Blood's 70th anniversary: arsenic—from poison pill to magic bullet

As part of a year-long celebration in honor of Blood*'s 70th anniversary, we are publishing a series of editorials written by past Editors-in-Chief of the journal. The authors reflect on their experience at* Blood *in light of the journal's publication history. Each of these special pieces will highlight and discuss the impact of one or more original research articles that had a significant influence on the field or that mark a pioneering scientific development in hematology that appeared in the journal during the author's term as Editor-in-Chief.*

As part of the American Society of Hematology (ASH) celebration of the 70th birthday of its flagship journal *Blood,* past editors were asked to reflect back on some articles published during their term. In thinking about this request, many articles published between 1993 and 1997 came to mind that impacted hematology greatly, but for me, none were more interesting than 2 papers from investigators in Shanghai and Harbin, China, published back-to-back in 1997, describing the mechanism of action and clinical results of treating patients with acute promyelocytic leukemia (APL) with arsenic trioxide (As_2O_3).[1,2] At the time, As_2O_3 treatment of leukemia was little known in Western medicine, despite many years of promising clinical studies in China. The new visibility of arsenic research created by these 2 *Blood* papers helped accelerate the development of As_2O_3 as a leukemia drug. Ultimately, As_2O_3 became widely used: first as a secondary therapy for APL patients, then as a primary therapy, and finally as part of the remarkably effective combination of all-*trans* retinoic acid (ATRA) and As_2O_3 as curative therapy for APL.

The notion that an arsenic compound might cure cancer was not an obvious one, and many readers doubtlessly reacted to these papers with interest and surprise. The 33rd element, arsenic (As), is a metalloid with semiconductor properties, found widely throughout the earth's crust and water. In nature, arsenic is found in both inorganic and organic compounds, with several sulfur compounds common enough to have names of their own, such as orpiment (As_2S_3) and realgar (As_4S_4). "White arsenic," or As_2O_3, can be readily produced by burning orpiment or realgar. Arsenic has been used in a variety of nonmedical applications, including in the manufacturing of electronic components; it has also been used as a paint pigment, a wood preservative, an insecticide, an herbicide, and (sadly) an agent of chemical warfare. Arsenic is a poison to humans, although it is possible that trace amounts have some biological value. Acute arsenic poisoning, typically through inhalation of dust, leads to damage to the gastrointestinal and nervous systems. Chronic arsenic poisoning, or arsenicosis, is usually the result of environmental contamination, such as that which formerly occurred in workers in the metal refining industry or those drinking contaminated water, and is associated with damage to the skin, liver, lungs, nervous system, and kidneys. The Environmental Protection Agency also classifies arsenic as a type A carcinogen, and exposure to arsenic is strictly regulated. Deliberate poisoning with arsenic was also once common, and a popular topic for detective stories. In past times, arsenic was both difficult to detect and easy to obtain because it was sold as a rat poison. It has been reported that in 19th-century England, one-third of all criminal poisonings involved arsenic.[3] Somewhere between 100 mg and 1 g of As_2O_3 was said to be plenty to "get the job done."

More interestingly, perhaps, is the fact that arsenic has been used as a medicine and remedy for at least hundreds, and possibly thousands, of years, usually in combination with other natural materials.[4] As 1 example of many, Hippocrates was said to use orpiment as an escharotic.[4] Topically, arsenicals were used for various skin conditions, especially acne and psoriasis. In 18th-century England, Thomas Fowler created a solution of 1% potassium arsenite ("Fowler's solution") that was used for >150 years as a "tonic" and in the late 1800s and early 1900s as a treatment for leukemia, especially chronic myelogenous leukemia.[4] In the early 1900s, arsenic became a popular treatment for syphilis, malaria, trichomoniasis, and other infectious diseases.

The history of As_2O_3 and APL is interesting. Sulfur salts of arsenic (including As_2S_3) were used in northern China in the 1960s and 1970s to treat acute leukemias.[5] In the early 1970s, a combination of As_2O_3, mercury chloride, and toad venom was reported as a successful treatment of cancers, including acute leukemias. Zhang Tingdong at Harbin Medical University reported that a medicine called "ailin I," composed of 99% As_2O_3 and 1% mercury by weight, had significant clinical activity; Zhang published several small studies in Chinese language journals (reviewed in Rao et al[5]). In the early 1990s, Zhang Peng and colleagues initiated studies with purified As_2O_3 without mercury, and reported complete remission (CR) rates in the range of 73%, and long-term survival of >28%, confirming that the active moiety in ailin I was As_2O_3. Side effects of arsenic were reported to be mild, particularly with regard to marrow suppression. By this time, the remarkable effects of ATRA (initially published in 1988 in *Blood* by Huang et al[6]) were well known, but almost all patients treated with ATRA alone relapsed, so there was an acute need for new therapies.

The 2 As_2O_3 papers published in *Blood* in 1997 extended the earlier published work in China, and brought international attention to these remarkable results. Starting with the clinical paper, Shen and colleagues reported the treatment of 15 patients with APL who had relapsed after initial responses to ATRA and chemotherapy.[2] Most patients had a t(15;17) karyotype or evidence of promyelocytic leukemia–retinoic acid receptor-α (PML-RARα) by polymerase chain reaction. Ten milligrams of As_2O_3 was given IV daily over 2 to 3 hours, and continued until CR or progression. A second 28-day course of arsenic was used for consolidation along with cytotoxic chemotherapy. Remarkably, 14 of 15 patients achieved CR, including 9 of 10 treated with As_2O_3 as a single agent. Hyperleukocytosis was less commonly observed than with ATRA, and severe cytopenias were not seen. The most common side effects were skin dryness or erythema, nausea, vomiting, and lassitude. At the time of publication, 10 of 15 patients remained in complete remission, and later studies from China suggested that a 3-year

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survival of 70% to 80% could be achieved with arsenic alone. These studies were rapidly confirmed in the West[7] and the value of As_2O_3 alone, in combination with chemotherapy, and later in combination with ATRA, has been extensively validated. Of note, Zhen-Yi Wang and Zhu Chen reviewed the development of both ATRA and As_2O_3 in a 2007 *Blood* article published in an issue to mark the 50th anniversary of ASH,[8] noting that discussions between Zhu Chen, Lauren Degos, and Sam Waxman about potential combination studies with ATRA and arsenic started as early as 1998, based on synergy being seen between the 2 agents in cell line experiments.

The clinical trial paper from Shen et al[2] was made all the more remarkable by its companion paper[1] on the mechanism of action of As_2O_3. Shen et al exposed NB4 cells, a human APL cell line expressing PML-RARα, to a wide range of concentrations of arsenic trioxide. Higher concentrations induced apoptosis, possibly associated with a downregulation of BCL2, while lower concentrations appeared to induce partial differentiation toward more mature myeloid cells. Similar effects were seen in primary APL cells cultured in vitro and in NB4 cells grown in mice. These studies confirmed and extended previous studies on NB4 cells by Chen et al.[9] Interestingly, the authors also reported that As_2O_3 was associated with a wave of differentiation in patients, about 3 weeks after initiation of therapy. However, potentially the most interesting finding was in Figure 6 of the article, where the authors showed that As_2O_3 treatment of NB4 cells resulted in almost complete loss of PML-RARα protein, and this was associated with a rapid return toward a more normal nuclear staining pattern of PML. These results suggested that, as far as APL is concerned, As_2O_3 was the second "targeted therapy" for acute leukemia in that it led specifically to the loss of the oncogene that causes APL. These results have been confirmed and extended over the last 2 decades, and it appears that a major mechanism of action is through direct binding of arsenic to PML, increasing sumoylation and ubiquitination of PML-RARα followed by rapid proteasomal degradation of the oncoprotein. The mechanism of action of arsenic has been the subject of many excellent reviews, including one by Zhu Chen and colleagues published in *Blood*.[10,11] Although enhanced degradation of PML-RARα appears to represent only part of the action of As_2O_3,[11] it is highly likely to be a critical part of the mechanism.

Overall, these 2 papers on As_2O_3 triggered enormous interest in the hematology community, and resulted in collaborations at both a research and a clinical level that spanned the globe. The result was an acceleration of the development of As_2O_3 as a drug, culminating in the current treatment strategy combining ATRA and As_2O_3 that likely cures the vast majority of these patients.[12] It has been a real pleasure to follow this field, and recall the initial impact of these 2 papers.

As an addendum, I was an Associate Editor of *Blood* for 5 years before becoming Editor-in-Chief. As I assumed that role in 1988, I "inherited" a paper still under review during the transition. The paper, by Huang et al, reported the remarkable clinical activity of ATRA in APL, which was found to induce complete remissions in 24 of 24 patients with APL, including 8 patients who were refractory to chemotherapy.[6] The reviewers had been concerned about many factors, including the definition of "complete response," but the real issue was that none of us were used to seeing papers reporting *clinical* results this good, despite excellent earlier in vitro laboratory studies showing that retinoids could induce terminal differentiation of APL cells.[13] In the end, we decided to publish the paper and let the hematology community see the results and begin confirmatory studies. And so they did.

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