Treatment of new cases of Acute Promyelocytic Leukemia With Arsenic Trioxide

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Running head: New cases of APL and Arsenic Trioxide

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Abstract

Introduction: Arsenic Trioxide is effective and approved for treatment of relapsed or refractory APL cases to ATRA but its effects in new cases of APL is not clear and needs long term follow up to disclose the role of this drug in treatment of APL in combination with chemotherapy/ATRA or alone.

Material and methods: we studied 111cases of APL (94 new case and 17 relapsed) diagnosed by morphological criteria and confirmed by cytogenetic and/or RT-PCR for the presence of PML/RARA fusion gene.

Arsenic Trioxide was infused as 0.15mg/kg/day doses, until complete remission by morphological criteria or till 60 days. In case of complete remission, after 28 days do rest, 0.15mg/kg/days Arsenic Trioxide was infused for an additional 28 days as consolidation. Also, we studied minimal residual disease by semi-sensitive RT- PCR on peripheral blood samples up to a year after complete remission.

Results: Complete remission was observed in 95 patients (85.6%) and median time to complete remission was 30 days. There was no significant difference between remission rate in new and relapsed cases.

During the induction phase, the most common cause of toxicity and mortality was APL differentiation syndrome (23 cases or 20.7%). Other toxicities were serosistis (7.2%) and hepatotoxicity (19.8%).

With a median follow up of 16.5(1-57) months for patients in complete remission, one and two year disease free survival (DFS) was 88.3% and 63.7%, respectively. We observed 24 relapses and 19 of them achieved second complete remission, again by Arsenic Trioxide. Median time to relapse was 17 months (4-33) and median time of second DFS after re-treatment with Arsenic Trioxide was 18 months. We observed a third and fourth remission for some patients, who relapsed, again by Arsenic Trioxide.

For patients in complete remission, one and three years survival was 95.5% and 87.6%, respectively.

Minimal residual disease was positive in 4 (8.3%) out of 48 cases up to a year after remission induction and 3 of these patients clinically relapsed.

Conclusion: Arsenic Trioxide is effective as a first line treatment of APL. Results of Arsenic Trioxide combination with chemotherapy/ATRA needs further study. Also it seems that Arsenic Trioxide is applicable for relapsed patients again and drug resistance is an unusual event.

Keywords: Arsenic Trioxide, APL

Acute promyelocytic leukaemia (APL) is a well-defined subtype of leukaemia with specific and peculiar characteristics. The most important characteristic of this disease is a translocation between chromosomes 15 and 17 in leukemic

cells and the presence of *PML/RARA* fusion gene product in affected cells.[1]

Today, accepted protocols for treatment of APL contain All-trans-retinoic acid (ATRA) ,which are usually combined with an antracycline.

Overall survival and 2-4 years event free survival are 70%-80% and 55%-85% respectively.[2-10]

Maintenance by ATRA with or without low dose chemotherapy improves the results and reduces relapse rate.[6, 8, 11]

Recently, some groups observed that, an ancient drug, Arsenic Trioxide is useful for treatment of resistant or relapsed cases of APL after treatment with ATRA.[12-15]

Arsenic Trioxide is the most active single agent against APL cells. It induces maturation and apoptosis in APL cells[1] and reduces microvascular density of bone marrow.

Soignet et al. showed that Arsenic Trioxide could induce high molecular remission rate, in relapsed APL cases.[16] Today Arsenic Trioxide is accepted for treatment of such cases and has improved the results of treatment of APL over stem cell transplantation.[17]

The role of this drug, in the induction of remission or consolidation phase of new cases of APL is less clear.

The purpose of our study is to define the efficiency and the safety of Arsenic Trioxide for treatment of new cases of APL and long term follow up.

Materials and Methods:

1- Acute promyelocytic leukemia was diagnosed by clinical manifestations, morphological FAB criteria, cytogenetic or Fluorescent Insitu Hybridisation (FISH) study for detection of t (15,17) and /or RT-PCR for *PML-RARA* transcript.

Table-1: Patient's characteristic

Sex	Female	60
	Male	51
Type of diseases	Relapsed	17
	New cases	94
Median age		27 (6-79) years
Median WBC at presentation		2050
Median hospitalisation time		32 days
highest WBC count for patients with hyperleukocytosis		49500/mm3 (10200-167700/mm3)

Between May 2000 and January 2005, 111 APL patients enrolled in our study. Fifteen patients were relapsed and 94 were new cases. Three patients relapsed after a previous stem cell transplantation (1 after allogeneic stem cell transplantation and 2 after autologous stem cell transplantation) and 14 patients relapsed after a previous treatment by ATRA and chemotherapy. None of the relapsed cases have used maintenance by ATRA or chemotherapy.

Hematologic and clinical characteristic of the patients are shown in table-1.

2- Arsenic Trioxide was prepared as 10mg/10 ml vials, manufactured by the pharmaceutical faculty of Tehran University of Medical Sciences and licensed by the food and drug division of ministry of health for this clinical trial.

3- This clinical trial was approved by a local ethical review board and consent form obtained before treatment.

4- Induction of remission: after diagnosis of APL according to the above criteria, Arsenic Trioxide was started immediately as a 2 hour intravenous infusion of 0.15mg/kg in 500ml dextrose water. Treatment continued until complete remission by morphological criteria or to a maximum of 60 days.

5- Supportive care during treatment: prothrombine time, activated partial thromboplastin time, Fibrin degradation product and fibrinogen were measured at the time of diagnosis and regularly during the treatment. In the presence of disseminated intravascular coagulopathy (DIC) fresh frozen plasma and platelet were transfused, if indicated. The White blood cell count and peripheral blood smear was observed daily. Liver and renal function was assayed regularly and fasting blood sugar, sodium, potassium, calcium, magnesium and urine analysis were tested twice weekly. The Electrocardiogram was studied and QTc was measured every other day. In case of QTc prolongation, Magnesium and Potassium was supplied. Patients weight was measured daily and diuretics prescribed for severe edema or weight gain. If the liver enzymes increased to more than 10 times the upper limit of normal or bilirubin to more than 5 mg/dl or creatinin to more than 2 mg/dl, Arsenic Trioxide was stopped for some days. After correction of abnormalities, drug administration was restarted with half the original dose and in-

creased to full dose rapidly. In cases with hepatic or renal complications or APL differentiation syndrome during induction of remission phase, we used full dose Arsenic Trioxide in the consolidation phase, without any significant observed complications.
The APL differentiation syndrome (defined as weight gain, fever, polyserositis and dyspnea with or without radiographic markers of pulmonary infiltration) was treated by Dexamethasone 10 mg twice daily, until patient symptom improvement. For patients whose APL differentiation syndrome worsened toward Adult Respiratory Distress Syndrome (ARDS) or pulmonary hemorrhage we tried immediate use of assisted ventilation with oxygen supplement and activated factor seven (Novo seven®) for some patients.
Peripheral blood smear was observed daily and Bone marrow was studied every 10 days for evaluation of remission and maturation.
After complete remission, treatment stopped and patients were discharged.

6- Consolidation: 28 days after complete remission, consolidation began as an out patient treatment. It consisted of 28 days infusion of 0.15mg/kg Arsenic Trioxide every day, 6 days a week. In this period, patients were visited every week and their CBC, liver enzymes, renal function and electrocardiogram were tested.

7- Follow up: after the consolidation, patients were visited every month and then every three months and their CBC, liver and renal function was studied.

8- Definition of outcome:
a. Complete remission was defined as its classic definition, neutrophil count more than 1500/mm^3; platelet count more than 100000/mm^3 and immature cells (promyelocytes and myeloblast) less than 5% of nucleated bone marrow cells.
b. Disease free survival: measured from the time of complete remission until relapse or censoring of data on patients.
c. Overall survival: measured from the time of diagnosis and beginning of the treatment by Arsenic Trioxide until the time of death or censoring of data on patients.

9- Minimal residual disease (MRD): 48 patients were evaluated within a one year period after complete remission for minimal residual disease (MRD) by RT-PCR on their peripheral blood samples by searching for mRNA of *PML-RARA* isoforms. Sensitivity of the test was 10^{-3}. Sequences of forward and reverse primers are shown in table-2.

Table-2: sequence of primers used for diagnosis and follow up for minimal residual disease by a nested-RT-PCR

Primer	Sequence (5′-3′)
M2	AGTGTACGCCTTCTCCATCA
M4	AGCTGCTGGAGGCTGTGGACGCGCGGTACC
R5	CCACTAGTGGTAGCCTGAGGACT
R8	CAGAACTGCTGCTCTGGGTCTCAAT

10- Statistical analysis: median values were measured for the of complete remission
and hospitalisation time. Disease free survival and the overall survival time was calculated by Kaplan-Meier's method.

Results:

1- Complete remission: complete remission was observed in 95 patients (85.6%). The median time to complete remission was 30 days (20-43 days). Median time of hospitalisation was 32 days for remission induction phase of treatment. Remission was observed in 82 new cases patients (86.3%) and 13(76.5%) relapsed cases. This difference wasn't statistically significant. Sixteen patients died due to the complications of their disease or treatment in the induction phase. The causes of death were cardiac arrest in two, APL differentiation syndrome in 8 (with pulmonary hemorrhage and/or acute respiratory distress syndrome), cerebral hemorrhage in three patients and disseminated,. Aspergilosis in one patient. Two patients did not respond and died due to disease progression after chemotherapy .The median time of death was 20 days (2-55 days) during the induction phase.
2- Hyperleukocytosis: The median white blood cell count was 2050/mm^3 at the time of diagnosis. In eighteen patients, white blood cell count was more than 10000/mm^3. Hyperleukocytosis (white blood cell count more than 10000/mm^3) happened in 65 patients during treatment. The median day for the beginning of hyperleukocytosis was 10 days (2-22) after starting the treatment and the median number of highest leukocyte count was 49500/mm3 (10200-

167700/mm^3). There was no significant difference between white blood cell counts at the onset between patients with and without hyperleukocytosis and it did not increase early mortality of treatment. We didn't observe any association between hyperleukocytosis and APL differentiation syndrome, remission rate and relapse rate. Overall survival and disease free survival was not significantly different for patients with and without hyperleukocytosis.

3- APL differentiation syndrome: this complication was observed in 23 patients (20.7%) and 10 of these patients succumbed during this complication (eight died due to APL differentiation syndrome and respiratory failure, one due to brain and pulmonary Aspergilosis and one due to intracranial hemorrhage)

We didn't observe any difference between new cases and relapsed subgroups. The death rate was significantly higher in patients with APL differentiation syndrome ($p<0.006$)

Disease free survival and overall survival analysis: Median follow up was 16.5(1-57) months. Relapse was observed in 24 patients (25.3%) in complete remission and median time to relapse after first complete remission was 17 months. One and two year disease free survival for patients in complete remission was 88.3% and 63.7%, respectively.

Disease free survival was similar for new cases and patients who were treated after relapse following previous ATRA and chemotherapy.

For patients who relapsed after the first course of Arsenic Trioxide treatment we restarted treatment with Arsenic Trioxide with the same schedule as the first treatment. Nineteen complete remissions were observed in this group (79.2%) and median time to relapse for this group was 18 months.

One and three year survival for patients in complete remission was 94.55% and 86.6%, respectively. (Fig-1)

Survival was similar for new cases of acute promyelocytic leukaemia and patients who were treated after relapse following previous ATRA and chemotherapy.

We couldn't find any independent risk factor for relapse and survival in complete remission patients, except positive minimal residual disease following remission induction or during follow up (*p*- value= 0.01 and <0.0001 respectively).

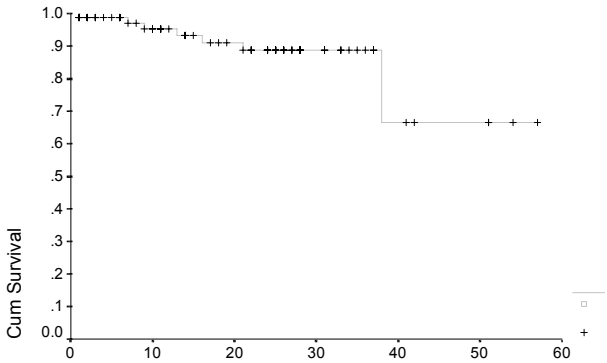

fig-1

overal survival of patients in complete remission

4- Molecular follow up of patients: 48 patients were followed by a semi-sensitive RT-PCR for detection of *PML-RARA* fusion gene mRNA during the first year after complete remission.

All patients were in complete haematological remission at the time of minimal residual disease study. RT-PCR was positive in 4 patients (8.3%) and three of them clinically relapsed.

Re-treatment of relapse: 24 patients who relapsed were treated again with the same treatment as before with the Arsenic Trioxide. Again we observed 19 complete remissions (79.2%) and 5 patients died during second remission induction by Arsenic Trioxide.

Discussion:

We observed that complete remission rate and one and two-year disease free survival of patients who were treated by Arsenic Trioxide were comparable to ATRA-chemotherapy regimen.

In the European APL study group experience, event free survival was 84% for patients who were treated by concomitant chemotherapy and ATRA and 77% for patients on sequential regimen[18].

With Arsenic Trioxide alone, overall survival and complete remission rate were comparable to the European group study, although we suggest addition of maintenance therapy by intermittent Arsenic Trioxide with or without oral chemotherapy, or, addition of chemotherapy to induction or consolidation phase to improve the results. This would be due to low observed resistant rate to Arsenic Trioxide after the first course of treatment.

ATRA without chemotherapy couldn't induce a durable remission,[19-22] so we suggest that Ar-

senic Trioxide is superior to ATRA for new cases and can induce a durable remission and good DFS. Also, long term treatment with ATRA can increase the metabolism of drug in the liver and the efficiency of ATRA decrease. So we can ask: is Arsenic Trioxide a good substitute for ATRA in new cases of APL?

In APL patients who were treated by Arsenic Trioxide, the most important limitation was APL differentiation syndrome.

Although it is possible to control this complication by corticostroids, better supportive care would be the use of activated factor VII in some patients with pulmonary hemorrhage, but sometimes it is fatal. This should be a subject for future studies to control this complication by early chemotherapy, use of ATRA in combination with Arsenic Trioxide and improve supportive care.

Minimal residual disease detection is another possible subject for study to improve the results of treatment and to start these patients early on Arsenic Trioxide.

It is proved that if MRD is negative two times after complete remission, risk of relapse is minimal.[5,23,24] Soignet et al showed that after the induction phase, 85% of patients were negative for MRD.[16] In our study MRD negativity was 91.7% of cases in peripheral blood up to a year after complete remission.

Also, we suggest that early treatment after detection of MRD may improve the results of Arsenic Trioxide and may prevent high mortality of relapse.[25-27]

Recently we studied patienty samples by a sensitive Real-time PCR and we could define a threshold for relapse(unpublished data) which will be useful for early detection and treatment to improve the results.

References:

1-Martin S. Tallman, Janet W. Andersen, Charles A. Schiffer, et al.
All-*trans* retinoic acid in acute promyelocytic leukemia: long-term outcome and prognostic factor analysis from the North American Intergroup protocol, Blood. 2002; 100:4298-4302.

2-Fenaux P, Castaigne S, Dombret H, et al. All transretinoic Acid followed by intensive chemotherapy gives a high complete remission rate and may prolong remissions in newly diagnosed acute promyelocytic leukemia: a pilot study on 26 cases. Blood. 1992;80:2176-2181.

3-Ohno R, Yoshida H, Fukutani H, et al. Multi-institutional study of all-trans retinoic acid as a differentiation therapy of refractory acute promyelocytic leukemia. Leukemia. 1993; 7:1722-1728.

4-Kanamaru A, Takemoto Y, Tanimoto M, et al. Alltrans retinoic acid for the treatment of newly diagnosed acute promyelocytic leukemia. Blood. 1995;85:1202-1206.

5-Mandelli F, Diverio D, Avvisati G, et al. Molecular remission in PML-RAR positive acute promyelocytic Leukemia by combined all-trans retinoic Acid and Idarubicin (AIDA) therapy. Blood. 1997; 90:1014-1021.

6-Sanz MA, Martin G, Rayon C, et al. A modified AIDA protocol with anthracycline based consolidation results in high antileukemic efficacy and reduced toxicity in newly diagnosed PML/RAR-positive acute promyelocytic leukemia. Blood. 1999;94:3015-3021.

7-Fenaux P, Le Deley MC, Castaigne S, et al. Alltrans retinoic acid in newly diagnosed acute promyelocytic leukemia: results of a multicenter randomized trial. Blood. 1993;82:3241-3249.

8-Tallman MS, Andersen JW, Schiffer CA, et al. Alltrans retinoid acid in acute promyelocytic leukemia. N Engl J Med. 1997;337:1021-1028.

9-Burnett AK, Grimwade D, Solomon E, et al. Presenting white blood cell count and kinetics of molecular remission predict prognosis in acute promyelocytic Leukemia treated with all-trans retinoic Acid: results of the randomized MRC trial. Blood.1999;93:4131-4143.

10-Fenaux P, Chevret S, Guerci A, et al. Long-term follow-up confirms the benefits of all-trans retinoic acid in acute promyelocytic leukemia. Leukemia. 2000;14:1371-1377.

11Fenaux P, Chastang G, Chevret S, et al. A randomized comparison of all-trans retinoic acid (ATRA) followed by chemotherapy and ATRA plus chemotherapy and the role of maintenance therapy in newly diagnosed acute promyelocytic leukemia. Blood. 1999;94:1192-1200.

12-Sun H, Ma L, Hu X, Zhang T. Treatment of acute promyelocytic leukemia by Ailing-1 therapy with use of syndrome differentiation of traditional Chinese medicine. Chin J Comb Trad Chin Med West Med. 1992;12:170-172.

13-Zhang P, Wang S, Hu X. Arsenic trioxide treated cases of acute promyelocytic leukemia. Chin Hematol. 1996;17:58-62.

14-Shen Z, Chen G, Ni J. Use of arsenic trioxide in the treatment of APL, clinical efficacy and pharmacokinetics in relapsed patients. Blood. 1997; 89:3354-3360.

15-Niu C, Yan H, Yu T, et al. Studies on treatment of acute promyelocytic leukemia with arsenic trioxide: remission induction, follow-up, and molecular monitoring in 11 newly diagnosed and 47 relapsed acute promyelocytic leukemia patients. Blood. 1999;94:3315-3324.

16-Soignet SL, Frankel SR, Douer D, et al. United States multicenter study of arsenic trioxide in relapsed acute promyelocytic leukemia. J Clin Oncol. 2001;19:3852-3860.

17-W.Y.Au, A.K.W.Lie, C.S.Chim etal. Arsenic trioxide in comparison with chemotherapy and bone marrow transplantation for treatment of relapsed acute promyelocytic leukaemia. Ann Oncol.2003:14;752-758

18-Martin S. Tallman, Chadi Nabhan, James H. Feusner, and Jacob M. Rowe. Acute promyelocytic leukemia: evolving therapeutic strategies

Blood. 2002;99:759-767
19Huang ME, Ye YC, Chen SR, et al. Use of alltrans retinoic acid in the treatment of APL. Blood. 1988;72:567-572.
20-Chen ZX, Xue YQ, Zhang R, et al. A clinical and experimental study on all-trans retinoic acidtreated acute promyelocytic leukemia patients. Blood. 1991;78:1413-1419.
21-Castaigne S, Chomienne C, Daniel MT, et al. Alltrans retinoic acid as a differentiation therapy for acute promyelocytic leukemia. I. Clinical results. Blood. 1990;76:1704-1709.
22-Castaigne S, Chomienne C, Daniel MT, et al. Retinoic acids in the treatment of acute promyelocytic leukemia. Nouv Rev Fr Hematol. 1990;32: 36-38.
23-Grimwade D, Howe K, Langabeer S, Burnett A, Goldstone A, Solomon E. Minimal residual disease detection in acute promyelocytic leukemia reverse transcriptase PCR: evaluation of PML-RAR alpha and RAR alpha-PML assessment in patients who ultimately relapse. Leukemia. 1996;10:61-66.
24-Diverio D, Rossi V, Avvisati G, et al. Early detection of relapse by prospective reverse transcriptase- polymerase chain reaction analysis of the PML/RAR-alpha fusion gene in patients with acute promyelocytic leukemia enrolled in the GIMEMA-AIEOP multicenter "AIDA" trial. GIMEMA-AIEOP Multicenter "AIDA" Trial. Blood. 1998;92:784-789.
25-Lo Coco F, Diverio D, Avvisati G, et al. Therapy of molecular relapse in acute promyelocytic leukemia. Blood. 1999;94:2225-2229.
26-Hoffman R, Haddad N, Sahar D, et al.. High-dose cytarabine therapy induces a molecular remission in APL patients who failed to achieve a complete response following treatment with ATRA and anthracyclines [abstract]. Blood. 1998;92:2517.
27-Lo Coco F, Diverio D, Falini B, et al.Genetic diagnosis and molecular monitoring in the management of acute promyelocytic leukemia. Blood. 1999;94:12-22.