Arsenic in cancer therapy

Jiong Hu[a], Jing Fang[a], Yin Dong[a], Sai Juan Chen[a] and Zhu Chen[a]

Arsenic, a natural substance that has been used as a drug for over 2000 years, has been revived because of its remarkable therapeutic efficacy in patients with acute promyelocytic leukemia (APL). Arsenic exerts a dose-dependent dual effect: it causes differentiation at low concentrations and apoptosis at relatively high concentrations. Specific degradation of the leukemogenic PML–RARα fusion protein induced by arsenic leads to the differentiation of leukemia cells. The arsenic-induced apoptosis occurs through direct effects on mitochondria, causing the release of apoptotic proteins into the cytosol and the activation of caspases. Preliminary *in vitro* studies have also extended the potential anti-cancer effect of arsenic to non-APL leukemias, lymphoid malignancies and other cancers. *In vitro* and *in vivo* studies demonstrate that arsenic exerts a broad spectrum of anti-cancer effects by induction of apoptosis, inhibition of cell proliferation, anti-angiogenesis and possible immunomodulation. Phase I and II clinical trials are underway to evaluate the feasibility, safety and potential effect of arsenic in various cancer types. *Anti-Cancer Drugs* 16:119–127 © 2005 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2005, **16**:119–127

Keywords: apoptosis, arsenic, cancer, leukemia

[a]Department of Hematology, Shanghai Institute of Hematology, Ruijin Hospital, Shanghai Second Medical University, Shanghai, China.

Sponsorship: This work was supported in part by Grants from the Chinese National High Tech Program (863), National Key Programs for Basic Research (973), National Natural Science Foundation of China, Shanghai Municipal Commission for Education, and the Shanghai Municipal Commission for Science and Technology.

Correspondence to Z. Chen, Shanghai Institute of Hematology, Ruijin Hospital, Shanghai Second Medical University, 197 Ruijin Road II, Shanghai 200025, China. Tel: +86 21 64377859; fax: +86 21 64743206; e-mail: zchen@stn.sh.cn

Received 31 August 2004 **Revised form accepted** 2 October 2004

Introduction

Arsenic is a natural substance that has been used as a drug for over 2000 years in both traditional Chinese medicine and the Western world. In the 19th century, a solution of potassium arsenite (Fowler's solution) was used for a variety of systemic illnesses including primary treatment of chronic myelogenous leukemia (CML) until replaced by radiation and cytotoxic chemotherapy in the middle of the 20th century [1,2]. Arsenic was rarely used as medicine since then, partly due to its low effect compared to modern chemo- and radiotherapy, but mostly because of concerns about the toxicity and potential carcinogenicity. For example, arsenic poisoning due to contamination in drinking water and industrial pollutants remains a serious public health problem in many parts of the world [3,4]. This old remedy has now been revived thanks to the recent discovery in China that it is specifically effective in the treatment of acute promyelocytic leukemia (APL) [5]. The accumulated experience since then has shown that a stable solution of arsenic trioxide [As_2O_3 (ATO)] given by i.v. infusion is remarkably safe and effective. Subsequent studies have shown great promise of arsenic as an anti-cancer therapy in various malignant diseases [6–9].

Arsenic in the treatment of APL

APL is a distinct type of acute leukemia with a characteristic reciprocal translocation between chromosomes 15 and 17 resulting in the chimeric gene encoding PML–RARα fusion protein [10]. Since 1986, the incorporation of all-*trans* retinoic acid (t-RA) in induction therapy has improved the complete remission rate in newly diagnosed APL up to 85–90%. Subsequent studies demonstrated that t-RA induced terminal differentiation of promyelocytes by specific modulation and degradation of PML–RARα fusion protein, releasing the dominant repression on RA signaling. In spite of the high remission rate with t-RA, prolonged treatment with t-RA as monotherapy failed to improve the long-term disease-free survival. Combinations of t-RA with standard cytotoxic drugs in induction, consolidation or maintenance therapy have led to improved survival in patients with APL. Although results with t-RA and chemotherapy were encouraging, a sizable proportion of patients relapsed, and usually became refractory and resistant to the t-RA therapy [11–13].

In 1992, a Chinese group from Harbin Medical University reported the successful application of ATO in the treatment of APL with i.v. administration of an arsenic-containing solution (1% of ATO + trace amount of mercury chloride). Complete remission was achieved in two-thirds of patients with 5- and 10-year survival at 50 and 18.8%, respectively [5]. In collaboration with the Harbin group, we reported the clinical efficacy of pure ATO solution as monotherapy in relapsed APL patients resistant to t-RA. ATO was given i.v. at a dose of 0.16 mg/kg/day and remission was obtained in 40 out of 47

0959-4973

relapsed patients after 17–76 (median 31) days. Side-effects include skin reaction (26.7%), gastrointestinal disturbances (26.7%), peripheral neuropathy and electrocardiographic (ECG) changes, which are moderate and tolerable in relapse patients. Mild (grade I, 14/47) to moderate (grade II, 1/47) increases of hepatic enzyme were observed among 15 out of 47 patients which was transient and responded well to either dose modification or concomitant hepatic protection therapy. Pharmacokinetic studies showed that plasma arsenic rapidly reached the peak level with the mean C_{pmax} at 6.85 μmol/l with $t_{1/2\alpha}$ and $t_{1/2\beta}$ at 0.89 and 12.13 h, respectively. A temporary increase of arsenic in hair and nails was documented with peak levels at 2.5–2.7 μg/g tissue, and a decline of arsenic was observed soon after withdrawal of arsenic therapy [6,7]. In our subsequent cohort of 11 newly diagnosed patients, remission was obtained in eight (72.7%) patients, while hepatic toxicity was documented in seven patients [7]. To avoid potential liver toxicity, we further explored the potential of lower daily dose arsenic at 0.08 mg/kg. Twenty cases of relapsed APL patients were enrolled for evaluation of clinical efficacy and pharmacokinetics study. Sixteen out of 20 (80%) patients achieved remission with mild liver dysfunction in four patients (20%) without any need of dose modification. The pharmacokinetics study demonstrated C_{pmax} of arsenic at 1.5–2.5 μmol/l during the whole induction therapy. These results indicated that the low-dose ATO (0.08 mg/kg) is as effective as the standard dose (0.16 mg/kg) and the subsequent clinical trials in newly diagnosed patients also showed good clinical response to ATO with acceptable side-effects [14]. More recently, another Chinese group reported their cohort of 129 APL patients (19 newly diagnosed APL, seven relapsed and 103 in complete remission) treated with tetra-arsenic tetra-sulfide (As_4S_4), an oral formula of arsenic. Remission was achieved in all 19 newly diagnosed and seven relapsed patients. Fourteen of 16 evaluable patients with newly diagnosed disease obtained complete cytogenetic and molecular response. The estimated disease-free survival (DFS) rates for 1 and 3 years were 86.1 and 76.6%. As_4S_4 was given to another of 103 patients in remission, and the estimated DFS rates for 1 and 6 years were 96.7 and 87.4%, respectively, with a median follow-up of 23 months (2–71 months). Treatment with As_4S_4 was well tolerated with only moderate side-effects, including asymptomatic prolongation of the corrected QT interval, transient elevation in liver enzyme levels, skin rash and mild gastrointestinal discomfort [15].

These clinical results and subsequent laboratory studies afford important insights into the mechanism of action of arsenic against APL. The net effect of ATO treatment is a dose-dependent dual effect in *in vitro* studies: ATO triggers apoptosis at relatively high concentrations (0.5–2.0 μmol/l) and induces partial differentiation at low concentrations (0.1–0.5 μmol/l) [16]. Degradation of the fusion protein, PML–RARα, is most likely the mechanism by which ATO induces cell differentiation in APL. ATO induces degradation of PML–RARα fusion protein over a wide range of concentrations (0.1–2.0 μmol/l). The wild-type PML protein is also degraded in ATO-treated cells, while the wild-type RAR proteins including RARα are not affected. Indeed, ATO treatment causes PML to localize to the nuclear matrix, where it becomes sumoylated and is degraded after recruitment of the proteasome. Degradation of PML–RARα as well as the wild-type PML releases the dominant repression effect of PML–RARα fusion protein on RA pathways and enhances the acetylation of histone, a process important for the transcriptional activation of RA targeted genes [16–18].

ATO-induced apoptosis, in contrast, occurs via a variety of mechanisms. In addition to the degradation of PML–RARRα and wild-type PML protein, the apoptotic effects of ATO occur through direct effects on mitochondria. ATO induces a loss of inner mitochondrial transmembrane potential ($\Delta\psi_m$), opens mitochondrial permeability transition (PT), releases cytochrome *c* and apoptotic proteins into the cytosol, and ultimately cause caspase activation, which is considered to be the executive phase of apoptosis [19]. Zheng *et al.* [20] further identified the voltage-dependent anion channel (VDAC) as the biological target of arsenic, responsible for eliciting cytochrome *c* release, since ATO induced $\Delta\psi_m$ reduction and cytochrome *c* release only in VDAC-expressing, but not in VDAC-deficient, cells. ATO-induced apoptosis is also thiol dependent and to some extent determined by intracellular glutathione (GSH) content since multiple thiol metabolism and/or GSH modulators exert an impact on the ATO-induced apoptosis. For example, ATO-induced apoptosis can be blocked by dithiothreitol (DTT), a disulfide bond-reducing agent, and can be enhanced by buthionine sulfoximine (BSO), an inhibitor of α-glutamylcysteine synthetase. In line with GSH content, the intracellular reactive oxygen species (ROS) content also plays a determinant role in ATO-induced apoptosis. ATO increases ROS content through various mechanisms such as opening of PT to release ROS and activation of NADPH oxidase to produce ROS. Although the ATO-induced differentiation is exclusively specific depending on the degradation of PML–RARα, whereas normal RA signaling is required, ATO-induced apoptosis, in contrast, can be independent of the presence of PML–RARα. A direct effect of ATO on mitochondria and induction of apoptosis at high concentration was documented in a variety of leukemia cell lines not expressing PML–RARα. The ATO-induced apoptosis is also PML independent because in cultures of murine bone marrow (BM) progenitor cells in which the PML gene was inactivated or not expressed, ATO could induce growth inhibition and apoptosis [21,22]. These data indicate that ATO-induced apoptosis is not limited to APL cells and arsenic can be used broadly for the cancer treatment.

Based on the dual effect of arsenic on APL cells and involvement of PML–RARα-dependent and -independent mechanisms, a potential additive and/or synergetic effect of arsenic and t-RA has been speculated and tested both in *in vitro* and *in vivo* studies. In leukemia transplantation and ascites models, it has been shown that the sequential treatment or combination of ATO and t-RA demonstrated a significant improvement in disease control and survival. Recently, we developed a protocol for newly diagnosed APL with a combination of t-RA and ATO as front-line therapy followed by consolidation chemotherapy and maintenance with sequential use of t-RA, ATO and low-dose chemotherapy [23,24]. A total of 61 patients were randomized into three treatment groups: t-RA monotherapy, ATO monotherapy and the t-RA + ATO combination. The primary data showed that all three groups obtained high remission rates (90% and above), while the combination of t-RA + ATO achieved rapid remission, approximately 1–2 weeks earlier than ATO and t-RA monotherapy, respectively. Evaluation of molecular response by quantitative real-time RT-PCR for PML–RARα transcripts demonstrated that the combination therapy achieved a more profound molecular response than monotherapy. Moreover, all 20 cases receiving combination therapy remained in remission, whereas seven of 37 patients within monotherapy groups relapsed with a median follow-up at 18 months (8–30 months) ($p < 0.05$) [25].

Arsenic in the treatment of hematological malignancies other than APL

The successful treatment of APL with arsenic is associated with the specific degradation of leukemogenic PML–RARα fusion protein. In more recent studies, the non-APL-specific induction of apoptosis by arsenic in a variety of leukemia cell lines and malignant lymphocytes has demonstrated a substantial growth inhibition and apoptosis in *in vitro* studies with a clinically achievable level of 1–2 μM ATO [26]. Subsequent *in vitro* and *in vivo* studies have been carried out to characterize the effectiveness of arsenic in various hematological malignancies, and multiple phase I and II clinical trials are also underway to evaluate its feasibility, safety and potential effect.

Activity of arsenic against BCR–ABL-positive leukemia

Solutions containing arsenicals have been used as therapeutic agents since ancient times for CML and this suggested that arsenic might have some inhibitory effect on proliferation of BCR–ABL-expressing cells [1]. Puccetti *et al.* [27] reported that ATO could induce apoptosis in BCR–ABL-positive lymphoblasts and CML cells. More interestingly, the effect of ATO was independent of BCR–ABL kinase activity because inhibition of tyrosine kinase by low-dose STI-571 did not affect the sensitivity of leukemia cells to ATO. In addition, the enforced expression of BCR–ABL in U937 cells dramatically increased the sensitivity to ATO. Thus, these data suggested that the inhibitory effect of ATO is not simply due to non-specific toxicity to the BCR–ABL leukemia cells. Instead, ATO-induced apoptosis is genetically determined by the presence of specific BCR–ABL fusion gene products, although the mechanism by which BCR–ABL mediates ATO-induced apoptosis remains unclear. Perkins *et al.* reported that following treatment with ATO of clinically achievable concentrations (0.5–2.0 μmol/l) for 7 days, growth inhibition (0.8–1.5 μmol/l) and apoptosis (2.0 μmol/l) were observed in BCR–ABL-positive acute leukemia HL-60/BCR–ABL and K562 cells. There was a declined expression of BCR–ABL at the protein level due to the inhibition of translation of BCR–ABL mediated by inhibition of ribosomal p70S6 kinase activity and ATO-induced acetylation of histones H3 and H4 [28].

ATO does not interfere with the constitutive kinase activity of BCR–ABL and the response to ATO is not influenced by the abrogation of BCR–ABL kinase activity. Thus, it seems that ATO and STI-571 can target the leukemia cell-specific BCR–ABL gene at complimentary, but different, levels: ATO at the protein level and STI-571 at the kinase activity, making STI-571 + ATO a reasonable combination for potential treatment. Porosnicu *et al.* [29] demonstrated that the co-treatment with ATO + STI-571 can induce significant apoptosis of HL-60/BCR–ABL and K562 cells with a profound reduction in Bcl-x_L, XIAP and Akt at the protein level, and Akt kinase activity. The synergistic cytotoxicity of ATO and STI-571 was documented also in other BCR–ABL-positive cell lines [30–32].

As_4S_4, shown to be effective in the treatment of APL, may have more advantages than ATO in clinical practice due to its easy oral intake [15]. Recently, we demonstrated that As_4S_4 at 1–3 μM exerted a synergistic effect with a suboptimal dose of STI-571 at 0.1–0.3 μM on both the K562 cell line and primary CML cells. As_4S_4 was able to induce G_2/M arrest, while STI-571 induces G_1 arrest. These two drugs also showed a synergetic effect targeting the BCR–ABL protein: a more profound reduction of protein level by Western blot analysis and inhibition of enzymatic activity levels of BCR–ABL by protein tyrosine kinase (PTK) activity assay was observed when cells were treated with As_4S_4 + STI-571 compared to A_4S_4 and STI-571 monotherapy (T. Ying, personal communication). These *in vitro* data strongly suggested a potential clinical application of the STI-571/arsenic combination on BCR–ABL-positive leukemia, and a phase I trial is underway to evaluate the feasibility and effectiveness of combination therapy with As_4S_4 + STI-571 (J. M. Li, personal communication).

Activity of Arsenic against multiple myeloma (MM)

Arsenic holds therapeutic promise in the treatment of MM, with primary data showing growth inhibitory and apoptotic effects of arsenic in myeloma cell lines and freshly isolated human myeloma cells [33]. At least two apoptotic signaling pathways are involved in myeloma cells. One is mediated by c-Jun NH_2-terminal kinases (JNKs), which are involved in apoptosis induced by irradiation which is unaffected by interleukin-6 (IL-6). A second mechanism is associated with the down-regulation of MAPK and P70 observed in dexamethasone-induced apoptosis, which is JNK independent and can be inhibited by IL-6 [34]. In myeloma cells, the ATO-induced inhibition of proliferation has been associated with induction of the p21 cyclin-dependent kinase inhibitor protein and addition of exogenous IL-6 does not overcome the arsenic-induced growth inhibition or apoptosis [33,35,36]. This may imply that the ATO can have a therapeutic effect on dexamethasone-resistant myeloma cells, and the combination of ATO with dexamethasone targeting both IL-6-sensitive and -insensitive apoptosis pathways might have additive or even synergistic effects.

In *in vitro* studies, 100% killing by ATO has been documented in some sensitive myeloma cell lines, but not all myeloma cells are sensitive to ATO. While the direct induction of apoptosis occurs mainly through a mechanism that involves the collapse of the mitochondrial transmembrane potential, the down-regulation of Bcl-2 and activation of caspase-3, the ROS potentiates the killing induced by arsenic, as demonstrated in leukemia and myeloma cells in which the critical modulator of the ROS (GSH) redox system plays an important role [22,37–39]. Depletion of intracellular levels of GSH by pretreatment or co-treatment with BSO and ascorbic acid (AA) enhanced the sensitivity of myeloma cells to ATO. Resistant cell lines can be converted into an ATO-sensitive phenotype treated with ATO + AA [40,41]. Interestingly, ATO-sensitive cells showed 100% apoptosis at 0.5 μM ATO, while all resistant cell lines required BSO to achieve 100% killing at the same concentration of ATO. The pretreatment of BSO for 24 h significantly reduce the intracellular GSH levels in myeloma cell lines. The intracellular GSH levels before and after BSO depletion correlated well with the difference in apoptosis induction following ATO treatment. The BSO- and ATO-induced cytotoxicity was attributable to the activation of the death signals through caspases-3, -8 and -9 [42].

In addition to the direct effects of arsenic on the malignant plasma cell clone, other mechanisms have been shown to be important for myeloma treatment. For example, arsenic exerts an anti-angiogenic activity targeting the BM microenvironment by inhibition of vascular endothelial growth factor (VEGF) produced in leukemia cell lines and reduction of capillary tubule formation of human umbilical vein endothelial cells [36,43]. In a murine fibrosarcoma model, ATO effectively causes the vascular shutdown which leads to tumor necrosis, although the results was observed using ATO at a higher concentration than is achievable clinically [44]. At clinically achievable doses, arsenic also acts in the BM microenvironment to decrease myeloma cell binding to BM stromal cells, inhibits IL-6 secretion induced by myeloma cell adhesion, and blocks proliferation of MM cells adherent to BM stromal cells [36]. More recent studies indicate a potential immunologic effect of ATO on MM [45]. The exposure of human myeloma-like cell lines and freshly isolated MM cells to arsenic resulted in increased killing mediated by lymphokine-activated killer cells (LAK). Treatment of both LAK cells and target myeloma cells with ATO selectively up-regulated the expression of adhesion molecules involved in cell–cell interactions such as CD38 and CD54, and their ligands CD31 and CD11a, respectively [45].

These data clearly indicate that arsenic exerts multiple activities towards the myeloma cells. More importantly, monotherapy and/or combination therapy of arsenic with BSO, AA and steroids has the potential to overcome the resistance of myeloma cells to corticosteroids, chemotherapy or even arsenic itself. These observations may have important implications for the treatment of refractory MM.

Recently, Rousselot *et al.* [46] documented the effectiveness of arsenic in the treatment of MM using a SCID mice transplantation model. In their study, 17 of 52 SCID mice developed human plasma cell tumor with i.p. injection of plasma cells from five myeloma patients after irradiation. Treatment with ATO (10 μg/g, i.p., 5 days/week) or phosphate-buffered saline as control was started when a sustained growth of the tumor cells was demonstrated. A significant decrease in human monoclonal immunoglobulin G (HuMIgG) concentration in mouse serum was observed in three of five mice in the ATO group, including two that achieved an apparent complete remission persisting up to 5 months after ATO discontinuation. A significant difference in survival was observed between control and ATO treated mice (113 and 158 days, respectively; $p = 0.01$).

Whether these observations of *in vitro* sensitivity of myeloma cell lines and *in vivo* effects of ATO treatment in mice models will translate into a clinically relevant effect in myeloma patients is still largely unknown. However, these data prompted investigations using ATO in clinical trials [47]. An initial phase II trial reported activity of ATO (0.15 mg/kg daily for 60 days) in 14 relapsed myeloma patients after at least one cycle of autologous transplantation and refractory to conventional

salvage therapy prior to the initiation of ATO therapy. Responses were noted in three patients, with one achieving 75% reduction in the monoclonal protein and the other two 50 and 25% reduction, respectively. Stable disease was observed in another eight patients [48]. Based on these preliminary results, a multicenter phase II trial in the US has been carried out to confirm the role of ATO in the management of relapsed or refractory myeloma with a higher dose and shorter schedule. Eight relapsed patients and 16 refractory patients were treated with 0.25 mg/kg ATO 5 days/week for 2 weeks followed by no therapy for 2 weeks in repeated 4-week cycles. Nine (43%) of 21 evaluable patients had an objective response as measured by a greater than 25% decrease in serum M-protein concentrations. One patient with refractory disease had a 50% decrease in plasmacytoma size, eight patients had stable disease and four had progressive disease at the first evaluation visit. Side-effects such as leukopenia/anemia, fatigue and transient increases in transaminase levels were observed, but no patients were withdrawn and no doses modification was required. These data suggested that ATO as a single agent demonstrates activity in relapsed and refractory myeloma, and that it was well tolerated [49]. An NCI/Cancer Therapy Evaluation Program-sponsored phase I/II trial of ATO + AA for relapsed/refractory MM is underway and the phase I component of trial has been completed. Six patients with stage IIIA relapsed/refractory myeloma were given 0.25 mg/kg/day ATO + 1000 mg/day AA for 25 days without any significant dose-limiting toxicity. The co-administration of AA did not alter the pharmacokinetics of ATO and elevated AA levels were associated with decreased intracellular GSH. Serial *in vitro* studies demonstrated a continued sensitivity of patient myeloma cells to ATO + AA. Finally, two patients (both with thalidomide-refractory disease) had partial responses and another four patients had stable disease. The primary data indicate that ATO + AA has acceptable toxicity and that there is promising evidence of activity in refractory/relapsed myeloma [40].

Activity of arsenic against lymphoma

The activity of arsenic against lymphoma was initially demonstrated by Zhu *et al.* [26] in their study on a panel of malignant lymphocytes. The therapeutic concentrations of ATO (1–2 μM) induced a substantial growth inhibition and apoptosis of most malignant lymphocytes tested, including SKW-3, Molt-4, BJAB, su-DHL-4, Raji, Nalm-6 and Namalwa. ATO-induced apoptosis was preceded by $\Delta\Psi_m$ collapse, and an inhibitor of glutathione synthesis, BSO-enhanced ATO-induced ATP depletion, $\Delta\Psi_m$ collapse and apoptosis. Subsequent *in vitro* studies confirmed the activity of arsenic against lymphoma cells and more importantly arsenic induces apoptosis equally in daunorubicin-resistant cells correlated with P-gp expression and non-resistant cells [50,51]. Primary clinical trials have been done in various types of lymphoma to explore the potential of ATO therapy alone or combined with other treatment options [52,53].

In cutaneous T cell lymphoma, Michel *et al.* [53] reported that ATO displays an apoptotic effect against cutaneous T cell lymphoma cell lines HuT-78, SeAx and Myla, and of Sezary cells from patients in a time- and concentration-dependent manner as demonstrated by Annexin-V staining, mitochondrial depolarization and DNA fragmentation. ATO-induced Sezary cell death involves activation of caspase-3, cleavage of poly ADP-ribose polymerase (PARP) and cytochrome *c* release, and was enhanced by the addition of AA (100 μM). ATO was also tested in two patients with cutaneous T cell lymphoma and gave a partial response in one case, whereas stability was observed in the second patient. These results demonstrate that ATO synergizes with AA to induce Sezary cell death at clinically achievable concentrations through a caspase-partially-independent pathway and provides a rationale for further *in vivo* studies on the therapeutic efficacy of ATO in cutaneous T cell lymphoma.

HTLV-I is the causative agent of adult T cell leukemia/lymphoma (ATL), which is an aggressive proliferation of mature activated T cells associated with a poor prognosis due to its intrinsic resistance to chemotherapy. Although zidovudine (AZT) and interferon (IFN)-α yield some responses in ATL patients and improve the prognosis, alternative therapies are required. Bazarbachi *et al.* demonstrated a high synergistic effect between IFN-α and ATO in ATL-derived cell lines, with a dramatic inhibition of cell proliferation, G_1 arrest and induction of apoptosis. Similar results were obtained with fresh leukemia cells derived from an ATL patient [54]. With more recent gene expression profiling studies, the ATO/IFN-α combination was shown to reduce HTLV-1 Tax protein expression and reverse the Tax-induced constitutive NFκB activation. ATO alone can induce a dramatic stabilization of IκBα and IκBε, without any impact on IκB kinase (IKK) activity and Tax degradation. The ATO/IFN-α combination can induced a late and massive down-regulation of cell cycle-regulated genes, concomitantly with Tax degradation by the proteasome and the cell death. These two events likely account for the potent and specific synergetic effects of ATO and IFN in ATL [55]. In a subsequent phase II clinical trial with a combination of ATO and IFN in seven patients with relapsed/refractory ATL (four acute and three lymphoma), four patients exhibited a clear initial response (one complete and three partial remissions). None of the seven patients could achieve the initially planned treatment for 56 days. The treatment was discontinued after a median of 22 days because of toxicity in three patients or disease progression in four patients. Although

six patients eventually died from progressive disease, the remaining patient is still alive and disease free at 32 months. The clinical data showed that arsenic/IFN-α treatment is feasible and exhibits an anti-leukemia effect in very poor prognosis ATL patients. Future studies should assess the optimization of arsenic therapy to maintain the anti-leukemia effect while reducing the toxicity and increasing the tolerability [52].

The effect of arsenic has also been tested in Hodgkin/Reed-Sternberg (HRS) cell lines L540, HDLM-2 and L1236 [56]. Arsenite ($NaAsO_2$) at 20 and 200 μM induced apoptosis of 22 and 70% cells, respectively, after 20 h. Arsenic rapidly down-regulated constitutive IKK as well as NFκB activity and induced apoptosis in HRS cell lines containing functional IκB proteins. The apoptosis was blocked by inhibition of caspase-8- and -3-like activity. In contrast, HRS cell lines with mutated, functionally inactive IκB proteins or with a weak constitutive IKK/NFκB activity showed no alteration of the NFκB activity, and were resistant to arsenic-induced apoptosis. In a subsequent mice xenotransplantation model, treatment of NOD/SCID mice with ATO (3.75 mg/kg) induced a dramatic reduction of xenotransplanted L540Cy Hodgkin tumors concomitant with the inhibition of NFκB activity induced by ATO. Thus, these data suggested that inhibition of NFκB contributes to arsenic-induced apoptosis and pharmacologic inhibition of the IKK/NFκB activity, either by ATO or other potential agents, and might be a powerful treatment option for Hodgkin lymphoma.

Arsenic in the treatment of non-hematological cancer

It has been well documented that arsenic has a direct apoptotic effect via targeting mitochondria with induction of loss of the inner mitochondrial transmembrane potential and release of cytochrome *c* into the cytosol, which leads to the activation of caspases in the executive phase of the apoptosis process. The direct anti-cancer effect of ATO has been broadly tested and documented in various human cancer cell lines.

In human pancreatic cancer cells MiaPaCa2 and PANC-1, ATO induced apoptosis at low and non-toxic concentrations [57]. The mechanism of apoptosis was shown to be activation of caspase-3, -7 and -9 by ATO together with cleavage of the downstream caspase-3 target PARP. Expression of the anti-apoptotic proteins Bcl-2 and Mcl-1 was decreased, while the Bax expression level was increased in a time-dependent manner. Flow cytometric analysis revealed changes of cell cycle distribution from G_0/G_1 phase arrest at 24 h to G_2/M phase arrest at 72 h following arsenic treatment. Arsenic also increased the expression of the p21 protein, and decreased the expression of cyclin A, B1 and D1, and markedly enhanced the expression of GADD45 and GADD153 in a time-dependent manner. Thus, these data clearly indicated that arsenic induced apoptosis in pancreatic cancer cells through activating the caspase cascade via the mitochondrial pathway, GADD expression and modification of cycle-regulating proteins. A similar observation was documented in other tumor cell lines such as renal cell carcinoma cell lines (ACHN, A498, Caki-2, Cos-7 and Renca) [58].

In addition to the mitochondrial toxicity, it has been shown that arsenic-induced apoptosis can be mediated by free radical species in leukemia cells via accumulation of H_2O_2 and that the ability of arsenic to induce apoptosis in cancer cells depends on the activity of the enzymes that regulate the cellular H_2O_2 content. One of these enzymes is glutathione-S-transferase (GST)-π, which plays an important role in the cellular efflux of ATO by catalyzing the conjugation of ATO with reduced GSH [21,22]. Since GST-π was found to be significantly increased in established arsenic-resistant or -tolerant cell lines, these data lead to the expectation that cancer cells that do not express GST-π would be highly sensitive to arsenic and modification of GST-π with arsenic may further increase the anti-cancer effect of ATO [59].

Human prostate cancer tissue is characterized by a frequent loss of GST-π, suggesting that ATO might be useful in the treatment of prostate cancer. Lu *et al.* [60] tested the potential of ATO in three prostate cancer cell lines: DU-145 and PC-3 (both cells express GST-π), and LNCaP cells (cell do not express GST-π). Surprisingly, ATO inhibited cell proliferation of DU-145 and PC-3 cells, but not of LNCaP cells, at concentrations below 1 μM and did not induce apoptosis in any of the three cell lines. At higher concentrations (10–20 μM), ATO induced apoptosis in LNCaP cells, but not in DU-145 or PC-3 cells and LNCaP cells transfected with GST-π. These data indicate that ATO inhibits cell growth and induces apoptosis through different mechanisms, and GST-π can be considered as a target when using ATO for treatment.

In human hepatoma-derived cell lines, it was demonstrated that the response to ATO was inversely related to the intracellular GSH and intensity of GSH synthesis. Human hepatoma cell lines such as SK-Hep-1, HepG2 and HuH7 present differences in sensitivity to arsenic. ATO at 1–2 μM reduced proliferation in a time- and dose-dependent way in SK-Hep-1 cell, while ATO at 2 and 4 μM inhibited proliferation in HepG2 cells. Depletion of GSH by addition of BSO can restore the cell sensitivity to arsenic [61]. Arsenic has been shown to be an active agent against bladder transitional carcinoma cell lines in the presence of BSO at non-toxic concentrations (up to 10 μM) [62]. Significant apoptotic events were observed

including the accumulation of sub-G_1 fractions and internucleosomal DNA breakdown, which were preceded by production of ROS, loss of mitochondrial membrane potential and activation of caspase-3. This strategy has been confirmed by Maeda *et al.* [63] using various cancer cell lines arising from prostate, breast, lung, colon, cervix, bladder and kidney cancers. BSO sensitized ATO-induced cytotoxicity in all investigated cancer cell lines including drug-resistant lines at clinically achievable concentrations of both drugs. More importantly, the BSO + ATO combination clearly showed more cytotoxicity to cancer cells compared with normal cells in *in vitro* assays. In *in vivo* studies using an orthotopic mouse metastasis model with inoculation of androgen-independent prostate cancer cells (PC-3) to the prostate of SCID mice, ATO alone provided a dose-dependent inhibition of both primary and metastatic lesions, while the addition of BSO markedly enhanced the inhibitory effect of ATO on tumor growth in both primary and metastatic lesions [64]. In terms of both tumor weight and number of metastases, combined treatment with 2 mg/kg/day ATO + BSO was more effective than 5 mg/kg/day ATO monotherapy. *In situ* terminal deoxynucleotidyl transferase-mediated nucleotide nick-end labeling (TUNEL) analysis of primary tumors showed more significant apoptotic cell death in mice with the combination therapy than control. Moreover, only the ATO + BSO combination therapy prolonged the survival rate significantly compared with the saline-treated mice. No marked damage to the liver, lungs or kidneys was observed in the combination therapy group compared to the control group. These *in vivo* data strongly indicated that the combination of ATO + BSO is superior to ATO monotherapy in growth inhibition, survival rate and side-effects. Although this approach is still experimental, the model of blocking H_2O_2-scavenging systems plus arsenic treatment can be expected to be a promising approach for clinical treatment. Future clinical studies are warranted to examine the clinical effect in advanced solid tumors, including relapsed drug-resistant ones.

An addition to the direct anti-cancer mechanism of ATO, an immunomodulatory effect has also been reported in breast cancer [65]. In human breast cancer cell lines MCF7, MDA-MB-231, T-47D and BT-20, ATO induced apoptosis at pharmacologically achievable concentrations. At low levels (0.5 μM), ATO was sub-apoptotic, but induced features of differentiation consisting of upregulation of ICAM-1 (CD54), a marker of mammary epithelial differentiation, and cell cultures appeared morphologically more organized. At high levels (2 μM and above), ATO rapidly induced apoptosis in MCF7 and MDA-MB-231 cells, while T-47D and BT-20 cells were partially resistant. It was demonstrated that ATO treatment augmented breast cancer cell lysis by LAK cells. This additional immunomodulatory effect of ATO associated with ICAM-1/lFA-1 interaction could translate into improved *in vivo* anti-tumor immunosurveillance.

Summary

The discovery of the remarkable therapeutic efficacy of arsenic in patients with newly diagnosed and relapsed APL stems from distinct features of the leukemic cell population carrying the PML–RAR-α chimeric gene product. Arsenic exerts a broad spectrum of biologic effects including induction of apoptosis, inhibition of cell proliferation, anti-angiogenesis and possible immunomodulation (Table 1). Preliminary *in vitro* studies have also extended its potential anti-cancer effect to other malignant diseases. The new therapeutic approach with arsenic must be pursued with caution since the concentration of arsenic *in vitro* necessary for inhibiting growth of cancer cell lines other than APL is generally higher than for APL cells. Furthermore, prolonged oral and/or i.v. administration for cancer therapy might lead to neurologic, cardiovascular, hepatic or renal toxicity. This toxicity is not covered by this review. Nevertheless, the described studies and ongoing clinical trials indicate that the future of arsenic therapy in malignant diseases is indeed promising.

Table 1 Potential mechanism of arsenic in various cancers

Potential efficacy in cancer	Biological effects	Mechanism of action
APL	induction of differentiation	altered nuclear distribution of PML: induces sumoylation, transfer to nuclear bodies and degradation of PML; degradation of PML–RARα transcripts
BCR–ABL-positive leukemia	inhibition of leukemia fusion protein	decline of BCR–ABL translation; degradation of BCR–ABL protein
APL; BCR–ABL-positive leukemia, myeloma, lymphoma, breast cancer cell line, prostate cancer cell line, pancreatic cancer cells, hepatoma-derived cell line	induction of apoptosis	elevated intracellular H_2O_2; mitochondrial collapse, cytochrome *c* release, opening of permeability transition pore; activation of the caspase cascade; decrease of Bcl-2 and increase Bax expression
Fibrosarcoma, myeloma	angiogenesis inhibition	diminished VEGF expression; inhibits endothelial cell proliferation in a dose- and time-dependent manner;
Breast cancer cells, myeloma	immunomodulation	upregulation of adhesion molecule; increase of tumor cell lysis by lymphokine-activated killer cells;
HRS	inhibition of NFκB activity	decrease of constitutive IKK as well as NFκB activity

References

1 Forkner CE, Scott TFM. Arsenic as a therapeutic agent in chronic myelogenous leukemia. *J Am Med Ass* 1931; **97**:3–5.

2 Waxman S, Anderson KC. History of the development of arsenic derivatives in cancer therapy. *Oncologist* 2001; **6**(**suppl 2**):3–10.

3 Kandel EV, LeRoy GV. Chronic arsenical poisoning during the treatment of chronic myeloid leukemia. *Arch Intern Med* 1937; **60**:846–866.

4 Chan PC, Huff J. Arsenic carcinogenesis in animals and in humans: mechanistic, experimental, and epidemiological evidence. *J Environ Sci Health C* 1997; **C15**:83–122.

5 Sun HD, Ma L, Hu XC. Ai-Lin 1 treated 32 cases of acute promyelocytic leukemia. *Chin J Integrat Chin West Med* 1992; **12**:170–172.

6 Shen ZX, Chen GQ, Ni JH, Li XS, Xiong SM, Qiu QY, *et al.* Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL): II. Clinical efficacy and pharmacokinetics in relapsed patients. *Blood* 1997; **89**:3354–3360.

7 Niu C, Yan H, Yu T, Sun HP, Liu JX, Li XS, *et al.* Studies on treatment of acute promyelocytic leukemia with arsenic trioxide: remission induction, follow-up, and molecular monitoring in 11 newly diagnosed and 47 relapsed acute promyelocytic leukemia patients. *Blood* 1999; **94**:3315–3324.

8 Soignet SL, Maslak P, Wang ZG, Jhanwar S, Calleja E, Dardashti LJ, *et al.* Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. *N Engl J Med* 1998; **339**:1341–1348.

9 Chen GQ, Zhu J, Shi XG, Ni JH, Zhong HJ, Si GY, *et al. In vitro* studies on cellular and molecular mechanisms of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia: As_2O_3 induces NB_4 cell apoptosis with downregulation of Bcl-2 expression and modulation of PML–RARRα/PML proteins. *Blood* 1996; **88**:1052–1061.

10 Huang ME, Ye YC, Chen SR, Chai JR, Lu JX, Zhoa L, *et al.* Use of all-*trans* retinoic acid in the treatment of acute promyelocytic leukemia. *Blood* 1988; **72**:567–572.

11 Chen Z, Wang ZY, Chen SJ. Acute promyelocytic leukemia: cellular and molecular basis of differentiation and apoptosis. *Pharmacol Ther* 1997; **76**:141–149.

12 Avvisati G, Tallman MS. All-*trans* retinoic acid in acute promyelocytic leukaemia. *Best Pract Res Clin Haematol* 2003;**16**: 419–432.

13 He LZ, Merghoub T, Pandolfi PP. *In vivo* analysis of the molecular pathogenesis of acute promyelocytic leukemia in the mouse and its therapeutic implications. *Oncogene* 1999; **18**:5278–5292.

14 Shen Y, Shen ZX, Yan H, Chen J, Zeng XY, Li JM, *et al.* Studies on the clinical efficacy and pharmacokinetics of low-dose arsenic trioxide in the treatment of relapsed acute promyelocytic leukemia: a comparison with conventional dosage. *Leukemia* 2001; **15**:735–741.

15 Lu DP, Qiu JY, Jiang B, Wang Q, Liu KY, Liu YR, *et al.* Tetra-arsenic tetra-sulfide for the treatment of acute promyelocytic leukemia: a pilot report. *Blood* 2002; **99**:3136–3143.

16 Chen GQ, Shi XG, Tang W, Xiong SM, Zhu J, Cai X, *et al.* Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL): I. As_2O_3 exerts dose-dependent dual effects on APL cells. *Blood* 1997; **89**:3345–3353.

17 Look AT. Arsenic and apoptosis in the treatment of acute promyelocytic leukemia. *J Natl Cancer Inst* 1998; **90**:86–88.

18 Shao W, Fanelli M, Ferrara FF, Riccioni R, Rosenauer A, Davison K, *et al.* Arsenic trioxide as an inducer of apoptosis and loss of PML/RAR alpha protein in acute promyelocytic leukemia cells. *J Natl Cancer Inst* 1998; **90**:124–133.

19 Cai X, Shen YL, Zhu Q, Jia PM, Yu Y, Zhou L, *et al.* Arsenic trioxide-induced apoptosis and differentiation are associated respectively with mitochondrial transmembrane potential collapse and retinoic acid signaling pathways in acute promyelocytic leukemia. *Leukemia* 2000; **14**:262–270.

20 Zheng YH, Shi Y, Tian CH, Jiang CS, Jin HJ, Chen JJ, *et al.* Essential role of the voltage-dependent anion channel (VDAC) in mitochondrial permeability transition pore opening and cytochrome *c* release induced by arsenic trioxide. *Oncogene* 2004; **23**:1239–1247.

21 Jing Y, Dai J, Chalmers-Redman RM, Tatton WG, Waxman S. Arsenic trioxide selectively induces acute promyelocytic leukemia cell apoptosis via a hydrogen peroxide-dependent pathway. *Blood* 1999; **94**:2102–2111.

22 Dai J, Weinberg RS, Waxman S, Jing Y. Malignant cells can be sensitized to undergo growth inhibition and apoptosis by arsenic trioxide through modulation of the glutathione redox system. *Blood* 1999; **93**:268–277.

23 Jing Y, Wang L, Xia L, Chen GQ, Chen Z, Miller WH, *et al.* Combined effect of all-*trans* retinoic acid and arsenic trioxide in acute promyelocytic leukemia cells *in vitro* and *in vivo*. *Blood* 2001; **97**:264–269.

24 Lallemand-Breitenbach V, Guillemin MC, Janin A, Daniel MT, Degos L, Kogan SC, *et al.* Retinoic acid and arsenic synergize to eradicate leukemic cells in a mouse model of acute promyelocytic leukemia. *J Exp Med* 1999; **189**:1043–1052.

25 Shen ZX, Shi ZZ, Fang J, Gu BW, Li JM, Zhu YM, *et al.* All-*trans* retinoic acid/As_2O_3 combination yields a high quality remission and survival in newly diagnosed acute promyelocytic leukemia. *Proc Natl Acad Sci USA* 2004; **101**:5328–5335.

26 Zhu XH, Shen YL, Jing YK, Cai X, Jia PM, Huang Y, *et al.* Apoptosis and growth inhibition in malignant lymphocytes after treatment with arsenic trioxide at clinically achievable concentrations. *J Natl Cancer Inst* 1999; **91**:772–778.

27 Puccetti E, Guller S, Orleth A, Bruggenolte N, Hoelzer D, Ottmann OG, *et al.* BCR–ABL mediates arsenic trioxide-induced apoptosis independently of its aberrant kinase activity. *Cancer Res* 2000; **60**:3409–3413.

28 Perkins C, Kim CN, Fang G, Bhalla KN. Arsenic induces apoptosis of multidrug-resistant human myeloid leukemia cells that express Bcr–Abl or overexpress MDR, MRP, Bcl-2, or Bcl-x_L. *Blood* 2000; **95**:1014–1022.

29 Porosnicu M, Nimmanapalli R, Nguyen D, Worthington E, Perkins C, Bhalla KN. Co-treatment with As_2O_3 enhances selective cytotoxic effects of STI-571 against Bcr–Abl-positive acute leukemia cells. *Leukemia* 2001; **15**:772–778.

30 La Rosee P, Johnson K, O'Dwyer ME, Druker BJ. *In vitro* studies of the combination of imatinib mesylate (Gleevec) and arsenic trioxide (Trisenox) in chronic myelogenous leukemia. *Exp Hematol* 2002; **30**: 729–737.

31 La Rosee P, Johnson K, Corbin AS, Stoffregen EP, Moseson EM, Willis S, *et al. In vitro* efficacy of combined treatment depends on the underlying mechanism of resistance in imatinib-resistant Bcr–Abl-positive cell lines. *Blood* 2004; **103**:208–215.

32 Nimmanapalli R, Bali P, O'Bryan E, Fuino L, Guo F, Wu J, *et al.* Arsenic trioxide inhibits translation of mRNA of *bcr–abl*, resulting in attenuation of Bcr–Abl levels and apoptosis of human leukemia cells. *Cancer Res* 2003; **63**:7950–7958.

33 Rousselot P, Labaume S, Marolleau JP, Larghero J, Noguera MH, Brouet JC, *et al.* Arsenic trioxide and melarsoprol induce apoptosis in plasma cell lines and in plasma cells from myeloma patients. *Cancer Res* 1999; **59**:1041–1048.

34 Munshi NC. Arsenic trioxide: an emerging therapy for multiple myeloma. *Oncologist* 2001; **6**(**suppl 2**):17–21.

35 Park WH, Seol JG, Kim ES, Hyun JM, Jung CW, Lee CC, *et al.* Arsenic trioxide-mediated growth inhibition in MC/CAR myeloma cells via cell cycle arrest in association with induction of cyclin-dependent kinase inhibitor, p21, and apoptosis. *Cancer Res* 2000; **60**:3065–3071.

36 Hayashi T, Hideshima T, Akiyama M, Richardson P, Schlossman RL, Chauhan D, *et al.* Arsenic trioxide inhibits growth of human multiple myeloma cells in the bone marrow microenvironment. *Mol Cancer Ther* 2002; **1**:851–860.

37 Yang CH, Kuo ML, Chen JC, Chen YC. Arsenic trioxide sensitivity is associated with low level of glutathione in cancer cells. *Br J Cancer* 1999; **81**:796–799.

38 Toyokuni S. Reactive oxygen species-induced molecular damage and its application in pathology. *Pathol Int* 1999; **49**:91–102.

39 McCafferty-Grad J, Bahlis NJ, Krett N, Aguilar TM, Reis I, Lee KP, *et al.* Arsenic trioxide uses caspase-dependent and caspase-independent death pathways in myeloma cells. *Mol Cancer Ther* 2003; **2**: 1155–1164.

40 Grad JM, Bahlis NJ, Reis I, Oshiro MM, Dalton WS, Boise LH. Ascorbic acid enhances arsenic trioxide-induced cytotoxicity in multiple myeloma cells. *Blood* 2001; **98**:805–813.

41 Bahlis NJ, McCafferty-Grad J, Jordan-McMurry I, Neil J, Reis I, Kharfan-Dabaja M, *et al.* Feasibility and correlates of arsenic trioxide combined with ascorbic acid-mediated depletion of intracellular glutathione for the treatment of relapsed/refractory multiple myeloma. *Clin Cancer Res* 2002; **8**:3658–3668.

42 Gartenhaus RB, Prachand SN, Paniaqua M, Li Y, Gordon LI. Arsenic trioxide cytotoxicity in steroid and chemotherapy-resistant myeloma cell lines: enhancement of apoptosis by manipulation of cellular redox state. *Clin Cancer Res* 2002; **8**:566–572.

43 Roboz GJ, Dias S, Lam G, Lane WJ, Soignet SL, Warrell Jr RP, *et al.* Arsenic trioxide induces dose- and time-dependent apoptosis of endothelium and may exert an antileukemic effect via inhibition of angiogenesis. *Blood* 2000; **96**:1525–1530.

44 Lew YS, Brown SL, Griffin RJ, Song CW, Kim JH. Arsenic trioxide causes selective necrosis in solid murine tumors by vascular shutdown. *Cancer Res* 1999; **59**:6033–6037.

45 Deaglio S, Canella D, Baj G, Arnulfo A, Waxman S, Malavasi F. Evidence of an immunologic mechanism behind the therapeutical effects of arsenic trioxide (As_2O_3) on myeloma cells. *Leuk Res* 2001; **25**:227–235.

46 Rousselot P, Larghero J, Labaume S, Poupon J, Chopin M, Dosquet C, *et al.* Arsenic trioxide is effective in the treatment of multiple myeloma in SCID mice. *Eur J Haematol* 2004; **72**:166–171.

47 Hussein MA. Trials of arsenic trioxide in multiple myeloma. *Cancer Control* 2003; **10**:370–374.

48 Munshi NC, Tricot G, Desikan R, Badros A, Zangari M, Toor A, *et al.* Clinical activity of arsenic trioxide for the treatment of multiple myeloma. *Leukemia* 2002; **16**:1835–1837.

49 Hussein MA, Mason J, Saleh NM, *et al.* Arsenic trioxide (Trisenox) in patients with relapsed or refractory multiple myeloma (MM): final report of a phase II clinical study. *Proc Ann Meet Am Soc Hematol* 2002; 5138 (abstr).

50 Zhu J, Okumura H, Ohtake S, Nakamura S, Nakao S. The molecular mechanism of arsenic trioxide-induced apoptosis and oncosis in leukemia/lymphoma cell lines. *Acta Haematol* 2003; **110**:1–10.

51 Hu XM, Hirano T, Oka K. Arsenic trioxide induces apoptosis equally in T lymphoblastoid leukemia MOLT-4 cells and P-gp-expressing daunorubicin-resistant MOLT-4 cells. *Cancer Chemother Pharmacol* 2003; **51**:119–126.

52 Hermine O, Dombret H, Poupon J, Arnulf B, Lefrere F, Rousselot P, *et al.* Phase II trial of arsenic trioxide and alpha interferon in patients with relapsed/refractory adult T-cell leukemia/lymphoma. *Hematol J* 2004; **5**:130–134.

53 Michel L, Dupuy A, Jean-Louis F, Sors A, Poupon J, Viguier M, *et al.* Arsenic trioxide induces apoptosis of cutaneous T cell lymphoma cells: evidence for a partially caspase-independent pathway and potentiation by ascorbic acid (vitamin C). *J Invest Dermatol* 2003; **121**:881–893.

54 Bazarbachi A, El-Sabban ME, Nasr R, Quignon F, Awaraji C, Kersual J, *et al.* Arsenic trioxide and interferon-alpha synergize to induce cell cycle arrest and apoptosis in human T-cell lymphotropic virus type I-transformed cells. *Blood* 1999; **93**:278–283.

55 Nasr R, Rosenwald A, El-Sabban ME, Arnulf B, Zalloua P, Lepelletier Y, *et al.* Arsenic/interferon specifically reverses 2 distinct gene networks critical for the survival of HTLV-1-infected leukemic cells. *Blood* 2003; **101**:4576–4582.

56 Mathas S, Lietz A, Janz M, Hinz M, Jundt F, Scheidereit C, *et al.* Inhibition of NFkappaB essentially contributes to arsenic-induced apoptosis. *Blood* 2003; **102**:1028–1034.

57 Li X, Ding X, Adrian TE. Arsenic trioxide induces apoptosis in pancreatic cancer cells via changes in cell cycle, caspase activation, and GADD expression. *Pancreas* 2003; **27**:174–179.

58 Hyun PW, Hee CY, Won JC, Oh PJ, Kim K, Hyuck IY, *et al.* Arsenic trioxide inhibits the growth of A498 renal cell carcinoma cells via cell cycle arrest or apoptosis. *Biochem Biophys Res Commun* 2003; **300**:230–235.

59 Lin X, Tascilar M, Lee WH, Vles WJ, Lee BH, Veeraswamy R, *et al.* GSTP1 CpG island hypermethylation is responsible for the absence of GSTP1 expression in human prostate cancer cells. *Am J Pathol* 2001; **159**: 1815–1826.

60 Lu M, Xia L, Luo D, Waxman S, Jing Y. Dual effects of glutathione-S-transferase pi on As_2O_3 action in prostate cancer cells: enhancement of growth inhibition and inhibition of apoptosis. *Oncogene* 2004; **23**: 3945–3952.

61 Oketani M, Kohara K, Tuvdendorj D, Ishitsuka K, Komorizono Y, Ishibashi K, *et al.* Inhibition by arsenic trioxide of human hepatoma cell growth. *Cancer Lett* 2002; **183**:147–153.

62 Pu YS, Hour TC, Chen J, Huang CY, Guan JY, Lu SH. Arsenic trioxide as a novel anticancer agent against human transitional carcinoma—characterizing its apoptotic pathway. *Anticancer Drugs* 2002; **13**:293–300.

63 Maeda H, Hori S, Ohizumi H, Segawa T, Kakehi Y, Ogawa O, *et al.* Effective treatment of advanced solid tumors by the combination of arsenic trioxide and l-buthionine-sulfoximine. *Cell Death Differ* 2004; **11**:737–746.

64 Maeda H, Hori S, Nishitoh H, Ichijo H, Ogawa O, Kakehi Y, *et al.* Tumor growth inhibition by arsenic trioxide (As_2O_3) in the orthotopic metastasis model of androgen-independent prostate cancer. *Cancer Res* 2001; **61**:5432–5440.

65 Baj G, Arnulfo A, Deaglio S, Mallone R, Vigone A, De Cesaris MG, *et al.* Arsenic trioxide and breast cancer: analysis of the apoptotic, differentiative and immunomodulatory effects. *Breast Cancer Res Treat* 2002; **73**:61–73.