

## High-resolution melting analysis for a reliable and two-step scanning of mutations in the tyrosine kinase domain of the chimerical *bcr-abl* gene

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Received: 12 March 2009 / Revised: 7 April 2009 / Accepted: 22 April 2009 / Published online: 23 May 2009  
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**Abstract** For relevant imatinib therapy against Philadelphia (Ph)-positive leukemias, it is essential to monitor mutations in the chimerical *bcr-abl* tyrosine kinase domain (TKD). However, there is no universally acceptable consensus on how to efficiently identify mutations in the target TKD. Recently, high-resolution melting (HRM) technology was developed, which allows gene scanning using an inexpensive generic heteroduplex-detecting dsDNA-binding dye. This study aimed to validate the introduction of HRM in a practical clinical setting for screening of mutations in sporadic sites of the chimerical *bcr-abl* TKD. All chimerical and wild-type *abl* TKD regions selectively amplified were used for HRM assays and direct sequencing. The HRM test had approximately 5–90% detection sensitivity for mutations. In contrast to mixture samples with mutant and wild-type cells, all mutant cell samples had indeterminate melting curves equivalent to those of the wild-type due to formation of only a homoduplex. This issue was improved by the addition of exogenous wild-type DNA after PCR. Subsequently, HRM results gave a high accordance rate of 97.8% (44/45 samples) compared to the sequencing data. The discordant results in one appear to be

due to unsuccessful amplification. Thus, HRM may be considered to be suitable for reliable scanning of mutations in the chimerical *abl* TKD in a clinical setting.

**Keywords** Ph · *bcr-abl* · Mutation · Melting analysis · HRM

### 1 Introduction

The chemical agent, imatinib, has a high therapeutic response for diseases carrying the chimerical *bcr-abl* gene. For example, the first imatinib treatment was reported to give approximately 80 and 60% complete molecular response rates in chronic myelogenous leukemia (CML) and Philadelphia (Ph)-positive acute lymphoblastic leukemia (ALL), respectively [1, 2]. However, in many cases, mutations in the chimerical *abl* tyrosine kinase domain (TKD) were revealed to precede resistance to imatinib, resulting in disease relapse and progression to advanced disease. At present, although it is known that there are several causative factors in resistance, such as expression of a rapid drug efflux protein and non-*bcr-abl*-dependent transformation involving the *src* family, TKD mutations in the chimerical gene are thought to play a major role in resistance acquisition [3]. Therefore, detection of mutations becomes essential in cases treated with imatinib. Moreover, mutations associated with imatinib therapy emerge as a Ph-positive subclone from minimal residual leukemia (MRD) even in the hematological remission period [4, 5]. This indicates the need for highly sensitive tests to detect only Ph-positive leukemic clones. To date, several methods to analyze mutations including direct and subcloning sequencing have been employed, but the respective methods have merits and demerits and are not

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always sensitive [6]. Recently, to analyze genetic variations (SNPs, mutations and methylations), a novel melting analysis called high-resolution melting (HRM) with an automated instrument and real-time PCR apparatus was used [7, 8]. HRM is used to characterize samples according to their dissociation profile as they transit from double-strand DNA (dsDNA) to single-strand (ssDNA). Therefore, mixture samples with mutant and wild-type cells are easily identified by differences in melting curve shapes. Mutant sequence variants produce a  $T_m$  shift compared with the wild-type [9]. In addition, it is a reliable- and closed-tube system without high-cost fluorescence probes [10, 11]. Thus, to introduce HRM assays in clinical settings to detect Ph-positive subclones with *bcr-abl* kinase domain mutations, the relevance and validation of the assay prior to direct sequencing was studied.

## 2 Materials and methods

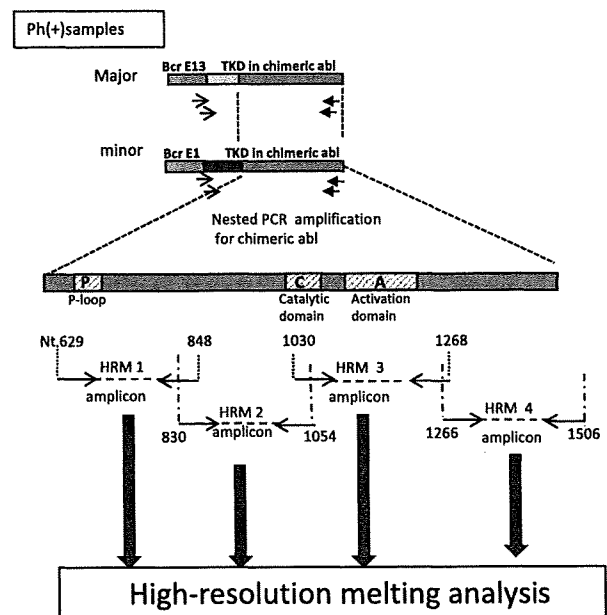
### 2.1 Samples and processing of cDNA

A total of 19 Ph-positive samples were used, consisting of 10 unlinked and already mutation-known specimens, 8 fresh practical samples from 6 patients with CML, 2 patients with ALL and one sample from a Ph-positive K562 cell line. All patients with Ph-positive leukemias were being treated with imatinib at 400–800 mg per day and had hematological remission, but were positive for *bcr-abl* real-time RT PCR. As controls, 16 peripheral blood samples from normal volunteers and 10 cell lines consisting of HTLV-1-associated cell lines (Hut102, KK1, KOB, OMT, MT2, SO4, ST1), T cell lines of Jurkat and MOLT4, and the monocytic line U937 were used.

Total RNA was extracted from total leukocyte guanidinium thiocyanate lysates using ISOGEN (Nippon gene, Toyama, Japan). cDNA was synthesized using oligo-dT primers and Superscript III reverse transcriptase (Invitrogen, Carlsland, CA, USA). Practical and stocked samples used in this study were applied under the approval (15040708) of the ethics committee and the condition of the criteria of the Japanese Association of Laboratory Medicine.

### 2.2 Study design for HRM assay in the chimerical *abl* TKD region

Our study for the detection of mutation was designed in a two-step manner: firstly, genetic alteration screening by HRM analysis (Fig. 1), using the LightCycler 480 (Roche Molecular System, Alameda, CA, USA) for HRM and real-time thermal cycling; secondly, only samples positively screened by HRM were directly sequenced. First of all, each sample was examined for *bcr-abl* chimerical status by



**Fig. 1** Study design of high-resolution melting (HRM) and the structure of the tyrosine kinase domain (TKD). Firstly, a part of chimerical *bcr-abl* TKD is selectively amplified by nested PCR. Using amplified chimerical *bcr-abl* products as a template, the second PCR for the HRM assay is performed, generating four intercalating dye amplicons, HRM-1, -2, -3 and -4. Then, HRM analyses were done by using the LightCycler Gene Scanning Application

the conventional method [12]. If positive for major or minor chimerical types, these *bcr-abl* kinase domains were selectively amplified, generating a fragment of 1504 bp for b2/a2 and 1579 bp for b3/a2, using primers previously reported [13].

For HRM analysis, PCR products of 50–250 bp length are recommended for best discrimination. Therefore, we applied a modified method previously reported by Poláková et al. [10], generating 4 amplicons, designated as HRM1, 2, 3 and 4 of 220, 225, 239 and 241 bp, corresponding to nt 629–848, 830–1054, 1030–1268, and 1266–1506 (NM\_005157), respectively. For HRM, a PCR reaction was performed in 20  $\mu$ l reaction volumes containing 1  $\mu$ l of 1/200 diluted template generated as described above, Master Mix, Taq DNA polymerase, dNTP mix, HRM dye, 3 mM  $MgCl_2$ , primers [10], and 1 M GC melt, according to the instructions of Roche Applied Science (Manheim, Germany). The PCR was monitored by real-time cycling and a strong fluorescent signal was generated only when bound to dsDNA, that is the touchdown PCR cycling and HRM conditions [14]. HRM melting curve data were obtained by slowly increasing the temperature, from 60 to 90°C at a rate of 100 acquisitions per 1°C. The melting status and changes in  $T_m$  value were analyzed using the Roche HRM algorithm (Gene Scanning Software, Roche Supplied Science, Manheim, Germany),

depicting graphs of fluorescence-normalized and temperature-shifted melting curves and difference plots. The cell line K562 was used as a wild-type reference sample.

### 2.3 Sequencing

In this study, to compare to accuracy of HRM analysis, regardless of the first-step negative samples, all of the samples used were confirmed by sequencing the regions of the selectively amplified chimerical *abl* TKD, as well as *abl* TKD from Ph-negative controls using a Big Dye terminator kit Ver 3.1 (Applied Biosystems, Carlsland, CA, USA) and the ABI Prism 3130 Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions.

## 3 Results

### 3.1 HRM assay validation

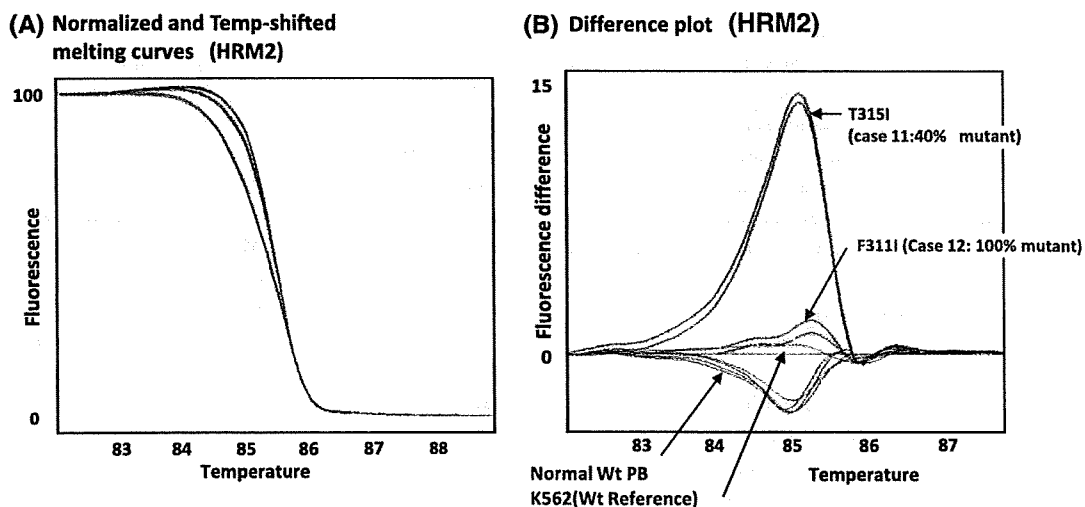
For HRM scoring, Ph(+) K562 was set up as a wild-type genotype and the 2 or 3 normal blood samples were monitored as negative controls. First of all, using three different samples with mutations, HRM analysis in duplicate was performed, generating constant positive melting curves both in terms of shape and peak height with a range of melting temperatures ( $T_m$ ) from 84 to 86°C (Fig. 2). On the other hand, 16 samples without the Ph-chromosome constantly produced the wild-type scanning profiles according to the Roche HRM program, as shown in Fig. 3 (normalized and temperature-shifted melting curves and difference plots).

Interestingly, as shown in Fig. 2b, no correlation was observed between fluorescence heights and the ratio of the mutant and wild-type: the peak was higher in mixture samples with mutant and wild-type cells than samples with only mutant clones. To address this strange relation, variable mixture samples with mutant and wild-type cells diluted by exogenous control DNA from wild-type cells were subjected to HRM assay. Figure 4 shows that samples containing only mutant cells (bottom graph) produced indeterminate signals with low peaks, but the mixture samples containing variable wild-type cell burden (5–90%) displayed apparently higher peaks, indicating the existence of mutation. This shows that 100% mutant samples may become false negative, indistinguishable from only wild-type patterns. To form only homoduplex, dsDNA in either mutant or wild-type DNA probably accounted for the indeterminate evaluation in all mutant or wild-type samples.

Accordingly, to avoid false negatives in samples with all mutant cells, we organized screen mutations using two divided samples; one was an original and the other mixed with exogenous wild-type cells, as shown in Fig. 5. Using this strategy, the test performance of HRM was examined in this study.

### 3.2 HRM assay results

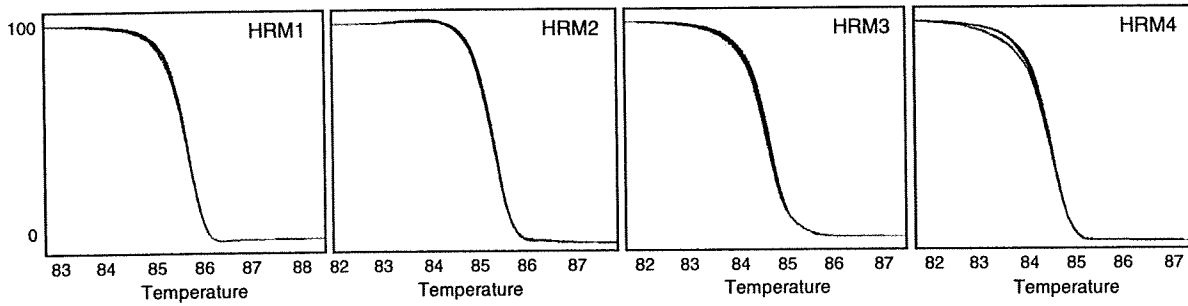
The HRM assay was blindly examined in duplicate by a single researcher and then compared to sequence data. As summarized in Table 1, the HRM test was positive for 13 (68.4%) of 19 Ph-positive leukemias, including a Ph-positive K562 cell line. Using the same amplicons as



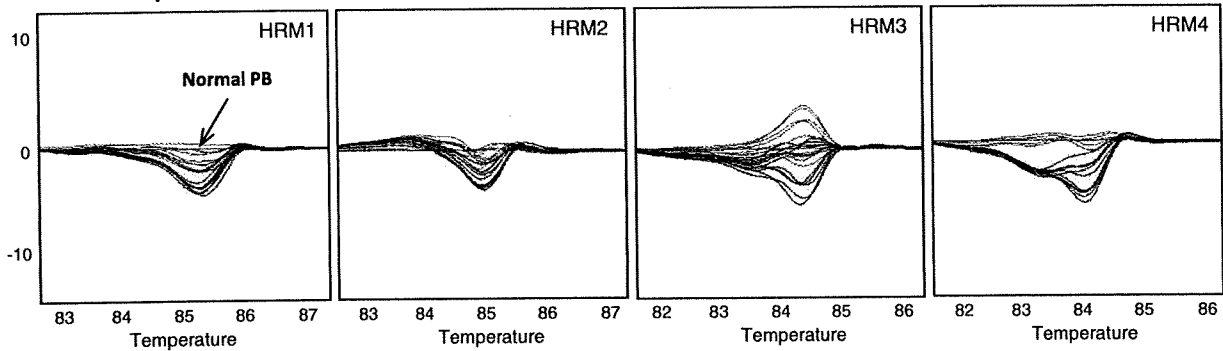
**Fig. 2** Validation of the HRM assay using samples with 100% mutant cells and samples with variable % mutant cells. Duplicate assays gave rise to the similar results, indicating the good

reproducibility in both normalized and temperature-shifted melting curves and difference plots. Ph-positive K562 cells were used as a wild-type (Wt) reference

**Normalized and Temp-shifted melting curves**



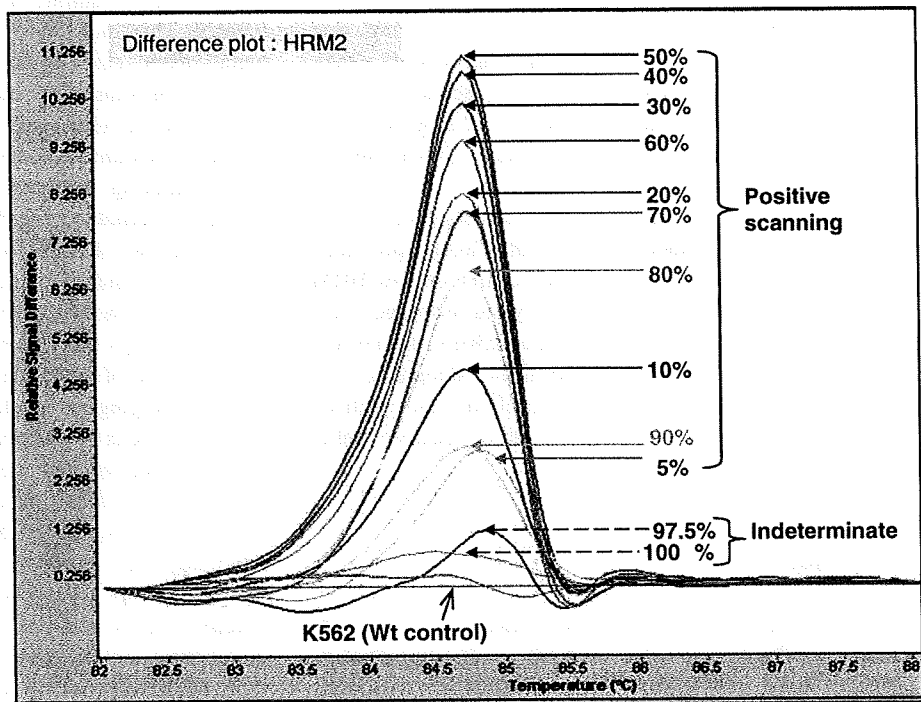
**Difference plot**

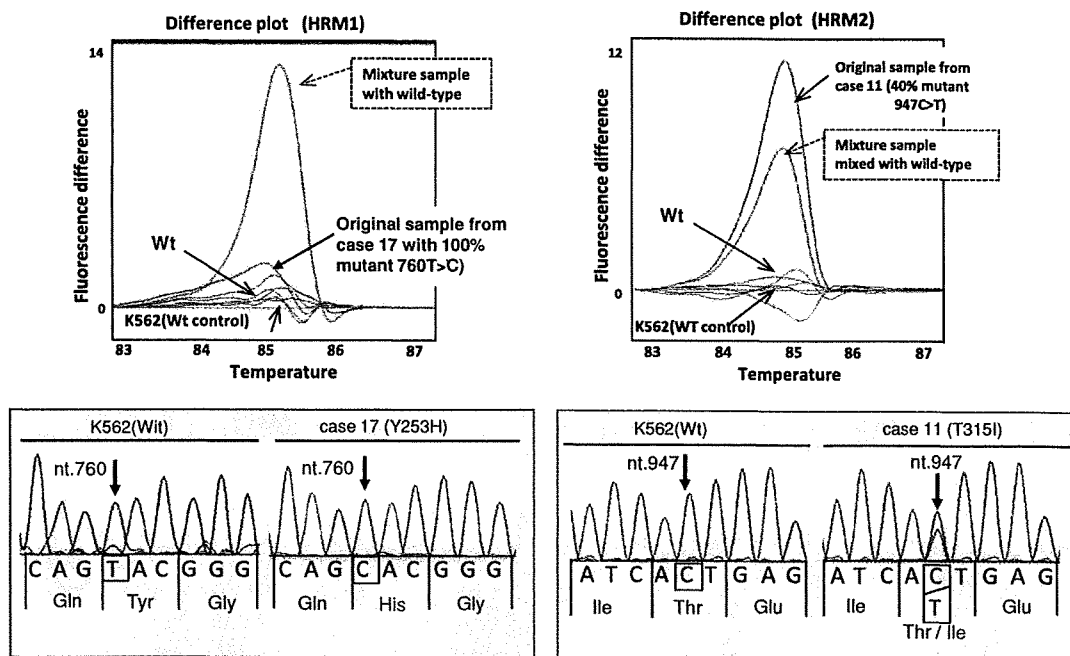


**Fig. 3** Validation of HRM test performance using negative controls with wild-type TKD demonstrated by direct sequencing in peripheral blood from 16 healthy persons and 9 hematopoietic cell lines, excluding the U937 cell line. All 4 HRM analyses were evaluated to

be wild-type by both normalized-temperature-shifted melting curves according to the Gene Scanning Application Algorithm. Normal peripheral blood from volunteers was used as a wild-type reference

**Fig. 4** Changes in the heights of peaks and  $T_m$  values depending on the difference in the mixture ratio of mutant and wild-type cells. HRM assay revealed indeterminate signals in samples with only mutant cells (around 100% mutant cell samples). On the other hand, the mixture samples diluted to 5% mutant cells produced typical positive scanning patterns, indicating that the best mixture ratio was 50 versus 50%





**Fig. 5** Representative cases (A and B) of HRM and sequencing analyses. The left panel showed that the addition of wild-type DNA into the 100% mutant (760 T > C) sample made discrimination easy.

On the other hand, the right panel showed that the change in the melting curves between the original (947 C > T) and the mix allowed discrimination

above, direct sequencing identified 14 missense mutations (73.6%) out of 19 Ph(+) leukemias. The positive and negative accordance rate of both tests was 94.7% among 19 Ph(+ or -) samples. The discrepancy in sample no. 5, negative in the HRM and positive in the sequencing, was expected before HRM analysis, because the PCR efficiency was not so good. As expected, the direct sequencing disclosed a problematic issue for PCR, in that a mutation (nt 838) existed within the annealing sequence (nt830-849) of the primer.

Next, of 26 Ph-negative samples consisting of 10 cell lines and 16 normal blood controls, HRM assays produced negative findings in all but one. The positive one for HRM was U937 derived from myelomonocytoid leukemic cells. The sequencing revealed a mutation of E308V, which was expected to be somatic as it is one of the oncogenes.

Conclusively, the accordance rate of the two methods was 97.8% in all 45 cases of Ph-positive/-negative leukemias and controls.

#### 4 Discussion

Most patients with Ph-positive leukemias, especially chronic CML, who receive imatinib as first-line therapy achieve good cytogenetic and molecular responses. However, long-term molecular studies suggest that around

25–30% of patients seem not to achieve successful responses and undergo disease progression. Major causes of imatinib resistance include the emergence of leukemic clones with mutations in the tyrosine kinase domain of *bcr-abl*. This indicates that it is necessary to screen for mutations in early phase of the emergence of mutation clones. Unfortunately, there is generally no acceptable consensus on when and by which technology the TKD mutations should be screened. At present, direct sequencing, denaturing high-performance liquid chromatography (D-HPLC), denaturing gradient gel electrophoresis (DGGE), allele-specific oligonucleotide-polymerase chain reaction (ASO-PCR) and pyro-sequencing are available, but the respective methods have merits and demerits for practical clinical settings [6]. A novel technology of HRM with the development of instruments and saturating intercalating dyes is emerging for the detection of nucleic acid sequence variations and is now applied in practical diagnostic settings [7, 15]. The two-step method allows to avoid the direct sequencing for the entire region of all samples.

In this study, the HRM in our system was shown to efficiently and simply differentiate mutations in the chimerical *bcr-abl* TKD region by using LightCycler technology and a software algorithm. In particular, it is noteworthy that mixture samples with mutant and wild-type cells were easily detectable with high sensitivity (approximately 5%). On the other hand, samples with all

**Table 1** Summary of the results on mutations examined by both HRM and direct sequencing analyses

Sample	Clinical dx	bcr-abl	HRM results	TKD	nt substitute	Exon
Unlinked						
1	CML	Major	Mut	Y253F	761 A > T	4
2	CML	Major	Mut	Y253H	760 T > C	4
3	CML	Major	Mut	E255 V	767 A > T	4
4	CML	Major	Mut	E255 K	766 G > A	4
5	CML	Major	NE	E279 K	838 G > A	5
6	CML	Major	Mut	T315I	947 C > T	6
7	CML	Major	Mut	F317L	954 C > A	6
8	CML	Major	Mut	M351T	1055 T > C	6
9	CML	Major	Mut	H396R	1190 A > G	7
10	CML	Major	Mut	F486S	1460 T > C	9
Fresh						
11	CML	Major	Mut	T315I	947 C > T	6
12	CML	Major	Mut	F311I	934 T > A	6
13	CML	Major	Mut	L370R	1112 T > G	7
14	CML	Major	Wt	Wt		
15	CML	Major	Wt	Wt		
16	CML	Major	Wt	Wt		
17	ALL	Minor	Mut	Y253H	760 T > C	4
18	ALL	Minor	Wt	Wt		
Cell lines						
19	K562	Major	Wt	Wt		
20	U937	(-)	Mut	E308V	926 A > T	5
21	KOB	(-)	Wt	Wt		
22	KK1	(-)	Wt	Wt		
23	ST1	(-)	Wt	Wt		
24	SO4	(-)	Wt	Wt		
25	OMT	(-)	Wt	Wt		
26	MT2	(-)	Wt	Wt		
27	Hut102	(-)	Wt	Wt		
28	Jurkat	(-)	Wt	Wt		
29	MOLT4	(-)	Wt	Wt		
Normal PB						
30-45		(-)	Wt	Wt		

Mut mutation, Wt wild-type, NE not evaluated

mutant cells often present with indeterminate low peaks in difference plots, causing confusing interpretations. This is probably the main defect in this HRM technology, resulting from a dependency on heteroduplex formation due to the mixture ratio of the mutant and wild-type. Therefore, to avoid false negatives with samples containing all mutant cells, it was shown that the addition of exogenous control DNA was useful (Figs. 4, 5). Actually, since the ratio of mutant cells in the samples is unknown in practical samples, we adopted an HRM assay system, which measures using a double feature: an original one and mixtures of the

mutant and exogenous wild-type DNA in the ratio of 1:1. Practical examples are shown in Fig. 5, indicating that the mixed sample (left panel) makes it easy to discriminate, whereas the change in the positive peak pattern was tolerable if a 40% mutant sample was diluted to 50%.

Finally, our HRM results were in agreement in all but one of 45 samples with an accordance rate of 97.8% compared with sequencing data. The discrepancy in the results in one sample was expected due to an accidental relation between the primer and mutation sites, as described above. The quality of HRM is thought to be highly dependent on real-time amplification, so that we are now revising part of the primer set and appropriate sequence length for HRM.

Clearly, this is a rapid, simple, accurate screening method using HRM technology for chimerical *bcr-abl* TKD mutations involved in resistance to imatinib. Since resistant Ph-subclones emerge from MRD and increase step by step in parallel with the long imatinib therapy duration, the HRM assay system is a suitable and useful method to better manage Ph-positive leukemias, for example, to decide on dose escalation or cessation of imatinib, alternation of new drugs or different therapies with dasatinib and bone marrow transplantation. Actually, we are applying this method in a routine clinical setting prior to sequencing to select only mutation-positive samples.

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## Comparative analysis of remission induction therapy for high-risk MDS and AML progressed from MDS in the MDS200 study of Japan Adult Leukemia Study Group

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Received: 12 August 2009/Revised: 2 December 2009/Accepted: 14 December 2009/Published online: 5 January 2010  
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**Abstract** A total of 120 patients with high-risk myelodysplastic syndrome (MDS) and AML progressed from MDS (MDS–AML) were registered in a randomized controlled study of the Japan Adult Leukemia Study Group (JALSG). Untreated adult patients with high-risk MDS and MDS–AML were randomly assigned to receive either idarubicin and cytosine arabinoside (IDR/Ara-C) (Group A) or low-dose cytosine arabinoside and aclarubicin (CA) (Group B). The remission rates were 64.7% for Group A (33 of 51 evaluable cases) and 43.9% for Group B (29 out of 66 evaluable cases). The 2-year

overall survival rates and disease-free survival rates were 28.1 and 26.0% for Group A, and 32.1 and 24.8% for Group B, respectively. The duration of CR was 320.6 days for Group A and 378.7 days for Group B. There were 15 patients who lived longer than 1,000 days after diagnosis: 6 and 9 patients in Groups A and B, respectively. However, among patients enrolled in this trial, intensive chemotherapy did not produce better survival than low-dose chemotherapy. In conclusion, it is necessary to introduce the first line therapy excluding the chemotherapy that can prolong survival in patients with high-risk MDS and MDS–AML.

For the Japan Adult Leukemia Study Group.

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**Keywords** MDS · MDS–AML · JALSG MDS200 · Induction therapy · HSCT

## 1 Introduction

Myelodysplastic syndrome (MDS) is a group of disorders in which abnormalities occur at the level of hematopoietic stem cells [1], leading to disturbance in the production of blood cells characterized by ineffective hematopoiesis [2], decrease in the number of peripheral blood cells and morphological/functional abnormalities in blood cells [3]. Allogeneic hematopoietic cell transplantation (allo-HCT) is the most effective curative therapy for acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) [4]. However, for patients with high-risk MDS (those with refractory anemia with excess of blasts in transformation (RAEB)-t and some patients with RAEB) and patients with acute myeloid leukemia progressed from MDS (MDS–AML), chemotherapy aimed at remission is being used. The reasons for this are that MDS often affects elderly people [5], suitable donors are not always available at the time of disease onset, the necessity of pretransplant conditioning chemotherapy is controversial [6, 7] with a lack of sufficient evidence, and the optimal timing for transplantation varies widely depending on disease type [8].

On the other hand, reduced-intensity conditioning has extended the use of allo-HSCT to patients otherwise not eligible for this treatment due to older age or frailty [9]. However, allo-HSCT using traditional myeloablative preparative regimens is not easily tolerated by the elderly or frailer patient, and may lead to prohibitive treatment-related mortality rates. Most patients treated in the past were younger and devoid of comorbid clinical conditions. Novel reduced-intensity regimens have recently made allogeneic transplants applicable to the elderly, providing the benefit of the graft-versus-leukemia effect to a larger number of patients in need [10].

Low-dose chemotherapy, which has been used in clinical practice for 20 years, reduces the number of myeloblasts, improves pancytopenia and induces remission not only in MDS patients but also in some MDS–AML patients [11]. Common antineoplastic agents used in low-dose chemotherapy include cytosine arabinoside (Ara-C), aclaurubicin (ACR), melphalan and etoposide. Nevertheless, despite improved Ara-C and regimens, the prognosis of AML in patients beyond 60 years of age remains dismal [4]. Low-dose antineoplastic drug therapy is still being used in some patients with MDS, which is common in elderly people, especially when the patient is at risk due to poor general condition or organ disorder [12].

The Japan Adult Leukemia Study Group (JALSG) previously conducted a pilot study for the treatment of

high-risk MDS and MDS–AML to compare low-dose monotherapy with low-dose Ara-C plus granulocyte colony-stimulating factor (G-CSF) and multiple drug therapy with Ara-C plus Mitoxantrone plus VP-16. Later, JALSG conducted studies using a single protocol (JALSG MDS96) in 1996, in which remission induction and post-remission therapies using Ara-C and IDR in patients with high-risk MDS (RAEB-t) and in those with MDS–AML were performed, after which the efficacy and safety of these therapies were evaluated [13]. Furthermore, a randomized controlled study (JALSG MDS200) of intensive chemotherapy (IDR/Ara-C) or low-dose chemotherapy (CA) for high-risk MDS was also performed by JALSG.

Here, we present and analyze the results of the JALSG MDS200 study to assess and evaluate the validity of the MDS200 protocol for MDS treatment.

## 2 Patients and methods

### 2.1 Patient eligibility

A total of 120 patients were initially registered into the JALSG MDS200 study between June 2000 and March 2005. They were assigned into two groups, namely, Groups A and B (Table 1). Patients aged 15 years or more and diagnosed as having high-risk RAEB with high International Prognostic Scoring System score [14], RAEB-t or MDS–AML were eligible for this study. MDS–AML denotes secondary AML transformed from MDS.

Other eligibility criteria were as follows: patients with a performance status (PS) of 0–2 (ECOG); patients whose key organs other than the bone marrow retain intact function; patients who have not undergone any chemotherapy, except for pretreatment that does not affect the outcome of the main therapy; and patients who have given informed consent. Informed consent was obtained after carefully explaining the protocol and before registration.

### 2.2 Study protocol

The MDS200 protocol (Fig. 1) was designed based on the results of MDS96, and involved a dose-attenuation plan and allowed a wider range of chemotherapy. Patients were randomly assigned to either Group A or B.

In therapy A, the dose was adjusted according to a dose attenuation plan based on the presence of risk factors. The following 3 factors were regarded as risk factors: (1) Age ( $\geq 60$  years), (2) hypoplastic bone marrow and (3) PS  $\geq 2$ . Patients with no risk factor received the standard dose, those with 1 risk factor received 80% of the dose and those with 2 or more risk factors received 60% of the dose (equivalent to the dose of MDS96). In therapy B, the use of

**Table 1** Characteristics of patients

Group	A (n = 53)	B (n = 67)	P value (A vs. B)
Age (range)	63 (23–77)	61 (32–81)	0.505
Gender			
Male	37	52	0.332
Female	16	15	
Disease type			
HR-RAEB	4	11	0.269
RAEB-T	22	29	
MDS-AML	27	27	
Infection			
Presence	10	11	0.726
None	43	56	
Karyotype <sup>a</sup>			
Good	23 (44.2%) n = 52	21 (33.9%) n = 62	0.524
Int	11 (21.2%)	15 (24.2%)	
Poor	18 (34.6%)	26 (41.9%)	
PB (range)			
WBC (/ $\mu$ L)	2,500 (700–64,240)	2,720 (600–43,700)	0.665
Hb (g/dL)	8 (4.7–12.6)	7.9 (4.4–12.7) n = 66	0.562
Plt (/ $\mu$ L)	5.8 (0.2–31.4)	5.9 (0.5–36.7)	0.363
BM (range)			
Blast (%)	30 (4–95) n = 51	24.2 (1.9–96) n = 66	0.171
Biochemical data (range)			
LDH (IU/L)	296 (132–882)	303.5 (111–906) n = 66	0.998
CRP (mg/dL)	0.5 (0–20.2)	0.35 (0–11.7) n = 66	0.292

Patients who met all of the inclusion criteria and did not meet any of the stated exclusion criteria were included the study. The disease types were classified by FAB classification

Statistical analysis between Group A and Group B was done using  $\chi^2$  test or Mann–Whitney *U*-test

*MDS* myelodysplastic syndrome, *HR-RAEB* high risk-refractory anemia excess of blasts with high International Prognostic Scoring System Score, *RAEB-T* refractory anemia excess of blasts in transformation, *MDS-AML* MDS overt leukemia, *WBC* white blood cell, *Hb* hemoglobin, *Plt* platelet, *LDH* lactate dehydrogenase, *CRP* C-reactive protein, *PB* peripheral blood, *BM* bone marrow

<sup>a</sup> Shows IPSS risk

**Remission induction therapy**

<b>Therapy A (IDR+Ara-C)</b>				day	1	2	3	4	5	6	7	
Ara-C	100mg/m <sup>2</sup>	continuous. iv.			↓	↓	↓	↓	↓	↓	↓	↓
IDR	12mg/m <sup>2</sup>	30 min. iv.			↓	↓	↓					
<b>Therapy B (CA therapy)</b>				day	1	2	3	4	5	6	7	.....14
Ara-C	10mg/m <sup>2</sup> /12h	subcutaneous injection			↓	↓	↓	↓	↓	↓	↓	↓
ACR	14mg/m <sup>2</sup> /day	30 min. iv.			↓	↓	↓	↓				

**Consolidation, maintenance and intensification therapies**

These therapies were performed in accordance with the JALSG MDS96 protocol both in groups A and B

**Fig. 1** Japan Adult Leukemia Study Group—myelodysplastic syndrome (JALSG MDS200 Protocol). In therapy A, the dose was adjusted according to a dose attenuation plan based on the presence of risk factors. The following 3 factors were regarded as risk factors: (1) Age ( $\geq 60$  years), (2) hypoplastic bone marrow and (3) PS  $\geq 2$ . Patients with no risk factor received the standard dose, those with 1

risk factor received 80% of the dose, and those with 2 or more risk factors received 60% of the dose (equivalent to the dose of MDS-96). In therapy B, the use of CAG therapy involving co-administration of G-CSF was allowed. *IDR* idarubicin, *Ara-C* cytosine arabinoside, *ACR* aclarubicin, *G-CSF* granulocyte colony-stimulating factor, *iv* intravenous injection, *min* minutes

CAG therapy involving the co-administration of granulocyte colony-stimulating factor (G-CSF) was allowed.

Untreated adult patients ( $\geq 15$  years) with MDS (RAEB, RAEB-t or MDS-AML) were randomly assigned to receive either IDR/Ara-C (Group A) or CA (Group B) [15]. Complete remission (CR) rate, CR duration, overall survival (OS) rate and disease-/relapse-free survival (DFS/RFS) rate were compared between the two groups.

Consolidation therapy and maintenance therapy were performed in accordance with JALSG MDS96 [13].

### 2.3 Evaluation of response

Response to treatment was evaluated in accordance with JALSG criteria [13]. CR was considered achieved when the following conditions remained for at least 4 weeks. For the bone marrow: blasts accounting for  $\leq 5\%$  of all cells; absence of blasts with Auer body; and presence of normal erythroblasts, granulocytes and megakaryocytes. For peripheral blood: absence of blasts; neutrophils  $\geq 1,000/\text{ml}$ ; platelets  $\geq 100,000/\mu\text{L}$ ; and no evidence of extramedullary leukemia. CR duration was defined as the duration from the day when CR is achieved to the day of relapse or death, OS or DFS as the duration from the day of initiation of treatment to the day of death and DFS as the duration in which CR patients survived without relapse. Patients who were treated with HCST were not censored at the date of transplantation. All toxicity was graded using the World Health Organization criteria [16].

### 2.4 Statistical analysis

The primary endpoint of this study is DFS. Assuming a 1-year DFS rate of 60% in the Group A and 40% in the Group B, this design required the randomization of 200 patients. Eligible patients were randomized according to age, sex and disease type. Differences in background factors (e.g., age, gender and disease type) between Groups A and B were statistically analyzed using the  $\chi^2$  test or Mann-Whitney *U*-test. Probability of OS and DFS were estimated according to the method of Kaplan and Meier.

## 3 Results

### 3.1 Recruitment of patients and suspension of the study

The initially registered 120 patients were assigned into two groups, namely, Groups A and B. The clinical characteristics of the registered patients are shown in Table 1. The present protocol was originally planned to recruit 200 patients for Groups A and B within 3 years. However, the recruitment pace was slower than expected and thus the

study period was extended from 3 years to 4.5 years. At the end of 2004, that is, after 4.5 years from the start of the study, the number of registered patients was only 113 in Groups A and B, which was 56.5% of the target number. At that point, the committee members discussed the progress of the MDS200 study and decided to suspend it at the end of March 2005. Since the final total number of patients did not reach the target number, we did not statistically compare DFS between Groups A and B, which was the primary endpoint of this study.

### 3.2 Characteristics of patients

There were no clear differences in the clinical characteristics of the patients between Groups A and B, such as FAB subtype, initial blood cell count, presence of infection, distribution in the karyotype group and biochemical data, as well as sex distribution (male/female ratio, 37/16 = 2.315 in Group A, and 52/15 = 3.467 in Group B).

### 3.3 Treatment outcome

The remission rates were 64.7% in Group A (33 out of 51 evaluable cases) and 43.9% in Group B (29 out of 66 evaluable cases). The 2-year overall survival (OS) rates were 28.1% in Group A and 32.1% in Group B, and the 2-year DFS rates were 26.0% in Group A and 24.8% in Group B. The mean duration of CR was 320.6 days (median: 213 days) in Group A and 378.7 days (median: 273 days) in Group B (Table 2). Reflecting the intensity of the remission induction chemotherapy, the period of WBC ( $<1,000/\mu\text{L}$ ) after the therapy was longer in Group A than in Group B (19 days and 4 days, respectively). There were more grade 3 or 4 adverse events during the remission induction therapy in Group A (19 out of 53 evaluable patients) than in Group B (13 out of 67 evaluable patients). This difference was mostly attributable to infectious episodes (17 patients in Group A and 4 patients in Group B). In terms of bleeding episodes, 1 patient in Group A and 2 in Group B had grade 3/4 adverse events. The numbers of

**Table 2** Treatment outcome (Group A vs. B)

	Group A ( <i>n</i> = 53)	Group B ( <i>n</i> = 67)
Remission rate (%)	64.7	43.9
Mean duration of remission (days)	320.6 (median: 213)	378.7 (median: 273)
2-Year survival rate (%)	28.1	32.1
2-Year disease-free survival rate (%)	26.0	24.8

The remission rates, 2-year overall survival (OS) rates and 2-year disease-free survival (DFS) rates are shown as percentages

early death in remission induction chemotherapy (death within 30 days) were 1 patient in Group A and 3 patients in Group B (Table 3). The cause of death in each group was infection or tumor progression. The completion rate of consolidation therapies were 37.3% in Group A (12 out of 33 evaluable cases), 37.9% in Group B (11 out of 29 evaluable cases). On the other hand, the maintenance therapies were completed 21.2% in Group A (7 out of 33 evaluable cases), and 15.2% in Group B (5 out of 33 evaluable cases). The numbers of dose attenuation in Group A were 30 patients of 100% dose, 21 patients of 80% or 60% dose and 2 patients of unknown.

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) was performed in 11 out of 50 patients (22%) in Group A and 19 out of 66 patients (28.8%) in Group B. Among those who received allo-HSCT, the transplantation

was performed during the first remission in 40%, 21% of patients in Groups A, B, respectively.

There were 15 patients who lived longer than 1,000 days after diagnosis: 6, 9 patients in Groups A, B, respectively. Regarding the transplantation among long-term survivors, 3 out of 6 patients were transplanted in Group A, 6 out of 9 in Group B. Comparing the achievement of CR among these patients in Groups A and B, all 6 patients in Group A achieved CR, but only 4 out of 9 patients in Group B achieved CR.

**4 Discussion**

In this MDS200 study, patients with high-risk MDS and AML transformed from MDS (MDS-AML) were treated with either intensive or low-dose remission induction therapy, followed by intensive post-remission therapy that was the same as in the JALSG MDS96 study [13].

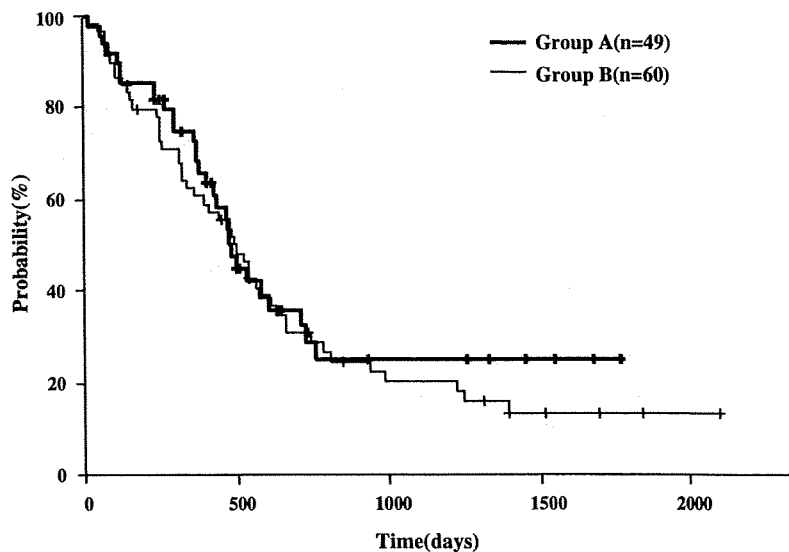
Although we did not perform statistical comparison of DFS or OS between these two treatment groups due to the insufficient number of patients enrolled, the results suggest that there was no significant difference, that is, survival curves were superimposable (Figs. 2, 3). Intensive chemotherapy similar to that for AML can produce a CR rate of 64.7% for high-risk MDS and MDS-AML patients, whereas low-dose induction therapy can result in a CR rate of 43.9%. However, among the patients enrolled in this trial, the difference in CR rate did not lead to better survival as described above. In terms of adverse events, patients who received intensive treatment had more grade 3 or 4 adverse events, particularly infectious events with a longer period of leukopenia. There was no increase in the number of patients succumbing to early death (death within 30 days after the

**Table 3** Toxicity of the induction therapy

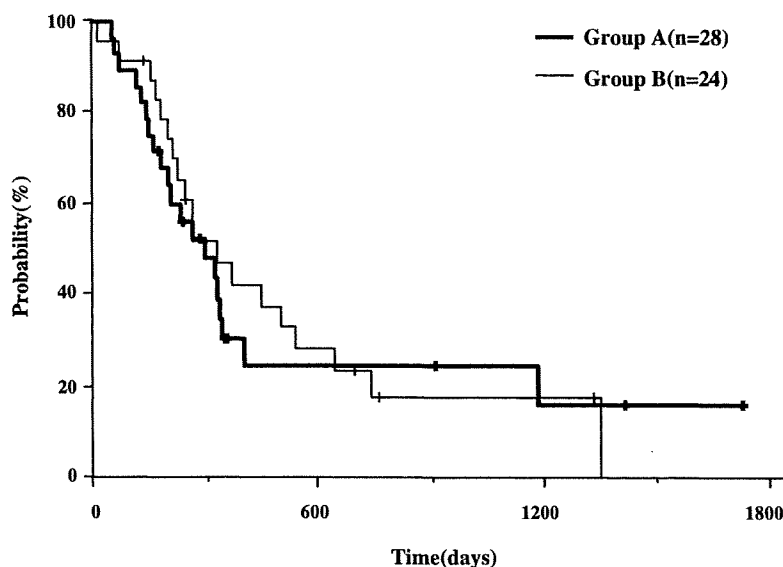
	A (n = 53) (range)	B (n = 67) (range)	P value (A vs. B)
Period of WBC <1,000 (day)	19 (0-44) n = 49	4 (0-50) n = 63	<0.0001
Toxicity (grade 3/4)			
Presence	19	13	0.427
Bleeding	2	1	ND
Infection	17	11	0.04
Others	2	2	ND
Early death (<30 days)	1	3	ND

Statistical analysis between Groups A and B was performed using the  $\chi^2$  test or Mann-Whitney U-test  
 ND not done

**Fig. 2** Overall survival. Survival was calculated from the date of the start of treatment to the date of death due to any cause or to the date of the most recent follow-up. These data were not censored at the time of HSCT. All randomized patients were not included this data in each group. Due to this reason, some patients were not known to be CR or not, but known to be alive or not



**Fig. 3** Disease-/relapse-free survival. RFS was calculated from the date of achieving complete remission to the date of relapse, death or the most recent follow-up. These data were not censored at the time of HSCT. All randomized patients were not included this data in each group. Due to this reason, some patients were not known to be CR state or relapse, but known to be alive or not



start of treatment) in Group A, suggesting that intensive treatment produced higher CR rate, and higher toxicity resulted in a similar survival rate with low-dose induction therapy at least during the early phase of treatment.

There are several reasons that could explain why no difference in survival rate was observed regardless of the difference in CR rate. One could be the similar post-remission therapy between Groups A and B, as demonstrated by the almost similar DFS curves among the two groups. Another reason could be the disease status at the time of transplantation for patients in the two groups. In Group A, 60% of the transplantation was performed during the period other than that covering the first CR; this was 79% in Group B. Allo-HSCT has been shown to have the strongest antileukemia effect, and this was also found in the current study in which 6 out of 15 long-term survivors received allo-HSCT in Groups A and B. From the viewpoint of transplantation, intensive treatment merely selected cases that were suitable for transplantation, as observed in the case of transplantation for relapsed AML patients [17]. There are arguments against remission induction therapy for MDS patients in that it does not affect post-transplant prognosis [6, 18]. In the results of JSHCT, the chemotherapy before undergoing allo-SCT is not necessary in patients with MDS [6]. A group from the Institute of Medical Science of Tokyo University performed umbilical cord blood stem cell transplantation without remission induction therapy in high-risk MDS patients aged not more than 55 years and obtained favorable results with reduced time from diagnosis to transplantation [19]. It is important to perform clinical studies based on the concept that HCST should be performed immediately after diagnosis without remission induction, and determine the types of patients

who would benefit from remission induction therapy prior to transplantation in terms of prognosis. In the present study, although suspended because of the insufficient number of patients enrolled, it appears that remission induction therapy with IDR and Ara-C did not produce better survival than that with low-dose chemotherapy despite higher CR rate. Therefore, it is suggested that CR rate is not a suitable surrogate marker for the evaluation of the outcome of chemotherapy for high-risk MDS and MDS-AML. In the latest reports, induction chemotherapy for patients with high-risk MDS and MDS-AML also provide no survival advantage [20, 21]. Considering the low survival rate of patients in this category, it is clearly necessary to introduce new strategies for the treatment of high-risk MDS and MDS-AML, such as molecular targeting agents and allo-HSCT with reduced-intensity conditioning regimens.

**Acknowledgments** We would like to thank the participating physicians in the Japan Adult Leukemia Study Group (JALSG) MDS200 study for their cooperation. This work was supported in part by grants-in-aid for Scientific Research from the Japanese Ministry of Education, Culture, Sport, Science, and Technology, and grants-in-aid for Cancer Research from the Japanese Ministry of Health, Labor, and Welfare.

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## Randomized trial of response-oriented individualized versus fixed-schedule induction chemotherapy with idarubicin and cytarabine in adult acute myeloid leukemia: the JALSG AML95 study

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Received: 11 May 2009 / Revised: 11 December 2009 / Accepted: 21 December 2009  
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**Abstract** A multicenter, prospective, randomized study was conducted to compare a response-oriented individualized remission induction therapy with a standard fixed-schedule induction therapy, using idarubicin (IDR) and cytarabine (Ara-C), in adult patients with acute myeloid leukemia (AML). Newly diagnosed patients with AML of age less than 65 were randomly assigned to receive either of the two schedules. Both groups received IDR (12 mg/m<sup>2</sup>)

for 3 days and Ara-C (100 mg/m<sup>2</sup>) for 7 days. In the individualized group, if the bone marrow on day 8 did not become hypocellular with less than 15% blasts, patients received additional IDR for one more day and Ara-C for 2 or 3 more days. Patients achieving complete remission (CR) received the same post-remission therapy. The CR rate was 79.4% for the individualized group ( $n = 209$ ) and 81.9% for the fixed group ( $n = 221$ ) ( $p = 0.598$ ). At a median follow-up of 81 months, 7-year predicted overall survival was 37% for the individualized group and 39% for

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the fixed group ( $p = 0.496$ ), and 7-year predicted event-free survival was 22% for the individualized group and 23% for the fixed group ( $p = 0.546$ ). Thus, the present study could not demonstrate any advantage of a response-oriented individualized induction therapy over a fixed-schedule induction therapy in this protocol setting.

**Keywords** Acute myeloid leukemia · Response-oriented individualized induction therapy · Idarubicin · Cytarabine

## 1 Introduction

In Japan, a response-oriented individualized induction therapy has been used for adult acute myeloid leukemia (AML) since the reporting of the success of DCMP two-step therapy using daunorubicin (DNR), cytarabine (Ara-C), 6-mercaptopurine (6MP) and prednisolone (PSL), by Uzuka et al. in the mid 1970s [1]. They reported a complete remission (CR) rate of more than 80% in adult AML, which is currently not surprisingly high but was remarkable in the mid 1970s even for a single institutional study. A subsequent multi-institutional study conducted at the Koseisho Leukemia Study Group using this DCMP two-step protocol could not replicate the high CR rate, but a subset analysis revealed the first-step alone could induce almost the same CR rate as the two-step therapy [2]. Accordingly, a response-oriented individualized induction therapy, the BHAC-DMP therapy, using enocitabine (BHAC), Ara-C, 6MP, and PSL, was developed, and Ohno et al. [3] reported more than 80% CR in adult AML by a single institutional study.

The multi-institutional AML87 study conducted by the Japan Adult Leukemia Study Group (JALSG) confirmed the high CR rate of response-oriented individualized BHAC-DMP therapy in adult AML, reporting an 80% CR

rate [4]. Subsequent JALSG studies, AML89 [5] and AML92 [6], also employed the response-oriented individualized induction therapy, and reported 81 and 77% CR rates, respectively, in adult patients of age less than 65 years with non-M3 type AML. These CR rates were around 10% higher than those reported from cooperative study groups in the USA and Europe, where fixed-schedule induction therapies are used [7]. Therefore, even though the necessity for a randomized study was seriously discussed among JALSG members, it was not possible to find any fixed-schedule regimen worth comparing with the present individualized therapy.

In the above 3 JALSG studies, DNR was used as one of the key drugs. However, in the late 1980s, a new DNR analogue, idarubicin (IDR), was introduced clinically, and in the early 1990s, one single [8] and 2 multi-institutional studies [9, 10] reported that IDR plus Ara-C regimens could produce 70–80% CR rates in adult AML by fixed-schedule therapy, which were significantly higher than the 58–59% CR rates of DNR plus Ara-C regimens.

Consequently, after IDR had been approved in Japan in 1995, a randomized study using IDR and Ara-C was conducted, comparing a response-oriented individualized induction therapy with a fixed-schedule therapy in previously untreated adult patients with AML.

## 2 Patients and methods

### 2.1 Patients

From August 1995 to December 1997, 437 newly diagnosed adult patients with AML, aged 15–64 years, were consecutively registered from 79 institutions, which participated in JALSG. The enrolled number of patients per hospital varied from 1 to 23 with median number of 4, and about 60% of patients were registered from major hospitals listed in the institutions of the authors.

AML was diagnosed by the French-American-British (FAB) classification at each institution. Peripheral blood and bone marrow smears from all registered patients were sent to Nagasaki University and examined by May-Giemsa, peroxidase, and esterase staining. Then, diagnosis was reevaluated by the central review committee. FAB-M3 was not registered in this study. Eligibility criteria included adequate functioning of the liver (serum bilirubin level  $< 2.0$  mg/dL), kidney (serum creatinine  $< 2.0$  mg/dL), heart, and lungs, and an Eastern Cooperative Oncology Group performance status between 0 and 3. Patients were not eligible if they had prediagnosed myelodysplastic syndrome (MDS), but were eligible if they had no definite diagnosis of MDS, as confirmed by bone marrow histological analysis even when they had a previous history of

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hematological abnormality. Cytogenetic analyses were performed at either laboratories in participating hospitals or authorized commercial laboratories according to standard methods of G-banding. Cytogenetic abnormalities were grouped by standard criteria and classified according to the MRC classification [11]. The protocol was approved by institutional review board of each hospital. Informed consent was obtained from all patients before registration.

2.2 Treatment regimens

Patients were assigned randomly to receive either a response-oriented individualized induction therapy or a fixed-schedule induction therapy, using a centralized telephone procedure. All patients received IDR (12 mg/m<sup>2</sup>/day, intravenously) from days 1 to 3 and Ara-C (100 mg/m<sup>2</sup>/day, by 24-h continuous infusion) from days 1 to 7. Examination of bone marrow on the day 8 was evaluated at each participating hospital and the decision was made by the attending physician in charge of the hospital. In the individualized group, bone marrow aspiration was performed on day 8, and if the marrow was not severely hypoplastic and had more than 15% blasts, additional IDR was given on day 8 and Ara-C on days 8 to 10, or if the marrow was severely hypoplastic and had more than 15% blasts, additional IDR was given on day 8 and Ara-C on days 8 and 9. If patients suffered from documented infection on day 8, cancellation of additional chemotherapy was permitted according to the judgment of the attending physician (Fig. 1). The main aim of the individualized therapy was to give highly intensive but not too toxic doses of anti-leukemia drugs, especially IDR, to make the bone marrow severely hypoplastic, reduce the percentage of blasts to less than 5% within 10 days and obtain CR by the first course of induction therapy. In the fixed-schedule group (fixed group), patients did not receive additional doses regardless of their marrow status at day 8. If patients did not achieve CR by the first course, the same induction

therapy was repeated at approximately 3- to 4-week intervals. If patients did not achieve CR with two courses, they were judged as failure cases.

All patients in both groups who had achieved CR planned to receive the same 3 courses of consolidation therapy. The first course consisted of mitoxantrone (MIT; 7 mg/m<sup>2</sup> by 30-min infusion on days 1–3) and Ara-C (200 mg/m<sup>2</sup> by 24-h continuous infusion on days 1–5). The second consisted of BHAC (200 mg/m<sup>2</sup> by 3-h infusion on days 1–7), DNR (50 mg/m<sup>2</sup> intravenously on days 1–3), 6MP (70 mg/m<sup>2</sup> orally on days 1–7), and etoposide (ETP; 100 mg/m<sup>2</sup> by 1-h infusion on days 1–5). The third consisted of BHAC (200 mg/m<sup>2</sup> on days 1–7) and aclarubicin (ACR; 14 mg/m<sup>2</sup> intravenously on days 1–7). Each consolidation course was given as soon as possible after WBC and platelet counts had recovered to more than 3,000/μL and 100,000/μL, respectively. Intrathecal methotrexate (15 mg), Ara-C (40 mg), and PSL (10 mg) were given after the second consolidation therapy for the prophylaxis of central nervous system leukemia.

After the completion of consolidation therapy, all patients planned to receive 6 courses of maintenance/intensification therapy every 2 months. The first course consisted of BHAC (170 mg/m<sup>2</sup> on days 1–5), DNR (40 mg/m<sup>2</sup> on days 1 and 4), and 6MP (70 mg/m<sup>2</sup> on days 1–7). The second consisted of BHAC (170 mg/m<sup>2</sup> on days 1–5) and MIT (5 mg/m<sup>2</sup> on days 1 and 2). The third consisted of BHAC (170 mg/m<sup>2</sup> on days 1–5), ETP (80 mg/m<sup>2</sup> on days 1, 3, and 5), and vindesine (2 mg/m<sup>2</sup> intravenously on days 1 and 8). The fourth consisted of BHAC (170 mg/m<sup>2</sup> on days 1–5), ACR (14 mg/m<sup>2</sup> on days 1–4) and 6MP (70 mg/m<sup>2</sup> on days 1–7), the fifth was the same as the first, and the sixth was the same as the third. Each course was given at 2-month intervals.

Best supportive care, including administration of antibiotics and platelet transfusion from blood cell separators, was given if indicated. When patients had life-threatening infections during neutropenia, the use of granulocyte colony-stimulating factor was permitted.

2.3 Response criteria and statistical analysis

CR was defined as the presence of all of the following: less than 5% of blasts in bone marrow, no leukemic blasts in peripheral blood, recovery of peripheral neutrophil counts over 1,000/μL and platelet counts over 100,000/μL, and no evidence of extramedullary leukemia. CR had to continue for at least 4 weeks, but the date of CR was defined as the first day when these criteria were fulfilled. Relapse was defined as the presence of at least one of the following: recurrence of more than 10% leukemic cells in bone marrow, any leukemic cells in peripheral blood, and appearance of extramedullary leukemia.

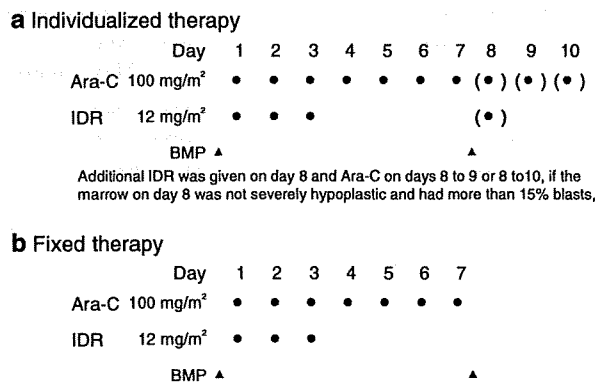


Fig. 1 Treatment scheme of induction therapy

Overall survival (OS) was calculated from the first day of induction therapy to death by any cause and censored at the last follow-up. Event-free survival (EFS) was computed from the first day of induction therapy to relapse or death by any cause and censored at the last follow-up, and the survival time of patients who did not achieve CR was defined as 0 days. Relapse-free survival (RFS) for patients who achieved CR was measured from the date of CR to relapse or death by any cause and censored at the last follow-up. Patients who underwent allogeneic bone marrow transplantation (BMT) were censored at the date of BMT or not censored according to the object of the analysis. Kaplan–Meier product-limit estimates were used to determine OS, EFS, and RFS. To test factors to predict CR,  $\chi^2$  test and Wilcoxon rank-sum test were used for univariate analysis and the multiple logistic regression model for multivariate analysis. For comparison of OS, EFS, and RFS, the log-rank test was used for univariate analysis and Cox's proportional hazard model for multivariate analysis. JMP software (SAS Institute Inc., Cary, NC, USA) was used for the analysis; *p* values less than 0.05 (two-sided) were considered statistically significant. Analysis was done on an intent-to-treat basis.

### 3 Results

#### 3.1 Patient population and characteristics

Of 437 patients registered, 7 patients were judged as ineligible by the central review committee because of other diseases: one refractory anemia with excess of blast, 5 mixed-lineage leukemia, and one acute lymphoblastic leukemia (ALL), with 430 patients considered evaluable. Two hundred nine patients received the individualized therapy and 221 the fixed-schedule therapy. Pretreatment characteristics are presented in Table 1. There were no major imbalances between the two randomized groups. Overall, the median age was 44 years, and 154 patients (36%) were of age 50 years or older. Cytogenetic analysis was reported in 414 patients (96%), and the cytogenetic prognostic groups were equally distributed in both arms.

#### 3.2 Overall treatment results

Of 430 evaluable patients, 347 (80.7%) achieved CR. Of 209 patients in the individualized group, 166 (79.4%) achieved CR, and of 221 in the fixed group, 181 (81.9%) obtained CR (*p* = 0.516) (Table 2). CR rates related to FAB classification, age, and cytogenetics are shown in Table 2, and there were no statistically significant differences between the two groups. In the individualized group, of 41 patients with favorable chromosomes, 39 (95%) achieved CR, of 133 with

**Table 1** Pretreatment characteristics

	Individualized group ( <i>n</i> = 209)	Fixed group ( <i>n</i> = 221)
Median age (range)	44 years (15–64)	44 years (15–64)
PS 0	34.9%	38.5%
PS 1	42.6%	45.2%
PS 2	14.4%	9.5%
PS 3	8.1%	6.8%
Leukocyte counts > 50,000/ $\mu$ L	17.7%*	29.9%*
Peroxidase positivity $\geq$ 50%	62.8%	64.2%
Presence of Auer body (%)	37.5%	46.1%
Presence of trilineage dysplasia	25.4%	21.2%
LDH $\geq$ 500 IU/L	65.9%	69.1%
Cytogenetics		
Favorable	19.6%	22.2%
Intermediate	63.6%	59.7%
Adverse	13.4%	14.0%
Unknown	3.3%	4.1%

\* *p* < 0.05

**Table 2** CR rates related to FAB classification, age, and cytogenetics

	All cases		Individualized group		Fixed group	
	No.	CR (%)	No.	CR (%)	No.	CR (%)
FAB						
M 0	16	62.5	8	62.5	8	62.5
M 1	80	85.0	41	85.4	39	84.6
M 2	192	82.3	95	77.9	97	86.6
M 4	108	78.7	55	80.0	53	77.4
M 5	20	90.0	5	100.0	15	86.7
M 6	8	50.0	2	50.0	6	50.0
M 7	6	66.7	3	66.7	3	66.7
Age						
15–19	40	90.0	19	100.0	21	81.0
20–29	65	78.5	29	75.9	36	80.6
30–39	71	81.7	41	75.6	30	90.0
40–49	100	83.0	45	77.8	55	87.3
50–59	105	77.1	53	79.2	52	75.0
60–64	49	77.6	22	77.3	27	77.8
Cytogenetics						
Favorable	90	93.3	41	95.1	49	91.8
Intermediate	265	80.8	133	78.9	132	82.6
Adverse	59	62.7	28	60.7	31	64.5
Unknown	16	75.0	7	71.4	9	77.8
Total	430	80.7	209	79.4	221	81.9

intermediate chromosomes, 109 (79%) achieved CR, and of 28 with adverse chromosomes, 17 (61%) achieved CR. In the fixed group, of 49 patients with favorable chromosomes,

45 (92%) achieved CR, of 132 with intermediate chromosomes, 109 (83%) achieved CR, and of 31 with adverse chromosomes, 20 (65%) achieved CR.

In the individualized group, 149 patients (71%) achieved CR after the first course, and 79 (38%) patients who had received additional chemotherapy during the first course, 56 (71%) achieved CR. In the fixed group, 159 (72%) achieved CR after the first course (Table 3; Fig. 2). CR rates between patients who had equal to or more than 15% of blasts in bone marrow on day 8 and those had less than 15% were not significantly different in the individualized group (75 and 63%, respectively;  $p = 0.09$ ), but were significantly different in the fixed group (81 and 56%, respectively;  $p < 0.001$ ).

Myelosuppression judged by the nadir of leukocyte counts and the period of leukocyte count less than 1,000/ $\mu$ L after the first course of induction therapy was significantly more severe in the individualized group, as shown in Table 4. Early death within 30 days occurred in 10 (4.8%)

in the individualized group and 4 (1.8%) in the fixed group ( $p = 0.105$ ). There were no statistically significant differences in the distribution or frequency of complications between the two groups.

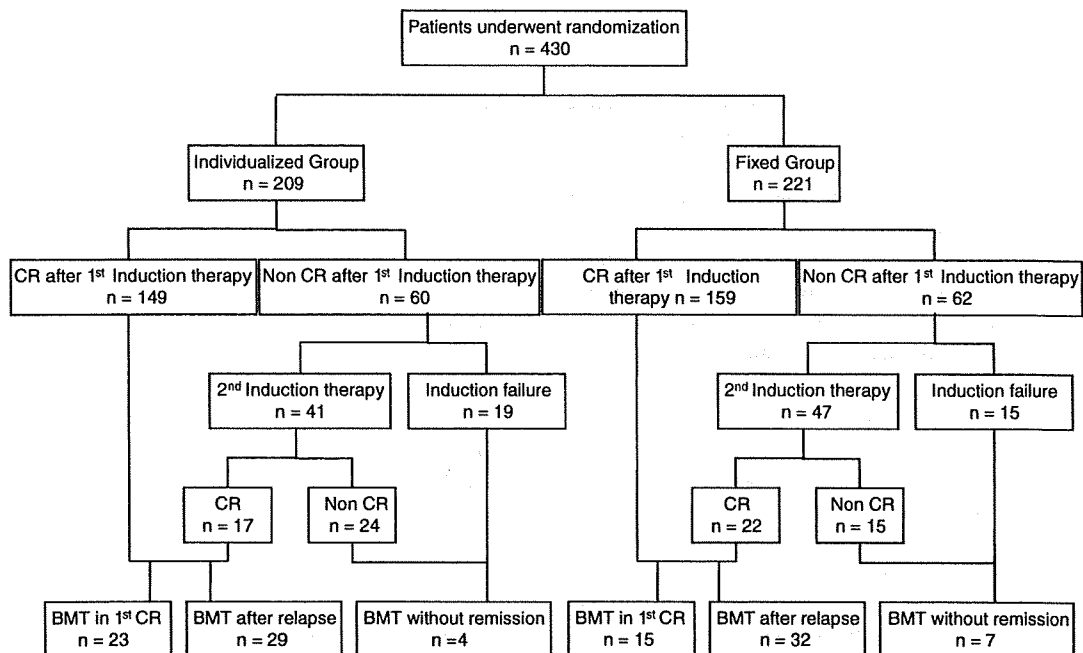
Significant favorable prognostic features for the achievement of CR were cytogenetic risk group (favorable or intermediate), blast peroxidase positivity of 50% or more, and pretreatment LDH value of less than 500 IU/L. These features were independent by the logistic regression analysis and not different between the two groups.

All courses of consolidation therapy were administered to 72% of patients in the individualized group and 80% in the fixed group ( $p = 0.087$ ), and all courses of maintenance therapy were administered to 36 and 41% ( $p = 0.365$ ), respectively. The most common reason for these cancellations was relapse in both groups (34 and 42 patients, respectively). The second common reason was BMT in the first remission (22 and 12 patients, respectively).

At a median follow-up of 81 months, 23 patients underwent BMT in the first remission, 29 after relapse and 4 without remission in the individualized group, and 15, 32 and 7 patients, respectively, in the fixed group. If patients who underwent BMT were censored at the date of transplantation to decrease the influence of BMT, 7-year predicted OS was 37% for the individualized group and 39% for the fixed group ( $p = 0.496$ ) (Fig. 3a), and 7-year predicted EFS was 22 and 23%, respectively ( $p = 0.546$ ) (Fig. 3b). If patients who underwent BMT were not censored, 7-year predicted OS was 35 and 35%, respectively

**Table 3** Effect of individualized induction therapy

	Patients (%)	CR after first course	
		n	%
Individualized group	209	149	71
Additional chemotherapy -	130 (62)	93	72
Additional chemotherapy +	79 (38)	56	71
Fixed group	221	159	72



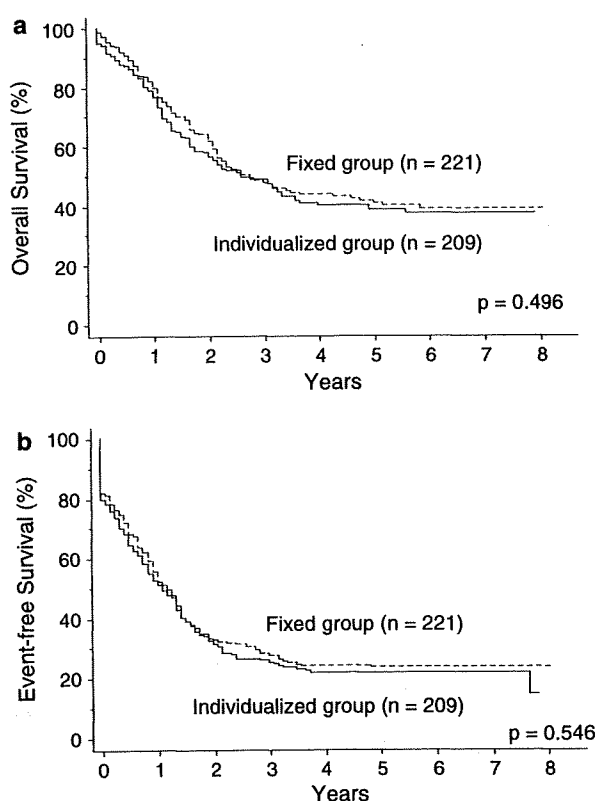
**Fig. 2** Flow diagram: study design and outcome

**Table 4** Comparison of treatment outcome

	Individualized group (n = 209)	Fixed group (n = 221)	p
CR rate (%)	79.4	81.9	0.516
After the first course	71.3	71.9	
After the second course	8.1	10.0	
Marrow blasts at day 8	12.9 ± 17.8%	11.1 ± 18.4%	0.021
Nadir of WBC <sup>a</sup>	328 ± 205/μL	394 ± 215/μL	0.0002
Period of WBC < 1,000/μL <sup>a</sup>	19.6 ± 9.8 days	17.8 ± 8.5 days	0.024
Days to CR <sup>a</sup>	38.9 ± 17.5	38.5 ± 16.2	0.802
Days till the consolidation therapy	49 ± 22	46 ± 18	0.157
Early death rate			
Within 30 days	4.8%	1.8%	0.105
Between 30 and 60 days	0.9%	1.4%	
Overall survival at 7 years	37%	39%	0.496
Event-free survival at 7 years	22%	23%	0.546

Data with ± denotes mean ± standard deviation

<sup>a</sup> After the initial course of induction therapy



**Fig. 3** Overall survival (a) and event-free survival (b). Predicted 7-year OS was 37% for the individualized group (n = 209) (solid line) and 39% for the fixed group (n = 221) (dotted line) (p = 0.496), and EFS was 22% for the individualized group (solid line) and 23% for the fixed group (dotted line) (p = 0.546)

(p = 0.840), and 7-year predicted EFS was 23 and 24%, respectively (p = 0.717). Significant adverse prognostic features for OS were absence of Auer body, cytogenetic

risk group (adverse), and age more than 30 years, and those for EFS were blast peroxidase positivity less than 50%, cytogenetic risk group (adverse), pretreatment LDH value equal or more than 500 IU/L, and FAB classification (M0, M6, or M7). When patients who underwent BMT were censored, RFS of CR patients was 27% for the individualized group and 29% for the fixed group (p = 0.712). Significant adverse prognostic features for RFS of CR patients were cytogenetic risk group (adverse) and FAB classification (M0, M6, or M7). There were no significant differences in these prognostic features between the two groups. However, among patients of age 50 years or older, the individualized group had significantly lower RFS (17%) than the fixed group (34%, p = 0.026), but there was no such difference of RFS (34 and 25%, respectively, p = 0.194) among patients of age less than 50 years.

#### 4 Discussion

Most drug therapies are generally carried out in a response-oriented and individualized manner. Physicians adjust the dosage and treatment period depending on the response of patient's disease to the administered drugs. The reason why cancer chemotherapy is generally carried out by fixed dosage and period is because myelosuppression, the most important toxic effect of cytotoxic drugs, appears 7–10 days after the discontinuation of drugs. Myelosuppression is usually judged by leukocyte or platelet counts in the peripheral blood. However, if it is judged by bone marrow itself it is possible to obtain information on myelosuppression directly and earlier. Although the present individualized therapy requires frequent bone marrow aspirations and a prompt decision by attending physicians, well-trained hematology oncologists have little difficulty in