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Acute Promyelocytic Leukemia

### Molecular remission with arsenic trioxide in patients with newly diagnosed acute promyelocytic leukemia


**Thirty six APML patients achieving hematologic remission with $As_2O_3$ were serially monitored using RT-PCR. Though only 5.5% achieved molecular remission at induction remission, 94.5% became negative during consolidation. At 20 months follow-up, 85% remain in remission but longer follow up studies are needed to monitor late relapses.**



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Arsenic trioxide ($As_2O_3$) achieves induction remission in 70-90% of patients with newly diagnosed acute promyelocytic leukemia (APL) with 65-70% long term remission rates[1,2] but there is limited data on molecular remission in these patients. We evaluated this aspect in 40 patients and describe our findings here. The study population was formed of 40 patients with t(15;17) APL treated with $As_2O_3$ between January 2000 and February 2004. $As_2O_3$ was administered in teh context of an institutional study protocol after obtaining ethical clearance in the first 5 patients, but since 2001, $As_2O_3$ has become standard therapy for patients who cannot afford treatment with ATRA. Intravenous $As_2O_3$, prepared in the hospital pharmacy at the cost of $0.5 per vial, was administered at a daily dose of 10 mg (adults) and 0.15 mg/kg/day (children) as per protocol (Figure 1). Full blood counts, coagulation parameters, as well as renal and hepatic function were closely monitored. Electrocardiograms were done if patient was symptomatic. Platelet and fresh frozen plasma transfusions were given to maintain platelet counts >20,000/mm$^3$ or if the patients had a coagulopathy. Bone marrow examination was done to assess remission on normalization of blood counts. The molecular monitoring was carried out by reverse transciption polymerase chain reaction (RT-PCR) to detect PML-RARα transcripts, as described by van Dongen *et al.*,[3] and was done at diagnosis, at hematologic remission, prior to consolidation therapy, twice during maintenance therapy (3 months apart) and subsequently every 6 months. This method, with nested amplification, has a sensitivity of $10^{-3}$ to $10^{-4}$. There were 23 males and 17 females, including 31 adults and 9 children (mean age 27.8 years; range: 6-60) with hypergranular APL. The median white cell count at diagnosis was $2.5\times10^9$/L (range: 0.6 to 58.9). Thirty-six patients (90%) achieved hematologic remission (HCR) at a median time of 42.6 days (range: 26-60) with 4 early deaths due to intracranial hemorrhage. Molecular remission was achieved in all at a median time of 83.9 days (range: 51-136). Though only 2 (5.5%) patients became PML-RARα transcript negative at HCR, another 25 (69.5%) had became negative at the start of consolidation without further treatment. Seven patients (19.5%) became negative at the end of consolidation while 2 (5.5%) became negative during maintenance therapy. Thirty-four patients (94.5%) were in molecular remission by the end of consolidation. As far as concerns toxicity, 20 patients (50%) had leukocytosis requiring addition of hydroxyurea with temporary discontinuation of $As_2O_3$ in 5 patients and prolonged neutropenia in 1 patient. Asymptomatic elevation of liver enzymes was noted in 7 (17.5%) patients. There were no cases of clinical cardiac toxicity. Isoform analysis showed that 29 patients (72.5%) were bcr-1-positive, 2 (5%) were bcr-2-positive and 9 (22.5%) were bcr-3 positive. The rate

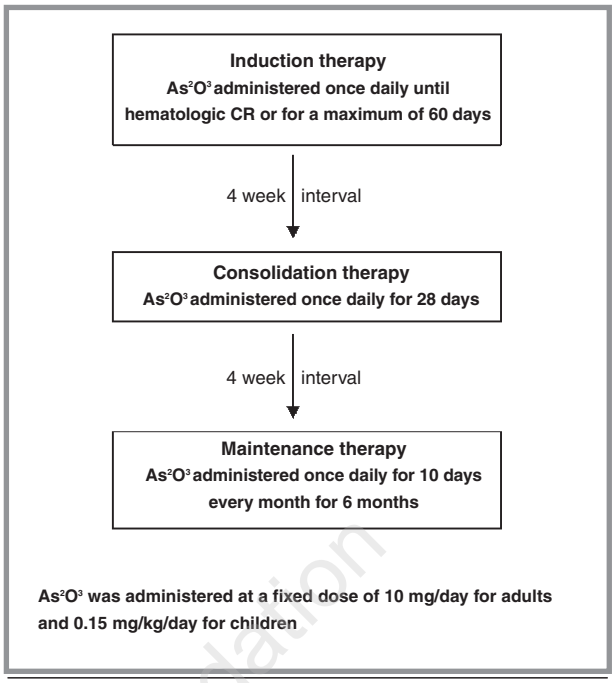


**Figure 1. Protocol for tretament of patients with APML with $As_2O_3$.**

**Table 1. Hematologic and molecular remission on treatment with $As_2O_3$.**

| | |
|---|---|
| Hematologic remission | 90% |
| bcr-1 isoform (n=29) | 89.6% |
| bcr-3 isoform (n=9) | 88.8% |
| bcr-2 isoform (n=2) | 100% |
| Time to hematologic remission (days) | 42.6 (25-60) |
| bcr-1 isoform | 46.5 (30-60) |
| bcr-3 isoform | 31 (25-37)* |
| bcr-2 isoform | 38.5 (33-44) |
| Time to molecular remission (days) | 83.9 (51-136) |
| bcr-1 isoform | 84.5 (51-119) |
| bcr-3 isoform | 67.5 (60-136)° |
| bcr-2 isoform | 84 (74-94) |

*$p < 0.001$; °$p=0.25$.

of HCR was similar in patients with all isoforms though the median time to HCR was significantly shorter in those with bcr-3 than in those with the bcr-1 isoform [31 vs 46.5 days] ($p<0.001$) with no significant difference was seen in the median time to molecular remission [67.5 days bcr-3, 84.8 days bcr-1] ($p = 0.2$). Two patients relapsed 6 and 7 months after treatment: one patient achieved a second complete remission on repeat treatment with a combination of $As_2O_3$ and ATRA while the second died of intracranial hemorrhage. At a median follow-up of 20.3 months (range: 4-53), thirty-four patients (85%) remain in remission with a leukemia-free survival of 94.5%. $As_2O_3$ achieves induction remission rates similar to those produced by treatment with ATRA or a combination of ATRA and $As_2O_3$ with similar numbers of patients achieving molecular remission by the end of consolidation therapy.[4,5,6] Trials in patients with relapsed APL have also shown 80-90% PCR negativity by the end of consolidation.[7,8] Interestingly $As_2O_3$ may show anti-leukemic efficacy for many days after the drug has been stopped, as suggested by the 70% of patients who became RT-PCR negative prior to starting consolidation despite being positive at the time of hematologic remission. There does not seem to be a major difference between patients with the bcr-1 or bcr-3 isoform but larger numbers need to be studied. There are, however, no data available on the significance of isoforms in APL patients primarily treated with $As_2O_3$. The median follow-up in our patients in our study is too short (20 months) to evaluate late relapses and the long-term significance of the various isoforms. These preliminary data show that all patients achieving hematologic remission on primary treatment with $As_2O_3$ also achieve molecular remission. Ninety-five percent of patients are in molecular remission by the end of consolidation with 85% achieving long-term remission. However, follow-up studies are needed to assess the durability of remissions in these patients.

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Acute Myeloid Leukemia

## Quantification of *DEK-CAN* fusion transcript by real-time reverse transcription polymerase reaction in patients with t(6;9) acute myeloid leukemia

**Real-time reverse transcription polymerase reaction (RT-PCR) was used to examine *DEK-CAN* transcript levels in serial samples from three patients with t(6;9) acute myeloid leukemia treated with intensive chemotherapy. All three patients achieved short first clinical remission, but without achieving RT-PCR negativity. DEK-CAN level significantly increased in two patients before relapse, while in the third a level of $2\times10^{-3}$ in remission bone marrow preceded relapse by 2 months.**

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The t(6;9)(p23;q34) translocation which produces the *DEK-CAN* fusion gene is detected predominantly (90%) in acute myeloid leukemia (AML) with FAB type M2 or M4 and associated with basophilia.[1-3] t(6;9) is associated with a poor prognosis.[2] There have been few studies to date using this aberration as a marker for monitoring minimal residual disease (MRD).[4-6] We have developed a highly sen-