Review Article

Arsenic Trioxide for Non Acute Promyelocytic Leukemia Hematological Malignancies: A New Frontier

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Abstract

Arsenic trioxide (As_2O_3) has been confirmed to be effective in the treatment of Acute Promyelocytic Leukemia (APL). Also, encouraging results have been reported in preclinical studies and pilot clinical trials of As_2O_3 in other hematological malignancies such as Multiple Myeloma (MM), Myeloplastic syndromes (MDS), T-cell leukemia-lymphoma and Chronic Myelogenous Leukemia (CML). However, conflicting findings have been reported in non-APL Acute Myeloid Leukemia (AML). The mechanisms of As_2O_3 activity are complex, with multiple modifications of cell growth and apoptosis control regulations of pro-survival and cell defense molecules, cell cycle arrest, glutathione redox system, p53-dependent apoptotic signals; telomerase activity and caspase pathway. It is now known that other mechanisms are also involved including the immunomodulatory and angiogenesis regulation. Recently there is a trend of investigating whether tetra-arsenic tetra-sulphide will be a better alternative for the arsenic trioxide. In summary this review describes emerging information that provides new insights for As_2O_3 as a broad spectrum chemotherapeutic agent in the treatment of hematological malignancies beyond APL.

Keywords: Arsenic trioxide; Leukemia; Mechanisms; Treatment

Introduction

Arsenic trioxide (As_2O_3) is a traditional folk medicine that has been used for over two thousand years in China [1]. During the 1970s, researchers working in China proposed As_2O_3 as part of the treatment for acute promyelocytic leukemia (APL) [2,3]. It has since been demonstrated that As_2O_3 can achieve complete remission rates that range from 70% to 90% in both newly diagnosed and relapsed APL patients [3-5]. Hence in the last decade, As_2O_3 has been widely accepted as an effective agent in both newly diagnosed and relapsed patients with cytogenetically confirmed APL. This effectiveness has been confirmed with a high Complete Remission rate (CR) and a low adverse effect rate with minimal myelosuppression [5].

The remarkable success of As_2O_3 in APL provides an impetus for the exploration of a possible role of As_2O_3 as an anti-cancer agent in other hematological cancers. Clinical studies of As_2O_3, singularly or in combination with other anti-tumor agents, revealed the potential wide applicability of this agent in patients with hematological malignancies beyond APL. These studies are supported by preclinical laboratory investigations indicating that As_2O_3 exhibits anti-tumor effects against cell lines derived from Multiple Myeloma (MM), Myelodysplastic Syndrome (MDS), non-APL Acute Myeloid Leukemia (AML), and lymphoma.

The first section of this review will discuss the various As_2O_3 anti-cancer mechanisms which are in common for many hematological malignancies. This will be followed by the second section of the tabulation of related clinical trials.

Mechanisms of action

The array of mechanisms that As_2O_3 achieves anti-cancer actions comprises induction of apoptosis; anti-proliferation; anti-angiogenic

activity and immunomodulation as highlighted in Figure 1. The understanding of these As_2O_3 – related mechanisms is developed through many preclinical studies that provide valuable insights on the molecular and genetic processes involved in these pathways.

Induction of apoptosis: As_2O_3 displays direct cytotoxic effects via caspase-9 and caspase-3 activation in MM cell lines U266 and MM1.S [6]; and via caspase 8 activation in leukemic cell lines [7]. This As_2O_3 –induced cytotoxcity has been shown to be related to both caspase-dependent and caspase-independent death signals in various myeloma cell lines [8]. Other than the caspase cascade system, p53
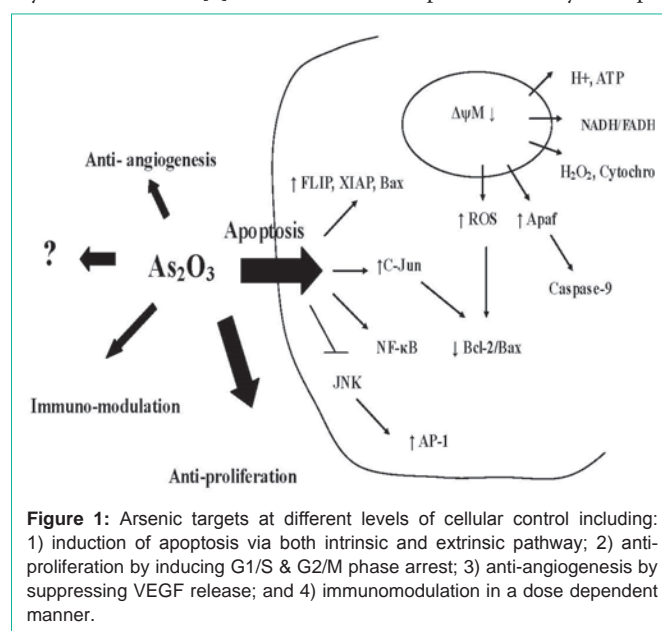
Figure 1: Arsenic targets at different levels of cellular control including: 1) induction of apoptosis via both intrinsic and extrinsic pathway; 2) anti-proliferation by inducing G1/S & G2/M phase arrest; 3) anti-angiogenesis by suppressing VEGF release; and 4) immunomodulation in a dose dependent manner.

has also been shown to be involved. For myeloma cells with mutated p53, As_2O_3 induced rapid and extensive (more than 90%) apoptosis in a time- and dose-dependent manner concomitant with arrest of cells in G2/M phase of the cell cycle [9]. This is in contrast to the wild-type p53 cells which are relatively resistant to As_2O_3 with maximal apoptosis of only about 40% concomitant with partial arrest of cells in G1 and up-regulation of p21 [9].

There are lines of evidence from various model systems delineating the effects of arsenic altering the function of several enzymes and signaling molecules, thus markedly influencing gene expression of apoptosis and proliferation. For instance, the transcription factor AP-1 is activated simultaneously with JNKs, which in turn phosphorylates many transcription factors that enhance expression of immediate early genes. As_2O_3 stimulates JNK activity by inhibiting constitutive JNK phosphates, of which the role in un-stimulated cells is to maintain low basal JNK activity.

Sustained activation of JNK played a critical role in As_2O_3-induced MM cell lines apoptosis [10]. As_2O_3 up-regulated Bax / Bim and 3 other pro-apoptotic BH3-only proteins (Noxa, Bmf, and Puma) and down-regulated 2 anti-apoptotic proteins Mcl-1 and Bcl-X (L) leading to apoptosis. Silencing of Bmf, Noxa, and Bim but not Puma significantly protected cells from As_2O_3-induced apoptosis. This data suggested that Noxa, Bmf, and Bim are necessary for As_2O_3-induced cell death in myeloma [11].

Other than myeloma, As_2O_3 has been reported to induce apoptosis in marrow cells from patients with Myelodysplastic Syndrome (MDS). It has been shown that As_2O_3 interferes with NF-κB; the protein that correlates with disease stage and flow scores [12]. The transcription of NF-κB-dependent anti-apoptotic proteins, such as FLIP Long, Bcl-xl, Bcl-2, and XIAP, can lead to the down-regulation of NF-κB-dependent anti-apoptotic genes. Constitutive NF-κB activity and deregulated FLIP levels may confer resistance to As_2O_3 in advanced-stage MDS. So in patients with more advanced MDS and high levels of FLIP, As_2O_3 was recommended to be used in combination with other agents capable of blocking FLIP amplification [13,14].

Furthermore, As_2O_3-induced apoptosis appears to be associated with the mitochondrial or intrinsic apoptotic pathway. It has been shown As_2O_3 relates to reduced mitochondrial transmembrane potential, enhanced generation of intracellular Reactive Oxygen Species (ROS), release of cytochrome c and Apoptosis-Inducing Factor (AIF) from mitochondria into cytoplasm. This in turn relates to the activation of caspases, and up-regulation of Bax and Bim expression. Exogenous glutathione (GSH) in a dose-dependent manner can reverse the As_2O_3-induced apoptosis, by enhancing oxidative stress [15,16].

As_2O_3 was also found to be active against acute megakaryocytic leukemia (AMKL, FAB-M7) Lam et al. [17] demonstrated that As_2O_3 is a potent agent against AMKL by activating the intrinsic (mitochondrial) pathway of apoptosis, which involved disrupting mitochondrial membrane potential, increased Bax/Bcl-2 ratio and caspase-9 activation, as well as the extrinsic apoptotic pathway mediated by Fas and caspase-8 activation. The multiple-signaling mechanism of As_2O_3 warrants it as a potential agent for the treatment of AMKL.

It has been reported that As_2O_3 can lead to the targeted degradation of AML1/MDS1/EVI1 oncoprotein [18]. It also can effectively overcome some known drug resistances in-vitro by showing significant cytotoxic effects on Daunorubicin (DNR)-resistant cell line K562/D1-9 which over expresses P-glycoprotein (Pgp); DNR and 1-beta-D-arabinofuranosylcytosine (Ara-C) double-resistant cell line HL60/AD which over expresses Multidrug Resistance-associated Protein (MRP1); and pre-B lineage leukemia cell line 697/Bcl-2 which was Bcl-2-transfected [19]. But higher As_2O_3 concentrations and combination treatments are required to reverse the resistance and suppress leukemia cells proliferation [19,20].

Anti-proliferative activity: As_2O_3 induces growth inhibition in a number of malignant hematological cell lines [21,22]. These effects appear to act through the cyclin and cyclin dependent kinases involved in the cell cycle control.

Our group has previously demonstrated that the inhibition of proliferation in myeloma cells by As_2O_3 is associated with demethylation of p15 and p16 and induction of the p21 cyclin-dependent kinase inhibitor protein [22]. As_2O_3 induced most of HS-Sultan cells, arrest at G0/G1 phase with a small fraction at G2/M phase and apoptosis occurred mainly in S phase. There was no expression of p15 and p16 mRNA in untreated HS-Sultan cells and 1.0 μM/L As_2O_3 could make them expressed after being exposed for 24 or 48 hours respectively. Expression of p12 mRNA was obviously elevated by As_2O_3 comparing with that of the control. The pharmacological mechanisms of As_2O_3 are partly due to the expression of CDKI p15, p16 and p21, and consequently affect cell proliferation cycle. In addition, G2/M phase arrest with disruption of the microtubule polymerization process is another possible mechanism in inhibiting cell proliferation by As_2O_3 [23].

Anti-angiogenic activity: As_2O_3 has also been shown to inhibit angiogenesis. Roboz et al [24] treated Human Umbilical Vein Endothelial Cells (HUVECs) with a variety of concentrations of As_2O_3 and showed that in a reproducible dose- and time-dependent sequence of events marked by change to an activated morphology, up-regulation of endothelial cell adhesion markers, and apoptosis of the HUVECs. In addition, treatment with As_2O_3 also caused inhibition of VEGF production in the leukemic cell line HEL, but not HL60 cell line which has previously been shown to be refractory to the effects of As_2O_3 [25]. Thus, the researchers postulated that the anti-angiogenesis effect of hematological malignancies may be due to As_2O_3 interrupting the reciprocal stimulatory loop between the leukemic cells and endothelium through inducing apoptosis of the endothelium and inhibiting VEGF production by leukemic cells. Using the Matrigel-induced capillary tube formation assay, the same research group continued to show that incubation of HUVECs with As_2O_3 prevented capillary tubule and branch formation in an in vitro endothelial cell-differentiation assay. This As_2O_3-induced down regulation of VEGF production has been shown in CML [26] and myeloma [6].

It is important to note that the ability of As_2O_3 to induce apoptosis or cause proliferation of the endothelium depends on the concentration of arsenite used. Barchowsky et al. [27] showed that incubation of second passage vascular endothelial cells with less than 5μ Marsenite for 4 hours increased incorporation of [3H] thymidine

into genomic DNA, while higher concentrations failed to stimulate or inhibit DNA synthesis. The researchers showed that within this 1 hour following addition of non-cytotoxic concentrations of arsenite, oxidants accumulated and thiol status increased; together with increased nuclear retention of NF-κB binding proteins and nuclear translocation of NF-κB. The same group has further shown that this arsenite-induced proliferation of primary porcine aortic endothelial cells MAPK-independent pathway for phenotypic change and proliferation in vascular cells [28].

Immuno-modulation: It is known that chronic arsenic poisoning affects the human immune function [29] with a decreased resistance against viral and bacterial infection associated with a decline in IL-1β and TNF-α release [30]. It also induced apoptosis on macrophages [31] and CD3+, CD56+ but not CD14+ cells [32]. This section aims to summarize the preclinical immunologic mechanisms of the effects of As_2O_3 in hematological malignancies other than APL.

As_2O_3 induced a marked increase in lymphocyte-activated killers mediated killing, which is possibly through the up-regulation of the CD38/CD31 and CD11a/CD54 receptor-ligand systems that increase recognition, adhesion, and lysis of targeted myeloma cells [33]. This data supported the notion that As_2O_3 has a role in the management of relapsed/refractory myeloma via the immunomodulatory mechanisms.

Natural Killer (NK) cells are important effect or immunological cells against tumor mediating through specific activating and inhibitory receptor-ligand pairs. It is now known that the induction of NKG2D ligands on tumor cells by various stresses will render them more sensitive to NK cell-mediated killing. As_2O_3 has been shown to up regulate NKG2D ligands on CML, APL and breast cancer cells and increase their susceptibility to NK cells induced lysis [34]. This increase in cytotoxicity was abolished by the addition of a blocking NKG2D monoclonal antibody, indicating that the action is mediated through up-regulation of NKG2D ligands. This study further supports that the immunomodulatory feature of As_2O_3.

Clinical trials on the use of arsenic trioxide in non-apl hematological malignancies

Multiple myeloma (MM): MM is a plasma cell neoplasm characterized with skeletal destruction, renal failure, anemia, and malignant proliferation of bone marrow plasma cells. Recent advances in the use of As_2O_3 as a single agent or in combination with other agents such as Ascorbic Acid (AA), melphalan, Bortezomib, or conventional regimens, are promising in patients with relapsed/refractory MM [6-9]. A significant number of preclinical experiments and clinical trials have been initiated based on various mechanisms (Table 1). We will first describe the clinical trials that use As_2O_3 as a single agent; then followed by a range of combinational therapeutics together with As_2O_3.

In a multi-center phase II trial [35] eight relapsed and sixteen refractory MM cases (median age 63 yrs) received As_2O_3 0.25 mg/kg intravenously, 5 days per week for 2 weeks followed by no therapy for 2 weeks, in repeated 4-week cycles [24]. There are 9 of 21 (43%) evaluable patients had an objective response as measured by a >25% decrease in serum M-protein concentrations including one patient with refractory disease with a 50% decrease in size of

the plasmacytoma. Eight patients had stable disease and four had progressive disease at the first evaluation visit. However, for advanced MM patients it was reported that As_2O_3 as a single agent did not produce any significant response [36].

In comparison to the use of As_2O_3 as a single agent, there are more clinical trials of combination chemotherapeutics with As_2O_3 in MM. For instance, in a phase I/II trial, six patients with stage IIIA relapsed/refractory myeloma were treated with As_2O_3 (0.25 mg/kg/day) + AA (1,000 mg/day) for 25 days (over a 35-day period) without dose-limiting toxicity [37]. Two patients (both with thalidomide-refractory disease) had partial responses; four patients had stable disease, suggesting that As_2O_3 + AA has acceptable toxicity and promising activity in refractory/relapsed myeloma. In a prospective multi-centre and phase II clinical trial [35] of MAC regimen (melphalan 0.1 mg/kg p.o., As_2O_3 0.25 mg/kg i.v. and AA 1 g i.v), objective responses occurred in 31 of 65 (48%) patients, including two complete, 15 partial and 14 minor responses. Median Progression-Free Survival (PFS) and Overall Survival (OS) were 7 and 19 months respectively, indicating that the steroid-free regimen was effective and well tolerated in heavily pretreated MM.

Based on preclinical results [38], a phase II trial of As_2O_3 combined with dexamethasone and AA was conducted in 20 relapsed/refractory MM patients [39]. Median PFS was 316 days in all the patients and 584 days in those with a response, similar to the result of Wu [40], showing the clinical efficacy and tolerability of the combination of As_2O_3, dexamethasone and ascorbic acid.

As_2O_3 has also been shown to enhance the sensitivity of MM cells to Bortezomib in five myeloma cell lines and primary cells from MM patients [41]. The synergistic activity of As_2O_3 and Bortezomib, melphalan and Ascorbic Acid (AA) has been shown by in vitro and in vivo using a Severe Combined Immunodeficient (SCID)-hum urine myeloma model [42]. In human trials, ABC regimen (As_2O_3/bortezomib/ascorbic acid) showed 27% objective response rate in heavily pretreated MM cases [43]. However, the addition of As_2O_3 to liposomal Doxorubicin, Vincristine and Dexamethasone (DVD) did not demonstrate improvement in newly diagnosed patients [44]. As_2O_3 has shown to inhibit the proliferation of myeloma cells by the down-regulation of Jag2 and Hes1 gene, as well as the up-regulation of tumor suppressor gene PTEN [45].

In summary, As_2O_3 as a single agent could not produce significant response in advanced MM patients, while the combination of As_2O_3 with AA, dexamethasone + AA or MAC regimen have shown promising activity with acceptable toxicity in heavily pretreated refractory/relapsed MM. The As_2O_3 +DVD regimen was not recommended with the availability of more effective front line therapies. Optimal design and combination are still needed to improve the clinical effect of As_2O_3 in MM.

Myelodysplastic syndrome (MDS): MDS is a heterogeneous bone marrow disorder predominantly affecting older adults, for whom the only curative therapy, BMT, is rarely an option. As_2O_3 was found to have therapeutic efficacy in patients with MDS (Table 2). The deregulation of apoptosis is one of the central events in the pathophysiology of MDS. As_2O_3 induces apoptosis through several mechanisms. Preliminary clinical results indicate that As_2O_3 has

Table 1: Clinical trials on the responses of As_2O_3 in the treatment of multiple myeloma.

Reference	Study Design & Regimen	Patients' Characteristics	Outcome 1: Complete/ Partial Objective Response(%)	Outcome 2: Minor Objective Response(%)	Outcome 2: Stablization (%)
Single agent					
Rousselot et al [36]	median duration 38 days (9-54); 0.15 mg/kg/day.	Relapsed or refractory (N=10)	0 (0%)	3 (30%)	4 (40%)
Hussein et al [77]	0.25 mg/kg/day for 2week on/ 2week off at least 1 4-wk cycle	Relapsed or refractory (N=24)	0 (0%)	8 (33%)	6 (25%)
Berenson et al [71]	twice-weekly, 0.25-0.35 mg/kg	Relapsed or refractory (N=11)	0 (0%)	3 (27%)	
Munshi et al [72]	APL induction regimen of 0.15 mg/ kg for 60 days	Relapsed or refractory (N=14)	0 (0%)	1 (7.1%)	2 (14.2%)
Combination regimens					
Berenson et al [35]	As_2O_3+AA+MEL	Relapsed or refractory (N=65)	2 CR+15 PR (26%)	14 (22%)	
Bahlis et al [37]	As_2O_3+AA	Relapsed (N=6)	1PR (17%)	1 (17%)	4 (67%)
Abou-Jawde et al [39]	As_2O_3+AA+DEX	relapsed or refractory (N=20)	2 NCR + 4 PR (30%)		10 (50%)
Hofmeister et al [43]	As_2O_3 +DVD	relapsed or refractory (N=11)	4 PR (36%)		6 (55%)
Berenson et al [44]	As_2O_3+ bortezomib +AA	22	2 PR (9%)	4 (18%)	9 (41%)

PL: acute Promyelocytic Leukemia; As_2O_3: Arsenic trioxide; AA: Ascorbic Acid; MEL: Melphalan; MDS: Myelodysplastic syndrome; Dex: Dexamethasone; DVD: liposomal doxorubicin, vincristine and dexamethasone. The inclusion and exclusion criteria of the patients in these studies please refer to each paper respectively.

Table 2: Clinical responses of either As_2O_3 alone or As_2O_3 in combination with other agents for treatment of MDS.

Reference	Treatment	Number of Patients	Response in hematolotical improvement (HI)	Transfusion independence
Vey et al [47]	As_2O_3 0.3 mg/kg/d x 5d, 0.25 mg/kg 2/w x 15w	114	13/50 (26%)LR 11/64 (17%)HR	12/75 (16%) became RBC: transfusion-independent; 8/28 (29%) became Platelet transfusion-independent;
List et al [49]	As_2O_3 0.25 mg/kg/d 2w on, 2w off	46	8/21(38%)LR 4/25(16%)HR	4/35 (11%) became transfusion-independent; and transfusion requirements in 3 others decreased by $\geq$50%
Schiller et al [50]	As_2O_3 0.25 mg/kg/d 2w on, 2w off	121 (1 cycle: N=70; 2 or more cycles of therapy N=51)	For 1 cycle: Patients with HI (major and minor responses) 11/32 (34%) for LR and 2/36 (6%) for HR For 2 cycles or more: Patients with HI (major and minor responses) 11/28 (39%) for LR and 2/22 (9%) for HR	33% became RBC: transfusion-independent;
Raza et al [51]	As_2O_3 + Thalidomide	28	7/28 (25%) of LR+4 HR	6/28 (21%) total transfusion independence and two had 50% reduction in PRBC transfusions.
Zheng et al [52]	As_2O_3 + AA + Thal	21 (only HR)	10/21 (48%) including 1CR, 1 PR	5/21 (24%) total SRBC transfusion independence

As_2O_3=Arsenic trioxide; AAL: Ascorbic Acid; MDS: Myelodysplastic Syndrome; LR: Lower-Risk Group; HR: Higher-Risk Group; CR: Complete Remission; PR: Part Remission. The inclusion and exclusion criteria of the patients in these studies please refer to each paper respectively.

activity in both lower- and higher-risk MDS [46-49]. Approximately, a third of patients experienced hematological improvement, with few complete or partial remissions.

In an updated phase II clinical trial [50], 121 patients received As_2O_3 (0.25 mg/kg/d) on 5 consecutive days per week for 2 weeks, followed by 2 weeks' rest (one cycle). The overall major hematological remission rates were 20% in Lower-Risk (LR) and 22% in Higher-Risk (HR) patients. One higher-risk patient achieved a complete remission (3%). Transfusion independence or reduction more than 50% occurred in 33% of patients. The overall median duration of hematological remission was 6.8 months (range, 2 to 40 months). Hematological adverse events included neutropenia, thrombocytopenia, and febrile neutropenia. Most common grade 3/4 non-hematological events were pneumonia, fatigue, hemorrhage, pain, and dyspnea and many of which may have no direct relationship with the treatment itself. Thus, it suggests that As_2O_3 mono-therapy has moderate activity against MDS, with a manageable adverse effect profile. A latest Phase II multi-center study confirmed the similar results [47].

Given the manageable toxicity, combination trials were attempted to further improve the clinical results in comparison to with As_2O_3 mono-therapy. In a study of As_2O_3 plus thalidomide [51], there are 7 of 14 available patients (25%) experienced hematological improvement, including one CR, confirming that full dose As_2O_3 can be safely administered in combination with other agents. Besides the combination of As_2O_3, retinoic acid and thalidomide therapy were also evaluated in higher risk MDS [52]. Twenty-one patients were administered 10mg/day As_2O_3 intravenously for 10 days, 40mg/day retinoic acid orally for 2 weeks and 100mg/day thalidomide orally for 4 weeks per cycle. After at least 2 treatment cycles, 10 patients showed hematological responses. One achieved complete response, one achieved partial response, and three patients achieved major hematological improvements. The efficacy rate was 24% (5/21), and the response rate was 48% (10/21). The schedule was well tolerated by all patients and toxicities were moderate and reversible.

Our recent case controlled clinical study of 22 patients with Myelodysplastic Syndromes (MDS) suggested that the combination of thalidomide and arsenic trioxide is effective and well tolerated [53]. Four patients (18.2%) with thalidomide/As_2O_3 treatment achieved remission, comparing to none in the control group (p<0.05). Fifteen

of 22 patients in the treatment group got hematologic improvement (68.2% vs. 27.3% in the control, p<0.05). Both PFS and OS were significantly prolonged (26 vs. 10 months, 36 vs. 16 months, p<0.05), while no severe adverse reactions were observed. These preliminary findings suggest that thalidomide/As$_2$O$_3$ combination treatment could be effective and safe option for MDS.

In summary, As$_2$O$_3$ can achieve hematological improvement in 22% to 26% of patients as a single therapy or in combination with other agents. Most treatment-related adverse events were mild to moderate. Preliminary data suggested that MDS patients whose cells express the EVI1 mutation may benefit more from As$_2$O$_3$ therapy [54]. Further studies of As$_2$O$_3$ on MDS with or without other agents will help to verify its role in this refractory disease. The initial experiences are encouraging, demonstrating that the potential for tri-lineage hematological improvement with mono-therapy of As$_2$O$_3$ may be sustained for prolonged periods even after treatment cessation.

Non-APL acute myeloid leukemia (AML): In a phase II trial of As$_2$O$_3$ treatment for relapsed/refractory/secondary AML [55], eleven patients with a median age of 77 years (56-90yrs) were given a daily dose of 0.25 mg/kg. Median survival following the first dose of As$_2$O$_3$ was 2.25 months (0.4-19mos). All subjects had progressive disease and there was no direct treatment-related mortality. Based on these results, single agent As$_2$O$_3$ is not recommended to be used as a treatment option for non-APLAML.

Yan et al [56] reported that of bortezomib at 32 nM and As$_2$O$_3$ at 1 mM synergistically induced apoptosis in some forms of acute leukemia cell lines and primary cells. These two drugs synergistically induced proteolytic activation of protein kinase C delta (PKCδ) with enhanced activation of two mitogen-activated protein kinases phospho-c-Jun NH(2)-terminal kinase and p38. The specific PKC inhibitor rottlerin markedly decreased bortezomib plus As$_2$O$_3$-induced apoptosis, suggesting that PKC plays an important role in bortezomib plus As$_2$O$_3$-induced apoptosis. The combined regimen of bortezomib and As$_2$O$_3$ might be a potential therapeutic remedy for the treatment of leukemia.

Various other strategies [57-60] have been explored to induce synergy apoptosis aimed at enhancing the anti-tumor effect of As$_2$O$_3$ on AML. Examples of which include an MEK1 inhibitor that activates the p73-p53AIP1 apoptotic pathway, and 17-Allylamino-17-demethoxygeldanamycin (17-AAG)43 which can abrogate the function of heat-shock protein Hsp90 and modulate cellular sensitivity to anticancer agents. This preliminary data provides us with a basis for studying the role of combined use of As$_2$O$_3$with other agents in the treatment of acute myeloid leukemia.

Chronic myeloid leukemia (CML): In the 1930's, As$_2$O$_3$ was reported to be effective in patients with Chronic Myelogenous Leukemia (CML) [1]. Arsenic together with irradiation was the treatment of choice for CML until busulfan was introduced in 1953.

As$_2$O$_3$ reduces bcr-abl levels and induces apoptosis in human myeloid leukemia cells that express bcr-abl. These effects are independent of bcr-abl kinase activity, since they occur when cells are pretreated with Imatinib, a tyrosine kinase inhibitor specific for the bcr-abl tyrosine kinase. Furthermore, As$_2$O$_3$ decreases proliferation of CML blasts but does not affect growth of peripheral CD34 progenitor

cells. Concurrent treatment of As$_2$O$_3$ with Imatinib causes a greater apoptotic response compared with using either agent alone [61-63].

Lymphoma/Lymphoblastic leukemia: It was previously reported that As$_2$O$_3$ with or without interferon-α (IFN-α), can lead to induction of apoptosis and growth inhibition on Human T-cell Leukemia Virus type 1 (HTLV-1)-associated adult T-cell leukemia/lymphoma (T-ALL/NHL) cells [64,65]. For non-HTLV-1 T-ALL, a French phase II trial of As$_2$O$_3$ combined with IFN-α or bortezomib therapy in seven patients with relapsed/refractory T-ALL showed Complete Remission (CR) in 1 patient and Partial Remission (PR) in 3 patients [64]. Though the case number was limited, this study did provide the important information that moderately-aggressive relapsed/refractory T-ALL patients are promising candidates, while those who are heavily-treated or presenting very aggressive T-ALL do not appear to benefit. Incorporation of apoptosis-inducing agents into current therapeutic regimens is an attractive strategy to improve treatment for drug-resistant or aggressive lymphoma/lymphoblastic leukemia. Further studies are needed to determine the efficacy of As$_2$O$_3$ treatment alone or in combination with IFN-αfor the treatment of T-ALL [66].

A phase II trial of As$_2$O$_3$ for the treatment of relapsed and refractory Acute Lymphoblastic Leukemia (ALL) reported the use of high dose As$_2$O$_3$ of 0.25 mg/kg/day intravenously for 5-7 days per week for up to 60 days. Of 11 patients eligible, eight had B-ALL, three T-ALL and two were Philadelphia chromosome-positive [67]. The median duration of therapy was 21 days (range 7-28). There were no responses noted. One patient died from infection and in the remaining patients the disease further progressed leading to death. The median survival was only 3.2 months (range 1.2-4.1). This study indicated that As$_2$O$_3$ is not effective as a single agent in the treatment of ALL, even with high dose. Novel combination strategy with other agents to improve the cytotoxic effect is therefore needed.

The combination of As$_2$O$_3$ and dexamethasone was tested in Glucocorticoid (GC)-resistant Acute Lymphoblastic Leukemia (ALL) [68]. Low-dose As$_2$O$_3$ markedly increased in vitro GC sensitivity of ALL cells derived from T-cell and precursor B-cell ALL patients with poor in vivo response to prednisone. In GC-resistant cell lines, this effect was mediated, at least in part, by inhibition of Akt and affecting downstream Akt targets such as Bad and the X-Linked Inhibitor of Apoptosis Protein (XIAP). Combination of As$_2$O$_3$ and dexamethasone resulted in increased bad and rapid down-regulation of XIAP, while levels of the anti-apoptotic regulator Mcl-1 remained unchanged. Expression of dominant-active Akt, reduction of Bad expression by RNA interference, or over-expression of XIAP abrogated the sensitizing effect of As$_2$O$_3$. The inhibitory effect of XIAP over-expression was reduced when the Akt phosphorylation site was mutated (XIAP-S87A). These data suggested that the combination of As$_2$O$_3$ and glucocorticoids can be advantageous in GC-resistant ALL.

Current information revealed that Primary Effusion Lymphoma (PEL) cells utilize activated NF-kB pathway for their survival [69]. In a subsequent study, the same group indicated that a dramatic inhibition of cell proliferation and induction of apoptosis was observed in cells treated by Velcade (PS-341), a proteosome inhibitor, and As$_2$O$_3$ plus IFN-a treated cells. This was associated with of the mitochondrial membrane potential, cytosolic release of cytochrome c, caspase

activation and the effects could be reversed by the pan-caspase inhibitor, z-VAD. Velcade and As_2O_3 plusIFN-α treatment abrogated NF-kB translocation to the nucleus and decreased the levels of the anti-apoptotic protein Bcl-X (L). Altogether, these results provide a rational basis for a future therapeutic use of Velcade in combination with As_2O_3 plusIFN-α in a specific group of lymphoma patients.

Childhood non-APL hematological malignances: Childhood APL with relapse responded equally as effective as the adult with the 0.15mg/m2 daily dose of As_2O_3 intravenously and the toxicity profile also showed similar pattern. But the data on the use of As_2O_3 on other non-APL hematological malignancies is very limited. It can be due to small number of patients that can be recruited because of the low incidence of childhood cancer compared to adults and the excellent upfront chemotherapy results for childhood leukemia/lymphoma.

A phase I study by Fox et al [70] applied As_2O_3 to children (median age 13 yrs, range, 2-19yrs) with refractory leukemia. As_2O_3 was administered intravenously over 2 hours, 5 d/wk for 20 doses/cycle. Patients with APL (n=13) received 0.15 mg/kg per day, and patients with other types of leukemia received 0.15 mg/kg per day (n=2) or 0.2 mg/kg per day (n=4). Nineteen of the 24 enrolled patients were fully evaluable for toxicity. Morphologic complete response was achieved in 85% of patients with APL, and disappointingly, no response was observed in all non-APL patients. Arsenic trioxide has also been tested in children with infiltrating astrocytic brain tumors which showed an acceptable safety profile but the efficacy could not be evaluated due to other confounding treatments were added [73].

In our in-vitro study, non-APL leukemic cells often required higher concentration of As_2O_3 to achieve satisfactory cytotoxic effects. The serum concentration should be in the range of 1 to 2μM/ml. However, in an pharmacokinetic study done on 13 children with refractory solid tumors by 0.15mg/m2 daily intravenously, most children could only achieved a peak drug concentration of <1μM/ml (range 0.6 - 1μM/ml) (GCF Chan, unpublished data). That partly explained why the clinical effects were suboptimal in previous trials.

Conclusion and Perspectives

This review describes As_2O_3 has potential therapeutic benefits in a variety of other hematological malignancies other than the known advantages for patients with APL. As_2O_3 acts through several signal transduction pathways in a range of target cells involved. The underlying mechanisms include apoptosis induction, anti-proliferation, angiogenesis inhibition and immuno-modulation. However, different types of hematological malignancies may have differences in the response pattern to As_2O_3.

As_2O_3 generally does not induce myelosuppression which is a good complement to conventional cytotoxic agents. This review describes phase I/II studies using combination of As_2O_3 with other anti-cancer therapeutic agents delivering promising results for MM, MDS and CML Whether such combination strategy may result in enhanced antitumor activity without significant additive side effects has yet to be confirmed.

For non-APL myeloid leukemia, T-ALL and lymphoma, the results of using As_2O_3 as a single agent are disappointing. Again, the combination of As_2O_3 with selected targeted therapeutic agents may

enhance the sensitivity of resistant blast cells to As_2O_3. What accounts for the relative poor cytotoxic response to As_2O_3 in these cells and how to reverse the underlying resistant mechanisms will help to develop new therapeutic targets.

Since most previous trials are single arm studies with small sample size, well designed randomized prospective clinical trials with good sample size are needed to verify these pilot study results and find out the best combination strategy of As_2O_3 for different diseases types in the future.

There is an emerging trend of using other arsenicals such as tetra-arsenic tetra-sulfide or realgar (As_4S_4) for the treatment of APL. For instance, a randomized, multicenter, phase III non-inferiority clinical trial further tested the efficacy and safety of an oral As_4S_4-containing formula named the Realgar-Indigo naturalis formula, comparing with intravenous As_2O_3 as both induction and maintenance therapies [74]. In this study, 242 newly diagnosed APL patients were randomly assigned (1:1) to oral RIF (60 mg/kg) or As_2O_3 (0.16 mg/kg) combined with all-trans retinoic acid (ATRA; 25 mg/m²) during induction therapy. After achieving CR, all patients received three courses of consolidation chemotherapy and maintenance treatment with sequential ATRA followed by either RIF or As_2O_3 for 2years. After median follow-up time of 39 months, DFS at 2 years was 98.1% in the RIF group and 95.5% in the As_2O_3 group. There were no significant differences in CR rate between the RIF and ATO groups (99.1% v 97.2%; P = 0.62) or the overall survival at 3 years (99.1% v 96.6%; P = 0.18). Also the rates of adverse events were similar. These results indicated that oral RIF plus ATRA is not inferior to intravenous As_2O_3 plus ATRA as first-line treatment of APL and may be considered as a routine treatment option for appropriate patients [74].

Mechanistic study has shown that As4S induced apoptosis in NB4-R1cells by increasing PP2A and reducing PML-RAR α expressions through the down regulation of SET protein expression [75]. Realgar is also reported to induce apoptosis and differentiation in ATRA-sensitive NB4 and ATRA-resistant MR2 cell lines simultaneously. Gene expression profiles indicated that genes influenced by realgar treatment were involved in the modulation of signal transduction, translation, transcription, metabolism and the immune response. Given its low toxicity, realgar is also believed to be a promising alternative reagent for the treatment of APL [76].

Acknowledgement

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