

# A Review of Arsenic Trioxide and Acute Promyelocytic Leukemia

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## **ABSTRACT**

Arsenic Trioxide is an old drug that has recently re-introduced into new medicine. It is very potent against a specific type of leukemic cells harboring translocation between chromosomes 15 and 17. It has been demonstrated that this drug is effective against all stages of acute promyelocytic leukemia, including for remission induction of relapsed cases, or as first-line treatment. It is also useful in the consolidation/maintenance phase of treatment. Many trials are ongoing to determine the best and optimum schedule for this drug as a single agent or in combination with other drugs. In the future, its indications might extend to other malignancies. In this review, we will study biologic effects of arsenic trioxide on APL cells and the results of clinical trials on the treatment of APL. We will also discuss the toxicity and minimal residual detection during patient follow-up.

**KEYWORDS:** APL, Arsenic Trioxide, Biology and treatment

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## **INTRODUCTION**

Arsenic trioxide (ATO) is very potent as a single agent against acute promyelocytic leukemia (APL). This old drug was reintroduced into new medicine by Chinese studies on APL treatment and rapidly approved by the FDA for relapsed cases. It can induce complete remission when used for remission induction with or without the addition of chemotherapy or all-trans-retinoic acid.

It is useful in new cases of relapsed APL and has been used as a remission inducer or in the consolidation phase of treatment for APL. Recently, this drug has undergone study for other hematologic and non-hematologic malignancies and will probably be useful in future, in combinations with other drugs in these disorders. In this article, we will discuss the biologic effects and clinical applications of ATO in the targeted therapy of leukemia treatment and the role of hematopoietic stem cell transplantation in the treatment of APL.

## **Biologic effects of ATO in APL**

A unique chromosomal aberration, t(15,17), is the major genetic abnormality in majority of APL patients, and PML-RAR $\alpha$  chimeric gene production has a central role in APL pathogenesis. Targeted therapies usually disrupt this fusion gene or its signaling pathways in cells to control disease. Different mechanisms are proposed for the effects of ATO on the NB4 or HL-60 cell lines or in leukemic cells in vivo.

## **Differentiation induction**

ATO can induce differentiation of the NB4 cell line through attack on PML moiety of PML-RAR $\alpha$  fusion products and disruption of this chimeric protein when applied to cell lines at low concentration (0.1-0.5 mmol). This effect is through SUMOylation of PML-RAR $\alpha$  then SUMOylated PML recruits RING finger protein 4 (RNF4), which is known as a SUMO-

dependent ubiquitin ligase and polyubiquitylated PML-RAR $\alpha$  can be degraded by ubiquitin-proteasome pathway.<sup>1</sup>

Disruption of PML-RAR $\alpha$  releases the differentiation blockade in APL by overcoming the dominant negative effects of the fusion protein on the normal functioning of PML and RAR $\alpha$ .<sup>2,3</sup>

During cell maturation, the differentiation antigens CD11b and CD33 were also modulated to some extent. Clinically, this effect can be observed as maturation of leukemic cells in peripheral blood and bone marrow. After beginning ATO we usually observe release of leukemic cells into peripheral blood in some patients and an increase of immature cells, which may increase leukemic cells to more than 100000/mm<sup>3</sup> (we observed some patients with an increase of leukemic cells to more than 200000/mm<sup>3</sup> after receiving ATO, with a gradual decline to normal counts).

During remission induction, bone marrow doesn't become hypoplastic, and bone marrow becomes mature without cytotoxicity to hematopoietic cells.

### Apoptosis of leukemic cells

Higher concentrations of ATO (achieved during the first hours after ATO infusion) can induce apoptosis in leukemic cells (concentrations between 0.5-2 mmol). This effect is exerted on leukemic cells through direct apoptosis induction in cells by ATO cytotoxic effects or indirectly through effects of ATO on different regulatory pathways in leukemic cells.

Actually Arsenic induces the formation of reactive oxygen species (ROS) and deplete GSH content of cells. It also directly damage to DNA and RNA. Arsenic can induce mitochondrial caspase system for induction of apoptosis.<sup>22</sup> Which can be prevented by Azidothymidine.<sup>23</sup>

### Another mechanisms

ATO has anti-angiogenesis effects and can reduce new vessels formed during leukemia transformation. Also ATO has some effects on telomere length<sup>7</sup> and telomerase activity. Also it has some effects on microvascular density of bone marrow during remission induction.<sup>17</sup> These effects are summarized in Table 1.

**Table 1: biological effects and mechanisms of action of ATO on APL**

Study	Mechanism of Apoptosis induction references
Chen et al <sup>3</sup>	Downregulation of Bcl-2 expression
Chen et al <sup>3</sup>	Modulation of PML-RAR $\alpha$ /PML proteins
Quignon et al <sup>4</sup>	Caspase-independent cell death by PML through Bax and p27 Kip1 recruitment to nuclear domain
Dai et al <sup>5</sup> , Jing et al <sup>6</sup>	Modulation of the glutathione Redox system and hydrogen peroxide-dependent pathway
Ghaffari et al <sup>7</sup> , Tarkanyi et al <sup>8</sup>	Telomere shortening and telomerase activity modification
Estrov et al <sup>9</sup>	Interleukin-1 B –induced activation of the nuclear transcription factor, NF- KB
Cai et al <sup>10</sup>	Mitochondrial transmembrane potential collapse
Kitamura et al <sup>11</sup>	Caspase-8 activation in a CD95-independent but GSH concentration-dependent manner
Park et al <sup>12</sup>	Caspase-3 activation and Bcl-2 phosphorylation
Hossain et al <sup>13</sup>	Acceleration of caspase activation by the inhibition of arsenite-induced, membrane rafts-dependent Akt activation
Zhu et al <sup>14</sup>	Upregulation of CD95/CD95L expression and activation of caspases-8 and -3
Cai et al <sup>15</sup>	Mitotic arrest
Davison et al <sup>16</sup>	JNK activation
Alimoghaddam et al <sup>17</sup>	Antiangiogenic effects
Teng et al <sup>18</sup>	Upregulation of Apaf1 and downregulation of PNAS-2
Joe et al <sup>19</sup>	Activating proapoptotic kinase Chk2
Glienke et al <sup>20</sup>	Downregulation of WT1 expression
Dbaibo et al <sup>21</sup>	Accumulation of cytotoxic levels of ceramide

## CLINICAL APPLICATIONS OF ATO IN THE TREATMENT OF APL

### ATO for relapsed APL after ATRA and chemotherapy

The first clinical trials on treatment of relapsed APL after resistance to ATRA were conducted by a group from China.<sup>24</sup> They observed that ATO can induce complete remission in 72% of patients. Then the Shanghai group<sup>25</sup> reported a 90% complete remission rate with ATO for treatment of relapsed APL. Another study on relapsed APL in the US<sup>26</sup> achieved a 92% complete remission rate in these patients, and PMLRARA disappeared in 8 of 11 patients in CR. In another multicenter study on relapsed APL following first line treatment by ATRA and chemotherapy, 40 patients received 0.15mg/kg ATO until disappearance of abnormal promyelocytes and myeloblast from bone marrow and peripheral blood.<sup>27</sup> Complete remission was observed in 85% and median time to complete remission was 59 days. We studied ATO in 31 relapsed cases of APL.<sup>28</sup> The median age of patients was 27 years old and the median WBC at presentation was 2000/mm<sup>3</sup> (500-44000). ATO was infused at 0.15mg/kg/day until disappearance of abnormal cells from bone marrow. Complete remission observed in 77% of treated cases and four patients died during remission induction. For patients in complete remission, relapse reoccurred in 10 cases. In these case 2 years LFS and OS were 54.6% and 81.1% respectively.

ATO in combination with chemotherapy and /or ATRA (as concomitant treatment during induction or sequentially during consolidation/maintenance) for relapsed cases of APL is another issue that needs more study. After CR with ATO, Kwong et al.,<sup>29</sup> used consolidation with idarubicin. Minimal residual disease (MRD) was positive in 10 cases that achieved CR with ATO, but they reverted to negative after 2 courses of consolidation with idarubicin, and seven patients out of 8 in molecular CR did not relapse during a median follow up 13 months.

ATRA synergizes with the effects of ATO for remission induction and, in cases that are resistant to ATO, may induce durable CR;<sup>30</sup> however, in a

small randomized trial,<sup>31</sup> the addition of ATRA to ATO did not improve remission rates or event free survival (EFS). The combination of ATO with ATRA and Gemtuzumab Ozogamicin in post remission consolidation by ATO was effective for APL patients in first relapse following ATRA treatment.<sup>32</sup> So not only ATO is effective for relapsed cases after ATRA and chemotherapy but it is also effective for relapsed cases following previous ATO treatment.<sup>33</sup>

ATO plus a proteasome inhibitor is another potential combination according to in vitro studies,<sup>34</sup> and the combination of ATO with an oxidative agent, such as ascorbic acid, may improve results of treatment. Because of the antiangiogenic effects of ATO<sup>17</sup> addition of an antiangiogenic agent may be improve results and need further study.

So ATO alone or in combination is effective for remission induction of relapsed cases of APL and finding the best possible schedule for this cases need further study to improve remission and also reduce early mortality in such cases.

Despite of high remission rate with ATO between relapsed cases of APL, relapse rate is high<sup>35</sup> and post-remission treatment is very important to prolong remission and achieve to possible cure which will discussed below.

### ATO in new cases of APL

The first report of ATO in new cases of APL was from China. Niu et al.,<sup>36</sup> used ATO for remission induction in 11 new cases of APL and observed a 72.7% CR rate, but hepatotoxicity was observed in 7 patients, including 2 deaths. Because of our experience and the updates of this study,<sup>33, 37</sup> we used ATO alone for remission induction and consolidation in APL patients. The remission rate was 85.8% and 5 years disease free survival (DFS) rates were 66.7%. We have observed some patients at 14 years of follow up with durable clinical and molecular remission with just one remission induction and one consolidation treatment with ATO alone. In patients who relapsed after treatment, re-induction was possible with ATO with the same remission rate, and in some patients, 3rd and 4th remission induction was possible with ATO alone. Molecular follow up of patients also showed good MRD negative rates.

Although the remission rate was good and patients could achieve to durable remission with one induction and one consolidation treatment with ATO, we increased the number of consolidations to 4 courses during 2 years after remission and we observed that the relapse rate decreased more.<sup>37</sup> Another group in India reported a good remission rate.<sup>38</sup> EFS and DFS were better than our first results.<sup>17</sup> We suggest that the better results in this trial was due to more consolidation (6 in comparison to one consolidation in our study). ATO in combination with ATRA has been used for remission induction. Remission rates improved marginally.<sup>39, 40</sup> The combination of ATRA and ATO with or without Gemtuzumab Ozogamicin studied in another trail from MD Anderson<sup>41, 42</sup> demonstrated good remission rates, DFS and OS. ATO not only push bone marrow to complete remission but also controls coagulopathy that is observed in APL. Thrombomodulin expression on the APL cell surface is significantly upregulated with treatment. The levels of P-selectin, soluble fibrin monomer complex (SFMC), and D-dimer (D-D) decrease after ATRA or ATO treatment.

Abnormal high expression of TF in APL cells is downregulated in patients treated with ATRA or ATO. So by these mechanisms bleeding symptoms are ameliorated during ATO or ATRA treatment<sup>43-46</sup> and usually coagulation abnormality control is seen during the 2nd to 3rd week of therapy. In our experience, use of activated factor VII can ameliorate life threatening hemorrhage. We were able to save some patients with severe pulmonary hemorrhage after sever APL maturation syndrome with dexamethasone, intensive respiratory support, and a 2-3 hour infusion of activated factor VII.<sup>47, 48</sup> The combination of ATRA and ATO in<sup>49-51</sup> has been studied in randomized clinical trials, although remission rates were similar between 61 cases randomized into three treatment groups (ATRA alone, vs. ATO alone vs. combination of ATRA and ATO), but speed of remission induction achievement was faster with the combination, tumor burden decreased more with ATRA/ATO, and relapse was less frequent. Finally in a randomized clinical trial effects of ATO in combination with ATRA compared with conventional chemotherapy and ATRA regimen in

low and intermediate risk APL patients.<sup>52</sup> Remission rate and survival were at least equal between two arms. So non-inferiority of this regimen in comparison with conventional regimens approved. Despite of success in treatments of APL by ATRA or ATO, early mortality rate remain high in community data bases.<sup>53, 54</sup> The reason of morality is hemorrhagic complications or APL differentiation syndrome.

The major toxicity of ATO treatment during remission induction is APL differentiation syndrome (fever, weight gain, polyserositis, respiratory distress, and pulmonary infiltrate and, in severe cases, respiratory failure, renal failure and pulmonary hemorrhage). This complication is observed in about a third of patients.<sup>33, 55</sup> The major cause of remission induction failure is APL differentiation syndrome.<sup>33</sup> APL differentiation syndrome occurs in patients with high or low initial WBC counts and corticosteroid administration is needed for control.<sup>56</sup>

Other toxicities include hepatotoxicity, nephrotoxicity, neurotoxicity, metabolic disturbance (hyperglycemia, hypomagnesemia, and hypokalemia), fluid retention, skin discoloration, xeroderma, and conjunctivitis. Usually, none of these complications jeopardize the successful control of disease by ATO. During the consolidation phase, occurrence of these complications is very rare, so it is possible that complications are due to toxic effects of the drug and toxic products of leukemic cells.

Another major complication reported is QTc prolongation and sometimes torsade de pointes arrhythmia, which can cause sudden cardiac-related death.<sup>57</sup> We have not observed a high rate of cardiac arrhythmia but we suggest monitoring of QTc at least every other day and control by Potassium and magnesium administration.

### Post remission therapy

After remission induction with ATO and/or ATRA or chemotherapy, MRD usually remains detectable, which predicts future relapse in absence of any effective treatment. In our experience MRD usually remains detectable after the first course of treatment with ATO alone, and

consolidation/maintenance treatment is needed to reduce the chance of relapse.

Previously we have used a second course of ATO alone after a one-month rest after hematologic complete remission as a 28-day course of ATO with the same daily dose as remission induction. With comparison of our results and Indian group results<sup>38</sup> and the observation that most relapses happen during the 12-18 months after remission induction, we increased the number consolidation courses to 4 and observed reduction of relapses. With this method outcome and relapse rate, improved.<sup>37</sup>

Other methods of consolidation after remission induction with ATO are use of ATRA with or without chemotherapy and Gemtuzumab Ozogamicin.<sup>41, 58-60</sup> Optimal consolidation/maintenance is unknown and needs clinical trials to determine, but any modality should keep MRD undetectable or below the threshold defined previously.<sup>61, 62</sup>

Another issue in the treatment of APL with ATO is the use of this drug as consolidation in the classic treatment of APL with ATRA and chemotherapy. Results of the North American Intergroup trial<sup>63</sup> or<sup>64</sup> the addition of 2 courses of ATO consolidation after CR with ATRA and chemotherapy significantly improved EFS and OS.

### Prognostic factors

There are several risk factors for APL treatment failure. These factors for treatment by ATRA and chemotherapy are high WBC and low platelet count before beginning treatment.<sup>65-67</sup> So design of treatment should be according to these factors.

In a study from China of 120 new cases of APL treated with ATRA/chemotherapy and ATO as post remission therapy, low WBC count at diagnosis and long isoform of PML-RAR $\alpha$  were associated with good OS,<sup>68</sup> and peak of WBC count during consolidation phase and ATO for post remission therapy were associated with good RFS. In this retrospective study persistence of negative PML-RAR $\alpha$  during follow up was associated with good RFS and OS. Other studies<sup>69, 70</sup> also suggested that short isoform of PML-RAR $\alpha$  is an important prognostic factor.

Complex karyotype of APL is another possible risk factor for APL treatment with ATRA and chemotherapy.<sup>71</sup> Expression of CD56 and flt3-ligand

mutation are other prognostic factors with ATRA and chemotherapy.<sup>72, 73</sup>

For treatment with ATO alone, an Indian group observed that WBC count less than 5000/mm<sup>3</sup> and platelet more than 20000/mm<sup>3</sup> at diagnosis are good prognostic factors and they<sup>74</sup> couldn't find Flt3-L mutation as independent risk factor in APL.<sup>73</sup> We couldn't find an association between PML-RAR $\alpha$  isoform and prognosis. We found that the only significant risk factor for relapse and OS was detection of MRD after treatment and during follow up.

The North America Intergroup trial also suggests that importance of high WBC count is not a factor with use of ATO in consolidation.<sup>75</sup> So we suggest that ATO should be included in treatment of high risk APL (as the only drug or in combination with other drugs), and detection of MRD any time after first consolidation during treatment should be a warning of ongoing relapse and the need for additional treatment modalities.

### Minimal residual disease

Minimal residual disease (MRD) is the most important prognostic factor for APL treated by different protocols. Detection rate of MRD and its importance is based on the method of MRD assay and the sensitivity of the test. The source of MRD study (bone marrow vs. peripheral blood) and time course of MRD positivity detection are also important factors for MRD study. Presence of continued MRD positive test results during follow up, a PCR negative test changing to positive, or an increasing titer of PML-RAR $\alpha$  copy number may predict ongoing relapse.

When MRD is negative by nested PCR, risk of relapse is very low, and detection of PML-RAR $\alpha$  increases the risk of relapse dramatically.<sup>76-81</sup> It is possible to detect residual PML-RAR $\alpha$  with a very sensitive PCR method (more than 10<sup>6</sup>) in patients with continued remission.<sup>82</sup> It is better to study MRD at least every three months for better prediction of relapse.<sup>83</sup>

Recently quantitative PCR has been studied for detection of MRD instead of conventional RT-PCR.<sup>62, 84, 85</sup> For patients treated with ATO, many remain MDR-positive after remission induction but convert to MRD negative after the consolidation phase of

treatment.<sup>33, 38, 61</sup> The rate of nested PCR positivity was 8.3% during first year of follow up in patients in complete remission, and many of them relapsed. We studied Q-PCR during follow up of APL treated with ATO<sup>61</sup> and defined a threshold of normalized Q-PCR level for prediction of relapse. When Q-PCR normalized to every 106 copies of G6PDH transcript, a PML-RAR $\alpha$  transcript level more than  $5 \times 10^2$  increases chance of relapse dramatically. FISH is another method of MDR detection and is effective for prediction of relapse.<sup>86</sup>

MRD detection in APL patients is mandatory and we should change the treatment modality for each patient treated with ATO when RT-PCR with a sensitivity about  $10^{-4}$  becomes persistently positive or Q-PCR level increases to more than the defined threshold.

#### **Hematopoietic stem cell transplantation (HSCT) for APL**

Before introduction with ATO, stem cell transplantation was the next step for relapsed cases of APL after treatment with ATRA and chemotherapy.<sup>87, 88</sup> Autologous hematopoietic stem cell transplantation was the preferred choice if MRD was negative and allogeneic transplantation was preferred for MRD positive patients who had a suitable donor. With introduction with ATO, HSCT is usually reserved for patients with persistent PML-RAR $\alpha$  after treatment with ATO or patients who relapsed after ATO remission induction.<sup>89</sup> We compared results of HSCT in our center in patients with APL who were treated with ATO alone. Results of APL treatment by these two modalities are comparable, and we suggest that HSCT should be reserved for patients who failed treatment with ATO.<sup>90</sup>

#### **Extramedullary relapse**

Extramedullary relapse of APL became more common after introduction with ATRA and ATO. We observed extramedullary relapse in the skin and CNS in patient's with multiple relapse.

Skin relapse presented as skin maculopapular lesions with purple to brown discoloration. These lesions are usually resistant to ATO alone and need chemotherapy with or without ATRA as well as ATO,

until disappearance of disease. Skin relapse can be an isolated relapse without bone marrow involvement, but PML-RAR $\alpha$  is usually positive at this stage. CNS relapse is also usually observed in multiple relapsed patients as meningeal involvement. At this phase, PML-RAR $\alpha$  of CSF is positive too. Although by systemic administration ATO achieved detectable levels in CSF,<sup>91, 92</sup> our group recommend intrathecal chemotherapy and systemic treatment. The role of radiotherapy<sup>93</sup> and detection of MRD in CSF and peripheral blood or bone marrow are other issues that need to be addressed in relapsed cases. Role of prophylactic intrathecal chemotherapy in APL is not clear, but it seems that high risk patients need some type of CNS prophylaxis.<sup>66</sup>

#### **CONCLUSION**

ATO is a very potent single agent against acute promyelocytic leukemia and it is time to move it to the first line treatment of APL. Recently NCCN accepted it in combination with ATRA as first line treatment for low and intermediate risk APL. Also it is the drug of choice for relapsed cases of APL or cases of treatment failure with ATRA and chemotherapy.

Combination of ATO and ATRA is at least equal to ATRA and chemotherapy in low and intermediate risk APL.<sup>52</sup> Also, the position of ATO in the treatment schedule of new cases (as remission inducer or as consolidation/ maintenance phase) needs more study and randomized trials to compare with conventional treatment with ATRA/chemotherapy.

The major causes of treatment failure with ATO are due to the delay of beginning treatment (especially in developing countries) and APL differentiation syndrome.<sup>53, 94</sup>

Prompt and immediate diagnosis and beginning treatment as soon as possible is recommended, and we need clinical trials for prevention and better control of APL differentiation syndrome.

Finally it seems that better education of APL treatment teams and implementation of technical and requirements in developing countries would improve results of treatment in less developed regions and would help better outcome for this potentially curable disease.<sup>95</sup>

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