A phase II trial of arsenic trioxide for relapsed and refractory acute lymphoblastic leukemia

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We designed a phase II trial of arsenic trioxide (AT) for the treatment of relapsed and refractory acute lymphoblastic leukemia (ALL). The dose administered was 0.25 mg/kg/day intravenously for 5-7 days per week for up to 60 days. Of 11 patients eligible, eight had B-cell and three T-cell ALL and two were Philadelphia chromosome-positive. The median duration of therapy was 21 days (range 7-28). One patient died of an infection. There were no responses. Ten patients have died. The median survival was 3.2 months (range 1.2-4.1). We conclude that AT is not active in the treatment of ALL.

Key words: acute lymphoblastic leukemia, relapse, arsenic trioxide.

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The treatment of relapsed acute lymphoblastic leukemia (ALL) in adults is unsatisfactory. The median duration of remission after treatment with chemotherapy is less than 6 months, and long-term survival is rare.[1,2] Arsenic trioxide (AT) is effective in the treatment of acute promyelocytic leukemia (APL). Remission rates of up to 90% have been achieved in patients with relapsed APL with 80% of patients becoming negative for the *PML/RARα* fusion transcript by polymerase chain reaction (PCR).[3]

Arsenic trioxide appears to function by causing apoptosis at higher concentrations and differentiation at lower concentrations.[4] It has demonstrated *in vitro* activity against tumor cell lines derived from lymphocytes and plasma cell[5,6] non-Hodgkin's lymphomas,[7,8] adult T-cell leukemia,[9] and non-M3 acute myeloid leukemia.[10] Akao *et al.* demonstrated that AT inhibited the cell growth of four B-cell leukemia lines, three of which were acute infantile leukemias expressing t(11;19) (q23;p13).[11] In these cell lines, AT induced apoptosis without evidence of differentiation as a result of activation of caspases and down-regulation of BCL-2.

An intravenous dose-ranging study of AT in advanced hematologic malignancies for 25 days per treatment course every 3-5 weeks showed that doses up to 0.25 mg/kg per day were well-tolerated.[12] We performed a phase II study to investigate the therapeutic potential of AT in relapsed and refractory ALL. Laboratory studies were done to determine whether the *in vitro* sensitivity of pretreatment ALL cells to AT-induced cytotoxicity and/or apoptosis would be predictive of clinical response.

Design and Methods

Patients

This phase II study was conducted under the auspices of the Leukemia Committee of the Eastern Cooperative Oncology Group (ECOG) and was approved by the Institutional Review Boards of the participating institutions according to the guidelines of the Declaration of Helsinki. Adult patients with ALL refractory to induction therapy or who had relapsed following chemotherapy or autologous bone marrow transplantation were eligible for treatment on this protocol. All patients met morphological and cytochemical criteria for a diagnosis of ALL, were centrally immunophenotyped by multiparameter flow cytometry and had to have >25% marrow blasts. All patients were tested for bcr-abl expression by PCR. Patients with a prior history of leukemic involvement of the central nervous system (CNS) were eligible if they had been treated and were disease-free. Additional eligibility criteria included an ECOG performance score of 0-2, and the following laboratory parameters: direct bilirubin <2.0 mg/dL, creatinine <2.0 mg/dL and liver enzymes less than or equal to two times the upper limit of normal.

Drug dosing

Arsenic trioxide was supplied by the National Cancer Institute. Patients received a daily 1-hour intravenous infusion of 0.25 mg/kg of actual weight in 100-500 mL of 5% dextrose in water. The drug was administered for 7 days per week in the first nine patients for a maximum of 60 days and continued until bone marrow blasts were less than 5%, until progression of disease or unacceptable toxicity occurred. The protocol was subsequently amended to allow the same daily dose of 0.25 mg/kg, but for five days per week for up to 60 days to make the treatment more convenient to administer and the last two patients were treated on this schedule. The protocol specified that if patients achieved a complete remission, they could receive up to five consolidation courses at the same dosage and schedule for up to 25 days. Electrolyte abnormalities were corrected and the drug was withheld if the QTc interval was more than 0.48 seconds and resumed if the QTc corrected to less than 0.48 seconds.

In vitro laboratory studies

Pre-treatment samples of leukemic blasts were recovered from the interface of sodium metrizimide density gradients (r=1.077 g/mL) and cultured in the presence and absence of 1 and 2 μM AT and harvested after 24 and 48 hours of incubation. Total and percent viable cells were determined by hemocytometer counts using trypan blue exclusion and apoptosis was assessed by determination of DNA content, including sub-G_1 DNA content, by flow cytometry with propidium iodine staining following the ethanol/high-molarity phosphate-citrate buffer procedure.[13]

Statistics

A two-stage statistical design was planned. If at least one response occurred among the first 12 eligible patients, then an additional 25 patients were to be entered in the trial for a maximum of 37 eligible patients. Four or more responses were considered to be a promising response rate and would be worthy of further investigation. The design had a one-sided error rate of 0.09 and 90% power.

Results and Discussion

Thirteen patients were enrolled. Two patients were found to be ineligible. One had leukemic CNS involvement and one had a bone marrow lymphoblast count of 16%. The main outcome analyses were done on the 11 eligible patients, and toxicity data were collected for all 13 treated patients. The characteristics of the patients are shown in Table 1. The median age was 38 years and the median leukocyte count was 5.9×10^9/L. The median percentage of blasts in the bone marrow was 88.5%.

Table 1. Characteristics of the 11 patients on-study.

	N (%)
Gender	
Male	9 (82%)
Female	2 (18%)
Performance Status	
0	1 (9%)
1	7 (64%)
2	3 (27%)
Age (years)	
Median	38
Range	21-73
Hemoglobin (g/dL), n=10	
Median	9.2
Range	8-11.3
Platelets ($\times10^9$/L)	
Median	37
Range	12-111
WBC ($\times10^9$/L)	
Median	5.9
Range	0.8-65
Blood blasts (%), n=5	
Median	41%
Range	0-94
Bone marrow blasts (%), n=10	
Median	88.5%
Range	49-95

Because this was a cooperative group study, details of the specific prior therapy the patients had received was not available, but it was known that eight of the evaluable patients had received a median of two prior regimens (range 1-6) before going on-study. The other three patients had received multiple prior therapies, but the exact number was not known.

Immunophenotypic analysis demonstrated that five patients had early pre-B ALL, two patients had pre-B, one had mature B, and three had immature T cell (surface membrane CD2 and CD3 negative and CD33 positive) ALL. One of the T-cell cases expressed surface CD56 with intracytoplasmic expression of CD3 consistent with a natural killer subtype. Myeloid antigen expression was rare.

The ECOG Cytogenetic Committee reviewed karyotype preparations and laboratory reports for nine patients (eight of whom were eligible for the study). Two patients had Philadelphia chromosome-positive ALL (both with e1a2 transcripts). The karyotype of one patient was normal. One other patient had one abnormal metaphase and 19 normal metaphases, but these results do not meet the minimum criterion for an abnormal clone. Each of the remaining five patients had a complex karyotype with both numerical and structural anomalies without evidence of any classical chromosome anomalies associated with ALL. Cytogenetic results at the time of diagnosis and at relapse for four patients analyzed were largely concordant.

The median duration of AT therapy was 21 days with

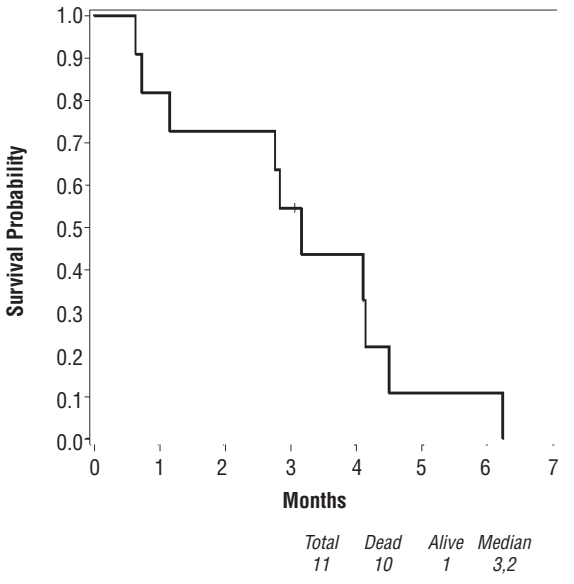

Figure 1. Kaplan-Meier estimate of overall survival of the 11 eligible cases.

Table 2. Treatment-related toxicity (n=13).

	Grade*		
Toxicity type	3	4	5
Hemoglobin	6	1	–
Leukocytes	4	3	–
Neutrophils	–	6	–
Platelets	3	5	–
Hypotension	–	1	–
Cardiac-other	1	–	–
Fatigue	1	–	–
SGPT	1	–	–
Febrile neutropenia	–	–	1
Hyperkalemia	1	–	–
Hypermagnesemia	1	–	–
Hypocalcemia	–	2	–
Hypokalemia	2	1	–
Hyponatremia	1	–	–
Abdominal pain	1	–	–
Dyspnea	2	1	–
Hypoxia	1	1	–
Pneumonitis/pulmonary infiltrates	1	–	–
Creatinine	–	1	–
Worst degree†	–	4	1

**According to NCI Common Toxicity Criteria; †excludes hematologic toxicities in calculation.*

a range from 7 to 28 days. None of the 11 eligible patients achieved a definite response. Five patients had no response. The response assessment was planned at day 28 of therapy. Six cases were unevaluable because of a lack of response assessment by day 28: three died before the response assessment, one went off therapy on day 13 due to toxicity, another stopped therapy on day 13 because of reported progression and one started non-protocol therapy before day 28. The median survival was 3.2 months (range 1.2 to 4.1 months) (Figure 1). All patients for whom follow-up is available have died. One patient has been lost to follow-up. The median progression-free survival was 2.8 months with a range of 1.2-4.1 months.

No unexpected toxicities were noted. Most patients experienced grade 3 or 4 hematologic toxicity. Electrolyte abnormalities were generally grade 3 or less. The only treatment-related death was from sepsis. No deaths of cardiac causes were reported. The toxicities are summarized in Table 2.

*In vitro l*aboratory studies were performed on six of nine submitted cases. Of interest, cell viability in the absence of AT from day 1 to day 2 fell from 0% to 51% (median 23%). In two cases there was a decline in viability and an increase in apoptosis with exposure to AT, but neither of these cases demonstrated any significant leukemic blast cytoreduction with AT therapy.

The rationale for this study was to explore the efficacy of AT in ALL because of *in vitro* data indicating an effect of this drug against ALL because of the impressive results seen with AT in APL. The dose of AT utilized was higher than that used in APL, but despite this did not result in any significant responses. Five patients did not have a documented response to the therapy at the initial assessment point of day 28 and were, therefore, taken off-study. Of the other six patients, three died before the day 28 assessment could be done, and the other three stopped therapy before the day 28 assessment could be completed because of toxicity or lack of efficacy. Thus, in these latter six patients it is clear that the AT therapy was not having any clinical benefit.

Although disappointing, the results seen in this trial are similar to those in subtypes of acute myelogenous leukemia other than APL in which AT is largely ineffective.[14]

A continuing effort to find new agents effective in the treatment of ALL is urgently needed. Recent studies incorporating imatinib mesylate into the treatment of Philadelphia chromosome-positive ALL,[15-17] the use of rituximab for CD-20-positive ALL[18,19] and nelarabine (506 U78)[20] for T-cell ALL are encouraging in this regard.

ML: designed the study, supervised the conduct of the trial and wrote the paper; SL: analyzed the data and performed the statistical analysis; JB: reviewed the hematopathology of the cases to confirm the diagnosis of ALL; GD: reviewed and confirmed the cytogenetic data; RG: designed and analyzed the in vitro laboratory studies; VJ: performed the in vitro laboratory studies and assembled the data; EP: performed and analyzed the immunophenotypic studies; JR: performed polymerase chain reaction assays for bcr-abl to confirm the diagnosis of Ph+ ALL; SR: accrued patients to the study; JM: assisted in the design and conduct of the trial; MT: assisted in the design and conduct of the trial and accrued patients to the study, he is the chair of the ECOG Leukemia Committee: he is also on the advisory board of Cell Therapeutics Inc., the company that markets arsenic trioxide in the United States of America. None of the other authors have any potential conflicts of interest.

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