From an old remedy to a magic bullet: molecular mechanisms underlying the therapeutic effects of arsenic in fighting leukemia

Sai-Juan Chen,1,3 Guang-Biao Zhou,2 Xiao-Wei Zhang,1,3 Jian-Hua Mao,1 Hugues de Thé,4 Zhu Chen1,3

1Shanghai Institute of Hematology and State Key Laboratory for Medical Genomics, Rui Jin Hospital Affiliated to Shanghai Jiao Tong University (SJTU) School of Medicine, 197 Rui Jin Road II, Shanghai, 200025; 2Division of Molecular Carcinogenesis and Targeted Therapy for Cancer, State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101; 3Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, 800 Dong Chuan Road, Shanghai 200240, China; 4Institut National de Santé et de Recherche Médicale, Centre National de Recherche Scientifique, Institut Universitaire d'Hématologie, Université Paris-Diderot UMR 944/7212, Equipe labellisée par Ligue contre Cancer, Service de Biochimie, Hôpital St. Louis, 2 avenue C. Vellefaux, 75475 Paris, CEDEX 10, France.

Correspondence: Sai-Juan Chen, Ph.D, or Zhu Chen, Ph.D, Shanghai Institute of Hematology, Rui Jin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, 197 Rui Jin Road II, Shanghai, 200025, China; e-mail: sjchen@stn.sh.cn or zchen@stn.sh.cn.
Abstract

Arsenic (As) had been used in treating malignancies from the 18th to mid-20th century. In the last three decades, arsenic was revived and shown to be able to induce complete remission and achieve, when combined with all-trans retinoic acid (ATRA) and chemotherapy, a 5-year overall survival of 90% in patients with acute promyelocytic leukemia (APL) driven by the t(15;17) translocation-generated PML-RARα fusion. Molecularly, arsenic binds thiol residues and induces the formation of reactive oxygen species, thus affecting numerous signaling pathways. Interestingly, arsenic directly binds the C3HC4 zinc finger motif in the RBCC domain of PML and PML-RARα, induces their homodimerization and multimerization, and enhances their interaction with the SUMO E2 conjugase Ubc9, facilitating subsequent sumoylation/ubiquitination and proteasomal degradation. Arsenic-caused intermolecular disulfide formation in PML also contributes to PML-As binding. ATRA which targets RARα moiety of PML-RARα, synergizes with arsenic in eliminating leukemia-initiating cells. Arsenic perturbs a number of proteins involved in other hematological malignancies, including chronic myeloid leukemia and adult T-cell leukemia/lymphoma, where it may bring new therapeutic benefits. The successful revival of arsenic in APL, together with modern mechanistic studies, has thus allowed a new paradigm to emerge in translational medicine.
Arsenic (As) is the 20th most abundant element in the earth crust.\textsuperscript{1} It is a chemical analog of phosphorus (P) and lies directly below P in the periodic table. A unique feature of arsenic is its extremely paradoxical abilities: it is toxic to humans, animals and plants, but is used instead of phosphorus by a recently isolated bacterium;\textsuperscript{2} It kills people, but saves lives; It can cause some cancers, but cures others such as the acute promyelocytic leukemia (APL);\textsuperscript{3} It is one of the oldest drugs in the world, but was progressively revived between 1970s to 1990s, because of its striking efficacy on APL which represented the most malignant type of acute leukemia.\textsuperscript{3} These paradoxical effects of arsenic reflect its unique metabolism and multiple properties.\textsuperscript{4} In this review, we address how arsenic rebuilds its reputation from a notorious poison to a new lease of life via mechanistic molecular and translational medicine studies.

**Arsenic in nature**

Arsenic is the 33rd element in the periodic table and exists ubiquitously in either inorganic or organic forms, pure metallic arsenic being rarely found in nature. Arsenic occurs in 5 different valence states, +V (arsenate), +III (arsenite), +I (arsonium metal), 0 (arsenic), and −III (arsine).\textsuperscript{5} Most arsenic compounds have no smell or special taste, and are white or colorless powders that do not evaporate. Arsenic gets into air when contaminated materials are burned. Inorganic arsenic occurs naturally in soils and sedimentary rocks such as minerals and ores containing copper or lead, and occurs in combination with many other elements especially oxygen, chlorine, and sulfur. There are three inorganic arsenic forms, namely, red arsenic (As\textsubscript{4}S\textsubscript{4}, also known as realgar), yellow arsenic (As\textsubscript{2}S\textsubscript{3}, also known as orpiment) and white arsenic (arsenic trioxide, ATO; As\textsubscript{2}O\textsubscript{3}). ATO is made by burning realgar or orpiment.\textsuperscript{6} Organic arsenic is arsenic compounds containing carbon that can be found in nature in water,
natural gas, and shale oil. Examples of organic arsenic are methylarsine (CH$_3$AsH$_2$), dimethylarsine [(CH$_3$)$_2$AsH], trimethylarsine [(CH$_3$)$_3$As], monomethylarsonic acid [CH$_3$AsO(OH)$_2$, MMA$^V$], monomethylarsenous acid [CH$_3$As(OH)$_2$, MMA$^{III}$], dimethylarsinic acid [(CH$_3$)$_2$AsO(OH), DMA$^V$], dimethylarsenous acid [(CH$_3$)$_2$AsOH, DMA$^{III}$], trimethylarsinic oxide [(CH$_3$)$_3$AsO, TMAO], tetramethylarsonium ion [(CH$_3$)$_4$As$^+$, TMA$^+$], arsobetaine [(CH$_3$)$_3$As$^+$.CH$_2$COO$^-$, AB], arsenocholine [(CH$_3$)$_3$As$^+$.CH$_2$.CH$_2$.OH, AC], and others. Darinaparsin is an organic arsenic composed of dimethylated arsenic linked to glutathione.

Regarding arsenic metabolism, an arsenic methyltransferase, encoded by a gene that was originally annotated as “CytI9” and subsequently As3mt, was identified in rat that catalyzes the methylation of arsenite, with S-adenosyl-L-methionine (AdoMet) as the methyl donor. Inorganic arsenic is metabolized by a sequential process involving a two-electron reduction of pentavalent arsenic to trivalent arsenic, followed by oxidative methylation to pentavalent organic arsenic. The postulated scheme is as follows: iAs$^V$ $\rightarrow$ iAs$^{III}$ $\rightarrow$ MAs$^V$ $\rightarrow$ MAs$^{III}$ $\rightarrow$ DMAs$^V$ $\rightarrow$ DMAs$^{III}$ $\rightarrow$ MAs$^{III}$. Of note, derivatives of MAs$^{III}$ and DMAs$^{III}$ are more toxic than either iAs$^V$ or iAs$^{III}$. Methyldimethyarsine oxide (MAs$^{III}$O) and to a lesser extent iododimethylarsine are more potent growth inhibitors and apoptotic inducers than iAs$^{III}$ in leukemia cells, and apoptosis is associated with greater hydrogen peroxide accumulation and inhibition of glutathione peroxidase activity. In vivo hepatic methylation of iAs$^{III}$ may contribute to ATO-induced apoptosis, but not differentiation of APL cells.

Arsenic is toxic to human health in that it can induce skin lesions, haemorrhagic gastroenteritis, cardiac arrhythmia, psychiatric disease, and cancers. Generally inorganic
arsenic species are more toxic than organic forms to living organisms, and arsenite is usually more toxic than arsenate. Exposure to ATO by ingestion of 70–80 mg has been reported to be fatal for humans.\textsuperscript{7,19} The World Health Organization (WHO) set the first International Drinking Water Standard for arsenic concentration at 200 $\mu$g/L in 1958, recommended lowering the standard to 50 $\mu$g/L in 1963, and further lowered the standard to 10 $\mu$g/L in 1993.\textsuperscript{20} Yet, millions of people worldwide ingest drinking water contaminated with arsenic at levels $>$100 $\mu$g/L.\textsuperscript{5,7,20}

The toxicity of trivalent arsenic is related to its high affinity for the sulfhydryl groups of biomolecules such as glutathione and lipoic acid and the cysteiny1 residues of many proteins and enzymes.\textsuperscript{7,21} Arsenic upregulates glutathione-related genes and enzyme activities and binds to sulphydryl groups.\textsuperscript{22,23} The formation of As\textsuperscript{III}–sulfur bonds results in various harmful effects by inhibiting the activities of enzymes such as glutathione reductase, glutathione peroxidases, thioredoxin reductase, and thioredoxin peroxidase.\textsuperscript{4,7,21} Because all of these enzymes regulate cellular redox status, by providing anti-oxidant defense, arsenic exposure leads to production of reactive oxygen species (ROS).\textsuperscript{4} Similarly, flavin enzymes such as NAD(P)H oxidase and NO synthase isozymes have been proposed to be involved in the generation of ROS associated with arsenic exposure.\textsuperscript{5} Arsenic also alters global histone H3 methylation.\textsuperscript{24} Consequently, arsenic affects many signal transduction cascades (e.g., activation of the epidermal growth factor receptor (EGFR) signal pathway\textsuperscript{25}), and activates (or inactivates) transcription factors such as AP-1\textsuperscript{26} and nuclear factor–erythroid 2–related factor 2 (Nrf2).\textsuperscript{27,28} Biomarkers of arsenic exposure include the total arsenic in urine,\textsuperscript{29} clastogenicity in peripheral lymphocytes, micronuclei in oral mucosa and bladder cells, and
induction of heme oxygenase. A potential susceptibility biomarker is variability in arsenic metabolism, which reflects polymorphisms in the genes that encode the arsenic metabolizing enzymes.29

**Arsenic as an old remedy**

Arsenic is one of the oldest drugs in the world. It was firstly mentioned by Hippocrates (460–370 BC) who used realgar and orpiment pastes to treat ulcers in Western medicine. In China, arsenic pills for the treatment of periodic fever were recorded in the Chinese Nei Jing Treaty (263 BC).30 Si-Miao Sun (581–682 AD) purified a medicine composed of realgar, orpiment and ATO in treating malaria,31 while Shi-Zhen Li (1518-1593 AD)32 in the Ming Dynasty described the use of ATO as a remedy for a variety of diseases in his pharmacopedia. Arsenic therapy was introduced to Europe by Avicennes (980–1037 AD) and Paracelsus (1493–1541 AD). In 1774, Lefèbure introduced an arsenic-containing paste proposed to be an “established remedy to radically cure all cancers”.6 Fowler’s solution (1% potassium arsenite, KAsO₂) was firstly described in 1845 and was used to treat anemia and rheumatism, psoriasis, eczematous eruptions, dermatitis herpetiformis, asthma, cholera and syphilis. In 1865, Fowler’s solution was the first chemotherapeutic agent used in the treatment of leukemia which produced some transient improvement.33-35 In 1931 Forkner and Scott, at Boston City Hospital, “rediscovered” Fowler’s solution for the treatment of chronic myeloid leukemia (CML), and arsenicals and irradiation remained the treatment of choice until busulphan was introduced in 1953.33-35

**Current use of arsenic in treating malignant neoplasms**

**Arsenic for APL**
APL, the M3 subtype of acute myeloid leukemia (AML M3), is characterized by the accumulation of abnormal promyelocytes in blood and bone marrow, the occurrence of fibrinogenopenia and disseminated intravascular coagulation, and the specific chromosomal translocation t(15;17)(q22;q21). The t(15;17) fuses the retinoic acid receptor α (RARα) gene on chromosome 17 to the promyelocytic leukemia (PML) gene on 15q, yielding the PML-RARα fusion protein which is the key driver of APL leukemogenesis. APL was firstly described by Hillestad in 1957 and was considered at the time the most fatal type of acute leukemia. The past 5 decades have witnessed tremendous advances in improving APL outcome from highly fatal to highly curable (Fig. 1). While chemotherapy (anthracyclines) shines the first light of hope, all-trans retinoic acid (ATRA), which triggers terminal differentiation of APL cells, achieves a complete remission (CR) rate of 90% (and references within these articles). While ATRA alone rarely cures APL patients, its combination with anthracyclines allowed a significant number of cures. ATO further prolongs survival of APL patients, especially those with relapsed or refractory disease, and cures a number of them as a single agent. Moreover, combined use of ATRA and ATO not only markedly enhances clearance of PML-RARα transcript, but allows the 5-year (yr) overall survival (OS) to reach 91.7% (Fig. 1). Mechanistically, both ATRA and ATO trigger catabolism of the PML-RARα fusion protein. However, ATO has no effect on APL driven by PLZF-RARα which is generated by t(11;17) and accounts for 1-2% of patients with APL. Thus the story of APL can serve as a model for the development of curative approaches for malignant disease.

Clinical efficacy
In the early 1970s, a group from Harbin Medical University in northeastern China tested Ailing-1 containing 1% ATO and trace amount of mercury chloride in a variety of cancers by intravenous administration. In the 1990s, Zhang and colleagues\textsuperscript{52} showed that Ailing-1 induced CR in 21 out of 32 APL patients with an impressive 10-yr survival rate of 30%. The efficacy of pure ATO in treating relapsed APL was then reported by Shanghai Institute of Hematology (SIH) in 1996 to 1999.\textsuperscript{53,54} Shen et al\textsuperscript{54} evaluated the therapeutic effect of ATO in the treatment of 15 APL patients at relapse after ATRA induced and chemotherapy maintained CR. ATO was administered intravenously at the dose of 10 mg/day. They showed that CR was achieved in 9 of 10 (90%) patients treated with ATO alone and in the remaining five patients with ATO in combination with low-dose chemotherapeutic drugs or ATRA. No bone marrow depression was encountered during ATO treatment.\textsuperscript{54} Niu et al\textsuperscript{53} reported that clinical CR was obtained in 8 of 11 (72.7%) newly diagnosed cases and 40 of 47 (85.1%) relapsed patients. They recommended that ATRA is used as first choice for remission induction in newly diagnosed APL cases, whereas ATO can be either used as a rescue for relapsed cases or included into multidrug consolidation/maintenance clinical trials. Furthermore, after CR achieved by using ATO alone, a molecular remission is obtainable in a relatively high proportion of the patients, from 72\%\textsuperscript{55} to 91\%\textsuperscript{56} in different multicenter studies, demonstrating that ATO is an effective drug for APL. Using ATO as a single agent, a good long-term remission can be obtained, as evidenced by a 5-yr event-free survival (EFS) of 69\%\textsuperscript{57} to 72.7\%\textsuperscript{58} in 2 recent reports (Table 1).

The efficacy of pure As\textsubscript{4}S\textsubscript{4} in APL was also investigated. In 1995, Huang et al\textsuperscript{59} reported remarkable results of the Realgar-Indigo Naturalis Formula, in which As\textsubscript{4}S\textsubscript{4} is the principle...
ingredient, in treating 60 APL patients including 43 newly diagnosed cases. In 2002, Lu et al. showed that of the 129 APL patients receiving As$_4$S$_4$, 103 (79.8%) cases achieved CR. In the newly diagnosed group (19 patients), the estimated disease free survival (DFS) rates for 1 and 3 years were 86.1% and 76.6%, respectively, with a median follow-up time of 13.5 months. They further showed that in 114 patients receiving As$_4$S$_4$ in combination with ATRA or chemotherapy (mitoxantrone and/or hydroxyurea), the estimated 4-yr DFS was 94%.

Mechanisms of action

PML-RAR$\alpha$ as a direct arsenic target

That ATO exerts drastic therapeutic effects against APL, but not other subtypes of AML (including variant APL driven by the PLZF-RAR$\alpha$ fusion), suggests a crucial link between its mechanism of action and PML-RAR$\alpha$, a potent transcriptional regulator that alters expression of ATRA or non-ATRA target genes. Indeed, it was rapidly shown that arsenic efficiently triggers the degradation of PML-RAR$\alpha$, through its PML moiety.

The wild-type PML and the PML moiety in fusion protein harbor the RBCC domain, which contains one RING and two B boxes (B box 1 and B box 2) motifs capable of binding metal (physiologically zinc) ions, and a coiled-coil (CC) motif mediating homodimer formation. Fig. 2A is a schematic representation of major domains of PML, RAR$\alpha$ and PML-RAR$\alpha$. In normal cells, PML proteins are the major components of spherical nuclear organelles designated nuclear bodies (NBs), which play a key role in regulation of apoptosis, epigenetic control of chromatin and transcriptional expression as well as storage/modulation of certain nuclear proteins. NBs structures are disrupted in APL cells, due to formation of PML/PML-RAR$\alpha$ heterocomplex. This yields a much larger number of tiny dots by
immunofluorescence analysis, illustrating the disorganization of this nuclear domain. The ATRA and arsenic-triggered degradation process is intimately coupled to changes in PML/PML-RARα localization. Indeed, in ATO-treated APL cells or cells transfected with PML, RARα, or PML-RARα, both PML-RARα and wild-type PML are quickly translocated to the nuclear matrix, sumoylated, ubiquitinated and subsequently degraded by the proteasome. In parallel, in arsenic-treated APL cells, dots of PML-containing proteins aggregated to form larger particles at the nuclear matrix, before their ultimate disappearance (Fig. 2B) and the cells committed to apoptosis or partial differentiation (Fig. 2C).

Because the wild-type PML and the PML moiety in the fusion protein harbor a number of adjacently located cysteine residues with metal-binding ability in its RBCC domain, we hypothesized that ATO might target PML/PML-RARα at this domain. We tested this possibility by biotin-arsenic/streptavidin pull down affinity assay and a red fluorescent organic arsenic compound ReAsH/immunofluorescent analysis. Zhang et al showed that ReAsH co-localized with PML/PML-RARα (Fig. 2D) and arsenic could directly bind the wild-type and fusion proteins. Consistently, deletion of RING domain in PML abolished ReAsH co-localization signal. The PML RING peptide containing C3HC4 (aa 57 – 91) zinc finger (ZF) was expressed and purified for refined arsenic binding analyses. By using MALDI-TOF mass spectrometry, it was found that one PML RING molecule without metal ions (apo-PML RING) could bind two arsenics at 1 to 2 μM concentrations of ATO. Arsenic bound PML RING through thiol groups of cysteines with the formation of As-S bonds, as evidenced by near-ultraviolet absorbance spectrometry assays. By using x-ray absorption spectra assay including extended x-ray absorption fine structure (EXAFS) and x-ray
absorption near-edge structure (XANES), the local structures of PML RING around metal ions within about 6 Å were obtained, and it was found that trivalent arsenics could coordinate to PML RING each via three conserved cysteines, in ZF1 with C60, C77 and C80 and ZF2 with C72, C88 and C91, as compared to the coordination by zinc in ZF 1 with C57, C60, C77, C80, and in ZF2 with C72, H74, C88 and C91 (Fig. 2E). In addition, the PML B box2 domain also directly bound arsenic in vitro, while its deletion led to a significant reduction of ReASH co-localization signals in cells.\textsuperscript{46,76} Of note, arsenic was able to competitively replace zinc in PML RING coordinated with zinc (zinc-PML RING) according to the NMR heteronuclear single-quantum coherence (HSQC) spectra.\textsuperscript{76} Upon binding to arsenic, PML RING underwent conformational changes and aggregation, most likely because of the formation of homodimer via inter-molecular As-S bonds, followed by oligomerization among these homodimers.\textsuperscript{46,76} In in vitro reconstituted conditions and mammalian two-hybrid assay in cells, arsenic binding to PML facilitated its interaction with the unique SUMO E2 conjugase Ubc9, leading to sumoylation at K65 and K160.\textsuperscript{77} K160 also mediated subsequent recruitment of 11S proteasome.\textsuperscript{78} A 3-D structure modeling reveals that the two ZFs in PML RING motif locate at the interface with Ubc9, providing a structural basis for the formation of PML/Ubc9 complex in the presence of arsenic (Fig. 2F). Recent studies indicate that RNF4, a ubiquitin E3 ligase containing SUMO interaction motif (SIM),\textsuperscript{74,79} could recruit sumoylated PML/PML-RARα and promote their proteasomal degradation.\textsuperscript{74,79} These results demonstrate that ATO controls the fate of the PML-RARα by directly binding PML (Fig. 2G), and at least partially explain why ATO is effective for APL.\textsuperscript{76}

In addition to formation of arsenic-cysteine bonds that favor aggregation, arsenic-induced
ROS also initiate intermolecular disulfide formation. Disulfide-linked PML or PML-RARα multimers become nuclear matrix-associated and form NBs. Thus, PML oxidation regulated NB-biogenesis. In that respect, non-arsenical oxidants also elicited PML-RARα multimerization, NB-association, degradation, and leukemia response in vivo. Critically, oxidants did not affect PLZF-RARα-driven APL, a genetic demonstration that PML is the key target. Arsenic can also bind other proteins including the ubiquitin E3 ligases c-CBL (Casitas B-lineage lymphoma) and SIAH1, both harboring RING finger motifs (Table 2).

Elimination of leukemia-initiating cells (LICs)

LICs are pluripotent, self-renewing, phenotypically primitive and mitotically quiescent cells which have been identified in acute and chronic myeloid and lymphoid leukemia subtypes. Their non-cycling status and inherent or acquired drug resistance mechanisms allow them to escape conventional and targeted therapies that effectively kill proliferating leukemia cell. In APL, PML-RARα is required and even minute amount of the oncoprotein allows LICs self-renewal in vivo. Zheng et al showed that in Sca1+/lin– murine hematopoietic stem cells retrovirally transduced with PML-RARα and LICs from PML-RARα mice, ex vivo treatment with arsenic overcomes the aberrant stem cell capacity of PML-RARα-positive LICs. Whereas transcriptional activation of PML-RARα upon effect of ATRA is likely to control differentiation, only the catabolism of the fusion protein triggers LICs eradication and long-term remission of mouse APL. ATRA induces differentiation of PLZF-RARα-driven mouse APL, but neither LICs clearance nor disease remission, explaining the clinical ATRA-resistance of this rare APL subtype. Importantly, the ATRA/ATO combination rapidly clears PML-RARα-positive LICs, resulting in APL eradication in murine models and
patients. Because anthracyclines produce ROS, AIDA (ATRA and Idarubicin) regimen may induce PML-RARα degradation and hence promote LICs clearance, resulting in dramatically prolonged survival.

Arsenic targeting of PML may also be important in non-APL setting. Indeed Ito et al showed that PML was required for hematopoietic stem cell maintenance, and in CML it appeared to be the factor that enabled LICs to maintain their quiescence—the inert state that prevented them from being destroyed by cancer therapies. Interestingly, ATO could reversibly decrease PML expression in LICs, suggesting that this agent may be of broader interest than previously thought. In gliomas, ATO seemed able to inhibit Notch pathway and deplete the cancer stem-like cell population. ATO was shown to antagonize the Hedgehog pathway by preventing ciliary accumulation and reducing stability of the Gli2 transcriptional effector. ATO also represses NFκB and β-catenin, facilitating elimination of LICs (Fig. 3).

**Cell differentiation, apoptosis and autophagy**

At cellular level, ATO exerts dose-dependent dual effects on APL cells in that it induces apoptosis through activating the mitochondria-mediated intrinsic apoptotic pathway at high concentration (1 to 2 μM), and promotes cell differentiation at low concentrations (0.25 to 0.5 μM) (Fig. 2C). The mechanisms of pro-apoptotic activity of ATO were scrutinized by many groups at gene/protein levels, and a large body of information has been gathered, including histone H3 phosphoacetylation at the Casp-10, the involvement of JNK signaling, anion exchanger 2 and GSTP1-1, the suppression of human telomerase reverse transcriptase (hTERT), C17 and c-Myc through Sp1 oxidation, the repression of NFκB activation and the down-regulation of Wt1 gene. Recently, a pathway composed of ATR...
(ataxia telangiectasia mutated and Rad3-related), PML, Chk2 and p53 has been proposed to mediate ATO-induced apoptosis. ATO upregulates a set of genes such as NADPH oxidase and 8-hydroxy-2’-deoxyguanosine (8-OHdG), and causes generation of ROS which play a role as a mediator to induce apoptosis through release of cytochrome c to cytosol and activation of caspases. However, while all those mechanisms likely contribute to chronic or acute arsenic toxicity, it is unclear what is their contribution to arsenic response in APL, because they are unlikely to be specific for APL cells.

Recent studies further showed that in APL cells, both ATRA and ATO induce autophagy via the mammalian target of rapamycin (mTOR) or MEK/ERK pathway, and autophagic degradation contributes significantly to proteolysis of PML-RARα. Inhibitors of autophagy or molecular targeting of Beclin 1 or Atg7 results in reversal of the suppressive effects of ATO on leukemic cells.

**Arsenic-based combinatorial therapeutic regimens**

**ATO/ATRA combination**

Rationales Combination treatment regimens have been carefully designed to conquer APL. In a *PML-RARα* mouse model and a human NB4 APL cell line-based ascites/leukemia mouse model, ATRA/ATO combination dramatically prolongs survival or even eradicates the disease. Both ATO and ATRA induce LICs loss in PML-RARα mouse APL, and combination of the two results in synergistic clearance of LICs through cooperative PML-RARα degradation. Moreover, ATRA was shown to be able to increase the expression of the cell membrane arsenic transporter AQP9, which facilitated arsenic uptake.

Though ATO alone induces partial differentiation, it synergizes with cyclic AMP (cAMP)
analogue 8-CPT-cAMP in inducing full maturation of ATRA-sensitive and ATRA-resistance cells. On the other hand, activation of cAMP signaling was shown to enhance LIC loss by ATRA. Interestingly, ATRA could rapidly trigger a marked increase in the intracellular cAMP level and cAMP-dependent protein kinase (PKA) activity. Therefore, a crosstalk may exist between ATO and ATRA signaling pathways through cAMP/PKA node.

Moreover, ATRA potentiates ATO-induced RXRα phosphorylation and cooperates with ATO to induce apoptosis, and it was shown that ATRA induced degradation while ATO antagonized catabolism of IκB. In the maturation-resistant NB4-R1 cells, ATRA exhibited antiproliferative properties through downregulation of telomerase and ATO enhanced this effect. When transcriptome/proteome approaches were used, Zheng et al reported that while ATRA mainly caused transcriptional remodeling, ATO induced a deeper change of proteome pattern. ATRA/ATO combination amplified RA signaling, as highlighted by molecules involving IFN, calcium, cAMP/PKA, MAPK/JNK/p38, G-CSF, and TNF pathways. ATRA/ATO combination strongly activated ubiquitin-proteasome pathway, and significantly repressed genes/proteins promoting cell cycle or enhancing cell proliferation. Interestingly, ATRA/ATO combination did not enhance the expression of stress-response-related genes including HSPA8, HSPCA, and AHSAI. Taken together, these results suggest that ATRA/ATO combination may cause a synergy in therapeutic efficacy, but not adverse effects.

Clinical efficacy A randomized clinical trial comparing ATRA/ATO combination and monotherapy was conducted in SIH from April 2001 to February 2003. Sixty one APL cases were randomized into three groups treated respectively with ATRA, ATO, and the
combination of the two. It was reported in 2004 that the three groups achieved same results in
terms of CR rates (≥90%) although the combination therapy group needed the shortest time
duration for remission induction. An obvious advantage of the combination therapy was that
it generated much less minimal residual disease after consolidation than the two other
therapeutic approaches as measured with real-time quantitative RT-PCR for PML-RARα.
After a follow-up of 8 to 30 months all patients in combination therapy group were in good
clinical remission, whereas 7 out of 37 cases in the two monotherapy groups relapsed
(p<0.05), demonstrating the superiority of the combination therapy. Under this circumstance,
the investigators from the SIH decided, from the ethical point of view, a termination of the
randomized grouping and only the arm of combination therapy should be extended. In 2009,
SIH reported the results of 85 patients administrated ATRA/ATO with a median follow-up of
70 months. Eighty patients (94.1%) entered CR. Kaplan-Meier estimated of the 5-yr EFS
and OS for all patients were 89.2%±3.4% and 91.7%±3.0%, respectively, and the 5-yr
relapse-free survival (RFS) and OS for patients who achieved CR (n = 80) were 94.8% ±
2.5% and 97.4% ± 1.8%, respectively. Upon ATRA/ATO, prognosis was not influenced by
initial white blood cell count, distinct PML-RARα types, or FLT3 mutations. The toxicity
profile was mild and reversible (see below). The results were confirmed by recent long-term
follow-up studies. Powell et al reported that out of the 244 patients who received
ATRA/ chemotherapy as induction and ATRA/ chemotherapy plus ATO as consolidation
therapies, 195 (80%) cases achieved a 3-yr EFS. Compared to the above trial using
ATRA/ATO/ chemotherapy as induction therapy, the slightly lower EFS rate of this study
might be due to the multi-center nature of the trial, or could reflect the advantage of
incorporating ATO into induction remedy for newly diagnosed APL. Taken together, ATRA/ATO/ chemotherapy combinatory regimen transforms APL from a highly fatal to a highly curable disease.

Realgar-Indigo Naturalis Formula ATRA/As$_4$S$_4$ combination also showed enhanced therapeutic efficacy in APL.$^{61}$ In traditional Chinese Medicine (TCM), combination therapy containing multiple drugs with distinct but related mechanisms has been advocated for more than 2,500 years by prescriptions called formulae in order to amplify therapeutic efficacies of each agent and minimize adverse effects.$^{30,119}$ Based on TCM theories, a patented Realgar-Indigo Naturalis Formula (RIF) was designed in 1980s,$^{59}$ in which a mined ore realgar was the principle element, while *indigo naturalis*, *salvia miltiorrhiza* and *radix pseudostellariae* were adjuvant components to assist effects of realgar. Multicenter clinical trials showed that a CR rate of 96.7%$^{120}$ to 98%$^{59}$ and a 5-yr OS rate of 86.88%$^{121}$ were achieved in APL patients receiving RIF, with moderate gastrointestinal discomfort and rash as main adverse effects. Realgar in combination with *indigo* also exhibited an extent of anti-APL activity.$^{122}$ Recently, the mechanisms of action of RIF were carefully dissected employing As$_4$S$_4$ (A), indirubin (I), and tanshinone IIA (T) as representatives of realgar, *indigo naturalis*, and *salvia miltiorrhiza*, respectively$^{123}$, and it was shown that ATI combination yielded enhanced therapeutic efficacies against APL in murine model. ATI combination caused synergetic effects and resulted in a much more profound differentiation of APL cells, potentiated ubiquitination and degradation of PML-RAR$\alpha$ oncoprotein, stronger reprogramming of myeloid differentiation regulators, and enhanced G1/G0 arrest compared to cells treated with mono- or bi-agents. Furthermore, T and I upregulated AQP9 and
facilitated transportation of arsenic into malignant promyelocytes, which in turn intensified arsenic-mediated PML-RARα degradation and therapeutic efficacies (Fig. 4). These results open a new window for a better understanding of the therapeutic strategies of other traditional formulae.

ATO in combination with MEK1 inhibition

Studies demonstrate that activation of the extracellular signal-regulated kinases 1/2 (ERK1/2) as well as of the kinases immediately upstream of ERK, known as mitogen-activated protein (MAP)/ERK kinases (MEKs) can confer a drug-resistant phenotype to cancer cells. For example, rapamycin and its analogs activate the MAPK pathway in solid tumor, imatinib increases the activity of p42/44 MAPK in CML CD34+ cells which contributes to incomplete elimination of CML progenitors, and FLT3 inhibitor-resistant cells show continued activation of PI3K/Akt and/or Ras/MEK/MAPK signaling pathways. Accordingly, MAPK/MEK inhibitors may be helpful to overcome drug-resistance in leukemic cells. Altman et al showed that in leukemia cells upon ATO treatment, the Akt kinase is phosphorylated/activated to regulate downstream engagement of mTOR and its effectors. Targeted disruption of Akt1/Akt2 genes or inhibition of mTOR strongly enhances ATO’s effects on leukemia cells. Treatment with ATO induces a MAPK-mediated PML phosphorylation which is associated with subsequent ubiquitination and proteasomal degradation. Lunghi et al reported that APL cells exploited the Ras-MAPK pathway to inactivate the proapoptotic protein Bad by phosphorylation at Ser112 and delay ATO-induced apoptosis. MEK1 inhibitors suppressed ERK1/2, dephosphorylated Bad and inhibited the ATO-induced increase of Bcl-xL, resulted in enhanced apoptosis and overcame
drug-resistance. Combined use of ATO and MEK1 inhibitors leads to induction of the p53AIP1 (p53-regulated apoptosis-inducing protein 1) in NB4 and K562 cell lines and primary cells from AML patients, and inhibition of tumor growth and elongation of survival in a human xenograft multiple myeloma (MM) model. These studies provide the framework for testing MEK1 inhibitor/ATO combination in patients with hematological malignancies.

**Arsenic in treating CML**

CML, a malignant myeloproliferative disease originated from pluripotential hematopoietic stem cells, is characterized by the Philadelphia (Ph) chromosome formed by translocation t(9;22)(q34;q11) which generates a chimeric fusion protein BCR-ABL with constitutively activated tyrosine kinase activity. Imatinib mesylate (IM; or Gleevec, Glivec or STI571), a rationally-designed BCR-ABL inhibitor, has demonstrated remarkable clinical efficacy which achieved an estimated 5-yr OS of 89% in 553 CML patients. However, IM and Dasatinib do not deplete LICs, while a proportion of patients develops IM-resistance, and patients with advanced stage disease respond initially but then relapse. Moreover, cardiotoxicity of IM was also reported.

Historically, ATO therapy was the first chemotherapeutic intervention for CML. Fowler’s solution was used to treat CML in 19th century and became the mainstream therapeutic reagent for leukemia. In the 1930s, the efficacy of arsenic in the treatment of CML established it as a primary therapeutic agent for this disease. Until the advent of modern chemotherapy, arsenic and radiation were the mainstays of treatment for patients with CML. Recently, arsenic was shown to be able to target PML and eradicate quiescent LICs in CML.
ATO inhibited translation of mRNA of BCR/ABL, resulting in attenuation of BCR/ABL levels and apoptosis of human leukemia cells. Zhang et al. reported that arsenic targets BCR-ABL via ubiquitination of key lysine residues, leading to its proteasomal degradation. Recently Mao et al. showed that arsenic could directly bind c-CBL, the E3 ligase of BCR-ABL, via the conserved cysteines including C381 at RING finger domain (Fig. 5), resulting in inhibition of c-CBL’s self-ubiquitination at K389 and subsequent proteasomal degradation. Consistent with these results, substitution of cysteine at 381 or lysine at 389 by alanine abrogated arsenic binding and c-CBL’s self-ubiquitination, respectively. Consequently, elevated c-CBL promoted ubiquitination of BCR-ABL at K1517, leading to degradation of the aberrant kinase (Fig. 5).

Arsenic exerts synergistic effects with IM in inducing apoptosis of CML cells and in prolonging survival of mice inoculated with CML cells. It was shown that arsenic and IM induce cell cycle arrest at G2/M and G1 phases, respectively. Arsenic and IM synergistically activate the endogenous and exogenous ER stress, leading to enhanced cell apoptosis. These discoveries provide rationales for a clinical trial to test the arsenic/IM combination therapy in CML.

Arsenic in treating other malignancies

Arsenic has been used in treating multiple myeloma (MM), myelodysplasia syndrome (MDS) and lymphoid malignancies including non-Hodgkin lymphoma, and displayed beneficial effects in some cases (Table 3). In adult T-cell leukemia/lymphoma (ATL)-derived cells, ATO reportedly synergized with interferon-α (IFNα) to induce cell cycle arrest and apoptosis through down-regulation of the HTLV-1 oncoprotein Tax and inactivation of
Clinically, arsenic/interferon therapy exhibited some efficacy in 7 refractory aggressive ATL patients, while in 10 newly diagnosed chronic ATL cases arsenic/interferon/zidovudine combination showed an impressive 100% response rate. Recent animal studies in lck-Tax transgenics that develop an ATL-like disease have recapitulated the therapeutic action of the arsenic/IFNα association, strongly suggesting that the latter is actually targeting Tax for degradation. Moreover, transplantation studies have demonstrated that Tax degradation is accompanied by loss of leukemia-initiating activity, but not short-term growth, providing a striking parallel with APL and suggesting that arsenic may promote catabolism of specific classes of oncoproteins.

There are 111 recently completed or ongoing clinical trials listed on www.clinicaltrials.gov evaluating ATO alone or in combination with other agents for treatment of cancers excluding APL. ATO is under investigation as treatment for a variety of solid tumors including lung cancer, hepatocellular carcinoma (HCC) and colorectal cancer. Limited clinical activity as a single agent has been reported in a small number of patients with HCC, melanoma, and renal cell carcinoma; ATO in combination with chemotherapy has shown promising activity in osteosarcoma and Ewing sarcoma (and references in this review article).

**Adverse effects of arsenic**

Although arsenic seems to be synonymous with poison, nearly all recent clinical trial results suggest that arsenic at therapeutic concentrations is generally well tolerated. No bone marrow depression and chemotherapy-associated secondary malignancy was observed with arsenic treatment. Sudden death was recorded in one study, and severe liver impairment was documented.
These toxicities might be due to a genetic basis with exceptional susceptibilities to arsenic toxicity in rare patients, exposure to anthracyclines or other cardiotoxic agents prior to ATO therapy and abnormal electrolyte levels, or other unidentified factors.\textsuperscript{53,57,157,158} Hyperleukocytosis, a retinoic-acid syndrome (or differentiation syndrome)-like clinical entity was also reported, and was shown to be driven by chemokine production induced by ATO or ATRA as single agent or in combination.\textsuperscript{159} In long-term studies,\textsuperscript{39,57,58,158} the toxicity profile of arsenic was mild and 24 months after the last dose of ATRA/ATO, patients had urine arsenic concentrations well below the safety limit. Mathews et al\textsuperscript{57} reported that despite counseling against pregnancy post-therapy, in view of the absence of data on impact of prior ATO therapy and teratogenicity, seven patients (four women and three men) have had eight normal babies.

**Perspectives**

As a traditional poison, inappropriate use of arsenic may kill people; as one of the oldest drugs in the world, its appropriate application cures some cancer types and saves lives. These facts clearly suggest that when considering how to control an emerging “bad factor”, one might try to find out its other side and the safe translation.

Arsenic is the most potent single agent against APL. The revival of arsenic by its application in treating APL is a unique story in cancer research. It also highlights some of the essential concepts in pharmacology, such as the key importance of the therapeutic ratio between normal cells and the target. It illustrates the power of combinations. Indeed, in APL, ATO/ATRA combination has exhibited drastically enhanced therapeutic efficacy compared to either single agent, transforming the fate of an otherwise highly fatal disease. In treating other
types of malignancies including solid tumors, rational combinatory regimens could be
designed to improve clinical outcome. For example, since arsenic binds and activates c-CBL
which controls signaling of EGFR, the therapeutic efficacy of ATO in combination with
EGFR inhibitor could be tested in non-small cell lung cancer and other related human
malignancies.
Authorship

Contribution: S.J.C, G.B.Z., X.W.Z, J.H.M, H.D.T. and Z.C. have all contributed to the writing of this manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Sai-Juan Chen, Ph.D, or Zhu Chen, Ph.D, Shanghai Institute of Hematology, Rui Jin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, 197 Rui Jin Road II, Shanghai, 200025, China; e-mail: sjchen@stn.sh.cn or zchen@stn.sh.cn.

Acknowledgement

We apologize to our many colleagues whose work could not be cited due to space restrictions. The authors thank Prof. Zhen-Yi Wang at SIH for his long term support, Dr. Laurent Degos from Hospital Saint Louis in Paris and Dr. Samuel Waxman from Mount Sinai Medical Center in New York for friendly long-term collaboration. This work was supported in part by the Chinese National Key Program for Basic Research (973; 2010CB529200) and National High Tech Program (863), National Natural Science Foundation of China, Shanghai Municipal Commission for Science and Technology and Shanghai Municipal Commission for Education, and Samuel Waxman Cancer Research Foundation.
References


47. Rego EM, He LZ, Warrell RP, Jr., Wang ZG, Pandolfi PP. Retinoic acid (RA) and As2O3 treatment in transgenic models of acute promyelocytic leukemia (APL) unravel the distinct nature of the leukemogenic process induced by the PML-RARalpha and PLZF-RARalpha oncoproteins. Proc Natl Acad Sci U S A 2000;97(18):10173-10178.


94. Pan XY, Chen GQ, Cai L, Buscemi S, Fu GH. Anion exchanger 2 mediates the action of


183. Chang JE, Voorhees PM, Kolesar JM et al. Phase II study of arsenic trioxide and ascorbic
Table 1. Outcome of APL patients treated with ATO-based regimens since 2006

<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>N</th>
<th>Regimen</th>
<th>CR, %</th>
<th>EFS, %*</th>
<th>DFS, %*</th>
<th>OS, %*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>Powell et al\textsuperscript{118}</td>
<td>244 (237       standard controls)</td>
<td>Induction: ATRA/CT; consolidation: ATRA/CT/ATO</td>
<td>90</td>
<td>80 (3 yr)</td>
<td>90 (3 yr)</td>
<td>86 (3 yr)</td>
</tr>
<tr>
<td>2010</td>
<td>Zhou et al\textsuperscript{58}</td>
<td>19 (age, ≤15 )</td>
<td>ATO</td>
<td>89.5</td>
<td>72.7</td>
<td>NR</td>
<td>83.9</td>
</tr>
<tr>
<td>2010</td>
<td>Mathews et al\textsuperscript{57}</td>
<td>72</td>
<td>ATO</td>
<td>86.1</td>
<td>69</td>
<td>80</td>
<td>74.2</td>
</tr>
<tr>
<td>2009</td>
<td>Dai et al\textsuperscript{160}</td>
<td>90</td>
<td>ATO+ATRA</td>
<td>93.3</td>
<td>92.2 (3 yr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>Ghavamzadeh et al\textsuperscript{61}</td>
<td>111</td>
<td>ATO</td>
<td>85.6</td>
<td>NR</td>
<td>63.7 (2 yr)</td>
<td>87.6 (3 yr)</td>
</tr>
<tr>
<td>2008</td>
<td>Hu et al\textsuperscript{39}</td>
<td>85</td>
<td>ATO+ATRA</td>
<td>94.1</td>
<td>89.2</td>
<td>NR</td>
<td>91.7</td>
</tr>
<tr>
<td>2007</td>
<td>Wu et al\textsuperscript{61}</td>
<td>114</td>
<td>A\textsubscript{5}S\textsubscript{3}+ATRA or A\textsubscript{5}S\textsubscript{3}+CT</td>
<td>94 (4 yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*5 yr, otherwise indicated. NR, not reported.
<table>
<thead>
<tr>
<th>Proteins and references</th>
<th>Disease or cell lines</th>
<th>Category</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>PML 76</td>
<td>APL</td>
<td>Phosphoprotein; scaffold protein</td>
<td>tumor suppressor; probable transcription factor</td>
</tr>
<tr>
<td>c-CBL 80</td>
<td>CML, K562 cells</td>
<td>Ubiquitin ligase</td>
<td>E3 proteolysis</td>
</tr>
<tr>
<td>SIAH1 80</td>
<td>CML, K562 cells</td>
<td>Ubiquitin ligase</td>
<td>E3 proteolysis</td>
</tr>
<tr>
<td>Trx R 162</td>
<td>MCF-7 cells</td>
<td>Oxidoreductase</td>
<td>Redox regulation</td>
</tr>
<tr>
<td>GSR 163</td>
<td>Arsenic intoxication</td>
<td>Oxidoreductase</td>
<td>Redox regulation</td>
</tr>
<tr>
<td>TPX-2 II 164</td>
<td>Ovary cells</td>
<td>Peroxidase</td>
<td>Redox regulation</td>
</tr>
<tr>
<td>PDI 165</td>
<td>fibrosarcoma cells</td>
<td>Oxidoreductase</td>
<td>Redox regulation</td>
</tr>
<tr>
<td>MTs 166</td>
<td>arsenic detoxication</td>
<td>Metallothioneins</td>
<td>Binding heavy metals</td>
</tr>
<tr>
<td>MTF1 167</td>
<td>arsenic detoxication</td>
<td>Transcription factor</td>
<td>Activation of metallothionein transcription</td>
</tr>
<tr>
<td>Keap1 27</td>
<td>hepal c1c7 cells</td>
<td>Phosphoprotein</td>
<td>Transcription regulation</td>
</tr>
<tr>
<td>Tubulins 168-170</td>
<td>K562 cells</td>
<td>Cell skeleton proteins</td>
<td>Structural subunit of microtubules</td>
</tr>
<tr>
<td>β-actin 169-171</td>
<td>K562 and MCF-7 cells</td>
<td>Cell skeleton proteins</td>
<td>Cytoskeleton</td>
</tr>
<tr>
<td>PPM1D 172</td>
<td>malignancies</td>
<td>Phosphatase; oncoprotein</td>
<td>Protein serine/threonine phosphatase; regulation of cell cycle</td>
</tr>
<tr>
<td>JNK phosphatase 36</td>
<td>carcinoma</td>
<td>Dual specificity protein phosphatase</td>
<td>Cellular signaling</td>
</tr>
<tr>
<td>Ikappa B 173</td>
<td>inflammation and carcinogenesis</td>
<td>Protein kinase</td>
<td>Regulation of NFκB pathway</td>
</tr>
<tr>
<td>Galectin-1 164,174</td>
<td>Ovary cells</td>
<td>Pyruvate kinase</td>
<td>Regulation of NFκB pathway</td>
</tr>
<tr>
<td>PKM2 169</td>
<td>MCF-7 cells</td>
<td>Phosphoprotein</td>
<td>Glycolysis</td>
</tr>
<tr>
<td>Hemoglobin 175</td>
<td>red blood cells</td>
<td>Globin family</td>
<td>Oxygen transport</td>
</tr>
</tbody>
</table>
Table 3. Clinical studies of arsenic in treating other malignancies.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Year</th>
<th>Authors</th>
<th>N</th>
<th>regimen</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM</td>
<td>2006</td>
<td>Berenson&lt;sup&gt;176&lt;/sup&gt;</td>
<td>65</td>
<td>ATO + AA + Melphan</td>
<td>2 CR; 15 PR; 14 MR</td>
</tr>
<tr>
<td></td>
<td>2006</td>
<td>Abou-Jawde&lt;sup&gt;177&lt;/sup&gt;</td>
<td>20</td>
<td>ATO + AA + Dexamethasone</td>
<td>6 PR;</td>
</tr>
<tr>
<td></td>
<td>2006</td>
<td>Wu&lt;sup&gt;178&lt;/sup&gt;</td>
<td>20</td>
<td>ATO + AA + Dexamethasone</td>
<td>2 PR; 6 MR</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>Berenson&lt;sup&gt;179&lt;/sup&gt;</td>
<td>22</td>
<td>ATO + AA + Bortezomib</td>
<td>2 PR; 4 MR</td>
</tr>
<tr>
<td>ATL</td>
<td>2004</td>
<td>Hermine&lt;sup&gt;153&lt;/sup&gt;</td>
<td>7</td>
<td>(Relapsed/refractory)</td>
<td>ATO + IFN</td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>Kchour&lt;sup&gt;154&lt;/sup&gt;</td>
<td>10</td>
<td>(Newly diagnosed)</td>
<td>ATO + IFN + Zidovudine</td>
</tr>
<tr>
<td>MDS</td>
<td>2006</td>
<td>Schiller&lt;sup&gt;180&lt;/sup&gt;</td>
<td>76</td>
<td>ATO</td>
<td>1 CR; 13 HI</td>
</tr>
<tr>
<td></td>
<td>2006</td>
<td>Vey&lt;sup&gt;181&lt;/sup&gt;</td>
<td>115</td>
<td>ATO</td>
<td>1 CR; 1 PR; 22 HI</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>Zheng&lt;sup&gt;182&lt;/sup&gt;</td>
<td>21</td>
<td>ATO + RA + Thalidomide</td>
<td>1 CR; 1 PR; 3 HI</td>
</tr>
<tr>
<td>Lymphoid malignancies</td>
<td>2009</td>
<td>Chang&lt;sup&gt;183&lt;/sup&gt;</td>
<td>16</td>
<td>ATO+AA</td>
<td>1 response</td>
</tr>
</tbody>
</table>

Abbreviation: MM, multiple myeloma; AA, ascorbic acid; ATL, adult T-cell leukemia/lymphoma; CR, complete response; PR, partial response; MR, minor response; HI, hematologic improvement, RA, retinoic acid.
Legends for figures

Fig. 1. A historical view of the treatment outcome (represented by 5 yr overall survival) of APL.

Fig. 2. Effects of arsenic on APL cells. (A) Schematic represents the structure of PML, RARα and PML-RARα. (B) The NB4 cells were treated with 1 µM ATO for indicated time points, and assessed by immunofluorescence staining with an anti-PML antibody (green). (C) Arsenic induces dual effects on APL cells. The NB4 cells were treated with indicated concentration for 48 hr, and stained with the Wright’s stain. (D) Colocalization of PML and PML-RARα with the fluorescent organic arsenical ReAsH in NB4 cells. (E) The schematic diagram of the structure of PML RING coordinated with zinc or arsenic. (F) Predicted structure of PML RING/Ubc9 complex. (G) A working model of the mechanism by which arsenic controls the fate of PML and PML-RARα.

Fig. 3. Arsenic targets critical pathways for the leukemia-initiating cells. Arsenic induces generation of ROS, perturbation of some signal pathways and modulation of transcriptional factors. Arsenic also activates MEK1/ERK pathway, while combination of MEK1 inhibitor (MEKi) and arsenic results in synergistic anti-tumor effects.

Fig. 4. Mechanisms of action of representative components of RIF in treating APL. As, As₄S₄; Ind, indirubin; Tan, tanshinone IIA.
Fig. 5. Effects of arsenic on c-CBL and BCR-ABL in CML cells. (A) Without Arsenic treatment, c-CBL is self-ubiquitinated and degraded in proteasome. (B) Arsenic treatment inhibits c-CBL self-ubiquitination and proteasomal degradation, and triggers ubiquitination of BCR-ABL at K1517 followed by degradation in proteasome.
Fig. 1
Fig. 2
Fig. 4
Fig. 5
From an old remedy to a magic bullet: molecular mechanisms underlying the therapeutic effects of arsenic in fighting leukemia

Sai-Juan Chen, Guang-Biao Zhou, Xiao-Wei Zhang, Jian-Hua Mao, Hugues de The and Zhu Chen