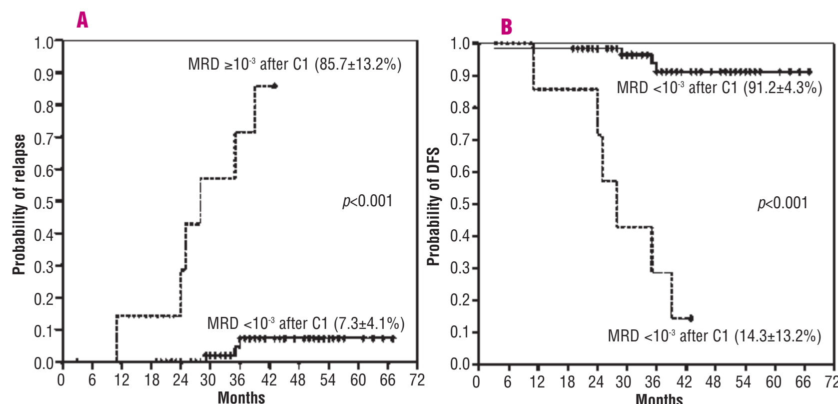

Figure 2. **Probabilities of relapse (A) and disease-free survival (B) according to MRD level after first consolidation. Patients with an MRD of greater than 10^{-3} after first consolidation had a poorer outcome in terms of relapse and disease-free survival than those with MRD of less than 10^{-3}. The dotted line indicates patients with an MRD of greater than 10^{-3} after first consolidation; the solid line indicates patients with MRD of less than 10^{-3}.**

had PCR-negative results after consolidation completion relapsed without any preceding molecular events, whereas four patients (100%) who had PCR-positive results relapsed 1 to 9 months later. These results indicate that serial MRD monitoring is required on a 3-monthly basis until the completion of the maintenance schedule. Recently, the Intergroup[16] reported that RQ-PCR based on peripheral blood samples is as informative as that on bone marrow (p<0.001). If confirmed, this would greatly facilitate clinicians' compliance with MRD monitoring requirements. The 5-year probabilities of relapse and disease-free survival were 17.3±5.4% and 81.5±5.4%, respectively (Table 1). To analyze the prognostic factors, we selected the MRD cut-off value showing the highest statistical significance (10^{-2} after induction, 10^{-3} after first consolidation). Interestingly, a significant correlation was found between MRD level after first consolidation and outcome. Six of the seven patients with an MRD of greater than 10^{-3} after first consolidation eventually relapsed, whereas only three of the 62 patients with an MRD of less than 10^{-3} underwent relapse. This translated into a better outcome in terms of the probabilities of relapse (85.7±13.2% versus 7.3±4.1%, p<0.001) and disease-free survival (14.3±13.2% versus 91.2±4.3%, p<0.001) (Figure 2). We suggest that the MRD level after first consolidation may reflect the presence of resistant leukemic clones and, thus, that the magnitude of MRD reduction at this stage is related to clinical outcome. Only limited information has been available concerning the significance of MRD level between consolidation courses. Our findings appear to be at variance with those of the MRC group,[3] which reported that PCR-positivity at any stage following induction is associated with an increased risk of relapse. This trend was found to be most useful following the third course of chemotherapy, when patients remaining PCR-positive had an increased risk of relapse compared to that of PCR-negative patients (57% versus 27%, p=0.006). In their study, some patients allocated to the short ATRA arm may have contributed to delayed leukemic cell clearance. This hypothesis is supported by the findings of the Intergroup study,[16] which showed that MRD level is lower in ATRA-induced complete remission than in chemotherapy-induced complete remission (p=0.018). In addition, the MRC group[3] determined PCR positivity using *PML-RARα* and *RARα-PML* assays with a sensitivity of 10^{-5}, which may also account for the discrepancy between their results and ours.

In summary, prospective MRD monitoring by RQ-PCR may allow us to identify subgroups of patients at high risk of relapse earlier during treatment. Further large series, studied with a prospective long-term follow-up and a standardized RQ-PCR protocol, are needed to validate our results.

SL was the main investigator involved in the design of the study, analysis of the data, interpretation of the results, and writing of the manuscript. Y-JK, K-SE, and C-KM performed the molecular laboratory work and contributed to drafting the paper. H-JK and S-GC contributed to the collection and analysis of the clinical data and to drafting the paper. J-WL, W-SM, and CCK supervised the study and critically reviewed the manuscript. The authors declare that they have no potential conflicts of interest. We thank the nurses dedicated to our chemotherapy and stem cell transplantation program, our fellows, house-staff, and medical technicians for their continued assistance. This work was supported by a Korea Research Foundation Grant (KRF-2004-013-E00011). Manuscript received October 7, 2005. Accepted February 17, 2006.

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