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ORIGINAL ARTICLE

Pharmacokinetics of arsenic species in Japanese patients with relapsed or refractory acute promyelocytic leukemia treated with arsenic trioxide

Shinya Fujisawa · Ryuzo Ohno · Kazuyuki Shigeno · Naohi Sahara · Satoki Nakamura · Kensuke Naito · Miki Kobayashi · Kaori Shinjo · Akihiro Takeshita · Yoshinari Suzuki · Hisakuni Hashimoto · Kenji Kinoshita · Masahito Shimoya · Toshikazu Kaise · Kazunori Ohnishi

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Abstract
Purpose To investigate the pharmacokinetics of arsenic species in Japanese patients with relapsed or refractory acute promyelocytic leukemia (APL) treated with arsenic trioxide (ATO) at a daily dose of 0.15 mg/kg.
Methods Inorganic arsenic (As^{III} and As^{V}) and the major metabolites monomethylarsonic acid (MAA^{V}) and dimethylarsinic acid ($DMAA^{V}$) in plasma and urine collected from 12 Japanese patients were quantified by HPLC/ICP-MS.
Results The plasma concentrations of As^{III} and As^{V} on day 1 reached the similar *C*max (12.4 ± 8.4 and 10.2 ± 3.9 ng/ml) immediately after completion of administration followed by a biphasic elimination. The $AUC_{0-\infty}$ of As^{V} was about twice that of As^{III}. The appearance of methylated metabolites in the blood was delayed. During the repeated administration, the plasma concentrations of inorganic arsenic reached the steady state. In contrast, the MAA^{V} and $DMAA^{V}$ concentrations increased in relation to increased administration frequency. The mean total arsenic excretion rate including inorganic arsenic and methylated arsenic was about 20% of daily dose on day1 and remained at about 60% of daily dose during week 1–4.
Conclusions This study demonstrates that ATO is metabolized when administered intravenously to APL patients and methylated metabolites are promptly eliminated from the blood and excreted into urine after completion of administration, indicating no measurable accumulation of ATO in the blood.

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S. Fujisawa · K. Shigeno · N. Sahara · S. Nakamura · K. Naito · M. Kobayashi · K. Shinjo · K. Ohnishi
Department of Medicine III, Hamamatsu University School of Medicine, Hamamatsu, Japan

R. Ohno
Aichi Cancer Center Hospital, Nagoya, Aichi, Japan

A. Takeshita
Department of Laboratory Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan

Y. Suzuki · H. Hashimoto
Pharmacy Department, Hamamatsu University Hospital, Hamamatsu, Japan

K. Kinoshita · M. Shimoya · T. Kaise
Laboratory of Environmental Chemodynamics, Tokyo University of Pharmacy and Life Science, Hachioji, Tokyo, Japan

K. Ohnishi (✉)
Division of Clinical Oncology, Hamamatsu University School of Medicine, 1-20-1, Handayama, Hamamatsu 431-3192, Japan
e-mail: kohnishi@hama-med.ac.jp

Introduction

Acute promyelocytic leukemia (APL) is a distinctive type of acute myelocytic leukemia (AML) characterized by chromosome translocations *t* (15; 17) and accounts for approximately 10–15% of all cases of AML. In the 1990s, investigators from China reported that arsenic trioxide (ATO) induces complete remission (CR) in patients with relapsed or refractory APL

[1–3]. After the Chinese reports, the pilot and multicenter studies of ATO were conducted in the United States in relapsed or refractory APL patients. Combining the results from the pilot and multicenter studies, the CR rate was 87% [4, 5]. At present, ATO is the first-choice treatment of relapsed or refractory APL. In Japan, we have previously reported the prospective study of ATO in APL patients [6]. ATO was administered by the same protocol used in the United States multicenter study [5] to 14 relapsed APL patients who had not responded to ATRA and conventional chemotherapy, and of these 11 (78%) achieved CR.

While the clinical effect of ATO against APL was established, its pharmacokinetics has yet to be clarified. ATO is methylated by methyl transferase in the liver to monomethyl and dimethyl arsenic compounds, including the major metabolites monomethylarsonic acid (MAA^V) and dimethylarsinic acid ($DMAA^V$), which are mostly excreted into urine [7, 8]. Recent investigation shows that, among inorganic arsenic and methylated metabolites, As^{III} could induce cytotoxity against NB4 cells that derived from APL, degradation of PML-RARα chimeric protein that formed as a consequence of the t (15; 17) and causes the pathogenesis of APL, and differentiation in NB4 cells [9]. However, in most reports on the pharmacokinetics of ATO to patients with APL and other hematological malignancies, the arsenic concentrations were measured as total arsenic including metabolites [3, 10–14]. Recently, since the HPLC/ICP-MS, which combines high performance liquid chromatography (HPLC) and inductivity coupled plasma mass spectrometry (ICP-MS), was used for the quantitation of arsenic, the pharmacokinetics of arsenic species has been analyzed in several reports [15, 16]. However, the pharmacokinetic data of arsenic species for Asian people are presently limited [16].

In the prospective study [6], we have collected the blood and urine from 12 APL patients, subsequently stored frozen. Using the freeze-stored samples, we determined inorganic arsenic (As^{III} and As^V) and the major metabolites MAA^V and $DMAA^V$ concentrations by the HPLC/ICP-MS method [16], and conducted a pharmacokinetic analysis.

Patients and methods

Patients and administration schedule

The prospective study [6] was conducted at the Hamamatsu University School of Medicine in 14 APL patients from March 1999 to March 2001 according to the United States multicenter study [5]. ATO (Trisenox®) was provided by PolaRx Biopharmaceuticals (New York, NY, USA) and later from Cell Therapeutics (Seattle, WA, USA). Eligibility criteria and treatment plan were previously described. [6] The protocol was reviewed and approved by the institutional review board of the Hamamatsu University Hospital. Written informed consent was obtained from patients before the treatment. During the induction treatment, ATO was administered intravenously over 2 h at a dose of 0.15 mg/kg in 500 ml of 5% of dextrose given once daily for cumulative maximum of 60 days. Patients who had achieved complete remission received one course of consolidation therapy with ATO for a cumulative total of 25 days, using the same dose and schedule as the induction therapy.

Samples used in the pharmacokinetic analysis

The blood and urine were collected from 12 patients during the induction treatment. Samples were also collected from two patients (patient 7 and 9) during the consolidation treatment. Sample of blood of 5 ml was collected on the first day of administration (day 1) and after 1, 2, and 4 weeks from the start of administration. The time points for blood collection were as follows. On day 1 and after 4 weeks: before administration, 1 h, at the end of the infusion, 4, 6, 12 (or 18) and 24 h after the start of administration. After 1 and 2 weeks: before administration, at the end of the infusion, and 4 h after the start of administration.

The urine was collected for 24 h on day 1, and after 1, 2, and 4 weeks. After each urine sample collection, urine volume was measured. The plasma obtained from blood and urine samples were stored frozen (–20 or –80°C) until analysis.

As the standard arsenic compounds, sodium arsenite (As^{III}), sodium arsenate (As^V), monomethylarsonic acid (MAA^V), dimethylarsinic acid ($DMAA^V$), trimethyl arsenoxide (TMAsO), arsenobetaine (AB), arsenocholine (AsCho), tetramethylarsonium (TetMAs) and arseno-sugar (AsS) were purchased from Tri Chemical Laboratories Inc. (Yamanashi, Japan).

HPLC/ICP–MS analysis

The quantification of arsenic was performed by HPLC/ICP–MS, which combines HPLC (LC PU611 VS GL Sciences Inc., Tokyo, Japan) and ICP-MS (ELAN DRC-*e* Perkin Elmer SCIEX Inc., Ontario, Canada). [16] Inertsil AS (150 mm × 2.1 mm, 3.0 mm; GL Sciences Inc.) was used as the HPLC column and an

ODS guard column (GL Sciences Inc.) was attached to allow direct injection of the biological samples. Plasma samples were prepared using a protein precipitation procedure with acetonitrile. Urine samples were diluted twice with column eluate (dilution factor = 1:1). Column eluate was developed with 10 mM sodium butanesulfonate, 4 mM tetramethyl ammonium hydroxide, 4 mM malonic acid, and 0.05% methanol at pH 3.0 with HNO_3 and the elution flow rate used was 0.2 ml/min. All samples were filtered using a 0.45 μm membrane filter before injection.

The arsenic detection was performed at *m/z* 75 by ICP-MS. Sample solution of 5 μl was injected onto the guard column and the amount of each arsenic compound was obtained from the calibration curve (the standard arsenic compound was diluted with water to 1, 5, 10, and 20 ppb As). The lower limit of quantification for each arsenic species (As^{III}, As^{V} and the methylated metabolites) was 0.1 ppb.

Pharmacokinetic analysis

On the basis of plasma concentrations, the following pharmacokinetic parameters were calculated by non-compartmental analysis. It was not possible to calculate the parameters in some patients due to incomplete blood sampling. For inorganic arsenic, the parameters on day 1 were obtained from 10–11 patients and those on week 4 obtained from six patients. For the methylated metabolites, the C_{max} and T_{max} were obtained from 11–12 patients and $AUC_{0-tlast}$ was obtained from 9 patients on day 1 while these parameters were determined in six patients on week 4.

1. Maximum concentration (C_{max}) and time to reach C_{max} (T_{max}): C_{max} and T_{max} were obtained from the measured values.
2. Elimination rate constant (λz): λz was obtained by linear regression of the linear part of the log plasma concentration-time curve in the elimination phase according to the least squares method. The data points (≥ 3 time points) were used to perform linear regression and the slope of the fitted line with the best correlation coefficient was used as the elimination rate constant.
3. Elimination half-life ($t_{1/2,\beta}$): $t_{1/2,\beta}$ was calculated as $Ln(2)/\lambda z$.
4. Area under the plasma concentration-time curve (AUC): the AUC up to the final measurable time point (t_{last}) was calculated by the trapezoidal method ($AUC_{0-tlast}$) and added to $C_{tlast}/\lambda z$ to calculate $AUC_{0-\infty}$, where C_{tlast} is the concentration at t_{last}.
5. Total clearance (CL_{tot}): CL_{tot} was calculated as dose/$AUC_{0-\infty}$ on day 1 or dose/$AUC_{0-tlast}$ on week 4.
6. Volume of distribution (Vz, Vss): Vz was calculated from dose/($\lambda z \times AUC_{0-\infty}$) and Vss from dose × MRT/$AUC_{0-\infty}$. The $AUMC_{0-tlast}$ up to t_{last} ($= \int_0^t t \times Cdt$) was calculated by the trapezoidal method and added to $Ct/kel^2 + Ct \times t/kel$ to calculate $AUMC_{0-\infty}$ and MRT was obtained from $AUMC_{0-\infty}/AUC_{0-\infty}$.

On the basis of urinary concentrations, the urinary excretion (% of dose) and the renal clearance (CL_{re}) were calculated from the following equations:

$$\text{Urinary excretion (\%)} = \frac{\text{Urinary concentration } (\mu g/ml) \times \text{Total volume (ml)}}{\text{Dose } (\mu g/\text{body})} \times 100$$

$$\text{Renal clearance (l/kg h)} = \frac{\text{Urinary excretion } (\mu g)/\text{body weight (kg)}}{\text{AUC (ng h/ml)}}$$

WinNonlin standard version 4.1 (Pharsight Co., Palo Alto, CA, USA) was used for the non-compartmental analysis. Other calculations were performed using Microsoft Excel 2000 (Microsoft Co., Redmond, WA, USA).

Results

Patient background and efficacy results

The background and outcomes of ATO therapy for 12 APL patients are shown in Table 1. Ten of 12 patients achieved complete remission. Six of 10 patients who achieved CR became negative in the post-treatment RT-PCR test. Patient 11 died of

Table 1 Patient background and efficacy results

Patient number	Age	Sex	As_2O_3 treatment (days)		Outcome	RT-PCR for PML-RARα[b]	CR duration (month)
			Induction	Consolidation			
1	36	F	54	25	CR	–	22+
2	61	M	40	25	CR	–	10
3	36	F	46	20	CR	–	12+
4	33	F	41	25	CR	–	12+
5	58	M	41	25	CR	–	9+
6	52	M	43	–	CR	+	6
7	57	M	43	25	CR	+	12
8	23	F	27	25	CR	–	8
9	62	M	60	25	CR	+	8
10	50	M	44	–	CR	ND	4
11	65	M	21	–	Early death[a]	NA	–
12	64	M	64	–	NR	NA	–

ND not done; *NA* not applicable; *CR* complete remission; *NR* no response

[a] Due to cerebral hemorrhage on day 21

[b] Reverse transcriptase (RT)-PCR assays of bone marrow mononuclear cells for PML-RARα were performed after the consolidation treatment

cerebral hemorrhage on day 21, and patient 12 was discontinued the administration on day 92 because ATO was ineffective.

For two patients (patient 7 and 8) the blood and urine were collected also during the consolidation treatment.

Pharmacokinetics of arsenic species on the first day of administration

The plasma concentrations of inorganic arsenic (As^{III} and As^{V}) on day 1 are shown in Fig. 1, and the pharmacokinetic parameters in Table 2. The plasma concentration of As^{III} reached C_{max} immediately after the administration in most of the patients, followed by a biphasic decline with a mean $t_{1/2,\beta}$ of 17 h after an initial distribution phase (up to 4–8 h after the start of administration). The mean C_{max} was 12.4 ± 8.4 ng/ml. The mean volume of distribution at steady state (Vss) was large (55.9 l/kg) suggesting extensive distribution throughout the body. The plasma concentration profile of As^{V} was similar to that of As^{III}. After reaching C_{max} of 10.2 ± 3.9 ng/ml, a biphasic decline was observed with a mean $t_{1/2,\beta}$ of 18.3 h. After the end of the infusion, the As^{V} plasma concentrations initially declined more slowly than those for As^{III}, and hence the $AUC_{0-\infty}$ of As^{V} was about twice that of As^{III} (As^{III}, 80.5 ± 39.8 ng h/ml; As^{V}, 155.1 ± 78.6 ng h/ml). In addition, the pharmacokinetic parameters for the inorganic arsenic concentrations (the total measured As^{III} and As^{V} values) were calculated, and the results indicated a C_{max} of 22.6 ± 11.4 ng/ml and $AUC_{0-\infty}$ of 211.8 ± 55.1 ng h/ml.

The plasma concentrations and the pharmacokinetic parameters of major metabolites (MAA^{V}, $DMAA^{V}$) on day 1 are shown in Fig. 2 and Table 2, being compared to the inorganic arsenic (As^{III} + As^{V}). The MAA^{V} and $DMAA^{V}$ concentrations were below the quantification limit until immediately after completion of administration, but a gradual increase was observed from 4 h after the start of administration reaching C_{max} in many patients at 24 h after administration, the final time point on day 1. The mean C_{max} values were 3.1 ± 1.6 and 5.4 ± 2.9 ng/ml, respectively. The plasma concentrations of arsenobetaine (AB), an organic

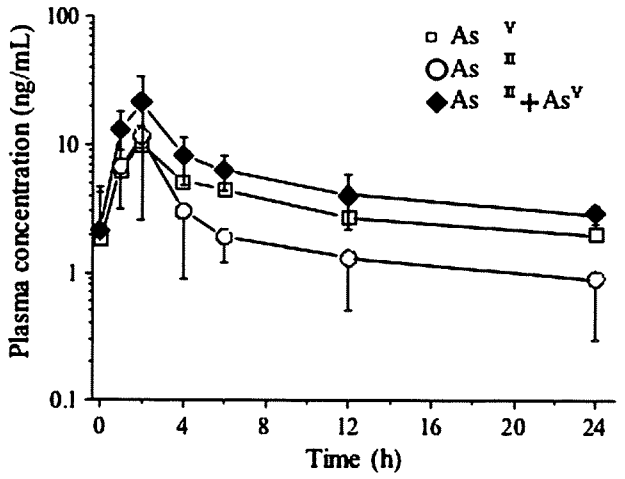

Fig. 1 Plasma concentrations of inorganic arsenic on day 1 of the repeated administration. The values shown in the figure were determined in 12 patients (N = 14; mean ± standard deviation). The values obtained during the induction and consolidation treatment were used for patient 7 and 8

Table 2 Pharmacokinetic parameters of inorganic arsenic (As^{III} + As^{V}) and its metabolites (MAA^{V}, $DMAA^{V}$) on day 1 and week 4

Arsenic species	Day		T_{max} (h)	C_{max} (ng/ml)	$t_{1/2,\beta}$ (h^{-1})	$AUC_{0-tlast}$ (ng h/ml)	$AUC_{0-\infty}$ (ng h/ml)	Vz (l/kg)	CLtot (l/kg/h)	Vss (l/kg)
Inorganic Arsenic[a] $As^{III}+As^{V}$	Day 1	Mean	1.9	22.6	15.4	138.6	211.8	15.2	0.7	14.3
		SD	0.7	11.4	9.2	32.4	55.1	6.7	0.2	6.3
	Week 4	Mean	2.0	23.2	24.2	233.3	474.8	12.8	0.8	12.5
		SD	0.3	10.2	12.5	92.8	192.6	10.6	0.5	10.4
As^{IIIa}	Day 1	Mean	1.9	12.4	17.0	52.8	80.5	44.0	2.3	55.9
		SD	0.7	8.4	19.0	21.2	39.8	27.5	1.3	55.9
	Week 4	Mean	1.6	10.1	24.8	82.6	190.6	41.5	2.7	39.7
		SD	0.5	6.6	12.3	55.7	189.9	28.6	1.7	28.1
As^{Vb}	Day 1	Mean	1.8	10.2	18.3	86.9	155.1	25.8	1.2	24.8
		SD	0.9	3.9	11.3	22.5	78.6	9.4	0.6	8.9
	Week 4	Mean	2.0	14.2	32.2	150.7	357.5	21.0	1.2	20.6
		SD	0.3	6.6	24.2	51.9	164.7	18.6	0.8	18.4
MAA^{Vc}	Day 1	Mean	18.0	3.1	–	48.7	–	–	–	–
		SD	6.4	1.6	–	12.9	–	–	–	–
	Week 4	Mean	3.9	10.9	–	174.2	–	–	–	–
		SD	6.5	4.7	–	66.1	–	–	–	–
$DMAA^{Vd}$	Day 1	Mean	20.1	5.4	–	83.1	–	–	–	–
		SD	6.3	2.9	–	30.8	–	–	–	–
	Week 4	Mean	5.6	21.4	–	374.1	–	–	–	–
		SD	9.2	12.3	–	214.5	–	–	–	–

– Not analyzable

T_{max} time to maximum concentration, C_{max} maximum concentration, $t_{1/2,\beta}$ apparent terminal half-life, $AUC_{0-tlast}$ area under the curve from time zero to the final measurable time point (t_{last}), $AUC_{0-\infty}$ area under the curve from time zero to infinity, Vz volume of distribution at the terminal phase, CL_{tot} systemic clearance, Vss volume of distribution at steady-state

The values obtained during the induction and consolidation treatment were used for patient 7, while the values obtained during the consolidation treatment were used for patient 8

[a] The values obtained from ten patients (N = 11) on day 1 and six patients (N=6) in week 4 were analyzed

[b] The values obtained from 11 patients (N = 12) on day 1 and six patients (N=6) in week 4 were analyzed

[c] The C_{max} and T_{max} were obtained from 12 patients (N=13) and $AUC_{0-tlast}$ was obtained from 9 patients (N=10) on day 1 while these parameters were determined in six patients (N=6) in week 4

[d] The C_{max} and T_{max} were obtained from 11 patients (N=12) and $AUC_{0-tlast}$ was obtained from 9 patients (N=10) on day 1 while these parameters were determined in six patients (N=6) in week 4

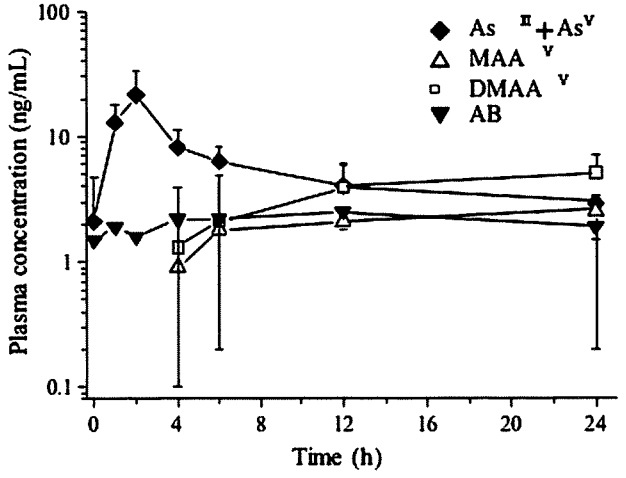

Fig. 2 Plasma concentrations of inorganic arsenic and methylated metabolites (MAA^{V}, $DMAA^{V}$, AB) on day 1 of the repeated administration. The values shown in the figure were determined in 12 patients (N = 14; mean ± standard deviation). The values obtained during the induction and consolidation treatment were used for patient 7 and 8

arsenic compound derived from seafood, remained almost constant (about 2 ng/ml) during the study period. Accordingly, the influence of arsenic derived from meals in this study was considered negligible.

For the 2 patients (patient 7 and 8) whose arsenic concentrations were determined both the induction and consolidation treatment, the plasma concentrations of As^{III}, As^{V}, and MAA^{V} had decreased below the quantitation limit during the washout period (69 days for patient 7; 37 days for patient 8) before the consolidation treatment, and only the $DMAA^{V}$ concentration of 1.9 ng/ml was detected in patient 8. For patient 7, the blood was collected 9 days after the completion of the induction treatment for 43 days receiving a total of 448 mg ATO, and the plasma concentrations were as follows: As^{V}—0, As^{III}—0, MAA^{V}—1.9 ng/ml, $DMAA^{V}$—8.3 ng/ml, indicating the complete disappearance of inorganic arsenic. These results indicate that most of the inorganic arsenic is

promptly metabolized to $DMAA^V$ and ATO is not accumulated in the blood.

Pharmacokinetics of arsenic species during the repeated administration

The mean plasma concentrations of inorganic arsenic ($As^{III} + As^V$) and its metabolites (MAA^V and $DMAA^V$) on day 1 and weeks 1, 2, and 4 during the repeated administration are shown in Fig. 3, and the pharmacokinetic parameters on day 1 and week 4 are in Table 2. In comparison with the levels on day 1, the C_{max} of inorganic arsenic on week 4 was similar but the elimination was delayed. As a result, the $AUC_{0-\infty}$ increased about twofold. However, the $AUC_{0-tlast}$ of inorganic arsenic on week 4 was similar to the $AUC_{0-\infty}$ on day 1, indicating that no marked change was observed in CL_{tot} during the repeated administration. The mean concentration profile from day 1 to week 4 indicated no increase in the C_{max} of inorganic arsenic related to administration frequency. These results suggest that the plasma concentration had reached the steady state. Alternatively, the plasma concentrations of MAA^V and $DMAA^V$ increased along with the increase in administration frequency during the repeated administration. The C_{max} and $AUC_{0-tlast}$ of both metabolites on week 4 were about four times those of the respective levels observed on day 1. The plasma concentrations of AB remained almost constant (about 2–4 ng/ml) during the repeated administration.

Urinary excretions of arsenic species

The urinary excretions (daily excretion rate, % of dose) of inorganic arsenic and methylated metabolites on day 1 and weeks 1, 2, and 4 during the repeated administration are shown in Table 3. The respective excretions of As^{III} and As^V on day 1 accounted for about 6%. During the repeated administration, the excretions increased and remained almost constant after week 1–4 (As^{III}: about 13–16%, As^V: about 7–8%), suggesting that the steady state was attained. A similar tendency was observed in the excretion rates of MAA^V and $DMAA^V$ on day 1 and during the repeated administration. The mean total arsenic excretion rate including inorganic arsenic and methylated arsenic was about 20% of daily dose on day1 and remained at about 60% of daily dose during week 1–4.

The CL_{re} values for As^{III} and As^V were about 10 and 6% of the CL_{tot} (shown in Table 2), respectively. These results indicate that renal excretion play no significant role in the elimination of inorganic arsenic, and suggest that hepatic elimination appears to be the main route of systemic clearance.

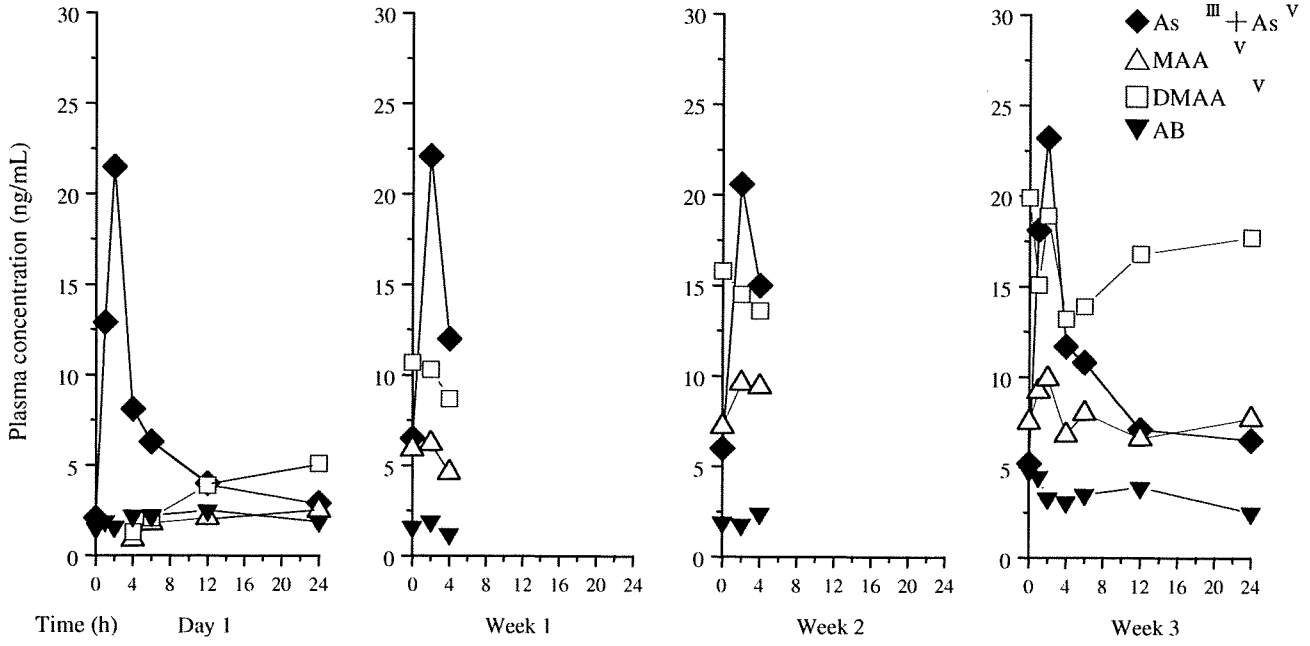

Fig. 3 The plasma concentrations of inorganic arsenic ($As^{III} + As^V$) and its metabolites (MAA^V, $DMAA^V$, and AB) on day 1 and weeks 1, 2, and 4. The values shown in the figure indicate mean values determined in 12 patients ($N = 14$) on day 1, in nine patients ($N = 10$) during week 1, in ten patients ($N = 12$) during week 2 and in seven patients ($N = 7$) during week 4. The values obtained in the induction and consolidation treatment were used for patients 7 and 8

Table 3 Urinary excretions of inorganic arsenic and methylated metabolites during the repeated administration

		Urinary excretions (% of dose)				CLre (l/kg/h)	
		Day 1	Week 1	Week 2	Week 4	Day 1	Week 4
As^{III}	Mean	6.5	16.2	12.8	13.7	0.20	0.32
	SD	4.9	10.1	8.3	9.6	0.10	0.14
As^{V}	Mean	5.6	7.3	8.2	6.8	0.07	0.07
	SD	5.9	8.6	12.1	8.2	0.06	0.06
MAA^{V}	Mean	5.0	17.4	12.9	19.6	0.11	0.17
	SD	2.5	11.2	5.9	10.0	0.07	0.10
$DMAA^{V}$	Mean	3.2	19.4	19.8	21.1	0.05	0.10
	SD	1.3	8.5	9.6	9.5	0.03	0.03
Total	Mean	20.4	60.3	53.7	61.1	–	–
	SD	7.4	25.1	22.6	28.5	–	–

The urine was collected for 24 h on each measurement day. The values obtained during the induction and consolidation treatment were used for patient 7 and 8. The urine samples obtained from nine patients ($N = 11$) on day 1, from nine patients ($N = 10$) during week 1, from nine patients ($N = 11$) during week 2 and from six patients ($N = 6$) during week 4 were analyzed

CL_{re} values were obtained from five to seven patients ($N = 6$–8) on day 1 and from four to five patients ($N = 4$–5) on week 4

Discussion

The plasma and urine concentrations of inorganic arsenic and methylated metabolites in APL patients treated with ATO were determined with HPLC/ICP–MS to clarify the pharmacokinetics of arsenic species. Until recently, arsenic was determined as the total arsenic content including metabolites in most of the reports on the pharmacokinetics of ATO. The current report therefore has considerable clinical meaning because it is the first study investigating the pharmacokinetics of arsenic species in as many as 12 Japanese patients with APL.

Arsenic is contained in foods and especially abundant in fish, shellfish, and seaweed. Arsenic compounds abundant in seafood are mainly organic such as arsenobetaine (AB), a trimethylarsenic compound. The toxicity of AB is extremely low and it is quickly excreted unmetabolized [17, 18]. Since the Japanese people ingest a large quantity of seafood, their urinary concentration of arsenic is higher as an ethnic characteristic. Then the influence of AB derived from meals cannot be ignored, especially for the Japanese. It is possible to determine AB separately from other arsenic compounds in plasma and urine by HPLC/ICP-MS. Therefore this method is very effective to analyze the pharmacokinetics of arsenic species in the Japanese APL patients.

In our study, As^{V} was detected in the plasma and urine at a concentration equivalent to or higher than that of As^{III} and the pharmacokinetic parameters of inorganic arsenic (As^{III} + As^{V}) were similar to those of As^{III} in the Westeners. [Remick et al. J Clin Oncol 2004; 22:2018 (abstract)]. The previous reports have shown that the As^{V} concentrations in plasma or urine after ATO administration are very low. [15, 16, 19] The conversion from As^{III} to As^{V} occurs as natural oxidation while arsenate reductase mainly contributes to the reduction from As^{V} to As^{III} [20]. In general, the pentavalent arsenic compound is more stable than the trivalent arsenic compound. Feldmann et al. [21] reported that As^{III}, As^{V}, MAA^{V}, and $DMAA^{V}$ remained stable up to 2 months in human urine at 4°C or –20°C, but that about 30% of As^{III} was oxidized to As^{V} in some urine samples after storage at 4°C for longer than 4 months. Del Razo et al. [22] analyzed the stability of arsenic compound in aqueous solution and urine at 4°C and reported that 20 and 21–32% of As^{III} were oxidized, respectively after 2 months. Considering that the plasma and urine samples analyzed in our study were stored frozen for about 5 years, it is highly likely that the As^{V} in the plasma and urine was generated by oxidation of As^{III} during the storage period.

During the repeated administration, the plasma concentrations of inorganic arsenic reached the steady state, whereas accumulation of MAA^{V} and $DMAA^{V}$ in the blood was related to administration frequency. These accumulations were observed also in the Westerners. The total arsenic concentrations reported previously [11, 12, 14] indicated a tendency to increase during the repeated administration of ATO to patients with APL and other hematological malignancies. The results obtained in our study, indicate most of the increase in the total arsenic concentrations can be attributed to the accumulation of methylated metabolites.

During the repeated administration, approximately 60% of the administered arsenic trioxide dose was

excreted in urine as inorganic arsenic and methylated species in Japanese patients. There was not marked difference in the urinary excretions of inorganic arsenic ($As^{III} + As^{V}$) between the Westerners and the Japanese. These results were similar to those in the reports for the excretion of arsenic in humans [15, 23, 24]. In addition, CL_{re} was much lower than CL_{tot} for inorganic arsenic, indicating that renal excretion plays no significant role in the elimination of inorganic arsenic. It is therefore considered that the plasma concentrations of inorganic arsenic do not increase in patients with impaired renal function, as shown by Remick et al. [J Clin Oncol 2004; 22:2018 (abstract)] These results suggest that there were no marked differences in the plasma profiles and urinary excretions of inorganic arsenic and methylated metabolites during the repeated administration of ATO between the Westerners and the Japanese APL patients.

The problem that should be considered when treating APL patients with ATO is chronic arsenic poisoning which is induced by the intratracheal and oral exposure to a comparatively small amount of arsenic for a long term (ten or more years). The symptoms demonstrated in chronic arsenic poisoning are disorders in the skin, mucosa, peripheral nerves, liver, and respiratory organs as well as skin and lung cancer. According to the previous reports, the total arsenic concentrations in the blood of inhabitants who drank well water contaminated with arsenic (5–410 ng/ml) were 2–42.1 ng/ml [25]. These results are similar to the plasma concentrations in this study. Though the liver function abnormalities and peripheral neuropathies occurred in patients of this study [6], they have improved after completion of administration. In the case of treatment for APL, the administration of ATO is not continued permanently or long-term and arsenic is promptly excreted during the washout period. Therefore, it is unlikely that the treatment with ATO results in chronic arsenic poisoning, although sufficient attention should be paid to the adverse reactions during the treatment.

In conclusion, the present study showed the pharmacokinetics of arsenic species in Japanese APL patients treated with ATO. The plasma concentrations of inorganic arsenic (As^{III} and As^{V}) reached the C_{max} immediately after completion of administration followed by a biphasic elimination. However, the appearance of methylated metabolites (MAA^{V}, $DMAA^{V}$) in the blood was delayed. During the repeated administration, the pharmacokinetics of inorganic arsenic reached the steady state but the concentrations of MAA^{V} and $DMAA^{V}$ increased in relation to administration frequency. ATO is metabolized when administered intravenously to APL patients and methylated metabolites are promptly eliminated from the blood and excreted into urine after completion of administration, consequently indicating no measurable accumulation of ATO in the blood. These results are considered important information for the current and future clinical use of ATO.

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Appendix

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Phase I Trial of FLAGM with High Doses of Cytosine Arabinoside for Relapsed, Refractory Acute Myeloid Leukemia: Study of the Japan Adult Leukemia Study Group (JALSG)

Shuichi Miyawaki,[a] Yasukazu Kawai,[b] Akihiro Takeshita,[c] Norio Komatsu,[d] Noriko Usui,[e] Yukihiro Arai,[f] Fumihiro Ishida,[g] Takeshi Morii,[h] Yasuhiko Kano,[i] Michinori Ogura,[j] Noriko Doki,[a] Ryuzo Ohno[k]

[a]Leukemia Research Center, Saiseikai Maebashi Hospital, Gunma, Japan; [b]Divison of Hematology & Oncology University of Fukui Hospital, Fukui, Japan; [c]Department of Medicine III, Hamamatsu University School of Medicine, Shizuoka, Japan; [d]Division of Hematology, Department of Internal Medicine, Jichi Medical University, Tochigi, Japan; [e]Division of Oncology & Hematology, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan; [f]Department of Hematology, Dokkyo Medical University School of Medicine, Tochigi, Japan; [g]2nd Department of Internal Medicine, Shinshu University School of Medicine, Nagano, Japan; [h]2nd Department of Internal Medicine, Nara Medical University, Nara, Japan; [i]Division of Hematology, Tochigi Cancer Center, Tochigi, Japan; [j]Department of Hematology and Cell Therapy, Aichi Cancer Center, Aichi, Japan; [k]Aichi Cancer Center, Aichi, Japan

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Abstract

This study was designed to determine the optimal high dose for cytosine arabinoside (ara-C) in combination with fludarabine, granulocyte colony-stimulating factor, and mitoxantrone (FLAGM) in adult patients with relapsed or refractory acute myeloid leukemia. Nine patients were enrolled at increasing dosage levels of ara-C (8, 12, and 16 g/m^2 per dose level). Ara-C and fludarabine were administered once a day at level 1, once or twice a day at level 2, and twice a day at level 3. All patients had grade 4 hematologic toxicity. The most common adverse events were of grade 2 or less, with nausea and vomiting being the most common (6 events), followed by diarrhea (5 events), and rash (5 events). Of the 13 grade 3 nonhematologic toxicities reported, the 2 most common were febrile neutropenia (6 events) and disseminated intravascular coagulation (3 events). No early deaths were observed. FLAGM with high-dose ara-C was considered safe for patients, and the recommended dosage of ara-C in this study was 2 g/m^2 every 12 hours for a total dose of 16 g/m^2.
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Key words: AML; Ara-C; FLAGM therapy; Cytarabine; High-dose ara-C; Phase I study

1. Introduction

Treatment for acute myeloid leukemia (AML) has improved over the years since the addition of cytosine arabinoside (ara-C) to anthracycline therapy, which has enabled 70% to 80% of patients to achieve complete remission (CR). Even patients treated with this combination show long-term survival rates of only approximately 30%, however, and relapses occur in many patients [1,2]. In patients who relapse or have refractory disease, salvage therapy is imperative for long-term survival [3]. One type of salvage therapy is high-dose ara-C. Rudnick and colleagues [4] reported that 1 to 7.5 g/m^2 of ara-C is effective in refractory AML patients. Miyawaki et al [5] conducted a phase II study in which 2 g/m^2 of ara-C administered every 12 hours for a total of 24 g/m^2 was shown to be effective in patients with relapsed and refractory AML.

Arabinosylcytosine 5′-triphosphate (ara-CTP) is a metabolite of ara-C; studies have shown that fludarabine, a purine nucleoside analogue, can augment ara-CTP

N.K. is currently at the Department of Hematology, University of Yamanashi, Yamanashi, Japan.

Correspondence and reprint requests: S. Miyawaki, Leukemia Research Center, Saiseikai Maebashi Hospital, 564-1, Kamisinden-cho, Maebashi, Gunma 371-0821, Japan; 81-27-252-6011; fax: 81-27-253-0390 (e-mail: miyawaki@mail.wind.ne.jp).

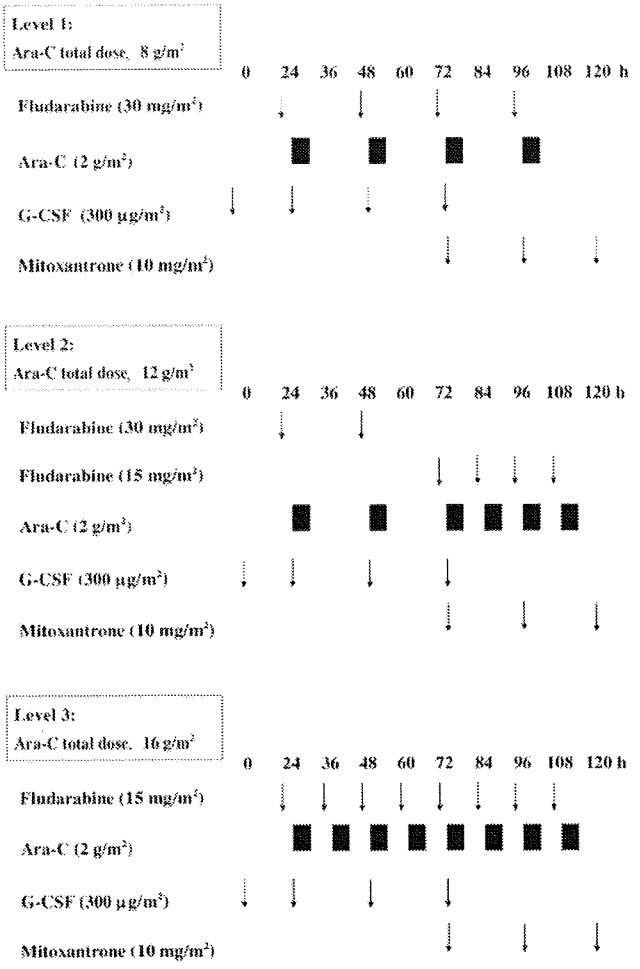

Figure 1. Dosing schedule for the 3 dosage levels. At all dosage levels, granulocyte colony-stimulating factor (G-CSF) was administered in 4 doses every 24 hours, beginning at the start of therapy, and mitoxantrone was administered in 3 doses every 24 hours, beginning 72 hours after the start of therapy. Ara-C (total dose per dosage level: 8, 12, and 16 g/m^2) was administered every 12 or 24 hours, beginning 24 hours after the start of therapy, and fludarabine (30 mg/m^2 once daily or 15 mg/m^2 twice daily) was administered 4 hours before each ara-C dose.

accumulation in leukemic cells [6-9]. This combination of fludarabine and ara-C was studied by Estey et al [10] in patients with newly diagnosed AML or myelodysplastic syndromes, and a CR rate of 53% was achieved. Other investigators also examined this combination therapy in patients with relapsed and refractory AML and achieved similar CR rates (28%-59%) [11-13]. The total-dose range of ara-C administered in these studies was 3 to 10 g/m^2. Because higher doses of ara-C have successfully been used to treat AML patients, the current phase I study was designed to determine the optimal high dose for ara-C in FLAGM, a combination with fludarabine, granulocyte colony-stimulating factor (G-CSF), and mitoxantrone, in patients with recurrent or refractory AML. In a high-dose ara-C regimen, ara-C is generally administered every 12 hours; therefore, fludarabine was administered twice a day. The optimal doses derived from the results of this study will be used in phase II studies.

2. Materials and Methods

The present study was conducted from October 2001 to June 2002 at 8 institutions belonging to the Refractory Leukemia Committee of the Japan Adult Leukemia Study Group (JALSG). Registration of the participants began after consent was obtained by the Ethics Committee or the Institutional Review Board of each institution.

2.1. Study Population

Patients who had recurrent AML (excluding M3 and hybrid leukemia) after a CR or who had failed 2 courses of standard induction therapy were enrolled in the study. M3 was excluded because this disease entity was treated with a specific regimen, and hybrid leukemia was excluded because it was not included in the AML category.

To be eligible, patients were required to meet the following criteria: having had an interval of ≥4 weeks before treatment; having a performance status of 0 to 2; being older than 18 years but younger than 65 years; having a life expectancy of ≥2 months and no major organ dysfunction (hemoglobin ≥9.0 g/dL; platelets ≥20,000 × 10^9/L; leukocytes ≥2000 × 10^9/L; total bilirubin ≤1.5 mg/dL; liver function tests ≤3 times the normal maximum value used by each institution; and serum creatinine ≤1.5 mg/dL); and having an arterial blood oxygen saturation ≥90%. All patients were required to provide written consent at the start of receiving the study medication.

2.2. Study Design and Treatment

As shown in Figure 1, 3 cohorts at 3 ara-C dosage levels (8 g/m^2, 12 g/m^2, and 16 g/m^2) were set in this study, and the administration of ara-C was started at 8 g/m^2 by the dose-escalation method. Each ara-C administration was given as a 3-hour infusion. G-CSF was subcutaneously administered at every dosage level at the start of treatment and was given every 24 hours for a total of 4 doses. Fludarabine (30 mg/m^2 once daily or 15 mg/m^2 twice daily) was administered as a 30-minute infusion 4 hours before the ara-C dose. The total fludarabine dose administered at each level was 120 mg/m^2. Lastly, 10 mg/m^2 mitoxantrone was administered as a 30-minute infusion for a total of 3 doses every 24 hours, beginning 72 hours after the start of therapy.

The sample size for each cohort was set at 3 patients. When the critical toxicity was observed in 1 of the 3 patients, 3 more patients were added to that cohort. When the critical toxicity was not seen in any of the 3 patients or was seen in 1 of the 6 patients, the dose was increased for the next cohort. Finally, when the critical toxicity was encountered in 2 of the 6 patients, the maximum tolerated dose was considered to have been reached in that cohort.

The treatment schedule for this study was derived from that of a phase II study in which 2 g/m^2 of ara-C was administered every 12 hours for a total of 12 doses

Table 1.
Demographic and Baseline Clinical Characteristics*

Dosage Level	Age, y	FAB Classification	Status	Karyotype (MRC Classification)	Duration of CR, mo	WBC, $\times 10^9$/L (blasts, %)	Nucleated Cell Count in BM, $\times 10^9$/L (blasts, %)
1	21	M4	Relapse 1	46,XY,del(12)(p?) (I)	11	9890 (47.0)	3.8 (40.4)
1	37	M2	Relapse 1	46,XY,t(6;9)(p23;q34),47,idem,+13 (I)	4	14,470 (50.0)	12.6 (78.8)
1	33	M2	Relapse 1	46,XY,del(1)(p?),add(3)(q21),add(5)(q22) 46,idem,add(7)(q32),add(9)(p13) 47,idem,+Y (P)	19	3800 (13.5)	19.7 (15.0)
2	57	M5b	Relapse 1	46,XY,t(2;3)(p23;q29) (I)	14	5770 (38.0)	7.5 (94.0)
2	60	M4	Relapse 2	46,XY (I)	18	20,630 (75.0)	7.6 (72.8)
2	29	M2	Relapse 1	46,XX,t(8;21)(q22;q22) (F)	24	2600 (34.0)	NA (30.0)
3	41	M5a	Relapse 1	46,X,add(Y)(p11),del(5)(p?),add(8)(q22) (I)	34	2100 (14.5)	8.9 (94.0)
3	51	M2	Relapse 1	46,XY (I)	16	5400 (0)	91.7 (10.4)
3	55	M4	Relapse 1	46,XY (I)	13	40,100 (80.0)	NA

*FAB indicates French-American-British; MRC, Medical Research Council; CR, complete remission; WBC, white blood cells; BM, bone marrow; I, intermediate; P, poor; F, favorable; NA, not available.

(total dose, 24 g/m^2) [5]. Treatment-related deaths occurred in 5 of 46 patients in that study. Therefore, fludarabine and mitoxantrone were given concurrently in the present study, and 16 g/m^2 was taken to be the maximum administrable dose of ara-C. If none of the 3 patients or 1 of the 6 patients showed critical toxicity at dose level 3, the trial was terminated without further increase in the dose.

2.3. Supportive Care

Inhaled amphotericin B, amphotericin B syrup, and nystatin were administered to neutropenic patients to prevent airway, oral, and esophageal fungal infections. Oral polymyxin B sulfate was administered to limit colonization in the gastrointestinal tract, and the prophylactic use of isoniazid was prescribed to patients who had a history of tuberculosis. Platelets were supplemented as needed to maintain a platelet count ≥20,000 × 10^9/L, and G-CSF was administered within the scope of the protocol guidelines.

2.4. Safety Evaluations and Study End Points

Safety was the primary study end point, and adverse events were graded according to the National Cancer Institute Common Toxicity Criteria. The critical toxicity was decided as follows: (1) grade 3 or higher nonhematologic toxicity (except for nausea and vomiting, loss of appetite, diarrhea, infection, or fever of grade 4); and (2) early death (defined as death occurring within 2 months after the start of treatment). The secondary end points included the type, degree, and frequency of adverse events of grade 1 or 2, and the efficacy of treatment. For the assessment of efficacy, the JALSG criteria were followed [2]. A CR was established when observations of fewer than 5% blasts in normocellular marrow were accompanied by a normal level of peripheral blood neutrophils (>1200 × 10^9/L) and a normal platelet count (>100,000 × 10^9/L). The definition of partial remission was established when a decrease of at least 50% in the percentage of blasts, to between 5% and 25%, was observed in the bone marrow aspirate.

Table 2.
Summary of Adverse Events*

	Dosage Level 1 (n = 3)					Dosage Level 2 (n = 3)					Dosage Level 3 (n = 3)				
	Grade, n					Grade, n					Grade, n				
	1	2	3	4	Total, n (%)	1	2	3	4	Total, n (%)	1	2	3	4	Total, n (%)
Diarrhea	2	0	0	0	2 (67)	0	1	0	0	1 (33)	2	0	0	0	2 (67)
DIC	0	0	1	0	1 (33)	0	0	0	0	0 (0)	0	0	2	0	2 (67)
Fever (allergy)	0	0	0	0	0 (0)	2	0	0	0	2 (67)	0	1	0	0	1 (33)
Hyperglycemia	0	0	0	0	0 (0)	0	0	0	0	0 (0)	0	0	1	0	1 (33)
Nausea/vomiting	3	0	0	0	3 (100)	0	2	0	0	2 (67)	0	1	0	0	1 (33)
Febrile neutropenia	0	0	2	0	2 (67)	0	0	1	0	1 (33)	0	0	3	0	3 (100)
Rash	1	0	0	0	1 (33)	0	2	0	0	2 (67)	1	1	0	0	2 (67)
Sepsis	0	0	1	0	1 (33)	0	0	0	0	0 (0)	0	0	0	0	0 (0)
SGOT elevation	0	0	0	0	0 (0)	0	0	1	0	1 (33)	1	0	0	0	1 (33)
SGPT elevation	1	0	0	0	1 (33)	0	0	1	0	1 (33)	2	0	0	0	2 (67)
Stomatitis	0	0	0	0	0 (0)	1	0	0	0	1 (33)	0	1	0	0	1 (33)

*DIC indicates disseminated intravascular coagulation; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase.

Table 3.
Overall Outcome*

Dosage Level	Early Death, n	Response			WBC Nadir, $\times 10^9$/L	Duration of WBC $<1000 \times 10^9$/L, d	Duration of Plt $>10 \times 10^{13}$/L, d	Death, n (%)
		CR, n	PR, n	Overall, n (%)				
1 (n = 3)	0	1	0	1 (33)	70, 100, 270	15, 36, 43	—, 75, —	3 (100)
2 (n = 3)	0	3	0	3 (100)	50, 150, 160	14, 18, 22	18, 22, 35	1 (33)
3 (n = 3)	0	1	2	3 (100)	100, 100, 110	15, 18, 30	22, 33, 38	0 (0)
Total	0	5	2	7 (78)	Median = 100	Median = 18	Median = 33	4 (44)

*CR indicates complete remission; PR, partial remission; WBC, white blood cells; Plt, platelets.

3. Results

3.1. Demographic and Baseline Characteristics

Nine AML patients were enrolled, and all were eligible for this study. Their demographics and baseline characteristics are shown in Table 1. The median age was 41 years (range, 21-60 years). Eight of the 9 patients were in their first relapse, and 1 patient had a karyotype aberration involving core-binding factor [14].

3.2. Safety

No early deaths occurred within 2 months after the start of treatment. Grade 4 leukopenia ($<1000 \times 10^9$/L) was seen in all patients. The median leukocyte count was 100×10^9/L (range, 50-270 $\times 10^9$/L), and the median period for which the count was $<1000 \times 10^9$/L was 18 days (range, 14-43 days). The leukocyte count and the period over which that count was $<1000 \times 10^9$/L were not related to the ara-C dose.

The most commonly reported adverse events were of grade 2 or less, with nausea and vomiting being the most common (6 events), followed by diarrhea (5 events) and rash (5 events) (Table 2). Of the 13 grade 3 nonhematologic toxicities reported, the most common were febrile neutropenia (6 events) and disseminated intravascular coagulation (DIC) (3 events). Of the 3 DIC events, 2 were considered to be related to AML, and 1 was related to cytomegalovirus infection. In addition, 1 case of grade 3 sepsis was seen. Because DIC is a type of hematologic toxicity and because sepsis is caused by an infection, these adverse events were judged not to fall under the heading of critical toxicities as defined in the present study. Grade 3 hyperglycemia was detected after the administration of steroid drugs, and grade 3 increases in serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase concentrations were seen after the administration of antibiotics. One patient developed hepatotoxicity 1 month following chemotherapy. This patient had experienced hepatotoxicity and skin eruptions caused by the same antibiotics during previous chemotherapy. Accordingly, these events were considered to have had no causal relationship to the FLAGM therapy. Therefore, no critical toxicity attributable to this study was seen in any cohort.

3.3. Response to Dosing Regimens

The overall response rate in the study was 78% (7 cases), and the overall responses are summarized in Table 3. One patient (33%) achieved CR at dose level 1, 3 patients (100%) achieved CR at dose level 2, and 1 patient (33%) achieved a CR at dose level 3. In addition, 2 patients at dose level 3 had a partial response, and 2 patients at dose level 1 showed resistant disease. During the follow-up period, 2 patients who received doses at level 1 died from progressive disease, and 2 patients (1 each from dose levels 1 and 2) died from complications arising from a transplant received after having achieved CR.

4. Discussion

With the goal of improving response rates and long-term survival in patients with AML, treatments with new drugs such as mitoxantrone [11-13] and idarubicin [15-17] have recently been added to FLAG therapy (fludarabine, ara-C, and G-CSF). Because idarubicin is used in Japan as induction therapy for AML, this study developed the FLAGM regimen to determine the optimal dose for high-dose ara-C.

The fludarabine dosage in this study was 30 mg/m^2 administered once a day or 15 mg/m^2 twice daily. This dosing regimen was based on the results of Gandhi et al [18], who determined that both regimens would maximize ara-CTP accumulation in AML blasts. Studies have shown that numerous central nervous system adverse events can occur at ara-C dosages of 3 g/m^2 administered twice daily for 6 days (total dose, 36 g/m^2) [19]. In our previous phase II study of ara-C in which 2 g/m^2 was administered twice daily for 6 days (total dose, 24 g/m^2), we observed 5 deaths that were attributable to the treatment among a total of 46 cases [5]. In the present study, when we considered that fludarabine and mitoxantrone were to be administered concurrently with ara-C, we strictly fixed the maximum dose at 16 g/m^2.

As expected, the main adverse events were hematologic toxicities and febrile neutropenia, but both were manageable with supportive care. Neither reduction of the leukocyte count nor prolongation of the period of leukopenia due to higher ara-C doses was observed. In a study with a dosing regimen similar to that in our study, Hanel et al [13] demonstrated that the median period during which the leukocyte count was $<500 \times 10^9$/L was 21 days (range, 4-51 days), which was similar to the present results (ie, $\leq 1000 \times 10^9$/L

leukocytes for 18 days; range, 14-43 days). In another study of high-dose ara-C in patients with relapsed or refractory AML, the median period during which the leukocyte count was $<1000 \times 10^9$/L was 19 days [5]; this period was also similar to our results. The nonhematologic toxicities were manageable, and no central nervous system toxicity was observed. Koller et al [12] conducted a study in which fludarabine, ara-C, and comparable doses of mitoxantrone were administered concurrently; hyperbilirubinemia was reported in approximately 60% of the patients. In the present study, although 1 case of grade 3 liver failure was reported, no adverse events of a high bilirubin concentration were observed. Clavio et al [11] treated poor-risk AML patients with the same drug combination and the same mitoxantrone doses that we used in our protocol, and they found no patient with hyperbilirubinemia, as was the case in our study. The difference between the protocol of Koller et al and those used in the study of Clavio et al and our study is the dosage of mitoxantrone administered. Given this difference, a low mitoxantrone dose may be closely correlated with an absence of patients with hyperbilirubinemia.

Although the CR rate achieved in this study was 56%, the number of patients was small. In another study of high-dose ara-C therapy, however, the reported CR rate was 45.7%, and the remission rate was 51.4%. Therefore, it is possible that the FLAGM therapy regimen used in this study is more efficacious than the regimen of high-dose ara-C therapy. We conducted a phase I study for the purpose of selecting doses of high-dose ara-C for FLAGM therapy in patients with relapsed or refractory AML. The results of the study showed a high degree of effectiveness at dose levels 2 and 3, and we observed no treatment-related mortality at any dosage level. Therefore, the treatment was considered well tolerated. At the ara-C dose that we presumed to be the maximum administrable dose, 16 g/m^2, we observed no critical toxicity attributable to the study, and we therefore concluded that the recommended dosage for ara-C for phase II clinical trials should be 2 g/m^2 administered twice daily for 4 days, for a total dose of 16 g/m^2.

This regimen is currently being evaluated in phase II studies. However, the safety of this regimen should be continually evaluated in the phase II study because of the relatively small number of patients included in the phase I study.

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achieved morphologic and cytogenetic remission after 2 months of treatment with lenalidomide therapy and a second patient who achieved a platelet response after only 1 month of therapy before receiving an unrelated allogeneic stem cell transplant. Both patients remain without any evidence of relapse with a maximum follow-up of 8 months.

Both radiation therapy and traditional DNA-interactive antineoplastics, such as alkylating agents and topoisomerase II inhibitors, are known genotoxins with the potential to induce MDS or acute myeloid leukemia (AML) that commonly harbors a chromosome 5q deletion with high frequency of evolution to AML and short overall survival.[4–6] Our findings indicate that lenalidomide has therapeutic potential in patients with secondary MDS with complex karyotype accompanied by chromosome 5q deletion.

M Melchert[1], C Williams[2] and A List[1]
[1]*Malignant Hematology, Moffitt Cancer Center and Research Institute, Magnolia Drive, Tampa, FL, USA and*
[2]*Kansas City Blood and Marrow Transplant Program, Suite, Kansas, MO, USA*
E-mail: melcheme@moffitt.usf.edu

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Recurrent chromosomal aberration at 12q15 in chronic idiopathic myelofibrosis with or without JAK2^{V617F} mutation

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Mutation of JAK2^{V617F} is currently known to play a potential role in the development of chronic myeloproliferative disorders (CMPD);[1] 78% (393/506) of polycythemia vera (PV) cases have JAK2^{V617F}, while only 43% (55/127) of reported chronic idiopathic myelofibrosis (CIMF) cases have JAK2^{V617F}.[2] Thus the question naturally arises whether the CIMF patients without JAK2^{V617F} might have another pathway towards myelofibrosis or a common pathogenic factor may exist with or without the

Table 1 JAK2-V617F status and cytogenetic results at the time of myelofibrosis

Case no.	*Age at diagnosis/sex*	*Cytogenetics at the time of myelofibrosis*	*JAK2 V617F*
Idiopathic myelofibrosis			
JAK2_0048	41/male	46,XY,**t(1;12)(p34;q15)**[10]	G/G
JAK2_0039	56/male	46,XY,del(11)(q13)[18]/46,XY[3]	G/G
JAK2_0057	78/female	46,XX[21]	G/G
JAK2_0098	67/male	46,XY,del(20)(q11)[5]/46,XY[3]	G/G
JAK2 0112	33/male	46,XY[23]	G/G
JAK2_0163	63/female	46,XX,**t(12;20)(q15;q11)**[7]/47,XX,+9[10]	G/T
JAK2_0036	54/male	46,XY,del(20)(q11)[2]/ 46,XY,idem,t(2;17)(q24;q22)[13]/ 46,XY,idem,i(17q)[5]	G/T
JAK2_0105	70/male	46,XY,add(9)(p21)[16]	G/T
JAK2_0148	56/male	46,XY[21]	T/T
Myelofibrosis with prior history of myelodysplastic syndrome			
JAK2_0021	70/male	46,XY,**t(4;12)(q27;q15)**[22][a]	G/T
Polycythemia vera developing myelofibrosis			
JAK2_0042	55/female	46,XX,del(7)(q22)[9]/45,X,add(X)(p22),−18[6]/ 46,XX[5]	G/T
JAK2_0061	60/female	46,XX,tan(1q12–1qter)[8]/46,XX[1]	G/T
JAK2_0065	43/female	43,XX,−1,−3,−7,−9,−10,−12,−13, −16,+5m[13]/46,XX[8]	T/T
JAK2_0118	46/female	46,XX[20]	T/T
JAK2_0141	62/male	46,XY[20]	T/T

Table 1 *Continued*

Case no.	*Age at diagnosis/sex*	*Cytogenetics at the time of myelofibrosis*	*JAK2 V617F*
Essential thrombocythemia developing myelofibrosis			
JAK2_0013	58/female	46,XX[13]	G/G
JAK2_0035[b]	49/male	46,XY,t(2;5)(p16;q14),add(11)(q23)[23]	G/G
JAK2_0005[b]	57/male	46,XY,+1,der(1;7)(q10;p10),del(20)(q11)[20]/ 46,XY,idem,add(18)(p11)[2]/46,XY[4]	G/T
JAK2_0034	67/male	46,XY,i(7q)[6]/48,XY,+8,+21[9]/46,XY[6]	G/T
JAK2_0054[b]	59/male	46,XY,+1,der(1;7)(q10;p10)[9]/46,XY[3]	G/T
JAK2_0055	76/male	46,XY,add(18)(p11)[20]	G/T
JAK2_0158	46/female	46,XX[17]	G/T

[a]This case was reported as myelodysplastic syndrome developing myelofibrosis with a 6-year interval;[5] however, the continuity of the disease is uncertain.[3]
[b]JAK2_0035 was UPN-12, JAK2_0005 was UPN-6 and JAK2_0054 was UPN-8 in Hsiao *et al.*[6]
Bold signifies chromosomal translocations involving 12 of 15 region.

presence of JAK2^{V617F}. During the series of mutational assay of JAK2^{V617F} in CMPD using the sequence-specific primer-single molecule fluorescence detection assay,[3] we studied cytogenetic changes in nine patients with CIMF, 51 with essential thrombocythemia (ET) and 34 with PV. Myelofibrosis developed in seven of 51 patients (13.7%) with ET and five of 34 patients (14.7%) with PV, and we compared clinical and cytogenetic changes between patients with CIMF and those with myelofibrosis developing in PV/ET.

The group of patients with myelofibrosis associated with PV/ET had high incidences of history of thrombosis (4/12 versus 0/9; $P=0.0542$), requirement of cytoreductive chemotherapy (12/12 versus 5/9; $P=0.0103$) and acute leukemia development (7/12 versus 1/9; $P=0.0274$) compared with the CIMF group. Depending on the status of JAK2^{V617F}, the group of patients with myelofibrosis associated with PV/ET with GT/TT mutation of JAK2 had a high incidence of chemotherapy requirement (10/10 versus 2/4; $P=0.0157$) and tended to have a frequent thrombosis history (4/10 versus 0/4; $P=0.1345$) (Supplementary Table 1). However, there was no particular difference in the percentage of abnormal karyotypes at the time of myelofibrosis according to CIMF diagnosis or the mutational status of JAK2^{V617F}. We also noted a high frequency of myelofibrosis development in patients with JAK2^{V617F} in PV (wild-type JAK2/heterozygous JAK2^{V617F}/homozygous JAK2^{V617F}: 0/9 versus 2/18 versus 3/7: $P=0.0460$), but not in ET (2/18 versus 5/30 versus 0/1: $P=0.7970$), in agreement with the report by Kralovics *et al.*[4]

In myelofibrosis patients, we noticed that two of the nine patients with CIMF had chromosome abnormalities at the 12q15 region; one had t(1;12)(p34;q15), while the other had t(12;20)(q15;q11?) (Table 1 and Figure 1). Another patient (JAK2_0021) reported as showing t(4;12)(q31;q21) had a prior history of myelodysplastic syndrome (MDS)-refractory anemia with a normal karyotype 6 years before myelofibrosis.[5] Re-assessment by the spectral karyotypic analysis revealed that this anomaly was t(4;12)(q27;q15) (Supplementary Figure 1). In contrast to the results of CIMF, no patients with myelofibrosis developing from PV/ET had 12q15 anomaly. Of the 12 patients with myelofibrosis associated with PV/ET, four had −7/7q−, including two with der(1;7)(q10;p10); both patients with der(1;7)(q10;p10) had a prior ET diagnosis[6] with heterozygous JAK2^{V617F} (Table 1). Of the nine patients with CIMF and one myelofibrosis associated with MDS, four had heterozygous and

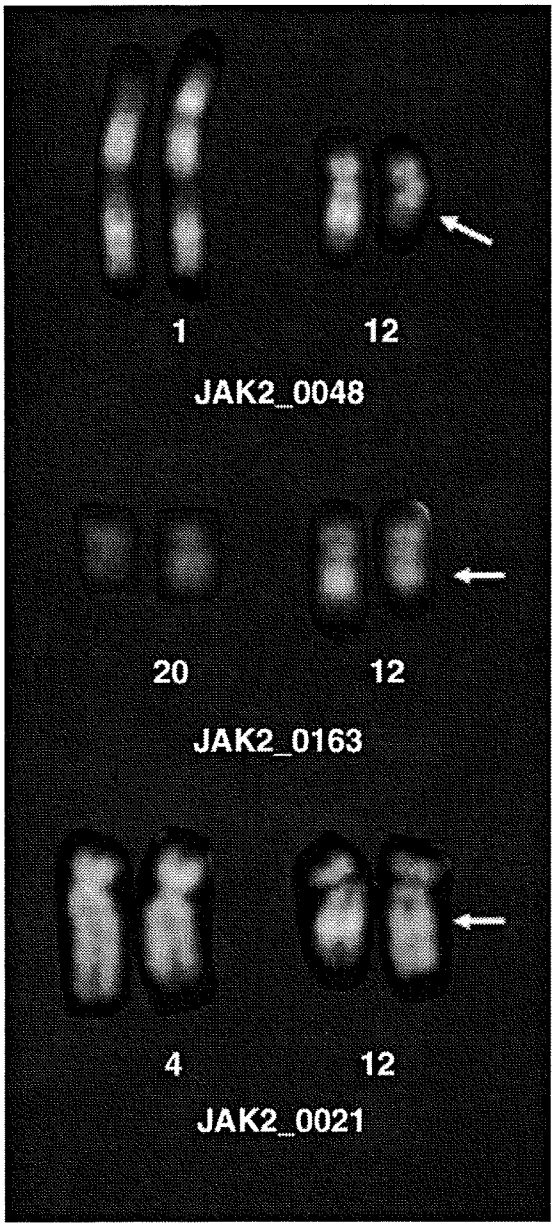

Figure 1 Partial quinacrine-banded karyotypes obtained from two patients with chronic idiopathic myelofibrosis (JAK2_0048 and JAK2_0163) and one patient with myelofibrosis with prior history of myelodysplastic syndrome (JAK2_0021) showing 12q15 anomalies, that is, t(1;12)(p34;q15), t(12;20)(q15;q11?) and t(4;12)(q27;q15). Arrows indicate possible breakpoint of 12q15.

one had homozygous $JAK2^{V617F}$; the 12q15 anomaly was detected in one patient with wild-type JAK2, while two had heterozygous $JAK2^{V617F}$.

Cytogenetic changes in CIMF are well documented: +8, del(20q), −7/7q−, del(11q) and del(13q) are known to be recurring nonspecific cytogenetic abnormalities, and some of them are also detectable in PV or ET patients. In the literature, Andrieux *et al.*[7] reported a possible role in the association between *HMGA2* and translocation involving 12q15 in CIMF. In the current study, we found that 12q15 anomaly does not depend on the JAK2 mutational status; thus genetic anomaly, independent to $JAK2^{V617F}$, may exist in CIMF, and molecular study on the 12q15 region, including *HMGA2*,[8] may disclose another pathogenetic pathway in CIMF. The 12q15 chromosomal abnormality was recurrently detected in patients with CIMF, while der(1;7)(q10;p10) was only noted in ET patients who had myelofibrosis with $JAK2^{V617F}$.[6] These findings clearly indicate that myelofibrosis among CMPD might be cytogenetically heterogeneous.

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K Ohyashiki[1], T Tauchi[1], M Kuroda[2], A Kodama[3] and JH Ohyashiki[4]
[1]*First Department of Internal Medicine (Hematology Division), Tokyo Medical University, Tokyo, Japan;*
[2]*Department of Pathology, Tokyo Medical University, Tokyo, Japan;*
[3]*Laboratory Medicine (Chromosome Unit), Tokyo Medical University, Tokyo, Japan and*
[4]*Intractable Immune System Diseases Research Center, Tokyo Medical University, Tokyo, Japan*
E-mail: ohyashik@rr.iij4u.or.jp

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Neighboring adipocytes participate in the bone marrow microenvironment of multiple myeloma cells

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Multiple myeloma (MM) is a hematological malignancy, characterized by the accumulation of monoclonal plasma cells in the bone marrow (BM). MM disease progression has been recently recognized as the result of an evolving crosstalk between different cell types within the BM. Although genetically abnormal plasma cells define the tumor compartment itself, the surrounding and interwoven stroma provides the supporting framework of the tumor. This framework includes extracellular matrix proteins, secreted growth factors and cellular interactions with fibroblasts, macrophages, endothelial cells, osteoblasts and osteoclasts.[1] Little attention has been given to another cell type present in the BM cavity: namely the adipocyte. These are absent in the BM of a new-born individual; however, their number increases with advancing age, resulting in adipocytic deposits occupying up to 70% of the BM cavity in elderly persons. MM is typically a disease of the elderly with a median age of diagnosis of 65 years and the incidence increases with age. Knowing that with advancing age, the BM cavity is filled with adipocytes and that MM cells closely interact with their neighboring cells, we assumed functional interactions between BM adipocytes and MM cells. We studied these interactions using the 5T33MM model and the human MM5.1 cell line. In this study, we further tried to characterize the secreted cytokines and explored the potential role of leptin in mediating the effects of adipocytes. We finally evaluated the expression of leptin receptor on both murine and human MM cells and tried to correlate this with different clinical parameters.

From the observation that MM cells, at interstitial disease stages, can be found in close contact with adipocytes, functional interactions between these cells are reasonable and prompted us to start *in vitro* tests. The murine BM adipocytic cell line 14F1.1 (obtained from Professor Zipori D, Department of Molecular Cell Biology, The Weizmann Institute of Science, Rehovot, Israel) and primary isolated human adipocytes were used. The 14F1.1 cells initially have a