Advances in the Management of Acute Promyelocytic Leukemia and Other Hematologic Malignancies with Arsenic Trioxide

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Key Words. Acute promyelocytic leukemia · All-trans-retinoic acid · Arsenic trioxide · Hematologic malignancy · Multiple myeloma

LEARNING OBJECTIVES

After taking all of the CME courses in this supplement the reader will be able to:

1. Describe the basic biology of various leukemias, multiple myeloma, and myelodysplastic syndrome (MDS).
2. Discuss new targeted treatment strategies for hematologic malignancies.
3. Understand the rationale for the use of nontraditional cytotoxic agents such as arsenic trioxide in the treatment of hematologic malignancies.
4. Examine the role of arsenic trioxide and other novel agents in early- versus accelerated-stage hematologic disease.
5. Discuss the preclinical and clinical efficacy of arsenic trioxide and various agents in treating acute promyelocytic leukemia, MDS, and multiple myeloma.

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ABSTRACT

Acute promyelocytic leukemia (APL), once considered the most devastating subtype of acute myeloid leukemia, is now the most treatable of all subtypes as a result of intensive research into its molecular pathogenesis. This research has led to a rational approach to treatment in which the use of the differentiating agent all-trans-retinoic acid (ATRA) has proven to be effective first-line treatment for inducing complete remission. Arsenic trioxide (ATO) is currently used to treat relapsed disease, further enhancing survival rates in a patient population for which limited salvage options exist. This review discusses the molecular mechanisms responsible for development of APL and the evolution of treatment options over the last three decades, including the major advances using ATRA and ATO in the last 12 years. The mechanism of action of ATO is also described in view of this agent’s potential for broader therapeutic application in a variety of hematologic malignancies. The Oncologist 2002;7(suppl 1):1-13

BIOLOGY AND MOLECULAR PATHOGENESIS OF ACUTE PROMYELOCYTIC LEUKEMIA

The morphologic and pathologic features of acute promyelocytic leukemia (APL) led to the recognition of this disorder as a specific subgroup of myeloid leukemias in the late 1950s [1]. With the establishment of the French-American-British classification system, APL was redefined as belonging to the acute myeloid leukemia (AML)-M3...
subgroup in 1976 [2]. Shortly thereafter, Rowley et al. detected a specific reciprocal translocation between chromosomes 15 and 17 in the vast majority of APL patients [3], and in 1980, investigators discovered that retinoic acid could induce the differentiation of myeloid cell lines [4]. Now, two decades later, elucidation of the molecular genetics of APL and the relationship between the genetic and pathologic features of this leukemia have increased our understanding of the disease process and provided a rational basis for therapy, which has few parallels in cancer biology.

The reciprocal translocation in APL, which reflects the disruption of the promyelocytic (PML) gene on chromosome 15 and the retinoic acid receptor-alpha (RARα) gene on chromosome 17 [5-7], results in the production of the chimeric proteins PML-RARα and RARα-PML. The mRNA for the former is found in nearly all patients with the t(15;17) translocation, whereas RARα-PML transcripts are detected in about two-thirds of patients [8, 9]. Few APL patients lack the characteristic t(15;17) translocation, but in those who do, the RARα gene is linked to the promyelocytic leukemia zinc finger (PLZF) gene on chromosome 11, the nucleophosmin (NPM) gene on chromosome 5, the nuclear mitotic apparatus protein (NuMA) gene on chromosome 11, or the signal transducer and activator of transcription 5b (Stat5b) gene on chromosome 17 (Fig. 1A) [10].

Disruption of the RARα gene by the translocation in APL alters the RARα protein and exerts a dominant negative effect [5, 11], modifying the transcription of primary target genes directly regulated by normal RARα (Fig. 1A). The fusion proteins interfere with transcription factors regulating other genes and exerting post-transcriptional effects [8]. These effects block myeloid differentiation and cause leukemic cell accumulation at the promyelocytic stage (Fig. 1B) [12].

The role of RARα in normal myeloid growth and differentiation was determined by experiments in which RARα was overexpressed in the APL cell line NB4. These cells displayed markedly decreased growth rates and more prominently expressed myeloid differentiation markers [13]. Identifying the primary target genes whose expression is altered by APL translocations and relating these changes to pathogenesis are major goals in APL research and will likely form the basis of more effective therapeutic strategies [14, 15].

Of the five genes known to fuse with the RARα gene in APL, the PML gene is the best characterized and is involved in more than 90% of cases of APL [10, 11]. Although PML is by far the most frequent partner of RARα in APL translocations, considerable insight into APL biology has been derived

\[ \text{Figure 1. Molecular pathogenesis of APL.} \]

**A** The reciprocal translocation of the retinoic acid receptor-alpha (RARα) gene on chromosome 17 with one of 5 different partner genes is the primary genetic lesion contributing to the pathogenesis of APL. The resulting fusion protein tightly associates with the nuclear corepressor (N-CoR)/histone deacetylase (HDAC) complex leading to transcriptional repression of target genes. Transcriptional repression is mediated by deacetylation of histones, resulting in a closed chromatin conformation denying transcriptional activator proteins access to DNA, in the promotor regions of target genes. **B** PML-RARα alters myeloid commitment by interfering with normal hematopoietic progenitor cell (HPC) and promyelocyte maturation by transcriptional modulation of specific hematopoietic gene expression programs.
from the study of alternative translocations, in particular the t(11;17) translocation associated with PLZF-RARα [16]. The leukemic blasts of patients with this translocation have a distinct morphologic identity and do not respond to treatment with all-trans-retinoic acid (ATRA) [16, 17]. Similar to PML, the fusion partners NPM, NuMA, and Stat5b do not appear to affect responsiveness to ATRA [18, 19].

PML was originally identified as a result of its translocation in APL [5-7]. Sequence analysis of this gene indicated the presence of a cysteine-rich region that resembles a zinc-finger DNA-binding domain and suggested the action of PML as a transcription factor [5]. Consistent with this possibility, the endogenous PML protein in normal cells was shown to be part of a novel macromolecular structure in the nucleus [20-22]. Expression of the PML-RARα fusion protein in leukemic cells disrupts the nuclear bodies, and the PML protein is dispersed into smaller fragments of the nuclear bodies. An important clue to the role of the nuclear bodies in regulating the growth and differentiation of APL cells was the discovery that the treatment of APL cells with ATRA could restore the aberrant PML in nuclear bodies to a normal appearance [23]. This may mean that the appropriate regulation of growth and differentiation of myeloid cells requires intact nuclear bodies. Within them, PML interacts with and recruits numerous proteins and antagonizes many of the processes required for the initiation, promotion, and progression of malignancy [24].

In normal cells, RARα represses the transcription of particular myeloid differentiation genes by recruiting histone deacetylases through a family of silencing mediators (SMRT and N-CoR). Nuclear corepressor-bound histone deacetylases are also tightly associated with PML-RARα and PLZF-RARα fusion proteins [25, 26]. The histones of actively transcribed chromatins contain acetyl groups that appear to relax specific segments of DNA and allow the binding of specific transcription factors [27, 28]. Histone deacetylases remove the acetyl groups and consequently block transcription.

When retinoic acid is added to the complexes, RARα and PML-RARα are dissociated from the nuclear corepressor histone deacetylase, whereas PLZF-RARα is not [29, 30]. This may explain the lack of response to ATRA in APL patients who exhibit the PLZF-RARα fusion gene. Paradoxically, the inhibition of histone deacetylase converts PLZF-RARα from an inhibitor of the retinoic acid signaling pathway to an activator. Thus, the association of the APL fusion proteins with histone deacetylase seems to be crucial to their transforming potential [25].

In one study, the histone deacetylase inhibitor sodium phenylbutyrate was successfully used to treat a patient with APL and restore the sensitivity of the patient’s cells to ATRA, thus illustrating the critical role of the PML-RARα fusion protein in sequestering the histone deacetylase complexes [31]. However, other APL patients in this study who were resistant to ATRA failed to respond to this therapy.

**Evolution of Treatment and Management of APL**

APL was once considered the most devastating subtype of AML because of its associated coagulopathy, which is complex and involves disseminated intravascular coagulopathy as well as fibrinolysis. Before the use of the anthracyclines and the limited use of proper supportive care, standard cytotoxic chemotherapy as induction treatment exacerbated coagulopathy, and approximately 10%-30% of patients with APL died of hemorrhage [32, 33]. Until about 12 years ago, treatment for APL was limited to chemotherapy with the anthracyclines alone or an anthracycline plus cytarabine (Ara-C) (Table 1) [34]. With the protocols available in the 1970s, complete remission (CR) rates of no more than 60% were reported [34]. Given the relatively high sensitivity of APL cells to the anthracyclines, the use of higher-dose chemotherapy—up to 300 mg/m² of daunorubicin (DNR)—and appropriate management of the APL-related coagulopathy improved the CR rate to between 70% and 80% during the 1980s [34]. Even with consolidation and maintenance therapy, the median duration of CR was no more than 1-2 years, with only 20%-45% of patients cured with chemotherapy alone and the remainder dying from hemorrhage or relapsed or refractory disease [34-38].

<table>
<thead>
<tr>
<th>Year</th>
<th>Management of APL</th>
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<tbody>
<tr>
<td>1967</td>
<td>Chemotherapy was used unsuccessfully against APL, a type of AML.</td>
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<tr>
<td>1973-1988</td>
<td>Anthracycline-based chemotherapy, with appropriate management of coagulopathy, yields CR rates of 50%-80% and longer DFS in patients with APL than with any other type of AML.</td>
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<tr>
<td>1988-2001</td>
<td>All-trans-retinoic acid, in combination with intensive chemotherapy, apparently cures ≥70% of patients.</td>
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<tr>
<td>2000-2001</td>
<td>ATO, alone and in combination, shows clinical efficacy, and is approved for the management of relapsed/refractory APL.</td>
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**Table 1. Evolution of the management of APL**

AML = acute myeloid leukemia; APL = acute promyelocytic leukemia; ATO = arsenic trioxide; CR = complete remission; DFS = disease-free survival.
Treatment of APL has improved to the point that APL is now the most curable subtype of AML. High concentrations of ATRA restore the gene expression and apoptosis program disrupted by the presence of PML-RARα. Since the introduction of ATRA therapy in 1987, its combination with anthracycline-based chemotherapy has increased the cure rate of APL to 70% or higher and more than doubled the 5-year survival rate achievable with anthracycline- or Ara-C-based therapies alone [37, 38].

**Clinical Use of ATRA Therapy**

Initial studies documenting the treatment of APL patients with ATRA were based on the premise that the induction of differentiation could serve as an alternative approach to cytotoxic regimens [39-41]. Studies from Shanghai showed that ATRA alone was able to induce CR in 23 of 24 APL patients; in the one unresponsive patient, CR was induced following treatment with ATRA and Ara-C [39]. In a subsequent study in France, ATRA at 45 mg/m² per day was administered to 22 patients, 16 of whom had relapsed at least once following conventional chemotherapy. Complete responses were observed in 14 patients. At remission, the t(15;17) translocation initially present in 20 patients was not found, and in vitro studies showed differentiation of cells, after the addition of ATRA, from 16 of the 18 patients from whom cells were taken [40].

In a larger trial, 49 newly diagnosed and 30 previously treated patients underwent ATRA therapy followed by cytotoxic chemotherapy for consolidation following the induction of remission. Overall, 88% of the newly diagnosed patients and 83% of the previously treated patients achieved CR, but, as in earlier trials, remissions achieved with ATRA therapy were of short duration, often lasting less than a year [9, 40, 42]. In a trial conducted at the Memorial Sloan-Kettering Cancer Center in New York, the median duration of remission was only 3.5 months, and 13 (100%) of 13 patients treated with ATRA alone relapsed within 10 months [43].

Investigators in Europe conducted clinical trials to assess the efficacy of ATRA combined with chemotherapy [44]. A pilot study was organized with 26 newly diagnosed APL patients who were treated with ATRA followed by intensive chemotherapy consisting of DNR and Ara-C. The total CR rate was 96% following treatment with ATRA alone (14 patients) or ATRA plus DNR (11 patients). The disease-free interval (DFI) and event-free survival (EFS) at 18 months were 87% and 77%, respectively, a considerable improvement over historical data, which showed a CR rate of 76%, a DFI of 59%, and an EFS of 48% at 18 months following treatment with chemotherapy alone [44].

The European APL 91 Group also conducted a randomized trial to compare chemotherapy regimens with and without ATRA. Although the CR rates between these study populations did not differ significantly (p = 0.08), 12-month EFS was estimated to be 79% in the patients receiving chemotherapy with ATRA and 50% in the patients receiving chemotherapy alone (p = 0.001). Furthermore, the estimated 12-month relapse rate was 19% and 40% in the two populations (p = 0.005), respectively, suggesting that the combination of chemotherapy and ATRA substantially reduced the relapse rate in patients with APL [44]. ATRA-based therapy also necessitated significantly fewer platelet and red blood cell transfusions and resulted in fewer days with fever, a reduced need for antibiotics, and shorter hospital stays [34].

Finally, initial studies with ATRA for induction therapy showed improvement of the coagulation disorders that often accompany APL and are frequently exacerbated by chemotherapy [34, 40].

In the aforementioned studies, the adverse effects experienced by ATRA-treated patients were generally well tolerated, and included headache, bone pain, elevated transaminase and triglyceride levels, and dryness of the skin and mucosal surfaces. One serious side effect is the retinoic acid syndrome [45], which is frequently associated with a rapid increase in leukocytes and is characterized by respiratory distress, unexplained fever, pulmonary infiltrates, pulmonary edema, pleural or pericardial effusions, hypotension, and acute renal failure [34, 46]. It affects 5%-27% of ATRA-treated patients, with an associated mortality of 5%-29% [46].

The incidence of the retinoic acid syndrome among patients enrolled in the National Cancer Institute Intergroup Protocol 0129 was 26% (44 of 167 patients). Patients were effectively treated with dexamethasone 10 mg twice daily, and the administration of ATRA resumed when the syndrome resolved [46]. Fenaux et al. observed that adding ATRA during induction chemotherapy reduced the incidence of the fatal retinoic acid syndrome in patients with high white blood cell counts [46, 47].

Two trials were conducted to demonstrate the utility of maintenance therapy in APL patients treated with ATRA [47, 48]. In one study, performed in North America, 174 patients were randomized to receive ATRA or one course of DNR and Ara-C as induction chemotherapy, followed by two courses of DNR and Ara-C for consolidation. These patients were further randomized to receive 1 year of continuous maintenance with ATRA or undergo observation only [48]. In patients receiving ATRA induction therapy, ATRA maintenance therapy substantially decreased the incidence of relapse compared with observation only (21% versus 39%). However, even patients who did not receive ATRA induction chemotherapy benefited from ATRA maintenance therapy, and patients who received chemotherapy followed by ATRA maintenance therapy demonstrated similar outcomes as those receiving ATRA.
induction followed by observation only [48]. Therefore, the inclusion of ATRA as part of a therapeutic regimen for APL was judged to be beneficial regardless of the phase (induction or maintenance) during which it was included.

The other study, conducted in Europe, confirmed the benefit of maintenance ATRA therapy [47]. In addition, Fenaux et al. showed that ATRA administered in conjunction with chemotherapy, followed by an intermittent ATRA maintenance schedule, was more effective at improving EFS than was continuous maintenance treatment [47].

To eliminate minimum residual disease (MRD), as measured by reverse transcriptase-polymerase chain reaction (RT-PCR), with ATRA therapy, it is now believed that ATRA should be combined with an anthracycline during the induction phase. It is also believed that maintenance therapy with ATRA is required. Further, it is accepted that patients must become RT-PCR-negative for a treatment regimen to be effective (i.e., so they will not relapse).

Many consider the AIDA (ATRA plus idarubicin) regimen, popularized by the Gruppo Italiano Malattie Ematologiche Maligne dell’Adulto cooperative group, to be the best single approach toward treating APL and minimizing relapse [49]. The AIDA regimen is administered until CR, followed by three polychemotherapy cycles of consolidation therapy. An analysis of hematologic and molecular relapse by RT-PCR indicated that 229 of 253 patients who participated in this study achieved hematologic remission. Of 139 cases evaluated for MRD, 84 (60.5%) remained RT-PCR negative [49].

**Reinduction with ATRA**

ATRA has been highly effective as reinduction therapy for patients who relapse after induction chemotherapy. Approximately 85%-90% of patients can achieve a prolonged second CR with ATRA followed by intensive consolidation chemotherapy and allografts or autografts [34]. However, relapse that occurs within 1 year after discontinuation of ATRA therapy may be resistant to reinduction with ATRA, in part because cytochrome P450 metabolism reduces peak serum ATRA concentrations and also because of other mechanisms of ATRA resistance, such as mutations in the RARα moiety of PML-RARα [34].

**Clinical Use of ATO Therapy**

The first observational studies suggesting that therapy incorporating arsenic trioxide (As₂O₃; ATO) could induce CR in newly diagnosed and relapsed APL patients were conducted in China in the early 1990s [50]. In these studies, ATO treatment yielded CR rates of 73% (in a series of 72 patients) and 5-year survival rates of 50% (in a series of 32 patients) [50, 51]. In a well-designed study from Shanghai, ATO induced CR in 85% of patients with APL who had been resistant to chemotherapy and ATRA [52].

The results of an open-label, dose-escalation pilot study in the U.S., involving 12 patients with relapsed APL, confirmed the findings of the study from China and provided some information on the mechanism of action of ATO in APL [53]. ATO doses of 0.06 to 0.20 mg/kg/day for 12-39 days of treatment induced CR in 11 (92%) of 12 patients who had relapsed after prior therapy. The drug was given until leukemic blasts and promyelocytes were no longer visible in the bone marrow.

Patients who achieved CR were eligible to receive a cycle of consolidation therapy consisting of 25 days of ATO 0.15 mg/kg/day administered over the course of 5 weeks, and up to four cycles of maintenance therapy at the same dosage. These were given at 3- to 6-week intervals over a period of about 10 months. Median time to achieve CR was 47 days (range, 24-83 days) and median duration of remission was 5 months (range, 1-9 months). After two courses of therapy, the PML-RARα fusion product was no longer detectable by RT-PCR in 8 of 11 patients tested.

Three patients relapsed during the second course of treatment; in none of these had the PML-RARα fusion product been eliminated. Flow cytometry in patients in remission revealed a population of bone marrow cells expressing surface antigens characteristic of both mature and immature myelocytes, CD11b and CD33, respectively. These cells were no longer positive for the PML-RARα fusion product and had begun to synthesize cysteine proteases (caspases), which are active in apoptosis. Loss of the PML-RARα fusion product, partial differentiation of promyelocytes, and induction of apoptosis all accompanied ATO administration.

One multicenter study investigated the efficacy of ATO as a single agent for patients who relapsed after, or were refractory to, therapy with an anthracycline and retinoic acid (Table 2) [54]. Of 40 patients enrolled, 21 were in first relapse, whereas 19 had experienced two or more relapses. Five patients (12%) had relapsed following bone marrow transplantation (BMT). ATO 0.15 mg/kg/day was administered until leukemic cells were no longer detectable in the bone marrow. Patients in CR received one cycle of consolidation therapy with ATO, at the same dosage, for as long as 25 days, and could receive up to four more 25-day maintenance cycles of ATO [54].

Overall, 85% of patients achieved CR; median time to marrow remission was 35 days, and median time to CR was 59 days. Of the 34 patients who achieved CR, 29 were evaluable for MRD by RT-PCR of the PML-RARα transcript. Twenty-five of these patients tested negative following induction (14 patients) or consolidation therapy (11 patients) [54]. The 18-month overall and relapse-free survival
estimates were 66\% and 56\%, respectively, suggesting that the responses can be durable [54].

Of the 21 patients alive at the time of this writing, 11 had received autologous (three patients) or allogeneic (eight patients) transplants following induction or consolidation therapy with ATO, and 10 had received ATO only in a postconsolidation setting [54]. Of the remaining 10 patients, nine received ATO maintenance following induction and consolidation [54]. The 85\% remission rate and durability of response established ATO as a highly effective therapeutic option for patients with APL. Additionally, preliminary data suggest that ATO maintenance therapy may be appropriate for relapsed APL patients who are not candidates for BMT and may be used prior to or after transplant to eliminate MRD [55].

Many of the adverse reactions reported following ATO therapy are similar to those seen with ATRA, including self-resolving leukocytosis [54, 56]. Other frequent ATO-related toxicities include skin rash, hyperglycemia, gastrointestinal reactions, peripheral neuropathy, and hypokalemia, all of which are manageable with symptomatic therapy and are self-resolving [54, 57]. Side effects prevalent with standard chemotherapy, including alopecia, mucositis, severe nausea and vomiting, and chronic myelosuppression, rarely occurred in patients treated with ATO.

Patients who present with leukocytosis or develop peak white blood cell counts of \( \geq 10,000/\mu l \) are at risk of developing a syndrome identical to the retinoic acid syndrome, referred to as the APL differentiation syndrome [54, 56]. In the multicenter trial, this syndrome occurred in 10 (25\%) of the 40 patients who received ATO induction therapy [54]. As with the retinoic acid syndrome, the prompt administration of corticosteroids can effectively reverse the initial symptoms and prevent the development of more severe symptoms [56].

ATO treatment can also result in QTc-interval prolongation. An analysis of 1,039 electrocardiograms showed that 68 (69\%) of 99 APL patients, from multiple studies, receiving ATO therapy demonstrated QTc-interval prolongation (\( \geq 450 \) msec in men and \( \geq 470 \) msec in women) [54, 58]. Cycle length returned to baseline between courses of ATO. QTc-interval prolongation can be managed by electrocardiographic monitoring and the replacement of magnesium and potassium to maintain serum levels above 1.8 mg/dl and 4 mEq/l, respectively.

In September 2000, the Food and Drug Administration approved Trisenox\textsuperscript{®} (ATO; Cell Therapeutics, Inc; Seattle, WA) for the induction of remission and consolidation in patients who are refractory to, or who have relapsed from, treatment with ATRA and chemotherapy.

Additional safety data on ATO have since been reported from the National Cancer Institute Compassionate Use Program, which showed that 6 of 57 evaluable relapsed APL patients treated with ATO experienced only nine drug-related serious adverse events [59]. Furthermore, more than 540 patients with a variety of hematologic cancers or solid tumors have been treated with ATO and demonstrated adverse events similar to those described in the Trisenox\textsuperscript{®} package insert, with no deaths being reported [60].

**DIFFERENTIATION MECHANISMS AND APOPTOTIC EFFECTS OF ATO ON MALIGNANT CELLS**

The ability of ATO to induce high rates of CR in patients with relapsed APL provides an impetus for determining the specific mechanisms of action that mediate the partial differentiation and apoptosis of APL cells. Morphologic changes indicating partial myeloid differentiation occur in vitro in the NB4 APL cell line following treatment with ATO at low concentrations (0.1-0.5 µM). After 10 days in the presence of ATO, the NB4 APL cell line demonstrates a decreased nuclear/cytoplasmic ratio, the appearance of cytoplasmic granules, the condensation of chromatin, and the partial disappearance of nucleoli [61]. ATO also modulates the expression of
cell surface differentiation antigens. Modulations include increasing the levels of CD11b, a mature myeloid marker, and decreasing the levels of CD33, an immature myeloid marker. Changes in differentiation markers are also observed in patients treated with ATO, but differentiation seemed incomplete in these patients, as a population of double-positive cells (CD11b+CD33+) persisted with continued exposure to ATO [53].

The partial differentiating effects of ATO on APL cells are mediated by targeting the leukemogenic fusion protein PML-RARα. In vitro studies with NB4 cells and cultured primary APL cells from patients show that ATO degrades both PML and PML-RARα [61]. This reduction releases the differentiation block in APL by overcoming the dominant negative effects of the fusion protein on the normal functioning of PML and RARα [62]. The resultant differentiating effects of ATO are incomplete compared with the effects of retinoic acid, suggesting that they induce different patterns of gene expression following the degradation of PML-RARα.

Shao et al. showed that each agent can inhibit the other’s function in NB4 cells [62]. Pretreatment of NB4 cells with ATO followed by treatment with retinoic acid inhibited retinoic acid-inducible type II transglutaminase expression and retinoic acid-induced differentiation. Conversely, ATRA pretreatment of NB4 cells inhibited ATO-induced apoptosis. However, the two agents act synergistically. In a transgenic mouse model of APL, ATO and retinoic acid combined to enhance the regression of established leukemia [63]. In another study, the combination of ATO and retinoic acid was more effective at prolonging survival in PML-RARα leukemic transgenic mice and nude mice transplanted with PML-RARα leukemic cells than was either drug alone [64].

High concentrations of ATO (0.5-2 µM) inhibit growth and reduce the viability of APL cells, mainly through apoptosis [23, 61]. Many mechanisms contribute to this effect. ATO treatment alters the cellular location of PML protein before its degradation and reestablishes normal PML functioning within the cell. In normal cells, PML is a growth- and tumor-suppressor protein involved in caspase-independent apoptosis [21, 65, 66].

Guo et al. showed that PML-mediated apoptosis occurs through p53 transcriptional activation that depends on the colocalization of p53 and PML within nuclear bodies or promyelocytic oncogenic domains; however, this association is disrupted in APL cells [66]. The PML-RARα fusion protein in APL cells forms heterodimers with PML and is responsible for disrupting normal PML localization, thus functioning as a dominant negative inhibitor of its function [20, 21]. APL cells stained for the presence of PML exhibit a speckled appearance marked by a number of micropunctates throughout the nucleus and cytoplasm, indicative of PML delocalization. After treatment with ATO, the micropunctates disappear and large aggregates, presumably reassociations of PML within domains, appear in the nucleus [23, 61, 62, 67].

Experimental evidence also suggests that ATO-induced apoptosis occurs independently of PML. A recent study with an ATO-resistant NB4 cell line showed that ATO still altered PML localization, without inducing apoptosis, by exhibiting the same PML staining pattern as normal NB4 cells after treatment [68]. Also, many cells lines that do not express PML-RARα remain sensitive to the apoptotic effects of ATO; these effects are comparable to those observed in NB4 cells [69]. Finally, cells from PML knock-out mice undergo apoptosis after treatment with ATO, further supporting the premise that apoptosis is induced independently of the presence of PML [69]. These observations suggest that additional mechanisms exist in ATO-sensitive cells regardless of the presence or activity of PML.

Additional intracellular targets of ATO have been identified and are discussed by Miller [70].

ATO-induced apoptosis is associated with the disruption of mitochondrial membrane potential (ΔΨm), in contrast to apoptosis induced by traditional chemotherapeutic agents, which indirectly compromise mitochondrial function. Therefore, ATO may bypass resistance mechanisms associated with indirect apoptotic pathways.

Sordet et al. showed that the 12-0-tetra-decanoylphorbol-13-acetate-induced differentiation of leukemic cells inhibited etoposide-induced apoptosis by inhibiting cell death pathways upstream of mitochondrial targets [71]. Yet, these leukemic cells were sensitive to ATO-induced apoptosis. Treatment of NB4 cells with 1 µM ATO for 1-3 days caused a time-dependent increase in ΔΨm disruption and was associated with apoptosis, as confirmed by decreased cell viability, morphologic changes, and the formation of DNA ladders in an agarose gel [72, 73]. Treatment with ATO also induced the release of cytochrome c, triggering the caspase cascade and the breakdown of substrates responsible for the orderly disassembly of the cell that is characteristic of apoptosis [72, 74]. In addition to inducing apoptosis through the activation of death-effector molecules such as the caspases, ATO may downregulate antiapoptotic molecules such as bcl-2, which may contribute to ATO sensitivity [23, 69].

The sensitivity of malignant hematopoietic cells to ATO-induced apoptosis varies. Several studies have shown that cellular variability in the glutathione redox system is responsible for arsenic sensitivity [75]. Supporting studies have shown that the treatment of APL cells with ATO is associated with an accumulation of intracellular hydrogen peroxide [72]. No such accumulation is observed in cells...
that are typically resistant to ATO-induced apoptosis, such as U937 cells. Therefore, the sensitivity of leukemic cell lines to ATO can be predicted by measuring the levels of enzymes involved in controlling intracellular hydrogen peroxide concentrations, such as GPx (important for the catabolism of hydrogen peroxide), as well as enzymes important for the removal of ATO, such as glutathione-S-transferase.

The influence that intracellular hydrogen peroxide levels have on modifying a cellular response to ATO also gives potential utility to agents that increase intracellular hydrogen peroxide concentrations to sensitize cells to ATO-mediated apoptosis. Agents such as buthionine sulfoximine (BSO) and ascorbic acid have no effect on cell growth or apoptosis when used alone. However, when administered in conjunction with ATO, they can modulate the activity of hydrogen peroxide-processing enzymes in malignant cells to increase sensitivity to ATO-induced apoptosis.

This effect was observed when ascorbic acid was used in combination with ATO to induce apoptosis in multiple myeloma (MM) cells [75]. Ascorbic acid acts as an oxidizing agent that also reduces cellular glutathione levels, possibly through the formation of hydrogen peroxide. Sensitivity to ATO-induced apoptosis with ascorbic acid or BSO has been found in many malignant hematologic cell lines, such as MM and ATO-resistant APL, and in primary cultures of cells harvested from patients with chronic lymphocytic leukemia (CLL) and chemoresistant myeloma [75-79].

ATO induces partial differentiation and apoptosis, not only in APL cells, but in a variety of malignant cell lines that include MM, lymphoma, B- and T-cell lymphoblastic, and megakaryocytic leukemia [80-84]. ATO-induced apoptosis in most cells occurs through a caspase-dependent pathway mediated by hydrogen peroxide and is enhanced by agents that alter the enzymes involved in maintaining intracellular hydrogen peroxide levels (Table 3). These observations support further investigation into the broader use of ATO in the clinical management of hematologic malignancies other than APL.

### Table 3. Mechanisms of action of ATO-induced apoptosis

- ATO induces apoptosis through a caspase-3 (Casp-32)-dependent pathway.
- ATO-induced hydrogen peroxide accumulation may be a trigger for apoptosis.
- Low levels of the detoxification enzyme glutathione-S-transferase and the hydrogen peroxide-scavenging enzymes, glutathione peroxidase and catalase, contribute to arsenic sensitivity.
- ATO may induce hydrogen peroxide accumulation through inhibition of glutathione peroxidase, and this effect can be enhanced by the addition of ascorbic acid.

ATO = arsenic trioxide.

### Table 4. Reports of ATO in other (non-APL) malignancies

<table>
<thead>
<tr>
<th>Malignancy</th>
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<tbody>
<tr>
<td>Myeloid leukemia</td>
<td>Wang et al. [69]</td>
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<td>Li and Broome [86]</td>
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<td>Perkins et al. [85]</td>
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<td>Bachleitner-Hoffman et al. [98]</td>
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<td>Lymphoma, lymphocytic leukemia</td>
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<td>Zhu et al. [82]</td>
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APL = acute promyelocytic leukemia; ATO = arsenic trioxide.

**Potential Clinical Use of ATO Therapy in Other Hematologic Malignancies**

Recent reports suggest a rationale for the evaluation of ATO as possible therapy in other hematologic malignancies (Table 4). Studies of ATO-induced apoptosis in malignant cell lines in vitro have revealed the involvement of a number of mechanisms. Apoptosis occurs independently of the presence of the PML-RARα fusion protein, as observed in a variety of myeloid cell lines treated with ATO, including an NB4 APL cell line that lacks PML-RARα [69].

In vitro studies revealed that ATO-induced apoptosis is mediated by the activation of caspase-3 and caspase-8 [89]. The caspase-8 inhibitor, Ac-IETD-CHO, reduced the proportion of NB4 cells that underwent apoptosis following exposure to 1 μM ATO. Caspase-3 activation was also associated with apoptosis of KOCL33 B-cell leukemia cells in response to ATO. The KOCL33 line carries a t(11;19) translocation involving the mixed-lineage leukemia gene and a mixed-lineage leukemia translocation gene of chromosome 19 (LTG19) fusion protein that may be leukemogenic through the inhibition of physiologic apoptosis [89].

ATO also induced apoptosis in LyH7 cells, a murine B-cell leukemia line that overexpresses the bcl-2 protein. Western blot analysis showed that the synthesis of the bcl-2 protein decreased in preapoptotic and early apoptotic cells. Electron microscopy revealed that mitochondrial bcl-2 content remained about the same in these cells but the content in the Golgi apparatus, endoplasmic reticulum, and nucleus declined. Mitochondrial bcl-2 may protect against ATO-induced apoptosis in some cells [89].

ATO at pharmacologic concentrations of 0.01-1 μM can induce plasma cell apoptosis in myeloma cell culture systems and in bone marrow isolated from MM patients [80].
Investigations with an MM cell line showed that 2 µM ATO induced apoptosis associated with the downregulation of bcl-2, loss of mitochondrial transmembrane potential, and increase of caspase-3 activity [90]. Exposure of MM cells to low doses of ATO was followed by a marked increase in lymphokine-activated killer-cell-mediated killing and upmodulation of CD38 and CD54, two molecules involved in cell-to-cell interactions [91].

An in vivo model to study MM was established in severe combined immunodeficient (SCID) mice implanted with human fetal bone (SCID-hu model) [92]. Human primary myeloma cells engrafted and proliferated when injected into the medullary cavity of the human bone graft on one side, and metastases were observed within the contralateral bone graft after 12 weeks. ATO-treated SCID-hu mice, inoculated with human primary myeloma cells, exhibited a reduced secretion of human immunoglobulin and decreased bone marrow plasmacytosis in the engrafted fetal bone compared with untreated mice.

The results obtained in cell culture studies and the SCID-hu mouse model prompted the design of ATO studies in humans with MM. A pilot study was conducted to determine whether ATO could induce complete or partial remission in refractory or relapsed disease, with duration of remission and potential treatment toxicity noted as well [93]. Ten patients (median age 56 years) were involved in the study. Most had stage III disease and were heavily pre-treated, many having undergone autotransplantation and demonstrating chromosomal abnormalities (indicating the presence of proliferative disease), extensive bone marrow involvement, and high β2-microglobulin and C-reactive protein levels. Reductions in serum and urine myeloma protein were the primary clinical end points of the study.

After a median of 24 doses of ATO 0.15 mg/kg/day for 30 days, two patients demonstrated 25% and 50% reductions in serum myeloma protein levels with a duration of response of up to 6 weeks, while a third patient exhibited a 75% reduction but died within 1 week due to disease-related complications. Seven patients did not respond; however, of these, one had stable disease for 6 months and two had reductions in serum myeloma protein of <25%. The other four had increases of >25% [93].

In this patient population, in which existing treatment options are limited, eight patients developed grade III to IV toxicities that included pneumonia (n = 4), deep vein thrombosis (n = 3), bone pain (n = 2), fatigue (n = 2), bacteremia (n = 1), and fungemia (n = 1) [93]. These toxicities were due, at least in part, to the patients’ condition (i.e., late-stage disease in patients who had received extensive therapy previously). Yet, these results showed the activity of ATO in relapsed disease. Larger studies are under way to confirm the role of ATO as a single agent or in combination therapy in patients with MM. Preliminary results of these studies are further described by Hussein [94].

Human myeloid leukemia cells that are resistant to other agents that induce apoptosis are sensitive to physiologic concentrations of ATO. Cell lines derived from human chronic myelogenous leukemia (CML) patients (e.g., K562, HL-60/Bcr-Abl) and HL-60 cells that overexpress bcl-2, bcl-XL, or multiple drug resistance proteins will undergo apoptosis when exposed to 0.8-2.0 µM ATO for 7 days [85]. Apoptosis in these cells is associated with a cytosolic accumulation of cytochrome c, loss of mitochondrial membrane potential, and caspase production.

Apoptosis of CML-derived cells is associated with a decline in Bcr-Abl protein levels. In a number of cell lines, apoptosis has occurred with hyperacetylation of the intracellular histones H3 and H4. Several studies suggest the potential effectiveness of ATO as a tumor cell-specific agent in the treatment of CML. ATO induced apoptosis in Philadelphia chromosome-positive but not negative cell lines [95]. The apoptosis that occurred was not affected by Bcr-Abl kinase activity and did not influence colony formation in cultures of peripheral CD34+ precursor cells.

At a meeting in Shanghai in October 2000, Hu et al. reported the interim results of a study of ATO treatment in 34 patients with CML. CR, as determined by peripheral blood cell counts, bone marrow aspiration, and pathologic examination of the spleen, was achieved by 25 patients (74%) and partial remission was achieved by seven patients (21%). The median duration of remission was 58 days [96].

ATO may also be used to manage adult T-cell lymphocytic leukemia. In transgenic mice that develop lymphocytosis and splenomegaly, ATO and ATO plus ascorbic acid significantly delayed the appearance of these morbidities, as compared with ascorbic acid alone or placebo [97]. As discussed earlier, potentiation of the apoptotic effects of ATO appears to occur when this agent is combined with agents that reduce glutathione, such as ascorbic acid. This was demonstrated by Bachleiter-Hoffman et al., who showed such an enhancement of apoptosis in fresh AML cells [98].

A French study reported the preliminary results of a trial in eight patients with relapsed or refractory adult T-cell leukemia, who had previously received zidovudine, lamivudine, interferon-α (IFN-α), and chemotherapy. A median of 20 days’ treatment with ATO or ATO plus IFN-α yielded CR in one patient and partial remission in three patients. Median survival was 3 months, and one patient remained alive for at least 20 months [99].

The ability of ATO to induce growth inhibition and apoptosis in a variety of tumor cell lines in vitro indicates the significance of its anticancer effect. Results in experimental
animal models and preliminary clinical studies have reinforced this significance. Clinical trials are ongoing, or planned, to test the efficacy of ATO in managing MM, myeloid leukemia, lymphoma, and lymphocytic leukemia (Table 5). Separate trials for patients with relapsed or refractory CLL, AML, CML, and IFN-α-refractory or -intolerant CML are also ongoing or planned. Another study is designed to treat patients with Philadelphia chromosome-positive acute lymphoblastic leukemia (ALL) or CML in blast crisis.

Porosnicu et al. have demonstrated that combination therapy induces significantly more apoptotic cells expressing bcr-abl than does treatment with STI571, the Bcr-Abl tyrosine kinase inhibitor, or ATO alone. In addition, it may be prudent to pursue combination therapy in clinical trials for patients with CML as well as patients with Philadelphia chromosome-positive ALL [100]. Further discussion of the role of ATO, alone or in combination with agents such as STI571, in CML is addressed by O’Dwyer [101]. Also, additional trials are planned to evaluate ATO in the treatment of patients with high- and low-grade lymphoma and Hodgkin’s disease who have failed or are ineligible for autologous transplantation [102]. Finally, ATO is being evaluated in patients with myelodysplastic syndromes (MDS), and preliminary results from clinical trials in this disease are discussed by List [103].

SUMMARY AND CONCLUSIONS
Remarkable progress has been made in identifying the molecular changes that alter cell developmental processes leading to leukemogenesis. In APL, therapies such as ATRA and ATO, which specifically target cells expressing the PML-RARα fusion protein, have been identified. Such therapies have significantly enhanced the efficacy of cytotoxic chemotherapy. Although treatment with ATRA has greatly improved patient survival, 20%-30% of patients still relapse following combination therapy that includes ATRA. Recent studies have demonstrated the efficacy and safety of ATO in treating patients with relapsed APL, and ATO is now considered the standard of care in relapsed/refractory APL. Unlike patients treated with ATRA alone, patients treated with ATO alone have a high rate (78%) of molecular conversion to PML-RARα negativity (i.e., molecular remission). The multiple mechanisms by which ATO can induce cell differentiation and apoptosis suggest its potential for clinical application alone or combined with other agents to manage a variety of malignancies. Preclinical and preliminary clinical data from small, open-label studies support the broader study of the efficacy of ATO in MM, chronic and acute myeloid leukemia, lymphoma, lymphocytic leukemia, and other hematologic malignancies.

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Table 5. Hematologic malignancies that may respond to ATO therapy

- Multiple myeloma
- Acute lymphoblastic leukemia
- Chronic lymphocytic leukemia
- Acute myeloid leukemia
- Chronic myelogenous leukemia (chronic phase and blast crisis)
- Myelodysplastic syndromes
- Hodgkin’s disease
- Non-Hodgkin’s lymphoma

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