Phase 1 trial and pharmacokinetic study of arsenic trioxide in children and adolescents with refractory or relapsed acute leukemia, including acute promyelocytic leukemia or lymphoma

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Arsenic trioxide (ATO) induces remission in 85% of adults with refractory acute promyelocytic leukemia (APL). We conducted a phase 1 trial of ATO in children (median age 13 y, range, 2-19) with refractory leukemia. ATO was administered intravenously over 2 hours, 5 d/wk for 20 doses/cycle. Patients with APL (n = 13) received 0.15 mg/kg per day, and patients with other types of leukemia received 0.15 mg/kg per day (n = 2) or 0.2 mg/kg per day (n = 4). Nineteen of the 24 enrolled patients were fully evaluable for toxicity. At 0.15 mg/kg per day, 2 of 15 patients experienced dose-limiting corrected QT interval (QTc) prolongation, pneumonitis, or neuropathic pain. At 0.2 mg/kg per day, 2 of 4 patients had dose-limiting QTc prolongation or pancreatitis. Non–dose-limiting toxicities included elevated serum transaminases, nausea, vomiting, abdominal pain, constipation, electrolyte imbalance, hyperglycemia, dermatitis, and headache. At 0.15 mg/kg per day, the median (range) plasma arsenic maximum concentration (C_{max}) was 0.28 μM (0.11-0.37 μM) and at 0.2 mg/kg per day, C_{max} was 0.40 and 0.46 μM; area under the concentration times time curve (AUC_{0-24}) was 2.50 μM-hr (1.28-3.85 μM-hr) and 4.37 μM-hr and 4.69 μM-hr, respectively. Morphologic complete response (CR) was achieved in 85% of patients with APL; no responses were observed in non-APL patients. ATO is well-tolerated in children at the recommended dose of 0.15 mg/kg per day. The response rate in children with relapsed APL is similar to the response rate in adults. This trial was registered as #NCT00020111 at www.ClinicalTrials.gov. (Blood. 2008; 111:566-573)

Introduction

Arsenic trioxide (ATO) induces complete remission (CR) in 70% to 85% of adults with newly diagnosed or relapsed acute promyelocytic leukemia (APL),[1-4] and these remissions are often durable.[5,6] In a multicenter trial in patients with relapsed APL, the 18 month overall and disease-free survival rates were 66% and 56%, respectively.[3] In adults with newly diagnosed APL, the addition of 2 courses of ATO consolidation therapy after remission induction significantly improved the event free and overall survival compared with the same standard therapy with daunorubicin, cytarabine, and oral tretinoin without ATO.[7]

Several mechanisms of action may contribute to the anticancer activity of ATO (for review, see Rojewski et al[8]). In APL cells[9] and cell lines[10,11] in vitro, low ATO concentrations (0.1 to 0.5 μM) resulted in degradation of PML-RARα proteins, relocation of PML into nuclear bodies, and partial differentiation, whereas higher concentrations (0.5 to 2 μM) induced apoptosis through the formation of reactive oxygen species (ROS), including hydrogen peroxide and superoxide, which results in oxidative stress, DNA damage[12-14] and activation of Jun N-terminal kinase (JNK), triggering apoptosis.[15,16]

We conducted a multi-institutional phase 1 trial of arsenic trioxide in children and adolescents with relapsed or refractory leukemia. Initially the trial was designed as a dose-escalation study with planned dose levels of 0.15, 0.2, and 0.25 mg/kg. ATO was administered intravenously daily every 5 days for 4 weeks (20 doses), with a 2-week break between treatment cycles and a maximum of 3 cycles. In September 2000, ATO (Trisenox) at a dose of 0.15 mg/kg daily for 60 doses was FDA approved for patients more than 5 years old with relapsed APL harboring the t(15:17) translocation. Our trial was subsequently amended to treat children with APL at dose level 1 (0.15 mg/kg), the FDA-approved dose, but to perform the planned dose escalation only in children with other leukemias or lymphoma. The objectives of our study were to evaluate the tolerability of ATO at the FDA-approved dose in children with APL, to determine the maximum tolerated dose (MTD) and dose-limiting toxicities (DLT) of ATO in children with non-APL leukemia, and to study the pharmacokinetics of ATO in children.

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Methods

Eligibility

Patients more than 2 years and less than 22 years of age with histologically confirmed recurrent or refractory leukemia or lymphoma were eligible for this study. Patients must have recovered from the toxic effects of prior therapy and have an Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2. Patients may not have received chemotherapy with any standard agent for at least 2 weeks prior to study entry, their last dose of retinoid therapy within 1 week prior to study entry, their last dose of limited field radiation therapy within 4 weeks prior to study entry, or colony stimulating factors within 1 week of study entry. Patients were required to have a normal serum bilirubin and a serum ALT less than or equal to twice the upper limit of normal, an age-adjusted normal serum creatinine or a creatinine clearance 60 mL/min per 1.73 m^2 or more, and serum electrolytes, calcium, and magnesium equal to or greater than the institutional lower limit of normal. Chronic oral mineral and electrolyte supplementation was acceptable. All patients were required to have a QT interval corrected for heart rate using Bazett's formula (QT_c) less than or equal to 0.48 seconds at the time of enrollment.

Patients were excluded if they (1) were currently receiving other investigational chemotherapeutic agents, (2) had clinically significant unrelated systemic illness, (3) were pregnant or breast-feeding, or (iv) had previously received arsenic trioxide. In addition, patients with non-APL diagnoses were excluded if they had central nervous system leukemia, seizures, cardiac dysrhythmias, or greater than grade 2 peripheral neuropathy.

Institutional review boards at participating institutions (the list of institutions is available as Document S1, on the *Blood* website; see the Supplemental Materials link at the top of the online article) approved the study. Informed consent was obtained in accordance with the Declaration of Helsinki from patients 18 years and older and from parents or legal guardians of children, with child assent as appropriate, according to individual institutional policies.

Treatment regimen and dose escalation

ATO (Trisenox) for intravenous infusion was supplied as 10-mg vials by the Cancer Therapy Evaluation Program, National Cancer Institute (NCI, Bethesda, MD). The drug was administered intravenously daily over 2 hours for 5 consecutive days per week for 4 weeks (20 doses), followed by a 2-week break. Patients with APL received 0.15 mg/kg per dose. Patients with non-APL diagnoses were enrolled on the dose escalation portion of the study. Planned dose levels were 0.15, 0.2, and 0.25 mg/kg per dose. Treatment cycles were repeated every 6 weeks if the patient had recovered to grade 1 or less from the ATO-related toxicities from the previous treatment cycle. In cycle 1, patients who had no DLT but who did achieve a complete remission and who did not have progressive disease after the initial 20 ATO doses were permitted to receive 10 additional doses of ATO prior to the 2-week break. A maximum of 70 ATO doses were administered in a total of 3 cycles.

For the dose escalation portion of the study, cohorts of 3 to 6 patients were treated at each dose level. When a minimum of 3 patients who were evaluable for toxicity completed 1 cycle of therapy at a dose level without evidence of DLT, subsequent patients were enrolled at the next higher dose level. Toxicity data from the initial patients with APL treated at the 0.15 mg/kg per day dose level were included with data from the patients with non-APL diagnoses to assess the safety of the starting dose level. No intrapatient dose escalation was permitted.

Toxicity monitoring

Monitoring for treatment-related toxicity included weekly physical examination and serum LDH, ALT, alkaline phosphatase, bilirubin, total protein, and albumin. Clotting parameters (prothrombin time/partial thromboplastin time and fibrinogen) were monitored weekly and complete blood counts, serum electrolytes, creatinine, calcium, magnesium and phosphorus, and uric acid were monitored twice weekly. Electrocardiograms were required prior to each cycle and after ATO infusion on days 3, 6, 11, and 16 of each cycle. Clinical and laboratory adverse events were graded according to the NCI Common Toxicity Criteria version 2.[17]

Definition of dose-limiting toxicity and maximum tolerated dose

Hematologic toxicity was monitored but not used to determine dose escalation or define the maximum tolerated dose. Nonhematologic dose-limiting toxicity (NH-DLT) was any grade 3 or 4 nonhematologic toxicity related to ATO or failure to recover to grade 1 or less toxicity or to baseline toxicity level (if higher than grade 1) by the end of the 6-week treatment cycle. Exceptions were grade 3 or 4 disseminated intravascular coagulopathy, grade 3 tumor lysis syndrome, grade 3 or 4 acute vascular leak syndrome (Retinoic Acid Syndrome/APL differentiation syndrome), grade 3 or 4 febrile neutropenia or any grade 3 infection, and grade 3 or 4 hypokalemia, hypomagnesemia, or hypophosphatemia related to administration of antifungal agents and corrected with intravenous or oral supplementation. The CTC v.2 toxicity grading was not used to determine whether QTc prolongation was dose limiting. Dose-limiting prolongation of the QTc interval was instead defined as a QTc greater than or equal to 0.45 seconds in association with ventricular arrhythmias (premature ventricular beats or sustained or nonsustained ventricular tachycardia) or prolongation of the QTc to greater than or equal to 0.49 seconds in the absence of other ventricular arrhythmias, unless the prolongation was associated with electrolyte abnormalities and was corrected on repeat EKG after correction of electrolyte abnormalities.

The MTD was determined from DLT occurring during the first treatment cycle. For patients in the non-APL cohort on the dose-escalation portion of the study, the MTD was defined as the dose level immediately below the dose level at which 2 or more patients at a dose level of 2 to 6 patients experienced a DLT. In the APL cohort treated with 0.15 mg/kg per dose, we planned to treat at least 3 patients who were 12 years of age or older and at least 3 patients younger than 12 years of age. ATO at the recommended dose of 0.15 mg/kg per dose was to be considered tolerable in the APL cohort if less than 20% of patients experienced a DLT.

Dose modification for toxicity

Patients in the non-APL cohort who experienced an ATO-related DLT could receive their subsequent course of treatment at the next lower dose level (or 0.10 mg/kg for patients treated at dose level 1), if in the judgment of the treating physician they had benefited from the prior treatment cycle. Patients who experienced DLT after a dose reduction could have a second dose reduction to the next lower dose level, but if DLT occurred after the second dose reduction, the patient permanently discontinued protocol therapy.

Patients with APL who experienced clinical benefit from 0.15 mg/kg per dose ATO but who experienced grade 3 or 4 nonhematologic dose-limiting toxicity had the drug withdrawn until the toxicity resolved to grade 1 or less but could be rechallenged with ATO at 0.10 mg/kg per day.

ATO was discontinued immediately in patients who experienced dose-limiting QTc prolongation. For patients with a QTc of 0.49 seconds or longer but without ventricular arrhythmias, the QTc must have returned to 0.48 seconds or less before resuming the drug at the next lower dose level. If patients experienced a ventricular arrhythmia, the drug was restarted at the next lower dose level after the ventricular arrhythmia resolved and the QTc was more than 0.01 seconds shorter than the QTc at which the arrhythmia occurred. If dose-limiting QTc prolongation recurred after dose reduction, ATO was discontinued and the patient was removed from the study. If the patient had QTc prolongation and concurrent hypokalemia, hypocalcemia, or hypomagnesemia, the electrolyte abnormalities were corrected with supplementation prior to restarting ATO. If the QTc interval normalized with correction of the electrolytes, then the drug was restarted without a dose reduction.

Pharmacokinetics

Heparinized whole blood samples were obtained prior to the ATO infusion, at the end of infusion, and 0.5, 1, 2, 4, 6, 10, 23, and 24 hours after the end of

the infusion of the first dose, then prior to the ATO dose on day 5 and day 20 of cycle 1. Plasma was separated by centrifugation within 2 hours and stored at −70°C. Total arsenic was measured using a modification of previously described methods[18,19] for atomic absorption spectrophotometry (A800; Perkin Elmer, Norwalk, CT). Briefly, 250 μL of plasma underwent liquid extraction with 20% trichloroacetic acid (Sigma, St Louis, MO). The recovery of arsenic from plasma using this extraction procedure was greater than 85%. Matrix modifier [5 μL, 1% PdCl/0.05% $Mg(NO_3)_2$] was added to 15 μL aliquots of the extracted samples, and each sample was analyzed in triplicate.

A Perkin Elmer EDL arsenic lamp at a wavelength of 193.7 nm and with a current of 330 mA, a slit width of 0.7 nm, and a furnace setting of 2000°C for 5 seconds was used to quantify total arsenic concentration in the extracted plasma samples. The plasma standard curve was linear from 0.05 to 6 μM. The lower limit of quantification (LLQ) was 0.05 μM and lower limit of detection was 0.025 μM. Intraday and interday coefficients of variation were less than 10% and 15%, respectively. This assay for total arsenic was performed at the Pediatric Oncology Branch, NCI. In addition, plasma elemental trivalent arsenic (As^{III}), elemental pentavalent arsenic (As^{V}), and the metabolites methylarsonic acid and dimethylarsinic acid were measured by Elemental Research (North Vancouver, BC) using anion exchange high performance liquid chromatography (HPLC) combined with inductively-coupled plasma mass spectrometry (ICPMS). Assay linearity was 5.0 to 500 ng/mL for each of the arsenic species. Inter- and intra-assay precision was within 12% for all arsenic species, except for the intra-assay accuracy of 19% for As^{III}.

Pharmacokinetic parameters were analyzed using model-independent methods. The area under the concentration times time curve ($AUC_{0\text{-}24}$) was calculated using the linear trapezoidal method.

Response assessment

A bone marrow biopsy and aspiration for morphology, cytogenetics, and flow cytometry was performed at baseline and prior to each treatment cycle. For patients with APL, real time–polymerase chain reaction (RT-PCR) for PML-RARα was requested at baseline, after dose 19 of ATO, and before each cycle. However, cytogenic and molecular response monitoring were not required. For morphologic response, a complete response was defined as an M_1 bone marrow ($< 5\%$ blasts) with no evidence of circulating blasts or extramedullary disease and normalization of peripheral blood counts (neutrophil count $\geq 1.5 \times 10^9$/L and platelets $\geq 100 \times 10^9$/L). For patients with APL, a complete cytogenic response was defined as normal karyotype by GTW banding. Negative RT-PCR for PML-RARα was considered a complete molecular response. A morphologic partial response was defined as an M_2 bone marrow ($\geq 5\%$ but $< 25\%$ blasts), no evidence of extramedullary disease, and normalization of peripheral blood counts (neutrophil count $\geq 1.5 \times 10^9$/L and platelets $\geq 100 \times 10^9$/L). Progressive disease was defined as an increase of at least 25% in the absolute number of circulating or bone marrow leukemic cells, the development of extramedullary disease, or other evidence of an increase in tumor burden

Results

Patients

Twenty-four children with recurrent or refractory leukemia were enrolled between July 2000 and January 2005 (Table 1), including 14 patients with relapsed APL, 13 of whom were evaluable. One patient with APL was found not to be eligible for the study after enrollment due to serum magnesium concentration less than the lower limit of normal at study entry. This patient did not experience toxicity attributed to ATO, but additional data from this patient has been excluded from this report. Of the 10 patients with leukemia or lymphoma enrolled in the dose escalation portion of the study, 4 had progressive disease prior to completing cycle 1 and were inevaluable. Six patients in the non-APL cohort (2 at dose level 1 and 4 at dose level 2) were fully evaluable for toxicity.

Table 1. Patient characteristics

Characteristic	Quantity
Overall	
Enrolled, no./evaluable, no.	24/19
Median age, y (range)	13 (2-21)
Male, no./female, no.	12/12
APL cohort	
Enrolled, no./evaluable, no.	14/13
Median age, y (range)	17 (4-21)
Male, no./female, no.	8/6
Dose level: 0.15 mg/kg	
First relapse, no. patients	13
Second or subsequent relapse, no. patients	1
Dose escalation (non-APL) cohort	
Enrolled, no./evaluable, no.	10/6
Median age, y (range)	9.5 (2-17)
Male, no./female, no.	4/6
Dose level: 0.15 mg/kg	
ALL, no. patients	3
AML, no. patients	1
Dose level: 0.2 mg/kg	
ALL, no. patients	2
AML, no. patients	3
B-cell lymphoma, no. patients	1

Of the 19 patients who were evaluable for toxicity, 2 received less than 1 cycle, 8 received 1 cycle, 3 received 2 cycles, and 6 received the protocol maximum 3 cycles of ATO therapy. All patients who received more than 1 cycle of therapy had APL. Of patients with APL (n = 13), 9 received 10 additional doses of ATO after cycle 1. In 4 APL patients with a body mass index greater than 30, the ATO dose was based on ideal body weight rather than actual body weight.

Toxicity

Hematologic toxicity was monitored during this trial but was not used to determine the MTD. For patients enrolled with an absolute neutrophil count (ANC) greater than 1.5×10^9/L (2 at dose level 1 and 3 at dose level 2), the maximum grade of neutropenia was grade 4 in 3 patients and grade 3 in 1 patient during cycle 1. For patients enrolled with a platelet count greater than 100×10^9/L (3 at dose level 1), grade 4 thrombocytopenia occurred in all 3 patients during cycle 1.

Nonhematologic toxicities that were possibly, probably, or definitely related to ATO and that were observed during cycle 1 are presented in Table 2. At the 0.15 mg/kg dose level, 2 of 15 patients experienced DLT. A 5-year-old female with APL (denoted as "†," patient 17 in Table 2) experienced grade 4 vascular leak (APL differentiation syndrome), hyperglycemia (grade 4), anorexia (grade 3), and neuropathic pain (grade 3). Her dose of ATO was reduced to 0.1 mg/kg and corticosteroids were administered without improvement. After grade 4 pneumonitis developed, ATO was discontinued. An 18-year-old female with APL experienced asymptomatic dose limiting prolongation of the QTc interval (0.535 seconds) after dose 17 on cycle 1. Her serum potassium was 2.62 mEq/L. After potassium supplementation and withholding ATO for 24 hours, the QTc interval was 0.490 seconds. After 3 ATO doses were withheld (72 hours) the QTc interval returned to baseline (0.460 seconds). She then resumed ATO therapy and received 10 additional doses without recurrence of QTc prolongation. During daily EKG monitoring, the QTc interval range was 0.452 to 0.480 seconds, the longest QTc interval was recorded after dose 4 (0.480 seconds). After completion of the 10 additional doses, her QTc interval was

Table 2. ATO related non-hematological toxicities during cycle 1

	Dose level (number of evaluable patients)							
	0.15 mg/kg (n = 15)				0.2 mg/kg (n = 4)			
Toxicity	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Allergy								
Hypersensitivity	—	—	1	—	—	—	—	—
Urticaria/hives	1	—	—	—	—	—	—	—
Cardiovascular								
Prolonged QTc	1*	—	—	—	1*	—	—	—
Sinus tachycardia	—	1	—	—	—	—	—	—
Hypotension	—	1	—	—	—	1	—	—
Vascular leak syndrome§	—	—	—	1†	—	—	—	—
Coagulation								
Prolonged PT	1	—	—	—	—	—	—	—
Prolonged PTT	1	—	—	—	—	—	—	—
Elevated fibrinogen	—	1	—	—	—	—	—	—
Constitutional								
Fever (no neutropenia)	1	1	1	—	—	—	—	—
Nasal congestion	1	—	—	—	—	—	—	—
Myalgia	—	1	—	—	—	—	—	—
Dermatological								
Rash/dermatitis	3	—	—	—	—	—	—	—
Dry skin	1	—	—	—	—	—	—	—
Gastrointestinal								
Anorexia	1	—	1†	—	—	—	—	—
Constipation	1	2†	—	—	—	—	—	—
Vomiting	5	—	—	—	—	—	—	—
Nausea	3†	1	—	—	1	—	—	—
Diarrhea	1	1	—	—	—	—	—	—
Pancreatitis	—	—	—	—	—	—	—	1‡
Stomatitis	1†	1	—	—	—	—	—	—
Typhlitis	—	—	1	—	—	—	—	—
Hepatic								
Elevated AST (SGOT)	5	1	1	—	—	—	—	1‡
Elevated ALT (SGPT)	4	1	1	—	—	1‡	—	—
Elevated GGT	—	1	—	—	—	—	—	—
Elevated alkaline phos	1	—	—	—	—	—	—	—
Hyperbilirubinemia	—	—	—	—	1‡	—	—	—
Infection§								
Infection (no neutropenia)	—	—	3	—	—	—	—	—
Febrile neutropenia	—	—	1	—	—	—	—	—
Metabolic								
Elevated Amylase	—	—	—	—	—	—	1‡	—
Hypokalemia§	1	—	1	—	—	—	—	—
Hyponatremia	2	—	—	—	—	—	1‡	—
Hypomagnesemia	4	1†	—	—	—	—	—	—
Hypocalcemia	3	2	—	—	—	—	—	—
Hypophosphatemia	1	1†	—	—	—	—	—	—
Hyperphosphatemia	1	—	—	—	—	—	—	—
Hypoglycemia	1	—	—	—	—	—	—	—
Hyperglycemia	3	1	1	1†	—	—	—	—
Elevated lipase	—	—	—	—	—	—	—	1‡
Hypoalbuminemia	1	—	1	—	—	—	—	—
Decreased total protein	1	—	—	—	—	—	—	—
Neurological								
Motor neuropathy	1	—	—	—	—	—	—	—
Sensory neuropathy	1	1	—	—	—	—	—	—
Ocular								
Conjunctivitis	1	—	—	—	1	—	—	—
Periorbital edema	—	—	—	—	1	—	—	—
Pain								
Abdominal cramping	1	1	—	—	—	—	—	1‡
Headache	3	—	—	—	—	—	—	—
Neuropathic pain	—	1	1†	—	—	—	—	—
Pulmonary, pneumonitis	—	—	—	1†	—	—	—	—

Data are numbers of patients with toxicity grades 1 through 4 in cycle 1.
— indicates none.
*Protocol-defined DLT
†Toxicities occurring in patient 17
‡Toxicities occurring in patient 23
§Excluded as DLT per protocol

Table 3. Non-hematological ATO-related toxicity in subsequent cycles

Toxicity	Grade	Patients, no.	Cycle no.
Cardiovascular			
Prolonged QTc	1	1	2
Tachycardia*	1	1	2
Hypotension*	3	1	2
Coagulation, elevated PTT	1	1	3
Constitutional, nasal congestion*	1	1	3
Gastrointestinal			
Vomiting*	1	1	2
Nausea*	1	2	2
Diarrhea*	1	1	2
Stomatitis*	1	1	2
Hepatic, elevated AST*	1	1	2
Metabolic			
Hypokalemia	1	1	3
Hypomagnesemia	1	1	2
Hypomagnesemia	3	1	2
Hypocalcemia	1	1	3
Hypocalcemia*	1	1	2
Hypocalcemia*	3	1	2
Hyperglycemia*	1	2	2
Hyperglycemia*	1	1	3
Neurological			
Motor neuropathy*	1	1	2
Tremor*	1	1	2
Tremor*	1	1	3
Pain			
Headache*	1	1	2
Pain (bone)*	1	1	2
Pulmonary, shortness of breath*	1	1	2

*Occurred in patients receiving ATO based on ideal body weight (actual doses 0.05 to 0.1 mg/kg).

0.452 seconds. Serum potassium was 3.2 to 3.7 mEq/L during the additional doses.

At the 0.2 mg/kg dose level, 2 of 4 patients experienced dose limiting toxicity. A 2-year-old child with AML developed QTc prolongation (0.550 seconds) after 17 doses of ATO. Serum potassium, magnesium, and calcium were normal. The drug was withheld, and the QTc interval normalized (0.420 seconds) within 24 hours. ATO dosing was subsequently resumed at 0.15 mg/kg. The patient completed the final 3 doses of cycle 1 but was removed from study due to progressive disease after cycle 1. A 17-year-old female with rapidly progressive B-cell lymphoma (denoted by "‡," patient 23 in Table 2) experienced pancreatitis (grade 4), abdominal cramping (grade 4), elevated lipase (grade 4), elevated amylase (grade 3), elevated serum AST (grade 3), hyponatremia (grade 3), elevated ALT (grade 2), and hyperbilirubinemia (grade 1) that were considered possibly related to ATO.

Nonhematologic toxicities attributed to ATO in second and third treatment cycles are presented in Table 3. Toxicities in subsequent cycles did not appear cumulative and most toxicities did not recur. Recurring toxicities included nausea and electrolyte abnormalities that were not more severe in subsequent cycles. Two patients who were obese and who were dosed based on ideal body weight (denoted by an "*" in the table) suffered most of the significant toxicities observed on subsequent treatment cycles.

Pharmacokinetics

Pharmacokinetic (PK) parameters were derived on 10 patients who received ATO 0.15 mg/kg per dose, 2 patients treated at 0.2 mg/kg per dose, and 2 patient dosed according to ideal body weight (0.07-0.1 mg/kg of actual body weight per dose). The terminal elimination half-life of total arsenic in plasma was greater than 24 hours. Results are presented in Table 4. Total arsenic concentration times time curves for representative patients are presented in Figure 1A.

Plasma samples were also analyzed for As^{III}, As^{V}, and the metabolites methylarsonic acid and dimethylarsinic acid. As^{III} was below the lower limit of detection (5 ng/mL, 0.07 μM) by 10 hours after the end of the infusion. As^{III} $AUC_{0\text{-}24\ hours}$ and the ratio of As^{III} AUC to total arsenic AUC is presented in Table 4. As^{III} concentration times time curves for representative patients are presented in Figure 1B. As^{V} was not detectable (less than 5 ng/mL, 0.07 μM) except in the 2 patients at 0.2 mg/kg dose level before and 1 hour

Table 4. Plasma pharmacokinetic parameters

Dose (mg/kg/d)/ patient ID no.	Age, y	Dose, mg/day	As^{III} $AUC_{0\text{-}24h}$, μM-h	Total Arsenic $AUC_{0\text{-}24h}$, μM-h	Total Arsenic C_{max}μm	Total Arsenic Before dose 2 μm	Total Arsenic Before dose 5 μm	Total Arsenic Before dose 20 μm	Ratio of As^{III} to total $AUC_{0\text{-}24}$
0.07, 18*	19	8.82	0.83	1.67	0.27	0.07	0.18	0.25	0.50
0.1, 20*	19	10	0.70	2.00	0.23	0.06	0.10	0.27	0.35
0.15									
1	17	14.8	2.06	3.23	0.22	0.10	0.26	0.39	0.64
2	13	5.8	1.90	3.00	0.21	0.11	ND	ND	0.63
4	14	10	1.80	2.89	0.26	0.11	<LLQ	<LLQ	0.62
5	9	3.8	1.84	3.44	0.37	0.12	0.21	ND	0.54
6	18	7.9	2.12	1.77	0.30	0.03	0.12	ND	1.22
10	17	7.9	2.75	3.85	0.26	0.17	0.35	ND	0.71
16	20	11	0.46	1.46	0.11	0.07	0.19	0.32	0.31
17	5	3.3	1.14	1.85	0.24	0.04	ND	ND	0.62
19	4	2.9	1.12	2.58	0.27	0.11	0.18	0.26	0.43
22	12	6	1.17	2.42	0.28	0.07	0.20	0.24	0.48
Median	13.5	7.0	1.82	2.74	0.26	0.11	0.20	0.26	0.62
Range	4-20	2.9-14.8	0.46-2.75	1.46-3.85	0.11-0.37	0.03-0.12	0-0.35	0-0.39	0.31-1.22
0.2									
8	16	12	2.72	4.77	0.40	0.16	0.58	ND	0.57
9	3	3.08	2.77	4.37	0.46	0.12	0.25	0.35	0.63

* These patients were treated at the 0.15 mg/kg/day dose level using ideal body weight to calculate the dose rather than the actual weight. Doses shown represent the dose per actual body weight.

ND indicates sample not drawn; and <LLQ, less than lower limit of quantification

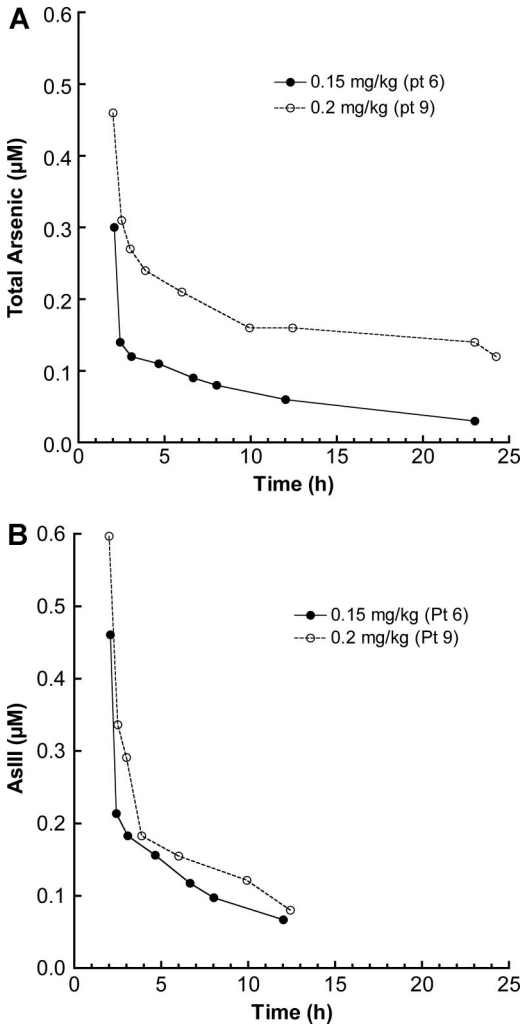

Figure 1. Total arsenic and As^{III} plasma concentrations for representative patients at the 0.15 and 0.2 mg/kg dose levels. Total arsenic (A) and As^{III} (B) plasma concentrations versus time.

after the end of the infusion. In these patients the As^{V} concentration ranged from 0.07 to 0.12 μM. At the 0.15 mg/kg (n = 3) and 0.2 mg/kg (n = 1) dose levels, 0.04 to 0.06 μM monomethylarsonic acid (MMA) was detectable in the 10- to 24-hour samples. Dimethylarsinic acid (DMA) was detected between 6 and 24 hours after completion of the infusion at concentrations ranging from 0.03 to 0.07 μM.

Although PK parameters are only available for 2 patients at the 0.20 mg/kg per day dose level, there was a dose proportional increase in the $AUC_{0\text{-}24h}$ for As^{III}, but a greater than expected increase in the $AUC_{0\text{-}24h}$ for total arsenic (67% increase in $AUC_{0\text{-}24h}$ with a 33% increase in the dose). Trough total arsenic concentrations on days 2, 5, and 20 reveal minimal plasma drug accumulation over the 4-week treatment cycle.

Dosing based on ideal body weight in patients with a BMI exceeding 30 resulted in an $AUC_{0\text{-}24h}$ for As^{III} and total arsenic that were below the median for the nonobese patients who were dosed based on actual body weight. If these obese patients had been dosed based on actual body weight, the predicted $AUC_{0\text{-}24h}$ for As^{III} would have been 1.8 μM-h and 1.1 μM-h, and the predicted $AUC_{0\text{-}24h}$ for total arsenic would have been 3.6 μM-h and 3.0 μM-h.

Response assessment

In the APL cohort (n = 13), one patient was removed from protocol therapy due to dose-limiting toxicity prior to completing cycle 1. Twelve patients with APL completed at least 1 cycle of ATO, and the complete morphologic response rate to ATO in children and adolescents was similar to the rate previously reported in adults (Table 5). Six of the APL patients who achieved complete morphologic and cytogenic response went on to receive bone marrow transplants. Two of these patients achieved molecular response prior to transplantation and 4 patients did not have RT-PCR evaluations reported. All patients with nonpromyelocytic leukemia had progressive disease.

Discussion

ATO at the FDA-approved dose of 0.15 mg/kg per day administered intravenously 5 days per week for 20 doses, followed by a 2-week break between treatment cycles for up to 70 doses, is well-tolerated in children and adolescents. Dose limiting toxicity included QTc prolongation, pneumonitis, neuropathic pain, and pancreatitis. QTc prolongation was dose limiting in 2 patients during cycle 1. In both patients, the QTc prolongation occurred during the fourth week of therapy (dose 17) and was not associated with ventricular arrhythmias. The QTc normalized upon cessation of ATO treatment and correction of electrolyte abnormalities, if present.

Frequent non–dose-limiting toxicities included elevated serum hepatic transaminases (37%), nausea/vomiting (26%), abdominal pain (10%), constipation (16%), hypomagnesemia (26%), hypocalcemia (26%), hyperglycemia (26%), dermatitis (26%), infection (16%), and headache (16%). Nine patients received 2 or more cycles (> 40 doses) of ATO. The toxicity did not appear to be cumulative in patients receiving multiple cycles with a 2-week drug-free interval between each cycle.

Despite dosing based on ideal body weight, there appeared to be an increased frequency of toxicities in patients who were obese. The apparent enhanced toxicity in this population is not accounted for by higher plasma concentrations of As^{III} or total arsenic or by enhanced accumulation of the drug. The $AUC_{0\text{-}24h}$ of As^{III} and total arsenic in the 2 obese patients who had PK sampling were below the median values for the nonobese patients treated at the 0.15 mg/kg per day dose level, and the trough concentrations in the

Table 5. Bone marrow response to ATO in patients (n=13) with APL

	Patients evaluated, no.	Patients achieving CR, no.	APL patients with CR, % of enrolled	Median doses to achieve CR, no. (range)
Morphological	13	11	85	20 (17-20)
Cytogenetic	11	9	69*	50 (20-70)
Molecular	7	3	23*	70 (40-70)

* Cytogenetic and molecular response evaluations were not required or performed in all APL patients. The response rate is calculated from number of enrolled APL patients (n=13). The number of patients evaluated by each method is listed in column 1.

obese patients on days 5 and 20 were similar to trough concentrations in the nonobese group. Capping of the ATO dose may be required to avoid excessive toxicity.

The acute toxicity profile of ATO in children is similar to that observed in adults. In clinical trials of ATO in adults with refractory cancers, toxicities included elevated hepatic transaminases, abdominal pain, musculoskeletal pain, peripheral neuropathy, hypokalemia, hypergylcemia, and dermatitis. Nearly 40% of adult patients treated with ATO experience cardiac conduction abnormalities, most commonly QT_c interval prolongation. In adults, QT_c prolongation of 30 to 60 milleseconds occurs in 37% of treatment courses and prolongation greater than 60 milleseconds occurs in 35% of treatment courses.[20] Rarely, QT_c prolongation can evolve into torsades de pointes, particularly when ATO is administered when the patient has concurrent hypomagnesemia, hypokalemia, or hypocalcemia.[21] In addition, up to 30% of adults treated with ATO experienced APL differentiation syndrome, clinically similar to retinoic acid syndrome, with fever, dyspnea, pleural effusion, pulmonary infiltrates, and weight gain.[22,23] In children and adolescents treated at the recommended dose on this study, 8% of cycles (3 of 37 cycles) were associated with prolonged QTc interval and 3% (1 of 37 cycles) with vascular leak syndrome (APL differentiation syndrome). Late effects of ATO in patients with APL or other hematologic malignancies have not been reported. However, chronic arsenic exposure from environmental sources results in peripheral neuropathy and dermopathy. The duration of exposure impacts the likelihood that these toxicities can be fully reversed.[24] In patients with APL receiving intravenous ATO, chronic toxicities, late effects and consequences of ATO prior to bone marrow transplant require additional studies.

As^{III} is the most reactive form of arsenic in biologic systems. In patients, As^{V} is reduced to As^{III} by arsenate reductase. Using S-adenosyl methionine (SAM) as the methyl donor and glutathione (GSH) as an essential cofactor, methyl transferases in the liver metabolize As^{III} to monomethylarsonic acid and monomethylarsinic acid (MMA) and then to dimethylarsonic acid (DMA). In our analysis, As^{V} was detectable transiently at the end of the infusion, As^{III} was measurable from end of infusion through 10 hours after infusion. MMA and DMA were detectable 6 hours after the start of infusion. The median (range) ratio of As^{III}: total arsenic exposure was 0.60 (0.31-1.20). Total arsenic was detectable in plasma for up to 24 hours after the end of the infusion. Comparison of trough total arsenic concentrations (prior to dose 2, 5, and 20) indicates minimal accumulation of arsenic during a cycle of therapy. Exposure to total arsenic was not dose proportional. Measurement of plasma arsenic concentration in adult clinical trials was performed using derivitization and gas chromatography, precluding comparison to our results.

The morphologic complete bone marrow response rate for patients with APL in our study was 85% and is similar to studies in adults. Morphologic responses in APL patients occurred after a median of 20 doses of ATO. Cytogenetic complete response was achieved in 9 patients after a median of 50 doses. Monitoring bone marrow by RT-PCR was not required but was reported in 7 patients; 3 converted to negative PT-PCR after a median of 70 doses of ATO. Remissions were achieved in patients treated based on actual body weight and ideal body weight.

In conclusion, ATO at the recommended dose of 0.15 mg/kg per dose is tolerable in children and adolescents with leukemia. The toxicity profile in this population is similar to previously published data in adult patients. Careful monitoring of QTc interval and correction of hypomagnesemia, hypokalemia, or hypocalcemia are warranted. For obese children and adolescents, ATO dosing using ideal body weight should be considered. In children with relapsed or refractory APL, morphologic response can be achieved with 20 to 30 doses of ATO, however, 70 doses were required to achieve negative RT-PCR in 3 of 7 patients.

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Authorship

Contribution: E.F. designed the trial, performed research, analyzed the data, and wrote the manuscript. R.I.R., B.C.W., G.H.R., J.J.M., and S.M.B. performed research. S.X., M.O.B., and W.G. performed research and analyzed data. F.M.B. and P.C.A. designed the trial, performed research and analyzed the data. All authors reviewed the manuscript prior to submission.

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References

1. Soignet SL, Maslak P, Wang ZG, et al. Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. N Engl J Med. 1998;339:1341-1348.
2. Shen ZX, Chen GQ, Ni JH, et al. Use of arsenic trioxide (As2O3) in the treatment of acute promyelocytic leukemia (APL): II. Clinical efficacy and pharmacokinetics in relapsed patients. Blood. 1997;89:3354-3360.
3. Soignet SL, Frankel SR, Douer D, et al. United States multicenter study of arsenic trioxide in relapsed acute promyelocytic leukemia. J Clin Oncol. 2001;19:3852-3860.
4. Lazo G, Kantarjian H, Estey E, Thomas D, O'Brien S, Cortes J. Use of arsenic trioxide (As2O3) in the treatment of patients with acute promyelocytic leukemia: the M. D. Anderson experience. Cancer. 2003;97:2218-2224.
5. Niu C, Yan H, Yu T, et al. Studies on treatment of acute promyelocytic leukemia with arsenic trioxide: remission induction, follow-up, and molecular monitoring in 11 newly diagnosed and 47 relapsed acute promyelocytic leukemia patients. Blood. 1999;94:3315-3324.
6. Shen ZX, Shi ZZ, Fang J, et al. All-trans retinoic acid/As2O3 combination yields a high quality remission and survival in newly diagnosed acute promyelocytic leukemia. Proc Natl Acad Sci U S A. 2004;101:5328-5335.
7. Powell B, Moser, B., Stck, W., Gallagher RE., Willman, C., Coutre, S., Feusner, JH., Appelbaum, FR., Tallman, MS., Larson, RA. Effect of consolidation with arsenic trioxide on event free survival ans overall survival among patients with newly diagnosed acute promyelocytic leukemia (APL): North American Intergroup Protocol C9710. Proc Am Soc Clin Oncol. 2007;25:Abstract 2.
8. Rojewski MT, Korper S, Schrezenmeier H. Arsenic trioxide therapy in acute promyelocytic leukemia and beyond: from bench to bedside. Leuk Lymphoma. 2004;45:2387-2401.

9. Chen GQ, Shi XG, Tang W, et al. Use of arsenic trioxide (As2O3) in the treatment of acute promyelocytic leukemia (APL): I. As2O3 exerts dose-dependent dual effects on APL cells. Blood. 1997; 89:3345-3353.
10. Zhu J, Koken MH, Quignon F, et al. Arsenic-induced PML targeting onto nuclear bodies: implications for the treatment of acute promyelocytic leukemia. Proc Natl Acad Sci U S A. 1997;94: 3978-3983.
11. Shao W, Fanelli M, Ferrara FF, et al. Arsenic trioxide as an inducer of apoptosis and loss of PML/RAR alpha protein in acute promyelocytic leukemia cells. J Natl Cancer Inst. 1998;90:124-133.
12. Jing Y, Dai J, Chalmers-Redman RM, Tatton WG, Waxman S. Arsenic trioxide selectively induces acute promyelocytic leukemia cell apoptosis via a hydrogen peroxide-dependent pathway. Blood. 1999;94:2102-2111.
13. Chou WC, Jie C, Kenedy AA, Jones RJ, Trush MA, Dang CV. Role of NADPH oxidase in arsenic-induced reactive oxygen species formation and cytotoxicity in myeloid leukemia cells. Proc Natl Acad Sci U S A. 2004;101:4578-4583.
14. Liu L, Trimarchi JR, Navarro P, Blasco MA, Keefe DL. Oxidative stress contributes to arsenic-induced telomere attrition, chromosome instability, and apoptosis. J Biol Chem. 2003;278:31998-32004.
15. Huang C, Ma WY, Li J, Dong Z. Arsenic induces apoptosis through a c-Jun NH2-terminal kinase-dependent, p53-independent pathway. Cancer Res. 1999;59:3053-3058.
16. Davison K, Mann KK, Waxman S, Miller WH, Jr. JNK activation is a mediator of arsenic trioxide-induced apoptosis in acute promyelocytic leukemia cells. Blood. 2004;103:3496-3502.
17. National Institutes of Health, NCICommon Toxicity Criteria version 2, http://ctep.info.nih.gov. Accessed November 15, 2007.
18. Kumana CR, Au WY, Lee NS, et al. Systemic availability of arsenic from oral arsenic-trioxide used to treat patients with hematological malignancies. Eur J Clin Pharmacol. 2002;58:521-526.
19. Nixon DE, Moyer TP. Arsenic analysis II: rapid separation and quantification of inorganic arsenic plus metabolites and arsenobetaine from urine. Clin Chem. 1992;38:2479-2483.
20. Barbey JT, Pezzullo JC, Soignet SL. Effect of arsenic trioxide on QT interval in patients with advanced malignancies. J Clin Oncol. 2003;21:3609-3615.
21. Westervelt P, Brown RA, Adkins DR, et al. Sudden death among patients with acute promyelocytic leukemia treated with arsenic trioxide. Blood. 2001;98:266-271.
22. Camacho LH, Soignet SL, Chanel S, et al. Leukocytosis and the retinoic acid syndrome in patients with acute promyelocytic leukemia treated with arsenic trioxide. J Clin Oncol. 2000;18:2620-2625.
23. Tallman MS, Andersen JW, Schiffer CA, et al. Clinical description of 44 patients with acute promyelocytic leukemia who developed the retinoic acid syndrome. Blood. 2000;95:90-95.
24. Mukherjee SC, Rahman MM, Chowdhury UK, et al. Neuropathy in arsenic toxicity from groundwater arsenic contamination in West Bengal, India. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2003;38:165-183.