Advances in Brief

Feasibility and Correlates of Arsenic Trioxide Combined With Ascorbic Acid-mediated Depletion of Intracellular Glutathione for the Treatment of Relapsed/Refractory Multiple Myeloma


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Abstract

Patients with multiple myeloma (MM) invariably relapse with chemotherapy-resistant disease, underscoring the need for new agents that bypass these resistance mechanisms. We have reported that ascorbic acid (AA) enhances the activity of arsenic trioxide (As2O3) against drug-resistant MM in vitro by depleting intracellular glutathione (GSH). These data led us to open a National Cancer Institute/Cancer Therapy Evaluation Program-sponsored Phase I/II trial of As2O3 + AA for relapsed/refractory MM. We now present the completed Phase I component of this trial. The primary objective of the trial's Phase I component was to assess whether the addition of AA affected the well-described toxicity profile of As2O3 alone. Correlative studies were undertaken of As2O3 and AA pharmacokinetics, the ability of AA to deplete intracellular GSH in vivo, and the development of arsenic resistance. Six patients with stage IIIA relapsed/refractory myeloma were studied. We found that 0.25 mg/kg/day As2O3 + 1000 mg/day AA could be given for 25 days (over a 35-day period) without dose-limiting toxicity. One episode of grade 3 hematological toxicity (leukopenia) and no grade 3 nonhematological toxicities (in particular, cardiac) were observed. The coadministration of AA did not alter the pharmacokinetics of As2O3 and elevated AA levels were associated with decreased intracellular GSH. Serial in vitro studies demonstrated continued sensitivity of patient myeloma cells to As2O3 + AA. Two patients (both with thalidomide-refractory disease) had partial responses; four patients had stable disease. In conclusion, we have found that As2O3 + AA has acceptable toxicity and that there is promising evidence of activity in refractory/refractory myeloma.

Introduction

MM remains an incurable disease with an overall 5-year survival of less than 30% (1). Even with intensive up-front treatment strategies with stem cell rescue, the majority of patients with MM eventually relapse with chemotherapy-resistant disease (2, 3). A major mechanism of multidrug resistance is overexpression of the mdr1 and mrp genes (4, 5). These drug efflux pumps protect the MM cell by preventing the intracellular accumulation of a wide range of chemotherapeutic agents. Pro-survival cytokines (interleukin 6, tumor necrosis factor α, vascular endothelial growth factor), stromal interactions, enhanced drug metabolism via the GSH redox pathway, alterations in drug targets (topoisomerase II enzyme mutations, tubulin gene mutation, glucocorticoid receptor down-regulation), and up-regulation of antiapoptotic genes (Bcl-2, Bcl-xL, Mcl-1) have also been reported as chemoresistant mechanisms in MM (6–10).

Median survival for chemotherapy-resistant/relapsed refractory MM is predictably short, in the order of months (6). Chemotherapy regimens for refractory and relapsed MM (e.g., VAD in melphalan-resistant disease, high-dose steroids, IFN-α, cyclophosphamide + topotecan, and so forth) typically have 10–30% response rates of short duration (11). The failure of “traditional” chemotherapy in relapsed and refractory MM is the rationale for testing novel agents that may act via unique tumoricidal mech-
anisms. The antiangiogenic agent thalidomide has demonstrated significant promise in refractory myeloma with an overall response rate of 32% (12), and is currently in wide clinical use for this cohort of patients.

The antitumor activity of arsenic trioxide (As$_2$O$_3$) was rediscovered in the modern era when Chinese groups reported that As$_2$O$_3$ induced CRs in >50% of APL patients that failed all-trans retinoic acid therapy (13, 14, 15), followed by United States clinical studies (using 0.06–0.2 mg/kg/day and cumulative doses of 160–515 mg) in APL demonstrating an 80–90% CR rate (16, 17). On the basis of these studies, As$_2$O$_3$ was approved by the Food and Drug Administration for APL, with the current recommended induction regimen of 0.15 mg/kg/day i.v. until CR or a maximum of 60 doses is reached. In APL, the reported As$_2$O$_3$-related toxicities were relatively mild, the most prevalent being skin rash, fatigue, nausea/vomiting/diarrhea, musculoskeletal pain, leukocytosis, liver function abnormalities, and “retinoic acid” syndrome. Although As$_2$O$_3$ does not appear to cause cardiomyopathy, 69% (18) to 100% (19) of patients had QTC prolongation during arsenic treatment, which was associated in some patients with nonsustained ventricular arrhythmias. Other groups have reported complete atroventricular block and torsade de pointes, and sudden death (20–22).

In MM, As$_2$O$_3$ has been shown to induce apoptosis in several cell lines (23) that was not blocked by the addition of the prosurvival cytokine interleukin-6 (24). We have also found that As$_2$O$_3$ induces apoptosis in the drug-resistant myeloma cell lines despite bcl-x$_L$ or mdr1 gene overexpression (10). In a small Phase II study of As$_2$O$_3$ alone in heavily pretreated refractory myeloma, Munshi et al. have reported a 23% response rate using As$_2$O$_3$ alone (0.15 mg/kg/day for 60 days) in nine patients (25).

The induction of apoptosis by As$_2$O$_3$ is likely mediated via the production of ROS that damage mitochondria (26, 27), because alterations in cellular antioxidant status modulate arsenic-induced cell death (28, 29). In addition to reducing ROS, which activates apoptosis. One such agent is AA. Although heralded as an antioxidant, AA also has prooxidant activities. In the plasma, it is rapidly oxidized to monohydroascorbyl and, further, to dehydroascorbic acid (DHA), which is then transported into the cell. AA is then regenerated from DHA via glutaredoxin, which converts GSH to GSH disulfide (33). This depletes intracellular GSH and increases cellular susceptibility to oxidative stress (34, 35). We have reported that AA-mediated GSH depletion augments As$_2$O$_3$-induced apoptosis in myeloma cells in vitro (10), and other groups have reported similar findings in in vitro and animal models of lymphoma and leukemia (29, 36, 37). Of particular relevance is the selective enhancement of As$_2$O$_3$-induced apoptosis in tumor cells (versus normal cells) by AA in vitro and, similarly, the lack of additional toxicity of As$_2$O$_3$ + AA compared with As$_2$O$_3$ alone in animal studies. The minimal toxicity of AA compares favorably with other GSH-depleting agents like BSO (38, 39, 40).

On the basis of these promising preclinical data, we initiated a NCI/CTEP-sponsored Phase I/II clinical trial of As$_2$O$_3$ and AA in relapsed and refractory MM. We now report the results of the Phase I component of this trial.

Material and Methods

Clinical Trial Design. This study (NCI 43/SCCC 20010) was designed as a combined Phase I/II trial. The goal of the Phase I component was to establish a dose of As$_2$O$_3$ (0.15 versus 0.25 mg/kg/day) that could be safely combined with AA (500 or 1000 mg/day). This dose would then be used in the Phase II component of the trial. Because the highest dose of As$_2$O$_3$ (0.25 mg/kg) tested has already been shown to be safe as a single agent (in APL studies), the primary objective of the Phase I component was to determine whether the addition of AA significantly changed the toxicity profile of As$_2$O$_3$ alone. The 1000-mg AA dose was predicted to give a plasma concentration of 100 μmol/liter, a level we found synergistic with As$_2$O$_3$ in vitro. Escalation of AA beyond 1000 mg is not likely to further increase efficacy because plasma ascorbate levels plateau at a dose between 500 to 1000 mg, and intracellular levels saturate at an even lower dose (41).

The Phase I study was designed only to examine specific dose levels and was not intended to establish the maximum tolerated dose (MTD). According to a “3 + 3” Phase I study design, a minimum of 6 patients (maximum of 18) were required to determine an As$_2$O$_3$ and AA dose level for which the incidence of DLT was less than 33%. DLT was defined as grade 3 or higher nonhematological toxicity or a grade 4 hematological toxicity occurring within the first course of treatment. Three patients were to be enrolled at the first dose level of 0.15 mg/kg/day As$_2$O$_3$ + 1000 mg/day AA. If no DLT were seen, three patients were to be enrolled at the next dose level of 0.25 mg/kg/day As$_2$O$_3$ + 1000 mg/day AA. The intermediate dose of As$_2$O$_3$, 0.20 mg/kg/day, and/or de-escalation of AA to 500 mg/day would occur only if more than 33% of patients treated at the highest doses of each agent experienced DLT.6

This trial was approved by the University of Miami Institutional Review Board and the CTEP, NCI. Informed consent was obtained from each subject.

Treatment. Patients were treated with As$_2$O$_3$ (provided by NCI/Division of Cancer Treatment and Diagnosis) as an i.v. infusion over 1–2 h followed by a 15-min i.v. infusion of AA (American Regent, Shirley, NY). Although AA can be given p.o., i.v. administration avoids the decreased gastrointestinal absorption that can be induced by As$_2$O$_3$ (diarrhea, mucositis, and so forth), yields higher peak-plasma levels, and allows for more accurate sequencing of AA-mediated GSH depletion with peak As$_2$O$_3$ levels. Treatment was administered on an outpatient basis except for the 1st week of the first cycle (to allow for initial toxicity assessment and pharmacokinetic studies). Both drugs were given Monday through Friday for 5 consecutive weeks followed by 2 weeks of rest (25 days of treatment over a 35-day time period constituted one cycle). Patients were allowed to receive a maximum of six cycles.

Patients were followed twice weekly for complete blood...
count with differential, electrolyte panel, calcium, magnesium, blood urea nitrogen, creatinine, glucose, total protein, albumin, hepatic transaminases, bilirubin, urine analysis. Serum K+ and Mg²⁺ levels were rigorously maintained above 3.8 and 1.8 mEq/liter, respectively. Twelve lead EKGs were done twice weekly to determine the QTc interval and monitor for arrhythmias or conduction defects. All of the toxicities were graded as defined by the NCI Common Toxicity Criteria (CTC v2.0). Additional studies for intracellular GSH, serum AA, and plasma elemental arsenic levels are detailed below. Serum protein electrophoresis and bone marrow biopsy were performed within 1 week of the completion of each cycle.

Disease response was defined as reported previously (16): CR was defined as the absence of detectable M protein and <5% plasma cells in the bone marrow. PR was defined as a >25% decrease in the amount of M protein or a 50% reduction in the plasma cells in the bone marrow. Responses were graded as a good PR if there was >50% reduction in M protein, or as a poor PR if there was <50% reduction. Stable disease was defined as a <25% change in M protein. A >25% increase in M protein was defined as progressive disease.

**Intracellular GSH Measurement.** GSH levels were determined as described previously (10) in PBMCs on day 1 (preinfusion and 1 h posttreatment), day 8, and day 33 (both postinfusion) of each cycle. GSH was measured in discontinuous density-gradient-purified PBMCs using the Calbiochem (San Diego, CA) GSH assay kit per the manufacturer’s instructions. Intracellular GSH was normalized to total cellular protein content (DC Protein Assay; Bio-Rad, Richmond, CA).

**AA and Elemental Arsenic Plasma Levels.** Serum AA levels were determined by capillary electrophoresis at the Mayo Clinic reference laboratory (Rochester, MN). Elemental arsenic blood levels were measured by mass spectrometry at Nichols Laboratory (San Juan, CA), with a lower limit of detection of 10 μg/liter. AA levels were measured on day 1 at 0, 1, 5, and 24 h postinfusion, on day 8, and on day 33. Elemental arsenic levels were measured on day 1 at 0 and 1, 5, and 24 h postinfusion, on day 8, and on day 33. Calculations of the serum concentration of the trioxide form of arsenic were done using the assumptions of Westervelt et al. (22), that the only source of measured plasma elemental arsenic is the administered As₂O₃ and that elemental arsenic is 76% of As₂O₃ by weight.

**In Vitro Sensitivity to As₂O₃ ± AA.** Patient bone marrow aspirates were obtained immediately before cycle 1 and within 7 days after the completion of each cycle. In vitro sensitivity of bone marrow mononuclear cells after 48-h exposure to As₂O₃, As₂O₃ + AA, or etoposide was determined and analyzed as described previously (10). Briefly, bone marrow mononuclear cells were isolated from aspirates by discontinuous density-gradient centrifugation. Cells (4 × 10⁶) were cultured in the presence or absence of As₂O₃ (2 μM) ± AA (100 μM) or of etoposide (10 μg/ml). After 48 h, cells were triple stained with a phycocerythrin-conjugated mouse antihuman CD38 antibody, a CyChrome-conjugated mouse antihuman CD45 antibody (both from Becton-Dickinson, San Jose, CA), and FITC-conjugated annexin V (Biovision, Mountain View, CA), or isotype-matched control antibodies. Plasma cells were identified on flow cytometry by their CD38high/CD45 low phenotype (6). Apoptotic cells were identified by annexin-V positivity. Flow cytometry was done on a FACSCan cytometer (Becton-Dickinson) using CellQuest software. Because there was patient-to-patient variation in the percentage of apoptotic myeloma cells in the untreated conditions (17.8 ± 7.5% (range, 6.1–31.5%)), the percentage apoptotic cells in the untreated conditions (background apoptosis) was subtracted from the drug-treated cultures results to generate the percentage apoptotic cells specifically attributable to treatment. Similar analysis was performed for apoptosis induced in normal bone marrow cells.

**Results**

**Patient Characteristics**

According to the escalation/de-escalation study design, six patients were required to achieve the primary objective of the Phase I component of this trial. All of the patients had failed or relapsed after multiple chemotherapy regimens, including three patients who had thalidomide-refractory disease. The median age was 54 (range, 40–65 years). All of the patients were stage IIIA (Durie-Salmon) with a mean β2-microglobulin of 4.78 mg/liter (range, 2.53–7.70). A total of three patients were treated at the 0.15-mg/kg/day As₂O₃ and 1000-mg/day AA dose level, and three patients were treated at the 0.25-mg/kg/day As₂O₃ and 1000-mg/day AA dose level. Patient demographics, disease characteristics, total dose of As₂O₃, and the number of cycles received are detailed in Table 1. A total of 21 cycles of As₂O₃ + AA were administered with a mean of 3.3 cycles/
Of note, four patients have exceeded the current recommended total As$_2$O$_3$ dose for APL.

**Plasma Elemental Arsenic Pharmacokinetics**

The pharmacokinetics of As$_2$O$_3$ were investigated at the 0.25 As$_2$O$_3$ mg/kg/day + 1000-mg/day AA dose level. Because there are no standard clinical assays for plasma As$_2$O$_3$ levels, we measured elemental arsenic plasma levels. The mean elemental arsenic levels on day 1/cycle 1 at 1, 5, and 24 h post-As$_2$O$_3$-infusion were 60.3 ± 19, 46 ± 12, and 47 ± 5.7 μg/liter, respectively (Fig. 1A). As indicated in Fig. 1, B and C, there is an accumulation of elemental arsenic in the blood as the treatment continues. On cycle 1/day 1, the mean elemental arsenic levels preinfusion (Fig. 1B) and 1 h postinfusion (Fig. 1C) were 68.3 ± 11.6 μg/liter and 119.5 ± 7.8 μg/liter, respectively, and on day 33, were 137 ± 36.8 μg/liter and 174.7 ± 28.7 μg/liter, respectively. After 2 weeks off treatment, the elemental arsenic levels were still detectable with mean preinfusion values of 40.5 ± 27.6 μg/liter on day 1/cycle 2, and 31.5 ± 12 μg/liter on day 1/cycle 3 (cycle 3 data not shown in Fig. 1). The mean postinfusion plasma elemental arsenic levels for cycle 2 and 3 were similar to those for cycle 1, with day-1/1-h values of 61 (cycle 2) and 81 ± 7 μg/liter (cycle 3), day-8 values of 133.5 ± 21.9 (cycle 2) and 115 ± 4 μg/liter (cycle 3), and day-33 values of 156 ± 46 (cycle 2) and 149.5 ± 6 μg/liter (cycle 3).

It is possible that any clinical effect of AA coadministration is mediated indirectly by effects on arsenic pharmacokinetics, as opposed to a direct effect of AA on intracellular GSH levels. In our study, the 1-h postinfusion elemental arsenic levels ($C_{1\text{-h postinfusion}}$) had a mean value of 60.3 μg/liter on day 1/cycle 1. A steady accumulation of elemental arsenic in the blood during treatment was observed, reaching a mean $C_{1\text{-h postinfusion}}$ of 174.7 μg/liter by day 33. The calculated mean $C_{1\text{-h postinfusion}}$ for the trioxide form of arsenic on days 1 and 33 are 79 μg/liter and 229 μg/liter, respectively, similar to values in the studies administering arsenic alone (14, 22). These findings suggest that the concomitant administration of AA does not significantly alter plasma levels of As$_2$O$_3$. 

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**Fig. 1** Elemental arsenic plasma levels. A, post-infusion elemental arsenic plasma levels on day 1/cycle 1. Blood samples were drawn at the times indicated and assayed for elemental arsenic. Arrows, As$_2$O$_3$ + AA infusions. B, preinfusion arsenic levels over cycle 1 and 2. Plateau elemental arsenic levels immediately preceding the infusion of As$_2$O$_3$ on days 1, 8, and 33 of each cycle. C, postinfusion arsenic levels over the first and second cycles. Plasma elemental arsenic levels were measured 1 h after the infusion of As$_2$O$_3$, as above.
The Effect of AA Administration on Intracellular GSH Levels

Although in vitro and animal studies demonstrate that AA can act as a chemosensitizer by depleting intracellular GSH, this ability (a central hypothesis of this trial) has never been clinically tested. Reliably measuring GSH changes in the myeloma cells themselves is not feasible with current assays, given the inadequate number of myeloma cells that can be purified from a bone marrow aspirate, the effect of the cell purification process itself on GSH levels, and the invasiveness of doing multiple bone marrow biopsies per cycle for serial assessment. However, previous studies using the GSH-depleting agent BSO have demonstrated that changes in the intracellular GSH levels in PBMCs paralleled changes in biopsied tumor cells (40), although this has not been specifically demonstrated for myeloma. We, therefore, measured intracellular GSH levels in PBMCs as a correlate to bone marrow myeloma cell GSH levels. Plasma AA levels were drawn simultaneously with the PBMCs for GSH assay.

All six of the patients received the 1000-mg AA dose. On day 1/cycle 1, the mean plasma AA levels at 0, 1, 5, and 24 h after the infusion were 49.8, 180, 66, and 46.5 μmol/liter, respectively. The mean AA level 1 h postinfusion on day 2 was 145.6 μmol/liter, on day 8 was 191.8 μmol/liter (62.5 before the infusion), and on day 33 was 145.2 μmol/liter. Increasing the As2O3 dose did not seem to affect AA pharmacokinetics, because there were no significant differences in AA C1-h postinfusion (P > 0.5 for all time points) in patients receiving the 0.15-mg/kg/day versus the 0.25-mg/kg/day As2O3 dose.

The effect of AA administration on intracellular GSH during the first three cycles is shown in Fig. 2. Overall, intracellular GSH decreased from day 1 to day 33 in 14 of the 19 cycles that we have complete data on. The mean percentage decline in GSH during cycle one; (from day 1 to day 33) was 13.1 ± 32.6%; during cycle two; was 60.7 ± 42.3%; and during cycle 3, was 64.1 ± 34%. The decline was most prominent when starting GSH levels were >25.5 nmol/mg total protein. Only patient 4 did not display this behavior. When GSH levels on day 1 were <25.5 nmol/mg total protein, increased AA levels had little effect on GSH through the rest of the cycle. Interestingly, GSH levels appeared to increase between cycles, significantly in some patients (patients 2, 5, and 6). However, these elevated GSH levels were substantially lowered by AA on day 8 and 33 of the subsequent treatment cycle.
As2O3 Phase II component of this trial (using the 0.25-mg/kg/day dose and typically developed in the 3rd week of a cycle. Grade 1–2 anemia was noted at both dose levels, but no transfusions were required at the completion of the first cycle. No thrombocytopenia was observed.

Nonhematological/Noncardiac Toxicity. Mild fatigue was seen in all of the patients treated. However, this did not seem to significantly impact the patients’ quality of life, and patients who were working before trial enrollment continued to do so during treatment. Mild-to-moderate nausea, dysgeusia with a metallic taste, and anorexia were the most common gastrointestinal toxicities. Reversible erectile dysfunction and loss of libido were observed in two patients receiving 0.25 mg/kg of As2O3. In both of these patients, serum testosterone levels were less than 60 mg/dl but returned to normal range after completion of the cycle. Mild-to-moderate sensory neuropathy was noted in two patients in each dosing group. Dry skin, pruritis, and diffuse maculopapular rash were the most frequent dermatological toxicities and were self-limited in the majority of the cases or were relieved with topical steroids. Patient 5 developed oliguric renal failure during his fourth cycle while receiving vancomycin for a nonneutropenic port infection. He subsequently required permanent hemodialysis and was taken off the study. Finally, patient 6 developed unilateral grade 2 sensorineural hearing loss during her sixth cycle of therapy.

Cardiac Toxicity. QTc prolongation has been consistently seen in the majority of APL patients treated with As2O3, with serious arrhythmias (particularly torsade de pointes) noted in some studies (21, 22, 42). Because the heart may be subjected to both acute and chronic toxicity from As2O3 + AA administration, we have followed each trial patient with twice-weekly EKGS for QTc prolongation (Fig. 3). Grade 1 QTc interval prolongation was observed in four patients. No dose reduction was necessary in any patients, and no treatment interruption was required. The QTc intervals returned to normal before the start of the next cycle, and there was no lengthening of baseline QTc intervals with cumulative cycles suggestive of chronic toxicity. The average QTc prolongation was 23.77 ± 2.95 ms for the 0.15-mg/kg/day group, and 35.79 ± 3.68 ms for the 0.25-mg/kg/day group. This compares favorably with the mean QTc prolongation of 63 ± 13 ms seen in APL patients treated with As2O3 alone (18). In 21 administered cycles, no episodes of supraventricular or ventricular arrhythmias were observed.

Serial Assessment of in Vitro As2O3 + AA Sensitivity

To determine whether resistance to arsenic ± AA developed over multiple cycles of treatment, we conducted serial in vitro assessments of patient bone marrow plasma cells apoptosis induced by As2O3 ± AA or by etoposide. Bone marrow was obtained before treatment and then after every cycle (we were unable to obtain bone marrow from patient 3). As seen in Fig. 4, primary plasma cells from all of the patients were initially sensitive to arsenic in vitro (20–40% apoptosis after 48 h). The sensitivity of plasma cells from patients 5 and 6 to As2O3 was decreased after cycle 1 but was restored by the addition of AA at 100 μmol/liter. Over subsequent cycles, we did not see

Table 2 Toxicity during the first cycle of treatment

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>% of patients in each treatment group of 3 patients that experienced the specific toxicity</th>
<th>As2O3 0.15 mg/kg + AA 1000 mg</th>
<th>As2O3 0.25 mg/kg + AA 1000 mg</th>
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<tbody>
<tr>
<td>General</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
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<td>100% (grade 1–2)</td>
<td>100% (grade 1–2)</td>
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<td>33% (grade 2)</td>
<td>33% (grade 2)</td>
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<tr>
<td>Peripheral edema</td>
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<td>33% (grade 2)</td>
<td>33% (grade 1)</td>
</tr>
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<td>33% (grade 1)</td>
<td>33% (grade 1)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>33% (grade 1)</td>
<td>66% (grade 1–2)</td>
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<td>0% (grade 2)</td>
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<tr>
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<td>66% (grade 1–2)</td>
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<tr>
<td>Prolonged QTc</td>
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<td>100% (grade 1)</td>
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<tr>
<td>Hematological</td>
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<tr>
<td>Anemia</td>
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<tr>
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<tr>
<td>Eye</td>
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<td>33% (grade 1)</td>
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</table>

* Toxicity is graded by the NCI Common Toxicity Criteria v2.0.

Toxicity

Compared with the experience with As2O3 alone in APL, factors that may have altered the toxicity profile in our trial included the addition of AA, the increased As2O3 dose (in the 0.25-mg/kg/day group), the schedule (25 days of treatment over a 35-day time period versus 60 consecutive days for APL), and the duration of treatment (a maximum of 150 total days of treatment versus 60 days for APL). All of the toxicities encountered for each dose group during the first cycle are shown in Table 2. No grade 3 or 4 nonhematological or grade 4 hematological toxicity occurred during cycle 1 in any of the patients. All of the toxicities resolved in the 2 weeks between cycles, except for the incomplete resolution of sensory neuropathy in patients 3, 4, and 5. However, these patients had preexisting sensory neuropathy secondary to prior therapy. Treatment was withheld during cycle 2 in patient 1 because of nonneutropenic sepsis from a surgical wound infection, which led to her death. We have subsequently treated eight additional subjects in the Phase II component of this trial (using the 0.25-mg/kg/day As2O3 + 1000 mg AA dose), and have found the same toxicity profile.7

7 N. J. Bahlis et al., unpublished observations.

Hematological Toxicity. In the first cycle, grade 2 leukopenia was observed in five patients and grade 3 leukopenia in one patient. This was more prevalent at the 0.25-mg/kg As2O3 dosage and typically developed in the 3rd week of a cycle.
further development of resistance to $\text{As}_2\text{O}_3 + \text{AA}$ by these *in vitro* assays.

Consistent with previous findings by our group and others (10, 26), normal bone marrow cells (CD38$^+$) were less sensitive than the myeloma cells to arsenic or the $\text{As}_2\text{O}_3 + \text{AA}$ treatment. For AA treatment alone, the mean percentage of apoptotic cells in the nonmalignant cell population was 1.10 to 3.43% (range, 5.27 to 6.23%), for arsenic treatment alone, the value was 5.96 to 7.78% (range, 3.1 to 27.95%), for $\text{As}_2\text{O}_3 + \text{AA}$, it was 12.75 to 12.29% (range, 3.18 to 37.28), and for etoposide, it was 7.70 to 8.37% (range, -1.6 to 25.43).

**Response and Current Status**

Although our Phase I trial was not designed to look at efficacy, the evaluation of response may give some indication of $\text{As}_2\text{O}_3 + \text{AA}$’s activity against refractory/relapsed myeloma. As seen in Table 3, two patients (patients 3 and 6) achieved major responses with 47.5 and 58% decreases, respectively, in their M protein. Neither had previously responded to thalidomide. Patient 3 had a decrease in M protein from 10.3 mg/dl to 5.6 mg/dl by cycle 3 but subsequently progressed after cycle 5 and was taken off the study. He did not receive further treatment and died 4 weeks after being taken off the study. Patient 6, after two cycles, had a decrease of M protein from 5.15 to 2.18 mg/dl, which remained stable at this level for her remaining four cycles, such that she completed the protocol (six cycles) without evidence of disease progression. Because she had residual disease, she is being maintained on thalidomide + dexamethasone.

Three other patients (patients 1, 4, and 5) had stable disease with less than 25% decrease in their M protein (~22%, +4%, and ~17%, respectively). Patient 1 developed nonneutropenic systemic candidiasis from an open surgical wound during her second cycle, which was the cause of her death. Patient 4 decided not to continue on the protocol after one cycle and was started on thalidomide. Patient 5 had his major decrease in M protein by cycle 2 with stable M proteins through cycle 4, but developed vancomycin-induced renal failure and was taken off the study. He had stable disease on thalidomide but suffered a fatal myocardial infarction (he had pre-existing coronary artery disease and had a bypass) 8 months after going off the study.

Patient 2 initially had light chain disease that later evolved into nonsecretory myeloma with 40% bone marrow plasma cells before trial enrollment. After three cycles of $\text{As}_2\text{O}_3 + \text{AA}$, the patient a stable degree of plasma cell infiltration in her bone marrow and no detectable urine light chains. The patient opted to go off study after her third cycle because of transportation problems and was started on pulse steroid therapy and thalidomide (200 mg). Her disease has subsequently progressed with a reappearance of urine light chains. She expired 3 months after she was taken off study while still on thalidomide.

**Discussion**

$\text{As}_2\text{O}_3$ alone has been shown *in vitro* and *in vivo* to be active against chemotherapy-resistant MM. We and others have demonstrated *in vitro* that AA can deplete intracellular GSH, increasing the efficacy of $\text{As}_2\text{O}_3$ by decreasing the cell’s ability to directly detoxify arsenic (via conjugation to GSH and export) and/or reducing the arsenic-generated ROS that cause cellular damage and induce apoptosis. These studies form the rationale for our NCI/CTEP Phase I/II trial using AA to modulate intracellular GSH and increase the efficacy of $\text{As}_2\text{O}_3$ in patients with refractory/relapsed MM. To our knowledge, this is the first clinical trial using AA as a GSH-depleting and chemosensitizing agent. The primary objective of the Phase I component of this study was to determine whether the addition of AA to $\text{As}_2\text{O}_3$ (at doses previously reported to have acceptable toxicity) significantly altered the acute and chronic toxicity profile reported for
As$_2$O$_3$ alone, and if so, how? Central to this objective were correlative studies determining whether coadministration of AA significantly changed the pharmacokinetics of As$_2$O$_3$ (compared with what has been reported for arsenic alone). The established acceptable dose of As$_2$O$_3$ + AA would then be used in the Phase II component of the trial. Because this Phase I component was designed to examine only two doses of arsenic and was not attempting to establish a maximally tolerated dose, this allowed us to enroll a small number of patients to achieve this objective.

Our second objective was to test the hypothesis that AA administration would deplete intracellular GSH in patients and to determine the characteristics of this depletion. The degree of GSH depletion may also correlate to toxicity and response. Our last objective was to initially assess the clinical efficacy of this approach.

### Table 3: Response and current status

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pretreatment</th>
<th>Best response (cycle achieved)</th>
<th>% change</th>
<th>Response</th>
<th>Current status</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP1</td>
<td>5,260</td>
<td>4,060 (2)</td>
<td>−22%</td>
<td>SD*</td>
<td>Expired (on-study, sepsis)</td>
</tr>
<tr>
<td>TP2</td>
<td>Nonsecretory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP3</td>
<td>10,300</td>
<td>5,400 (3)</td>
<td>−47.5%</td>
<td>PRb</td>
<td>Expired (on-study, relapsed MM)</td>
</tr>
<tr>
<td>TP4</td>
<td>8,480</td>
<td>8,840 (1)</td>
<td>+4%</td>
<td>SD</td>
<td>Off study (on thalidomide)</td>
</tr>
<tr>
<td>TP5</td>
<td>3,800</td>
<td>3,020 (4)</td>
<td>−22%</td>
<td>SD</td>
<td>Expired (off study, MI*)</td>
</tr>
<tr>
<td>TP6</td>
<td>5,150</td>
<td>2,180 (6)</td>
<td>−58%</td>
<td>PRb</td>
<td>Completed protocol (6 cycles) (on thalidomide + dexamethasone)</td>
</tr>
</tbody>
</table>

* SD, stable disease (<25% change in M protein); MI, myocardial infarction.

b Poor PR, 25–50% reduction in M protein; good PR, ≥50% reduction in M protein.

Fig. 4 Serial in vitro assessment of apoptosis induced in patient bone marrow plasma cells by As$_2$O$_3$ ± AA or by etoposide. Patient bone mononuclear cells were obtained at the time points indicated, and cultured in the absence or presence of As$_2$O$_3$ (As), As$_2$O$_3$ + AA (As+AA), or etoposide (Etop). The CD38$^{high}$/CD45$^{low}$/annexin V$^+$ phenotype was used to quantitate the degree of plasma cell apoptosis.
combination, and conversely to assess the development (and mechanisms) of As2O3 resistance in vitro.

Compared with the experience of As2O3 alone in APL, factors that may alter the toxicity profile in our myeloma trial include the disease itself, the addition of AA coadministration, increased As2O3 dosage (in the 0.25 mg/kg/day group), schedule, and duration of treatment. All of these factors may alter the plasma concentration of As2O3 and, thus, toxicity. To assess this, we determined arsenic pharmacokinetics in the 0.25-mg/kg/day As2O3 group. Our mean C1.h postinfusion As2O3 on days 1 and 33 were 79 µg/liter (0.4 µmol/liter) and 229 µg/liter (1.16 µmol/liter), respectively. Similarly, in APL patients, Shen et al. (14) reported day 1/cycle 1 C1.h postinfusion values ranging from 1.8 to 2.2 µmol/liter after a single 10-mg i.v. dose of As2O3 (0.14 mg/kg for a 70-kg patient). In contrast to our findings, this study did not demonstrate an accumulation of plasma arsenic by day 30/cycle 1. In another study, however, Westervelt et al. (22) recently reported that a dosing regimen of 0.10 mg/kg/day in APL patients resulted in barely detectable day 1/cycle 1 mean Cmax As2O3 levels of <0.26 µmol/liter, which rose to a mean Cmax of 0.95 µmol/liter by day 28, indicating an accumulation of blood arsenic over time. Thus our As2O3 plasma levels appear to be similar to those in previous studies, with the caveats that we used a higher dose but a “discontinuous” (weekdays only) schedule, and our small patient numbers may have limited the ability to discern even relatively large (50%) changes in drug disposition. Our findings are also similar to trials in solid tumors using As2O3 alone at doses from 0.15 to 0.30 mg/kg/day8 for both the mean day-1 postinfusion plasma levels and the accumulation of plasma arsenic over time. Together, these findings suggest that the concomitant administration of AA does not significantly alter the plasma pharmacokinetics of arsenic compared with the administration of As2O3 alone. Additional pharmacokinetic studies are ongoing in our Phase II trial.

We found that i.v. administration of 1000 mg of AA yielded a mean plasma level of 180 µmol/liter at 1 h, above the 100 µmol/liter concentration that depletes intracellular GSH in our in vitro studies (10). Correlative measurements in PBMCs demonstrated that AA administration can deplete intracellular GSH levels, most significantly when starting levels are >25.5 mmol/mg total protein. These results suggest that AA does not further deplete beyond a lower threshold (making an arsenic-sensitive cell more sensitive) but can deplete elevated GSH levels back to this threshold and maintain arsenic sensitivity. AA administration was also able to deplete the elevated GSH levels seen at the beginning of cycle 2 and/or cycle 3. The cause of this elevated GSH is currently being studied, but may represent a compensatory cellular response to GSH depletion during the previous cycle. It has been reported that GSH depletion by BSO induces γ-glutamylcysteine synthetase gene expression, the rate-limiting enzyme in GSH synthesis (43). Interestingly, the one patient who had high starting levels of GSH that were not affected by AA administration (patient 4) was also the least responsive to treatment. Because there was no clear evidence that pretreatment GSH levels predicted response in our patients, this suggests it is the degree of AA-mediated GSH depletion that may be the clinically relevant parameter. Further correlation of GSH depletion (e.g., a threshold value or percentage depletion) to specific toxicity and clinical response is being evaluated in the Phase II component of this trial.

The toxicities of As2O3 + AA at both the 0.15-mg/kg and 0.25-mg/kg As2O3 doses were largely limited and reversible. Compared with the reported toxicities of As2O3 alone (e.g., 0.15 mg/kg/day for APL), we did not see evidence of increased toxicity attributable to AA coadministration. Only erectile impotence appears to be unique to our trial. Consistent with the APL studies, the major toxicity was fatigue, although this was generally mild. No grade 3 or 4 nonhematological toxicity was observed in any patient. Grade 3 myelosuppression (asymptomatic) was seen in only one patient at the end of the first cycle. Interestingly, in our trial some of the toxicities reported in APL patients were either absent [dyspnea, chest pain, hyperglycemia, liver dysfunction, ventricular arrhythmias, leukocytosis, and retinoic acid syndrome (these last two may be unique to APL)] or diminished in incidence and/or severity (fluid retention/edema, QTc prolongation, fever, headache, abdominal pain, and cough).

Cardiac arrhythmia and sudden death were recently reported in two small studies of As2O3 alone in APL patients (21, 22) but not in a larger multicenter trial (17). We have not observed any arrhythmias to date. Asymptomatic QTc prolongation (all <500 ms) was seen in four patients and did not require dose modification or treatment interruption. Rigorous efforts to avoid hypokalemia and hypomagnesemia may have contributed to the absence of cardiac arrhythmias detected in our study.

With a median follow-up of 6.6 months (2–12 months), the only evidence of chronic/cumulative toxicity in the four patients who have received three or more cycles has been one case of mild sensineural hearing loss. This has been reported as an uncommon toxicity of the medical use of As2O3 (22) and is more frequently associated with chronic environmental exposure (44). Three of these patients have received >1200 mg total dose of As2O3 (versus ~600 mg recommended total dose for APL). In particular, we have not seen clinical or EKG evidence of cumulative cardiac toxicity. Together, these findings indicate that coadministration of AA does not increase the toxicity of As2O3 and that As2O3 + AA is well tolerated. Clearly, longer follow-up is needed to determine any serious chronic toxicity of As2O3 + AA, although the lack of evidence for this in the patients who have received >1200 mg of arsenic is promising.

To assess potential correlates and mechanisms of response or resistance to As2O3, we serially examined the in vitro sensitivity of patient bone marrow plasma cells to As2O3, As2O3 + AA, or etoposide (as an unrelated control). In all six of the pre-cycle-1 samples, the addition of AA did not substantially enhance As2O3-induced apoptosis. However, in the four patients who demonstrated decreased plasma cell sensitivity to As2O3 after cycle 1 (however slight), the addition of AA restored As2O3-induced apoptosis to (or above) pretreatment levels. In comparison, the one patient whose myeloma cells remained sensitive to arsenic (patient 2) continued to have relatively little augmentation by AA. If arsenic resistance is mediated primarily through increased intracellular GSH, these findings would sug-

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8 S. Soignet, personal communication.
gest that a major clinical benefit of AA is preventing this resistance. By these in vitro assays, we did not see evidence for the development of arsenic resistance at the myeloma cell level over multiple courses of treatment. This is largely consistent with our clinic responses, in which only one of five patients receiving more than one cycle relapsed while receiving As$_2$O$_3$ + AA (patient 3, receiving the 0.15-mg/kg/day As$_2$O$_3$ dose). Unfortunately, we were unable to obtain marrow samples from this patient at the time of relapse to assess the nature of the resistance.

Overall, two of six patients (both with thalidomide-refractory disease) achieved a PR, and four patients had stable disease. These results suggest that As$_2$O$_3$ + AA has activity in heavily pretreated myeloma patients and, of note, in patients with thalidomide-resistant disease. Interestingly, three of the four patients who had an initial decrease in M protein maintained this decrease as long as they were on treatment. The fact that the patients who had an initial decrease in M protein maintained this decrease as long as they were on treatment. The fact that the disease response plateaued indicates that an equilibrium between arsenic response and resistance was established. Because our in vitro assays did not find evidence that the myeloma cells themselves became resistant, this suggests that host factors may be involved. Because arsenic pharmacokinetics did not vary significantly between cycles (suggestive of changes in metabolism/excretion), factors such as bone marrow stroma:myeloma interaction may play an important role.

In conclusion, the combination of As$_2$O$_3$ (0.25 mg/kg/d) and AA (1000 mg/d) appears to have acceptable toxicity with promising activity in this cohort of patients. We did not find that AA coadministration altered arsenic pharmacokinetics or increased toxicity, even though it clearly modulated intracellular GSH levels. Whether AA coadministration actually protects against some arsenic-induced toxicities may be ascertainable when results from the ongoing trials of arsenic alone in MM are reported. The ongoing Phase II component of this study will establish biochemical and cellular correlates of efficacy, toxicity, and the development of arsenic resistance.

References


Feasibility and Correlates of Arsenic Trioxide Combined with Ascorbic Acid-mediated Depletion of Intracellular Glutathione for the Treatment of Relapsed/Refractory Multiple Myeloma


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