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Arsenic Trioxide – An Old Drug Rediscovered

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Abstract

Over the last 17 years, clinical trials conducted worldwide have demonstrated the efficacy of arsenic trioxide (As_2O_3) in the treatment of relapsed acute promyelocytic leukemia (APL). Currently, the role of As_2O_3 in front-line therapy is under investigation. Recent trials in the US have demonstrated that the addition of As_2O_3 to standard treatment regimens improves survival outcomes in patients with APL and may allow a reduction in cytotoxic chemotherapy exposure. As_2O_3 has also shown efficacy in other malignancies, particularly multiple myeloma and myelodysplastic syndromes. Therapeutic doses of As_2O_3 are well tolerated, with no evidence of long-term toxicity. Adverse events include APL differentiation syndrome, electrocardiographic abnormalities, and mild elevations in liver enzymes. This review highlights trials investigating the role of As_2O_3 in induction and consolidation for newly diagnosed APL, as well as its role in other hematologic malignancies. The chemistry, mechanisms of action, and clinical side effects of As_2O_3 are also discussed.

Keywords

Arsenic trioxide (As_2O_3); Acute promyelocytic leukemia (APL); Differentiation; Apoptosis; Reactive oxygen species; Induction and consolidation chemotherapy; Multiple myeloma; T-cell lymphotropic virus type I (HTLV-I)-associated adult T-cell leukemia-lymphoma (ATL); Myelodysplastic syndromes (MDS)

Introduction

Rediscovery of an old drug

Arsenic has been used in medicine for more than 2400 years for a variety of ailments including ulcers, the plague, and malaria.[1] In 1878, potassium arsenite was reported to have an anti-leukemic effect and was used for this purpose in the late 19th and early 20th centuries until it was replaced by busulfan in the 1950s.[2–4]

In the modern era, interest in arsenic as a chemotherapy was rekindled after it was identified as an active ingredient in traditional medicines in China.[5] Researchers evaluated arsenic compounds for the treatment of various cancers and in 1992 published the results of a trial in which intravenous administration of arsenic trioxide (As_2O_3) produced a complete response

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Dr. Gore: Received As_2O_3 from Cephalon, Inc., as part of a phase 2 trial of As_2O_3 in consolidation therapy of APL.
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(CR) in 21 (66%) of 32 patients with acute promyelocytic leukemia (APL).[5,6] In two subsequent studies, Zhang et al reported that As_2O_3 induced a CR in 22 (73%) of 30 newly diagnosed and 22 (52%) of 42 relapsed APL patients,[5,7] and Shen et al. observed a CR in nine (90%) of 10 relapsed APL patients.[8]

As_2O_3 for relapsed APL

Based on the results from the Chinese studies, a US pilot study was conducted in patients who had relapsed after one or more courses of all-*trans*-retinoic acid (ATRA) and anthracycline-based chemotherapy.[9] Most patients had experienced multiple relapses and 58% were resistant to retinoid therapy. Eleven of 12 patients achieved a CR with As_2O_3 alone. Eight of these 11 responders also achieved a molecular remission, as defined by the absence of detectable PML-RARα fusion gene expression.

Following the success of the pilot study, a larger, multicenter, single-arm trial was conducted in patients who had relapsed after ATRA-based therapy. More than one third of these patients had multiple relapses and were heavily pretreated (including five patients with prior BMT). CR was achieved in 34 (85%) of 40 patients (Table 1).[10] All patients who achieved a CR also showed evidence of elimination of the t(15;17), as measured either directly by traditional cytogenetics or by assays using FISH or RT-PCR for PML/RAR-α. Many of the remissions were durable. The estimated two-year overall survival (OS) and relapse-free survival (RFS) rates for patients in first relapse were 77% and 58%, respectively. These results are somewhat confounded because eleven patients underwent bone marrow transplant in remission after induction or consolidation with As_2O_3. Among the 27 patients who did not receive transplant, one-third remained alive with duration of remission ranging 22 to 42 months. This pivotal trial provided support for the approval of As_2O_3 in patients with APL who failed to respond to or relapsed following ATRA/anthracycline therapy.

Mechanisms of action

As_2O_3 affects multiple cellular functions via different molecular targets (summarized in Fig. 1). Although the fundamental mechanism is the favorable chemical reaction between arsenic and thiol groups within a protein, the final outcome depends on the cell type as well as the dose and duration of arsenite exposure. For example, in APL cells, As_2O_3 at low concentrations (<0.5 μM) induces differentiation; at higher concentrations (0.5–2.0 μM) it causes apoptosis.[14,15]

Chemistry of As_2O_3

Trivalent arsenic (As^{III}) in As_2O_3 or arsenite ($As[OH]_3$) and pentavalent arsenic (As^V) in arsenate ($HAsO_4^{2-}$) are the two biologically significant forms of arsenic. Although As^V disrupts cellular processes as a phosphate (HPO_4^{2-}) mimic, the interaction of As^{III} with the thiol (or sulfhydryl) groups (-SH) of proteins with a high cysteine content is the basic reaction that underlies the multiple mechanisms of action of this chemotherapeutic agent.[16] In this reaction, the valence orbitals of arsenic (As) have a better overlap and energy match with those of sulfur (S) than with those of oxygen (O), leading to the formation of an As^{III}-thiolate bond and the release of water, as demonstrated in the following equation[17]:

$$As^{III} - OH + RSH \leftrightarrows As^{III} - SR + H_2O$$

Vicinal thiols (i.e., thiols bonded to adjacent carbon atoms) have a high affinity for As^{III} (Fig. 2). Thus, proteins that contain cysteine residues that are conformationally constrained and favorably positioned will have a higher affinity for As^{III}.[18]

Stimulation of differentiation

Empirically, treatment of APL cells with As_2O_3 leads to their terminal differentiation *in vitro* and *in vivo*. APL cells are uniquely sensitive to As_2O_3 due to the expression of the PML-RARα fusion protein; however, the mechanism by which arsenic trioxide treatment induces terminal differentiation remains somewhat speculative. In normal myeloid cells, PML protein is localized to macromolecular structures in the nucleus (nuclear bodies), where PML antagonizes many processes required for the initiation and progression of malignancy.[19] In leukemic cells, the PML-RARα fusion protein blocks the expression of genes required for normal differentiation. The fusion protein disrupts the nuclear bodies, and the PML protein is dispersed into smaller organelles.20 PML contains a cysteine-rich region that is hypothesized to interact with As^{III}, resulting in the degradation of PML-RARα fusion protein.[21] Furthermore, As_2O_3-induced histone acetylation has been reported to promote differentiation via alteration in gene transcription.[22]

Induction of apoptosis

Caspases are intracellular cysteine proteases that are key components in classic apoptosis. Caspase activation occurs in response to various types of mitochondrial damage and proapoptotic stimuli, which cause cytochrome c (normally sequestered between the mitochondrial inner and outer membranes) to be released into the cytosol, where it binds and activates Apaf-1, which in turn activates procaspase-9.[23–25] Caspase-9 cleaves procaspase-3, and downstream of caspase-3, the apoptotic program branches into a multitude of subprograms, the sum of which results in the ordered dismantling and removal of the cell.26

As_2O_3 induces dose-dependent apoptosis in APL and hematopoietic non-APL as well as tumor and non-malignant cell lines.[27,28] The activation of the caspase cascade, the decrease of the mitochondrial membrane potential $\Delta\Psi_m$, and the production of reactive oxygen species (ROS) all play roles in induction of As_2O_3-induced apoptosis.[29] As_2O_3 also promotes apoptosis via down-regulation of Bcl-2 expression30 by prolongation of the cell cycle and cell cycle arrest of malignant cells31,[32] and by opening the permeability transition pore complex (PTPC) in mitochondrial membranes by directly binding to thiol groups in the PTPC.[33]

Accumulation of ROS

ROS are damaging to DNA, RNA, proteins, and lipids, and include free radicals such as hydroxyl ($OH^{\bullet}$) or superoxide ($O_2^{-}\bullet$) and molecules such as hydrogen peroxide (H_2O_2). Mutations in nuclear or mitochondrial genes encoding components of the mitochondrial electron transport chain can lead to the accumulation of electrons along the chain. These electrons are captured by O_2 to form superoxide, which is converted to hydrogen peroxide. The protective buffering systems against ROS include glutathione (GSH), thioredoxin, superoxide dismutase, catalase, and glutathione peroxidase.[34]

As_2O_3 can increase cellular levels of ROS via several targets. It can prevent ROS detoxification by inhibiting antioxidant enzymes like glutathione peroxidase, which has a thiol group required for its activation. The level of intracellular GSH, which titrates arsenic by forming $As(GS)_3$ complexes, as well as the activities of related antioxidant enzymes, are major factors for sensitivity to As_2O_3. The sensitivity of APL to As_2O_3-induced apoptosis is closely related to its relatively poor antioxidant capacity.[35] Furthermore, proteins with high sulfhydryl-disulfide oxidation potentials may be particularly susceptible to redox regulation, and the affinity of As_2O_3 for sulfhydryl groups on these redox-sensitive proteins explains many of its ROS-related effects.[36] Finally, through the use of gene expression profiling, interference RNA, and genetically engineered cells, Chou and colleagues reported that

NADPH oxidase, an enzyme complex required for the normal antibacterial function of white blood cells, is a major target of arsenic-induced ROS production.[37]

Inhibition of NFκB

NFκB is a transcriptional factor promoting cell survival with an important role in many cancer cells. Activation of NFκB depends on the integrity of the IκB kinase (IKK); upon phosphorylation by IKK, the inhibitory protein IκB releases NFκB for translocation to the nucleus. As_2O_3 has been shown to inhibit IKK by binding to cysteine-179 in the activation loop of the enzyme catalytic subunit. Although cysteine-179 is not located in the vicinity of another cysteine within the IKK primary structure, it has been suggested that another cysteine may come within a critical distance of cysteine-179 upon the folding of the polypeptide chain or the dimerization of the catalytic subunits, thus providing a high-affinity target for arsenite.[38]

As_2O_3 selectivity for APL cells

To understand the selective cytotoxicity of As_2O_3 against human APL, Dilda et al. screened the yeast deletion strains for sensitivity or resistance to the drug.[39] A prominent sensitive mutant was missing Hog1, a MAP kinase. The most resistant mutant lacked the plasma-membrane glycerol and arsenite transporter, Fps1. Hog1 and Fps1 control the response to osmotic stress in yeast through regulation of glycerol production and plasma membrane flux, respectively. Based on these results, the investigators tested the APL cell line NB4 for impaired osmoregulation and found that the APL cells did not produce glycerol in response to osmotic stress and underwent apoptotic cell death. Moreover, the glycerol content of NB4 and differentiated NB4 cells correlated with the level of As_2O_3 uptake and the sensitivity of the cells. Additionally, NB4 cells accumulated more As_2O_3 than did non-APL cells and were generally more sensitive to the drug. The investigators concluded that the selectivity of As_2O_3 for APL cells relates, at least in part, to impaired osmoregulation and control of drug uptake.

Efficacy of As_2O_3 in newly diagnosed APL

The standard of care for newly diagnosed APL is differentiation therapy with ATRA plus anthracycline-based chemotherapy.[40] This combination results in high response rates and prolonged survival (Table 1).[11–13] Incorporating As_2O_3 into the initial treatment of APL may further reduce the relapse rate and provide a more tolerable treatment option for patients who are not candidates for chemotherapy.

As_2O_3 as induction therapy

As_2O_3 has demonstrated clinical activity in patients with newly diagnosed APL, producing CR in 83–86% of patients and 3-year OS rates of 79–86% (Table 2).[41,42] Demonstrated efficacy, combined with evidence of synergy between As_2O_3 and ATRA, has fueled interest in combination therapy. A small randomized study in China evaluated the efficacy and safety of induction with ATRA, As_2O_3, or ATRA plus As_2O_3, followed by consolidation with three courses of conventional chemotherapy (Table 2).[43] Response rates were similar between treatment groups; however, relapse was significantly less frequent in patients receiving combination therapy as compared to either agent alone ($P = .038$). This improved response translated into a 2-year disease-free survival (DFS) advantage. After completion of the randomized phase of the study, the ATRA-plus-As_2O_3 arm remained open and long-term efficacy and safety data were collected (Table 2).[44] Among 80 patients who achieved CR, four (5%) had relapsed after a median follow-up of 70 months; all four were central nervous system (CNS) relapses.

A US phase 2 trial examined whether combination therapy with ATRA and As_2O_3 could replace anthracycline-based chemotherapy for newly diagnosed APL.[45] Low-risk patients (white blood cell [WBC] count $<10 \times 10^9$/L) received ATRA plus As_2O_3 as induction and post-remission therapy; high-risk patients (WBC count $\geq 10 \times 10^9$/L) also received a single dose of gemtuzumab ozogamicin. It was hypothesized that this approach might be advantageous for patients who are unable to tolerate conventional chemotherapy, particularly elderly patients. Outcomes were similar to those observed with standard ATRA and chemotherapy combinations (Table 2). The overall response rate was 92% and responses were durable. After a median follow-up period of 23 months, relapse was documented in three (4%) of 75 responders, all of whom had high-risk disease. In patients aged 60 years and older, a CR rate of 83% was observed, compared with 95% in patients younger than 60 years ($P = .17$).

As_2O_3 in consolidation

The only phase 3 trial of As_2O_3 (the North American Intergroup protocol C9710, presented to date only in abstract form) evaluated the addition of As_2O_3 in first CR prior to standard consolidation therapy for newly diagnosed 537 eligible adults and pediatrics patients with APL.[46] This study demonstrated that administration of As_2O_3 (0.15 mg/kg/d for 5 days each week for 5 week for two cycles, cycle 2 after two weeks rest), as the first consolidation, prior to subsequent consolidation with ATRA (45 mg/m^2 × 7d) and chemotherapy (daunorubicin 50 mg/m^2 × 3d; 2d for age < 15 yr) significantly improved event-free survival (EFS) (81% vs 66%, p=0.0007) in adults compared to consolidation with ATRA and chemotherapy only. Three-year OS was higher in the As_2O_3 group, albeit not statistically significant (86% vs 79%, p=0.063). These improvements were presumably due to a decrease in the relapse rate, although DFS has not yet been reported. It is noteworthy to mention that in this study patients who did not receive As_2O_3 appear to have had lower EFS and OS than historical controls treated with ATRA and chemotherapy; indeed, the survival rate in the As2O3 arm was similar to the best published data using ATRA plus chemotherapy. Full analysis of this critical study will require publication of the final manuscript.

Based on the preliminary report, there was remarkably no significant difference in DFS between patients with WBC count greater or less than 10,000/mL in As_2O_3 group. On the other hand, patients with WBC > 10,000/mL, who did not receive As_2O_3 had significantly worse DFS compared with patients with WBC < 10,000/mL (p=0.0016). This finding suggests a major advantage of As_2O_3-based consolidation compared to non-As_2O_3 containing regimens in which patients with high WBC are much more likely to relapse. There were no differences in grade 3 or 4 hematologic or non-hematologic toxicities between the two groups.

A recent phase 2 trial in the US assessed whether the incorporation As_2O_3 into consolidation therapy would allow a reduction in chemotherapy exposure without compromising patient outcomes.[47] Enrolled patients (45 analyzed) received a single consolidation cycle with As_2O_3 (0.15 mg/kg/day, Monday – Friday, beginning on day 8, for 30 doses), daunorubicin (60 mg/m^2/day days 1–3), and cytarabine (0.667 mg/m^2/day continuous infusion days 1–3) after achieving CR with ATRA plus chemotherapy. Survival outcomes (EFS, DFS, and OS) were comparable to other treatment regimens that included more extensive chemotherapy, including the As_2O_3 treatment arm of the C9710 phase 3 trial (Table 2). Of 37 patients who received consolidation therapy, only one (3%) patient suffered a relapse (in the CNS) after a median follow-up of 1.8 years. Thus, OS was 88% ± 5% and leukemia-free survival was 90 ± 6%. Secondary myelodysplastic syndromes (MDS) or acute myeloid leukemia (AML) were not reported, although the longest follow-up in this study is only 5.5 years and median follow-up 2.7 years. These results indicate that a reduction in anthracycline exposure and associated toxicities may be possible while maintaining a low incidence of relapse.

Based on the current data from the As_2O_3-based clinical trials, which indicate that first-line arsenic trioxide therapy markedly decreases the incidence of relapse in de novo APL, and high DFS can be achieved with much lower doses of anthracycline than currently being used, we strongly recommend the routine incorporation of As_2O_3 into the first-line consolidation therapy of de novo APL.

As_2O_3 in maintenance

In the study by Soignet et al [10], those patients who remained in CR after receiving their consolidation course of As_2O_3 were given the option to receive up to four additional cycles of As_2O_3 therapy on a dose schedule similar to consolidation on a separate protocol. Eighteen patients received additional As_2O_3 as maintenance. Nevertheless, to date there is no published randomized trial evaluating the effect of As_2O_3 in maintenance setting. Meanwhile, until further studies are designed and completed, maintenance therapy for 1 to 2 years with intermittent ATRA with or without 6-mercaptopurine and methotrexate is recommended by us and many others, particularly in patients with high-risk disease.

Efficacy of As_2O_3 in other malignancies

Multiple myeloma (MM)

In preclinical studies, As_2O_3 inhibited the proliferation of MM cell lines at pharmacological (micromolar) concentrations.[31,48] As_2O_3 treatment of cultured bone marrow mononuclear cells from MM patients selectively induced apoptosis in myeloma cells while sparing most myeloid cells.[48] Initial clinical studies of As_2O_3 as a single agent in heavily pretreated relapsed or refractory patients demonstrated a ≥25% reduction in serum levels of M-protein in 21–33% of patients, with durations of response ranging from 1 to 74+ weeks.[49–51] Disease stabilization occurred in an additional 7–40% of patients. This modest clinical efficacy of As_2O_3 as a single agent prompted trials in combination with other therapies.

As discussed in the mechanism of action section, sensitivity to As_2O_3 *in vitro* is inversely proportional to cellular levels of the antioxidant GSH, which can attenuate the effects of increased ROS.[35] Pharmacological depletion of GSH by ascorbic acid (AA) enhances the cytotoxic effects of As_2O_3 in MM cell lines, providing a rationale for combination therapy.[52] A phase 1 trial demonstrated that As_2O_3 plus AA was tolerable and demonstrated potential clinical activity (two of six patients had partial responses [PR]).[53] Further clinical studies are necessary to determine the efficacy of this combination, particularly in light of more recent *in vitro* evidence suggesting that AA may protect cells against arsenic cytotoxicity.[54]

Several trials have evaluated As_2O_3 in combination with existing MM therapies, including melphalan, dexamethasone, and bortezomib, in relapsed patients. In a small study of 10 patients treated with As_2O_3, ascorbic acid, and melphalan (MAC), a response (≥25% reduction in serum M-protein) was observed in all patients, with treatment durations of 13–104 weeks.55 Notably, six of these 10 patients had received melphalan previously: four as conditioning for stem cell transplantation (SCT) and two as part of a melphalan-prednisone regimen during which disease progression occurred. In a subsequent phase 2 study, MAC treatment produced a response in 31 (48%) of 65 patients who had failed at least two prior regimens; two CR, 15 PR, and 14 minor responses were observed.56 Median progression-free survival (PFS) was 7 months and median OS was 19 months. In another randomized phase 2 trial, the addition of As_2O_3 to AA and high-dose melphalan was safe and well tolerated as a preparative regimen for autologous hematopoietic stem cell transplantation.[57] This study was not powered to detect differences in efficacy between arms; overall, a response was observed in 41 (85%) of 48 patients including 12 (25%) CR, with a median PFS of 25 months. The combination of As_2O_3, AA, and dexamethasone has demonstrated

efficacy in patients with relapsed or refractory MM in two phase 2 trials. In a US study, six (30%) of 20 patients achieved a PR, including two near-CR (>90% decrease in M protein); 80% of patients demonstrated at least stable disease.[58] Similarly, a European trial showed a response in eight (40%) of 20 patients, including two PR.59 Median PFS was 4 months and median OS was 11 months. The combination of As_2O_3, AA, and the proteasome inhibitor bortezomib was examined in a phase 2 trial for relapsed MM patients, following a report of *in vitro* synergy.60,[61] Six (27%) of 22 patients showed a clinical response to the combination, including two PR; an additional nine (41%) patients had stable disease. Median PFS was 5 months, with 1-year PFS and OS rates of 34% and 74%, respectively. The specific contribution of As_2O_3 to the efficacy of these combination therapies cannot be isolated without randomized trials.

Myelodysplastic syndromes (MDS)

Several *in vitro* studies have demonstrated apoptosis in MDS cells exposed to As_2O_3.[27,62,63] MDS cells are under increased oxidative stress,[64] which may confer As_2O_3 sensitivity. In two phase 2 clinical studies involving 191 patients, single-agent As_2O_3 was associated with hematologic improvement in 26–34% of patients with lower-risk (low or intermediate-1 international prognostic scoring system [IPSS] risk groups) MDS and 6–17% of those with higher-risk (intermediate-2 or high IPSS risk groups) MDS.65,66 Schiller et al. observed one (3%) CR and Vey et al. observed one (<1%) CR and one (<1%) PR in higher-risk patients. Major responses were observed in all hematologic lineages in both trials.

As_2O_3 plus thalidomide was investigated based on the hypothesis that this combination would target both the MDS clone and the bone marrow microenvironment. This combination was evaluated in 28 patients with MDS (12 lower-risk, 16 higher-risk).[67] Seven patients (25%) responded, including one CR and two trilineage responses. Three of five patients with high baseline levels of the poor prognostic marker EVI1 responded to the combination; this observation was supported by experiments *in vitro*, which demonstrated greater As_2O_3 sensitivity in cells expressing high levels of EVI1. A subsequent trial of As_2O_3, thalidomide, and retinoic acid in 21 higher-risk patients demonstrated responses in 10 patients (48%), including one CR and one PR.[68]

Other hematologic malignancies

Several trials have demonstrated promising results in AML and human T-cell lymphotropic virus type I (HTLV-I)-associated adult T-cell leukemia-lymphoma (ATL). A phase 1/2 trial examined As_2O_3 in combination with low-dose cytarabine in 61 previously untreated AML patients aged 60 years or older.[69] A CR was achieved in 21 patients (34%), including 15 (30%) of 50 patients with secondary AML. Median survival was 5.2 months, with a median follow-up of 9.8 months. A randomized phase 3 trial of this combination versus low-dose cytarabine alone is under way (www.clinicaltrials.gov). For relapsed/refractory ATL patients, As_2O_3 in combination with interferon alpha (IFNα) was examined in a small (N = 7) phase 2 trial.[70] Despite significant toxicity that prevented completion of therapy, one patient achieved a CR lasting more than 32 months, and three patients achieved transient PR (median duration 1 month). In a separate report involving four patients with relapsed/refractory ATL, As_2O_3 plus IFNα produced one PR (duration of 8 months) and one stable disease, while As_2O_3 alone produced no response.[71] In a recent phase 2 trial of As_2O_3 and IFNα plus zidovudine in 10 newly diagnosed ATL patients, a 100% response rate was observed, including seven CR.[72] Most toxicities were grade 1 or 2; grade 3 neutropenia occurred in 3 patients and grade 3 thrombocytopenia was observed in 2 patients. As_2O_3 has demonstrated limited activity in non-Hodgkin lymphoma and chronic lymphocytic leukemia,[73] and no clinical benefit in patients with acute lymphoblastic leukemia.[74]

Solid tumors

As_2O_3 is under investigation as treatment for a variety of solid tumors including bladder cancer, glioma, breast cancer, hepatocellular carcinoma (HCC), cervical cancer, colorectal cancer, esophageal cancer, germ cell tumors, liver cancer, lung cancer, and melanoma (www.clinicaltrials.gov). Limited clinical activity as a single agent has been reported in a small number of patients with HCC,[75] melanoma,[76,77] and renal cell carcinoma[78]; As_2O_3 in combination with chemotherapy has shown promising activity in osteosarcoma and Ewing sarcoma.[79]

Safety and toxicity

Arsenic is well known as a toxic agent. Inorganic arsenic has been classified by the US Department of Health and Human Services, the International Agency for Research on Cancer, and the US Environmental Protection Agency as a known carcinogen. Chronic exposure to low levels of environmental arsenic has been reported to increase the incidence of skin, liver, bladder, and lung cancers.[80] Other potential signs of arsenic poisoning include peripheral neuropathy, cardiomyopathy, and renal failure.[81]

Despite its reputation as a poison, as a therapeutic entity As_2O_3 has been generally well tolerated. When administered intravenously at a dosage of 0.15 mg/kg/day, leukocytosis, gastrointestinal disorders (e.g., nausea, vomiting, diarrhea), fatigue, fever, headache, cough, and dyspnea are commonly observed. Common potentially serious toxicities include APL differentiation syndrome (APLDS) and electrocardiogram (ECG) abnormalities.[82]

APL differentiation syndrome

Previously known as “retinoic-acid syndrome,” APLDS may present during remission induction with ATRA or As_2O_3 therapies as a complex of signs and symptoms, including fever, dyspnea, hypotension, weight gain, acute renal failure, and lung infiltrates, and is usually treated with high-dose corticosteroids.[83] In the pivotal trial of As_2O_3 in APL, APLDS occurred in 10 (25%) patients; in three of these patients, APLDS was considered to be serious.[10] Therapy with As_2O_3 was briefly interrupted (for 1–5 days) in eight patients. Notably, all patients affected by APLDS achieved a CR. A similar incidence and severity of APLDS was reported with combined ATRA and As_2O_3; in the M. D. Anderson phase 2 trial, 13 patients (16%) developed APLDS, and all cases were successfully managed by withholding ATRA and administering corticosteroids.[45] In a study comparing As_2O_3, ATRA, and the combination, the incidence of APLDS-associated hyperleukocytosis was not increased with As_2O_3 plus ATRA; however, one death in the combination arm was attributed to APLDS.[43,44]

Cardiac events

ECG abnormalities, including prolonged QT interval and complete atrioventricular block, have been reported with As_2O_3 treatment. QT prolongation (defined as ≥450 msec for males and ≥470 msec for females) was seen in 63% of patients in the pivotal trial and led to temporary discontinuation of As_2O_3 therapy in one patient (3%).[10] In the phase 2 study by Ravandi et al., As_2O_3 was discontinued in five patients (6%) due to adverse cardiac events including atrial arrhythmias and myocardial infarction.[45] ECG and electrolyte monitoring is recommended prior to and during arsenic therapy. Serum potassium levels should be kept above 4 meq/L and magnesium concentrations above 1.8 mg/dL.

Liver and kidney impairment

No significant hepatotoxicity was reported in the pivotal trial in relapsed patients.[10] Hu et al. reported transient grade 1 or 2 liver dysfunction in 75% of patients during induction with

ATRA and As_2O_3; no grade 3 or 4 toxicity was observed, and treatment was not discontinued in any patient.44 Ravandi et al. noted grade 3 elevations in liver enzymes in two of 82 patients (2%), but treatment was not discontinued.45 Grade 3 renal failure occurred in one (3%) patient during the pivotal trial10 and was reported in four (5%) patients by Ravandi et al.45

Secondary malignancies

Carcinogenesis is a major concern associated with long-term exposure to arsenic. In the study published in December 2008 in the Proceedings of the National Academy of Sciences (PNAS) on long-term efficacy and safety of ATRA/As_2O_3-based therapy in newly diagnosed APL patients who started being accrued to the trial in 2001[44], no secondary carcinoma, including skin cancer, was observed. One male transiently tested positive for carcinoembryogenic antigen (CEA), and a mild, unsustained increase in CA125 in a female patient was recorded. Moreover, arsenic concentrations in the urine of patients who had ceased As_2O_3 treatment for 24 months were below the safety limits recommended by government agencies in several countries or regions.

Secondary malignancies were not reported in the short-term trials US registration trial[10] or the phase 2 trial of As_2O_3 plus ATRA.[45] Reduced exposure to or avoidance of chemotherapy in As_2O_3 patients may reduce the risk of secondary MDS and AML, which have been previously associated with APL treatment.[84]

Arsenic retention and long-term toxicity

The trial by Hu et al. included extensive screening of APL patients for long-term effects of arsenic exposure following completion of therapy.[44] Laboratory studies (blood counts, electrolyte panels, urinalysis, liver function tests, and serum tumor marker analysis) and physical examinations (ECGs, echocardiograms, chest x-rays, dermatologic and neurologic consults, and nerve conduction velocity tests) were performed in 33 patients during therapy and at a minimum of 2 years after receiving the last dose of As_2O_3. At the final follow-up, physical exam and laboratory results were similar between patients and healthy controls. No tumors or skin lesions were detected. ECGs, echocardiograms, and chest x-rays were normal. Toxicology analyses in these patients found that As_2O_3 levels in plasma and urine dropped after termination of As_2O_3 treatment to levels only slightly higher than those in healthy controls, and were within recommended US safety limits.

Pharmacokinetics

In a study of 12 Japanese patients receiving As_2O_3 (0.15 mg/kg IV over 2 hours), the mean maximum plasma concentration of inorganic arsenic (As^{III} and As^{V}) of 22.6 ± 11.4 ng/mL occurred at completion of the infusion, then declined biphasically, with an initial distribution phase followed by an elimination phase with a terminal half-life of 17 hours.[85] The volume of distribution was large (55.9 L/kg), indicating extensive distribution throughout the body. In a separate case report of a patient with relapsed APL and meningeal infiltration, As_2O_3 was observed to cross the blood-brain barrier when administered intravenously; arsenic levels in cerebrospinal fluid were approximately 14% of levels in blood.[86]

The major arsenic metabolites identified in humans are the methylated arsenics, methylarsonic acid (MAA^{V}) and dimethylarsinic acid ($DMAA^{V}$).[87–89] In the Japanese study, plasma levels of MAA^{V} and $DMAA^{V}$ increased gradually over 24 hours after the first administration of As_2O_3, reaching peak levels of 3.1 ± 1.6 ng/mL and 5.4 ± 2.9 ng/mL, respectively.[85] The contribution of these metabolites to the therapeutic effect of As_2O_3 is unknown. In APL, chronic lymphocytic leukemia, and leukemia/lymphoma cell lines, methylated arsenic derivates were shown to induce apoptosis, though they did not induce

differentiation in APL cells.[90] Plasma concentrations of inorganic arsenic did not increase with repeated administration; however, the concentrations of both major metabolites were approximately four times higher during week 4 of treatment compared with day 1.[85] Arsenic content in hair and nails also increased gradually with repeated treatment and reached levels 5–7 times higher than pretreatment levels.[8]

Approximately 60% of the daily dose of arsenic is excreted in the urine, including inorganic and methylated arsenic; other pathways of excretion, such as biliary, may also contribute to As_2O_3 elimination.[85,87]

Conclusions

As_2O_3 has demonstrated remarkable efficacy in APL. The use of As_2O_3 in induction or consolidation strategies reduces the relapse rate and improves survival in patients with APL, especially in high-risk patients.[43,44,46] The addition of As_2O_3 to ATRA/chemotherapy regimens may allow a reduction in chemotherapy exposure and associated toxicities without compromising cure rates.[47] In select patients, chemotherapy may be eliminated altogether, providing an alternative treatment option for patients who cannot tolerate anthracyclines.[45] Although As_2O_3 has demonstrated less robust efficacy in other malignancies, promising activity has been demonstrated when As_2O_3 is combined with other agents. As_2O_3 is generally well tolerated, both as a single agent and in combination, with manageable adverse effects and no documented evidence of long-term toxicities. Additional comparative trials will be necessary to determine which combinations of available therapies provide the greatest benefit with the least toxicity in patients with APL.

Practice Points

At the Sidney Kimmel Comprehensive Cancer Center (SKCCC) at Johns Hopkins University, patients with APL are treated as follows:

- Whenever possible, they are enrolled in clinical trials.
- Outside of clinical trials, patients should be treated according to a published treatment protocol, from induction through maintenance and post-maintenance monitoring.
- Remission can be achieved with ATRA plus an anthracycline. The use of 60 days of ATRA (rather than administration of ATRA until normalization of hemogram) may lead to a higher level of PML/RARα molecular negativity.
- If WBC $\geq 20 \times 10^9$/L, hydroxyurea is added to maintain WBC $< 20 \times 10^9$/L, with dexamethasone (10 mg IV, twice daily for 14 days) as prophylaxis against APLDS.
- At SKCCC, we believe that an As_2O_3-containing post-remission (i.e., consolidation) chemotherapy regimen results in high relapse-free and overall survivals (Table 2).
- In patients unable to tolerate anthracyclines, remission can be induced with ATRA plus As_2O_3 daily until absence of leukemia in bone marrow is documented, or for a maximum of 60 days.
- Maintenance therapy appears important and should be completed according to the elected treatment protocol.

- Treatment of relapsed disease (determined by two consecutive positive PML/RARα findings by qualitative RT-PCR within 4 weeks) should be individualized per patient.

Research Agenda

- There are 87 recently completed or ongoing clinical trials listed on www.clinicaltrials.gov evaluating As_2O_3 alone or in combination with other agents for treatment of APL, AML, multiple myeloma, MDS, primary myelofibrosis, hepatocellular carcinoma, metastatic melanoma, CNS tumors, and breast, lung, colorectal, and kidney cancers.
- In APL, the role of chemotherapeutic CNS prophylaxis, in all patients or high-risk patients, needs to be addressed in clinical trials.
- In APL, the role of maintenance therapy, particularly in patients who are treated with As_2O_3, also needs to be addressed by future research.

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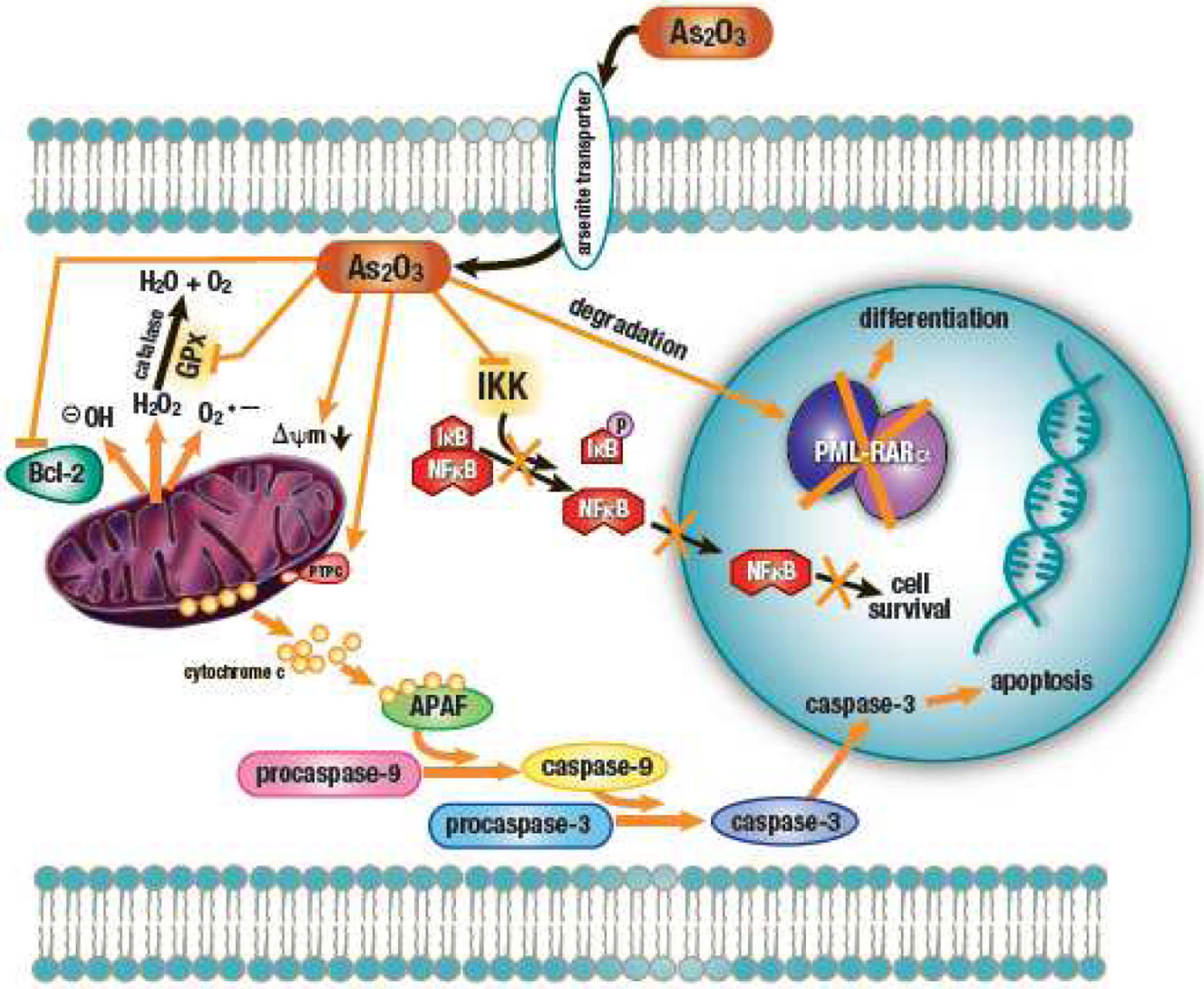

Figure 1. As_2O_3 targets multiple cellular pathways

In APL cells, arsenic trioxide (As_2O_3) restores differentiation by degrading the PML-RARα fusion protein. However, As_2O_3 has additional targets that are present in multiple cancer cell types. As_2O_3 targets the mitochondria, decreasing the mitochondrial membrane potential ($\Delta\Psi m$) via multiple specific targets including Bcl-2 and the PTPC. This change in potential results in the release of cytochrome C, which activates the caspase cascade. It also results in increased release of ROS from the mitochondria. ROS levels are increased further by As_2O_3 inhibition of the antioxidant enzyme GPx. As_2O_3 also inhibits activation of the cell-survival factor NFκB via inhibition of IKK, the kinase responsible for releasing NFκB that is sequestered in the cytoplasm.

Abbreviations: APAF, apoptotic peptidase activating factor; GP_x, glutathione peroxidase; IKK, IκB kinase; PTPC, permeability transition pore complex; ROS, reactive oxygen species.

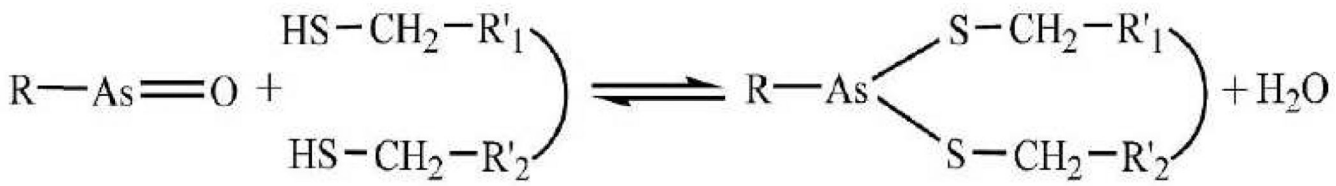

Figure 2.
Reaction of vicinal sulfhydryl groups in a protein structure with trivalent arsenic.

Table 1

Current therapies for APL

Study	N	Regimen	CR, %	CIR, %	EFS, %	DFS, %	OS, %
Initial therapy							
PETHEMA – LPA99[11]	251	**I:** ATRA + CT (IDA) **C:** 3 courses CT (IDA, MXN, IDA; low-risk pts) or 3 courses ATRA + CT (intermediate/high-risk pts) **M:** Low-dose CT (MCP/ MTX) + intermittent ATRA (every 3 mos) for 2 yrs	90	8.7, 3-yr	NR	90, 3-yr	85, 3-yr
European APL Group – APL 2000[12]	340	**I:** ATRA + CT (DNR ± ara-C) **C:** 2 courses CT (DNR ± ara-C) **M:** Low-dose CT (MCP/ MTX) + intermittent ATRA (every 3 mos) for 2 yrs	96	8.4, 2-yr	84.5, 2-yr	NR	91.9, 2-yr
JALSG – APL97[13]	283	**I:** ATRA (low-risk pts) or ATRA + CT (IDA + ara-C; intermediate/high-risk pts) **C:** 3 courses CT (MXN + ara-C, ara-C+ etoposide + DNR, ara-C + IDA) **M:** Randomized to observation or intensified maintenance CT (6 courses CT every 6 wks)	94	NR	NR	68.5, 6-yr	83.9, 6-yr
Second-line therapy							
Soignet et al.[10]	40	**I:** As_2O_3 (daily until BM remission or 60 days) **C:** As_2O_3 (25 doses in 35 days) **M:** Up to 4 additional consolidation courses of As_2O_3	85	NR	NR	56, 1.5-yr (RFS)	66, 1.5-yr

Abbreviations: APL, acute promyelocytic leukemia; ara-C, cytarabine; As_2O_3, arsenic trioxide; ATRA, all-*trans*-retinoic acid; BM, bone marrow; C, consolidation therapy; CIR, cumulative incidence of relapse; CR, complete response; CT, chemotherapy; DFS, disease-free survival; DNR, daunorubicin; EFS, event-free survival; I, induction therapy; IDA, idarubicin; JALSG, Japan Adult Leukemia Study Group; M, maintenance therapy; MCP, mercaptopurine; MXN, mitoxantrone; MTX, methotrexate; NR, not reported; OS, overall survival; PETHEMA, Programme de Estudio y Tratamiento de las Hamopatias Malignas; RFS, relapse-free survival.

Table 2

Clinical studies of arsenic trioxide in first-line therapy for APL[a]

Study	N	Regimen	CR	EFS	DFS (or RFS)	OS
As_2O_3 in induction						
Mathews et al.[41]	72	**I:** As_2O_3 (daily until CR or 60 days) **C:** As_2O_3 (daily for 4 wks) **M:** As_2O_3 10 d per mo for 6 mos	86%	75%, 3-yr	87%, 3-yr	86%, 3-yr
Ghavamzadeh et al.[42]	193	**I:** As_2O_3 (daily until CR or 60 days) **C and M:** 1 or 4 courses As_2O_3 (daily for 4 wks)	83%	NR	69%, 3-yr	79%, 3-yr
Shen et al.[43]	61	**I:** (A) ATRA or (B) As_2O_3 or (C) ATRA + As_2O_3 until CR **C:** (all patients) 3 courses CT[b] **M:** (A) ATRA or (B) As_2O_3 or (C) ATRA then As_2O_3 followed by low-dose CT (MCP or MTX) for 5 cycles	A: 95% B: 90% C: 95%	NR	A: 68%, 2-yr B: 89%, 2-yr C: 100%, 2-yr	NR
Hu et al.[44]	85	**I:** ATRA + As_2O_3 (daily until CR) **C:** 3 courses CT[b] **M:** ATRA then As_2O_3 followed by low-dose CT (MCP or MTX) for 5 cycles	94%	89%, 5-yr	95%, 5-yr (RFS)	92%, 5-yr
Ravandi et al.[45]	82	**I:** ATRA + As_2O_3 (+ GO in high-risk patients) until BM remission or 85 days **C and M:** 7 cycles ATRA + 4 cycles As_2O_3	91% (CR/CRi)	83%, 5-yr	NR	84%, 5-yr
As_2O_3 in consolidation						
Powell et al.[46] (C9710)	481	**I:** ATRA + CT (DNR + ara-C) **C:** (randomized) (A) 2 courses As_2O_3, then 2 courses ATRA + CT (DNR) or (B) 2 courses ATRA + CT (DNR) **M:** (randomized) ATRA ± low-dose CT (MCP + MTX)	A: 89% B: 89%	A: 81%, 3-yr B: 66%, 3-yr *P* = .0007	NR	A: 86%, 3-yr B: 79%,3-yr *P* = .063
Gore et al.[47]	45	**I:** ATRA + CT (DNR) for 60 days **C:** 1 course As_2O_3 + CT (DNR + ara-C) **M:** ATRA (low/intermediate-risk patients) or ATRA + low-dose CT (MCP + MTX) (high-risk patients)	91%	76%, 2.7-yr	90%, 2.7-yr	88%, 2.7-yr

Abbreviations: APL, acute promyelocytic leukemia; As_2O_3, arsenic trioxide; ATRA, all-*trans*-retinoic acid; ara-C, cytarabine; BM, bone marrow; C, consolidation therapy; CR, complete response; CRi, complete response without full platelet recovery; CT, chemotherapy; DFS, disease-free survival; DNR, daunorubicin; EFS, event-free survival; GO, gemtuzumab ozogamicin; I, induction therapy; M, maintenance therapy; MCP, mercaptopurine; MTX, methotrexate; NR, not reported; OS, overall survival; RFS, relapse-free survival.

[a] All patients were newly diagnosed with APL.

[b] Each course consisted of 3 consecutive regimens: DA (DNA + ara-C), ara-C pulse, HA (homogarringtonine + ara-C).