Original Article

Phase 1 Study of Arsenic Trioxide, High-Dose Cytarabine, and Idarubicin to Down-Regulate Constitutive Signal Transducer and Activator of Transcription 3 Activity in Patients Aged <60 Years With Acute Myeloid Leukemia

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BACKGROUND: Constitutive activation of signal transducer and activator of transcription-3 (STAT3) was detected in blasts from approximately 50% of patients with acute myeloid leukemia (AML) and was correlated with an adverse outcome. In vitro treatment of AML blasts with arsenic trioxide (ATO) down-regulated STAT3 activity within 6 hours associated with a reduced viability within 48 hours. **METHODS:** A phase 1 clinical trial to evaluate the biologically effective dose and/or the maximally tolerated dose (MTD) of ATO in vivo in conjunction with high-dose cytarabine (Hidac) and idarubicin (Ida) in patients with AML aged <60 years was conducted. Data were compared with 117 historic AML patients who had received treatment with Hidac/Ida. **RESULTS:** In total, 61 patients were enrolled onto 11 different dose levels (from 0.01 to 0.65 mg/kg ideal body weight). The MTD was 0.5 mg/kg. Compared with historic controls, patients who received ATO/Hidac/Ida, although they had similar pretreatment characteristics, had better overall survival ($P = .039$). **CONCLUSIONS:** ATO priming may have improved the outcome of patients aged <60 years with AML who received Hidac/Ida. The current data suggested that ATO may enhance the effect of chemotherapy. The authors concluded that further studies of this novel combination are warranted. ***Cancer*** **2011;117:4861–8.** © *2011 American Cancer Society.*

KEYWORDS: acute myeloid leukemia, signal transducer and activator of transcription 3, arsenic trioxide, treatment, phase 1.

Acute myeloid leukemia (AML) in patients aged <60 years is characterized by complete remission rates of 70% to 80% after 1 or 2 induction treatments, and the overall cure rate is only 35% to 45%.[1-3] We previously demonstrated that the constitutive activation of signal transducer and activator of transcription 3 (STAT3) protein in AML blasts is associated with short disease-free survival,[4] and this STAT3 activation is down-regulated in vitro by arsenic trioxide (ATO) in association with inhibited protein tyrosine kinase activities.[5] Therefore, we investigated whether ATO could reduce constitutive STAT3 activity in vivo. Because ATO as a single agent proved to be ineffective in AML treatment,[6] we

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The first author was the principal investigator of this protocol, oversaw patient accrual, contributed to the care of the patients, followed data collection, reviewed the data, and wrote the article The second author performed the statistical analyses. The third author constructed the database. The fourth author served as the research coordinator for this protocol. The fifth author reviewed the pathology specimens. The sixth and seventh authors reviewed all karyotype analyses. The eighth author oversaw the single nucleotide polymorphism analysis. The ninth author was the principal investigator on the high-dose cytarabine and idarubicin trials, served as a coinvestigator on this protocol, and contributed to the care of the patients. The 10th author contributed to the care of the patients. The 11th author was a senior coinvestigator on this protocol. All authors reviewed and approved the final article.

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determined its effect when administered over 1 hour followed by our previously described induction regimen of high-dose cytarabine (Hidac) (3 g/m^2 [1.5 mg/m^2 for patients aged $\geq$50 years] every 12 hours for 12 doses] and idarubicin (Ida) (12 mg/m^2 for 3 doses).[7]

Currently, ATO is approved by the US Food and Drug Administration for the treatment of relapsed acute promyelocytic leukemia (APL) at a dose of 0.15 mg/kg. In these patients, the beneficial action of ATO is observed more in its promoting degradation of promyelocytic leukemia (PML) fusion products with oncogenic activities rather than in attenuating protein kinase activities. ATO doses as high as 0.3 mg/kg were studied in myelodysplastic syndromes[8]; however, to the best of our knowledge, no phase 1 dose escalation in combination with intensive chemotherapy was ever conducted in AML.

ATO is methylated intracellularly to methylarsonic acid (MMA) and dimethylarsonic acid (DMA), and a genetic polymorphism exists in the monomethylarsonic acid (*MMA*) V reductase gene, which is involved in the biomethylation of ATO.[9] Therefore, we examined whether any single nucleotide polymorphism (SNP) in this pathway was associated with the toxicities observed in patients who were enrolled onto this trial.

To evaluate the potentially measurable, immediate biologic effect of ATO, we studied patient samples before and after ATO administration for the relative level of phosphortyrosine STAT3. Treatment-related reduction was planned to become the biologically effective dose (BED) for a cohort of 10 patients. Alternatively, if a maximum tolerated dose (MTD) had been achieved before the BED, then the study would have been terminated. Finally, to verify that results from this phase 1 trial were at least as good as the results from other phase 2 trials for newly diagnosed AML in patients aged <60 years, we compared patient outcomes with outcomes in a historic cohort of 117 patients with newly diagnosed AML who received Hidac/Ida alone.

MATERIALS AND METHODS

Patient Eligibility

Patients with newly diagnosed AML, excluding APL, ages 18 years and 59 years with an Eastern Cooperative Oncology Group performance status of 0 to 3 were eligible. Other eligibility criteria included the presence of $\geq$10% blasts in peripheral blood (to allow for BED determination), adequate hepatic function (total bilirubin, aspartate aminotransferase, and alkaline phosphatase levels $\leq$2 times the upper limit of normal [ULN]) and renal function (serum creatinine 1.5 times the ULN), no prior chemotherapy for AML except hydroxyurea, and absence of uncontrolled intercurrent illnesses, including infections, cardiac conditions, and other organ dysfunction. Patients with an absolute QT interval >460 msec in the presence of serum potassium >4 millequivalents per liter and magnesium >1.8 mg/dL were excluded. The outcome of these patients was compared with the outcome of 117 patients with newly diagnosed AML patients aged <60 years who were enrolled onto clinical trials of Hidac/Ida alone between September 1, 1991 and January 9, 2008. All patients signed informed consent form according to institutional guidelines and in compliance with the Declaration of Helsinki.

Study Design and Treatment Plan

This study used a classic 3 + 3, phase 1 design. The starting dose of ATO was 0.01 mg/kg ideal body weight (IBW), which was administered once intravenously over 1 hour; followed by Hidac 3 g/m^2 (1.5 mg/m^2 for patients aged $\geq$50 years) administered intravenously every 12 hours for 12 doses; and Ida 12 mg/m^2 for 3 doses administered intravenously after the third, fifth, and seventh Hidac doses. Granulocyte–colony-stimulating factor (G-CSF) was administered subcutaneously 12 hours after the completion of Hidac at a dose of 5 mg/kg daily until the patient achieved an absolute neutrophil count (ANC) $\geq 5 \times 10^9$/L, or the peripheral blood blast count rose $\geq$10%, or there was a life-threatening toxicity which we believed was related to G-CSF. The ATO dose was escalated to 0.05 mg/kg IBW, 0.1 mg/kg IBW, 0.15 mg/kg IBW, 0.2 mg/kg IBW, 0.25 mg/kg IBW, 0.3 mg/kg IBW, 0.33 mg/kg IBW, 0.42 mg/kg IBW, 0.5 mg/kg IBW, and 0.65 mg/kg IBW. Consolidation treatment and allogeneic transplantation were offered according to standard practice at Roswell Park Cancer Institute.

Dose-Limiting Toxicity and Escalation Rules

A dose-limiting toxicity (DLT) was defined as the occurrence of any clinically relevant and drug-related, grade $\geq$3, nonhematologic, noninfectious toxicity despite maximum supportive care or lack of hematologic recovery within 42 days from initiation of therapy that was attributable to drug effect rather than refractory leukemia. Also, for hepatic, cardiac, gastrointestinal, dermatologic, central nervous system, and pulmonary complications, grade 3 toxicity was regarded as expected, whereas grade $\geq$4 toxicity was counted toward the DLT. Three patients were

entered at each dose level. If no DLT was observed, then patients were treated at the next higher dose level. If 1 DLT was observed, then 3 more patients were treated at that dose level. The MTD was defined as the dose that produced grade 3 to 4 toxicity at least in 2 of 6 patients treated. The recommended dose for future phase 2 studies would be the dose level immediately before the MTD.

Response and Toxicity Criteria and Statistical Methods

Response criteria were standard.[10] Complete remission (CR) was defined as <5% blasts in a normocellular bone marrow, an ANC $\geq 1 \times 10^9$/L, and a platelet count $\geq 100 \times 10^9$/L. Treatment failure was defined as related to resistant disease (the patient survived for $\geq$1 week after completing therapy, and peripheral blood smear and/or bone marrow revealed persistent AML), complications of aplasia (the patient who survived for $\geq$1 week after completing therapy died while cytopenic, and the last postremission bone marrow was aplastic or hypoplastic without evidence of leukemia), or indeterminate cause (patients died <1 week or $\geq$1 week after completing therapy, the most recent peripheral blood smear did not reveal persistent leukemia, and bone marrow examination was not performed subsequent to therapy). Persistent cytopenia without leukemia was defined as persistent neutropenia and/or thrombocytopenia despite a bone marrow aspirate and biopsy that met criteria for CR. Relapse was defined as the reappearance of blasts in the blood or $\geq$5% blasts in the bone marrow that was not attributable to another cause (eg, bone marrow regeneration) or biopsy-proven leukemia relapse in nonmedullary site. The BED was determined by the relative decrease in constitutive STAT3 activity after ATO treatment as indicated by Western blot analysis (see below).

Toxicity was graded on a scale from 0 to 5 using the National Cancer Institute Common Terminology Criteria of Adverse Events (NCI-CTCAE) version 3.0. Survival and response durations were analyzed by the Kaplan-Meier method.

Information on the associations between 9 SNPs and 112 NCI-CTCAE categories was tabulated. Each SNP with sufficient variability (>5 observations in at least 2 categories) was used in logistic regression to predict DLT both alone and adjusted for dose level. No correction for multiple testing was done in this exploratory analysis.

The Kaplan-Meier method and the log-rank test were used to compare survival distributions (time from diagnosis to death) of patients in the current study and the historic study. No censoring for transplantation was conducted.

SNP Analysis

DNA was extracted from 57 cryopreserved bone marrow or peripheral blood samples using Gentra PureGene DNA extraction kits (Gentra Systems, Inc., Minneapolis, Minn) and was genotyped for 9 SNPs in the *MMA* V reductase gene by using the iPLEX software (Sequenom Inc., San Diego, Calif). Duplicate aliquots for approximately 10% of the samples were distributed randomly throughout the plates for quality-control purposes. Controls for genotype and 2 "no template" controls also were included on each plate. All genotyping results were reviewed manually for quality control.

Western Blot Analysis

Western blot analysis was performed as we described previously[5] with minimal modification. Briefly, peripheral blood mononuclear cells from patients who had at least 10% blasts before and immediately after the completion of ATO administration were isolated and immediately extracted. Aliquots of extract containing 20 μg proteins were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels. The relative amount of STAT3 phosphorylated on tyrosine 705 (PY705-STAT3) compared with the total amounts of STAT3 and actin was quantified by immunoblotting. In addition, samples were hybridized for phosphorylated and total STAT5, total STAT1, and phosphorylated mitogen-activated protein kinase (ERK1/2). Extracts of the HEL cell line (purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) that displayed constitutive STAT3 activity served as internal references in all analyzes. To account for background reaction of the immune reagents, the lowest level of PY705-STAT3 detection was set at <2%. Band intensity >2% was defined as constitutive activity. The BED was expressed as the percentage reduction in phosphorylated STAT3 band intensity as determined by densitometry analysis[4] between pre-ATO and post-ATO administration in 3 patient samples at a dose level.

RESULTS

Patient Characteristics and Treatment Administration

The characteristics of the 61 treated patients are listed in Table 1. All patients completed the planned treatment.

The number of patients treated at each dose level and the observed DLT are provided in Table 2. Because of concerns for patient safety, the first ATO increments were relatively small. When a CR rate of approximately 70% was met at the initial dose levels, 30% dose increments followed.

Table 1. Comparison of Patient Characteristics Between the Arsenic Trioxide/Hi-Dose Cytarabine/Idarubicin Arm and the Hi-Dose Cytarabine/Idarubicin Arm

Characteristic	ATO/ Hidac/ Ida	Hidac/ Ida	*P*
No. of Patients	61	117	
Median age, y	47	45.5	.7
Men:Women, %	41:59	62:38	
AML presentation, %			.2
De novo	87	78	
Secondary	13	22	
Therapy-related	62	31	
AHD[a]	13	58	
Both	25	8	
Karyotype, %[b]			.9
Favorable	14	13	
Intermediate	51	48	
Unfavorable	35	39	
Median WBC count, $\times 10^9$/L	17.8	9.0	.07
CR rate, %	72	66	.4
Eight-wk mortality rate, %	9.4	6.6	.6
Median follow-up, mo	17.4	19.4	.2

ATO indicates arsenic trioxide; Hidac, hi-dose cytarabine; Ida, idarubicin; AML, acute myeloid leukemia; AHD, antecedent hematologic disorder; WBC, white blood cell; CR, complete remission.

[a] AHD included patients who had a history of myelodysplastic syndrome or myeloproliferative neoplasm.

[b] See Byrd JC, Mrozek K, Dodge RK, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). *Blood*. 2002;100:4325-4336.[11]

DLTs and MTDs

The DLT of supraglottitis and acute respiratory distress syndrome were achieved at 0.65 mg/kg IBW. Therefore, the MTD was defined as 0.5 mg/kg IBW. In total, 3 additional patients were accrued at that dose level. The most common nonhematologic, drug-related adverse events (Table 3) were infection and skin (ranging from alopecia, bruising, dry skin, rash, and hives). Myelosuppression was expected with this regimen, and myelosuppression-associated complications included febrile neutropenia in 38 patients (62%). Three deaths occurred within the first 30 days of starting treatment. The cause of death in all 3 patients was multiorgan failure.

SNP Analysis

None of the SNP variables were predictive of the DLT. It is noteworthy that dyspnea and neutropenic fever were mildly associated with reference SNP 4925 (rs4925) ($P < .1$) (Table 4).

Biologic Activity

Samples from 48 patients were analyzed, and 36 samples had constitutive STAT3 activity, whereas 12 samples did not. A decrease in STAT3 activity between pre-ATO and post-ATO treatment was noticed in 1 patient sample at a dose of 0.33 mg/kg IBW (Fig. 1, samples 06-1351 and 06-1354).

Comparison With Historic Controls

A retrospective comparison with the historic control group of 117 patients who had newly diagnosed AML aged <60 years who received induction therapy with Hidac/Ida alone revealed similar pretreatment characteristics, CR rates, 8-week mortality (Table 1), and toxicities

Table 2. Arsenic Trioxide Dose Escalation and Dose-Limiting Toxicities

Dose Level	Dose per mg/kg IBW[a]	No. of Patients	Dose-Limiting Toxicities
1	0.001	6	Grade 4 LVEF and Grade 3 Rash
2	0.005	6	Grade 4 ARDS
3	0.1	3	
4	0.15	6	No count recovery by D 42 followed by a remission BM
5	0.2	3	
6	0.25	10	No count recovery by D 42 followed by a remission BM
7	0.3	3	
8	0.33	6	Grade 3 right upper extremity deep vein thrombosis
9	0.42	6	Grade 3 altered mental status
10	0.5	6	
11	0.65	6	Grade 4 supraglottitis and grade 4 ARDS

IBW indicates ideal body weight; LVEF, left ventricular ejection fraction; ARDS, acute respiratory distress syndrome; BM, bone marrow.

Table 3. Comparison of Grade 1 Through 5 Adverse Events Between the Arsenic Trioxide/Hi-Dose Cytarabine/Idarubicin Arm and the Hi-Dose Cytarabine/Idarubicin Arm

	Grade, %										
	ATO/Hidac/Ida, n=61					Hidac/Ida, n=117					
Toxicity	1	2	3	4	5	1	2	3	4	5	P^a
Nausea/vomiting	11.5	29.5	1.6			34.2	16.2	2.6	1.7		>.1
Mucositis	6.5	31.1	3.3			46.2	12.8	6.8	0.9		>.1
Diarrhea	19.7	24.6	27.9			36.8	35.9	16.2	2.6		>.1
Infection		3.3	60.7	4.9		0.9	4.3	43.6	8.5	3.4	>.1
Skin		24.6	55.7			12.0	33.3	18.8	0.9		<.0001
Liver	13.1	23	6.6	1.6		9.4	9.4	17.1	8.5		>.1
Pulmonary	14.8	3.3	19.7	8.2	4.9	10.3	4.3	12.0	7.7	3.4	>.1
Cardiac	6.6	21.3	14.8	3.3		12.8	10.3	13.7	5.1	0.9	>.1
Neurologic	13.1	11.5	9.8	1.6		12.0	20.5	15.4	6.0		>.01

ATO indicates arsenic trioxide; Hidac, hi-dose cytarabine; Ida, idarubicin.
[a] *P* values were calculated using the Wilcoxon-Mann-Whitney rank-sum test.

Table 4. Association Between Reference Single Nucleotide Polymorphism 4925 and Toxicities[a]

	SNP in No. of Patients		
Toxicity	A	C	AC
Dyspnea[b]			
False	7	16	27
True	0	7	0
Neutropenic fever[b]			
False	4	21	20
True	3	2	7

A indicates adenine; C, cytosine.
[a] $P<1$.
[b] Grade ≥ 3.

(Table 3), except that skin reactions were more common in the ATO/Hidac/Ida cohort, and neurologic toxicities were more common in the Hidac/Ida cohort. The consolidation treatments differed between the 2 cohorts (Table 5), with significantly more patients in the control group receiving etoposide and cyclophosphamide as their consolidation regimens. A similar percentage of patients proceeded to undergo allogeneic transplantation in first CR and beyond between the 2 cohorts (49% vs 42% for the ATO/Hidac/Ida cohort vs the Hidac/Ida cohort, respectively; $P = .3$). Finally, these 2 cohorts had similar median follow-up, but the overall survival of patients who received ATO/Hidac/Ida was significantly longer compared with that of patients who received Hidac/Ida alone (39.9 months vs 17.6 months; $P = .036$) (Fig. 2). The survival of patients in the historic control group was similar whether they were treated before or after January 1, 2000 ($P = .8$). A total of 45 patients from the historical controls and all study patients were treated after January 1, 2000; there was a trend toward better survival in the ATO/Hidac/Ida cohort compared with the Hidac/Ida cohort ($P = .07$), although the difference most probably did not reach statistical significance because of the smaller denominator. Even after removing those patients who received etoposide and cyclophosphamide from the analysis, the difference in survival between the 2 cohorts maintained statistical significance ($P = .034$; data not shown).

DISCUSSION

In this study, we demonstrated that increasing the ATO dose up to 0.5 mg/kg IBW in combination with intensive chemotherapy is safe and well tolerated. The 30-day mortality rate was only 4.9%. This compares favorably with other large studies, in which approximately 3%[3] to 5%[1] 30-day mortality rates were reported for similar age cohorts. Finally, although concerns regarding the long-term toxicities associated with ATO may arise, significantly higher cumulative ATO doses are used in APL patients without known long-term consequences.[12]

A polymorphism in the human *MMA* V reductase gene is associated with changes in urinary arsenic profiles.[13] Our SNP findings, although exploratory in nature because of the small patient population, are hypothesis provoking. To our knowledge, the association between allelic aberration and the occurrence of dyspnea and neutropenic fever has not been described previously. We propose that larger trials studying the use of ATO, like in patients with APL, could look into these associations, because several groups recently demonstrated a correlation between genetic variation in arsenic metabolism and arsenic-related diseases.[14-16]

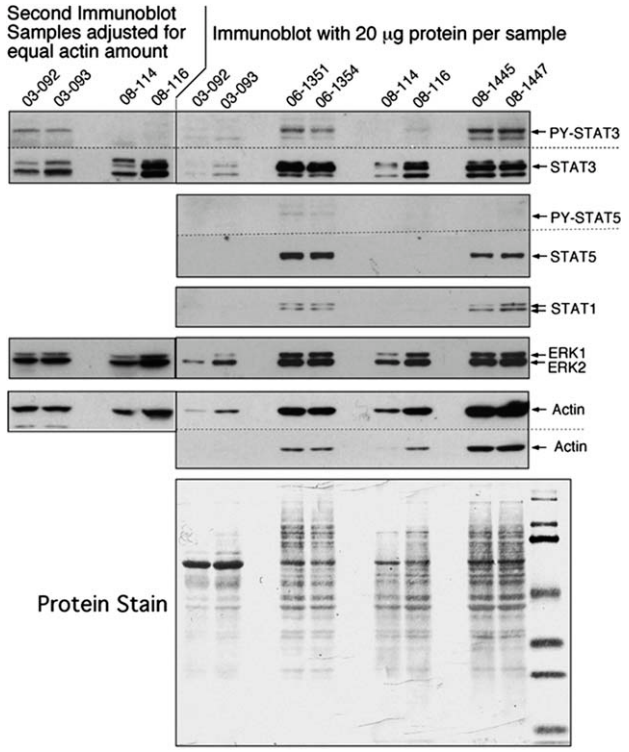

Figure 1. Arsenic trioxide (ATO) down-regulates constitutive signal transducer and activator of transcription 3 protein (P-STAT3) in samples from a patient with acute myeloid leukemia (AML). The numbers represent pre-ATO and post-ATO samples. When actin levels were not equal, immunoblotting was repeated, as indicated on the left for samples 03-092, 03-093, 08-114, and 08-116. Samples were hybridized for phosphorylated (PY) and total signal transducer and activator of transcription 3 (STAT3) and STAT5, total STAT1, mitogen-activated protein kinase 1 (ERK1) and ERK2, and actin. Protein staining is shown below to further demonstrate equal loading.

Table 5. Consolidation Treatments for the Arsenic Trioxide/Hi-Dose Cytarabine/Idarubicin Arm and the Hi-Dose Cytarabine/Idarubicin Arm

Treatment	ATO/Hidac/Ida, %	Hidac/Ida, %	*P*
Consolidation therapy			<.001
VP/CY (2.4 or 3.6)[b]	3	47	
Hidac (alone or with Ida)[a]	69	20	
Hidac followed by VP/CY	5	5	
Hidac followed by SCT	21	1	
VP/CY followed by SCT	0	5	
SCT alone	3	21	

ATO indicates arsenic trioxide; Hidac, hi-dose cytarabine; Ida, idarubicin; VP, etoposide; CY, cyclophosphamide; SCT, stem cell transplantation.

[a] The Hidac dose was 3 g/m^2 over 3 hours×6 doses on Days 1, 3, and 5; for Hidac with Ida, the Hidac dose was 3 g/m^2 (1.5 mg/m^2 for patients aged ≥50 years) given intravenously every 12 hours×12 doses, and the Ida dose was 12 mg/m^2×3 doses given intravenously after the third, fifth, and seventh Hidac doses.

[b] For VP/CY-2.4, the VP dose was 2.4 g/m^2 given as a continuous infusion over 34.3 hours, and the CY dose was 50 mg/kg of ideal body weight over 2 hours daily for 3 days; VP/CY-3.6, the VP dose was 3.6 g/m^2 given as a continuous infusion over 51.4 hours, and the CY dose was 50 mg/kg of ideal body weight over 2 hours daily for 4 days.

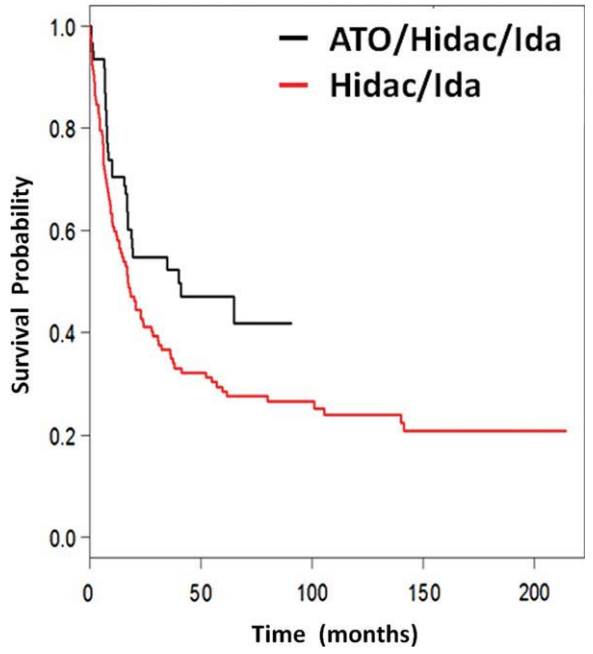

Figure 2. These Kaplan-Meier curves illustrate overall survival estimates for patients who received arsenic trioxide/high-dose cytarabine/idarubicin (ATO/Hidac/Ida) versus patients who received Hidac/Ida.

Finally, we did not observe a consistent ATO dose-dependent decrease in PY705-STAT3 levels. One possible explanation for this is the relatively short exposure followed by the administration of high-dose chemotherapy. Our in vitro work[5] demonstrated an ATO effect after 6 hours of incubation. However, we believe that in vivo exposure lasting longer than 1 hour may be inappropriate for patients aged <60 years with newly diagnosed AML who are treated with curative intent. The other possible explanation is that in vitro administration reaches higher blast exposure compared with in vivo administration. It is noteworthy that an ATO dose of 4 μM in vitro, which is sufficient to down-regulate constitutive STAT3 activity, is approximately equivalent to an in vivo dose of 0.15 mg/kg,[17] and this level is sufficient to differentiate APL cells. However, this dose may not be sufficient to down-regulate constitutively activated STAT3 in vivo within a 1-hour exposure period. White blood cell analysis could not have been performed at a later time point because of the exposure to Hidac/Ida. If a more effective, immediate reduction in constitutive

STAT3 activity is necessary for improved outcome, then either a longer exposure, the use of a heat-shock protein 90 inhibitor, or combining the 2 could be applied, because we recently demonstrated that heat-shock 90 inhibitors synergize with ATO to down-regulate constitutive STAT3 activity.[18,19] Therefore, testing the role of a heat-shock protein 90 inhibitor may be a potential future trial for AML patients. Other possibilities to down-regulated constitutive STAT3 activity include small-molecule peptidomimetics, antisense oligodeoxynucleotides, and short interference RNA.[20]

It is noteworthy that our current results demonstrated improved outcome for patients who received ATO/Hidac/Ida compared with patients who received Hidac/Ida alone in this nonrandomized comparison. Although this improved outcome may be explained by earlier detection or improvements in supportive care over the periods when these 2 sequential studies were conducted, we propose a different explanation. Specifically, it was demonstrated recently that ATO targets quiescent leukemia-initiating cells.[21,22] Furthermore, combining ATO with cytarabine significantly increased the efficacy of cytarabine to induce apoptosis and eradicate the leukemia-initiating cells.[21] In summary, we propose that the addition of ATO may have improved the outcome of patients with newly diagnosed AML aged <60 years who received treatment with Hidac/Ida. These results merit confirmation in a randomized phase 3 trial. Additional studies of the potential of ATO to prime leukemia-initiating cells as a means of enhancing chemotherapy effects are warranted.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

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