Supplementary Information for

Arsenic Trioxide Replacing or Reducing Chemotherapy in Consolidation Therapy for Acute Promyelocytic Leukemia (APL2012)

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Supplementary Information Text

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Synopsis of APL2012 Protocol

A randomized, open-label, multi-center study of all-trans retinoic acid and arsenic trioxide combined with chemotherapy for newly-diagnosed acute promyelocytic leukemia
Objectives: **The primary objective** of the trial is to compare the disease-free survival (DFS) in the two arms of each risk groups. **The secondary objectives are:** overall survival (OS), hematologic complete remission (hCR) after induction therapy, molecular complete remission (mCR) after consolidation therapy, early death, cumulative incidence of relapse (CIR), and hematologic and non-hematologic toxicities during consolidation therapy. **Study Design:** Randomized, open-label, multicenter, prospective pragmatic trial.
Populations: <u>Inclusion criteria:</u> (1) Patients with newly diagnosed acute promyelocytic leukemia (APL) confirmed by molecular analysis. (2) Between 18 and 65 years of age. (3) Normal liver and renal function: a bilirubin level of 35μmol/L or lower, an alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level below 2 times of normal upper limit, a creatinine level of 150μmol/L or lower. (4) Normal cardiac function. (5) Eastern Cooperative Oncology Group (ECOG) performance status score of 0-4. (6) Written informed consent signed by patients or their family members. <u>Exclusion criteria:</u> (1) Previously treated APL patients. (2) Patients with central nervous system (CNS) infiltration at diagnosis. (3) Patients allergic to any agents in the protocol. (4) Liver or renal dysfunction. (5) Severe heart disease (acute myocardial infarction or heart failure). (6) QT corrected (QTc) interval over 450mm in electrocardiogram (ECG) (7) Concurrent other active malignancies at time of study entry. (8) Tuberculosis or HIV infection.

(9) Patients not understand or obey the study protocol.

(10) Age <18 and >65.

(11) Pregnant or breast feeding women.

(12) Contraindication to anthracyclines.

(13) Drug addiction or psychiatric disorders.

(14) Enrolled in other clinical trials.

(15) Other situations that prevent patients from the study.

Screening

Screening procedure is set to start within 14 days before enrollment. Once have signed the informed consents, the subjects will take following tests: complete blood cell (CBC) count, coagulation function, liver and kidney function, ECG, bone marrow smear, chromosome (can be replaced by fluorescence in situ hybridization [FISH]), PML-RARa fusion gene, and bone marrow flow cytometry is recommended.

Early intervention is allowed in this study during screening phase since it is highly recommended in acute onset to avoid high rate of death. For suspected APL patients, ATRA with an oral dose of 25mg/m^2/d is recommended with no need of bone marrow smear report. When confirmed as APL morphologically, ATO at the dose of 0.16mg/kg/d (10mg/d maximum) shall be administered. In other words, patients may have been treated with induction therapy before enrollment, while it is not necessary for ATRA and ATO to be started simultaneously.

Induction Therapy

Newly diagnosed APL patients are divided into three risk groups according to the white blood cell (WBC) count and platelet (PLT) count ahead of treatment, namely low-risk group (WBC $\leq 10 \times 10^9$/L and PLT $> 40 \times 10^9$/L), intermediate-risk group (WBC $\leq 10 \times 10^9$/L and PLT $\leq 40 \times 10^9$/L) and high-risk group (WBC $> 10 \times 10^9$/L).

For low -risk group:

• ATRA 25mg/m^2/d given orally till hematologic complete remission (hCR).

• ATO 0.16mg/kg/d (10mg/d maximum) I.V. drip till hCR.

For intermediate-risk group:

- ATRA 25mg/m^2/d given orally till hCR.
- ATO 0.16mg/kg/d (10mg/d maximum) I.V. drip till hCR.
- Idarubicin (IDA) 8mg/m^2/d or daunorubicin (DNR) 45mg/m^2/d I.V. for 3-4 days (IDA or DNR are used only when WBC $\geq 10 \times 10^9$/L during induction).

For high-risk group:

- ATRA 25mg/m^2/d given orally till hCR.
- ATO 0.16mg/kg/d (10mg/d maximum) I.V. drip till hCR.
- IDA 8mg/m^2/d or DNR 45mg/m^2/d I.V. for 3-4 days.

Dosage of drugs:

- The dosage of ATRA is accurate to 10mg. Rounding method is adopted.
- The dosage of ATO is accurate to 1mg (10mg/d maximum). Rounding method is adopted.
- The dosage of IDA and DNR is accurate to 1mg. The total dose of IDA is required to reach the corresponding dose.

Supplementary notes for induction therapy:

- For Low-risk group, whenever WBC $\geq 10 \times 10^9$/L during induction therapy, hydroxyurea at an oral dose of 20-50mg/kg/d will be given till WBC $< 10 \times 10^9$/L.
- For intermediate-risk group, chemotherapy (IDA or DNR) is given only when WBC $\geq 10\times 10^9$/L during induction therapy. If WBC count is less than 10×10^9/L during the whole induction phase, chemotherapy (IDA or DNR) is omitted.
- For high-risk group, chemotherapy (IDA or DNR) shall be given as soon as possible. If the leukocyte count is still on the rise after 3 doses of IDA or DNR, the forth dose shall be given, otherwise the forth dose is omitted.
- Daily CBC test, coagulation test (every other day) and QTc measurement by ECG (weekly) are recommended according to previous clinical experience.

Randomization

Patients are recommended to take the first bone marrow analysis at the end of induction therapy, usually about 30 days from the initial treatment. Patients at each risk group who have achieved hCR testified by bone marrow analysis are randomly assigned to two arms before consolidation therapy.

Randomization is supported by Shanghai Clinical Research Center (SCRC). A block randomization is applied with stratification according to Sanz risk by each participating hospital. The information of randomization is put into opaque sealed randomization envelopes and distributed to each hospital. Once a patient has achieved hCR, the investigator shall open the corresponding random envelope of the patient and allocate the patient into the study group or the reference group according to the randomization assignment.

Patients of low-risk APL are randomly assigned to ATRA plus ATO arm (as study group) or ATRA plus chemotherapy arm (as reference group).

Patients of intermediate-risk APL are randomly assigned to ATRA plus ATO arm (as study group) or ATRA plus chemotherapy arm (as reference group). However, intermediate-risk patients whose WBC count is consistently well below 10×10^9/L during the whole induction therapy are assigned to the study group without randomization.

Patients of high-risk APL are randomly allocated into ATRA plus ATO and anthracycline arm (as study group) or ATRA plus anthracycline and cytarabine arm (as reference group).

Consolidation Therapy

For low-risk patients:

1) Study group: two courses of ATRA and ATO combination therapy.

• ATRA 25mg/m^2/d given orally on days 1-14.

• ATO 0.16mg/kg/d (10mg/d maximum) I.V. drip on days 1-28*.

2) Reference group: two courses of ATRA and IDA or DNR therapy.

• ATRA 25mg/m^2/d given orally on days 1-14.

• IDA 8mg/m^2/d or DNR 45mg/m^2/d I.V. on days 1-3.

There is a 2-week interval between the two courses.

For intermediate-risk patients:

1) Study group: three courses of ATRA and ATO combination therapy.

• ATRA 25mg/m^2/d given orally on days 1-14.

• ATO 0.16mg/kg/d (10mg/d maximum) I.V. drip on days 1-28*.

2) Reference group: two courses of ATRA and IDA/DNR therapy.

• ATRA 25mg/m^2/d given orally on days 1-14.

• IDA 8mg/m^2/d or DNR 45mg/m^2/d I.V. on days 1-3.

There is a 2-week interval between the two courses.

For high-risk patients:

1) Study group: two courses of ATRA and ATO plus anthracycline, and one course of ATRA plus ATO.

Course 1 and 2:

• ATRA 25mg/m^2/d given orally on days 1-14.

• ATO 0.16mg/kg/d (10mg/d maximum) I.V. drip on days 1-14.

• IDA 8mg/m^2/d or DNR 45mg/m^2/d I.V. on days 1-3.

Course 3:

• ATRA 25mg/m^2/d given orally on days 1-14.

• ATO 0.16mg/kg/d (10mg/d maximum) I.V. drip on days 1-14.

2) Reference group: two courses of ATRA plus anthracycline and Cytarabine (Ara-C), and one course of ATRA plus mid-dose Ara-C.

Course 1 and 2:

• ATRA 25mg/m^2/d given orally on days 1-14.

• IDA 8mg/m^2/d or DNR 45mg/m^2/d I.V. on days 1-3.

• Ara-C 150mg/m^2/d I.V. drip on days 1-7.

Course 3:

• ATRA 25mg/m^2/d given orally on days 1-14.

• Ara-C 1g/m^2 I.V. drip every 12 hours for 6 times on days 1-3.

There is a 2-week interval between the two courses.

*An interval of 7-14 days shall be allowed in the middle of 28-day ATO treatment.

Supplementary notes for consolidation therapy:

• For high-risk patients with delayed recovery (over 6 weeks from the 1st day of the former

chemotherapy) of peripheral blood cell counts, the dose of Ara-C in the reference group consolidation course 1 and 2 shall be reduced from 150mg/m^2/d to 100mg/m^2/d, while the dose of Ara-C in reference group consolidation course 3 and the dose of all agents in the consolidation of study group is unadjusted.

- Molecular analysis is applied to all patients after consolidation therapy. Patients with mCR shall receive maintenance therapy courses.
- For low- and intermediate-risk patients who fail to achieve mCR after consolidation therapy at two successive times of detection within one month apart, they will be enrolled crossly in the high-risk group for a further consolidation therapy of three courses (i.e. patients in the study group at low- and intermediate-risk shall receive the therapy of reference group at high-risk, and conversely, those in the reference group receive the therapy of study group at high-risk). After that, patients who are still positive for PML-RARa shall be withdrawn from the study and selected to salvage treatment.
- Patients of high-risk APL who fail to achieve mCR after consolidation therapy at two successive times of detection within one month apart, shall be withdrawn from the study and selected to salvage treatment.

Maintenance Therapy

For low- and intermediate-risk patients:

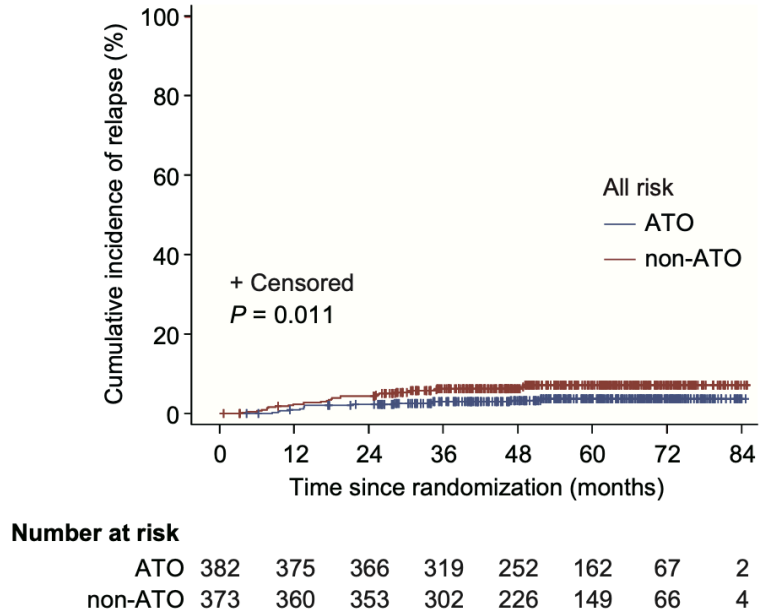
- ATRA 25mg/m^2/d given orally for 14 days (d1-14) on and 14 days (d15-28) off.
- ATO 0.16mg/kg/d (10mg/d maximum) I.V. drip for 14 days (d29-42) on and 14 days (d43-56) off.
- ATO 0.16mg/kg/d (10mg/d maximum) I.V. drip for 14 days (d57-70) on and 14 days (d71-84) off.

About 3 months (84 days) for each cycle of ATRA-ATO-ATO sequential treatment, and 3 cycles in total.

For high-risk patients:

- ATRA 25mg/m^2/d given orally for 14 days (d1-14) on and 14 days (d15-28) off.
- ATO 0.16mg/kg/d (10mg/d maximum) I.V. drip for 14 days (d29-42) on and 14 days (d43-56) off.
- ATO 0.16mg/kg/d (10mg/d maximum) I.V. drip for 14 days (d57-70) on.

• Methotrexate (MTX) 15mg/m^2/week for 4 weeks (d71-98), orally taken or I.V. About 3.5 months (98 days) for each cycle of ATRA-ATO-ATO-MTX sequential treatment, and 5 cycles in total.
Estimated sample size: 738 cases (total) **Duration of recruitment:** 5 years **Follow-up time:** 36 months after the last patient recruited



Number at risk

ATO	382	375	366	319	252	162	67	2
non-ATO	373	360	353	302	226	149	66	4

Fig. S1. Kaplan-Meier plot of cumulative incidence of relapse of patients in ATO or non-ATO groups.

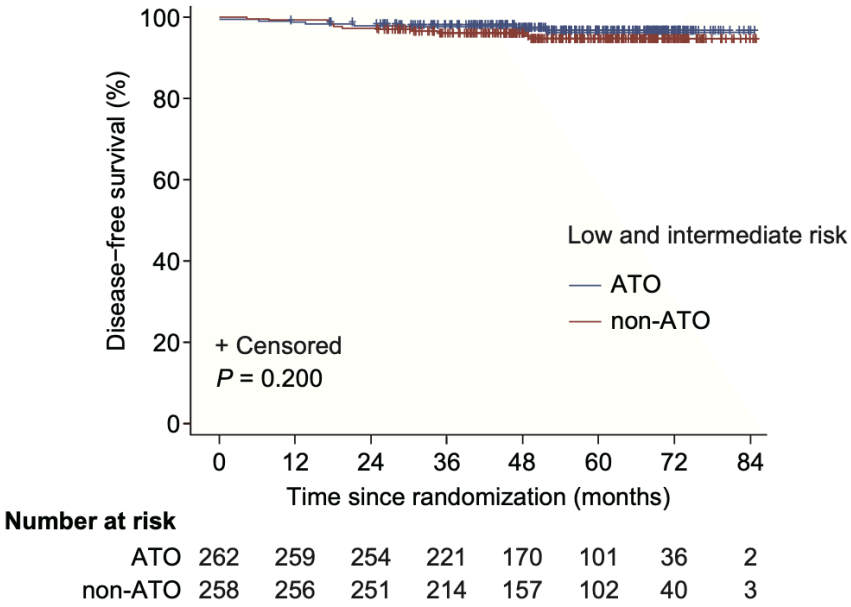


Fig. S2. Kaplan-Meier plot of disease-free survival of patients in ATO or non-ATO groups when low- and intermediate-risk categories are combined.

Table S1. Causes of death unrelated to acute promyelocytic leukemia.

Patient (n = 8)	Age (years)	Group	Risk	Time from CR to events (months)	Cause of death	Response at time of death
1	63	non-ATO group	High	17.0	sAML	mCR
2	38	ATO group	Intermediate	21.4	sAML	mCR
3	50	ATO group	Intermediate	28.1	sAML	mCR
4	50	ATO group	Intermediate	46.8	Lung Cancer	mCR
5	50	non-ATO group	Low	48.5	Gastric Cancer	mCR
6	19	non-ATO group	High	3.6	Hepatic Failure of Unknown Reason	mCR
7	36	ATO group	High	34.4	Suicide due to Depression	mCR
8	55	ATO group	Intermediate	51.7	Cerebral Hemorrhage*	mCR

*The patient experienced cerebral hemorrhage during the induction and consolidation period. The treatment was discontinued after the third course of consolidation and the patient died of the third cerebral hemorrhage at 51.7 months after complete remission. No evidence of central nervous system infiltration was observed before death.

Table S2. Sensitivity analysis for non-inferiority test (intention-to-treat population).

		ATO group 3-year DFS No. (%)	non-ATO group 3-year DFS No. (%)	3-year DFS difference Probability % (95% CI)	*P* value
Scenario 0	non-inferiority analysis	319/332 (96.1)	302/326 (92.6)	3.45 (–0.07-6.97)	< 0.001
Scenario 1	All lost to follow-up were considered disease free.	327/340 (96.2)	308/332 (92.8)	3.41 (–0.05-6.86)	< 0.001
Scenario 2	All lost to follow-up were considered disease progress.	319/340 (93.8)	302/332 (91.0)	2.86 (–1.15-6.87)	< 0.001
Scenario 3	Those lost to follow-up in ATO group were considered progress, and in non-ATO group considered disease free.	319/340 (93.8)	308/332 (92.8)	1.05 (–2.73-4.83)	0.001

Table S3. Clinical outcomes of low-/intermediate-risk (chemotherapy-replacing) and high-risk (chemotherapy-reducing) patients.

	Total (n = 755)	ATO group (n = 382)	non-ATO group (n = 373)	HR (95% CI)	*P* value
	Probability, % (95% CI)	Probability, % (95% CI)	Probability, % (95% CI)		
3-year disease-free survival					
Low and intermediate risk	97.1 (95.6-98.5)	98.1 (96.4-99.8)	96.0 (93.7-98.5)	0.57 (0.22-1.44)	0.200
High risk	90.4 (86.6-94.3)	93.2 (88.7-97.9)	87.4 (81.5-93.8)	0.52 (0.22-1.24)	0.14
7-year disease-free survival					
Low and intermediate risk	95.8 (94.0-97.7)	96.9 (94.5-99.2)	94.8 (91.9-97.8)	0.57 (0.22-1.44)	0.200
High risk	90.4 (86.6-94.3)	93.2 (88.7-97.9)	87.4 (81.5-93.8)	0.52 (0.22-1.24)	0.14
7-year overall survival					
Low and intermediate risk	97.9 (96.5-99.4)	97.9 (95.9-100)	98.0 (96.0-100)	0.98 (0.25-3.92)	0.977
High risk	94.9 (91.9-97.9)	94.5 (90.3-98.9)	95.3 (91.3-99.4)	1.11 (0.34-3.64)	0.864
7-year cumulative incidence of relapse					
Low and intermediate risk	2.7 (1.5-4.6)	0.82 (0.25-2.68)	4.52 (2.24-9.07)	0.18 (0.04-0.81)	0.025
High risk	7.6 (4.9-11.7)	5.1 (2.5-10.4)	9.9 (6.1-16.1)	0.50 (0.19-1.35)	0.173

Kaplan-Meier method was used to estimate the survival above. Treatment protocol of ATO group (Low risk: ATRA+ATO, Intermediate risk: ATRA+ATO, High risk: ATRA+ATO+IDA/DNR). Treatment protocol of non-ATO group (Low risk: ATRA+IDA/DNR, Intermediate risk: ATRA+IDA/DNR, High risk: ATRA+IDA/DNR+Ara-C).

NA，not applicable.

Table S4. Sensitivity analysis for non-inferiority test (per-protocol population).

		ATO group 3-year DFS No. (%)	non-ATO group 3-year DFS No. (%)	3-year DFS difference Probability % (95% CI)	*P* value
Scenario 0	non-inferiority analysis	286/296 (96.6)	276/297 (92.9)	3.69 (0.12-7.26)	< 0.001
Scenario 1	All lost to follow-up were considered disease free.	291/301 (96.7)	278/299 (93.0)	3.70 (0.17-7.24)	< 0.001
Scenario 2	All lost to follow-up were considered disease progress.	286/301 (95.0)	276/299 (92.3)	2.71 (–1.19-6.60)	< 0.001
Scenario 3	Those lost to follow-up in ATO group were considered progress, and in non-ATO group considered disease free.	286/301 (95.0)	278/299 (93.0)	2.04 (–1.76-5.84)	< 0.001

Table S5. Clinical outcomes of per-protocol analysis.

Outcomes	ATO group (n=340) Probability, % (95% CI)	non-ATO group (n=335) Probability, % (95% CI)	HR (95% CI)	*P* value
3-year disease-free survival				
Low risk	100	94.6 (89.6-99.9)	*NA*	0.01
Intermediate risk	97.3 (94.7-99.9)	96.2 (93.2-99.2)	0.88 (0.27-2.90)	0.838
High risk	94.2 (89.9-98.8)	88.9 (82.9-95.3)	0.50 (0.19-1.35)	0.172
Total	97.0 (95.2-98.9)	93.6 (91.0-96.3)	0.46 (0.23-0.95)	0.036
7-year disease-free survival				
Low risk	100	90.7 (83.7-98.2)	*NA*	0.01
Intermediate risk	96.3 (93.2-99.6)	96.2 (93.2-99.2)	0.88 (0.27-2.90)	0.838
High risk	94.2 (89.9-98.8)	88.9 (82.9-95.3)	0.50 (0.19-1.35)	0.172
Total	96.6 (94.6-98.6)	92.7 (89.8-95.7)	0.46 (0.23-0.95)	0.036
7-year overall survival				
Low risk	100	96.4 (91.5-100)	*NA*	0.147
Intermediate risk	98.2 (95.8-100)	98.6 (96.7-100)	1.02 (0.14-7.24)	0.985
High risk	93.8 (89.1-98.8)	96.8 (93.2-100)	1.88 (0.47-7.51)	0.373
Total	97.3 (95.4-99.2)	97.5 (95.7-99.4)	1.12 (0.41-3.09)	0.825
7-year cumulative incidence of relapse				
Low risk	0	7.3 (3.0-17.9)	*NA*	< 0.001
Intermediate risk	1.4 (0.3-5.5)	3.8 (1.7-8.3)	0.36 (0.07-1.78)	0.209
High risk	4.8 (2.2-10.6)	8.1 (4.5-14.7)	0.58 (0.19-1.76)	0.336
Total	2.1 (1.1-4.2)	5.9 (3.7-9.3)	0.36 (0.15-0.86)	0.021

Kaplan-Meier method was used to estimate the survival above. Treatment protocol of ATO group (Low risk: ATRA+ATO, Intermediate risk: ATRA+ATO, High risk: ATRA+ATO+IDA/DNR). Treatment protocol of non-ATO group (Low risk: ATRA+IDA/DNR, Intermediate risk: ATRA+IDA/DNR, High risk: ATRA+IDA/DNR+Ara-C).

NA，not applicable.