Translational Research on the Curative Therapy of Acute Promyelocytic Leukemia

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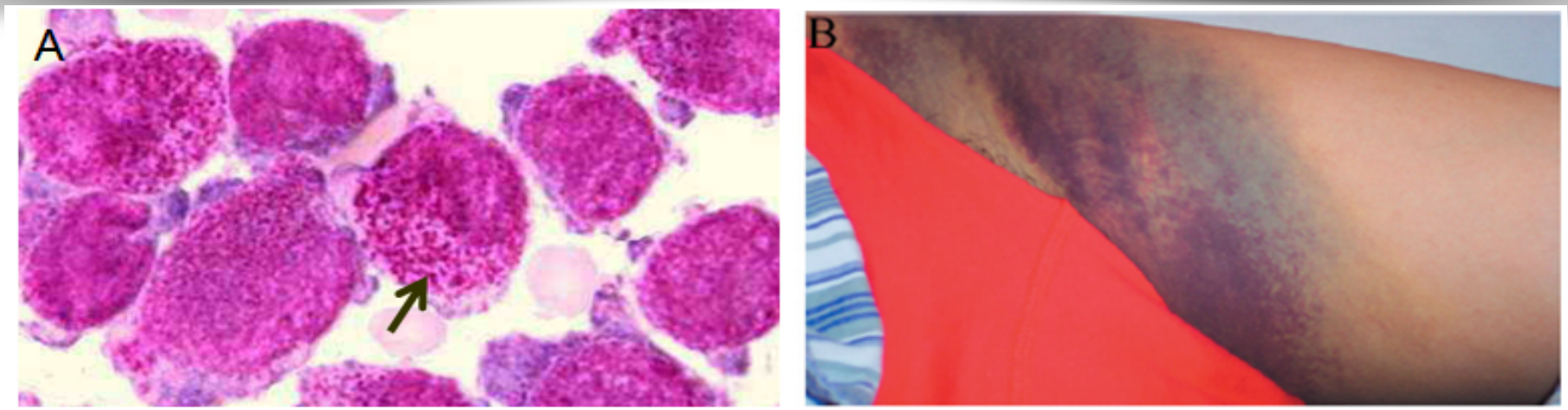
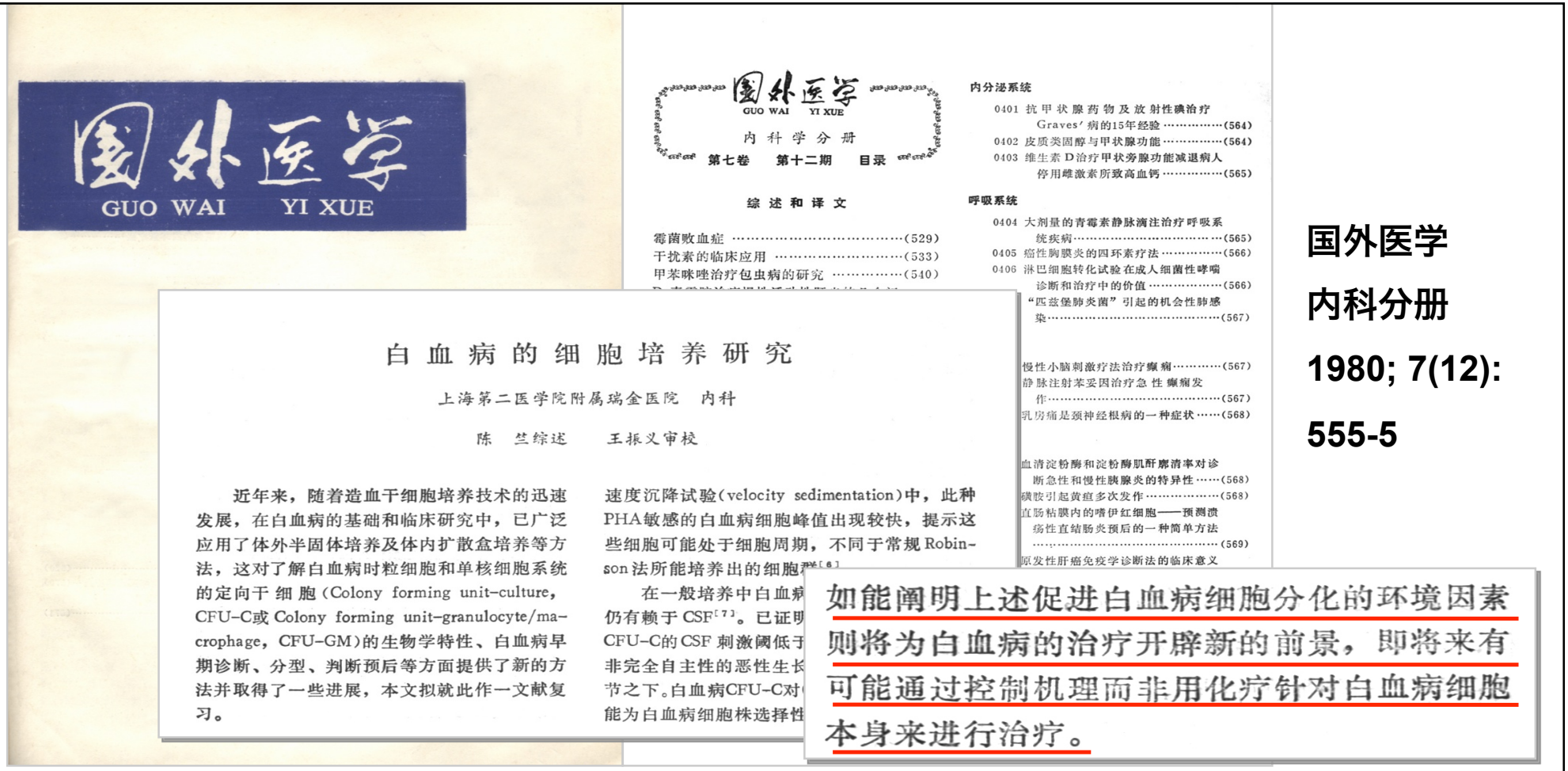
Clinical Features of APL



- M3 subtype of acute myeloid leukemia (AML); 10-15% of AML cases (high frequency in Latinos from Europe or S/C America; 19% in Chinese) ; once the most malignant form of acute leukemia
- Chemotherapy (CT) lead to CR in 70% cases, with median survival of 1-2 years.
- Severe bleeding syndrome, often deteriorated by CT
- Incidence of APL is constant over human lifespan, suggesting one rate-limiting mutation: Translocation t(15;17)(q22;q21)



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内科学分册
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原发性肝癌免疫学诊断法的临床意义

白血病的细胞培养研究

上海第二医学院附属瑞金医院 内科

陈 竺综述 王振义审校

近年来，随着造血干细胞培养技术的迅速发展，在白血病的基础和临床研究中，已广泛应用了体外半固体培养及体内扩散盒培养等方法，这对了解白血病时粒细胞和单核细胞系统的定向干细胞(Colony forming unit-culture, CFU-C或Colony forming unit-granulocyte/macrophage, CFU-GM)的生物学特性、白血病早期诊断、分型、判断预后等方面提供了新的方法并取得了一些进展，本文拟就此作一文献复习。

速度沉降试验(velocity sedimentation)中，此种PHA敏感的白血病细胞峰值出现较快，提示这些细胞可能处于细胞周期，不同于常规Robinson法所能培养出的细胞[6]

在一般培养中白血病仍有赖于CSF[7]。已证明CFU-C的CSF刺激阈低于非完全自主性的恶性生长节之下。白血病CFU-C对能为白血病细胞株选择性

如能阐明上述促进白血病细胞分化的环境因素则将为白血病的治疗开辟新的前景，即将来有可能通过控制机理而非用化疗针对白血病细胞本身来进行治疗。

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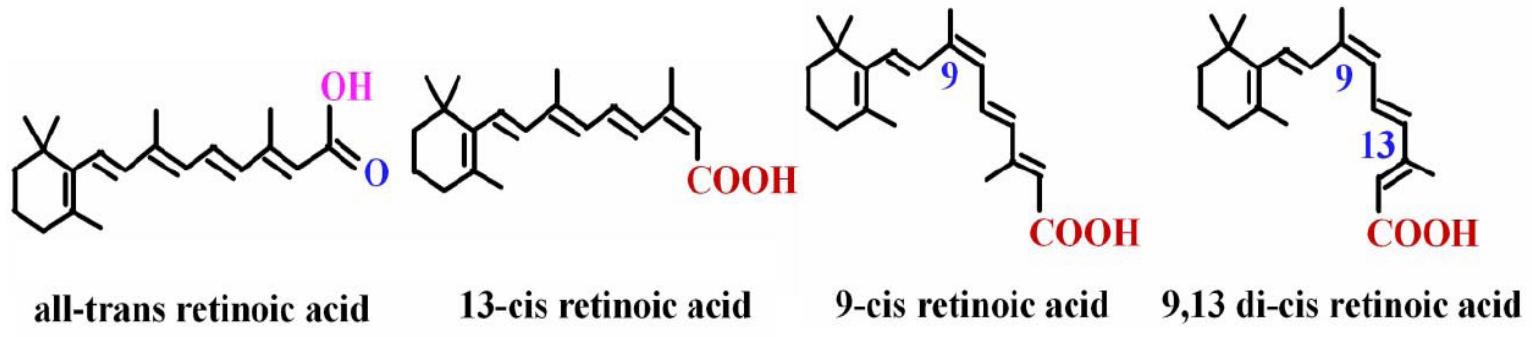
1980; 7(12):
555-5

Objectives set by SIH in 1980:

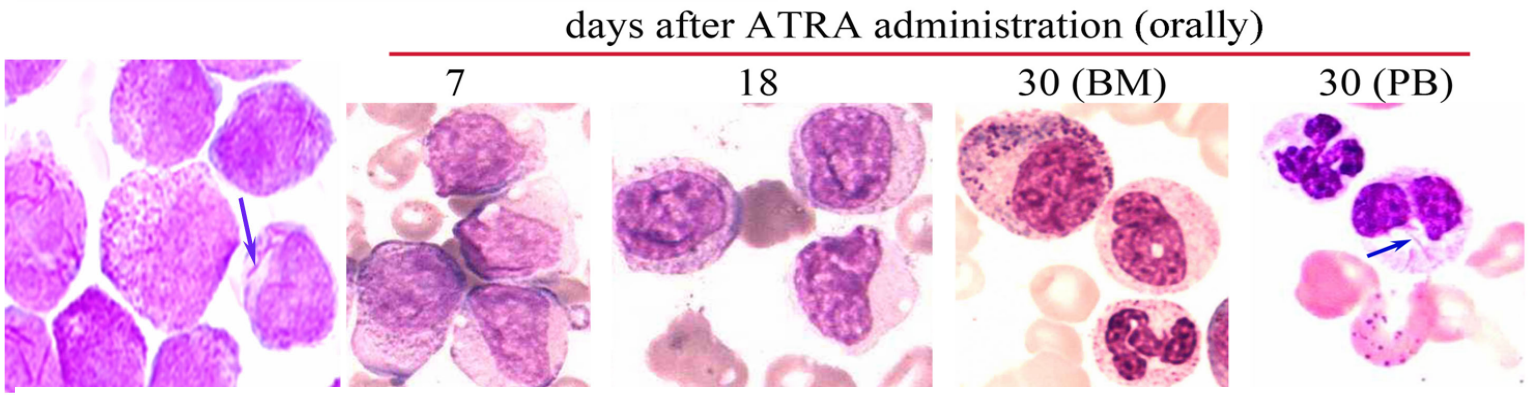
Identify factors promoting leukemia cell differentiation;

Explore treatment of leukemia with regulatory mechanisms

A Turning Point in the Treatment of APL:
Application of All-trans Retinoic Acid (ATRA)



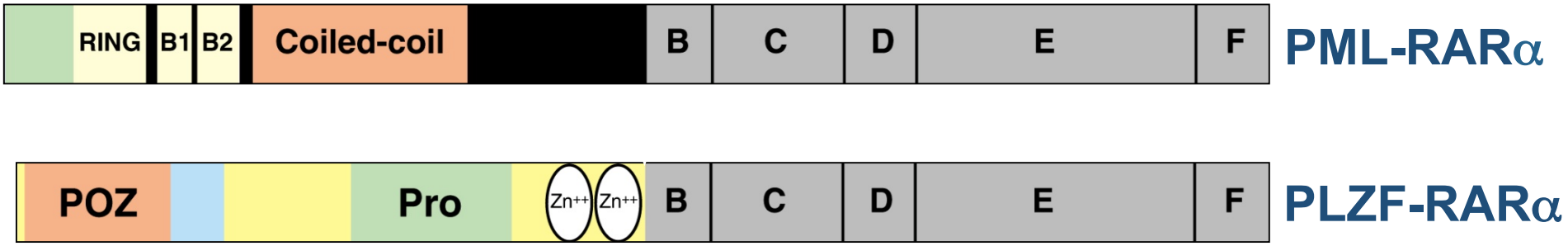
all-trans retinoic acid | 13-cis retinoic acid | 9-cis retinoic acid | 9,13 di-cis retinoic acid



The first clinical trial from Shanghai: CR achieved in 23/24 cases. Significantly reduced coagulopathy during remission induction.

Bedside to Bench translation: cloning of fusion genes in APL including PML-RARα and variant fusions such as PLZF-RARα.

PML-RARa and PLZF-RARα Fusions in Acute Promyelocytic Leukemia



Clinical relevance:

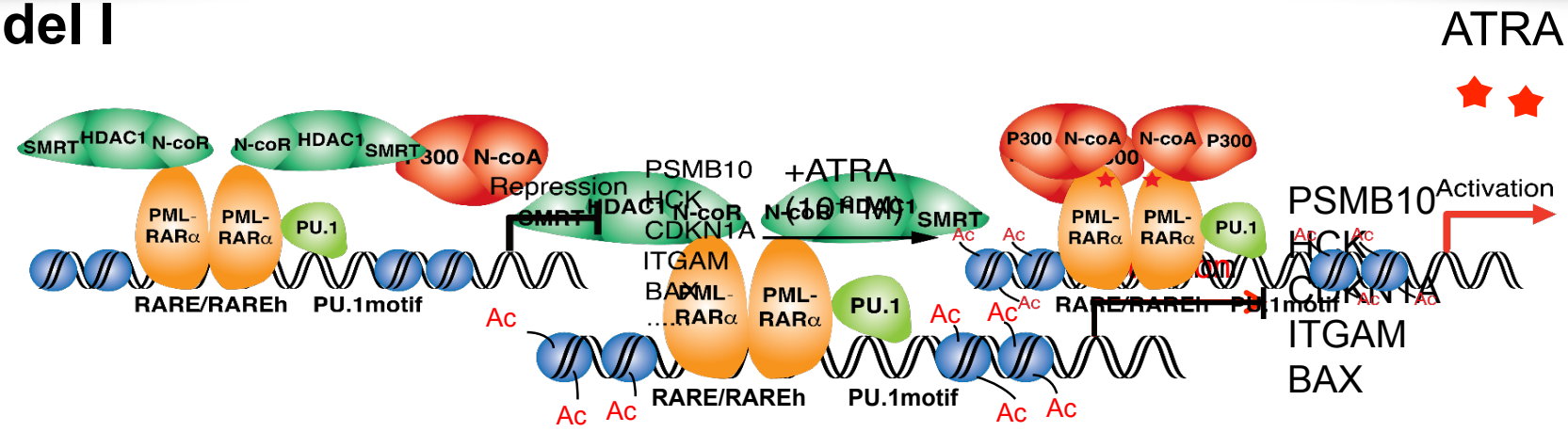
- t(15;17)(q22;q21) APL with PML-RARα: response to ATRA in overwhelming majority of patients
- t(11;17)(q23;q21) APL with PLZF-RARα: resistance to ATRA

Leukemogenesis

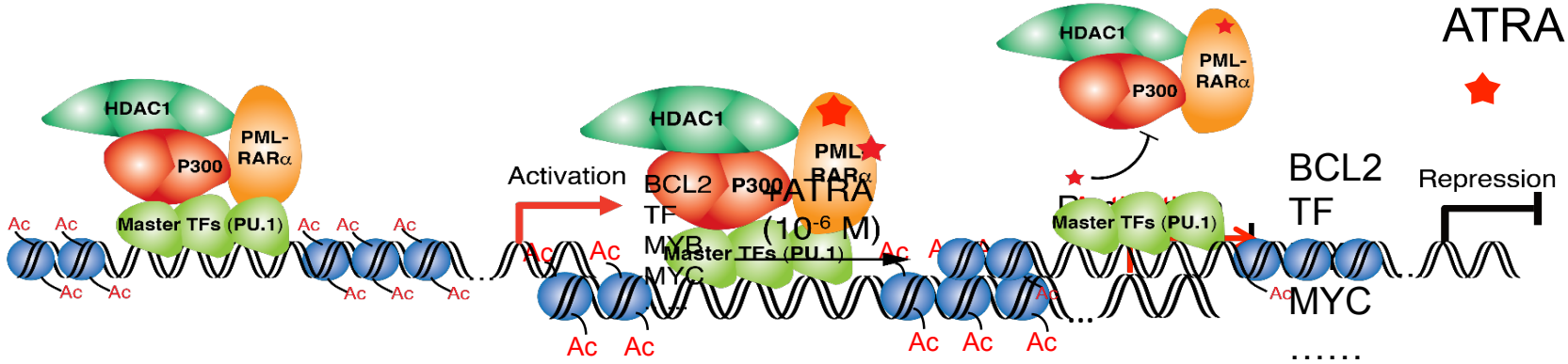
- Both *PML-RARα* and *PLZF-RARα* are leukemogenic in transgenic mice

Mechanisms Underlying Molecular Pathogenesis of APL and Its Response to ATRA

Model I



Model II



Model III

PML-RARα degradation via proteosome pathway upon ATRA binding

Challenges of ATRA Treatment for APL

Retinoic acid syndrome (RAS) in 5-20% of cases

Measures: ATRA at 25mg/m2; chemotherapy in the presence of hyperleukocytosis; careful use of dexamethasone

Resistance to ATRA after long time use in most cases

- **Catabolism of drug or decreased delivery to nucleus**
- **Appearance or selection of mutations in PML-RARα, especially in the LBD of RARα; additional cytogenetic and genetic abnormalities along with leukemia clonal evolution**
- **Inability of ATRA with conventional formulation to eliminate leukemia-initiating cells in most cases**

Measures: incorporation of chemotherapy

Long-term Survival of APL Patients with ATRA→CT / ATRA+CT

Author	Year	No.	Protocol	OS%	DFS%	Others
Hu et al	1999	120	A→DA	52.5(5Y)		RFS 34(5Y)
Tallman et al	2002	350	A→DA	69(5Y)	69(5Y)	
Sanz et al	2008	560	A+IDA	82(5Y)	84(5Y)	
Adès et al	2010	122	A→DA	81.8(10Y)		EFS 64.4(10Y)
		184	A+DA	85(10Y)		EFS 76.3(10Y)
Sanz et al	2010	402	A+IDA	89(4Y)	90(4Y)	
Lo-Coco et al	2010	445	A+IDA	87(6Y)	86(6Y)	
Avvisati et al	2011	761	A+IDA	76.5(12Y)	70.8(12Y)	CR 94.3%
Burnett et al	2013	142	A+ADE	84(5Y)	81(5Y)	CR 93%

Hu et al. Int J Hematol. 1999; 70: 248-60.
Tallman et al. Blood. 2002; 100: 4298-4302.
Sanz et al. Blood. 2008; 112: 3130-3134.
Adès et al. Blood. 2010; 115: 1690-6.
Sanz et al. Blood. 2010; 115: 5137-46.
Lo-Coco et al. Blood. 2010; 116: 3171-9.
Avvisati et al. Blood. 2011; 117: 4716-25.
Burnett et al. Leukemia. 2013; 27: 843–851.

Arsenic (As) : a Brief Introduction

- **Challenge: relapse in 30-50% APL patients after long time exposure to ATRA/CT**

  Arsenic: the 33rd element in the periodic table; inorganic or organic forms

- **Arsenic can be used as a drug**

  Hippocrates (460-370 BC) used realgar and orpiment pastes to treat ulcers

- **GE Hong (284-364) recorded that arsenic in realgar could be used as disinfector. SUN Simiao (581-682) used arsenic pills to treat periodic fever or malaria. LI Shizhen (1518-1593) used arsenic to treat many diseases**

  Arsenic trioxide (ATO) was used to treat chronic myeloid leukemia (CML) but was discarded in 1930s

- **Work of Ting-Dong Zhang et al in 1970s-1980s: Treatment of myeloid leukemia with ATO and mercury**

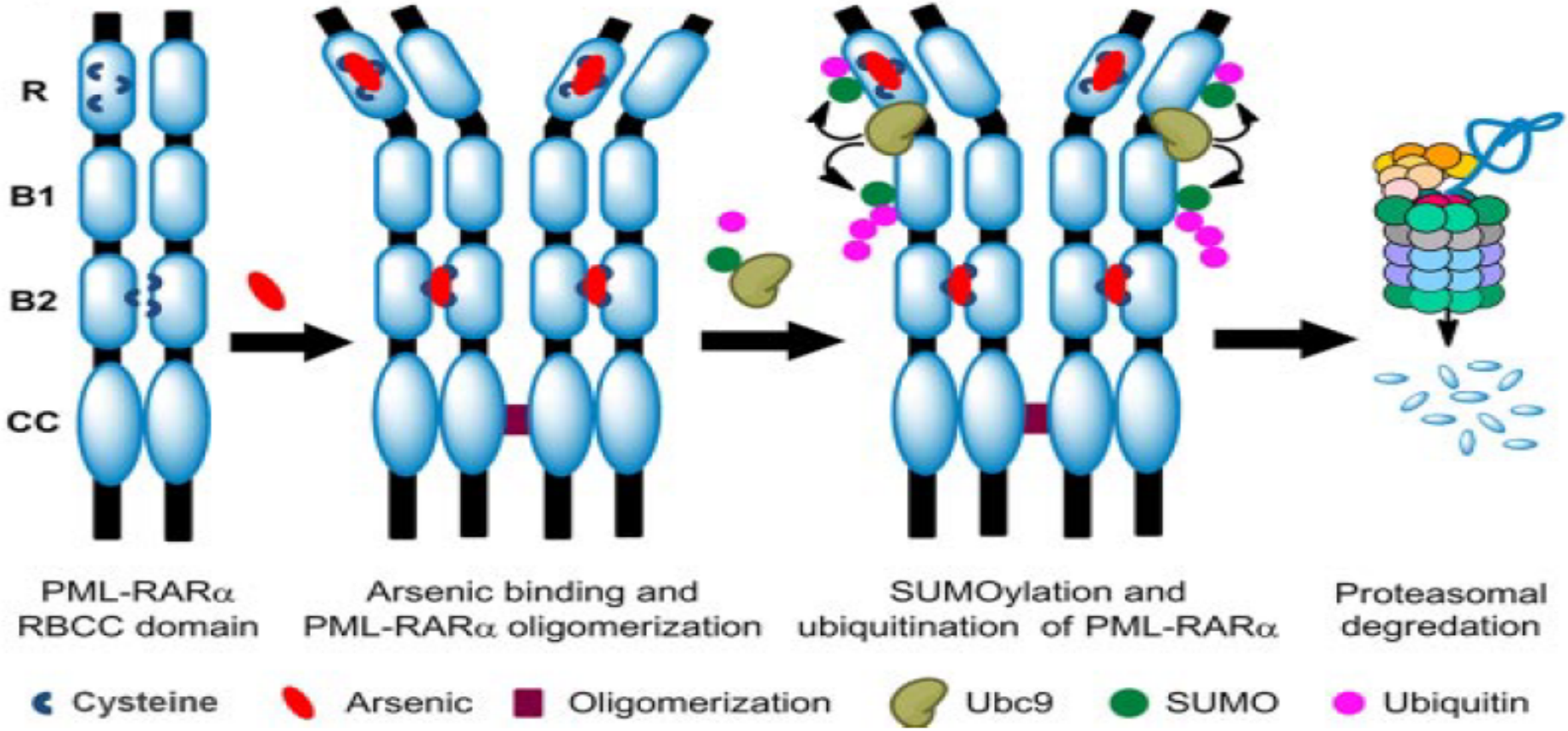
Hippocrates

GE Hong

A Working Model for Arsenic Triggered Degradation of PML-RARα and PML

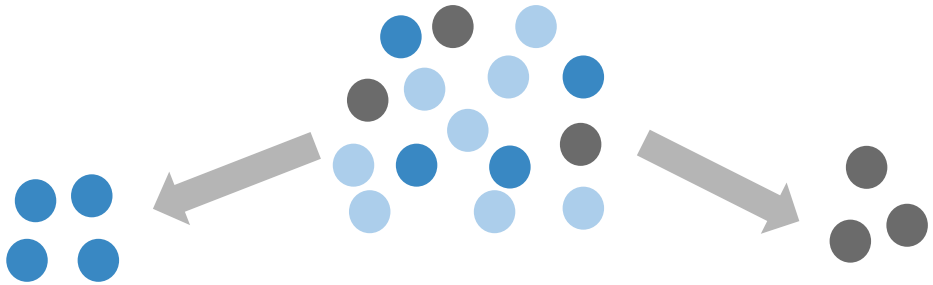
Early study of ATO effect on APL cells: Apoptosis or cell differentiation in a dose-dependent manner; Dose/time-dependent PML-RARα degradation

Molecular mechanism of ATO-induced PML-RARα degradation



Zhang et al. Science. 2010; 328:240-3. Jeanne et al. Cancer Cell. 2010; 18:88-98.

ATO in Relapsed/refractory APL



Differentiation syndrome in a few cases

Measure: adding of CT

Other side effects of ATO

- **Low degree liver dysfunction;**
- **Prolongation of Q-T interval on ECG;**
- **Gastrointestinal symptoms;**
- **Skin reaction;**

Measures: reduction of doses or temporary withdraw of drug

ATO+ATRA in Relapsed/refractory APL

- **SIH conducted controlled study with pure ATO and achieved CR in 40/47 (85%) relapsed APL patients, including 5/5 (100%) relapsed APL with ATO+ATRA, and 8/11 (73%) newly diagnosed APL patients**

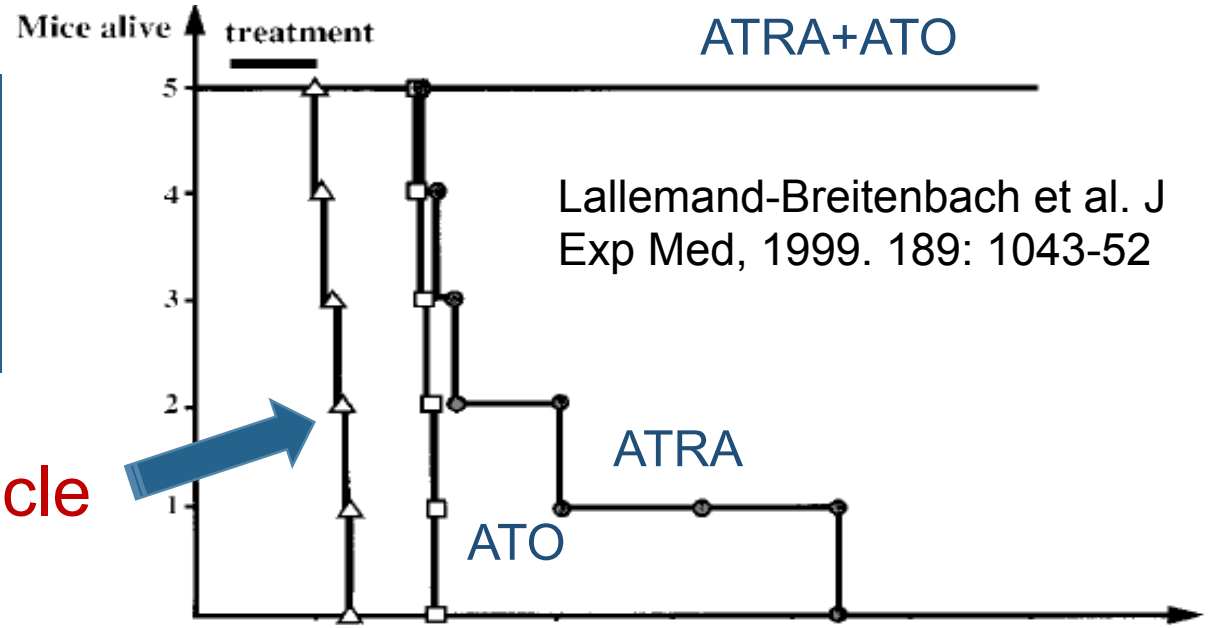
Table 2. Efficacy of As_2O_3 Treatment in APL

Group	Treatment	Case Numbers	CR (%)	Days to CR (Medium)
Newly diagnosed patients	As_2O_3	7	6 (85.7%)	30 to 36 (35)
	As_2O_3 + Chemo	4	2 (50.0%)	36 to 36 (36)
Relapsed patients	As_2O_3	31	26 (83.9%)	17 to 76 (30)
	As_2O_3 + Chemo	11	9 (81.8%)	25 to 63 (35)
	As_2O_3 + ATRA	5	5 (100.0%)	19 to 46 (39)

Molecular remission induced by ATO is more durable than that achieved by ATRA in newly diagnosed patients

Niu et al. Blood. 1999; 94: 3315-3324.

Synergistic Effects of ATRA and ATO: Animal Studies



Lallemand-Breitenbach et al. J Exp Med, 1999. 189: 1043-52

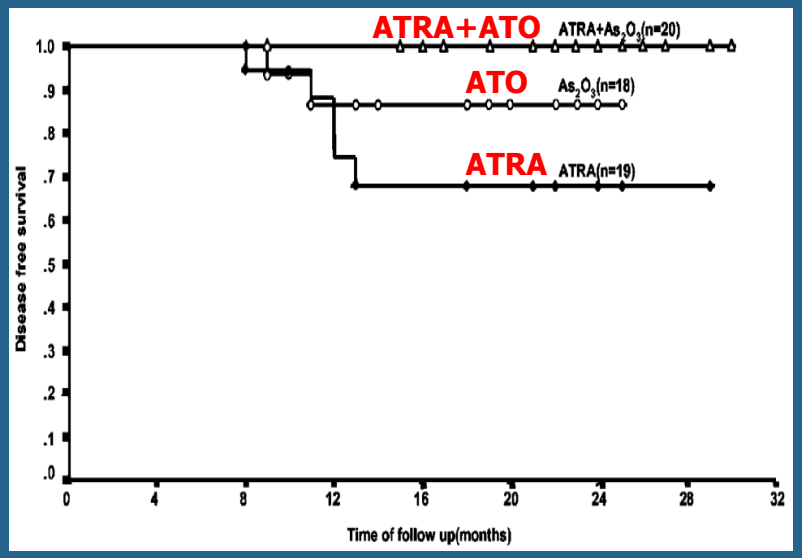
Jing et al. Blood, 2001;97:264-9.

Table 1. Effects of combined or sequential use of As_2O_3 and ATRA on survival time of SCID mouse–NB4 cell ascites model

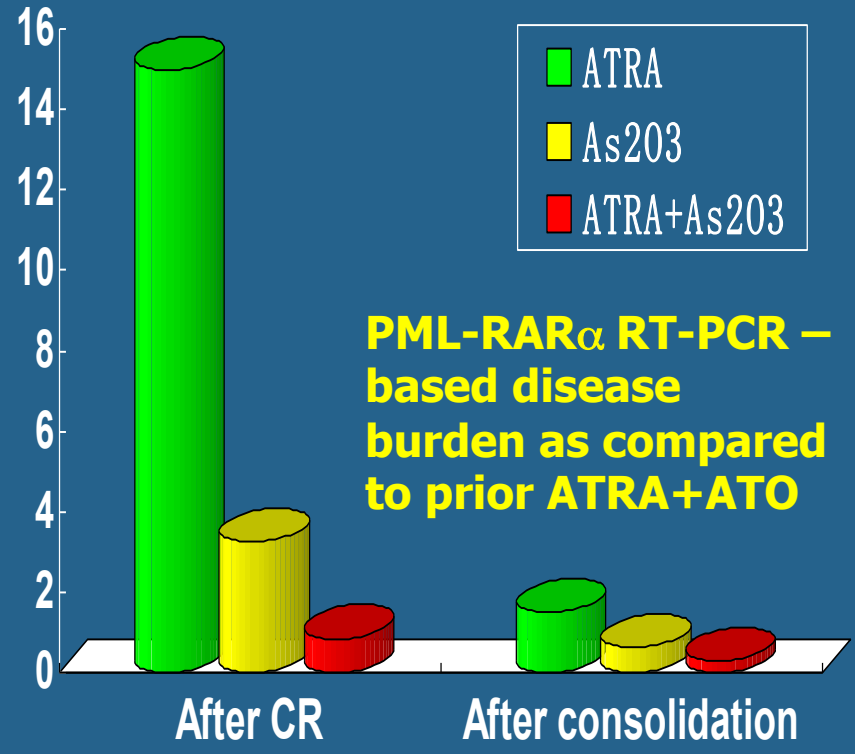
Protocol no.		Sample no.	Mean survival (d ± SD)	% Prolongation compared with control
A	Vehicle	15	21.2 ± 1.6	
B	ATRA	15	29.6 ± 3.5	39.9%*
C	ATO	9	28.6 ± 3.1	35.2%*
D	ATRA+ATO	11	36.4 ± 5.1	71.9%*
E	ATO+ATRA	12	38.5 ± 4.8	81.9%*

ATRA+ATO Combination Therapy in Newly Diagnosed APL

Apr 2001 to Feb 2003; N=61

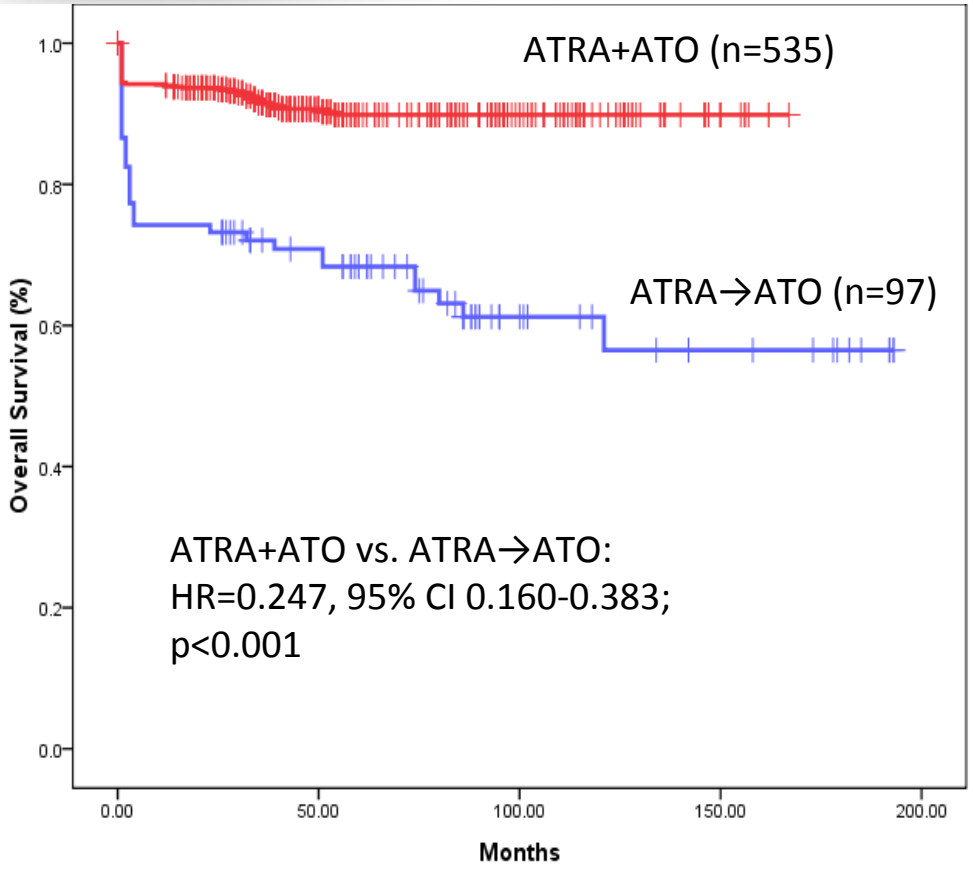


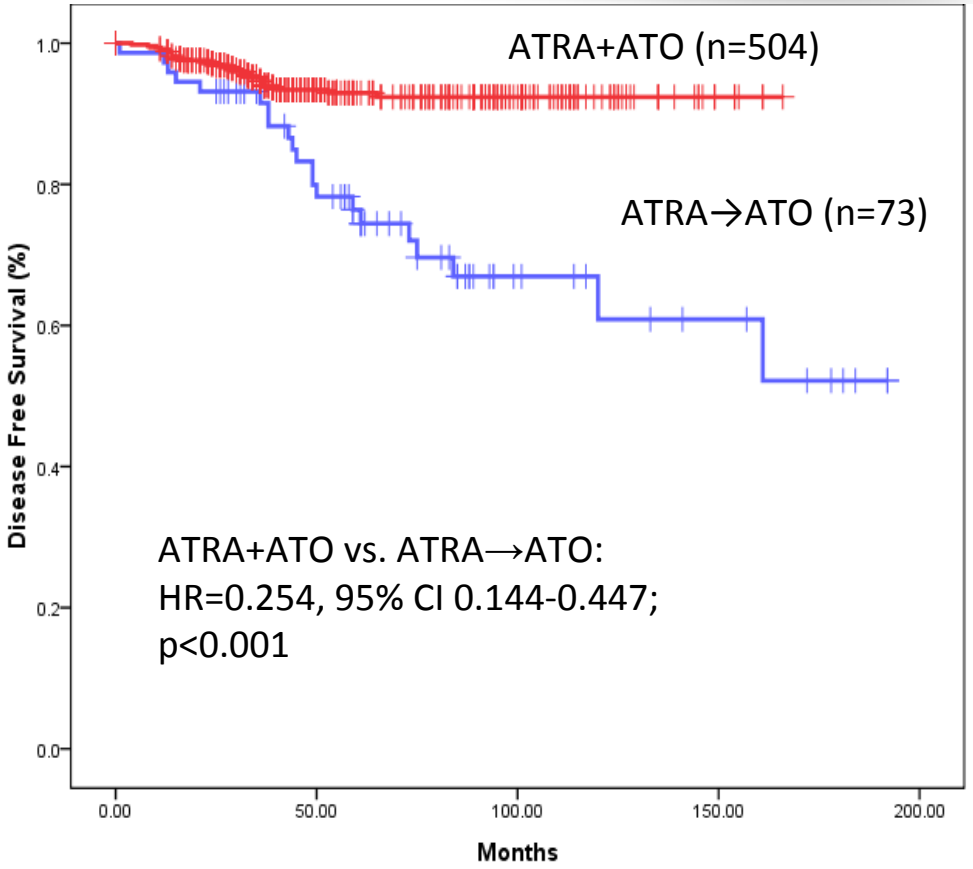
Kaplan-Meier DFS survival curves.



Shen et al. PNAS. 2004, 101(15): 5328-35.

Long-term Efficacy of ATRA+ATO: Synergistic Targeting of PML-RARα in Newly Diagnosed APL





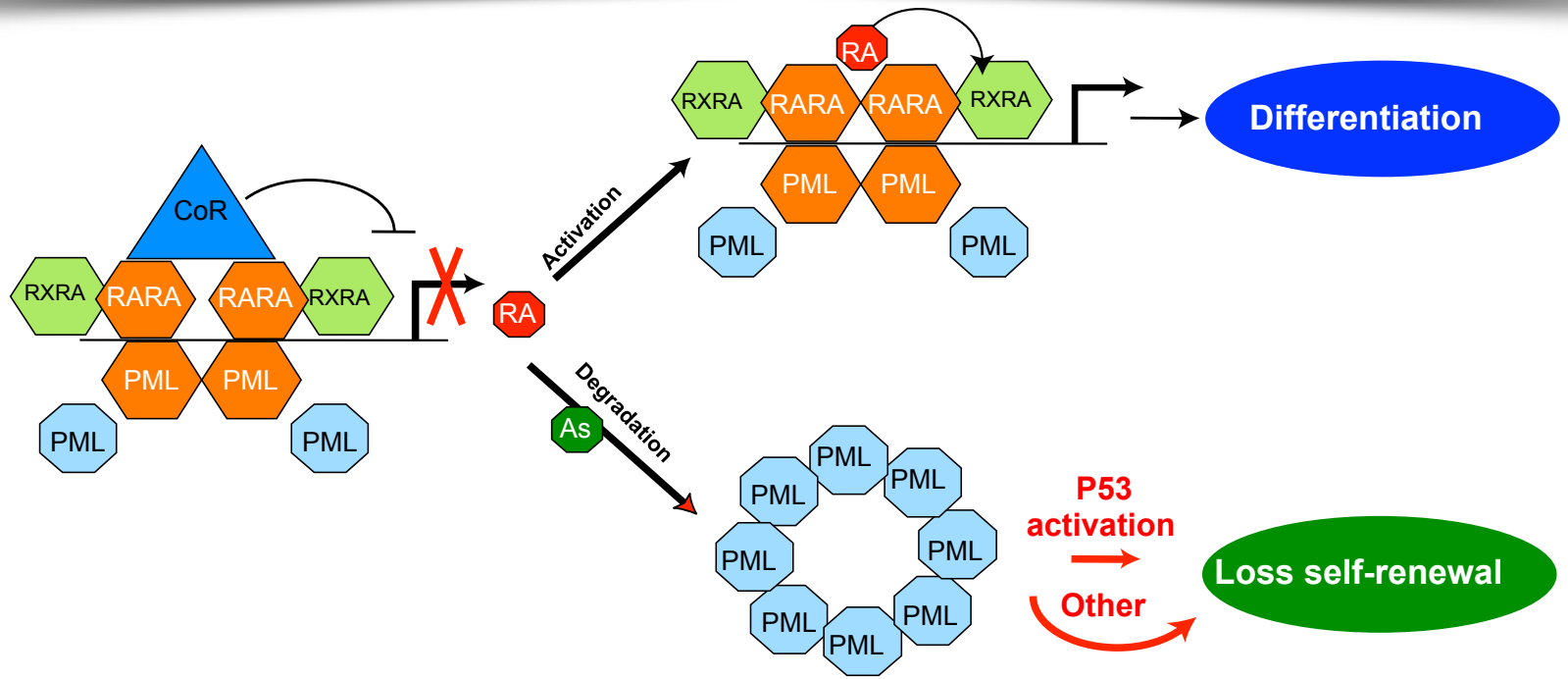
Shen, et al. EBioMedicine. 2015; 2: 563–571.

Long-term Follow-up of ATRA+ATO Based Treatment for APL

Author	Year	No.	Protocol	DFS (EFS)%	Risk & DFS%
Powell et al	2010 (3Y)	244	ATRA+ATO+CT	90	
Shen et al	2015 (5Y)	535	ATRA+ATO+CT	92.9	Low: 96.3 Intermed:93.4 High: 87.8
Burnett et al	2015 (4Y)	116 vs 119	ATRA+ATO+GO ATRA+IDA	91 vs 70	
Iland et al	2015 (5Y)	124	ATRA+ATO+IDA	95	
Zhu et al	2015 (10Y)	217	ATRA+ATO+CT	87.0	Non-high: 90.6 High: 73.1
Platzbecker et al	2017 (4Y)	127 vs 136	ATRA+ATO vs ATRA+IDA	97.3 vs 82.6	Low and intermediate

In 2014, ATRA/ATO synergistic targeted therapy was recommended by the USA National Comprehensive Cancer Network (NCCN) as the first choice for APL treatment.

ATRA+ATO Combination Therapy in Newly Diagnosed APL: Molecular & Cellular Basis



Hugues de The, Pier Paolo Pandolfi, Zhu Chen

The two agents target respectively the N- and C-terminal of PML-RARα. Functionally ATRA mainly regulates gene expression network related to differentiation while arsenic mainly modulates the key protein pathways involved in apoptosis and self-renewal. Transient restoration of PML NB activates P53.
Combination therapy induces a quicker degradation of the fusion protein and may more effectively eliminate leukemia-initiating cells (LIC) in APL

Optimized Treatment of APL: Risk Stratification

M.D. Anderson Cancer Center

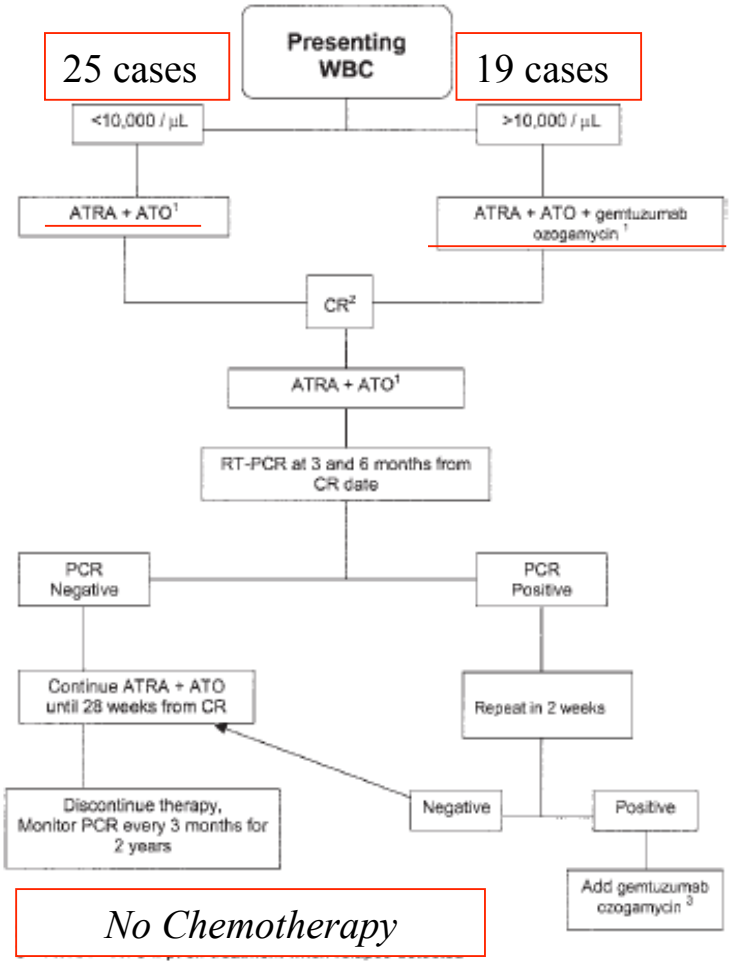
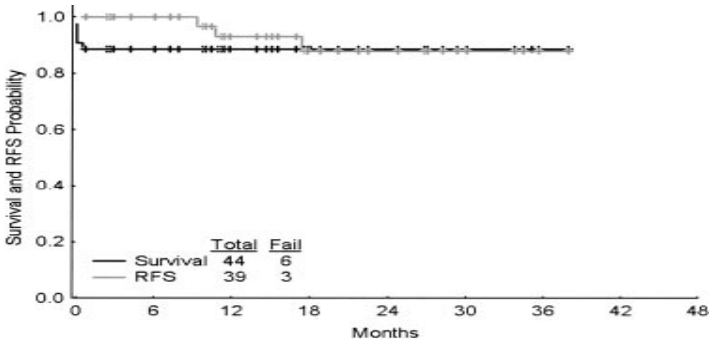


Table 1. Results of induction therapy

Patients	No. patients	CR, no. (%, 95% CI)	Median time to CR, d (range)
All	44	39 (89, 75-96)	28 (19-48)
Low risk	25	24 (96, 80-100)	28 (19-48)
High risk	19	15* (79, 54-94)	32 (22-41)



- ATRA plus ATO may serve as an alternative to chemotherapy in low-risk untreated APL.
- Combined with GO, may improve outcome in high-risk patients.

Estey et al. Blood. 2006; 107: 3469-73.

Optimized Treatment of APL with ATRA+ATO Saving Chemotherapy: Low-to-intermediate Risk Cases

Updated data showed better long-term remission in ATRA+ATO group

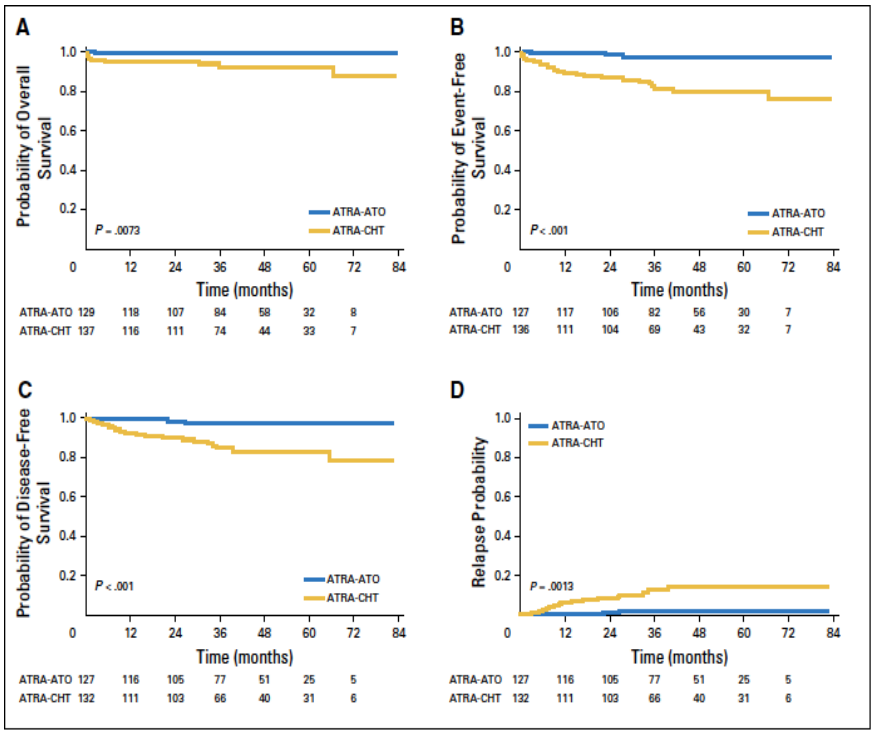
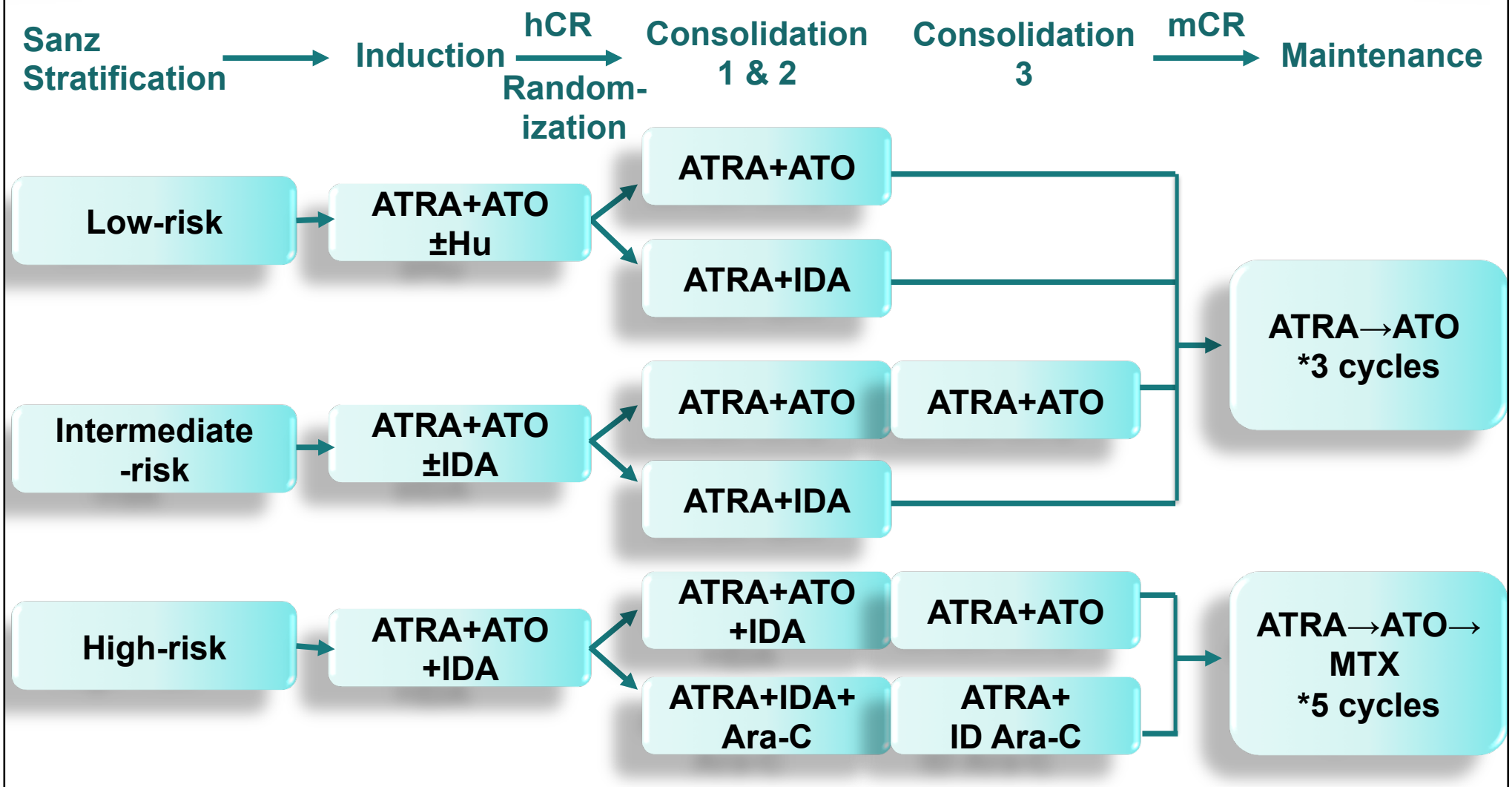


Table 4. Outcome Results

Outcome and Treatment Arm	Probability, % (95% CI)		*P*
	24 Months	50 Months	
Overall survival			.0073
ATRA-ATO	99.2 (97.7 to 100)	99.2 (97.7 to 100)	
ATRA-CHT	94.8 (91.1 to 98.6)	92.6 (87.9 to 97.5)	
Event-free survival			< .001
ATRA-ATO	98.3 (95.9 to 100)	97.3 (94.3 to 100)	
ATRA-CHT	86.8 (81.1 to 92.8)	80.0 (72.9 to 88.0)	
Disease-free survival			< .001
ATRA-ATO	98.3 (95.9 to 100)	97.3 (94.3 to 100)	
ATRA-CHT	89.4 (84.1 to 95.0)	82.6 (75.6 to 90.3)	
Cumulative incidence of relapse			.0013*
ATRA-ATO	0.9 (0 to 2.7)	1.9 (0.0 to 4.5)	
ATRA-CHT	8.2 (3.3 to 13.2)	13.9 (7.1 to 20.6)	

Platzbecker et al. J Clin Oncol. 2016 Jul 11. pii: JCO671982.

Optimized Treatment: Update of APL 2012 Trial Treatment Protocol



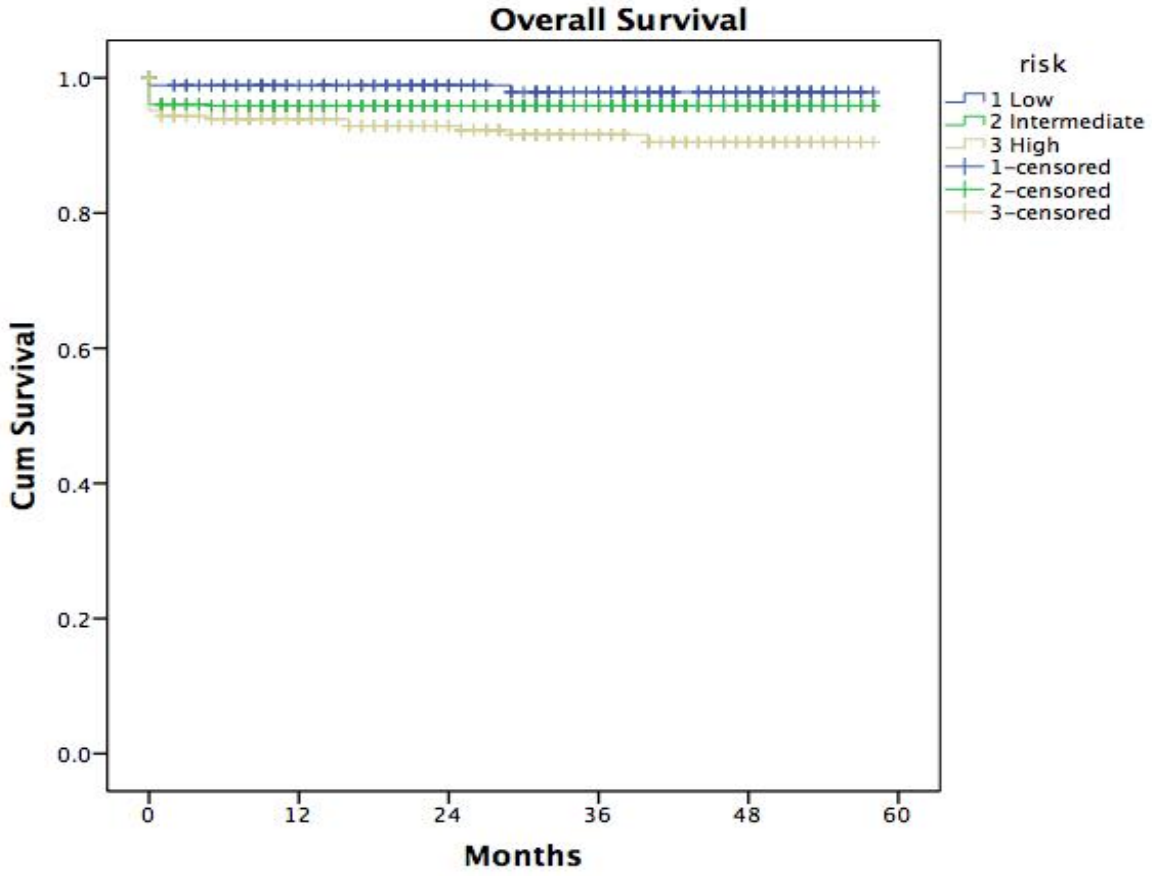
Update of APL 2012 Trial: Remission Induction and Analysis of Early Death Cases

949 cases eligible for analysis

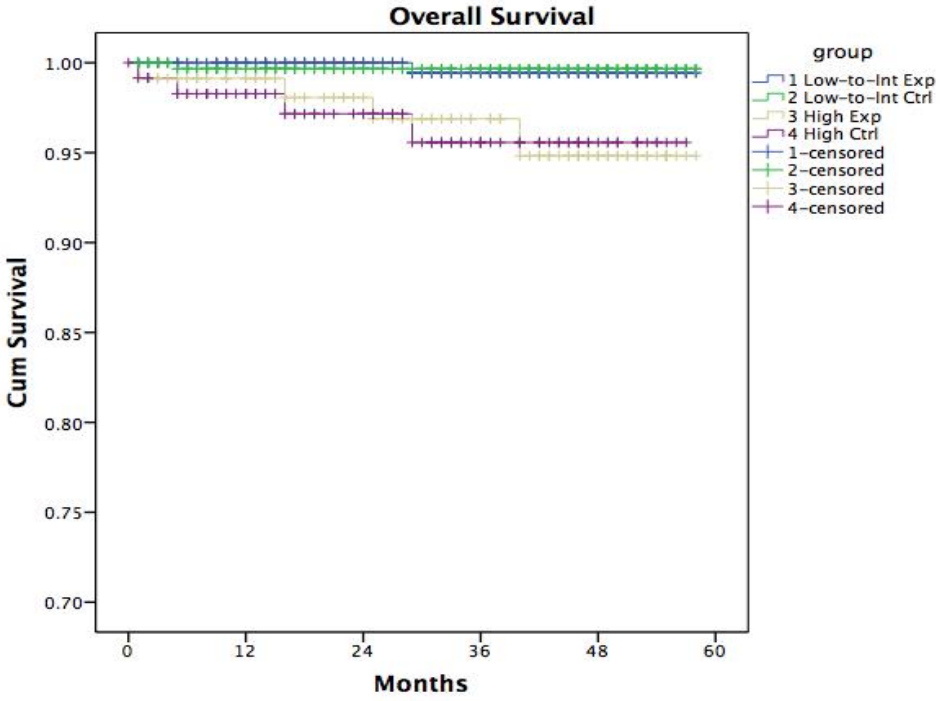
- CR rate： 96.6% (910/942)
- Early death： 3.4% (32/967)
  - Cause of early deaths:
    - Cerebral hemorrhage： 15 cases
    - Infection： 7 cases
    - Cerebral Infarction： 2 cases
    - Differentiation syndrome： 1 case
    - DIC： 1 case
    - MODS： 2 case
    - Pneumorrhagia： 2 case
    - Not clear： 2 case

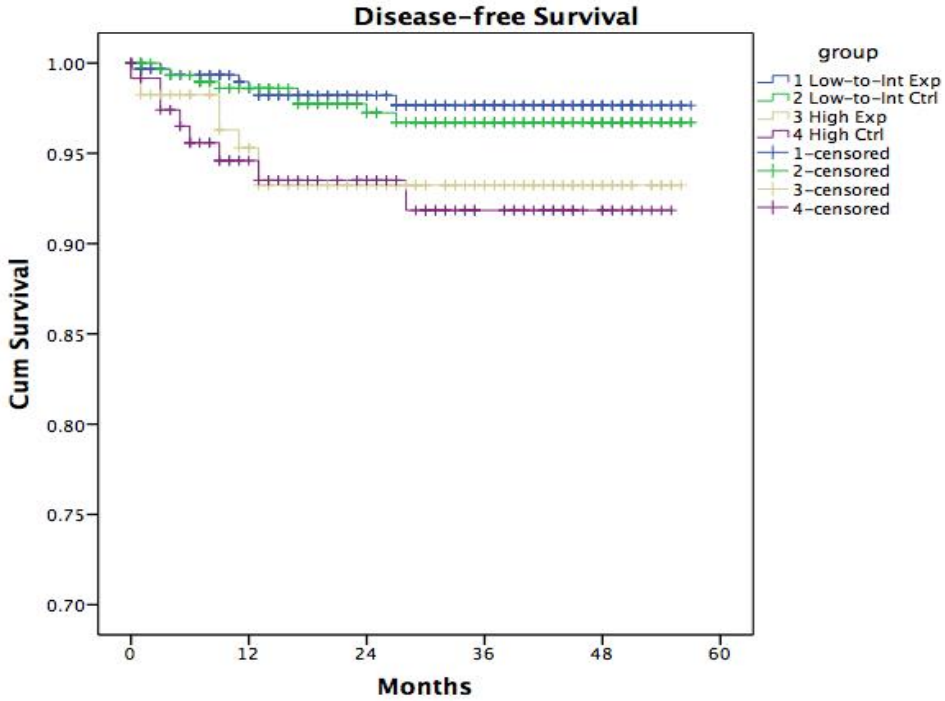
Update of APL 2012 Trial: Overall Survival in Different Risk Groups

- Median follow-up：
  32 months (0-58).
- 4-year OS:
  - Low-risk: 97.9%
  - Intermediate-risk: 95.9%
  - High-risk： 90.5%
  - Low-Intermediate >High (*P*=0.006)



Update of APL 2012 Trial: Post-remission OS/DFS in Different Risk Groups with Distinct Treatment Protocols





4y OS	Low-to-int risk %	High risk %	*P* value
Exp	99.4	94.7	0.011
Ctrl	99.7	95.6	0.007
P value	0.990	0.923	

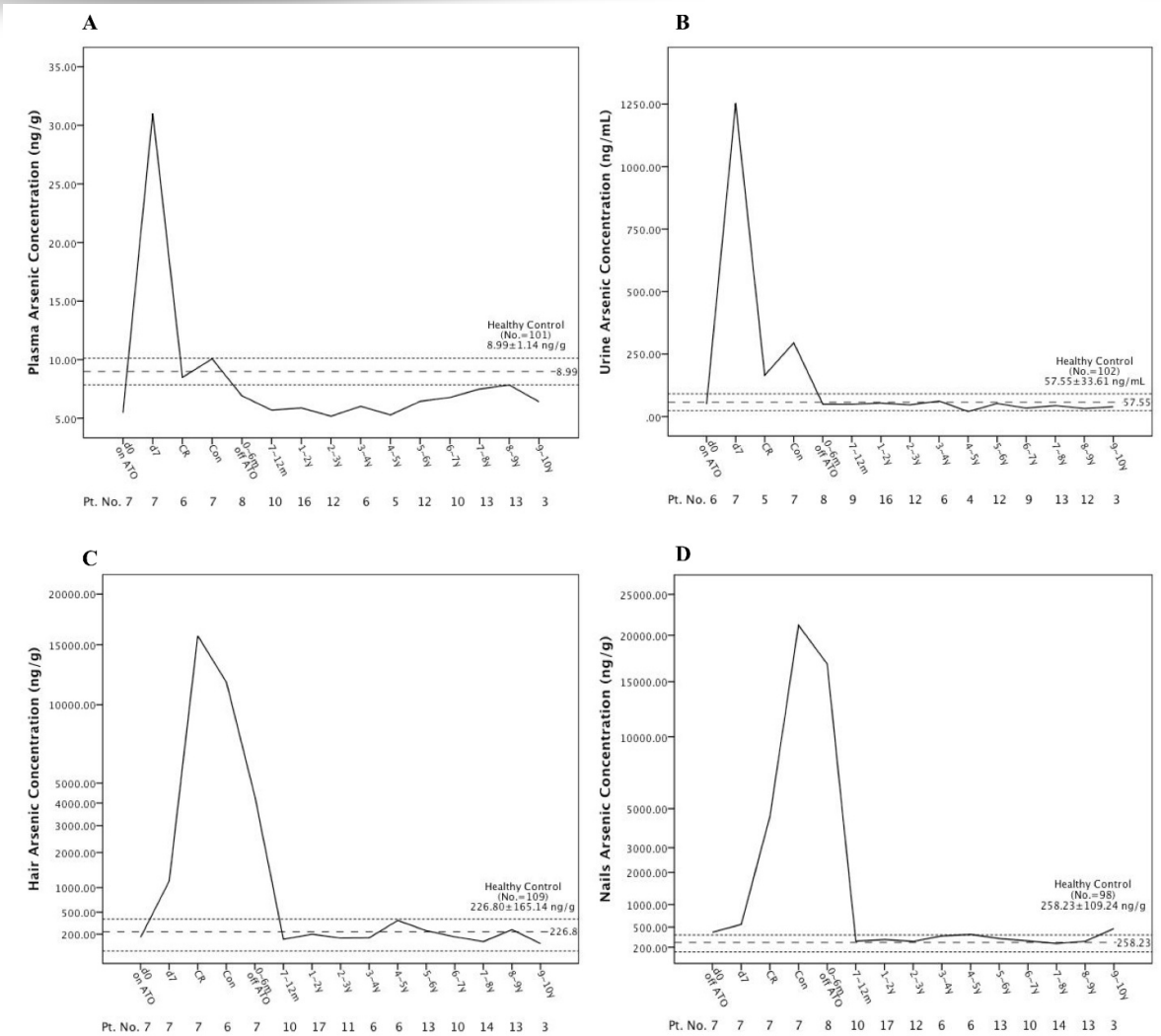
4y DFS	Low-to-int risk %	High risk %	*P* value
Exp	97.6	93.2	0.027
Ctrl	96.7	91.8	0.032
P value	0.591	0.770	

Long Term Safety Evaluation of APL Patients off ATRA+ATO Treatment (n=112) as Compared to Healthy Controls (n=112)

Abnormalities	Patients, n (%)	Healthy controls, n (%)	*P* value
Lower WBC count	1 (0.9)*	0	1.000
Cardiovascular events			
Elevated myocardial enzymes	5 (4.5)†	1 (0.9)	.212
Long Q-T interval	0	0	NA
T wave change	14 (12.5)	15 (13.4)	.842
Echocardiogram abnormality	1 (0.9)‡	0	1.000
Liver dysfunction			
Liver dysfunction, grade 1¶	17 (15.2)	2 (1.8)	<.001
Hepatic steatosis	48 (42.9)	20 (17.9)	<.001
Kidney and GI dysfunction			
Elevated creatinine	0	0	NA
Albuminuria	1 (0.9)‡	0	1.000
Fecal occult blood test	0	0	NA
Diabetes	6 (5.4)	5 (4.5)	.757
Neurological disorders	1 (0.9)§	1 (0.9)‖	1.000
Potential secondary tumor			
Elevated serum tumor markers	3**	NA	NA
Thoracic neoplasm on CXR	0	0	NA
Abdominal neoplasm on BUS	0	0	NA
Skin lesion	8 (7.1)††	5 (4.5)‡‡	.391
Breast cancer	1 (0.9)	0	1.000

Zhu et al. Blood. 2016 Jul 11. pii: blood-2016-02-699439.

Long Term Safety Evaluation of APL Patients off ATRA+ATO Treatment: Residual Arsenic Levels



	Patients	Controls	*P* value
Arsenic retention, mean ± SD			
Plasma, ng/g	6.43 ± 1.56	8.99 ± 1.14	<.001
Urine, ng/mL	45.28 ± 33.28	57.55 ± 33.61	.004
Hair, ng/g¶¶	195.43 ± 202.25	198.23 ± 133.16	.340
Nails, ng/g¶¶	294.65 ± 158.94	338.80 ± 212.27	.761

Arsenic concentration in plasma (A), urine (B), hair (C) and nails (D) of patients off ATO for more than 6 months showed no significant retention.

Zhu et al. Blood. 2016 Jul 11. pii: blood-2016-02-699439.

Challenges Still Existing in the Treatment for APL

How to further reduce the early death rates?

How to deal with cases with high risk of relapse?

How to make the effective ATRA/arsenic therapies available worldwide?

Reduce Early Death Rate in the Treatment of APL

Principle: To treat every APL case as an emergency case

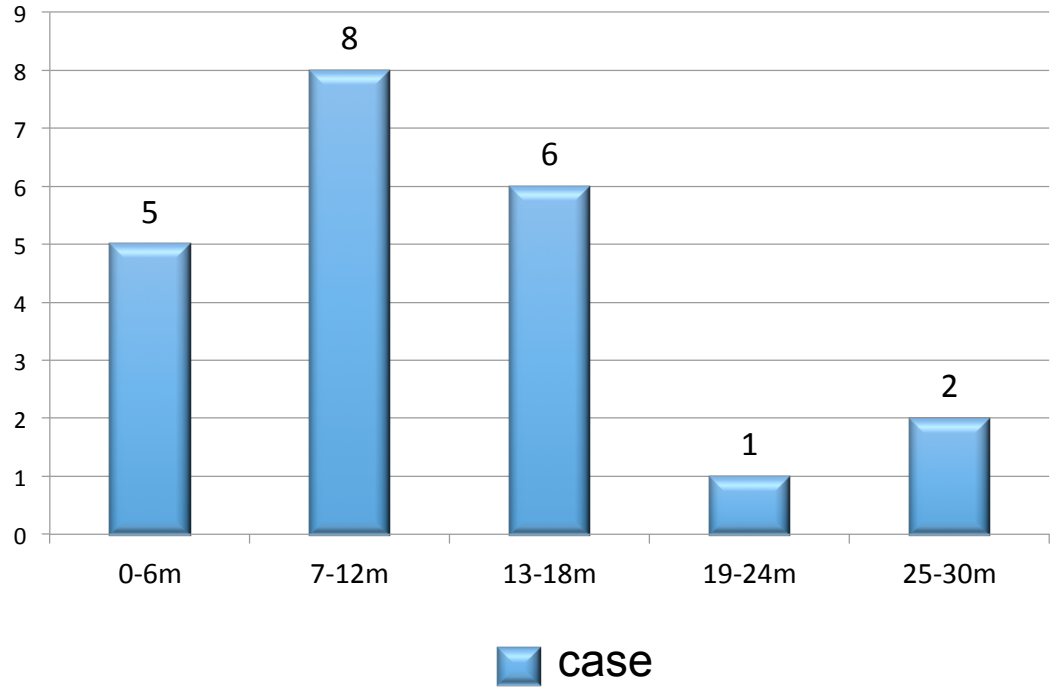
Practice:

- To make a diagnosis and to use ATRA+ATO as early as possible
- To correct bleeding tendency as much as possible in high risk patients
- To control infection more efficiently
- To prevent APL cell differentiation syndrome

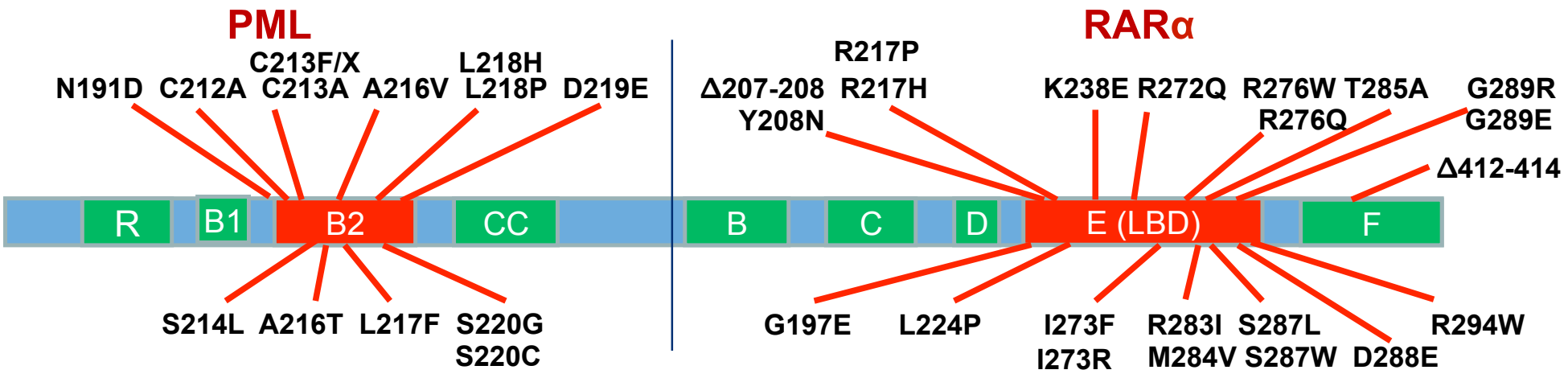
Update of APL 2012 Trial
Relapse/refractory Cases

- **A total of 25 patients were relapsed/refractory (including patients with persistent positive PML/RARa after consolidation therapy)**

- **Low-risk: 2.2%**
  (4/182)
- **Intermediate-risk：2.0%**
  (9/460, including 1 case not reaching mCR after consolidation)
- **High-risk: 4.8%**
  (12/248，including 2 cases not reaching mCR after consolidation)
- ***P* value: 0.074**



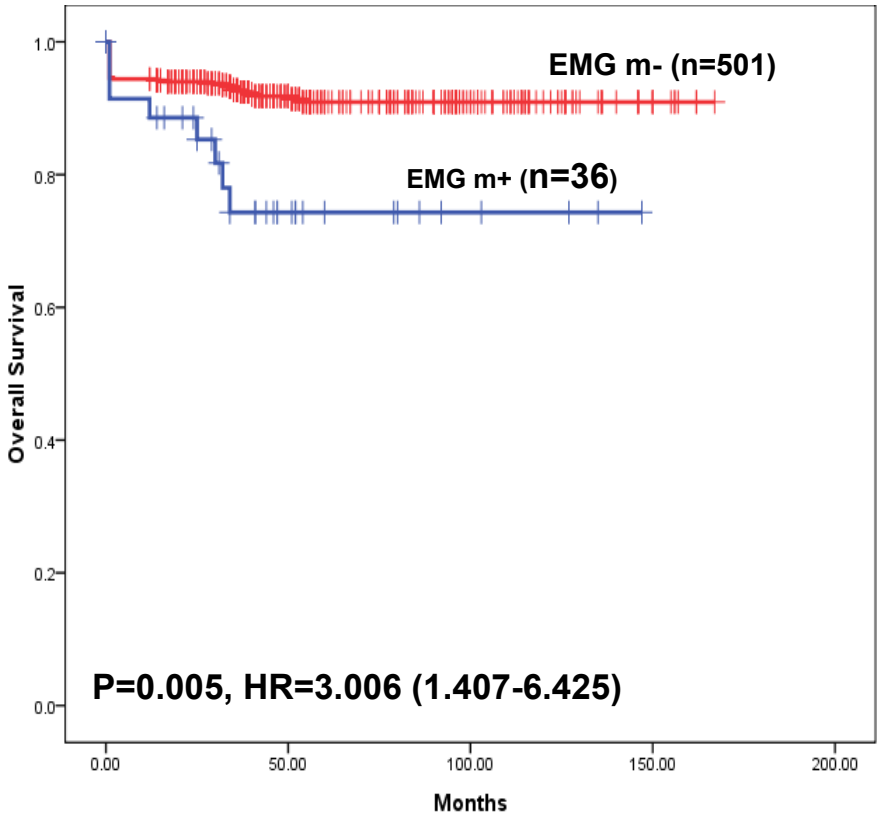
PML-RARα Mutations as Basis of Relapse

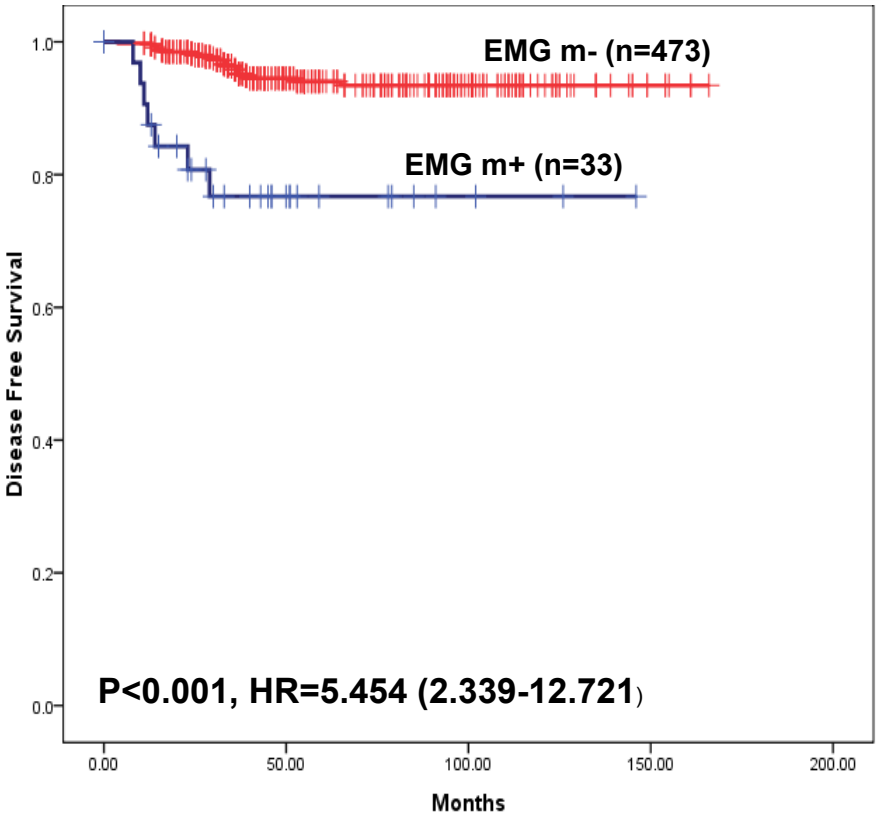


Hematopoietic stem cell transplantation is indicated

Goto, et al. Blood, 2011
Huang et al. NEJM 2014
Sheng et al. Ebiomedicine 2015
Madan et al. Leukemia 2016

Is It Possible to Predict Risk of Relapse? Trial of Using Molecular Markers

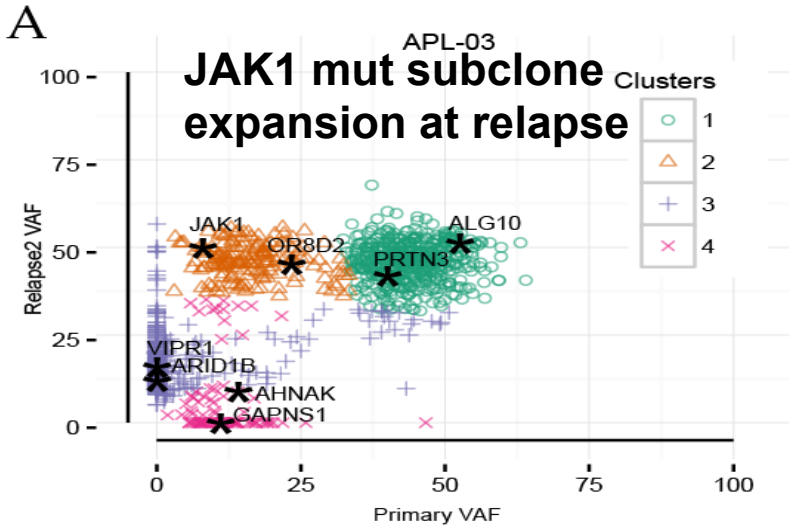


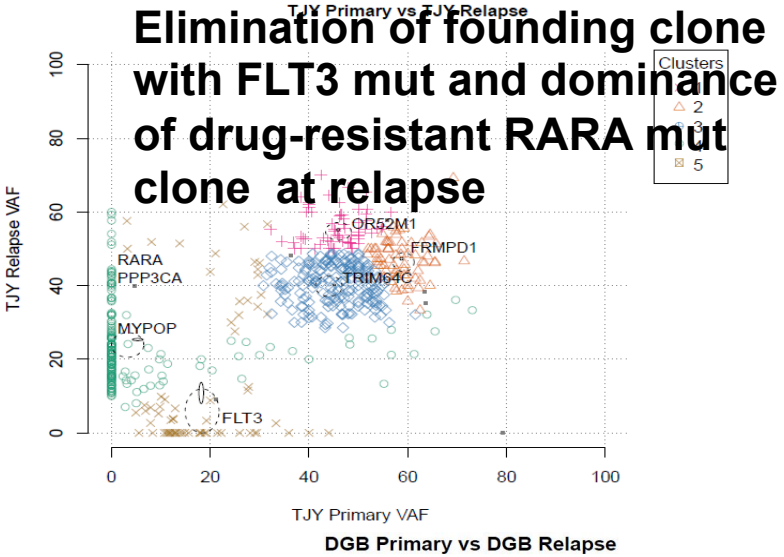


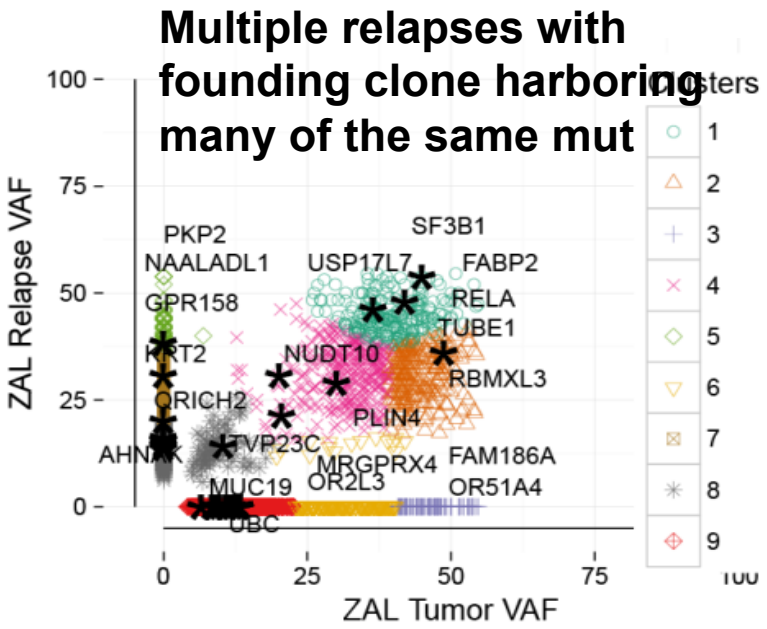
Shen, et al, EBioMedicine. 2015; 2: 563–571.

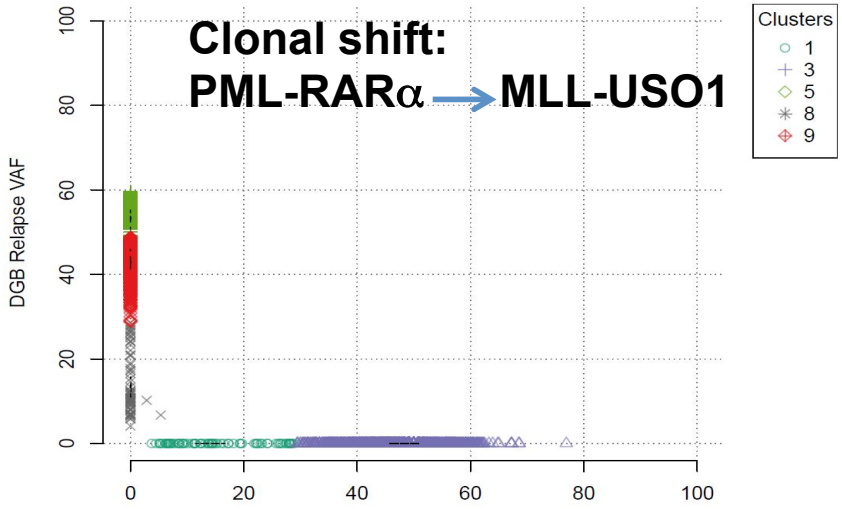
EMG: Epigenetic Modifier Genes (DNMT3A, TET2, ASXL1, IDH1/2)
More studies are required to drew conclusions

Genomic Analysis of Clonal Evolution Patterns during APL Relapse

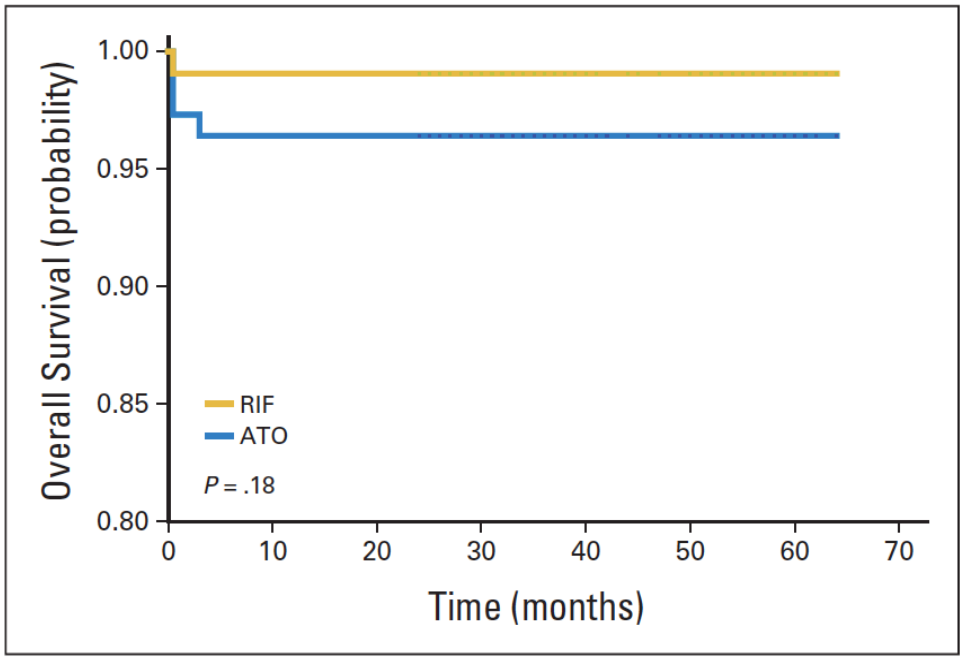


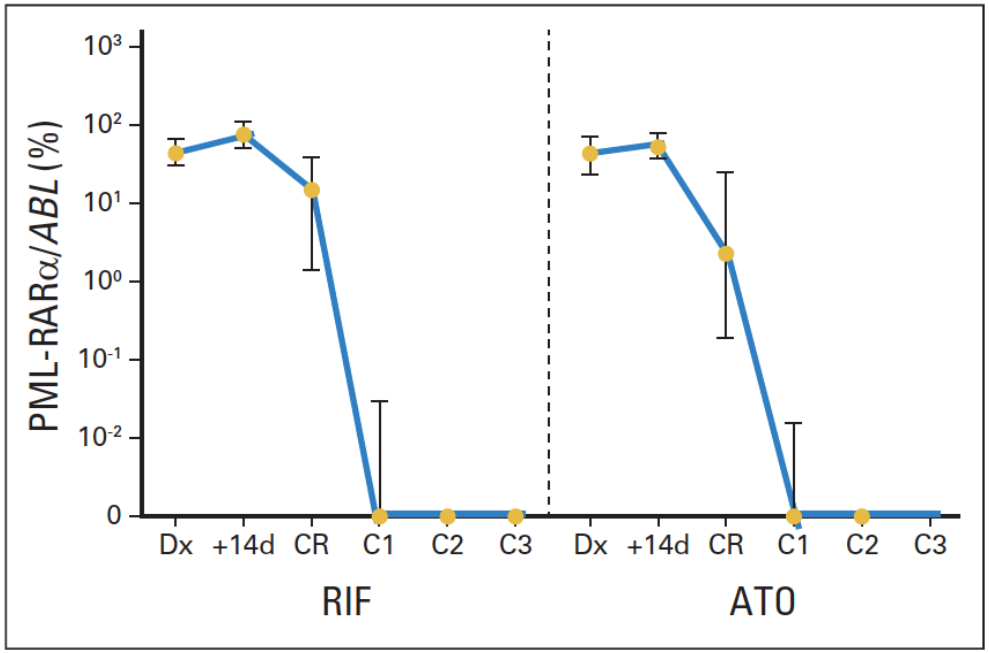






Cost-effectiveness Study: Oral Tetra-Arsenic Tetra-Sulfide (Realgar-Indigo Naturalis Formula, RIF)





Median follow-up time: 39 months.
3-year OS:
99.1% (RIF)
96.6% (ATO)
P=0.18

At the end of consolidation therapy, the PML-RARα transcript was undetectable in any patient in both RIF group and ATO group.

Zhu et al. J Clin Oncol. 2013; 31:4215-4221.

APL as a Model of Translational and Precision Medicine: "East meets West" to Promote Global Health

- **Significance of driver mutations in hematologic malignancies:**

  Development of disease stratification biomarkers and drug targets

- **Synergistic targeting of driver oncoprotein:**

  A strategy to more effectively target the leukemogenic molecules and the leukemia-initiating cells and to avoid the development of drug resistance by cancer cells

- **Convergence of TCM and western biomedical sciences:**

  Genomics and systems biology pave the way for East to meet West so that mankind can benefit more disease relief

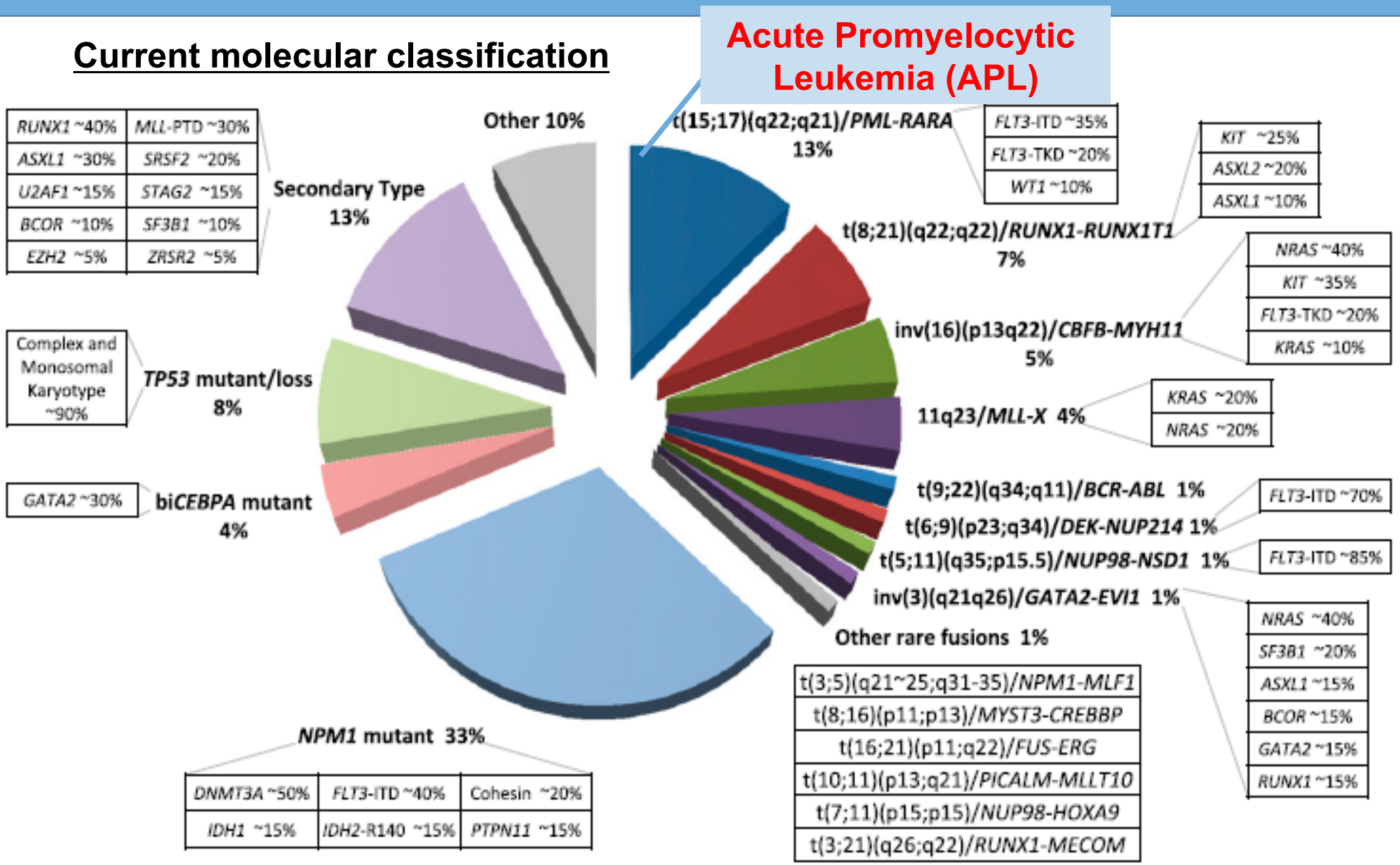
- **New dimensions of precision medicine:**

  Establishment of cost-effective system of universal coverage

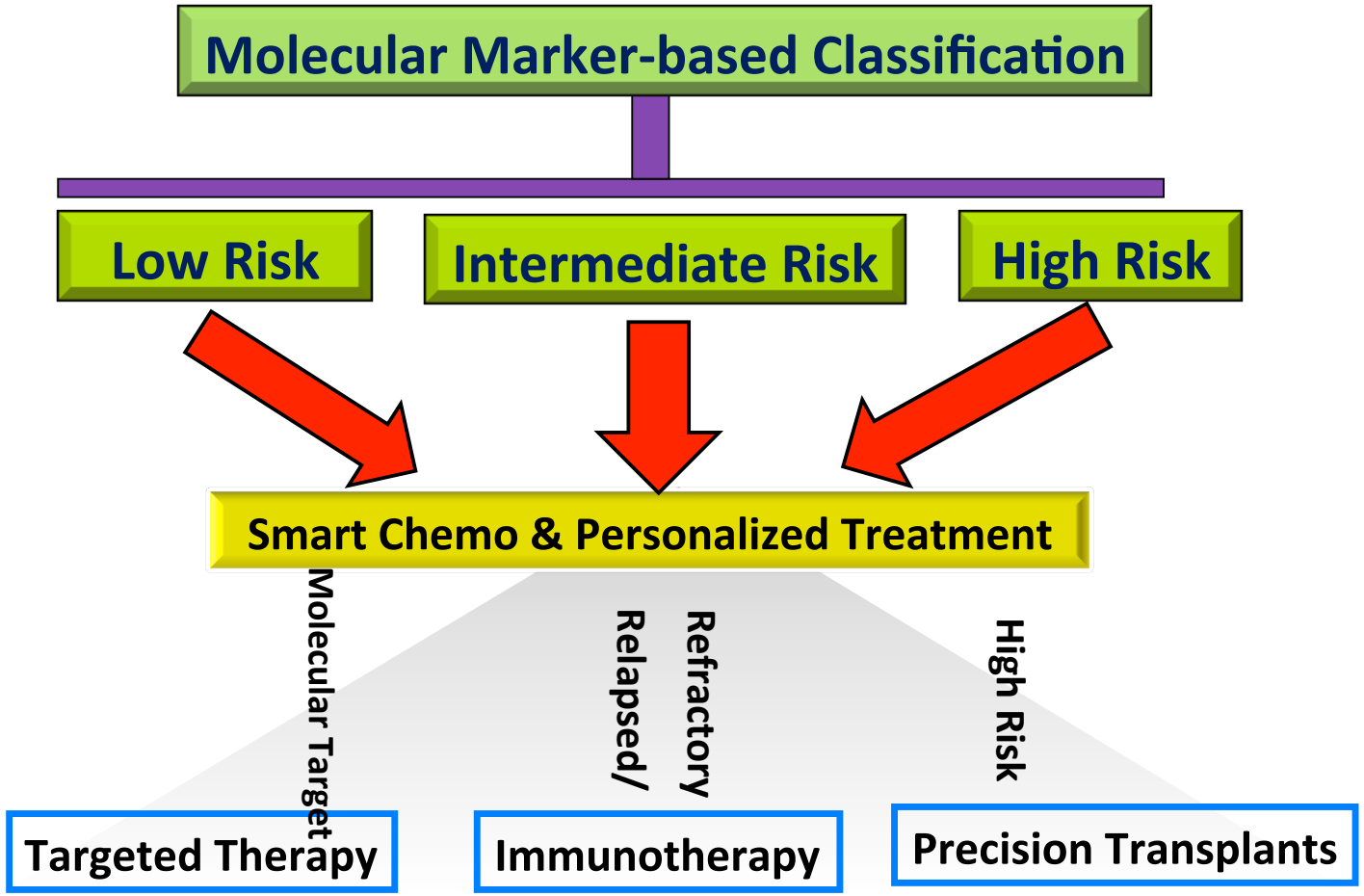
  International cooperation to promote global health

Challenges of Precision Therapy for Acute Myeloid Leukemia (AML)

Current molecular classification



Towards Cure of All Acute Myeloid Leukemia Subtypes: Molecular Marker-based Classification, Prognosis and Precision Therapy



Marco Polo, 1254-1324, was an Italian explorer. His well-documented travels to China were some of the most influential in world history, and did much to kickstart the European age of exploration.

Marco Polo is probably the most famous Westerner traveled on the Silk Road. He excelled all the other travelers in his determination, his writing, and his influence. His journey through Asia lasted 24 years. He reached further than any of his predecessors, beyond Mongolia to China. He became a confidant of Kublai Khan (1214-1294). He traveled the whole of China and returned to tell the tale, which became the greatest travelogue.


MARCO POLO RIDES THE SILK ROAD TO CHINA
Marco Polo's incredible 5,000-mile journey took him halfway across the world. On the way, he followed the network of tracks that snaked through deserts and across mountains to the court of the great Kublai Khan.
1. Marco, his father, and his uncle set sail from **Venice**.
2. They receive letters for the Khan from Pope Gregory X at **Acre**.
3. They collect holy oil in **Jerusalem**.
4. Leaving **Hormuz**, the Polos join a caravan traveling north.
5. Marco falls ill in the **mountains,** where he notices that fire burns less well.
6. Braving the deadly **Taklimakan Desert**
7. Through the **Great Wall of China**
8. They arrive at Kublai Khan's summer palace at **Shangdu**.
MONGOLIA
ARAL SEA
A caravansary
BLACK SEA
CASPIAN SEA
MEDITERRANEAN SEA
Great Wall of China
Peking (Beijing)
Himalayas
CHINA
ARABIA
INDIA
SOUTH CHINA SEA
A proposal for a Silk Road AML Project: An investigators-initiated international cooperation to improve the outcome of all types of AML
Welcome the 2017 ELN Recommendation for APL!

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