Pathogenesis, diagnosis and monitoring of Acute Promyelocytic Leukemia

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7th INTERNATIONAL SYMPOSIUM ON ACUTE PROMYELOCYTIC LEUKEMIA

Rome, 24-27 September 2017

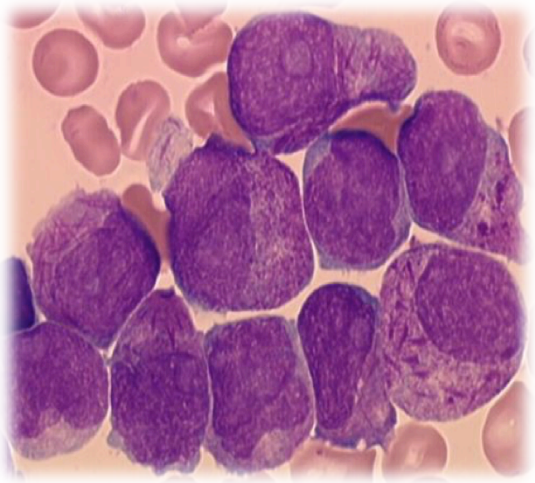
Outline

- Pathogenesis & biology of APL
- Diagnosis
- Monitoring of minimal residual disease

APL main presenting features

- Usually abrupt onset, with rapidly progressing coagulopathy (medical emergency)
- Life-threatening hemorrhagic events
- Infiltration by leukemic promyelocytes

1977. t(15;17) as a disease hallmark

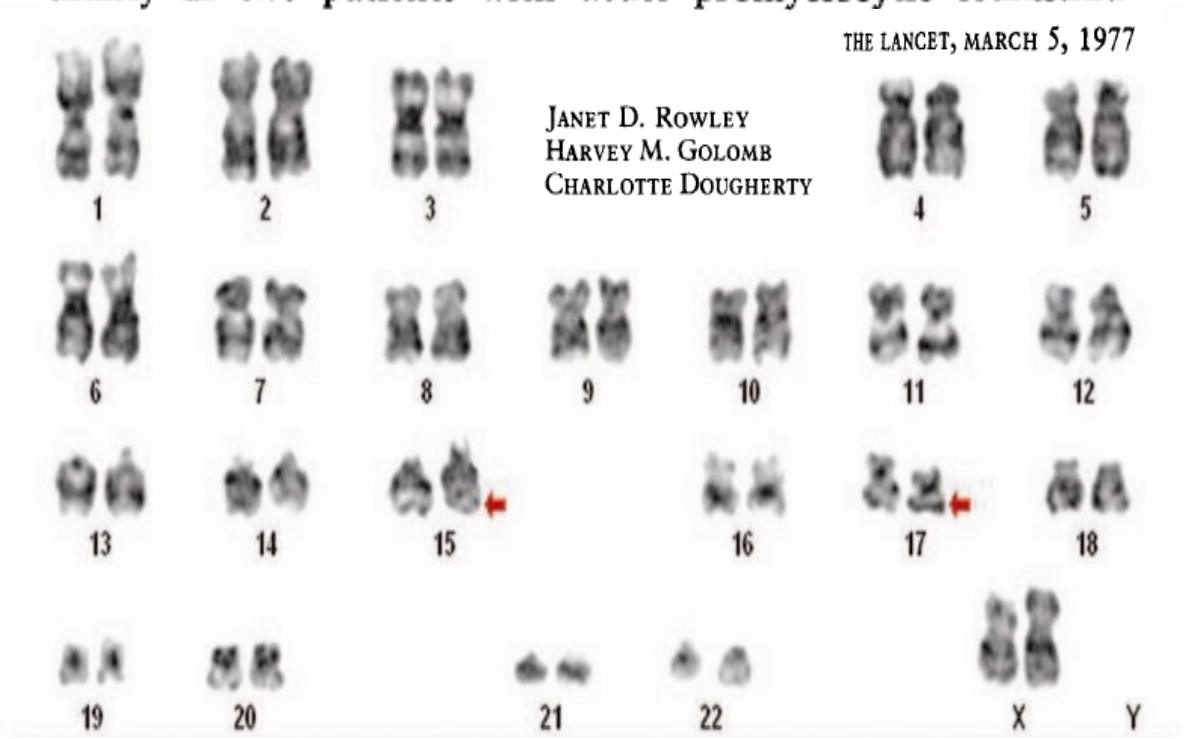


15/17 TRANSLOCATION, A CONSISTENT CHROMOSOMAL CHANGE IN ACUTE PROMYELOCYTIC LEUKAEMIA

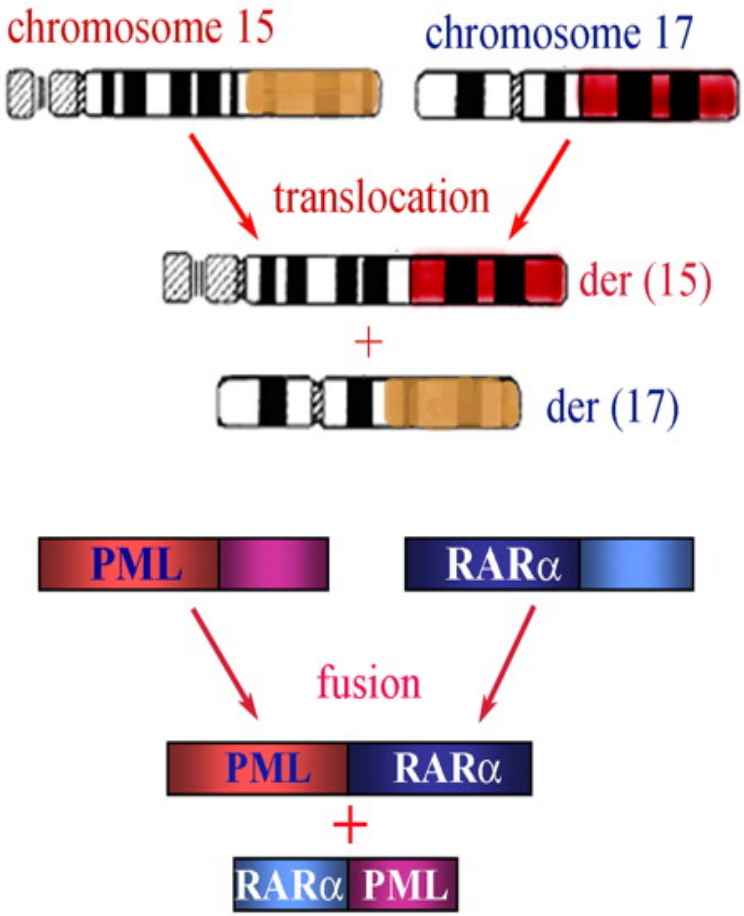
SIR,—We have described a similar chromosomal abnormality in two patients with acute promyelocytic leukæmia

THE LANCET, MARCH 5, 1977

JANET D. ROWLEY
HARVEY M. GOLOMB
CHARLOTTE DOUGHERTY



Molecular pathogenesis



The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor α gene to a novel transcribed locus

Hugues de Thé*, Christine Chomienne†, Michel Lanotte‡, Laurent Degos§ & Anne Dejean*

Molecular Analysis of Acute Promyelocytic Leukemia Breakpoint Cluster Region on Chromosome 17

JULIAN BORROW, AUDREY D. GODDARD, DENISE SHEER, ELLEN SOLOMON

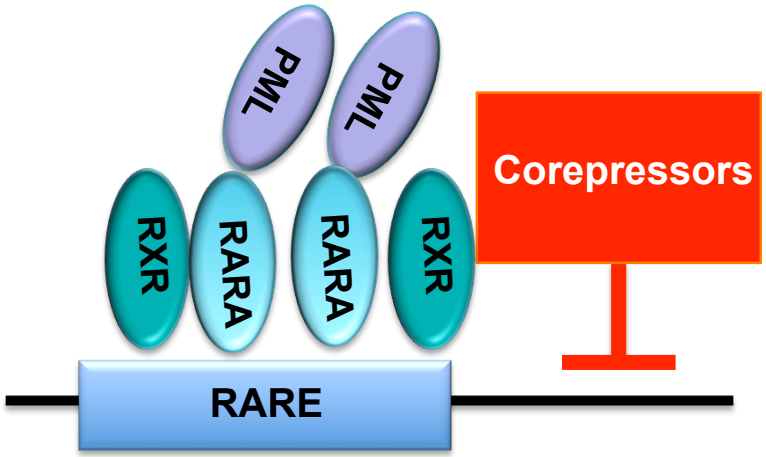
J. Borrow, A. D. Goddard, E. Solomon, Somatic Cell Genetics Laboratory, Imperial Cancer Research Fund, London WC2A 3PX, United Kingdom.
D. Sheer, Human Cytogenetics Laboratory, Imperial Cancer Research Fund, London WC2A 3PX, United Kingdom.

SCIENCE, VOL. 249

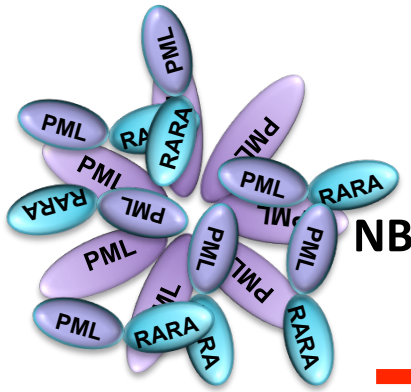
Rearrangements and Aberrant Expression of the Retinoic Acid Receptor α Gene in Acute Promyelocytic Leukemias

By Letizia Longo,* Pier Paolo Pandolfi,* Andrea Biondi,‡ Alessandro Rambaldi,§ Amedea Mencarelli,* Francesco Lo Coco,‖ Daniela Diverio,‖ Luigi Pegoraro,¶ Giancarlo Avanzi,¶ Antonio Tabilio,* Daniela Zangrilli,** Myriam Alcalay,* Emilio Donti,* Fausto Grignani,* and Pier Giuseppe Pelicci*

PML-RARA oncoprotein functions

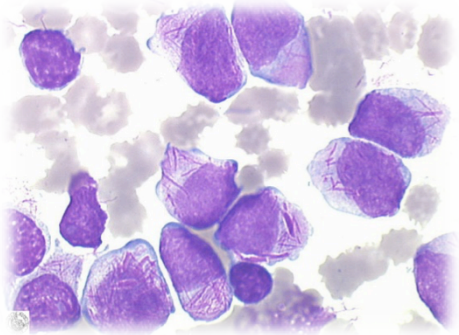


Transcriptional repression of RARA target genes



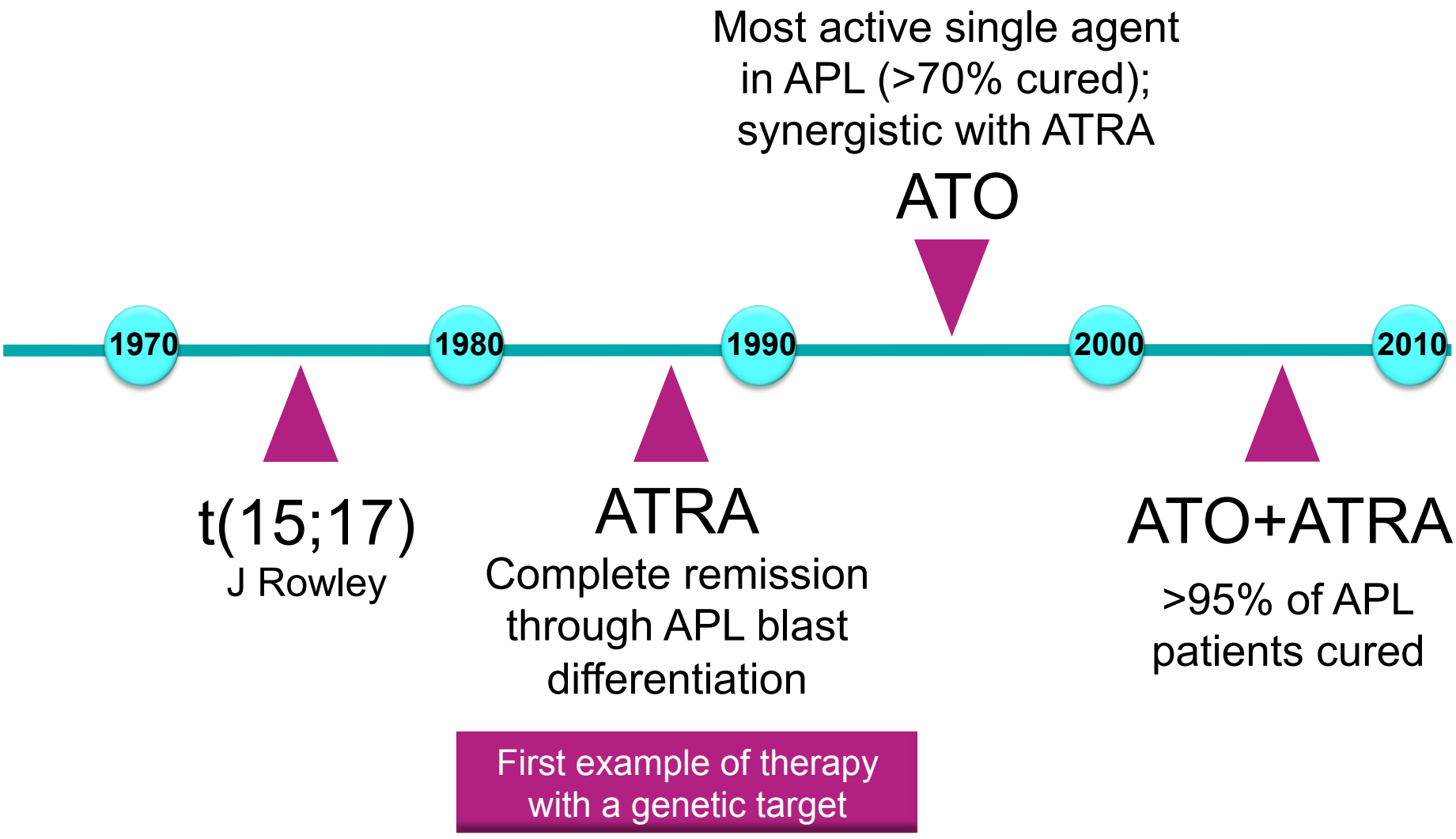
NB disruption

Tumor repression, Apoptosis
Senescence, DNA repair



MYELOID DIFFERENTIATION BLOCK

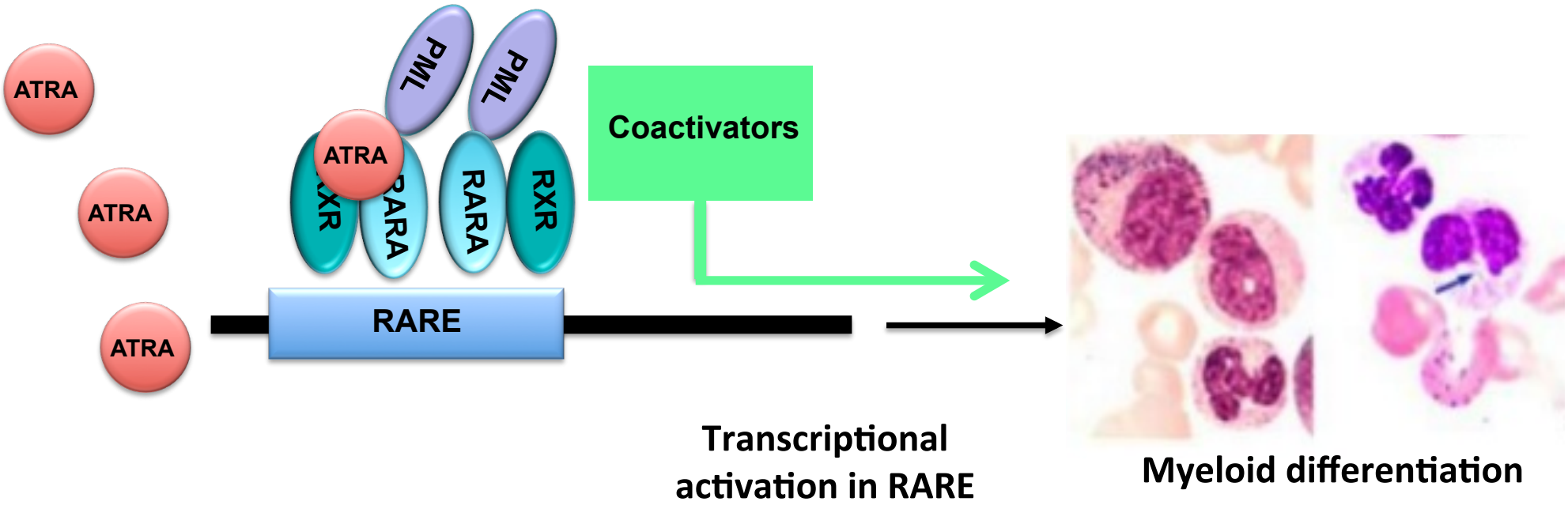
Targeted therapies in APL



PML/RARα and ATRA

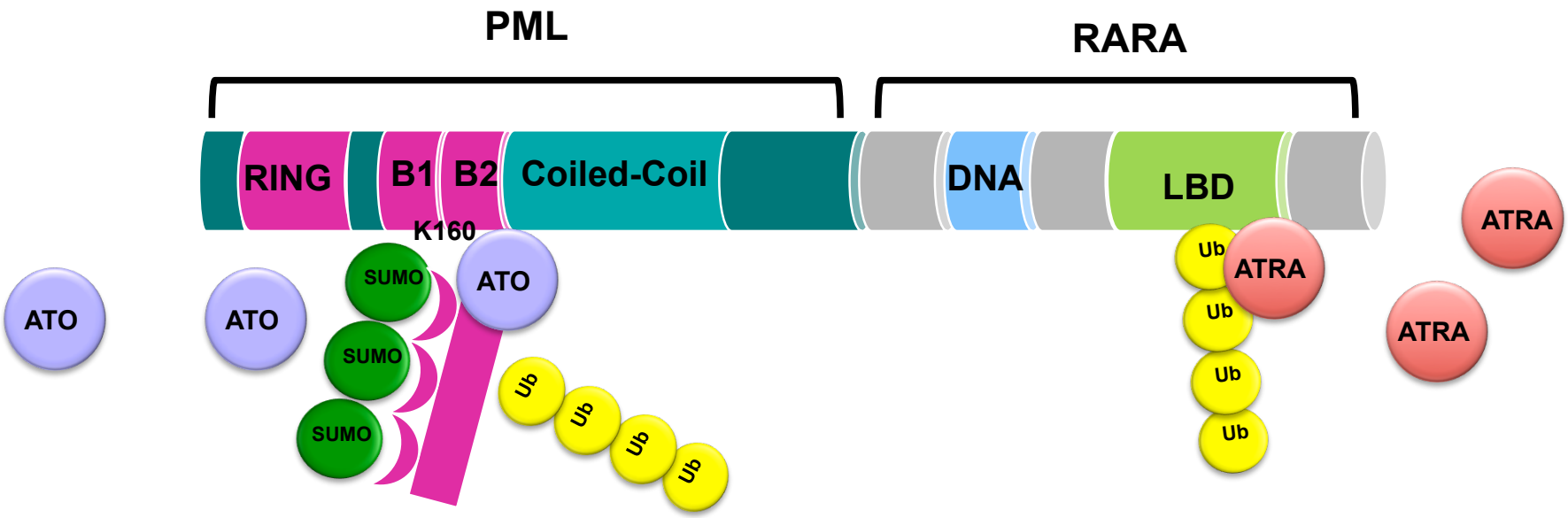
A model for differentiation therapy

Derivative of Vitamin A, at pharmacological dosis able to induce CR in >90% of APL patients (although short-lasted in the majority of cases)



PML/RARα, ATRA and ATO

Synergistic effect of the two agents



Proteasomal degradation

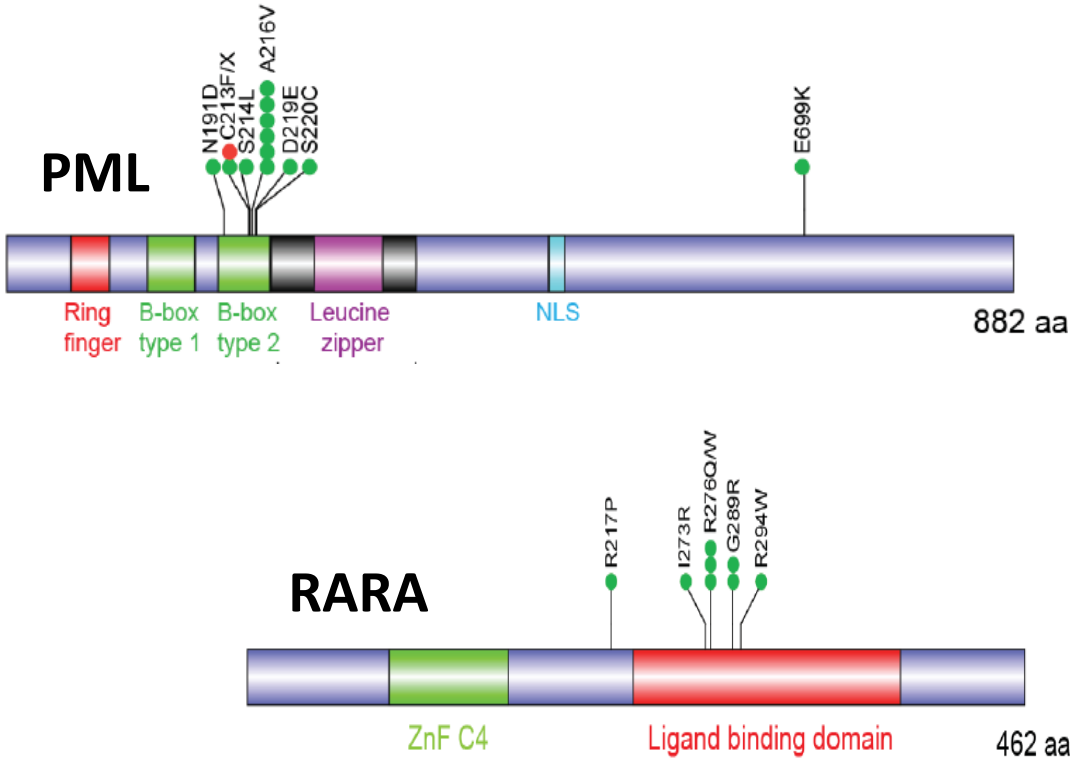
NB reformation

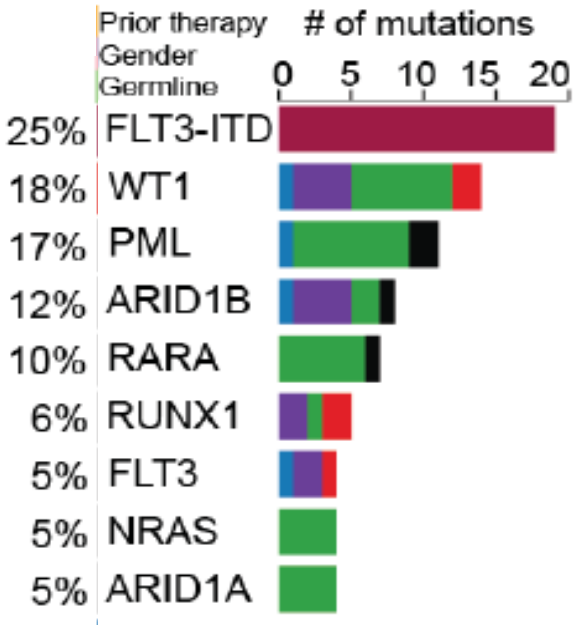
Transcriptional reactivation of RARA target genes

APL cure

Landscape of somatic mutations at APL relapse

PML and RARA mutations





Zhu, NEJM 2014; Lehmann-Che, NEJM 2014; Lou, Annals of Hem 2015; Chendamarai, Plos One 2015; Madan *et al*. Leukemia 2016;Iaccarino BJH 2016

The PML/RARα fusion protein

Why so important in diagnosis and treatment

- Unique to APL
- Strongly correlated with pathogenesis
- Targeted by specific therapies
- Detection predicts response to ATRA & ATO

Outline

- Clinical & biological background
- Diagnosis
- Monitoring of minimal residual disease

blood

2009 113: 1875-1891
Prepublished online Sep 23, 2008;
doi:10.1182/blood-2008-04-150250

Management of acute promyelocytic leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet

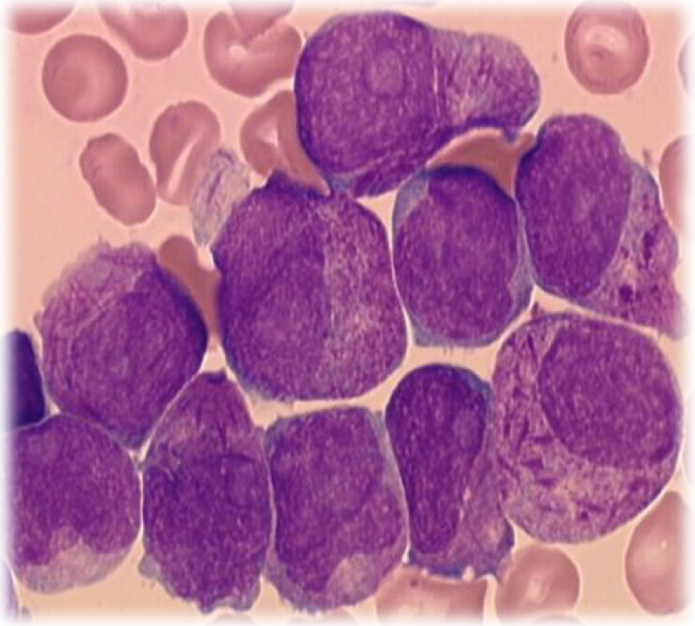
Miguel A. Sanz, David Grimwade, Martin S. Tallman, Bob Lowenberg, Pierre Fenaux, Elihu H. Estey, Tomoki Naoe, Eva Lengfelder, Thomas Büchner, Hartmut Döhner, Alan K. Burnett and Francesco Lo-Coco

Recommended actions in case of APL suspicion

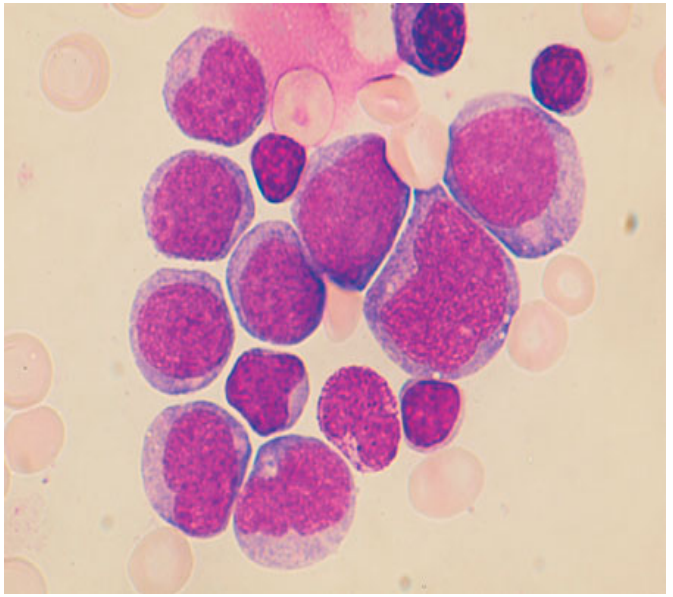
Consider the disease as a medical emergency

- Start treatment with ATRA
- Start intensive transfusion support
- Send a marrow sample to reference lab for genetic diagnosis

Morphology (can be easy, can be tough)



- Atypical promyelocytes
- Bilobed nuclei
- Cytoplasmic granules, Auer rods

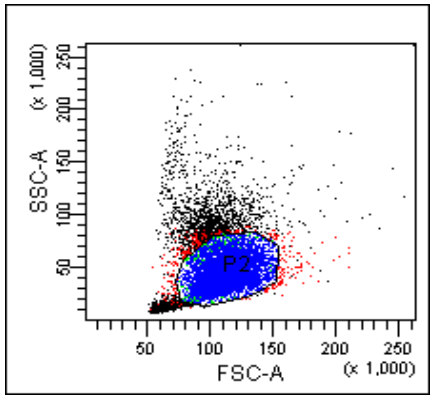


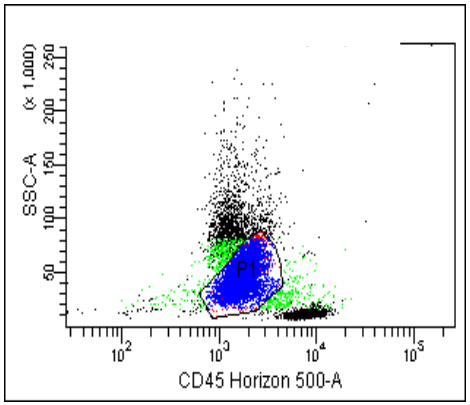
25% of APLs

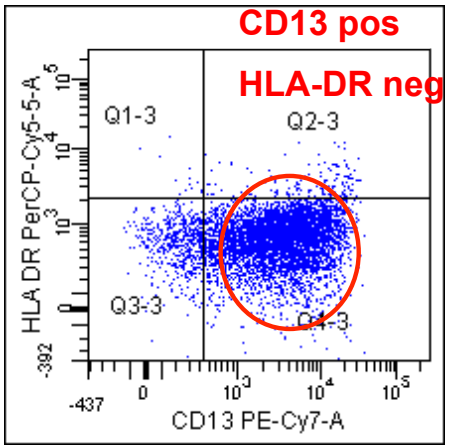
- Hypogranular
- Bilobed nuclei
- Monocytoid elements

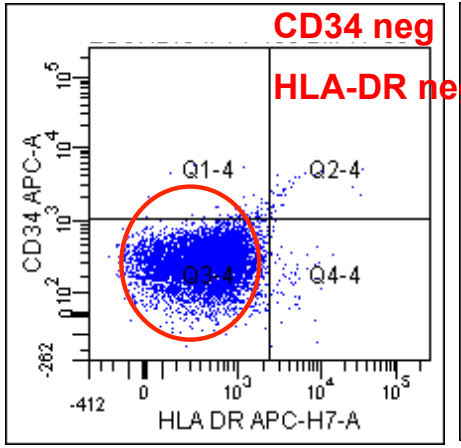
Suspected Acute Promyelocytic Leukemia: the role of immunophenotype

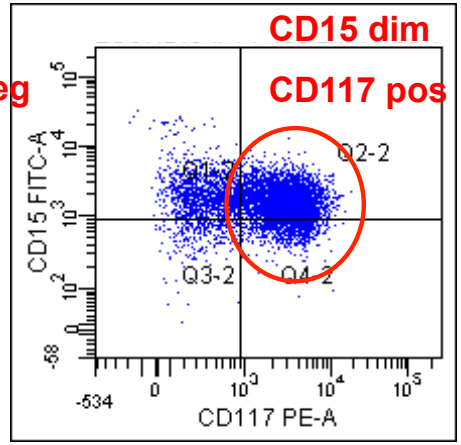
Suspect of APL may arise from **flow cytometry analysis**

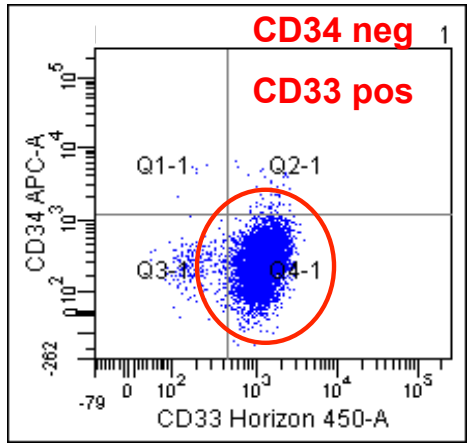












Immunophenotypic features of APL

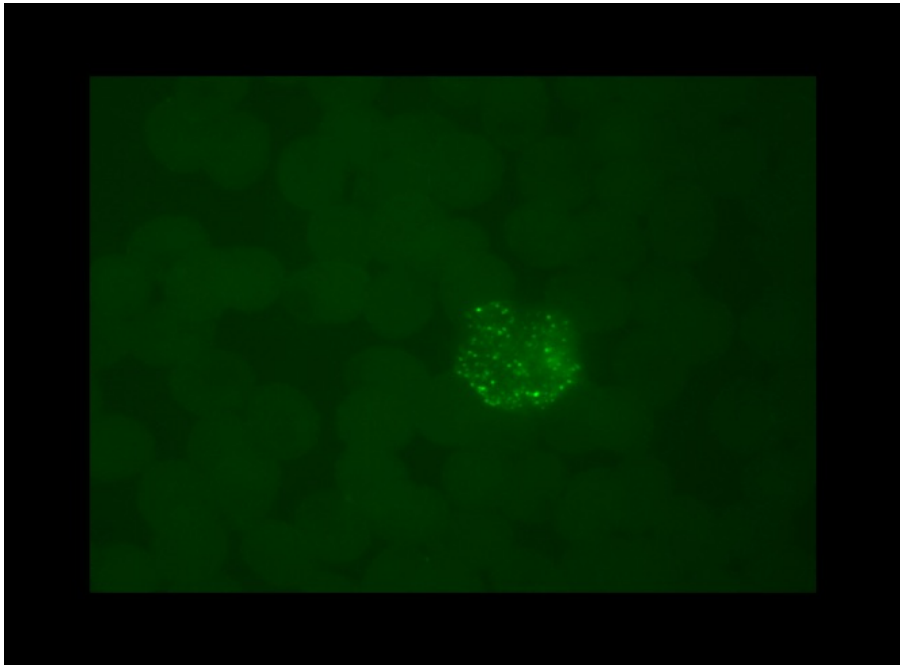
- CD34 and HLA-DR frequently absent
- Strong and highly homogeneous CD33 expression
- Heterogeneous expression of CD13
- Absence or very low expression of CD15, CD11a-b-c
- Expression of CD56 and CD2 (M3v)

Methods for Genetic Diagnosis
Pros and Cons

Technique	Target	Advantages	Pitfalls
Karyotype for t(15;17)	chromosomes	Specific	Time consuming; false negatives
FISH for PML/RARα	DNA	Specific; rapid	Poor sensitivity; No information on the *PML/RARA* isoform
anti-PML MoAb	Protein	Rapid; low cost	No information on the *PML-RARα* isoform
RT-PCR for PML/RARα	RNA	Specific; rapid	Artifacts; Contaminations

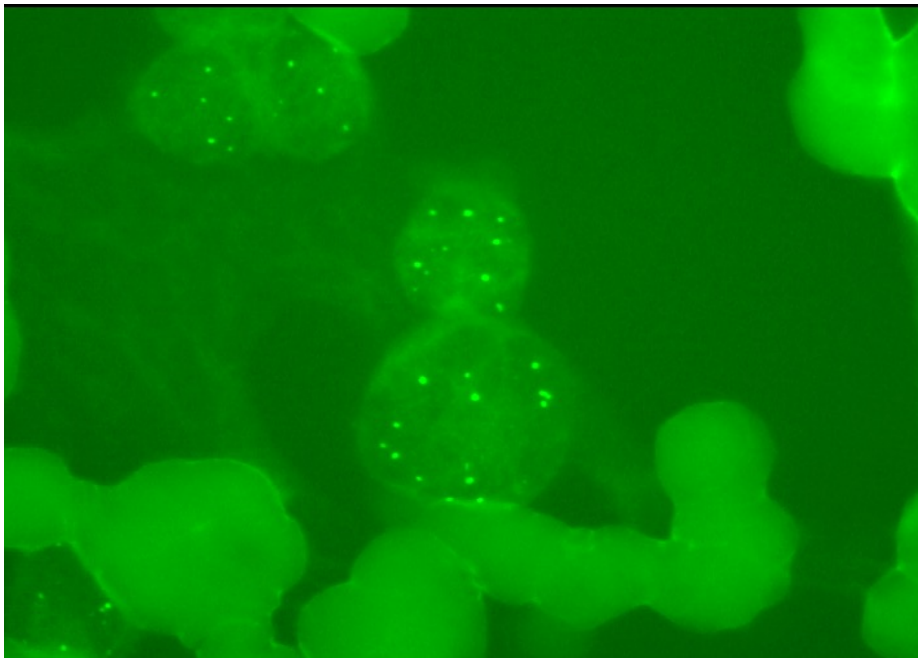
Patterns of PML nuclear staining

Microgranular pattern
PML/RARα +ve APL



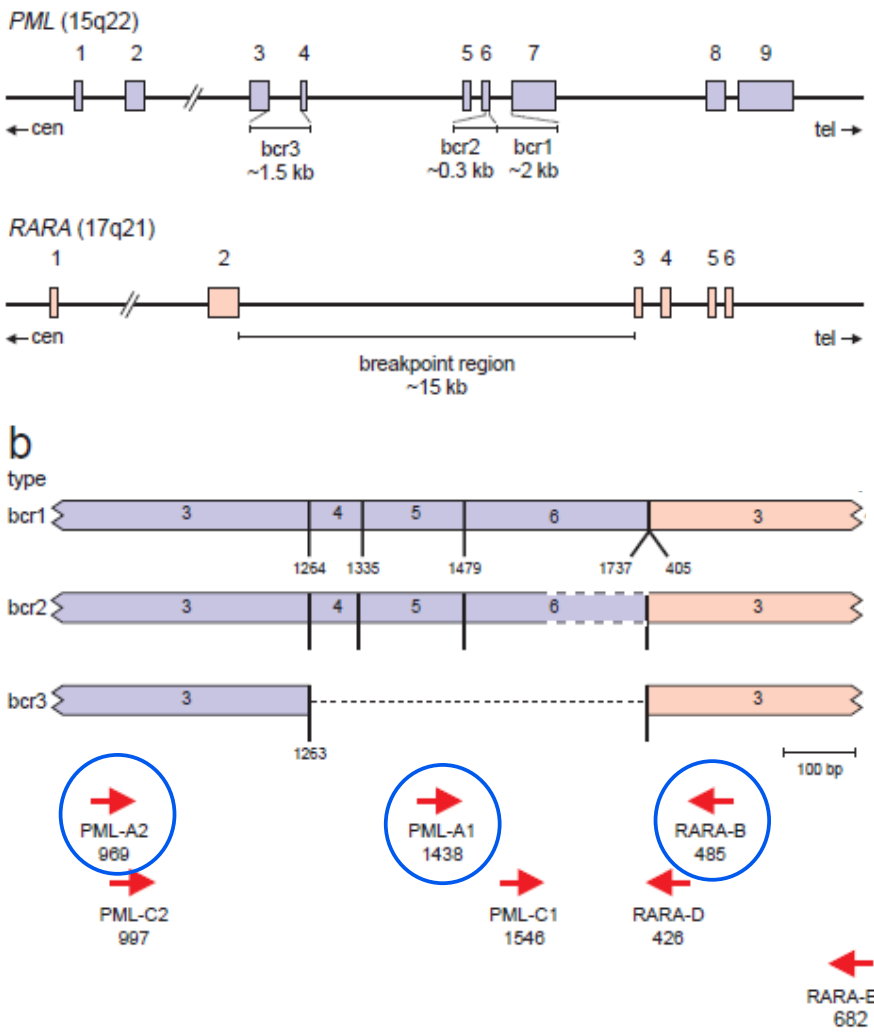
ATRA / ATO-responsive

Nuclear body pattern
PML/RARα -ve AML



ATRA / ATO resistant

RT-PCR amplification of *PML/RARα* transcripts



bcr1 (long) transcript **55 %**

bcr2 (variable) transcript **5 %**

bcr3 (short) transcript **40 %**

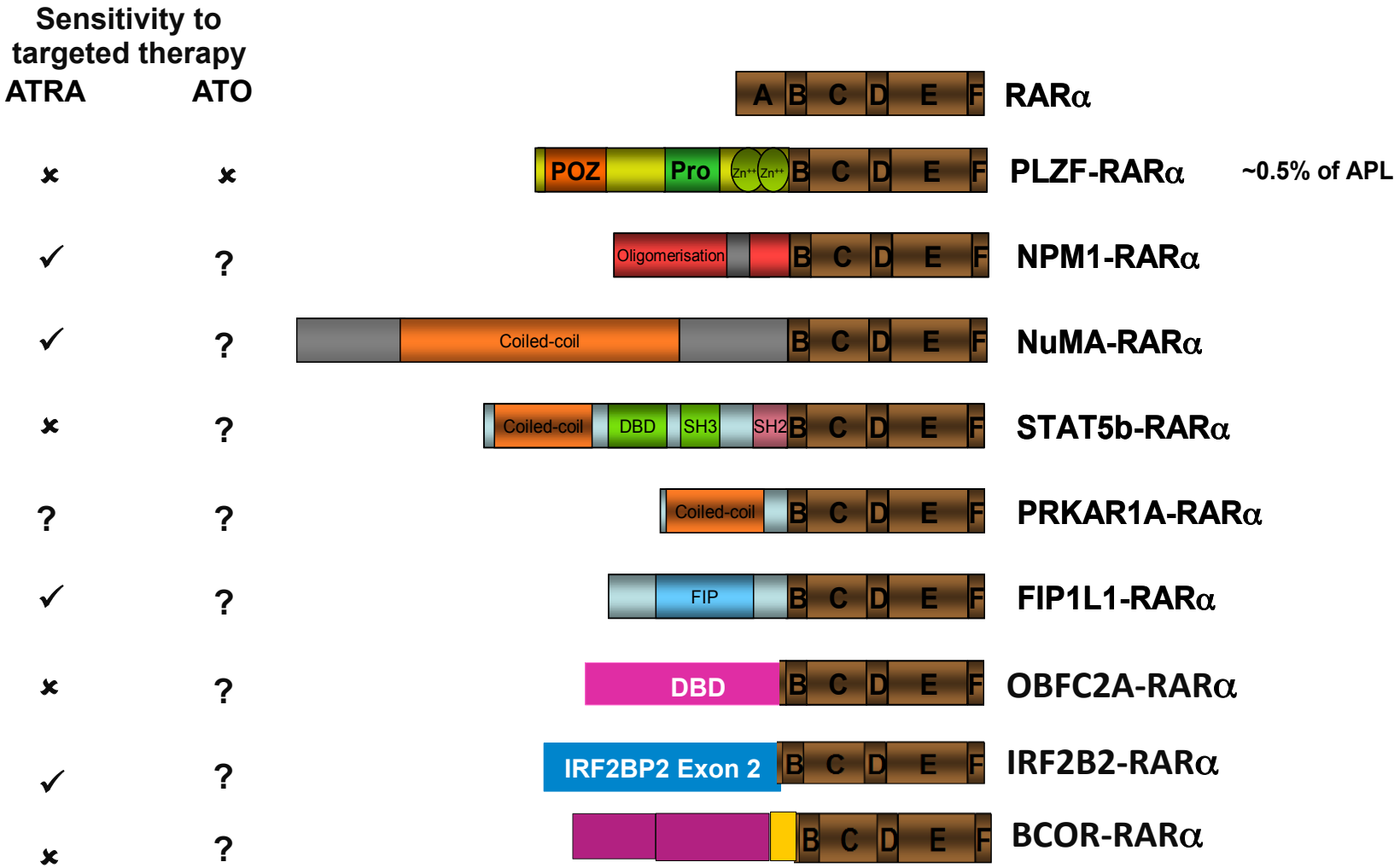
JM Van Dongen et al, Leukemia 1999

Recommended diagnostic work-up for APL

Sanz et al. (LeukemiaNet) Blood 2009; BSH guidelines, BJH 2006

- Diagnostic confirmation at genetic level is mandatory: PML-RARα predicts response to ATRA and/or ATO
- FISH or PML staining allow rapid diagnosis (valid for pt eligibility for ATRA- or ATO-based protocols).They do not define the type of PML/RARα fusion
- Always send sample to a reference molecular biology lab for RT-PCR of PML/RARα (required as baseline for MRD)

Gene fusions in APL variants: Implications for targeted therapy



Grimwade *et al*. Cancer Treat Rep. 2009; Yian et al. Natl Comp Canc Netw 2015; Yamamoto Y, Blood 2010

Outline

- Clinical & biological background
- Diagnosis
- Monitoring of minimal residual disease

The PML/RARα fusion protein

Why so important in diagnosis and treatment

- Unique to APL
- Strongly correlated with pathogenesis
- Targeted by specific therapies
- Detection predicts response to ATRA & ATO
- Ideal marker for residual disease monitoring

SPECIAL ARTICLE

Revised Recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia

By Bruce D. Cheson, John M. Bennett, Kenneth J. Kopecky, Thomas Büchner, Cheryl L. Willman, Elihu H. Estey, Charles A. Schiffer, Hartmut Doehner, Martin S. Tallman, T. Andrew Lister, Francesco Lo-Coco, Roel Willemze, Andrea Biondi, Wolfgang Hiddemann,

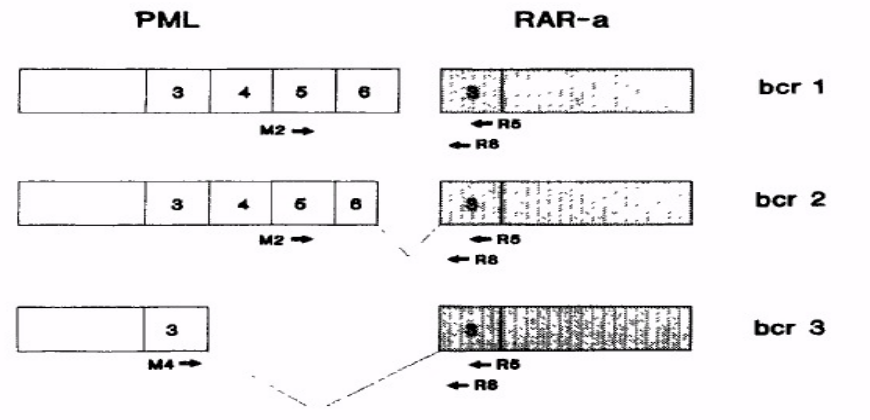
Table 1. Revisions in Current AML Guidelines

1. Recommendations for storage of viable blasts
2. Definitions of de novo and secondary AML
3. Implications of dysplasia
4. Use of WHO definitions
5. Importance of flow cytometry
6. Prognostic relevance of bone marrow cytogenetics and molecular genetics (eg, FLT-3 mutations and PTD of *MLL* gene)
7. Molecular remission of APL
8. Indications for central pathology review
9. Leukemia-free state as a response criterion
10. CRc and CRm as response criteria
11. 1,000/μL neutrophils as threshold for CR
12. Elimination of 4-week requirement for CR
13. Relapse requiring 5%-20% blasts in the bone marrow
14. No requirement for cellularity in CR definition
15. Late MDS as criterion for recurrence
16. RFS and OS as primary end points

Cheson *et al.* J Clin Oncol 2003

PCR monitoring in APL: early studies

Molecular evaluation of residual disease as a predictor of relapse in acute promyelocytic leukaemia

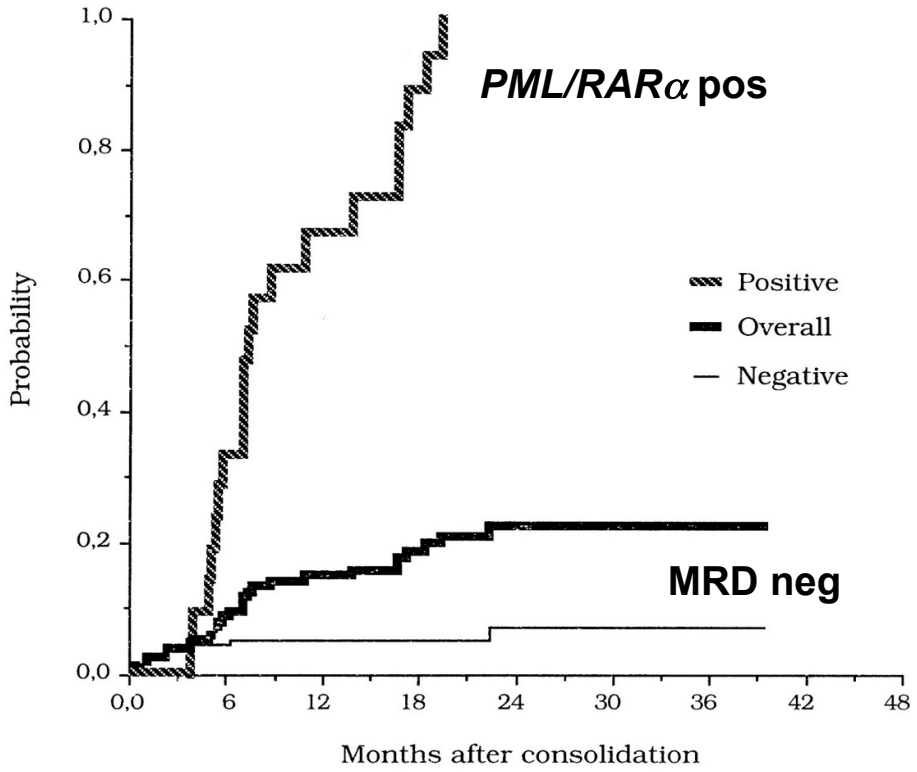


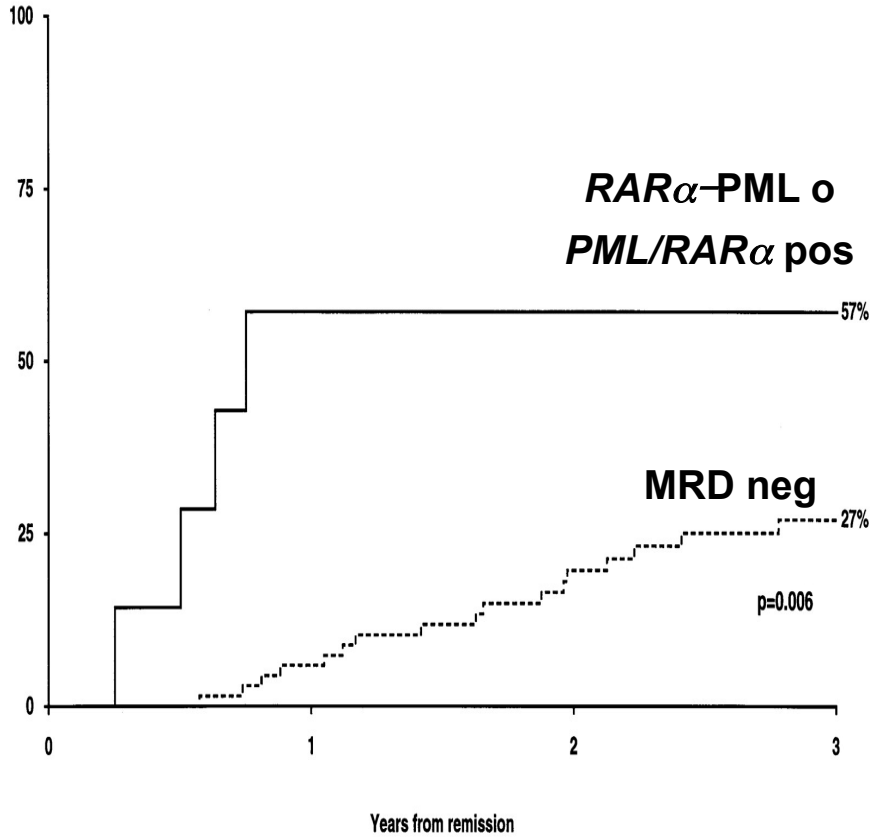
- ATRA therapy alone did not induce molecular remission
- Patients treated with ATRA+CHT in long-term clinical remission had consecutively negative RT-PCR tests

Miller et al.Proc Natl Acad Sci USA 1992 ; Lo-Coco *et al.* Lancet 1992; Martinelli G BJH 1995; Laczika K et al. Leukemia 1994; Grimwade et al , Leukemia 1996.

Risk of relapse according to RT-PCR status

Post-consolidation time point

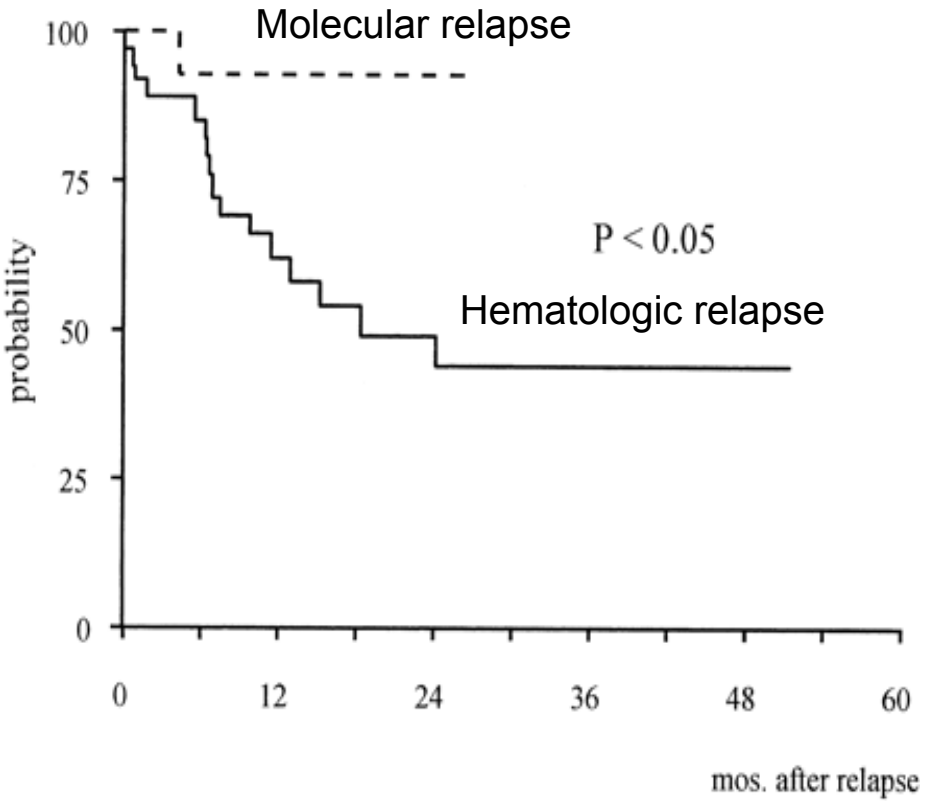




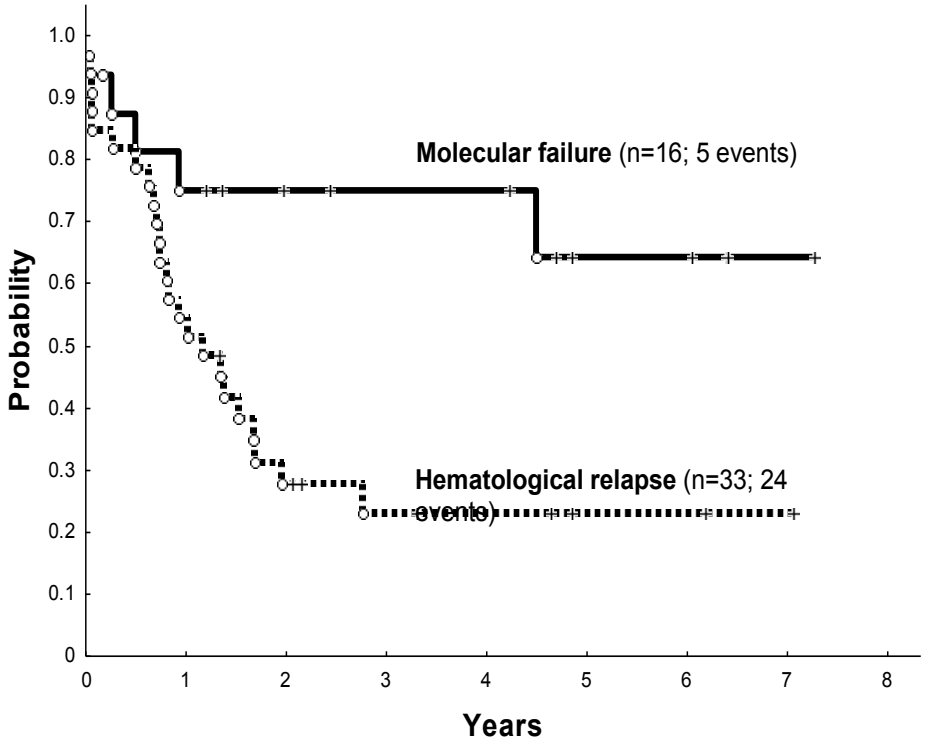
Diverio, et al. Blood 1998; Burnett et. Al, Blood 1999

Salvage Therapy for Relapsed APL

Treatment in molecular vs hematologic relapse



Lo-Coco *et al*. Blood, 1999

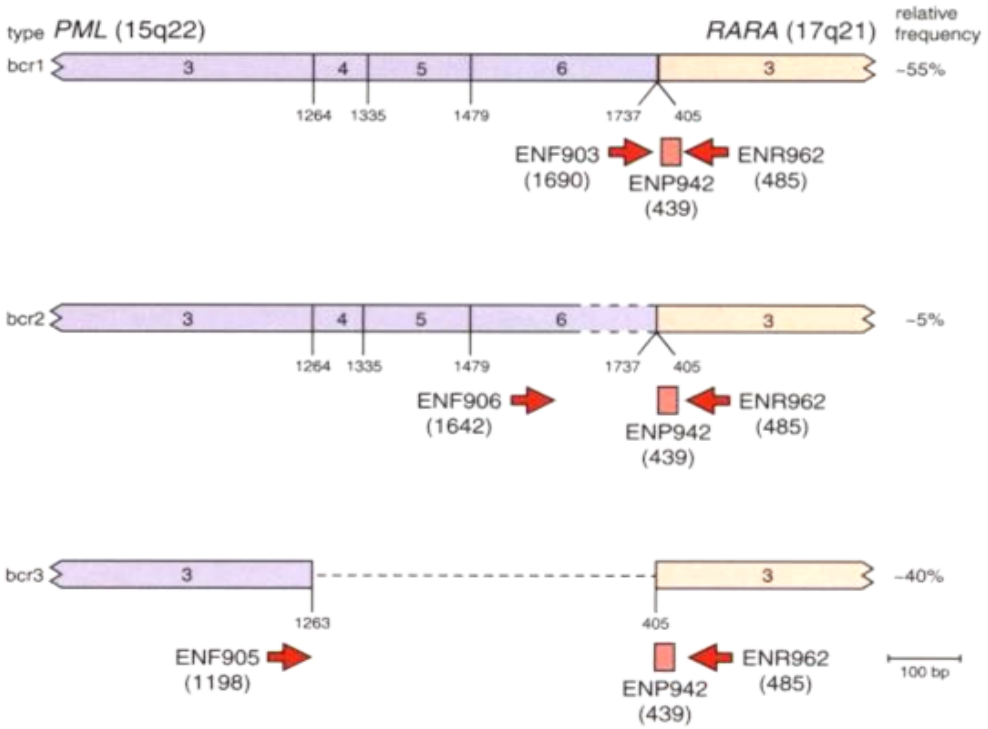


Esteve *et al*. Leukemia, 2007

Real-time Q-PCR for PML-RARA (EAC)

LEADING ARTICLE

Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia – A Europe Against Cancer Program



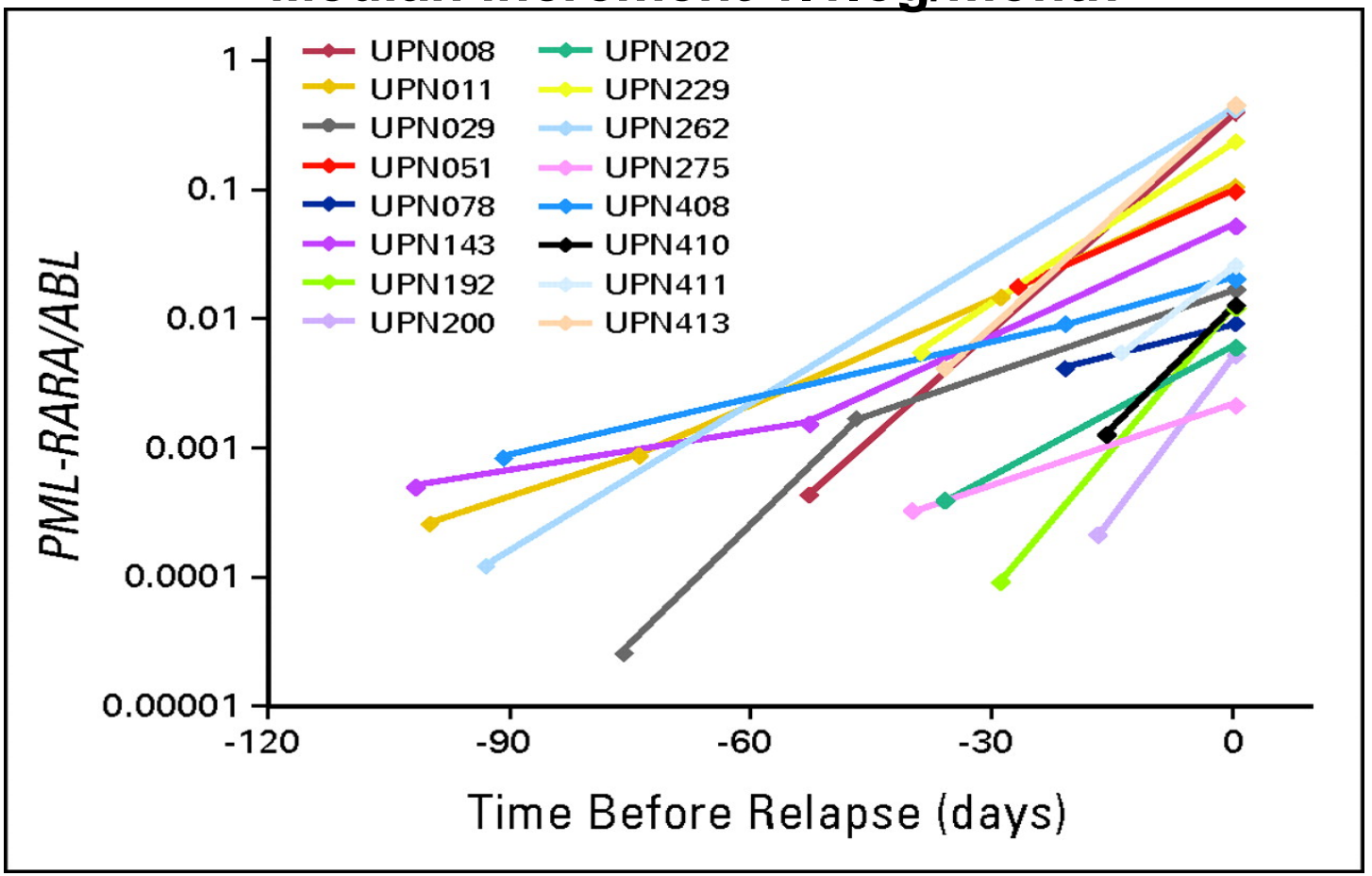
- Sensitivity
- Less prone to contaminations
- Reliable results in low-quality samples

Gabert *et al*, Leukemia 2003

Kinetics of molecular/frank relapse in APL

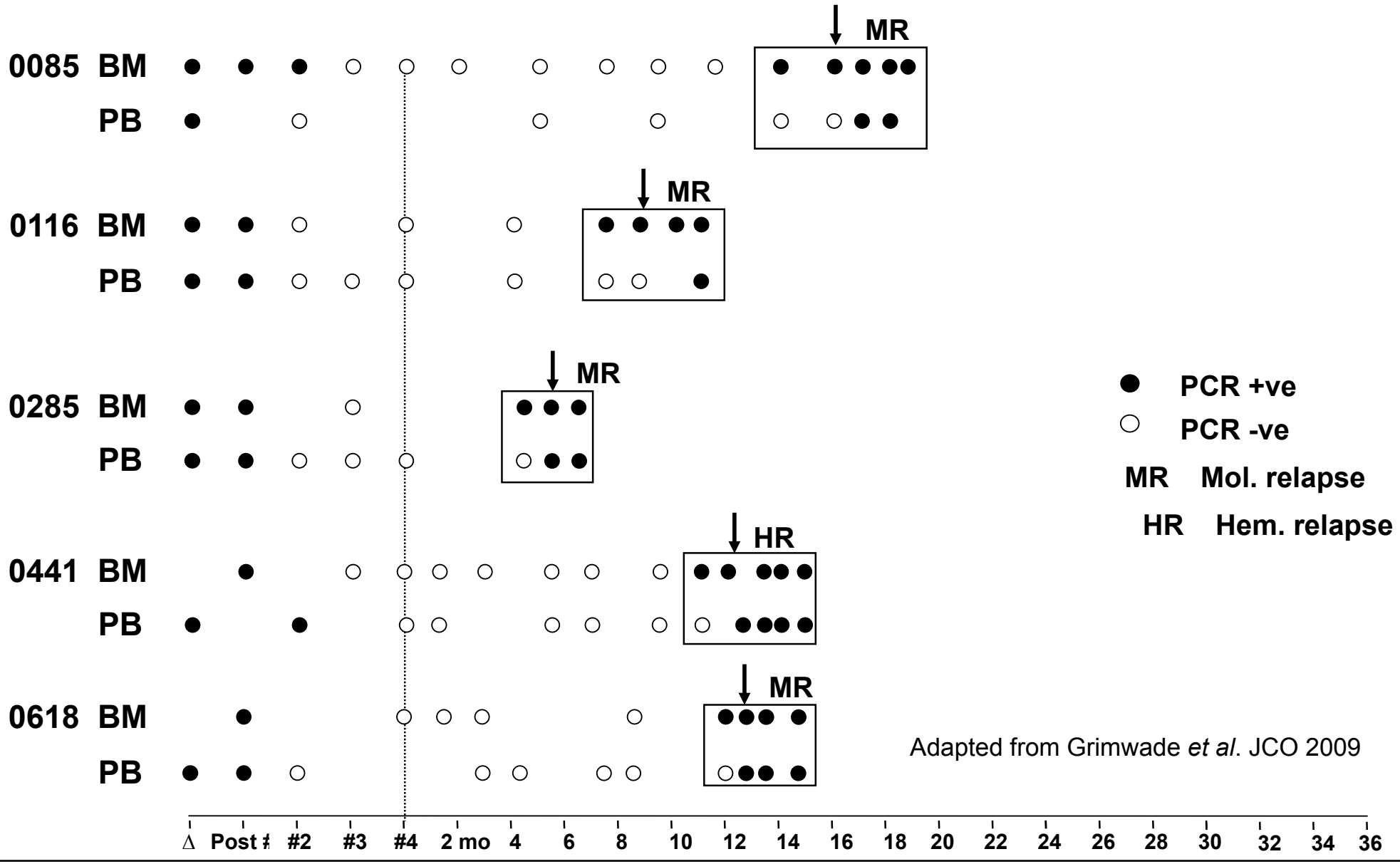
Implications for optimal sampling time-points

Median increment 1.1log/month



Grimwade *et al*. JCO 2009

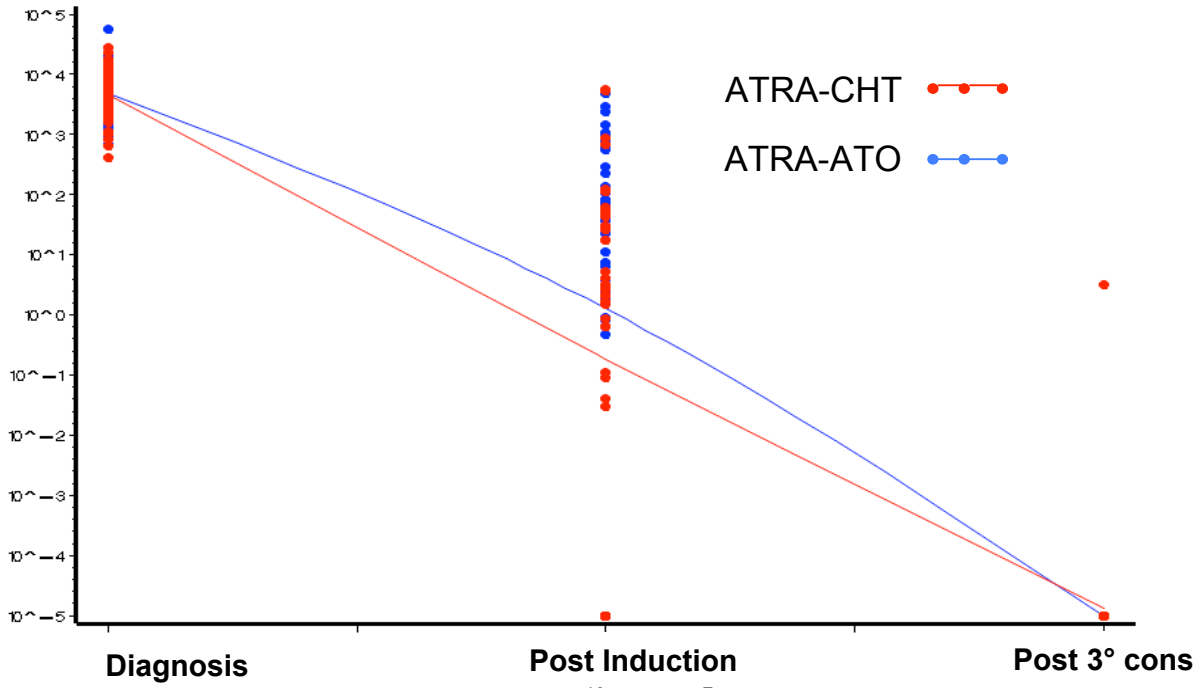
Sampling source: blood or marrow for MRD monitoring?



Adapted from Grimwade *et al*. JCO 2009

Q-PCR in ATO-ATRA treated patients

Effective clearance of PML-RARA



Log-reduction PML/RARα				
	Diagn-post ind	P-value	Post ind- post cons	P-value
ATRA-ATO	2.94	0.018	6.34	0.0024
ATRA-CHT	3.43		5.33	

- Similar clearance in ATO-ATRA and ATRA-CHT
- Low relapse % in ATO-ATRA pts questions cost-effectiveness of MRD monitoring

Cicconi *et al.* Leukemia 2016

Summary of Recommendations for Molecular Monitoring of APL

- Send samples to reference labs experienced with molecular testing of this rare disease
- Use of standardized reference methods (Gabert et al Leukemia 2003; Grimwade et al. J Clin Oncol 2009)
- Bone marrow sampling more informative than PB
- RQ-PCR advantageous over RT-PCR to better assess sample quality and to investigate kinetics of MRD

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Prof. MT Voso
Prof. W. Arcese

Prof. A Venditti
Prof. S. Amadori
M. Consalvo
P. Panetta
P. Curzi
D. Fraboni