

## Chapter 2

# An Evidence-based Perspective of Arsenic Trioxide (As<sub>2</sub>O<sub>3</sub>) for Cancer Patients

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**Abstract** The ancient drug, arsenic, has remarkable efficacy in the treatment of relapsed acute promyelocytic leukemia (APL) and it is highly likely that a regimen for treatment of APL that does not require any traditional chemotherapy drugs will be developed in the future. Arsenic trioxide (white arsenic or As<sub>2</sub>O<sub>3</sub>) was approved by the United States Food and Drug Administration for being used in the treatment of relapsed/refractory APL in 2000. This success has led to exploration of its use in other malignancies. As<sub>2</sub>O<sub>3</sub> interacts with multiple molecular targets and signaling pathways. The resultant effect depends on factors, including cell type, and the dose and duration of As<sub>2</sub>O<sub>3</sub> exposure. Understanding the molecular and biological basis of these effects will promote the rational and optimal application of As<sub>2</sub>O<sub>3</sub> in diseases other than APL. A series of clinical trials with As<sub>2</sub>O<sub>3</sub> has confirmed its benefit in the therapy of APL, although its role in the treatment of other malignancies remains to be determined. Careful attention to the clinical management of patients on As<sub>2</sub>O<sub>3</sub> therapy can significantly lessen the risk of major side effects. The administration of As<sub>2</sub>O<sub>3</sub> can be done safely if careful attention to electrolyte abnormalities and electrocardiographic monitoring is undertaken. Here we provide an overview of the mechanism of action of arsenic and summarize its development in the treatment of APL and other malignant disorders.

## 2.1 Introduction

Arsenic trioxide (white arsenic or As<sub>2</sub>O<sub>3</sub>) has been used in medicine for more than 2,400 years for a variety of ailments including ulcers, the plague, and malaria (Waxman and Anderson 2001). In 1878, potassium arsenite was reported to have an anti-

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leukemic effect and was used for this purpose in the late nineteenth and early twentieth centuries until it was replaced by busulfan in the 1950s (Kwong and Todd 1997). 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 (Sears et al. 1988; Cutler and Bradford 1878). Researchers evaluated arsenic compounds for the treatment of various cancers and published the results of a trial in which intravenous administration of  $\text{As}_2\text{O}_3$  produced a complete response (CR) in 21 (66%) of 32 patients with acute promyelocytic leukemia (APL) (Wang and Chen 2008; Sun et al. 1992). In subsequent studies, Zhang et al. (1996) reported that  $\text{As}_2\text{O}_3$  induced a CR in 22 (73%) of 30 newly diagnosed and 22 (52%) of 42 relapsed APL patients, and Shen et al. (1997) observed a CR in nine (90%) of 10 relapsed APL patients.  $\text{As}_2\text{O}_3$  induces a high rate of complete remissions (87%) in this disease,  $\text{As}_2\text{O}_3$  was approved by the United States Food and Drug Administration for use in the treatment of relapsed/refractory APL in 2000 (Waxman and Anderson 2001; Grimwade et al. 2009). Treatment of APL patients with  $\text{As}_2\text{O}_3$  is associated with the disappearance of the promyelocytic leukemia (PML)-RAR $\alpha$  fusion transcript, the gene product of the chromosomal translocation t(15;17) characteristic of APL (Grimwade et al. 2009; Miller et al. 2002).

In contrast to Western medicine, traditional Chinese medicine (TCM) does not focus on a single target but multiple targets involved in a particular disease condition by applying diverse modalities. One major reason for reluctance of Western academia towards TCM is due to the lack of clinical studies of TCM recipes. This situation is changing recently, and a number of clinical studies were conducted on  $\text{As}_2\text{O}_3$  providing convincing evidence for the first time to gain credibility and reputation outside China. Clinical trials with  $\text{As}_2\text{O}_3$  remedies focus on three major fields in cancer research: (1) improvement of poor treatment response rates towards standard chemotherapy and radiotherapy, (2) reduction of severe adverse effects of standard cancer therapy, and (3) unwanted interactions of standard therapy with  $\text{As}_2\text{O}_3$ . Appropriate quality assurance and control of  $\text{As}_2\text{O}_3$  products as well as sustainable production methods are pre-conditions for the implementation of  $\text{As}_2\text{O}_3$  in cancer therapy at an international level.  $\text{As}_2\text{O}_3$  has also shown efficacy in other hematological malignancies and solid tumors (Emadi and Gore 2010). Therapeutic doses of  $\text{As}_2\text{O}_3$  are well tolerated, with no evidence of long-term toxicity. Adverse events include APL differentiation syndrome, electrocardio-graphic abnormalities, and mild elevations in liver enzymes. This chapter highlights trials investigating the role of  $\text{As}_2\text{O}_3$  in induction and consolidation for different cancers in view of the evidence based medicine. The chemistry, mechanisms of action, and clinical side effects of  $\text{As}_2\text{O}_3$  are also discussed.

## 2.2 History of Arsenicals Medicinal Use and Their Metabolism

Arsenic is a semimetal commonly found in soil, water, and air. Common inorganic and organic forms of arsenic are listed in Table 2.1. Often, arsenic complexes with sulfur as red arsenic ( $\text{As}_2\text{S}_2$ ), also called realgar or sandaraca, or as yellow arsenic

**Table 2.1** Arsenic in nature

Arsenic forms	Chemical formula	Other names
Red arsenic	As <sub>2</sub> S <sub>2</sub>	Realgar, sandaraca
Yellow arsenic	As <sub>2</sub> S <sub>3</sub>	Arsenikon, aurumpigmentum, orpiment
White arsenic	As <sub>2</sub> O <sub>3</sub>	–
Phenylarsineoxide	C <sub>6</sub> H <sub>5</sub> AsO	–

(As<sub>2</sub>S<sub>3</sub>), also called orpiment or auripigment. White arsenic (As<sub>2</sub>O<sub>3</sub>) is produced by heating realgar. Arsenic is rarely found in a pure state; rather, it exists in both trivalent and pentavalent oxidation states as a chemically unstable sulfide or oxide, or as a salt of sodium, potassium, or calcium. Trivalent arsenicals, including sodium arsenite and the more soluble As<sub>2</sub>O<sub>3</sub>, inhibit many enzymes by reacting with biological ligands that possess available sulfur groups. Being pentavalent, arsenic is recognized as an uncoupler of mitochondrial oxidative phosphorylation. Although most inorganic arsenic that is ingested is eliminated fairly rapidly in the urine, a small amount may be modified by methylation to monomethylarsonic acid and to dimethylarsinic acid, a process referred to as biotransformation. Whereas these enzymatic reactions are considered to be detoxifying, some organic arsenic metabolites may actually contribute to the cytotoxicity of arsenic (Mantadakis et al. 2008; Antman 2001).

Arsenic has also long been demonstrated to have anticancer activity in some cases in the old days in China. In TCM, As<sub>2</sub>O<sub>3</sub> was recorded in the Compendium of Materia Medica by one of the greatest physicians and pharmacologists Li Shi-Zhen (1518–1593). Medical use of arsenic and its derivatives dates back more than 2,400 years to ancient Greece, China, and Rome. Arsenic was considered as both a therapeutic agent and a poison. Hippocrates administered orpiment and realgar as an ulcer remedy; Dioscorides used orpiment as a depilatory. Arsenic has also been used to treat the plague, malaria, and cancer, and to promote sweating. Physicians prescribed arsenic for both external and internal use throughout the eighteenth century. Arsenides and arsenic salts were key ingredients in antiseptics, antispasmodics, antiperiodics, caustics, cholagogues, hematinics, sedatives, and tonics (Miller et al. 2002). Approximately 60 different arsenic preparations have been developed and distributed during the lengthy history of this agent. More than 20 of these preparations were still in use at the end of the nineteenth century, including Aiken’s Tonic Pills, Andrew’s Tonic, and Arsenauero. When physicians first boiled arsenous acid with an alkali in the late 1700s and produced a water-soluble compound, the administration of medicinal arsenic changed radically from generally external to primarily internal (Haller 1975; Jackson and Grainge 1975; Chan and Huff 1997; Bernstam and Nriagu 2000). In 1786, Thomas Fowler, a physician to the General Infirmary of the County of Stafford, England, recommended use of potassium arsenite for the treatment of intermittent fever. Fowler’s Solution gained great renown and was used to treat many conditions, including paralytic afflictions, rheumatism, hypochondriasis, epilepsy, hysteria, melancholia, dropsy, rachitis, heart palpita-

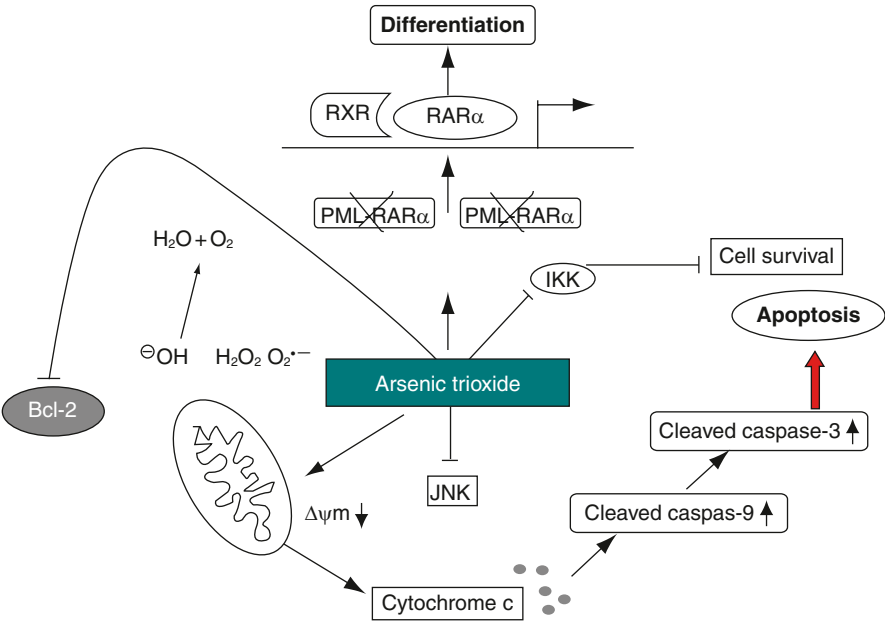
tions, convulsions, syphilis, ulcers, cancer, and dyspepsia. In 1911, Fowler's Solution was used as a treatment for pernicious anemia, asthma, psoriasis, pemphigus, and eczema. In 1910, additional experimentation with the properties of arsenic led Paul Ehrlich, the German physician and founder of chemotherapy, to the discovery of an organic arsenical, salvarsan. Arsphenamine was the standard therapy for syphilis for nearly 40 years before it was replaced by penicillin (Haller 1975; Jackson and Grainge 1975; Chan and Huff 1997; Bernstam and Nriagu 2000). Arsphenamine was also believed to be an effective treatment for trypanosomiasis. As above mentioned, arsenic has been proven recently to be highly effective in the treatment of APL; its use induces and maintains complete remissions, with a less toxic profile than traditional chemotherapy (Waxman and Anderson 2001). The role of arsenic in the treatment of other malignancies and hematologic disorders is much less clear but continues to be explored in combination with other modalities of therapy.

Arsenic was nicknamed "The Mule" not only for its dependability in many therapeutic regimens but also for the stubborn persistency with which it was used and the inconstant nature of its toxic capacity (Haller 1975; Jackson and Grainge 1975; Chan and Huff 1997; Bernstam and Nriagu 2000). Although arsenic was found to be beneficial in many disease states and side effects or later repercussions of therapy were inconsistent from patient to patient, concerns among medical professionals about toxicities associated with arsenic use, especially long-term use, surfaced in later years. The International Agency for Research on Cancer (IARC) first evaluated the carcinogenicity of arsenic and arsenic compounds in 1973. It found a "causal relationship between skin cancer and heavy exposure to inorganic arsenic in drugs, drinking water with a high arsenic content, or in the occupational environment". However, experimental studies with arsenic in animals were considered inadequate, and the causative role of arsenic remained largely unclear. In 1979, the agency classified arsenic and certain arsenic compounds as Group 1 carcinogens, those that were "carcinogenic to humans", on the basis of epidemiological studies of the relationship between exposure to arsenic compounds and skin cancer *via* occupation, ingestion, or medical use. In 1980, re-evaluation of the data determined that exposure to arsenic and arsenic-containing compounds were a cause of lung cancer in humans. Finally, in 1987, support for arsenic as a cause of human cancers was judged as adequate on the basis of "limited evidence of carcinogenicity in experimental animals" (Haller 1975; Jackson and Grainge 1975; Chan and Huff 1997; Bernstam and Nriagu 2000). Dermatological manifestations of arsenic use were commonly observed in patients with prolonged arsenic exposure. Long-term ingestion of Fowler's Solution produces signs of chronic arsenic intoxication. In one study, palmar and plantar keratoses occurred in all of the patients evaluated, although the minimum time to onset was 15 years (median, 24 years) after beginning arsenic treatment. Basal cell carcinoma developed in a majority of these patients, with the same time to onset. Other manifestations included carcinoma *in situ*, squamous cell carcinoma, breast adenocarcinoma, and colon carcinoma, all appearing within a minimum of 28–63 years after treatment. The precise amounts of arsenic these patients received over time is not known, because small amounts of

arsenic are present in the environment, and there are any number of possible routes of exposure. Nonetheless, despite the anecdotal nature of the evidence, the keratoses and carcinomas that developed were attributed to past and prolonged arsenic intake (Haller 1975; Jackson and Grainge 1975; Chan and Huff 1997; Bernstam and Nriagu 2000).

2.3 Mechanisms of Action of  $\text{As}_2\text{O}_3$

Exactly how  $\text{As}_2\text{O}_3$  mediates its clinical efficacy is not fully understood. Two main mechanisms of action of  $\text{As}_2\text{O}_3$  have been identified from both *in vivo* and *in vitro* studies: promotion of APL cell differentiation observed at low levels of  $\text{As}_2\text{O}_3$  and induction of apoptosis observed at high levels of  $\text{As}_2\text{O}_3$  (Fig. 2.1). It has become clear that  $\text{As}_2\text{O}_3$  interferes with a variety of cellular processes by targeting numerous different intracellular molecules and thereby disrupting key signal transduction mechanisms and producing programmed cell death. These findings underscore the importance of understanding how differences in cell types or cellular environments might affect the action of  $\text{As}_2\text{O}_3$  (Davison et al. 2002).



**Fig. 2.1** Cellular targets of Arsenic trioxide ( $\text{As}_2\text{O}_3$ ).  $\text{As}_2\text{O}_3$  targets multiple pathways in malignant cells resulting in apoptosis or in the promotion of the differentiation program

### 2.3.1 Differentiation and Oncogenic Proteins

As<sub>2</sub>O<sub>3</sub> has potent cytotoxic and antitumor activities *in vitro* and *in vivo*, there has been extensive research focused on the identification of the mechanisms by which it generates its effects on target cells. Degradation of the fusion protein, PML-RAR $\alpha$ , is most likely the mechanism by which As<sub>2</sub>O<sub>3</sub> induces cell differentiation in APL cells. Degradation of PML-RAR $\alpha$  allows malignant promyelocytes to overcome their maturation block (Novick and Warrell 2000). In clinical trials, cell samples taken from APL patients treated with As<sub>2</sub>O<sub>3</sub> suggest that partial differentiation of the maturation-arrested leukemia cells contributes to the therapeutic effect (Soignet et al. 1998). As<sub>2</sub>O<sub>3</sub> degrades the fusion protein and induces differentiation in APL cells whether or not they are resistant to retinoic acid. However, there is conflicting evidence whether As<sub>2</sub>O<sub>3</sub> can synergize with retinoic acid *in vitro* or *in vivo*. The partial differentiation effects of As<sub>2</sub>O<sub>3</sub> appear to be unique to APL because of the direct effect of As<sub>2</sub>O<sub>3</sub> on PML-RAR $\alpha$  degradation (Chen et al. 1997; Jing et al. 2001). Empirically, treatment of APL cells with As<sub>2</sub>O<sub>3</sub> leads to their terminal differentiation *in vitro* and *in vivo*. APL cells are uniquely sensitive due to the expression of the PML-RAR $\alpha$  fusion protein; however, the mechanism by which As<sub>2</sub>O<sub>3</sub> 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. In leukemic cells, the PML-RAR $\alpha$  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. PML contains a cysteine-rich region that is hypothesized to interact with As III, resulting in the degradation of PML-RAR $\alpha$  fusion protein. Furthermore, As<sub>2</sub>O<sub>3</sub>-induced histone acetylation has been reported to promote differentiation *via* alteration in gene transcription (Dyck et al. 1994; Chen et al. 1996; Wang et al. 1998; List et al. 2003). The degradation of PML-RAR $\alpha$  is a major mechanism of As<sub>2</sub>O<sub>3</sub> activity in APL, but it may also target other oncogenic proteins. As<sub>2</sub>O<sub>3</sub> treatment of human T-cell leukemia lymphoma virus (HTLV-1) containing acute T-cell leukemia cells resulted in degradation of Tax (a transactivator protein activated by HTLV-1) leading to apoptosis. Decreased levels of Bcr-Abl protein in K562 cells and other Bcr-Abl expressing cell lines has also been documented after As<sub>2</sub>O<sub>3</sub> treatment.

### 2.3.2 Apoptosis

As<sub>2</sub>O<sub>3</sub>-induced apoptosis, in contrast, occurs *via* a variety of mechanisms, which appear to be independent of the presence of PML-RAR $\alpha$ . The apoptotic effects of As<sub>2</sub>O<sub>3</sub> occur, in part, through direct effects on mitochondria. An important initial cellular event that occurs during treatment of target cells with As<sub>2</sub>O<sub>3</sub> involves elevation of reactive oxygen species (ROS). Such generation of ROS appears to be regulated, at least in part, by activation of nicotinamide adenine dinucleotide phosphate oxidase and ni-

tric oxide synthase isozymes. Also, arsenic-containing compounds are potent modulators of the thioredoxin system that includes thioredoxin, thioredoxin reductase, and nicotinamide adenine dinucleotide phosphate. The thioredoxin system controls, to a large extent, intracellular redox reactions, regulates apoptosis, and protects cells from stress damage, and the ability of arsenic-containing compounds to target and block thioredoxin reductase may be important in the induction of its pro-apoptotic effects.

### 2.3.2.1 Reactive Oxygen Species

Over-production of ROS is linked to the induction of apoptosis by  $\text{As}_2\text{O}_3$ . Accumulation of hydrogen peroxide leads to decreases in the mitochondrial membrane potential, resulting in cytochrome *c* release and activation of the caspase cascade. This appears to be a common mechanism of induction of cell death in diverse cellular backgrounds. There is extensive evidence implicating arsenic-dependent, ROS-mediated activation of caspases in various types of malignant cells. These include cells of APL origin, human T cell lymphotropic virus I-infected T cell lines and primary adult T cell leukemia cells (Nishikawa 2008; Dai et al. 1999; Sen 1998), multiple myeloma cells, and different types of solid tumor cells. However, caspase-independent death pathways have been also reported to be activated by arsenic in myeloma cells and may mediate pro-apoptotic signals (Nishikawa 2008; Dai et al. 1999; Sen 1998). Other recent work has implicated the c-Jun N-terminal kinase (JNK) as an essential component of  $\text{As}_2\text{O}_3$ -dependent apoptosis (Nishikawa 2008; Dai et al. 1999; Sen 1998). It was demonstrated that activation of JNK occurs in an  $\text{As}_2\text{O}_3$ -inducible manner in cells of APL origin and that  $\text{As}_2\text{O}_3$  resistance correlates with defective activation of the JNK pathway. Notably, in these studies, it was also shown that pharmacological inhibition of JNK significantly decreases  $\text{As}_2\text{O}_3$ -dependent growth inhibition and apoptosis, but it does not protect cells from the effects of chemotherapy (doxorubicin) (Nishikawa 2008; Dai et al. 1999; Sen 1998).

### 2.3.2.2 Bcl-2

A further mitochondria-related effect is the ability of  $\text{As}_2\text{O}_3$  to down-regulate *Bcl-2* expression. This has been associated with  $\text{As}_2\text{O}_3$ -mediated growth inhibition and apoptosis in a variety of cell types, although it has not been a universal finding (Lu et al. 1999; Zhang et al. 1998).

### 2.3.2.3 NF- $\kappa$ B

NF- $\kappa$ B is a transcriptional factor promoting cell survival with an important role in many cancer cells. Activation of NF- $\kappa$ B depends on the integrity of the I $\kappa$ B kinase (IKK); upon phosphorylation by IKK, the inhibitory protein I $\kappa$ B releases NF- $\kappa$ B for translocation to the nucleus.  $\text{As}_2\text{O}_3$  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 (Kapahi et al. 2000).

### **2.3.3 Anti-proliferative/Cell Cycle Effects**

Interference with cell cycle progression is a major factor in the growth inhibition caused by  $\text{As}_2\text{O}_3$  as it impairs proliferation and may lead to apoptosis or sensitize cells to differentiation. The cell cycle effects of  $\text{As}_2\text{O}_3$  vary considerably depending on the concentration and cell type. It has been associated with both a prolongation of the cell cycle as well as cell cycle arrest of malignant hematopoietic cells in the G1 and G2/M phases (Park et al. 2001).

### **2.3.4 Angiogenesis Inhibition**

Angiogenesis is a vital process for the growth and survival of solid tumors and a consistent pathological feature in hematological malignancies. The bone marrow of patients with conditions including acute and chronic leukemias, myelodysplastic syndrome, non-Hodgkin lymphoma (NHL), and multiple myeloma demonstrate increased microvascular density and vascular endothelial growth factor (VEGF) levels. In a mouse fibrosarcoma model, a single dose of  $\text{As}_2\text{O}_3$  induced apoptosis of new blood vessel endothelial cells and ischemic necrosis of the tumor while sparing normal tissues. Human umbilical vein endothelial cells have also been used as a model to study the anti-angiogenic effects of  $\text{As}_2\text{O}_3$ . In this system,  $\text{As}_2\text{O}_3$  induced the activation of endothelial cells, up-regulation of endothelial cell adhesion molecules, inhibition of capillary tubule growth and vessel branching, apoptosis of endothelial cells, and inhibition of VEGF production.  $\text{As}_2\text{O}_3$  also inhibited VEGF production in the human erythroleukemia leukemic cell line. It is thought that a reciprocal positive feedback loop exists between leukemic cells producing VEGF and the stimulated, rapidly proliferating endothelial cells producing leukemic cell growth factors (including granulocyte macrophage colony stimulating factor, interleukin IL-6, IL-7, and IL-10), and that  $\text{As}_2\text{O}_3$  may disrupt this loop (Roboz et al. 2000).

## **2.4 From Bedside to Bench, Then to Bedside Again**

The clinical results achieved with  $\text{As}_2\text{O}_3$  in APL have prompted investigations into the mechanisms of action by which arsenic produces clinical benefit. This “bedside to bench” approach has shown that  $\text{As}_2\text{O}_3$  has multiple targets. Chen et al. (1996) determined that, at low concentrations,  $\text{As}_2\text{O}_3$  promotes differentiation of APL cells

and, at higher concentrations, triggers apoptosis and down-regulates Bcl-2 expression. Subsequently, an intense research effort focused on whether these effects were unique to APL cells or to a more general response by different types of neoplastic cells (Xu et al. 2009; Jutooru et al. 2010; Lee et al. 2010; Subbarayan et al. 2010; Tingting et al. 2010; Wu et al. 2010). Considerable pre-clinical evidence supports the potential of As<sub>2</sub>O<sub>3</sub> against a number of different malignancies. Studies in cultured cells show that it inhibits growth and promotes apoptosis in myeloid leukemia, multiple myeloma, lymphoma, and lymphocytic leukemia, and solid tumor cell lines. As<sub>2</sub>O<sub>3</sub> exerts its cytotoxic effects on neoplastic cells by inhibiting proliferation, inducing apoptosis, and promoting cellular differentiation. Anecdotal and preliminary clinical data from China suggest a broad therapeutic potential for As<sub>2</sub>O<sub>3</sub> in the treatment of cancer. These findings support further investigation of the clinical utility of As<sub>2</sub>O<sub>3</sub> for the treatment of hematologic and solid tumor malignancies other than APL (Wang et al. 1998; Perkins et al. 2000; Yoo et al. 2009; Zhang et al. 2009; Zhao et al. 2009).

### 2.4.1 Clinical Trials in Hematologic Malignancies

There are several diseases in which As<sub>2</sub>O<sub>3</sub> have been studied in clinical trials. Hematologic malignancies studied include APL, multiple myeloma, acute myeloid leukemia (AML), acute lymphoblastic leukemia, adult T-cell leukemia/lymphoma (ATL), myelodysplastic syndrome, NHL, and chronic myeloid leukemia (Dai et al. 1999; Zhu et al. 1999; Zhou et al. 2010; Munshi 2001). In a recent study, a total of 19 children (< or = 15 years of age) with newly diagnosed APL were treated with single-agent As<sub>2</sub>O<sub>3</sub> for remission induction and post-remission therapy. The results suggested that seventeen of the children (89.5%) achieved complete hematologic remission, and two early deaths occurred from intracranial hemorrhage. As<sub>2</sub>O<sub>3</sub>-induced leukocytosis was observed in 13 (68.4%) patients. Other As<sub>2</sub>O<sub>3</sub>-related toxicities were minimal and transient. Post-remission As<sub>2</sub>O<sub>3</sub> therapy has continued for 3 years; the most commonly side effect was As<sub>2</sub>O<sub>3</sub>-induced neutropenia. With a median follow-up of 53 months (range, 23–76 months), the calculated 5-year overall survival and event-free survival were 83.9% and 72.7%, respectively, which are comparable with results achieved by the use of all-*trans* retinoic acid (ATRA) plus chemotherapy, which is the standard therapy for APL. No chronic arsenic toxicity or second malignancies were found during the follow-up period, and arsenic retention was not significant in patients off treatment more than 24 months. As<sub>2</sub>O<sub>3</sub> resistance was observed in only one patient with a complex karyotype. The results indicate the high efficacy and safety of single-agent As<sub>2</sub>O<sub>3</sub> regimens in the treatment of children with *de novo* APL.

Here we also summarize the clinical trials of As<sub>2</sub>O<sub>3</sub> in other hematologic malignancies (Table 2.2). ATL is resistant to chemotherapy and carries a dismal prognosis particularly for the acute and lymphoma subtypes. Chronic ATL has a relatively better outcome, but poor long-term survival is noted when patients are managed with a watchful-waiting policy or with chemotherapy. Clinically, arsenic/interferon therapy

**Table 2.2** Clinical studies of Arsenic trioxide ( $As_2O_3$ ) in hematologic malignancies other than acute promyelocytic leukemia

Hematologic malignancies	Phase	N	Regimen	Adverse effects	Results	References
AML	I/II	64	$As_2O_3$ 0.25 mg iv + low-dose cytarabine twice daily	Neutropenic fever was observed in >80% of patients and 41% of patients had bacteremia. Non-hematologic toxicity generally was mild and reversible, included fatigue, nausea, diarrhea, rash, peripheral edema, and elevated transaminases	Improve responses in elderly AML patients compared with either agent alone	Roboz et al. (2008)
Relapsed or refractory lymphoid malignancies	II	17	$As_2O_3$ 0.25 mg/kg iv + AA 1,000 mg iv for 5 days/1st week followed by twice weekly infusions during week 2–6 (repeated every 8 week)	Hematologic toxicities were the most commonly reported	Generally well tolerated but had limited activity in patients	Chang et al. (2009)
Relapsed or refractory MM	I/II	22	$As_2O_3$ 0.125 or 0.250 mg/kg + bortezomib 0.7, 1.0, 1.3 mg/m <sup>2</sup> + AA 1 g iv on day 1, 4, 8, 11 of a 21-day cycle for max 8 cycles	One occurrence of grade 4 thrombocytopenia was observed. One patient had asymptomatic arrhythmia and withdrew from the study	Well tolerated by most patients and produced preliminary signs of efficacy with 27% objective response rate	Berenson et al. (2007)
Relapsed or refractory MM	II	31	Melphalan 0.1 mg/kg po + $As_2O_3$ 0.25 mg/kg iv + AA 1 g iv on day 1–4 of week 1, $As_2O_3$ + AA twice weekly during week 2–5 and no treatment during week 6 of cycle 1	Specific grade 3/4 haematological (3%) or cardiac adverse events occurred infrequently. Frequent grade 3/4 non-haematological adverse events included fever/chills (15%), pain (8%) and fatigue (6%)	A new therapeutic option for patients with relapsed/refractory MM	Berenson et al. (2006)
MM	II	20	$As_2O_3$ + dexamethasone + AA	The regimen was well tolerated with most adverse events being mild or moderate	Showed the clinical efficacy and tolerability of this combination	Abou-Jawde et al. (2006)

Table 2.2 (continued)

Hematologic malignancies	Phase	N	Regimen	Adverse effects	Results	References
MM	II	24	As <sub>2</sub> O <sub>3</sub> 0.25 mg/kg/day for 5 days/1st 2 week of each 4-week cycle	Sixteen patients had grade 3 or 4 neutropenia and one required antibiotics	Active and reasonably well tolerated as a single-agent even in patients with late-stage, relapsed/refractory MM	Hussein et al. (2004)
Myeloid leukemia	II	11	As <sub>2</sub> O <sub>3</sub> 0.25 mg/kg/day	Myelosuppression was the major adverse effect, most likely due to disease progression rather than drug-related	All subjects had progressive disease and there was no direct treatment-related mortality	Parmar et al. (2004)
MM	II	14	As <sub>2</sub> O <sub>3</sub> iv	Although well tolerated, in these patients with extensive prior therapy, 11 developed cytopenia, five associated with infectious complications and three developed deep vein thromboses	Support further investigation of this novel drug for the treatment of patients with relapsed/refractory MM	Munshi et al. (2002)
Relapsed/refractory myeloma	I/II	6	As <sub>2</sub> O <sub>3</sub> 0.25 mg/kg/day + AA 1,000 mg/day for 25 days over a 35-day period	One episode of grade 3 hematological toxicity (leukopenia) and no grade 3 non-hematological toxicities (in particular cardiac) were observed	Two patients with thalidomide-refractory disease had partial responses; four patients had stable disease. This regimen has acceptable toxicity and promising evidence of activity in refractory/relapsed myeloma	Bahlis et al. (2002)

AA: ascorbic acid, AML: acute myeloid leukemia, As<sub>2</sub>O<sub>3</sub>: Arsenic trioxide, MM: multiple myeloma

exhibits some efficacy in refractory aggressive ATL patients. Promising results were obtained from Phase II study of the efficacy and safety of the combination of  $As_2O_3$ , interferon alpha, and zidovudine in newly diagnosed chronic ATL. Among 10 newly diagnosed chronic ATL patients, an impressive 100% response rate was observed, including seven complete remissions, two complete remissions but with more than 5% circulating atypical lymphocytes, and one partial response. Responses were rapid and no relapse was noted. Side effects were moderate and mostly hematologic. The results suggested that treatment of chronic ATL with arsenic, interferon-alpha, zidovudine is feasible and exhibits an impressive response rate with moderate toxicity (Kchour et al. 2009). The addition of  $As_2O_3$  to low-dose cytarabine was also reported to improve responses in elderly patients who had AML compared with either agent alone, and a randomized trial of the combination *versus* single-agent low-dose cytarabine is ongoing.

The utility of  $As_2O_3$  in the treatment of a number of lymphoid malignancies is also being evaluated as well. At Memorial Sloan-Kettering Cancer Center, patients with relapsed or refractory intermediate- or high-grade NHL are being treated with  $As_2O_3$ , 0.25 mg/kg/day, 5 days a week for 5 weeks. In a trial at Mount Sinai Hospital (New York, NY, USA), patients with relapsed or refractory low-grade NHL or chronic lymphoid leukemia (CLL) are eligible for treatment with  $As_2O_3$ . Investigators at Northwestern University (Evanston, IL, USA) have undertaken a trial of  $As_2O_3$  0.25 mg/kg/day for 60 days in patients with relapsed or refractory Hodgkin's disease. MD Anderson Cancer Center has investigated  $As_2O_3$  in patients with relapsed or refractory CLL. Clinical evaluation of  $As_2O_3$  for the treatment of multiple myeloma is under way too. Combination therapy with  $As_2O_3$  and ascorbic acid is also being studied in patients with relapsed or refractory multiple myeloma. The addition of ascorbic acid is linked to observations that, at least in part, the glutathione redox system mediates the inhibition of growth and induction of apoptosis that follow exposure to  $As_2O_3$ . Earlier work showed that ascorbic acid decreases glutathione levels and renders malignant cells, but not normal cells, more sensitive to  $As_2O_3$ -induced apoptosis. In a mouse model, ascorbic acid enhanced the anti-lymphoma effect observed in response to arsenic treatment, without additional toxicity. Abundant pre-clinical evidence shows that  $As_2O_3$  inhibits growth and promotes apoptosis in many different cancer cell types. This broad mechanism of action supports a potential for clinical activity in numerous neoplastic diseases other than APL and provides a basis for further clinical evaluation of  $As_2O_3$  in hematologic malignancies and solid tumors. The dose and dosing regimen required for clinical response in such cancers may be different from those for APL (Dai et al. 1999; Zhu et al. 1999; Zhou et al. 2010; Munshi 2001).

#### **2.4.2 Clinical Trials in Solid Tumors**

Based on promising pre-clinical data, clinical trials to examine the potential of  $As_2O_3$  for the treatment of solid tumors are under way or in the final planning stages (Table 2.3). But almost all the clinical trials indicated single-agent  $As_2O_3$

**Table 2.3** Clinical studies of Arsenic trioxide (As<sub>2</sub>O<sub>3</sub>) in solid tumors

Tumor type	Phase	N	Regimen	Adverse effects	Results	References
Metastatic melanoma	II	10	As <sub>2</sub> O <sub>3</sub> 0.25 mg/kg/day for 5 days followed by 0.35 mg/kg/day twice a week	Grade 3 toxicity included neutropenia, fatigue, abdominal pain and arthralgia. Grade 4 toxicity did not occur	Generally well tolerated, but no tumor regression was observed	Kim et al. (2005)
Advanced metastatic melanoma	II	21	As <sub>2</sub> O <sub>3</sub> 0.32 mg/kg/day for 4 days in week 1 followed by 0.25 mg/kg/day twice a week for 6 weeks followed by 1 week rest	Possible treatment-related grade 3 of 4 toxicities included idiopathic thrombocytopenic purpura (1) and elevated lactate dehydrogenase (1)	Well tolerated and had limited activity in patients with metastatic melanoma	Tarhini et al. (2008)
Metastatic melanoma	II	11	As <sub>2</sub> O <sub>3</sub> 0.25 mg/kg/day+AA 1,000 mg for 5 days and then twice weekly at 0.35 mg/kg during an 8-week cycle	Common grade 1 and 2 side effects included nausea and vomiting (10), fatigue (6), edema (6), rash (6) and elevated AST or ALT (6). Grade 3 and 4 side effects included nausea and vomiting (3), elevated AST or ALT (2), seizure (1) and renal failure (1)	No responses were seen in the 1st 10 evaluable patients leading to early closure of the study	Bael et al. (2008)
Pancreatic adenocarcinoma refractory to gemcitabine	II	13	As <sub>2</sub> O <sub>3</sub> 0.3 mg/kg for 5 days every 28 days	Anemia (50%) and leukopenia (25%), fatigue and thrombosis (17%), prolongation of the QTc interval (1) occurred in patients	Has no activity in pancreatic cancer patients who develop progressive disease after gemcitabine	Kindler et al. (2008)

Table 2.3 (continued)

Tumor type	Phase	N	Regimen	Adverse effects	Results	References
Hepatocellular carcinoma	II	29	As <sub>2</sub> O <sub>3</sub> 0.16–0.24 mg/kg/day for 5–6 days/week for 3–4 week followed by 1-week rest	Fatigue, rash, abdominal pain and nausea are reported	Not active against advanced hepatocellular carcinoma	Lin et al. (2007)
Metastatic renal cell carcinoma	II	16	As <sub>2</sub> O <sub>3</sub> 0.3 mg/kg/day iv for 5 days every 4 week	The most common toxicity observed was grade 2 elevations in liver function tests (36%), anemia (21%), renal insufficiency (14%), rash (7%) and diarrhea (7%)	Best response was stable disease in three patients, but did not achieve a complete or partial response in metastatic renal cell carcinoma	Vuky et al. (2002)
Advanced head and neck cancer	I	11	As <sub>2</sub> O <sub>3</sub> 10, 20, 30 mg/week a day prior to hyperthermia	No amplification of toxicities due to radiation or hyperthermia was evident	Patients who received 30 mg of As <sub>2</sub> O <sub>3</sub> weekly showed non-serious acute toxicities and patients without prior treatment showed better response	Huilgol (2006)

AA: ascorbic acid, AdenoCA: adenocarcinoma, As<sub>2</sub>O<sub>3</sub>: Arsenic trioxide

was generally well tolerated; however, no tumor regression was observed in most of the solid tumor patient situation. Future clinical trials should evaluate  $\text{As}_2\text{O}_3$  in combination with other anticancer drugs that may improve its clinical activity in solid tumor.  $\text{As}_2\text{O}_3$  cytotoxicity and apoptosis induction has been demonstrated with numerous solid tumor cell lines, including human melanoma, hepatocellular carcinoma (HCC), gastric carcinoma, etc. A recent second-line, Phase II, single-arm study of  $\text{As}_2\text{O}_3$  was conducted recently in patients with inoperable American Joint Committee on Cancer stage IV melanoma. Twenty-one patients (median age, 63.8 years) were accrued in the study. All had stage IV melanoma including M1a (two patients), M1b (six patients), and M1c (13 patients) disease. Among 17 evaluable patients, one patient (6%; 95% confidence interval (CI), 0–29%) achieved a partial response lasting 7 months, and 10 patients (59%) had disease stabilization after at least one cycle, but all eventually developed disease progression. The median time to disease progression was 17 weeks (95% CI, 11–38 weeks) and the median survival was 13 months (95% CI, 12–26 months). Their results suggested that  $\text{As}_2\text{O}_3$  as tested in the current trial was found to be well tolerated and had limited activity in patients with metastatic melanoma. The application of this agent in combination with either chemotherapy or agents that target recognized critical signaling and anti-apoptotic pathways of melanoma has not yet been performed (Tarhini et al. 2008). There is no effective therapy for patients with metastatic pancreatic cancer who fail initial therapy with gemcitabine. Previous studies had indicated that  $\text{As}_2\text{O}_3$  has potent anti-proliferative and pro-apoptotic effects in pancreatic cancer cell lines. A multicenter Phase II trial in patients with advanced pancreatic cancer who experienced disease progression on or after a gemcitabine-containing regimen was conducted in USA. Thirteen patients were enrolled between December 2002 and November 2003. Twenty-four cycles were administered (median 2; range 1–2). In this study,  $\text{As}_2\text{O}_3$  0.3 mg/kg was administered intravenously over 1 hour daily for 5 consecutive days every 28 days. Re-staging computed tomography scans were obtained every two cycles. Their results indicated that 13 patients were enrolled between December 2002 and November 2003. Twenty-four cycles were administered (median 2; range 1–2). There were no grade 3/4 hematologic toxicities; grade 1/2 anemia and leukopenia occurred in 50% and 25% of patients, respectively. Grade 3 toxicities included fatigue and thrombosis in 17% of patients. Only one patient developed a prolongation of the QTc interval. There were no objective responses. Median progression-free survival was 1.6 months (95% CI, 1.2–1.9). Median survival was 3.8 months (95% CI, 1.6–6.8) (Kindler et al. 2008). Despite promising *in vitro* data,  $\text{As}_2\text{O}_3$  has no activity in pancreatic cancer patients who develop progressive disease after gemcitabine.

Early reports also indicated that single-agent  $\text{As}_2\text{O}_3$  was not active against advanced HCC (Lin et al. 2007). As all the clinical trials indicated, despite promising *in vitro* data,  $\text{As}_2\text{O}_3$  has no activity in solid tumor patients who develop progressive disease. Multicenter Phase II trials are feasible in this patient population, and novel agents are clearly needed.

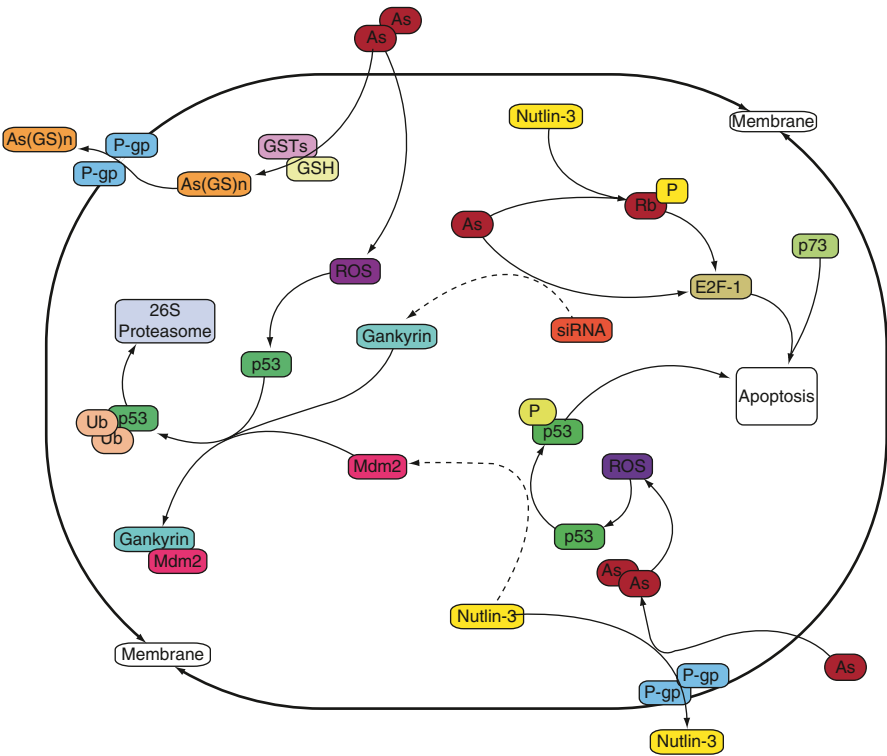
## 2.5 Mechanisms of Resistance to $\text{As}_2\text{O}_3$

As an ancient TCM,  $\text{As}_2\text{O}_3$  has been successfully used as a therapeutic agent for leukemia. Drug resistance and toxicity are major concerns with the treatment. Although a significant anticancer effect of  $\text{As}_2\text{O}_3$  had been reported in pre-clinical and clinical trials, failure of chemotherapy was reported frequently in patients and some cell lines. Among the reasons anticancer drugs fail, the most common is acquired drug resistance. Resistance of cancer cells to  $\text{As}_2\text{O}_3$  continues to be a major clinical obstacle to the successful treatment of cancer. At present, the anticancer drug resistance is considered as a multifactorial phenomenon involving several major mechanisms, such as decreased uptake of water-soluble drugs, increased repair of DNA damage, reduced apoptosis, altered metabolism of drugs, and increased energy-dependent efflux of chemo-therapeutic drugs that diminish the ability of cytotoxic agents to kill cancer cell, changes in glutathione transferase expression and topoisomerase II. Causes of cancer-specific drug resistance are currently believed to be linked to the random drug-induced mutational events (genetic hypothesis), to the drug-induced non-mutational alterations of gene function (epigenetic hypothesis), and recently, to the drug-induced karyotypic changes. Unfortunately, the key determinants of this phenomenon remain largely unknown (Lehnert 1998; Andrew et al. 2004; Glasspool et al. 2006; Iwasa et al. 2006; Duesberg et al. 2007; Fojo 2007; Roberti et al. 2006).

Increasing efflux of anticancer drugs is most commonly encountered in laboratories and clinical medication mediated by membrane transporters. The ABC (ATP-binding cassette) transporter family is well known in this area, especially such members as ABCB1 (P-gp, P-glycoprotein), MDR1 (multidrug-resistance gene 1), ABCC1 (multidrug-resistant protein 1, MRP1), ABCC2 (MRP2) and ABCG2 (breast cancer resistance protein, BCRP). The MRPs have been functionally characterized as the property of ATP-dependent export pumps for conjugates with glutathione (GSH), glucuronate, or sulfate. With respect to arsenic compounds, GSH S-transferase (GST) has been reported to conjugate GSH to arsenicals and several GST isoforms and genetic polymorphisms might influence arsenic metabolism and susceptibility. It should be pointed out that the arsenic-GSH conjugates are the substrates of some ABC transporter proteins, which efflux them outside mammalian cells. In  $\text{As}_2\text{O}_3$ -resistant human leukemia cell line K562/AS-3, the main mechanism of tolerance seems to be drug efflux by MRP1. Chronic exposure to low-concentration  $\text{As}_2\text{O}_3$  or methylated arsenicals can increase the expression of P-gp, MRP1 and MRP2 at both the protein and mRNA level. However, there is no established arsenic-resistant human solid tumor cell line (Keppler et al. 1998; Kala et al. 2000; Ejendal and Hrycyna 2002; Deeley et al. 2006; Schläwicke Engström et al. 2007). Therefore, which of the ABC members contribute to arsenic resistance by actively pumping arsenic compounds outside solid tumors derived from the human digestive tract is still uncertain.

It is known that  $\text{As}_2\text{O}_3$  induces DNA damage and p53 accumulation and that  $\text{As}_2\text{O}_3$ -induced apoptosis is required for p53 (Jiang et al. 2001; Filippova and Duerksen-Hughes 2003). Reducing the expression or inhibiting the function of p53 may

abrogate the apoptosis of tumor cells induced by  $\text{As}_2\text{O}_3$ . Several signaling pathways are involved in the regulation of p53; of these, ubiquitylation is the most common way for the degradation of p53 through the binding of MDM2 to p53 (Yang et al. 2004). Previously, studies further verify that gankyrin promotes both mono- and poly-ubiquitylation of p53 by MDM2 in a p300-independent manner (Higashitsuji et al. 2005). Direct interaction between gankyrin and MDM2 is necessary to enhance p53 ubiquitylation and gankyrin facilitates the binding of MDM2 to p53 *in vivo* and *in vitro*. This suggests that gankyrin could be a key point in the regulation of p53 and may contribute to the establishment of resistance to  $\text{As}_2\text{O}_3$  in tumor cells. In a previous study, Chen et al. (2009) established two arsenic-resistant solid tumor cell lines, HepG2/AS and SGC7901/AS to investigate the reasons of resistance. Their results indicate that HepG2/AS seems to efflux arsenic compounds mainly by ABCB1, ABCC1, and ABCC2, whereas SGC7901/AS seems to efflux arsenic compounds mainly by ABCB1. Inactivation of ABCB1 by higher concentration of verapamil can restore the sensitivity of  $\text{As}_2\text{O}_3$  in HepG2/AS and SGC7901/AS. Increasing the levels of gankyrin and MDM2 may enhance the degradation of p53 and the phosphorylation of Rb, resulting in abrogation of apoptosis (Fig 2.2). A better



**Fig. 2.2** The probable mechanisms of Arsenic trioxide ( $\text{As}_2\text{O}_3$ ) in solid tumor cells (e.g., HepG2/As) and the role of Nutlin-3 or siRNA gankyrin in overcoming this resistance

understanding of arsenic resistance in solid tumor cells may provide novel targets for treating arsenic-resistant tumors and promote screening of suitable patients. To evaluate whether inhibition of transporters or activation of p53 enhance the sensitivity to  $As_2O_3$  in liver cancer cells, Chen et al. (2009) used siRNA against gankyrin and molecular inhibitor nutlin-3 to reactivate p53. It was found the down-regulation of gankyrin or nutlin-3 could not inhibit cell proliferation in arsenic resistant cells. The combination of nutlin-3 with  $As_2O_3$  showed significant lethal effect in sensitive and resistant cell lines. Nutlin-3 increased intracellular arsenicals through the inhibition of drug efflux of P-gp and induced significant apoptosis in sensitive and resistant cell lines when combined with  $As_2O_3$ . The combination of nutlin-3 and  $As_2O_3$  induced the aggregation of p-p53 (ser392) in nucleus and activated E2F-p73 pathway in resistant cells. This therapeutic alliance showed effective anticancer effect in all the liver cancer cells and could be a more potential way in reversal of drug resistance (unpublished data).

## 2.6 MicroRNA and $As_2O_3$ in Cancer Research

MicroRNAs (miRNAs) are a class of endogenous, small, single stranded non-coding RNA molecules. They negatively regulate protein expression by binding to the 3' untranslated regions (3'UTR) of mRNAs and inhibiting translation or inducing mRNA degradation. MiRNAs have been shown to regulate a wide range of biological functions such as cell proliferation, differentiation, development, signaling pathways, apoptosis, and cell death. Currently, extensive studies have indicated the existence and importance of another mechanism of non-mutational regulation of gene function mediated by means of short non-coding RNA. As the name implies, miRNAs are small RNAs usually 19–23 bp in length or shorter, that are produced in all mammalian cells. Lacking the ability to encode a protein, these single-stranded miRNAs bind mainly to the 3'UTR of protein encoding mRNAs through sequences that are imperfectly complementary. The consequences of miRNA binding are that either the bound mRNA is silenced or degraded, resulting in reduced levels of the protein encoded by the mRNA. Aberrant levels of miRNA have been reported in a variety of human cancers (Cho 2010a). They have been shown to have both diagnostic and prognostic significance and to constitute a novel target for cancer treatment (Cho 2010b). Recently the evidence of the roles for miRNAs in determining drug sensitivity/resistance has been emerging (Bartel 2004; Lim et al. 2005; Lu et al. 2005; Calin and Croce 2006; Sevignani et al. 2006; Bushati and Cohen 2007; Barbarotto et al. 2008).

In a previous study, Meng et al. (2010) used miRNA microarrays and studied the alteration of miRNA expression profile in HCC cells after  $As_2O_3$  treatment. Their results showed that  $As_2O_3$  did alter specific miRNA expression in HCC cells, among which we found *miR-29a* might play an important role. Furthermore, they predicted and verified target genes of *miR-29a* which might be involved in the mechanisms of  $As_2O_3$  therapy. In this study, treatment of HepG2 cells with  $As_2O_3$

increased the expression of *miR-29a* and also down-regulated the expression of its target gene *PPMID*, and the anti-*miR-29a* inhibitor suppressed the effect of As<sub>2</sub>O<sub>3</sub> on *PPMID*, which indicated that one of the important pathways of As<sub>2</sub>O<sub>3</sub> on cancer cells is As<sub>2</sub>O<sub>3</sub> → *miR-29a* → *PPMID* → *Wip-1* → *p53* → cancer cell growth inhibition and apoptosis. It is exciting to speculate that *miR-29a* may represent the first non-coding miRNA to be related to As<sub>2</sub>O<sub>3</sub> treatment in cancers. High level of *miR-29a* may render the cancer cells more susceptibility to As<sub>2</sub>O<sub>3</sub> treatment (Meng et al. 2010). *MiR-21*, one of the most prominent miRNAs in the genesis and progression of many human cancers, has been rarely characterized in myelogenous leukemia. As<sub>2</sub>O<sub>3</sub> was successfully used in the treatment of APL. However, cytotoxicity or insensitivity is a major concern in the successful treatment of leukemia. Using a specific precursor pre-*miR-21* or anti-*miR-21* oligonucleotide (AMO-*miR-21*), a group studied the sensitivity of HL60 and K562 cells to As<sub>2</sub>O<sub>3</sub>. It was found that there was somewhat synergistic effect of AMO-*miR-21* and As<sub>2</sub>O<sub>3</sub> in growth inhibition and apoptosis promotion. Meanwhile, enforced pre-*miR-21* expression increased resistance to As<sub>2</sub>O<sub>3</sub>, nevertheless not affecting cell growth alone. So, this suggested *miR-21* by targeting *PDCD4* may play a functional role in modulating As<sub>2</sub>O<sub>3</sub>-induced cell death, and strategy using AMO-*miR-21* and its combination with As<sub>2</sub>O<sub>3</sub> may be useful as a myelogenous leukemia therapy. Further study suggested that AMO-*miR-21* sensitized leukemic K562 cells to As<sub>2</sub>O<sub>3</sub> by inducing apoptosis partially due to its up-regulation of *PDCD4* protein level. The combination of As<sub>2</sub>O<sub>3</sub> and As<sub>2</sub>O<sub>3</sub>-*miR-21* presents therapeutic potential for CML (Gu et al. 2011; Li et al. 2010).

## 2.7 As<sub>2</sub>O<sub>3</sub> and Cancer Stem Cells

The existence of a small population of “cancer-initiating cells” is responsible for tumor maintenance has been firmly demonstrated in leukemia. This concept is currently being tested in solid tumors. Leukemia-initiating cells, particularly those that are in a quiescent state, are thought to be resistant to chemotherapy and targeted therapies, resulting in disease relapse (Ito et al. 2008). AML is a stem cell disease. The inefficient targeting of the leukemic stem cells is considered responsible for relapse after the induction of complete hematologic remission in AML. APL is a subtype of AML characterized by the t(15;17) translocation and expression of the PML/RAR $\alpha$  fusion protein. Treatment of APL with ATRA induces complete hematologic remission, but not molecular remission (CMR), because the fusion transcript remains detectable, followed by relapse within a few months. Arsenic induces high rates of complete hematologic remission and CMR followed by a long relapse-free survival. After comparing the effects of ATRA and arsenic on PML/RAR $\alpha$ -positive stem cell compartments, it was found that in contrast to ATRA, arsenic abolishes the aberrant stem cell capacity of PML/RAR $\alpha$ -positive stem cells. Arsenic had no apparent influence on the proliferation of PML/RAR $\alpha$ -positive stem cells, whereas ATRA greatly increased the proliferation of these cells. Furthermore, ATRA induces prolif-

eration of APL-derived stem cells, whereas arsenic inhibits their growth. These data suggest a relationship between the capacity of a compound to target the leukemia-initiating cell and its ability to induce long relapse-free survival (Zheng et al. 2007). Cancer stem-like cells are potential targets for treatment of glioblastoma multiforme due to their role in tumorigenesis and recurrence. In a recent study, the inhibitory effect of  $\text{As}_2\text{O}_3$  on cancer stem-like cells (CSLCs) of glioblastoma multiforme was investigated in human glioma cell lines (U87MG, U251MG, and U373MG) *in vivo* and *in vitro*. Immuno-fluorescence staining and flow cytometry revealed that the percentage of Nestin-positive cells in the aforementioned cell lines was diminished by 12%, 14%, and 7%, respectively, after treatment with  $2\text{ }\mu\text{M}$   $\text{As}_2\text{O}_3$ . Furthermore, they used soft-agar in U87MG and tumor xenografts in nude mice to demonstrate the ability of  $\text{As}_2\text{O}_3$  to inhibit the formation of tumor in the three cell lines. These results indicate the negative regulation of CSLCs by  $\text{As}_2\text{O}_3$ . In addition, a Western blot analysis revealed decreased levels of Notch1 and Hes1 proteins due to  $\text{As}_2\text{O}_3$  treatment. It was concluded that  $\text{As}_2\text{O}_3$  has a remarkable inhibitory effect on CSLCs in glioma cell lines *in vivo* and *in vitro*; in addition, the mechanism of CSLC inhibition involves the deregulation of Notch activation (Zhen et al. 2010).

## 2.8 Synergy of Arsenic with Other Agents

Because of the many pathways involved in mediating the effects of arsenic, the potential exists for synergism with other agents to provide enhanced therapeutic benefits. As mentioned earlier,  $\text{As}_2\text{O}_3$  shows effects distinct from those of ATRA in APL cells and has clinical efficacy in patients with ATRA-resistant APL. The combination of ATRA and  $\text{As}_2\text{O}_3$  may be synergistic or antagonistic *in vitro*, whereas *in vivo* the combination or sequential use of the agents has been reported to accelerate tumor regression by enhancing both differentiation and apoptosis in some but not all of the models (Zhou et al. 2007). Furthermore, combination therapy may allow for administration of lower doses of  $\text{As}_2\text{O}_3$ , minimizing toxicity and potential drug antagonism.

Observations that perturbations in cellular methyl metabolism modulate the cytotoxicity of arsenic have led to the suggestion that methotrexate may act synergistically with  $\text{As}_2\text{O}_3$ . The combination of  $\text{As}_2\text{O}_3$  with IFN- $\alpha$  has activity in adult T cell leukemia cell lines *in vivo* and *in vitro* (Mahieux and Hermine 2005). Potential synergy of  $\text{As}_2\text{O}_3$  and vitamin C has been shown *in vitro* and *in vivo* by several groups (Berenson et al. 2007; Subbarayan et al. 2007), and additional studies of the mechanism both *in vitro* and in the clinic appear warranted. Ascorbic acid is not the only agent affecting intracellular redox to synergize with arsenic *in vitro*. The profound increases in arsenic sensitivity *in vitro* associated with glutathione depletion by buthionine sulfoximine suggest a need for experiments in animal models using this or related agents as well (Han et al. 2008).

Enhancement of radiation response is possible to be achieved by combination treatment with therapeutic drugs. Previously, it has been shown that  $\text{As}_2\text{O}_3$  in combination with ionizing radiation enhanced radiation response in cervical cancer

cells. In a study, molecular mechanism of synergistic enhancement of radiation response in combination with As<sub>2</sub>O<sub>3</sub> was further investigated. The combination treatment of HeLa cells induced translocation of Bax to the mitochondria and a marked phosphorylation of Bcl-2. p38 MAPK and JNK were also found to be activated in response to the combination treatment. Pre-treatment of PD169316, a p38 MAPK specific inhibitor, completely attenuated the combination treatment-induced mitochondrial relocation of Bax, and altered Bcl-2 phosphorylation. Moreover, pre-treatment of SP600125, JNK specific inhibitor, clearly attenuated Bcl-2 phosphorylation, but did not affect Bax translocation to the mitochondria. In addition, N-acetyl-L-cysteine, a thiol-containing antioxidant, completely blocked p38 MAPK and JNK activations, Bax relocation, and Bcl-2 phosphorylation. These results indicate that activation of p38 MAPK is specifically required for translocation of Bax to the mitochondria, and both JNK and p38 MAPK are involved in phosphorylation of Bcl-2 in response to combination treatment with gamma-radiation and As<sub>2</sub>O<sub>3</sub>, and that ROS is a critical regulator of p38 MAPK and JNK activations. The molecular mechanism elucidated in this study may provide insight into the design of future combination cancer therapies to cells intrinsically less sensitive to radiation treatment (Chun et al. 2002; Kang and Lee 2008).

## 2.9 Safety and Tolerability

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 arsenic exposure can result in a variety of skin manifestations including hyperpigmentation, keratosis, squamous cell carcinoma, and bowenoid lesions. Other potential signs of arsenic poisoning include peripheral neuropathy, cardiomyopathy, and renal failure. Despite its reputation as a poison, as a therapeutic entity As<sub>2</sub>O<sub>3</sub> has been generally well tolerated. When administered intravenously at a dosage of 0.15 mg/kg/day, leukocytosis, gastrointestinal disorders (e.g. nausea, vomiting, and diarrhea), fatigue, fever, headache, cough, and dyspnea are commonly observed. Common potentially serious toxicities include APL differentiation syndrome (APLDS) and electrocardiogram (ECG) abnormalities. Previously known as “retinoic-acid syndrome,” APLDS may present during remission induction with ATRA or As<sub>2</sub>O<sub>3</sub> 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. In the pivotal trial of As<sub>2</sub>O<sub>3</sub> in APL, APLDS occurred in 10 (25%) patients; in three of these patients, APLDS was considered to be serious. Therapy with As<sub>2</sub>O<sub>3</sub> 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<sub>2</sub>O<sub>3</sub>; in the MD Anderson Phase II trial, 13 patients (16%) developed APLDS, and all cases were successfully managed by withholding ATRA and administering corticosteroids.

In a study comparing  $\text{As}_2\text{O}_3$ , ATRA, and the combination, the incidence of APLDS-associated hyperleukocytosis was not increased with  $\text{As}_2\text{O}_3$  plus ATRA; however, one death in the combination arm was attributed to APLDS. ECG abnormalities, including prolonged QT interval and complete atrioventricular block, have been reported with  $\text{As}_2\text{O}_3$  treatment. QT prolongation (defined as  $\geq 450$  ms for males and  $\geq 470$  ms for females) was seen in 63% of patients in the pivotal trial and led to temporary discontinuation of  $\text{As}_2\text{O}_3$  therapy in one patient (3%). In the Phase II study by Ravandi et al. (2009),  $\text{As}_2\text{O}_3$  was discontinued in five patients (6%) due to adverse cardiac events including a trial arrhythmias and myocardial infarction. 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 (Soignet et al. 2001; Shen et al. 2004; Hu et al. 2009; Ravandi et al. 2009).

Liver function test abnormalities can commonly occur and typically cause hepatitis, with increases in aminotransferases (rarely greater than five times normal) beginning about 5–10 days after drug administration. Peripheral neuropathy in a “glove and stocking” distribution can occur in up to 10% of patients. Carcinogenesis is a major concern associated with long-term exposure to arsenic. In the study published in PNAS in 2008 on long-term efficacy and safety of ATRA/ $\text{As}_2\text{O}_3$ -based therapy in newly diagnosed APL patients who started being accrued to the trial in 2001, no secondary carcinoma (including skin cancer) was observed. One male transiently tested positive for carcinoembryogenic antigen, and a mild unsustained increase in CA125 in a female patient was recorded. Moreover, arsenic concentrations in the urine of patients who had ceased  $\text{As}_2\text{O}_3$  treatment for 24 months were below the safety limits recommended by government agencies in several countries or regions. Interestingly,  $\text{As}_2\text{O}_3$  therapy has been associated with frequent varicella zoster reactivation, which developed in 26% of patients within a year of treatment with no preferential dermatomal involvement or systemic spread. Another study reported an increased incidence of herpes simplex infection after  $\text{As}_2\text{O}_3$  therapy. This increased incidence of viral infections is thought to be related to apoptosis of T helper lymphocytes by  $\text{As}_2\text{O}_3$ . Finally, there is concern that chronic exposure to arsenic could increase the risk of secondary malignancies as a result of DNA damage. An increased number of structural chromosomal abnormalities have been seen in rats chronically exposed to arsenic. A report of solid tumors (including nasopharyngeal carcinoma and colonic adenocarcinoma) developing after treatment with  $\text{As}_2\text{O}_3$  has elevated this concern, although retrospective analysis suggested that these cancers may have been present prior to or shortly after the start of  $\text{As}_2\text{O}_3$  therapy (Soignet et al. 2001; Shen et al. 2004; Hu et al. 2009; Ravandi et al. 2009).

## 2.10 Conclusions and Future Perspectives

The therapeutic uses and potential of arsenic-containing compounds have been evolving over centuries, starting with the empiric use of arsenic in ancient times up to the current United States Food and Drug Administration approval of  $\text{As}_2\text{O}_3$  for the treatment of APL in humans. Despite the well-known toxicities and side ef-

fects of arsenic compounds, the prospects for arsenic use in the treatment of human diseases remain high. The evolution of our understanding of how arsenic mediates biological responses over the last decade has led to new studies aimed at establishing conditions for the selective enhancement of its antitumor properties *in vitro* and *in vivo*. It is possible that the next phase in the medical use of arsenic compounds will involve selective applications to malignancies with distinct molecular profiles that define arsenic sensitivity and/or combinations with other agents that target cellular pathways which negatively control arsenic responses. The usefulness of such approaches remains to be established over the next several years. Independently of the outcome of such studies and on the basis of historical considerations and the ongoing evolution in the field, one can argue with a degree of certainty that arsenicals will continue to be the focus of intense research investigations in the near and distant future.

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