When science meets medicine:

Acute Promyelocytic Leukemia:

From highly fatal to highly curable!

Acute Promyelocytic Leukemia

- First described by Hillestad (Sweden) in 1957
  - Promyelocyte morphology and severe bleeding
  - Most malignant leukemia
- FAB: AML M3
- WHO 2008: AML with recurrent genetic abnormalities
  - APL with t(15;17)(q22;q12);(*PML-RARA*)
- 10% of all AML cases (≈1300/year in US)

Clinical features

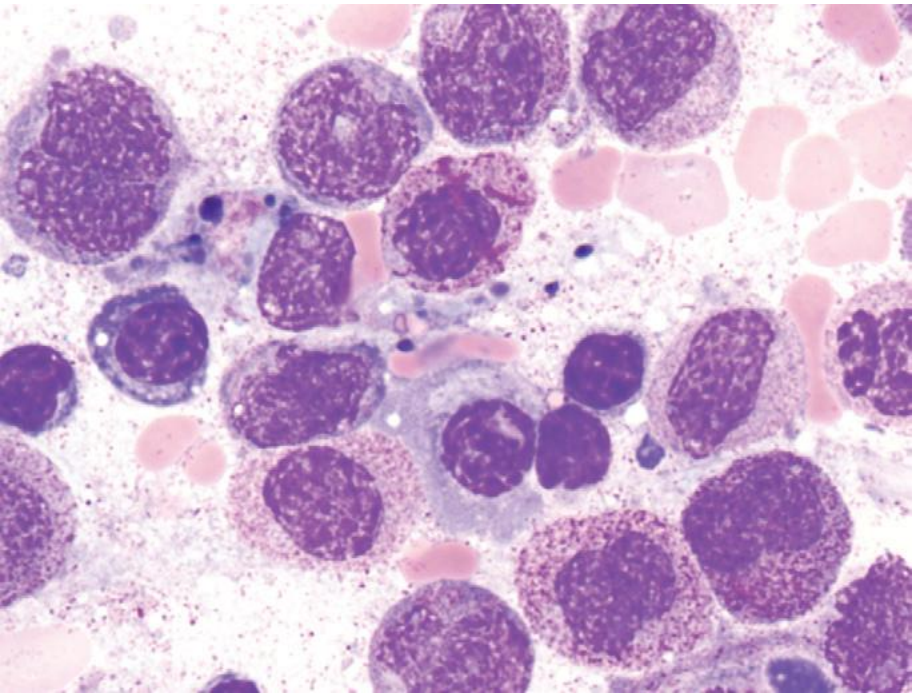
- Young adults
- Presentation:
  - Weakness, fatigue, fever
    - Anemia and neutropenia
  - Bleeding
    - Thrombocytopenia, fibrinolysis (↑ annexin II→ plasmin)
  - DIC
    - esp. microgranular variant
    - ↑ Tissue factor and cancer procoagulant.

High early mortality rate due to intracranial bleeding or DIC.
Prompt diagnosis is crucial.

Diagnosis

- Morphology (peripheral blood smear and/or bone marrow)
- Cytochemistry
- Immunofluorescence staining for PML
- Flow cytometry
- FISH
- RT-PCR for fusion gene product
- Conventional Cytogenetics

Morphology



►Elaine S. Jaffe, et al. Hematopathology. 1st edition. 2011

Immunophenotype

Flow cytometry:

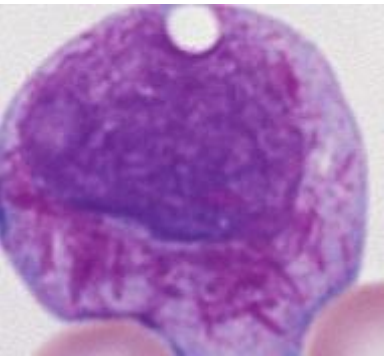
Blast markers :HLA-DR-, CD34-,

Myeloid markers: CD33 +(bright, homo), CD13+ (heter) and MPO+

CD117+, CD64+

Granulocytic markers: CD15- and CD65-

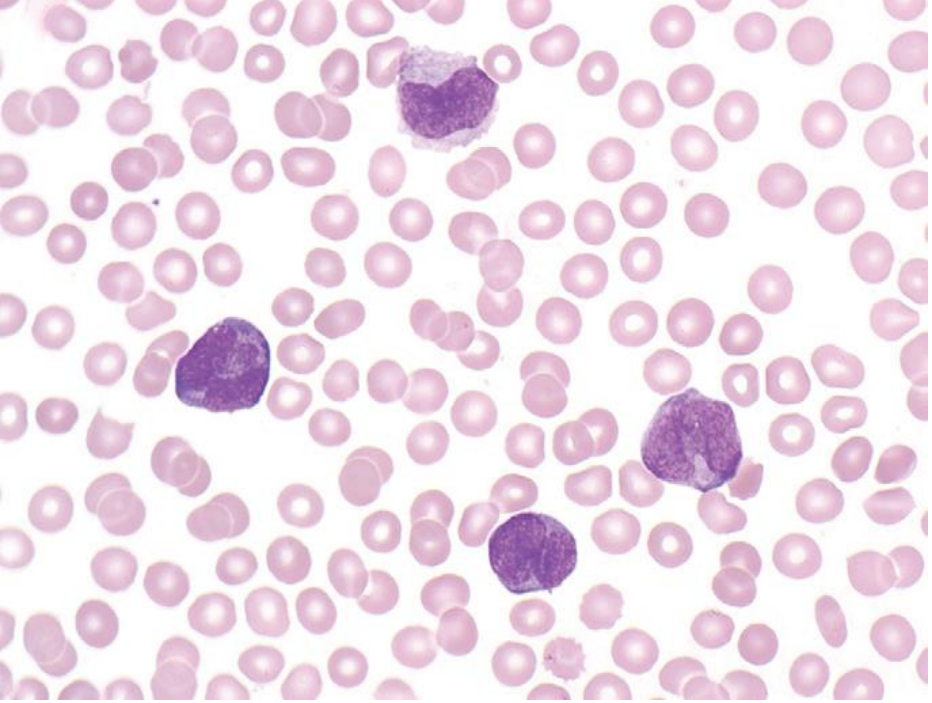
CD56+: unfavorable prognosis

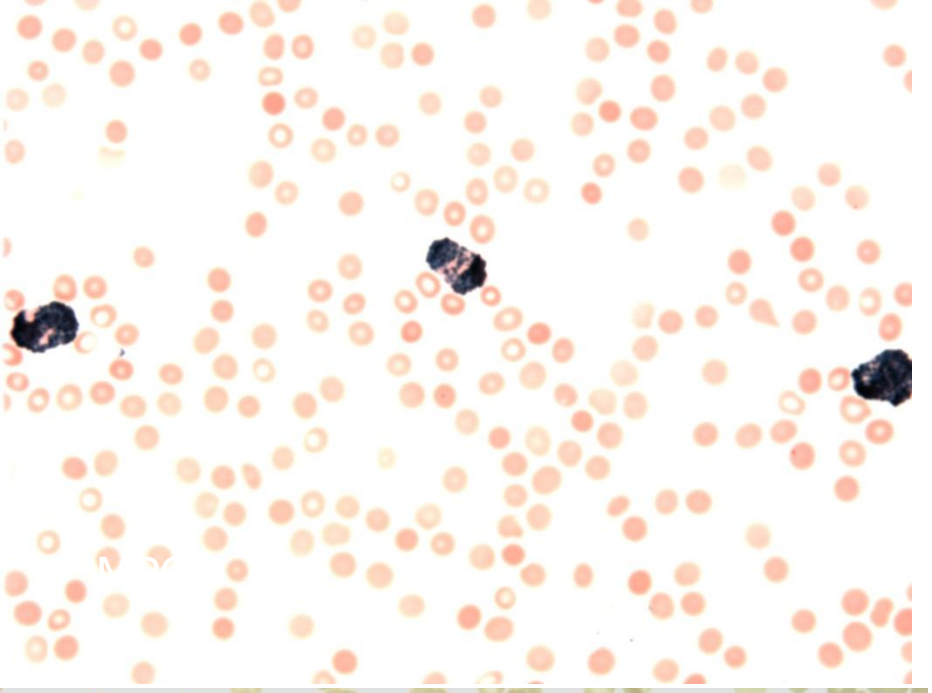


Leukopenia

DD: agranulocytosis with arrested maturation at the promyelocyte stage

Keys: agranulocytosis has normal platelet count and hemoglobin, marrow is not hypercellular, the nuclear features of neoplastic promyelocytes not present, no Auer rods.





Elaine S. Jaffe, et al. Hematopathology. 1st edition. 2011

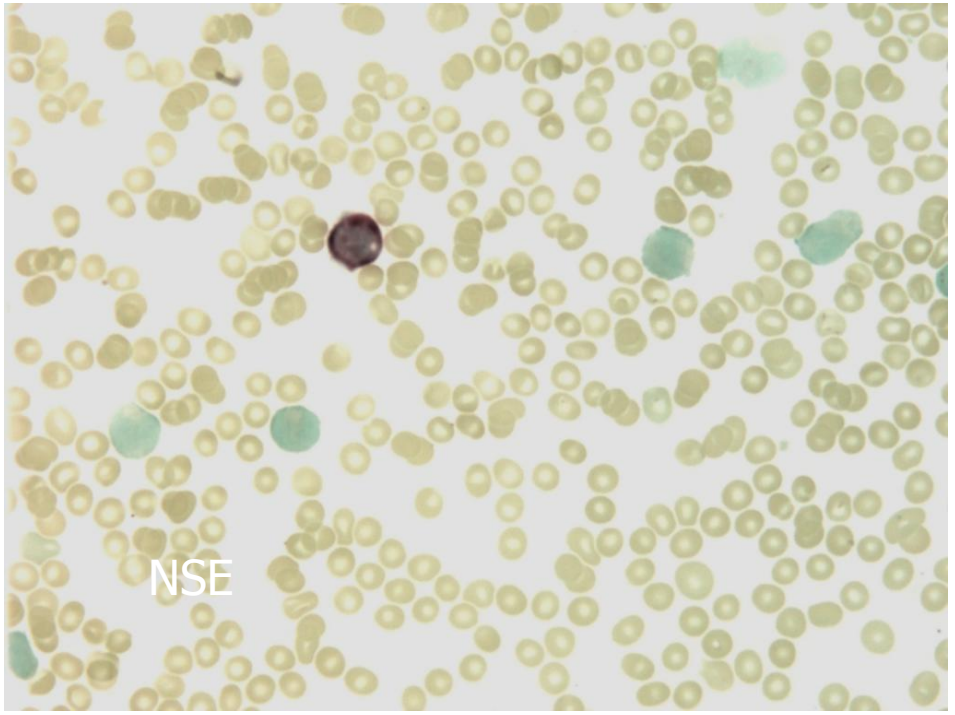
Leukocytosis

DD: Acute monocytic leukemia

Keys:

APL: positive for MPO, Sudan black B, Negative for NSE

AML: negative for MPO, Sudan black B positive for NSE



APL microgranular variant vs Acute monocytic leukemia

- Hematology alalyzer, Perox window

Flow cytometry

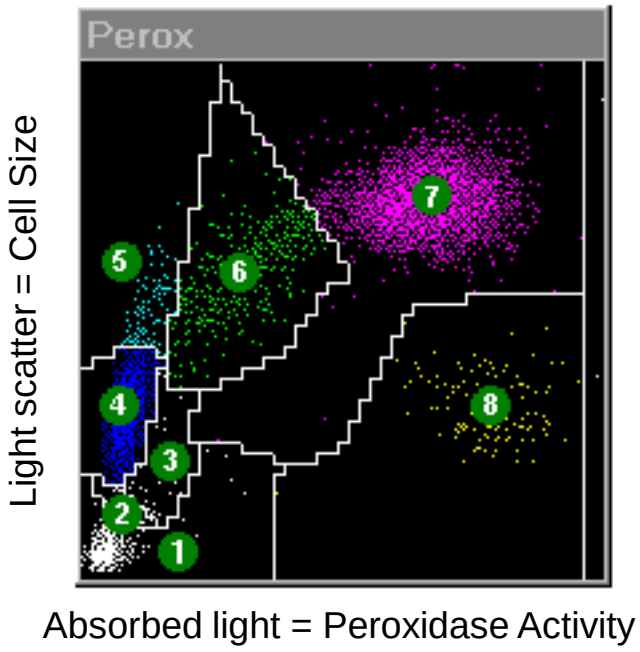
Blast markers :HLA-DR-, CD34+/-,

Myeloid markers: CD33 +(bright, homo), CD13+ (heter) and MPO+

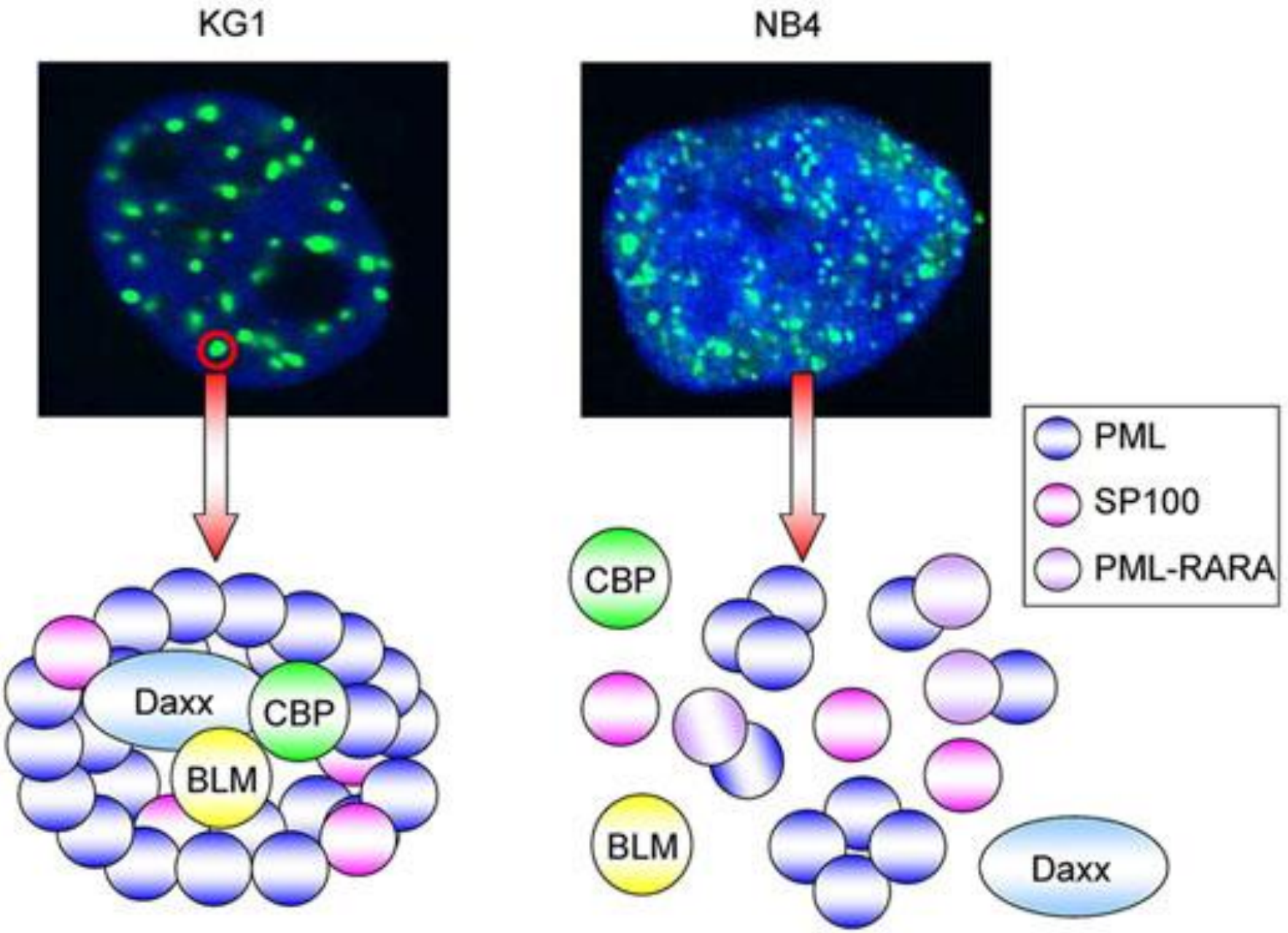
CD117+, CD64+, CD2+

Granulocytic markers: CD15- and CD65-

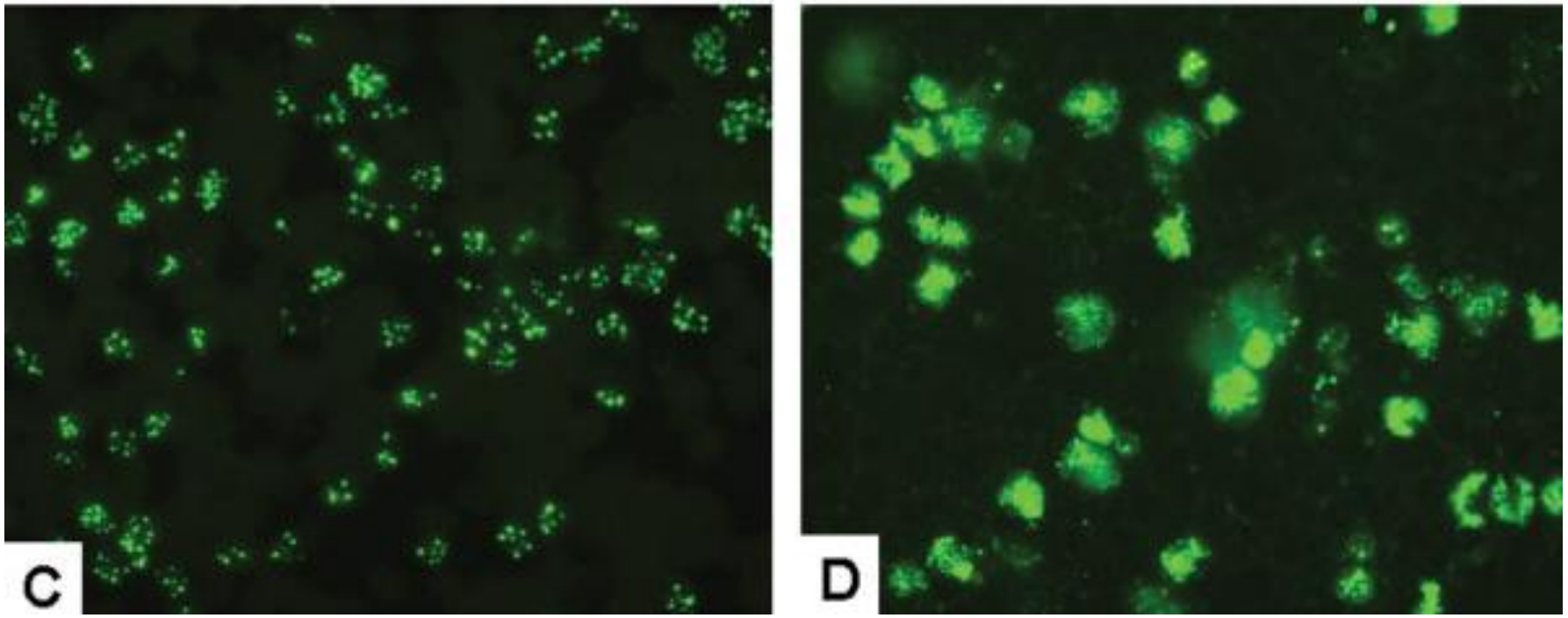
CD56+: unfavorable prognosis



Immunofluorescent staining of promyelocytic leukemia (PML)



Anti-PML staining

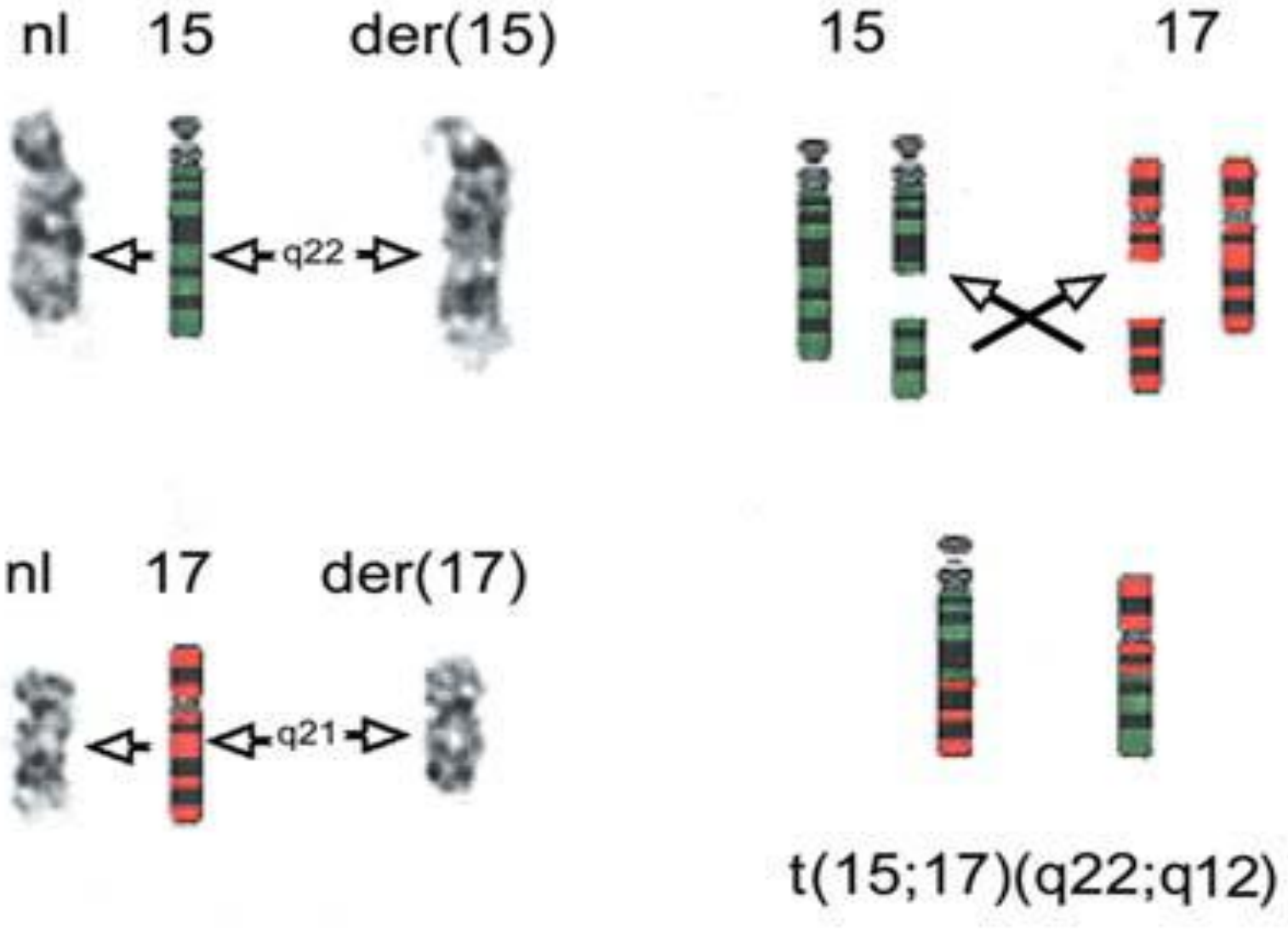


The sensitivity and specificity of the test were 98.9% and 98.7%, respectively. CONCLUSIONS: PML immunofluorescence staining is a rapid (<4 hours turnaround time) and reliable frontline diagnostic approach that can facilitate initiation of targeted therapy, particularly in clinical settings where cytogenetic and molecular testing are not readily available.
Cancer 2010;116:369–76.

Conventional Cytogenetics

- Sensitivity: 90%
- Can detect t(15; 17) (q22; q21) and variants
- Need culture, long TAT

- t(15; 17) (q22; q12); PML-RARA



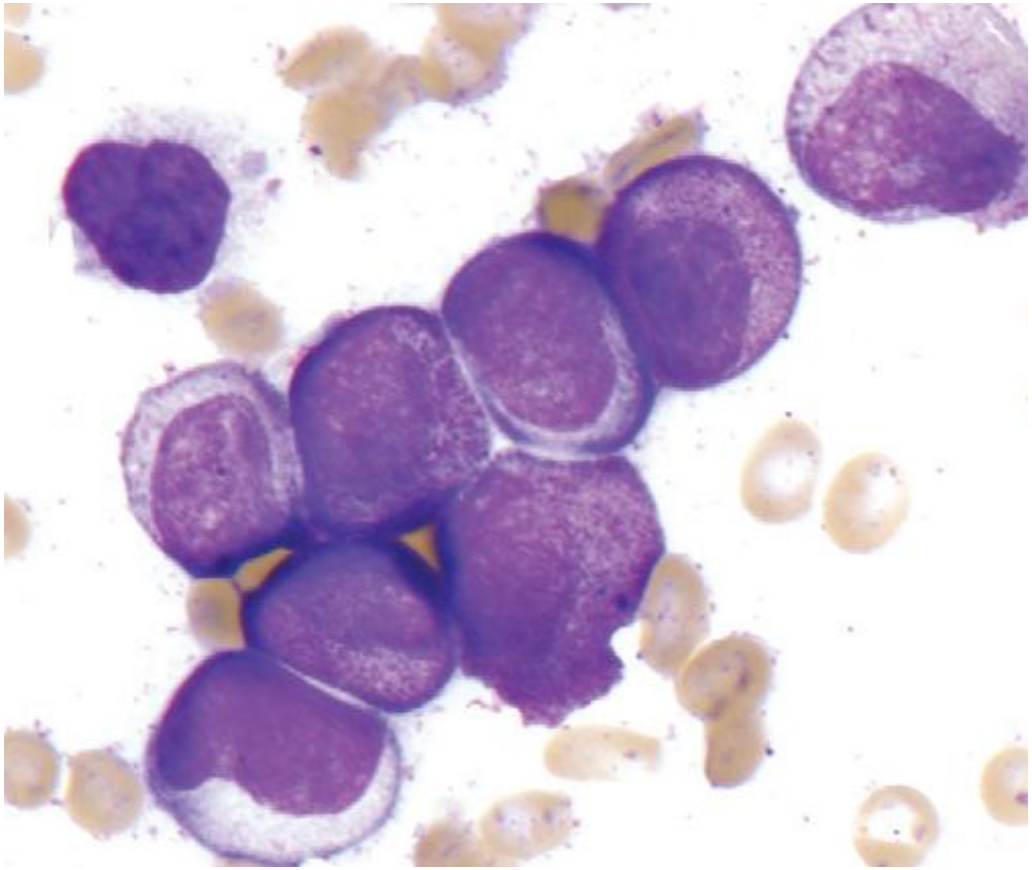
Acute Promyelocytic Leukemia with Variant *RARA* Translocations

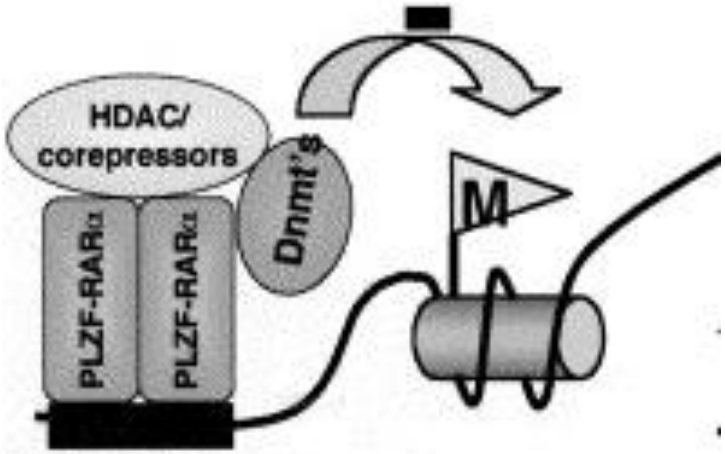
Translocation	Frequency (%)	Fusion Product (X-RARα)	Function "X" Gene	Retinoid Sensitive	Chemotherapy Sensitive
(15;17) (q22,q21)	95	PML-RARα	Transcriptional factor	+	+
(11;17) (q23,q21)	<5	PLZF-RARα	Developmental/differentiation control	-	-
(5;17) (q35,q21)	<1	NPM-RARα	Ribonucleoprotein maturation and transport	+	+
(11;17) (q13,q21)	<1	NuMA-RARα	Structural role in mitosis, apoptosis, and interphase nuclear matrix	±	±
(17;17) (q11,q21)	<1	STAT 5b-RARα	Signal transduction, transcriptional factor	-	?

+, sensitive; -, not sensitive; ±, may be sensitive; NPM, nucleophosmin; NuMA, nuclear mitotic apparatus; PLZF, promyelocytic leukemia zinc finger; PML, promyelocytic leukemia; RARα, retinoic acid receptor-α; STAT 5b, signal transducer and activator of transcription 5b; "X," RARα partner gene.

Acute Promyelocytic Leukemia with Variant *RARA* Translocations

T(11;17)(q23;q12) (*ZBTB16-RARA*; formerly known as *PLZF-RARA*)





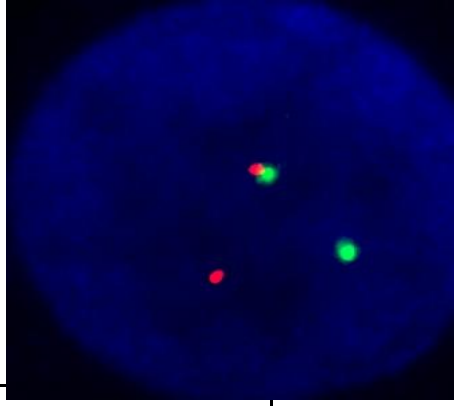
- Elaine S. Jaffe, et al. Hematopathology. 1st edition. 2011

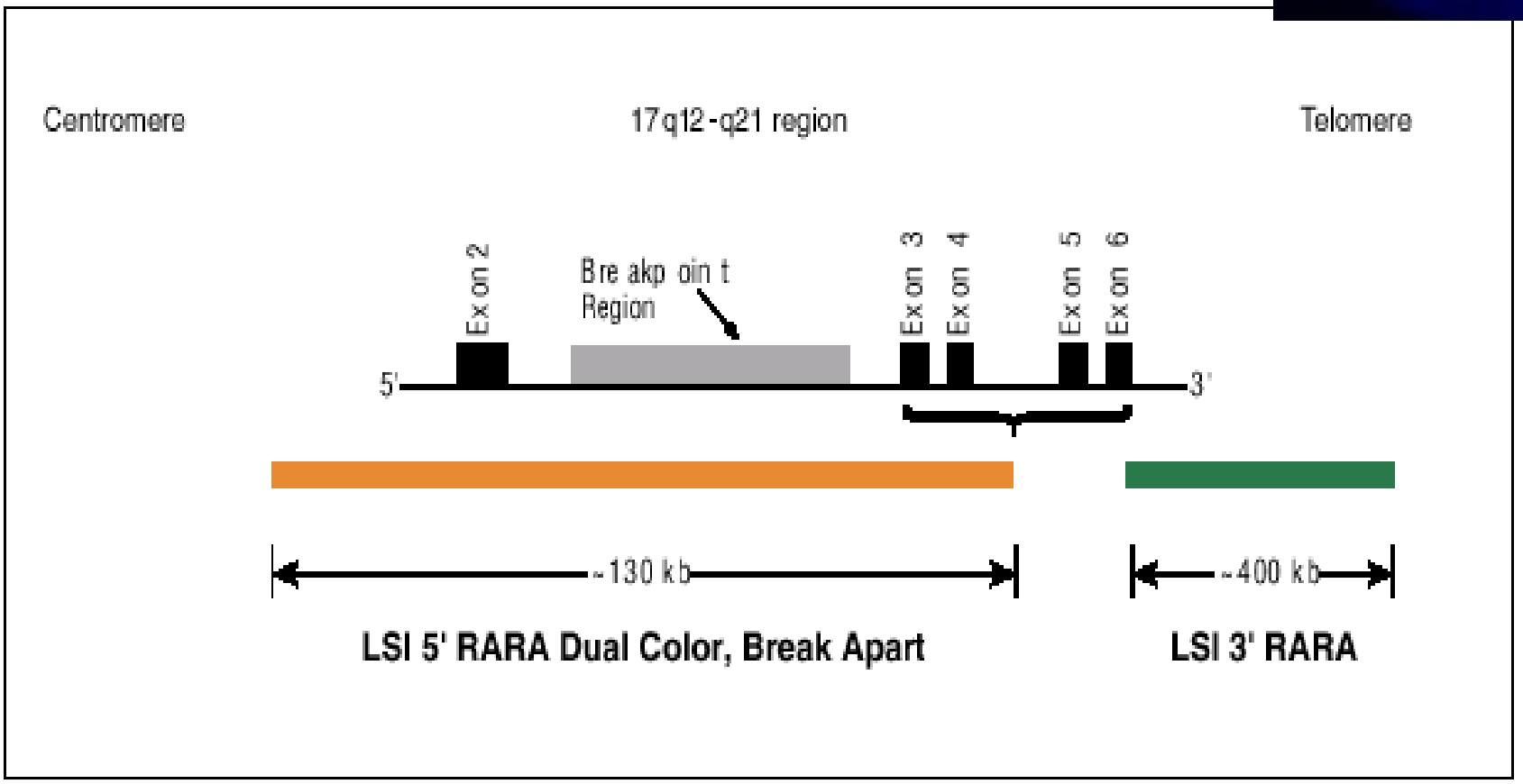
Other cytogenetic abnormalities

- +8
- i(17)(q10)
- Complex abnormality
- No prognostic significance

FISH

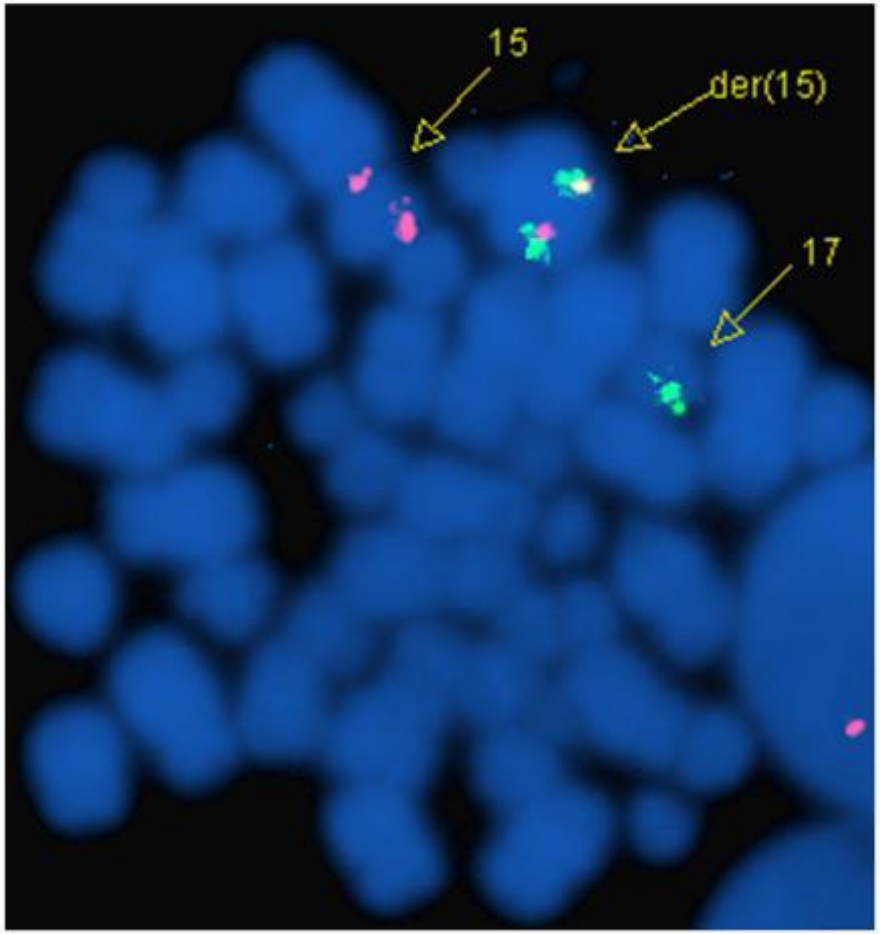
RARA Dual Color Break Apart Probe

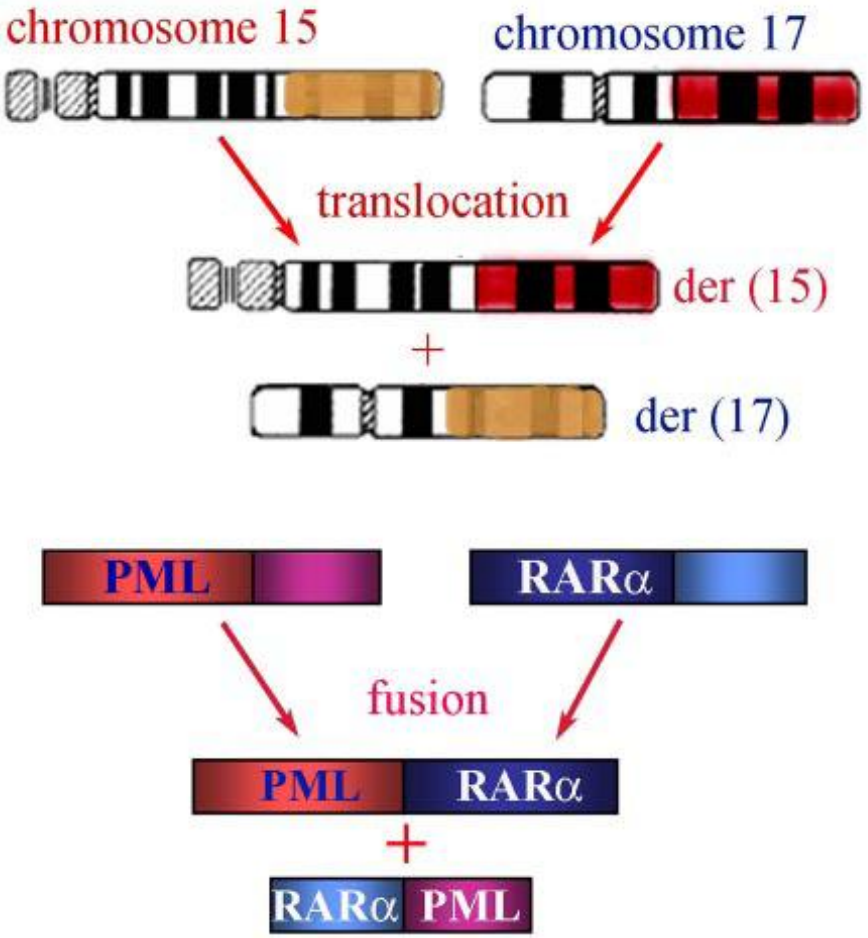




http://www.vysis.com/AnalyticSpecificReagents(ASR)_59424.asp?PrintPage=true&ProdID=32-191011

Dual Color fusion Probe





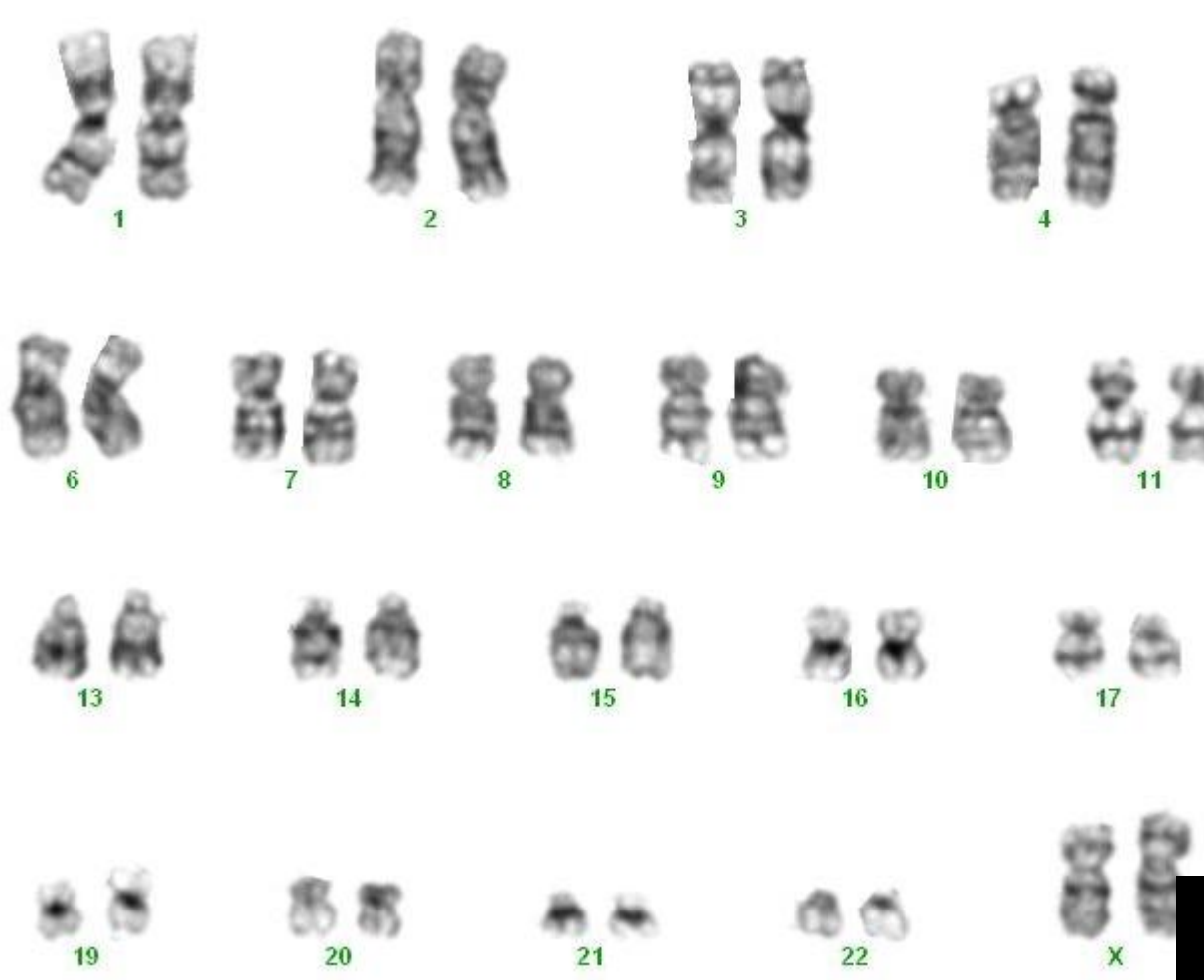
Cryptic Submicroscopic Types

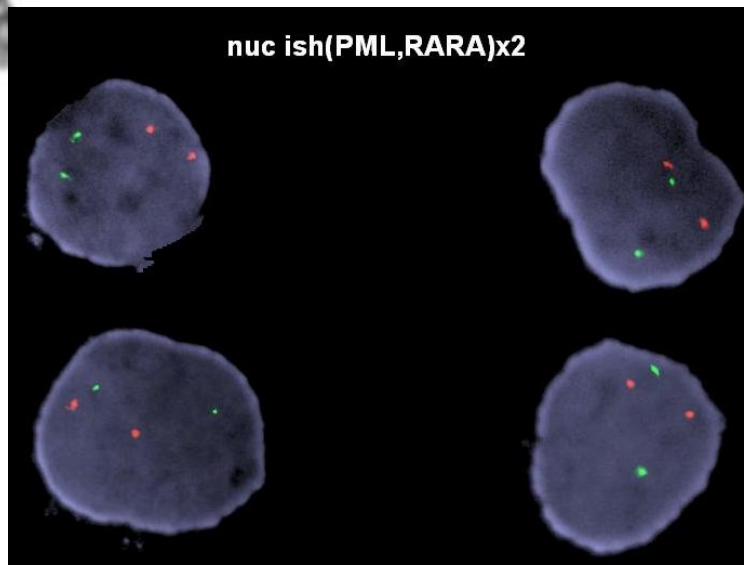
Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Identification of PML/RARα fusion gene transcripts that showed no t(15;17) with conventional karyotyping and fluorescent in situ hybridization

A. Choughule[1,3], S. Polampalli[1], P. Amre[2], S. Shinde[3], S. Banavali[1], K. Prabhash[1], R. Nair[1], P.G. Subramanian[3], S. Gujral[3] and P.M. Parikh[1]

Genetics and Molecular Research 8 (1): 1-7 (2009)

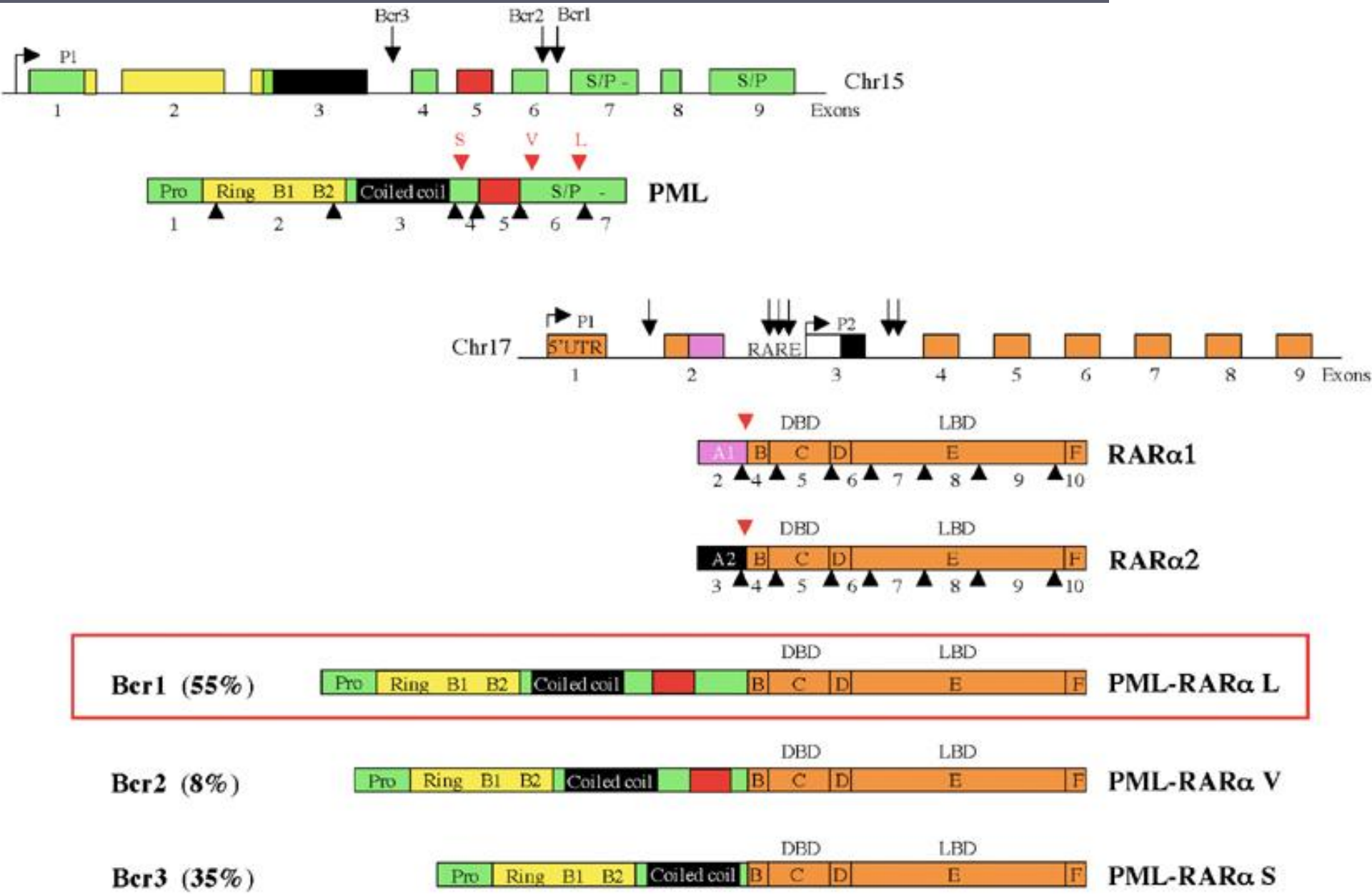

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X


nuc ish(PML,RARA)x2

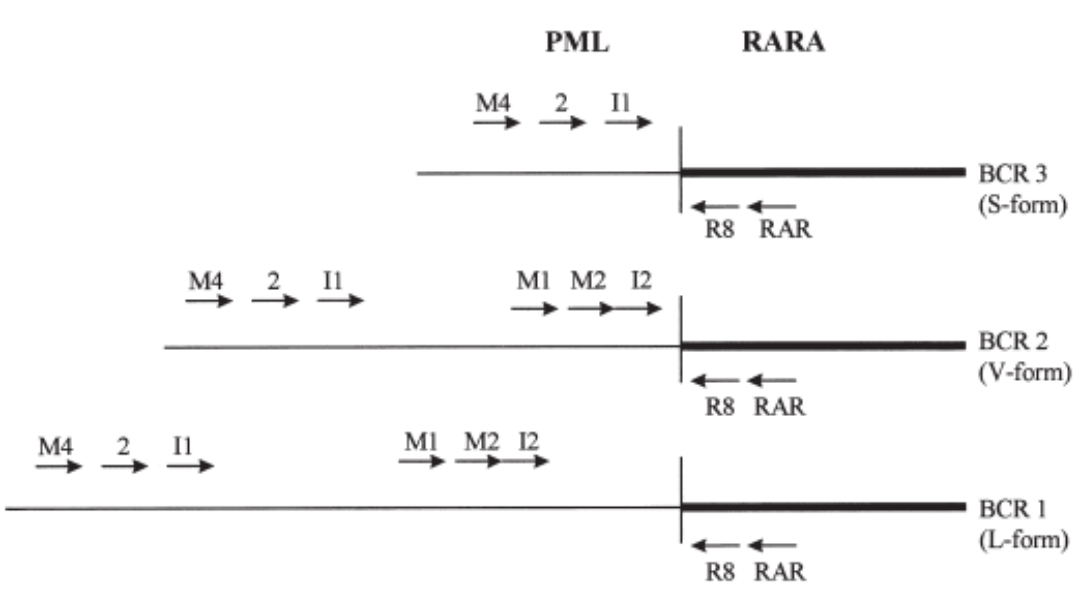
Rt-PCR for PML-RARa transcript

- Relative fast turnaround time
- More sensitive
- Can be quantitated (Real time Pt-PCR)
- Isoform determination
- Predict relapse
  - $>10^{-3}$ s/p 1 cycle consolidation, high relapse rate

BREAK POINT POSITIONS IN PML-RARα



Tussié-Luna M I et al. **Pro-proliferative function of the long isoform of PML-RAR involved in APL.** Oncogene (2006) 25, 3375–3386



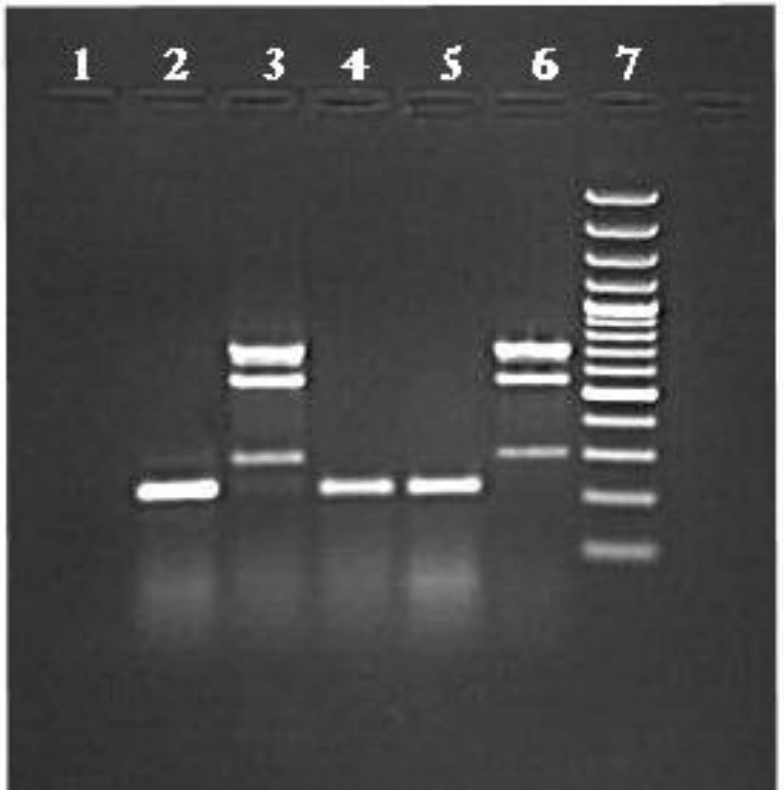
Primers:

M4 [7]: AGCTGCTGGAGGCTGTGGACGCGCGGTACC; M1: AGTCAGTGCC CGGGGCACAC;
RAR: AGGGCTGGGCACTATCTCTTC; 2 [4]: TGTGCTGCAGCGCATCCGCA;
M2[7]: AGTGTACGC CTTCTCCATCAAAG; R8 [7]: CAGAACTGCTGCTCTGGGTCTCAAT;
I1(internal probe): GTGCAG AGGATGAAGTGCTA;
I2 (internal probe): AGGCCCTTCCTATGGAGA

PCR products:

Second round Primers	Bands sizes
2 + R8	S-form: 248 bp V-form: 578bp*, 319 bp L-form: 722, 578, 319 bp
Band 319 bp may not be amplified in some samples with V or L-form	
M2 + R8	V-form: 180 bp* L-form: 324 bp
* This size may vary in different patients with the V-form	

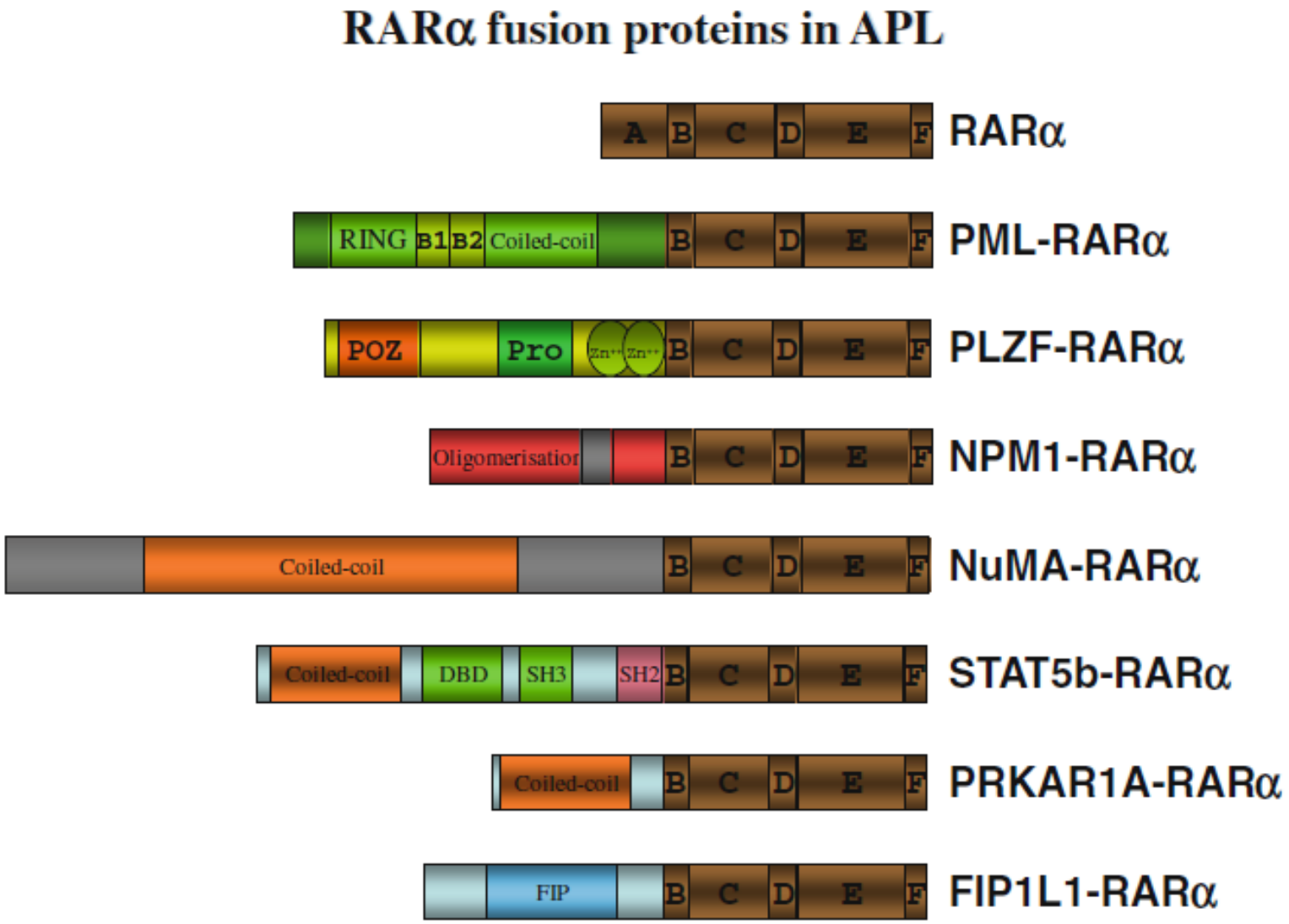
Leukemia (1998) 12, 1349–1354

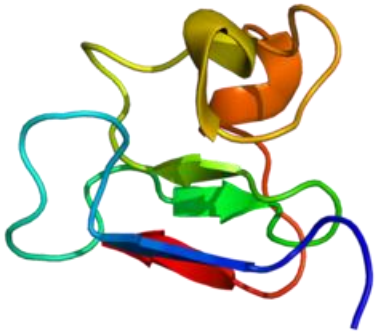


Genetics and Molecular Research 8 (1): 1-7 (2009)

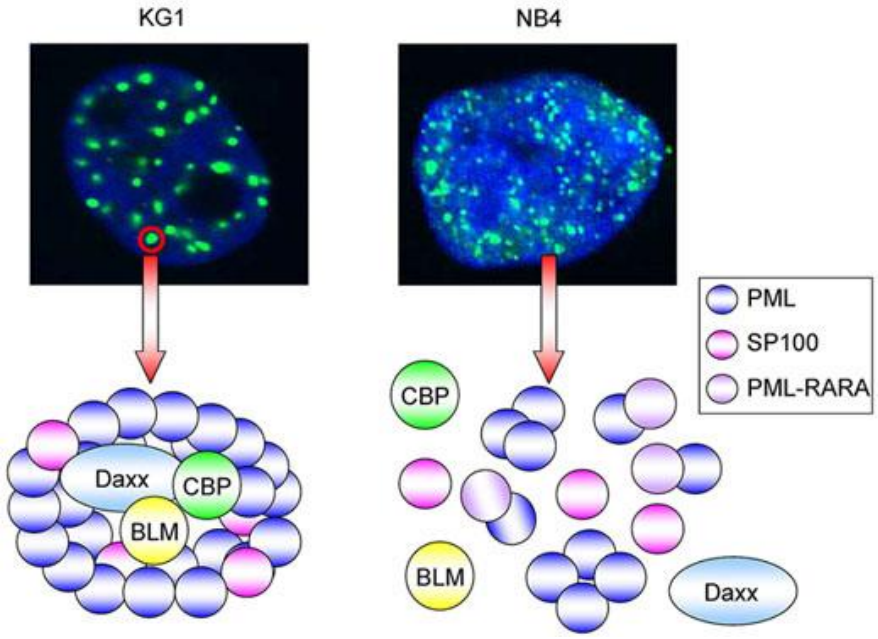
Short form associated with M3v, leukocytosis and poor prognosis

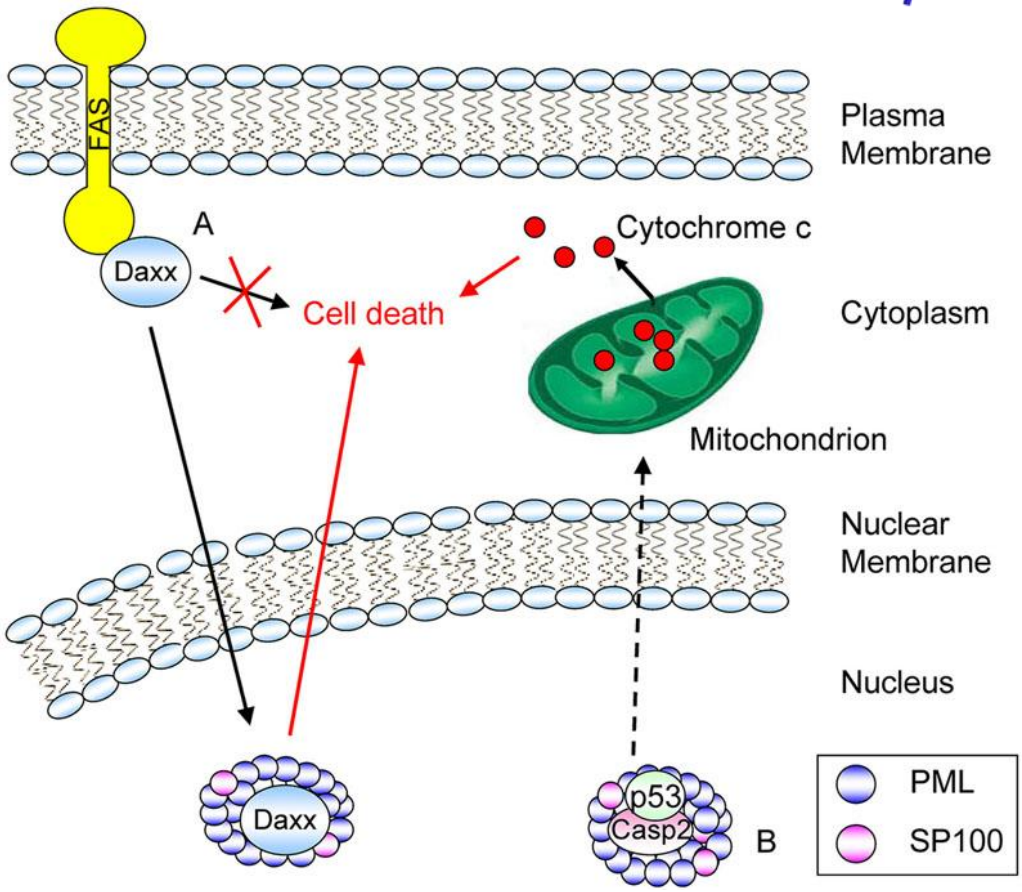
Molecular leukemogenesis





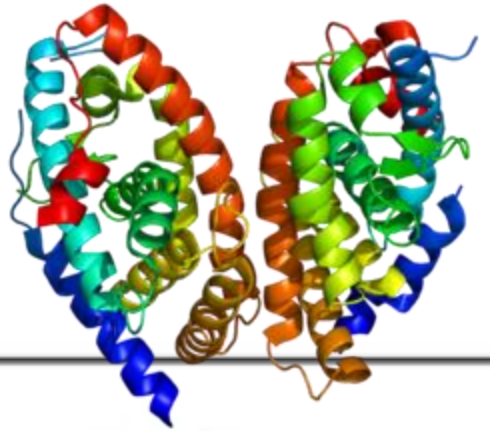
Promyelocytic Leukemia Protein (PML)



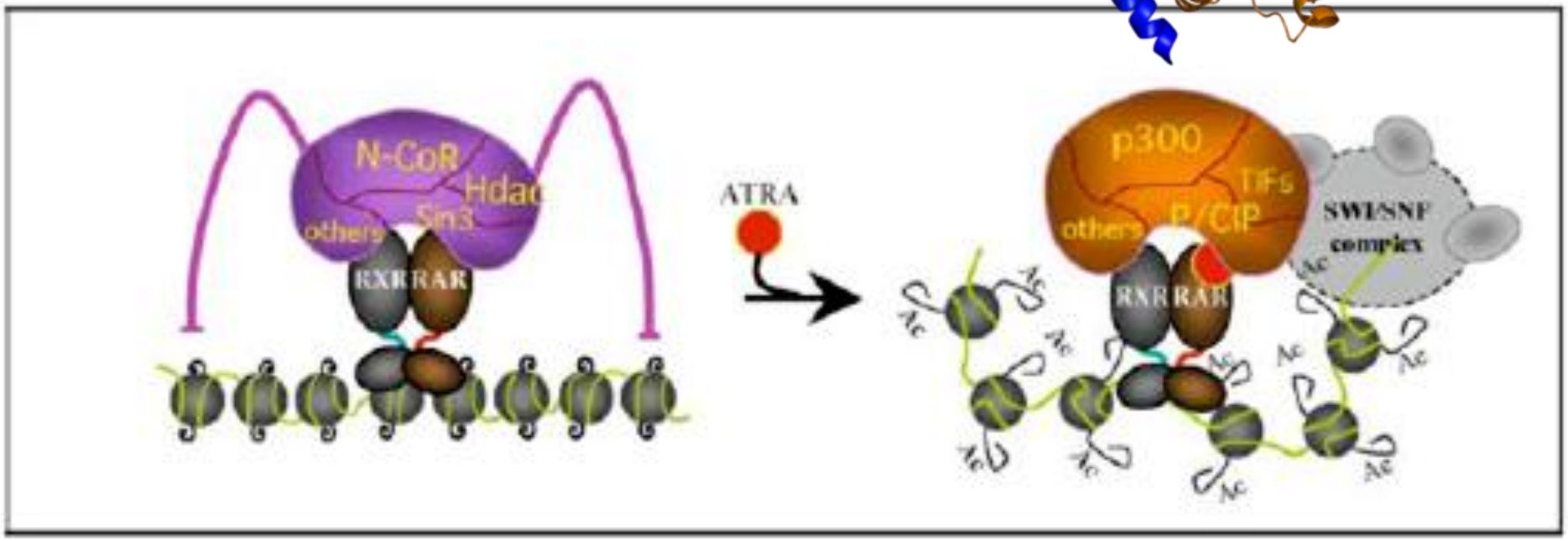


Nicola J.M. Brown, Michal Ramalho, Eva W. Pedersen, Eva Moravcsik, Ellen Solomon, David Grimwade. PML nuclear bodies in the pathogenesis of acute promyelocytic leukemia: active players or innocent bystanders? Frontiers in Bioscience 14, 1684-1707, January 1, 2009

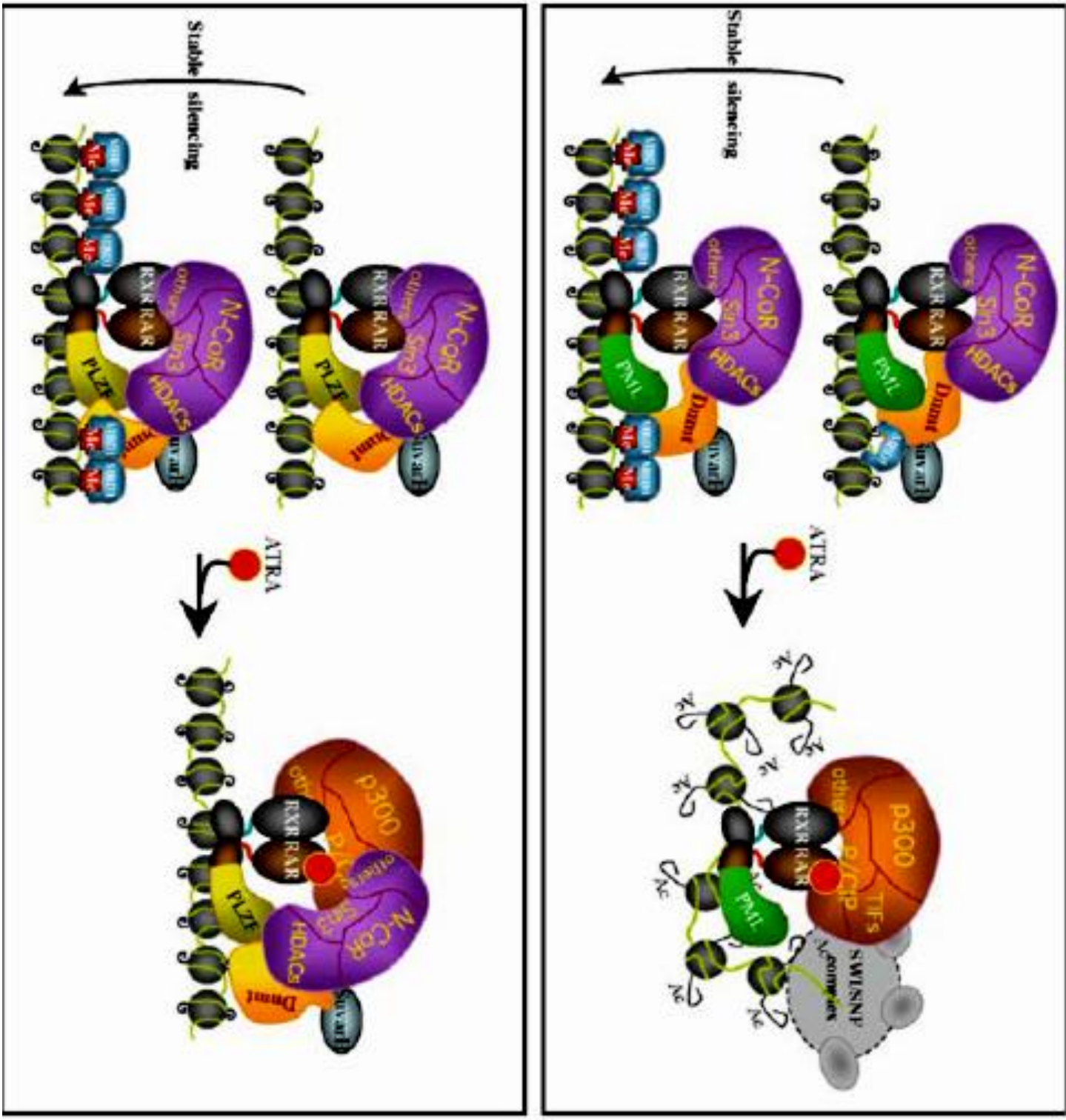
PML-RARα



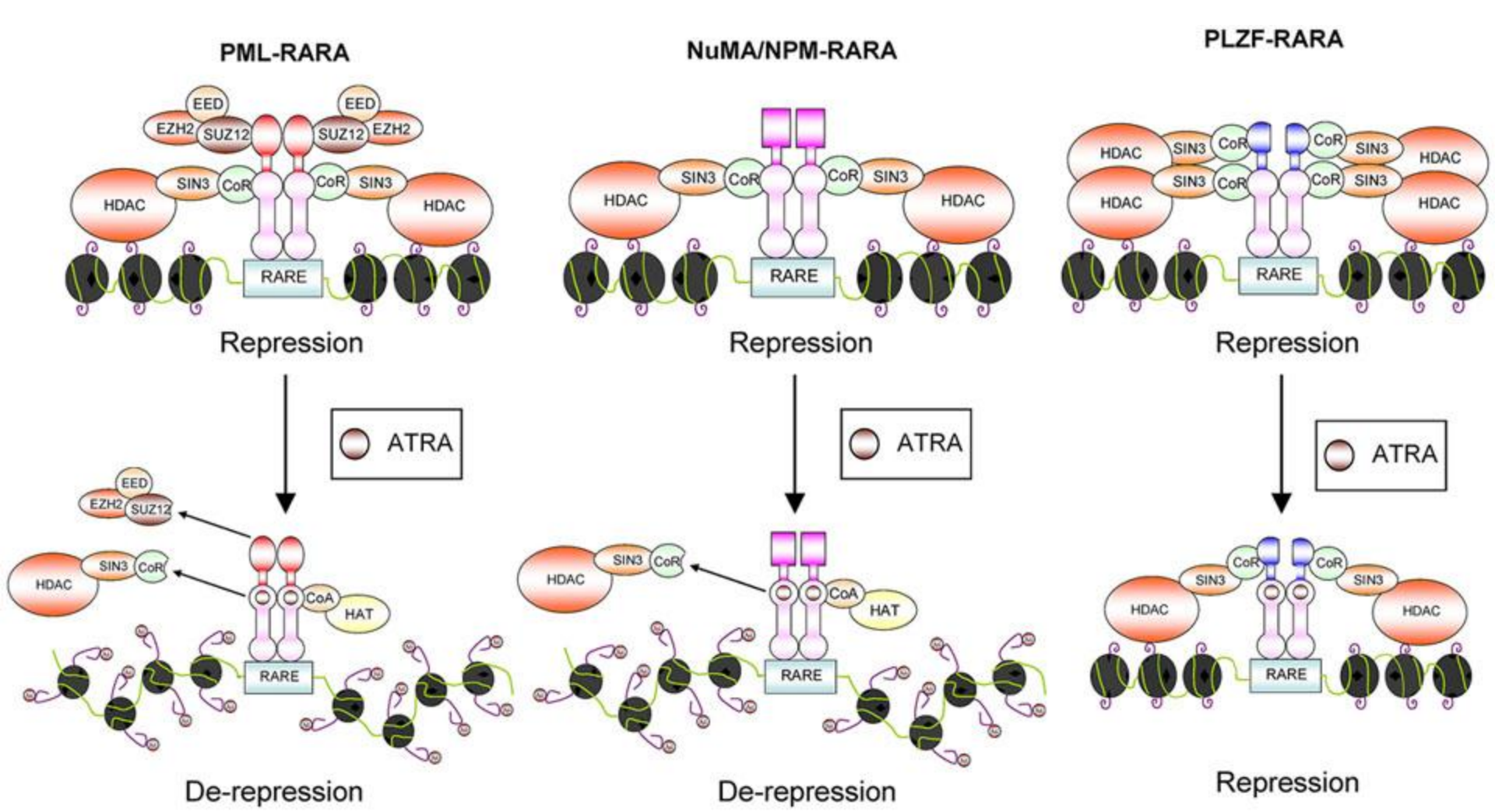
Retinoic acid receptor alpha (RAR-α)



David Grimwade, et al. Acute Promyelocytic Leukemia: A Paradigm for Differentiation Therapy. Acute Myelogenous Leukemia: *Genetics, Biology and Therapy*. 2010.

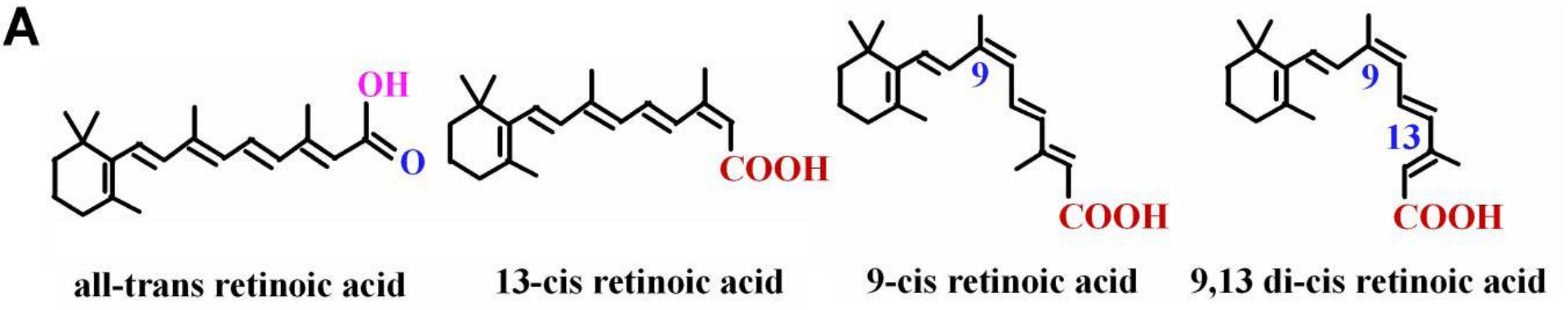


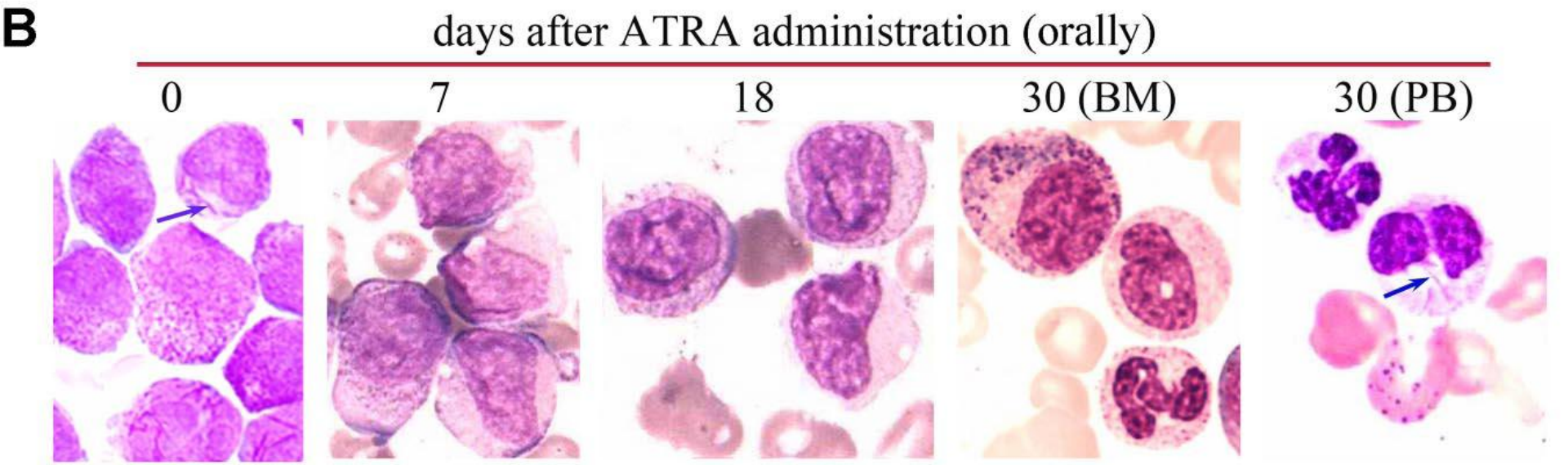
David Grimwade, et al. Acute Promyelocytic Leukemia: A Paradigm for Differentiation Therapy. Acute Myelogenous Leukemia: *Genetics, Biology and Therapy*. 2010.



Nicola J.M. Brown, Michal Ramalho, Eva W. Pedersen, Eva Moravcsik, Ellen Solomon, David Grimwade. PML nuclea bodies in the pathogenesis of acute promyelocytic leukemia: active players or innocent bystanders? Frontiers ir Bioscience 14, 1684-1707, January 1, 2009


RUIJIN HOSPITAL
SHANGHAI JIAO TONG UNIVERSITY SCHOOL OF MEDICINE
R J
1907
10mg 400片
维 甲 酸 片
上海瑞金医院
上海第二医科大学附属
RUIJIN HOSPITAL AFFILIATED TO SHANGHAI NO.2 MEDICAL UNIVERS

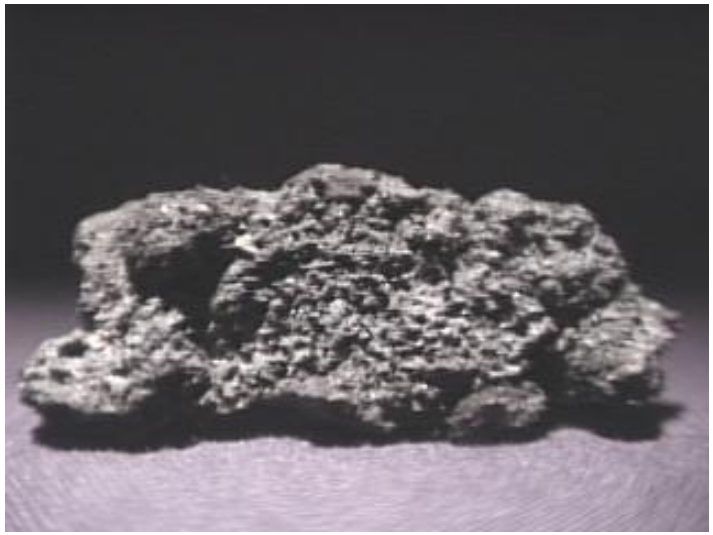


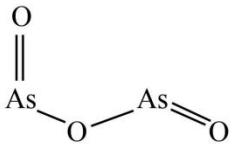


Zhen-Yi Wang and Zhu Chen. Acute promyelocytic leukemia: from highly fatal to highly curable. BLOOD, 2008, 111(5): 2505-2515

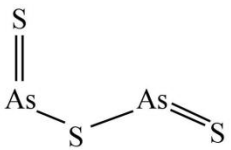
Arsenic Trioxide (ATO)

Taming an evil with a toxic agent

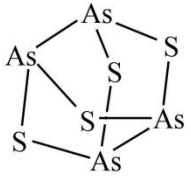




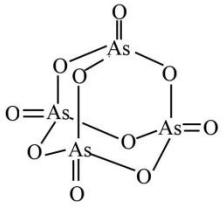
Arsenic trioxide
(white arsenic)



Arsenic trisulfide
(orpiment, yellow arsenic)

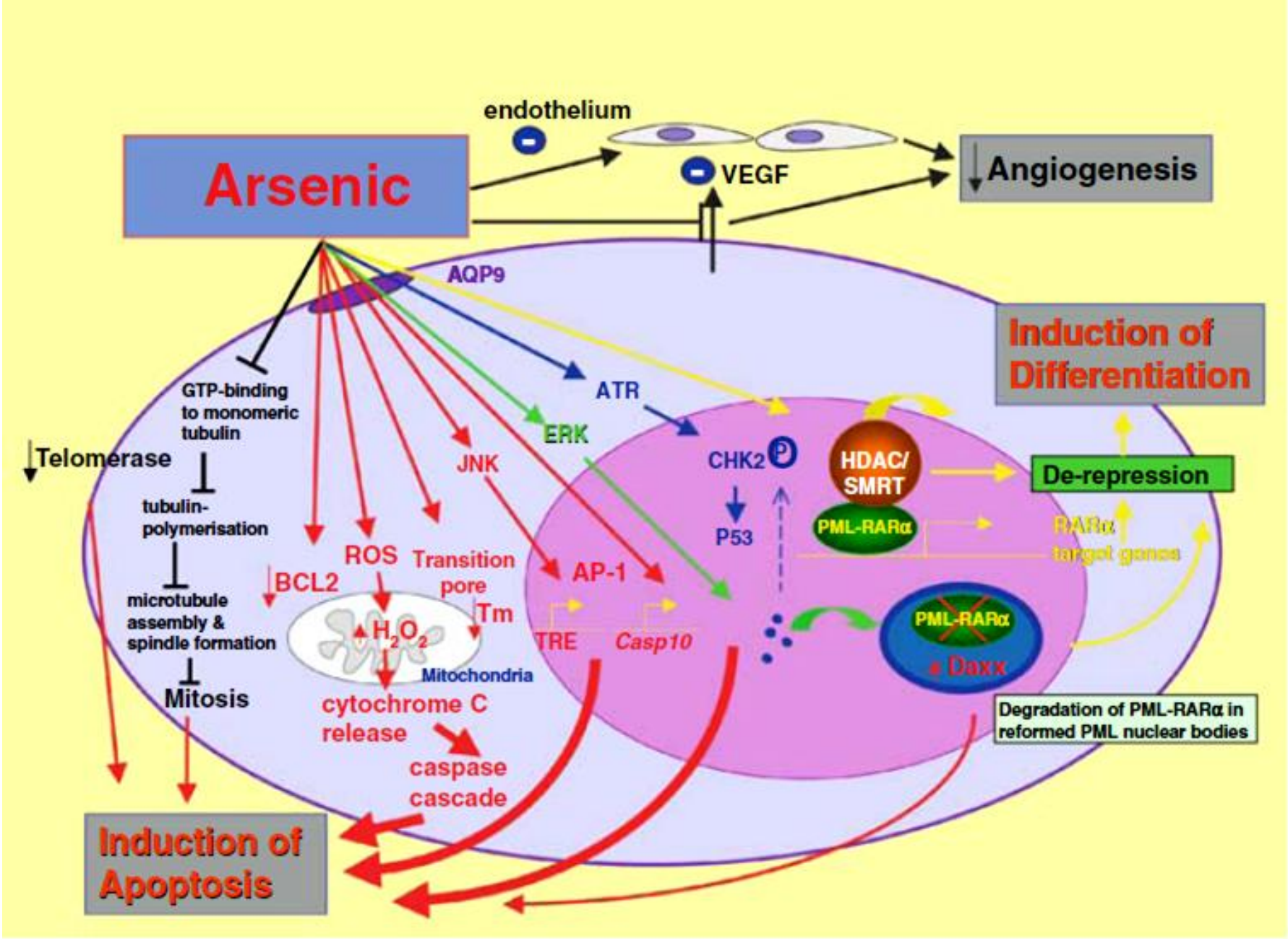


tetra-arsenic tetra-sulfide
(realgar, red arsenic)

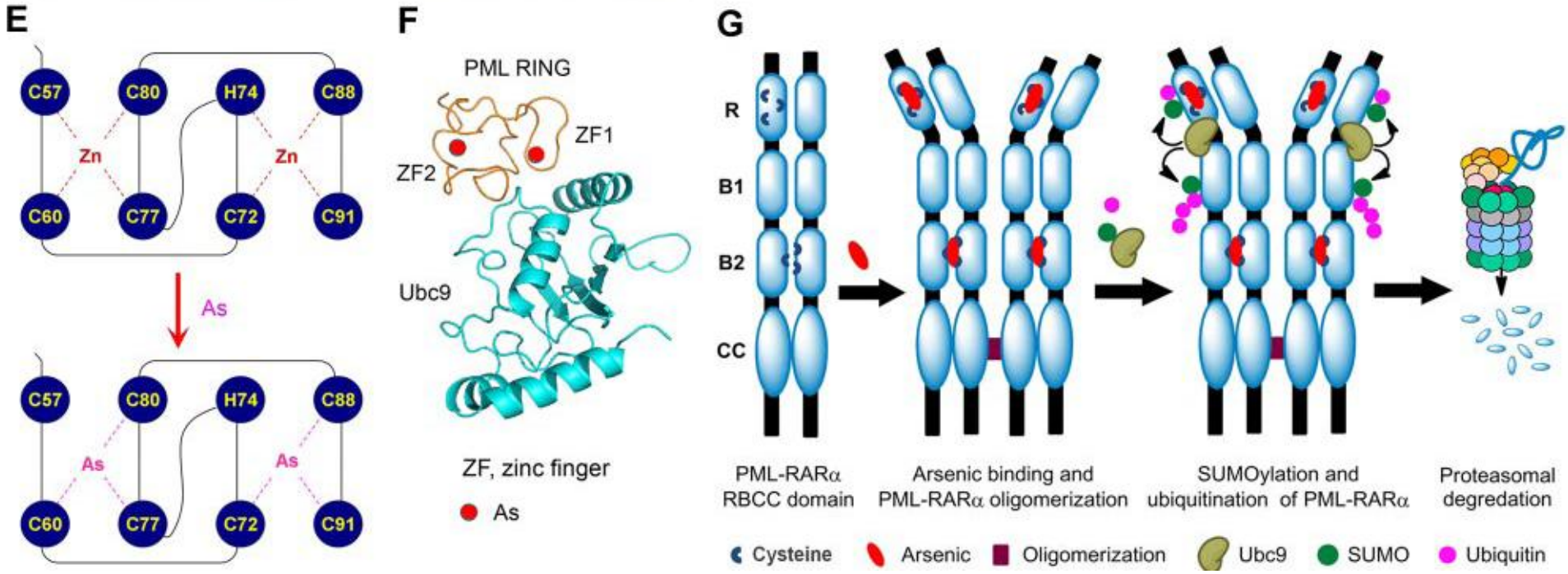


Arsenic pentoxide

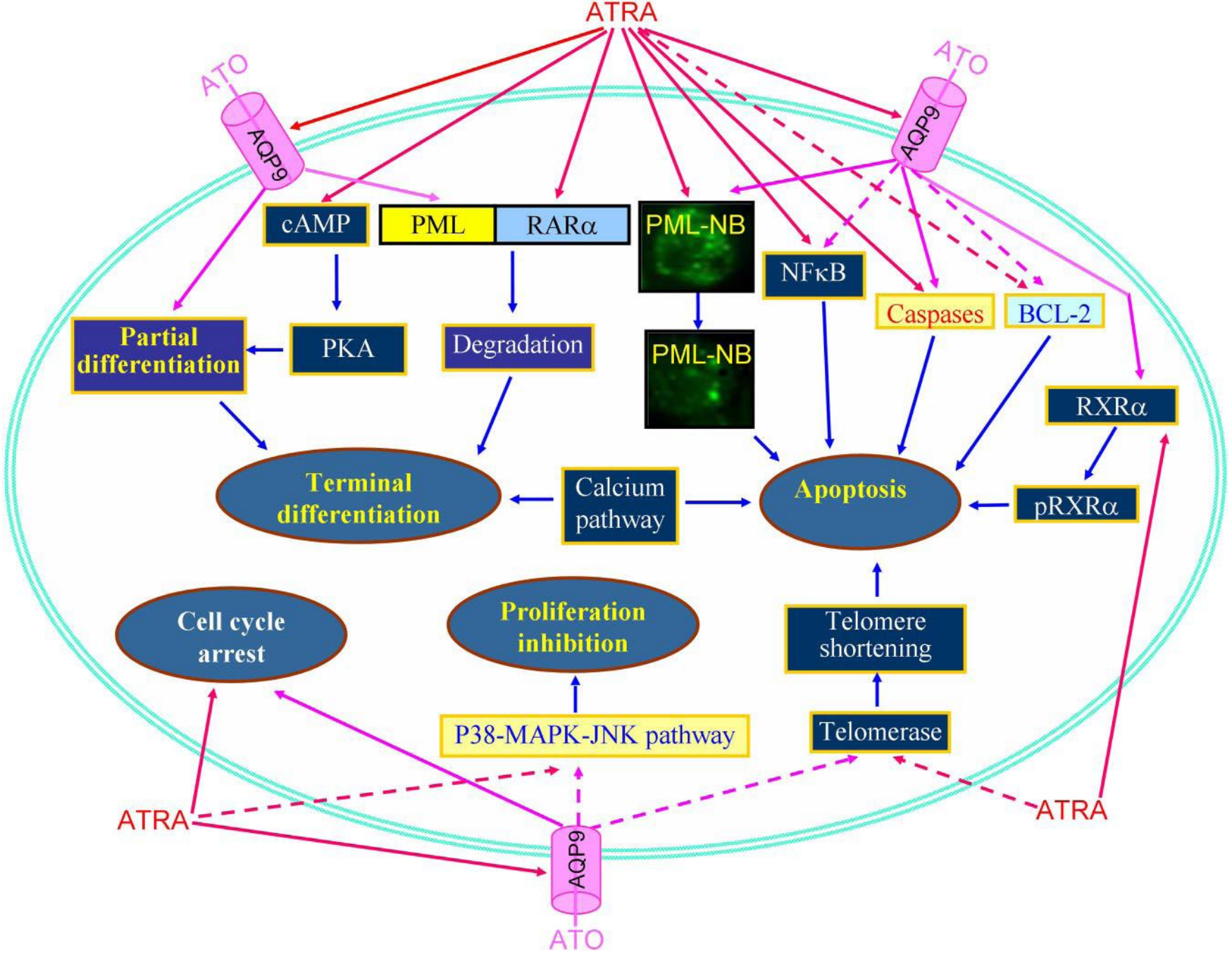
- Mentioned by Hippocrates (460-370 BC) for treatment of skin ulcer.
- Treatment of malaria-associated periodic in Chinese Treaty NeiJing (263 BC).
- Early 1970s, a group from Harbin Medical University in northeastern China identified ATO as an active ingredient from an anticancer remedy.
- In 1992, Sun et al. reported that, by administration (intravenous) of a crude solution of ATO composed of 1% ATO with a trace amount of mercury chloride, 66%(21/32) APL patients entered CR with an impressive 30% survival rate after 10 years.



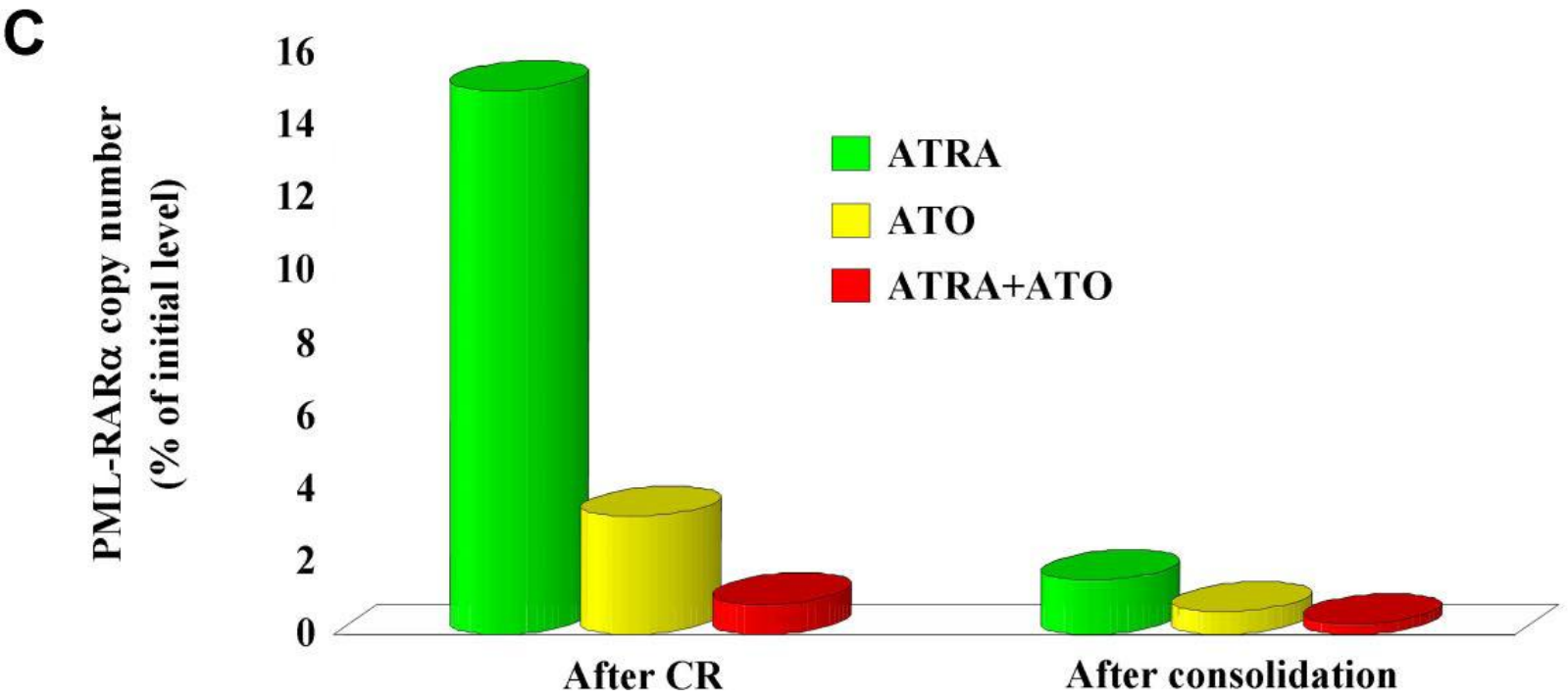
David Grimwade, et al. Acute Promyelocytic Leukemia: A Paradigm for Differentiation Therapy. Acute Myelogenous Leukemia: *Genetics, Biology and Therapy*. 2010.



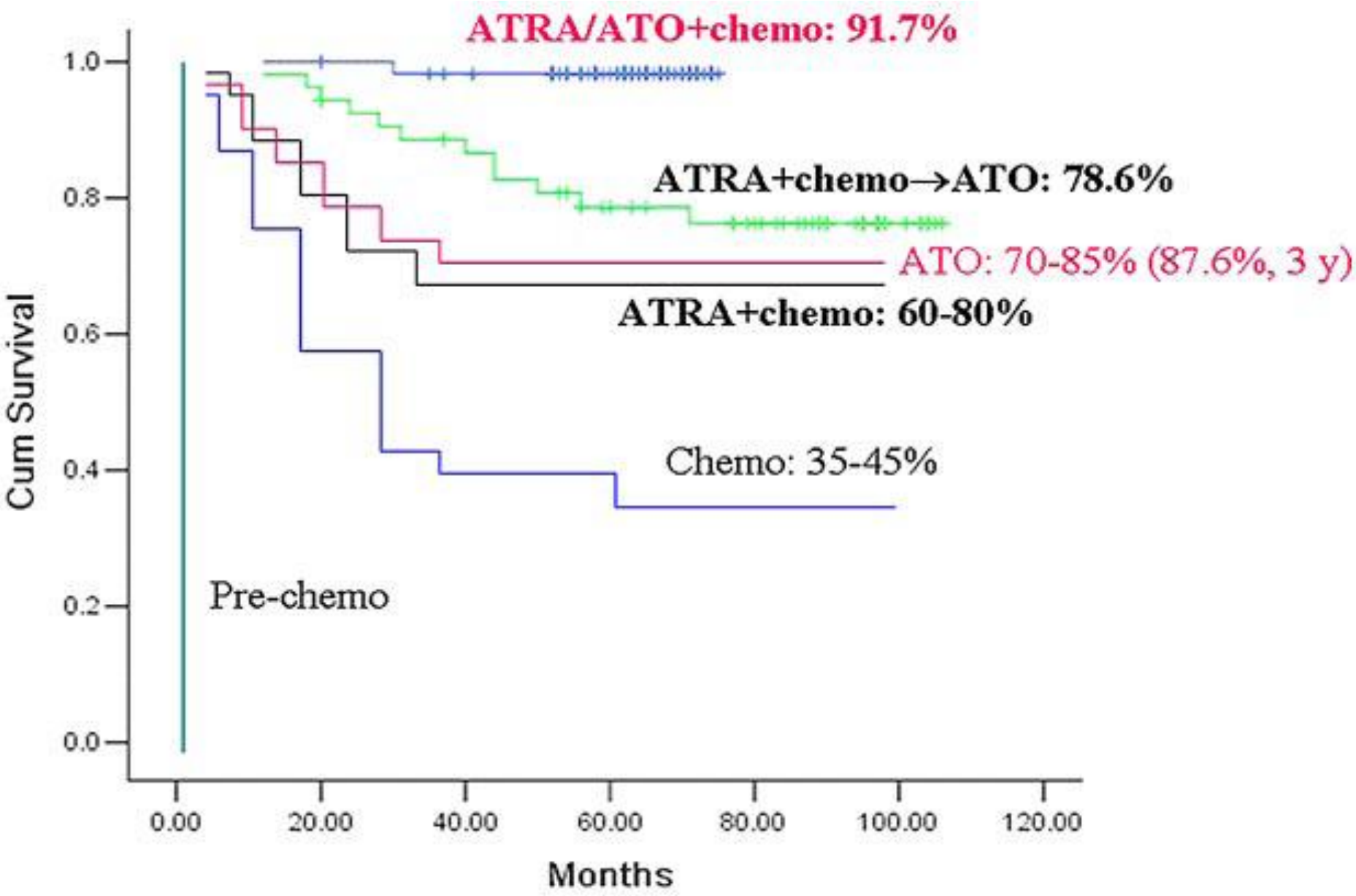
Chen SJ, et al. From an old remedy to a magic bullet: molecular mechanisms underlying the therapeutic effects of arsenic in fighting leukemia. Blood 2011 117:6425-6437



Zhen-Yi Wang and Zhu Chen. Acute promyelocytic leukemia: from highly fatal to highly curable. BLOOD, 2008, 111(5): 2505-2515



Zhen-Yi Wang and Zhu Chen. Acute promyelocytic leukemia: from highly fatal to highly curable. BLOOD, 2008, 111(5): 2505-2515



Chen SJ, et al. From an old remedy to a magic bullet: molecular mechanisms underlying the therapeutic effects of arsenic in fighting leukemia. Blood 2011 117:6425-6437

inside blood
23 AUGUST 2012 | VOLUME 120, NUMBER 8

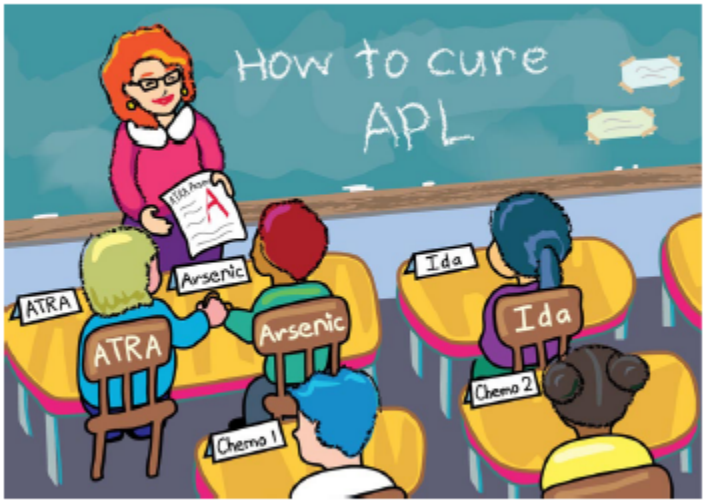
CLINICAL TRIALS

Comment on Iland et al, page 1570

ATRA plus arsenic gets another "A" in APL

Jae H. Park MEMORIAL SLOAN-KETTERING CANCER CENTER

In this issue of *Blood*, Iland et al report that the addition of arsenic trioxide during induction and consolidation can substantially reduce the amount of chemotherapy and the duration of consolidation to achieve excellent outcomes in patients with newly diagnosed acute promyelocytic leukemia (APL; see figure).[1]



The combination of ATRA and arsenic with varying amounts of chemotherapy has been studied in newly diagnosed APL patients. Iland et al report one of the best outcomes observed in APL studies using ATRA plus

clinical trials combined ATRA and As_2O_3 with minimal chemotherapy, and observed an outcome comparable to that reported with ATRA plus anthracycline-based chemotherapy.[8,9]

Here, Iland and colleagues report the outcome of 124 newly diagnosed APL patients treated with ATRA and As_2O_3 both during induction and consolidation with limited chemotherapy exposure.[1] In the study, patients with de novo APL received induction consisting of ATRA, intravenous As_2O_3, and 4 doses of age-adjusted idarubicin (maximum cumulative dose = 48 mg/m^2). All patients regardless of presenting white blood cell (WBC) counts received prednisone as prophylaxis for APL differentiation syndrome. CR was achieved in 95% of patients with an early death rate of 3.2% (defined as death during induction). Notably, the trial included only 2 consolidation cycles consisting of ATRA and intravenous As_2O_3, administered on continuous schedule during consolidation 1 and 5 d/wk during consolidation 2. All patients achieved molecular CR by the end of consolidation, and all received maintenance therapy for 2 years consisting of intermittent ATRA, oral methotrexate, and 6-MP. Two of the 112 patients who completed consolidation and maintenance therapy relapsed, with an impressive 98% disease-free survival rate at 2 years. Sanz

FLT3 ABNORMALITIES

- **FMS-like tyrosine kinase-3 (FLT3) is class III receptor tyrosine kinase**
- **Expressed in primitive hematopoietic cells and activated by a ligand (FL cytokine)**
- **Commonest mutation in AML – 40% of cytogenetically normal AML**
- Internal tandem duplication in the juxtamembrane domain and point mutation in the tyrosine kinase II domain can be detected in 25% to 45% of APL patients.
- FLT-3 inhibitor SU11657

Treatment Approaches

- What is the risk-stratification based on the Sanz criteria? (WBC and platelet count)
- Induction
- Consolidation
- Maintenance
- Relapse

Risk Stratification

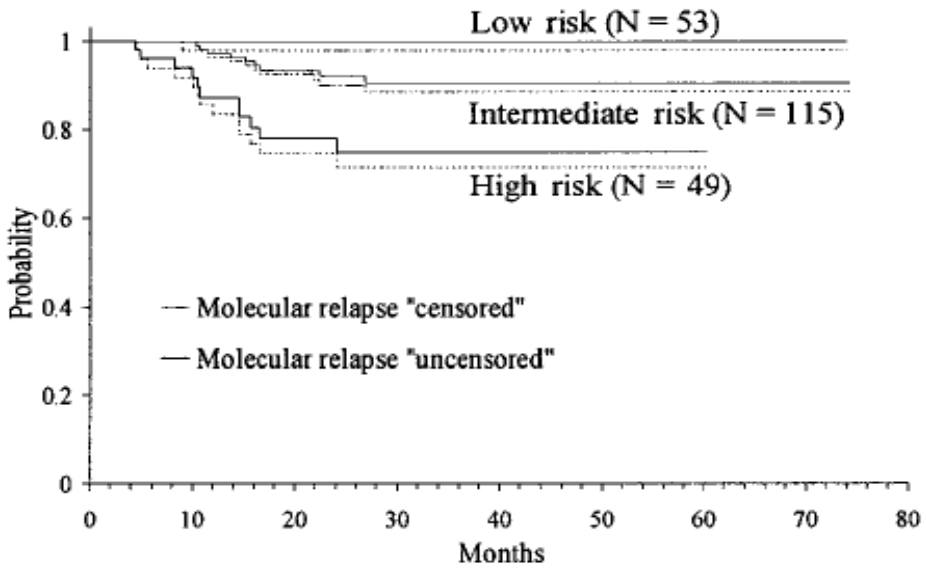


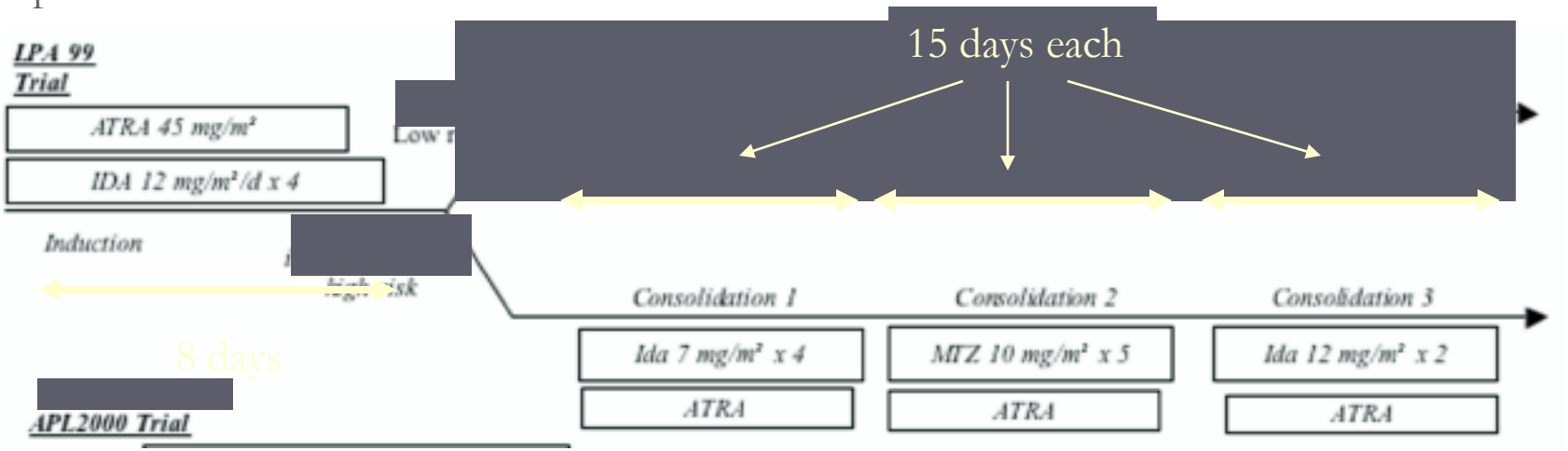
Figure 5. Kaplan-Meier product-limit estimate of RFS according to the risk groups defined by the predictive model.

- Low: WBC < 10,000 and platelets > 40,000
- Intermediate: WBC < 10,000 and platelets < 40,000
- High: WBC > 10,000

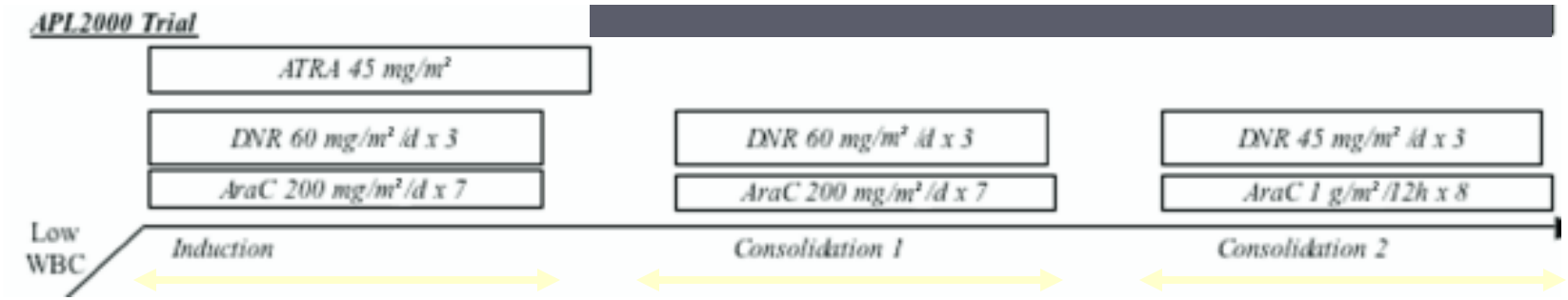
Miguel A. Sanz, et al. Definition of relapse risk and role of nonanthracycline drugs for consolidation in patients with acute promyelocytic leukemia: a joint study of the PETHEMA and GIMEMA cooperative groups.Blood 2000 96:1247-1253

Low/Intermediate Risk Disease Induction and Consolidation Options

Spanish PETHEMA

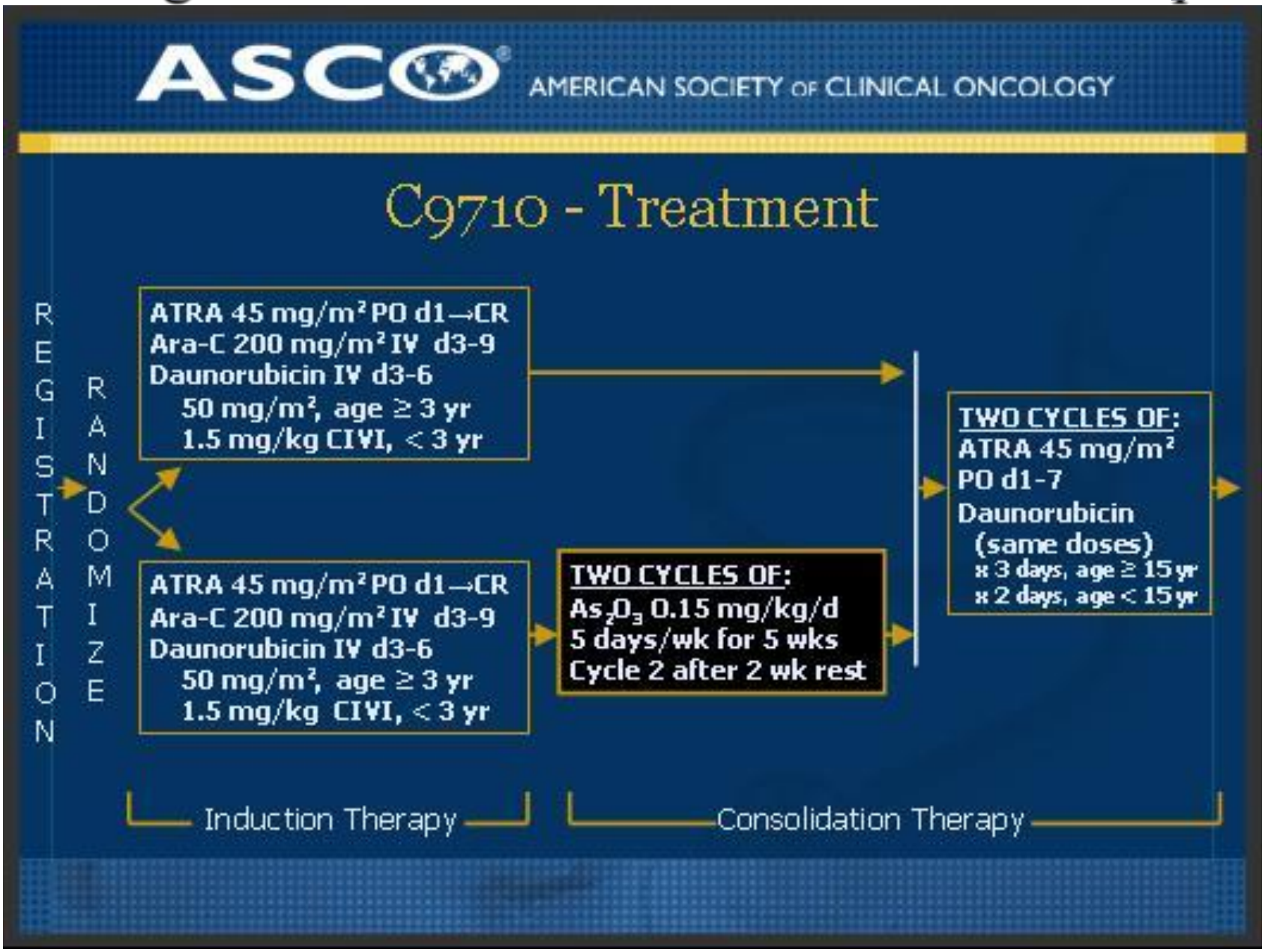


French-Belgian-Swiss APL Group



Ades, Blood, 2008

High Risk Induction and Consolidation-Option 1



Powell, ASCO 2007, North American Intergroup Results

Assessment of response

- BM biopsy
- Morphology complete remission
- Cytogenetic complete remission
- Molecular complete remission
- Morphology complete remission with incomplete blood count recovery
- Partial remission
- No response

Post-Consolidation Therapy

- Rt- PCR for PML-RARa after consolidation
- Negative?
  - Maintenance with 1-2 years of ATRA +/- 6MP and methotrexate
  - Rt-PCR every 3 months for 2 years
- Positive?
  - Repeat within 4 weeks
  - Treat for relapse if still positive

Relapsed APL

- Treat with ATO +/- ATRA
- Strongly consider CNS-directed treatment with intrathecal chemotherapy
- Morphologic remission?
  - Yes
    - PCR Negative-Auto SCT vs. ATOc x 6 cycles
    - PCR Positive-Allo SCT
  - No
    - Allo SCT

NCCN AML 2010

Summary

- APL has a unique and specific translocation t(15;17), resulting in PML/RARa
- Abnormal RARa fusion protein plays a central role in APL leukemogenesis
- ATRA and ATO, to modulate and/or degrade the fusion protein PML/RARa furnishes the first model of molecular target–based induction of differentiation and apoptosis
- High CR rates (90%-94%) and high 5-year DFS rates (90%) using ATRA/ATO/CT in APL
- Achievements result from the collaboration between researchers and clinicians

References:

- Steven H. Swerdlow, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th edition 2008
- Elaine S. Jaffe, et al. Hematopathology. 1st edition. 2011
- David Grimwade, Anita R. Mistry, Ellen Solomon, and Fabien Guidez. Acute Promyelocytic Leukemia: A Paradigm for Differentiation Therapy. Acute Myelogenous Leukemia: *Genetics, Biology and Therapy*. 2010.
- Zhen-Yi Wang and Zhu Chen. Acute promyelocytic leukemia: from highly fatal to highly curable. BLOOD, 2008, 111(5): 2505-2515
- Julien Ablain and Hugues de The. Revisiting the differentiation paradigm in acute promyelocytic leukemia. *Blood*. 2011;117(22):5795-5802
- Sai-Juan Chen, et al. From an old remedy to a magic bullet: molecular mechanisms underlying the therapeutic effects of arsenic in fighting leukemiaBlood 2011 117:6425-6437
- Miguel A. Sanz, et al. Definition of relapse risk and role of nonanthracycline drugs for consolidation in patients with acute promyelocytic leukemia: a joint study of the PETHEMA and GIMEMA cooperative groups:Presented in part at the 41st meeting of the American Society of Hematology, New Orleans, LA, December 3-7, 1999.Blood 2000 96:1247-1253