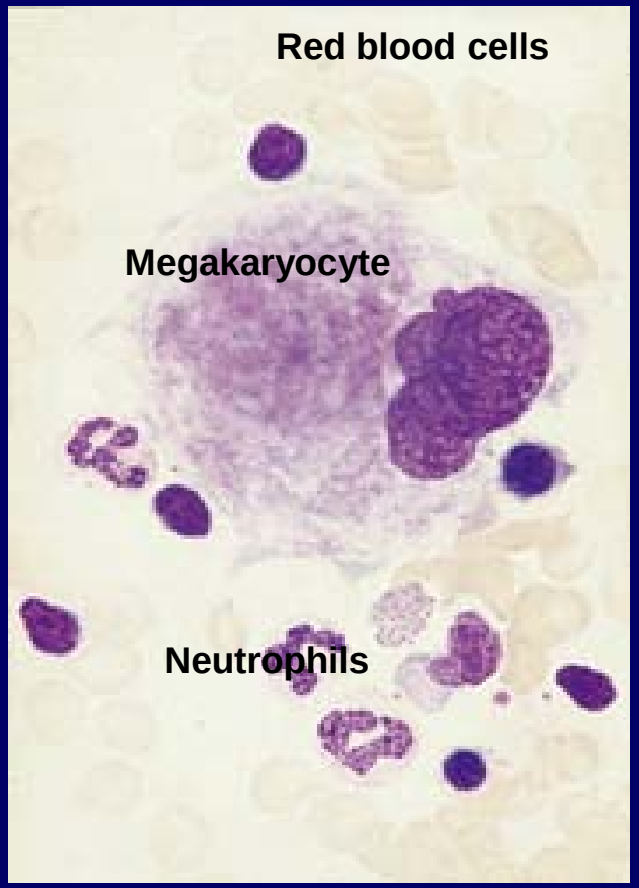
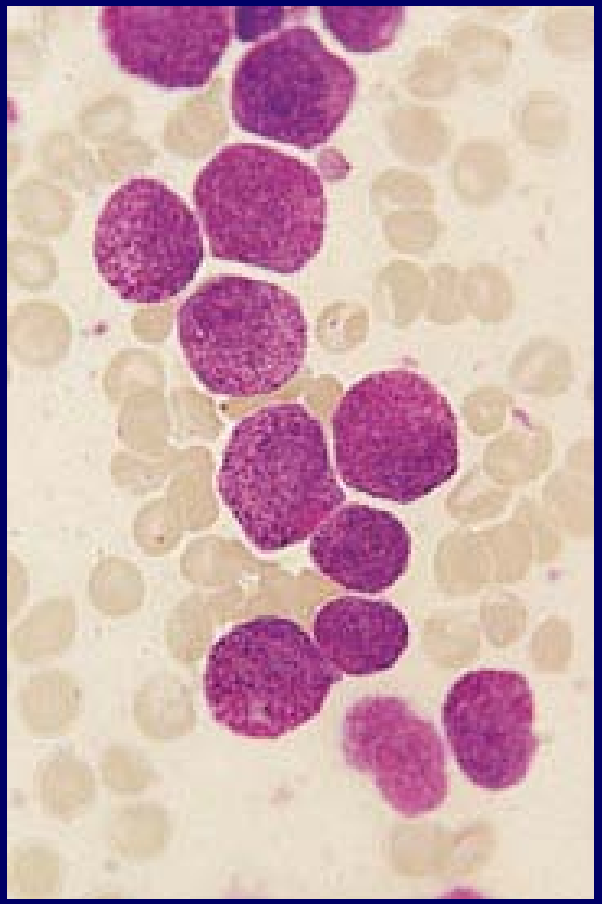
Acute promyelocytic leukaemia (APL)



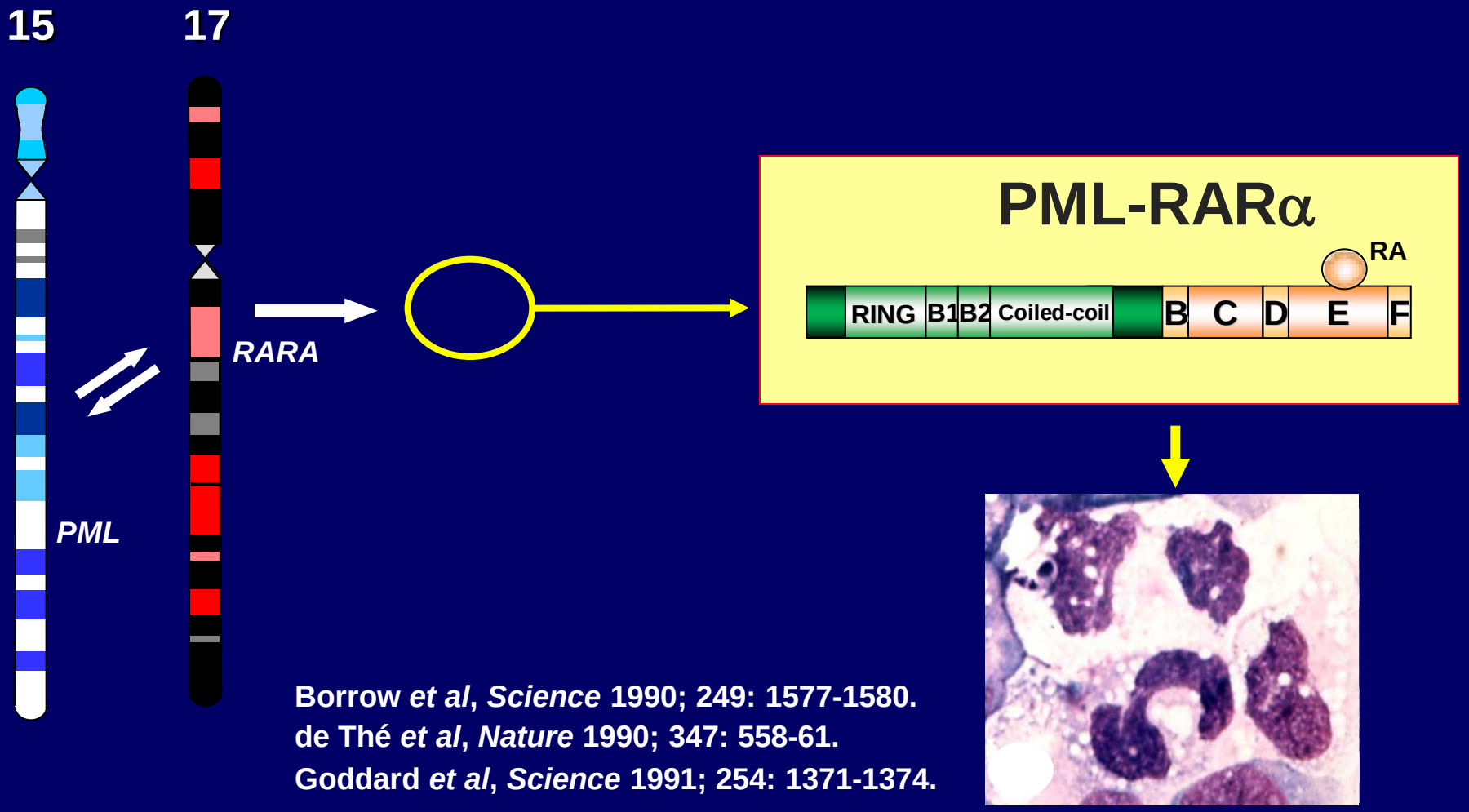
Normal bone marrow



Leukaemic bone marrow (APL)

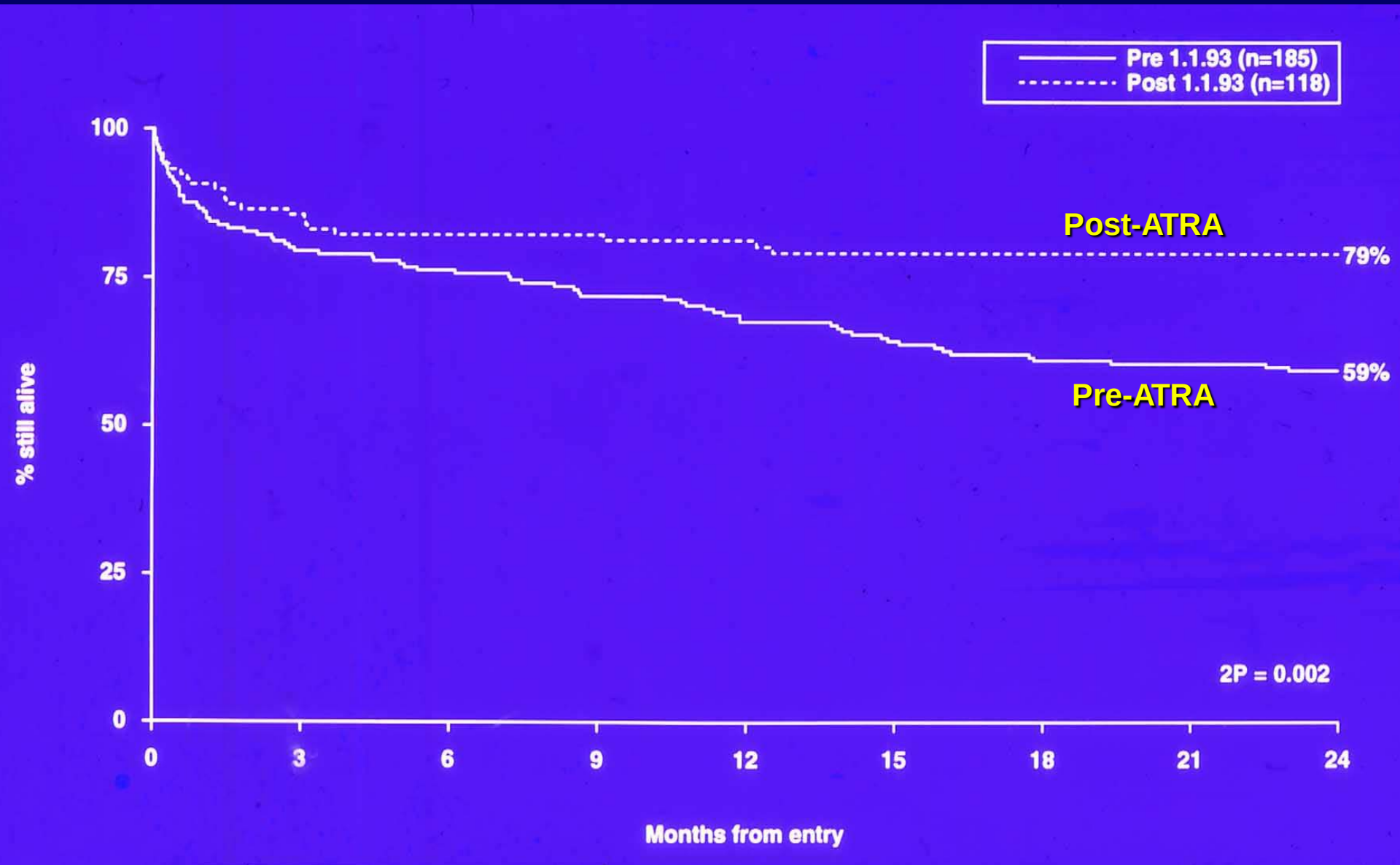
First success for molecularly targeted therapy:

Targeting of PML-RARα oncoprotein formed by t(15;17) in APL

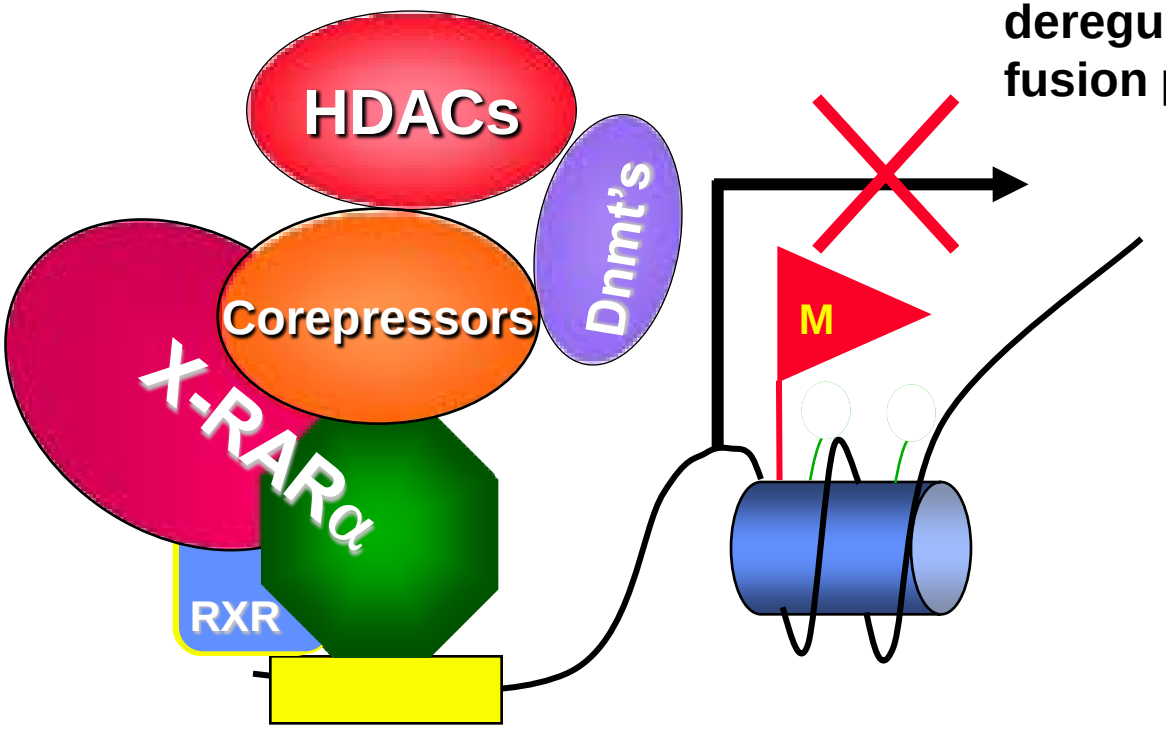


Borrow *et al*, *Science* 1990; 249: 1577-1580.
de Thé *et al*, *Nature* 1990; 347: 558-61.
Goddard *et al*, *Science* 1991; 254: 1371-1374.

Impact of molecularly targeted therapy – all *trans*retinoic acid (ATRA) on outcome in APL



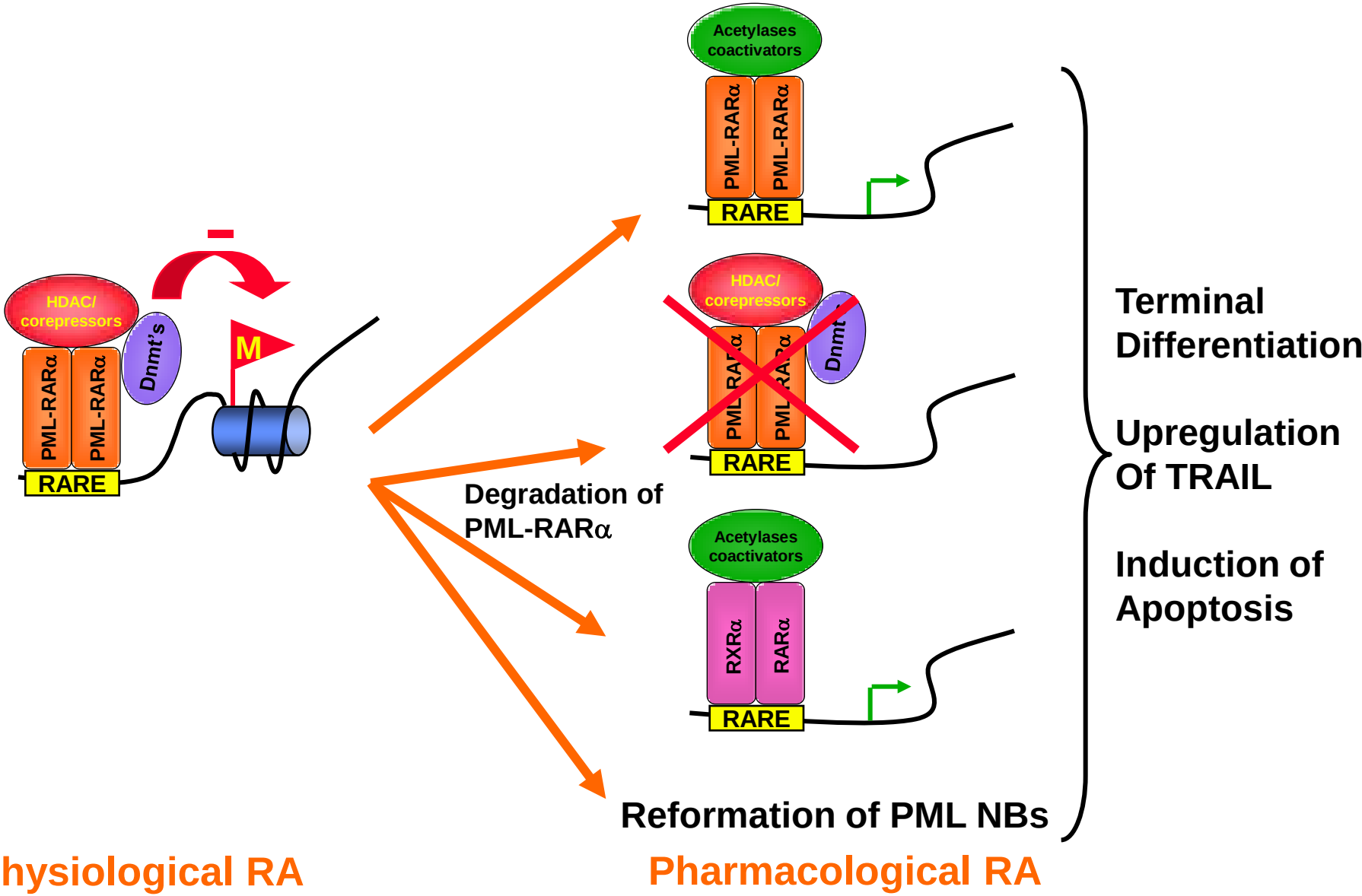
Repression of target genes implicated in myeloid differentiation as a common mechanistic theme in APL



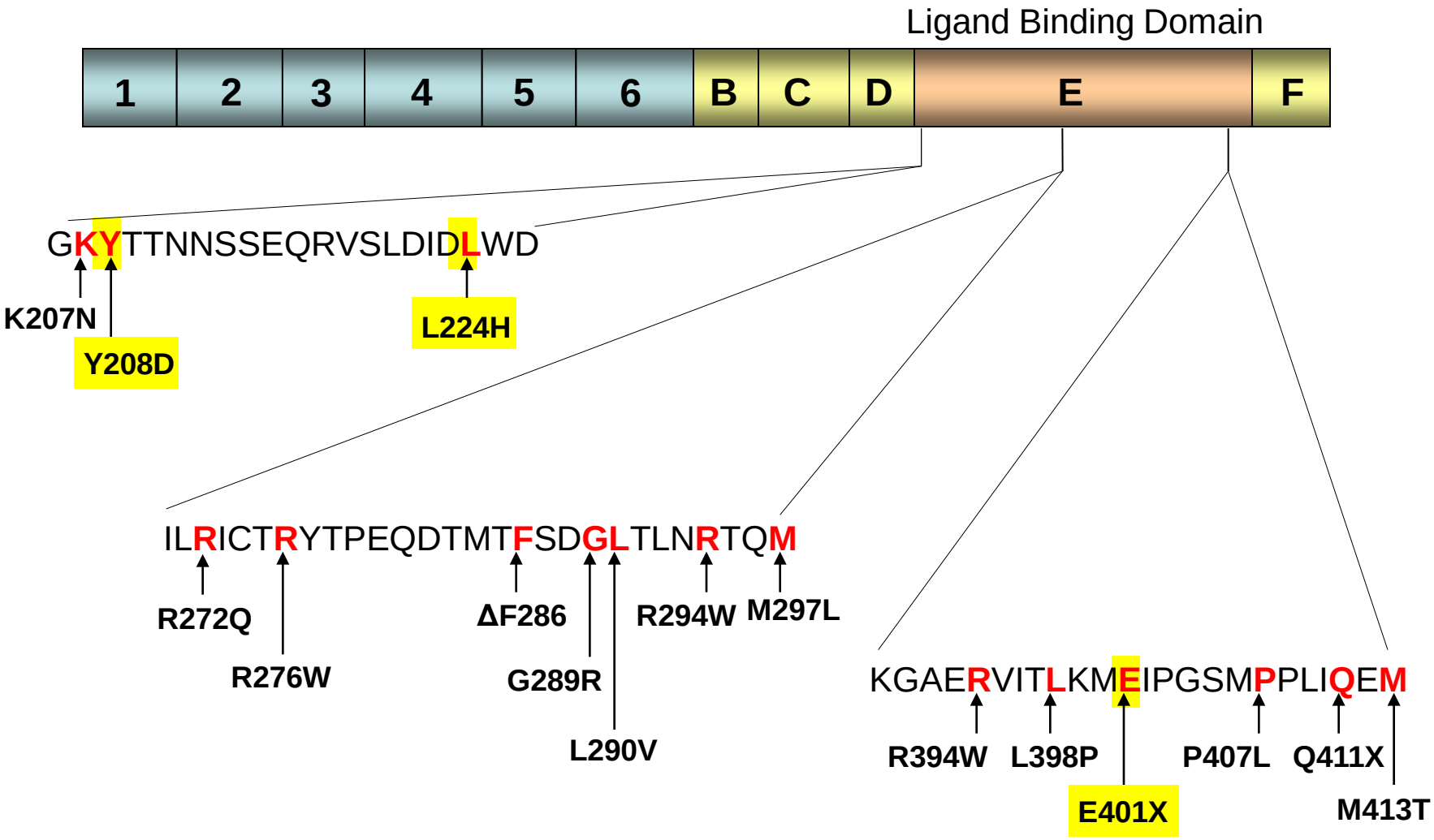
Genes involved in haematopoietic differentiation potentially deregulated by APL-associated fusion proteins

Hox
C/EBPβ
RARβ2
p21$^{WAF/CIP}$

Mechanisms of ATRA activity in PML-RARα associated APL



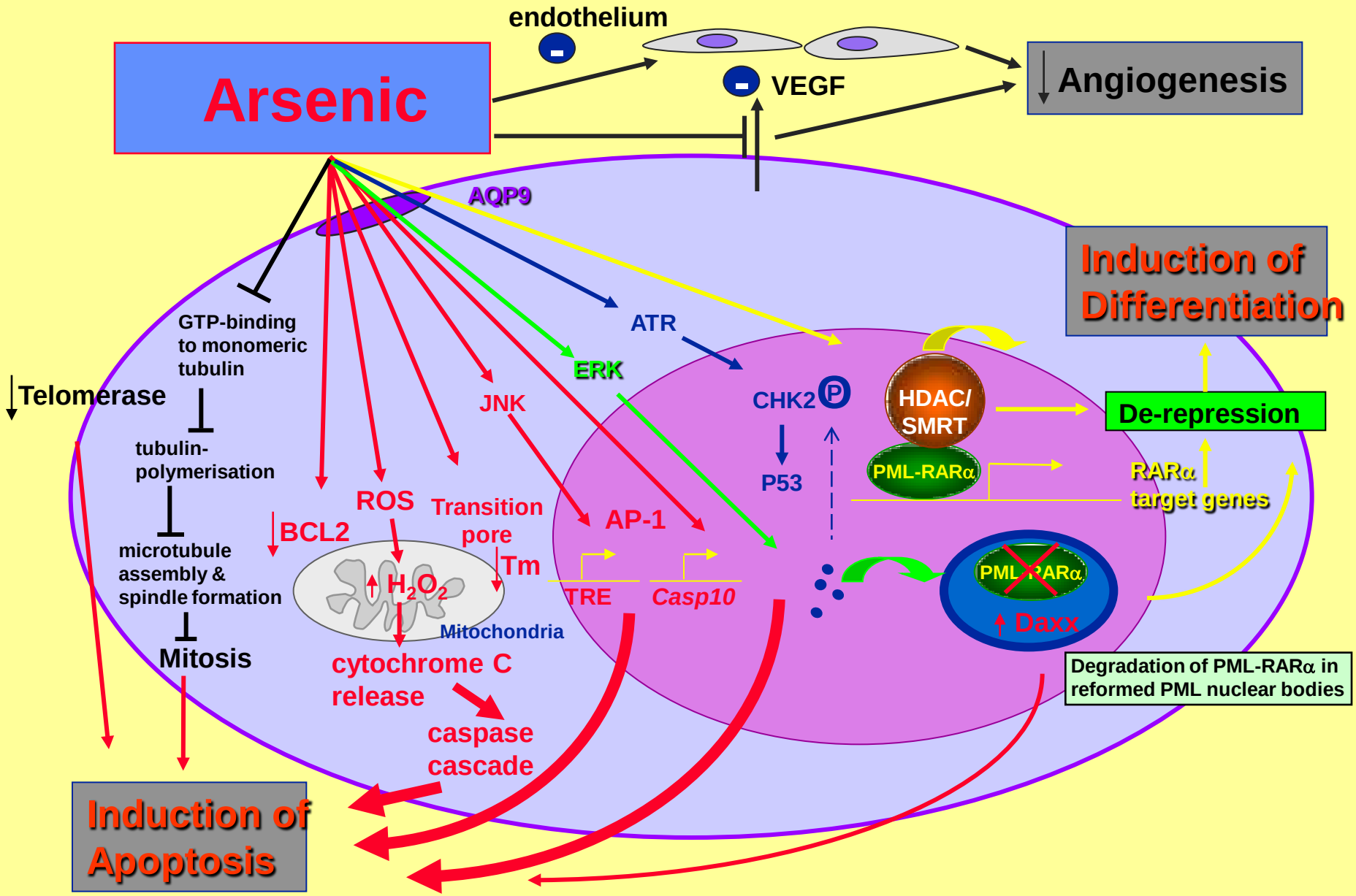
Mutations in PML-RARα Ligand-Binding Domain (LBD) Found in ATRA-resistant Cell Lines and/or APL Patients



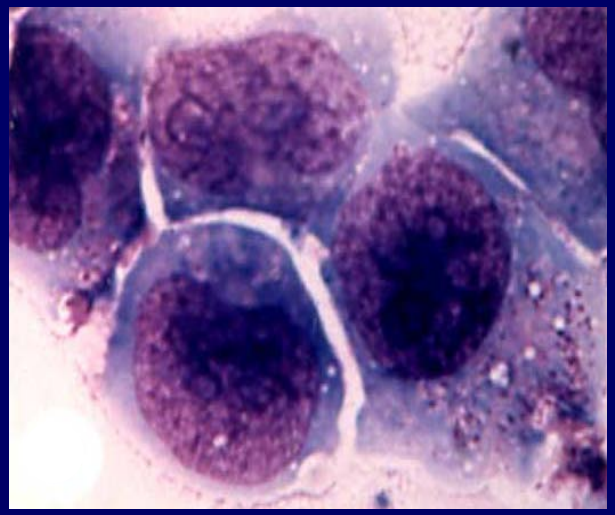
Arsenic activity in APL

- **Effective in PML-RARα associated APL, including RA resistant cases**
- ***In vivo* response reflects *2* dose dependent activities:**
  - **Apoptosis (0.5-2μM)**
    - **Triggered by alteration in mitochondrial membrane potentials & transition pore permeability**
      - **dithiol oxidation or cross-linking**
      - **Induction of reactive oxygen species**
  - **Partial differentiation (0.1-0.5μM)**

Potential mechanisms of arsenic activity in APL

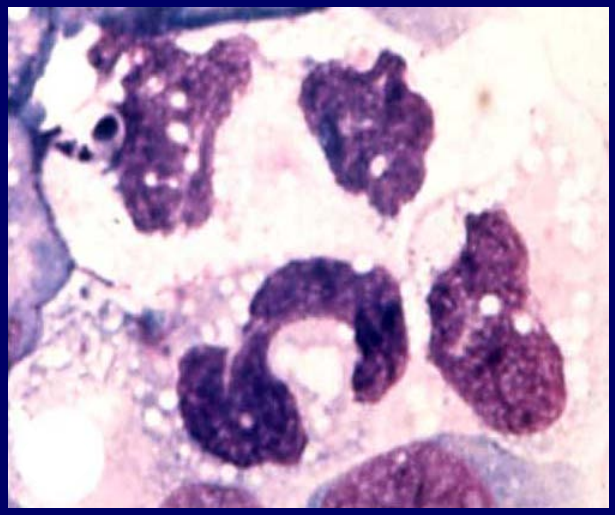


Impact of PML-RARα fusion protein on nuclear architecture and its reversal by targeted therapies

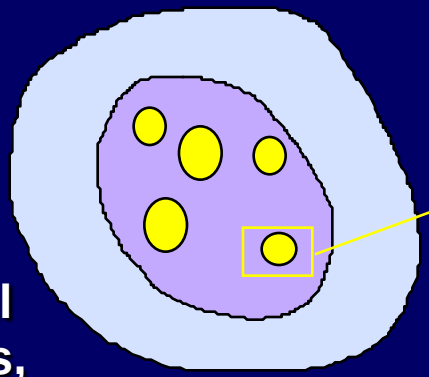


ATRA

Arsenic

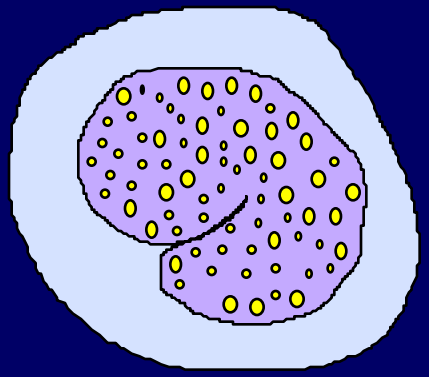


Disruption of PML nuclear bodies in t(15;17) associated APL

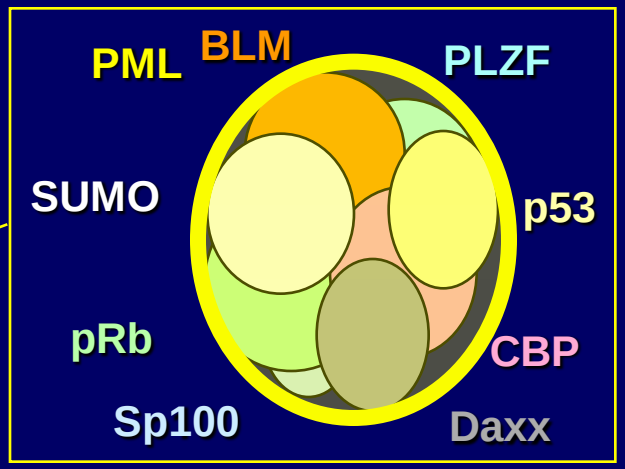


Normal tissues, Other leukaemias

ATRA
Arsenic

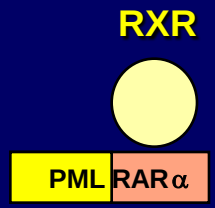


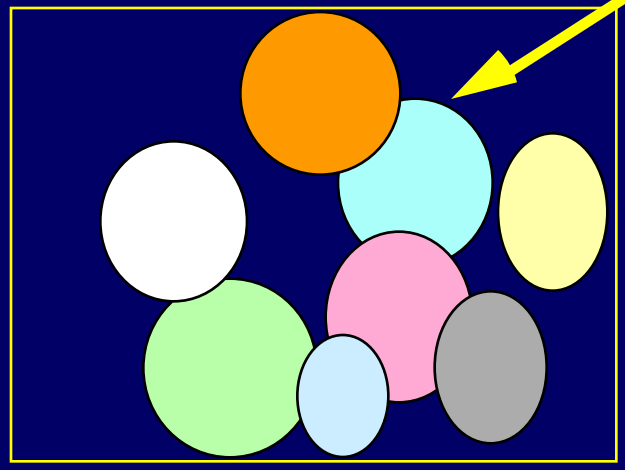
t(15;17) APL



NBs implicated in regulation of:

- Apoptosis
- Senescence
- Growth suppression
- Genomic stability



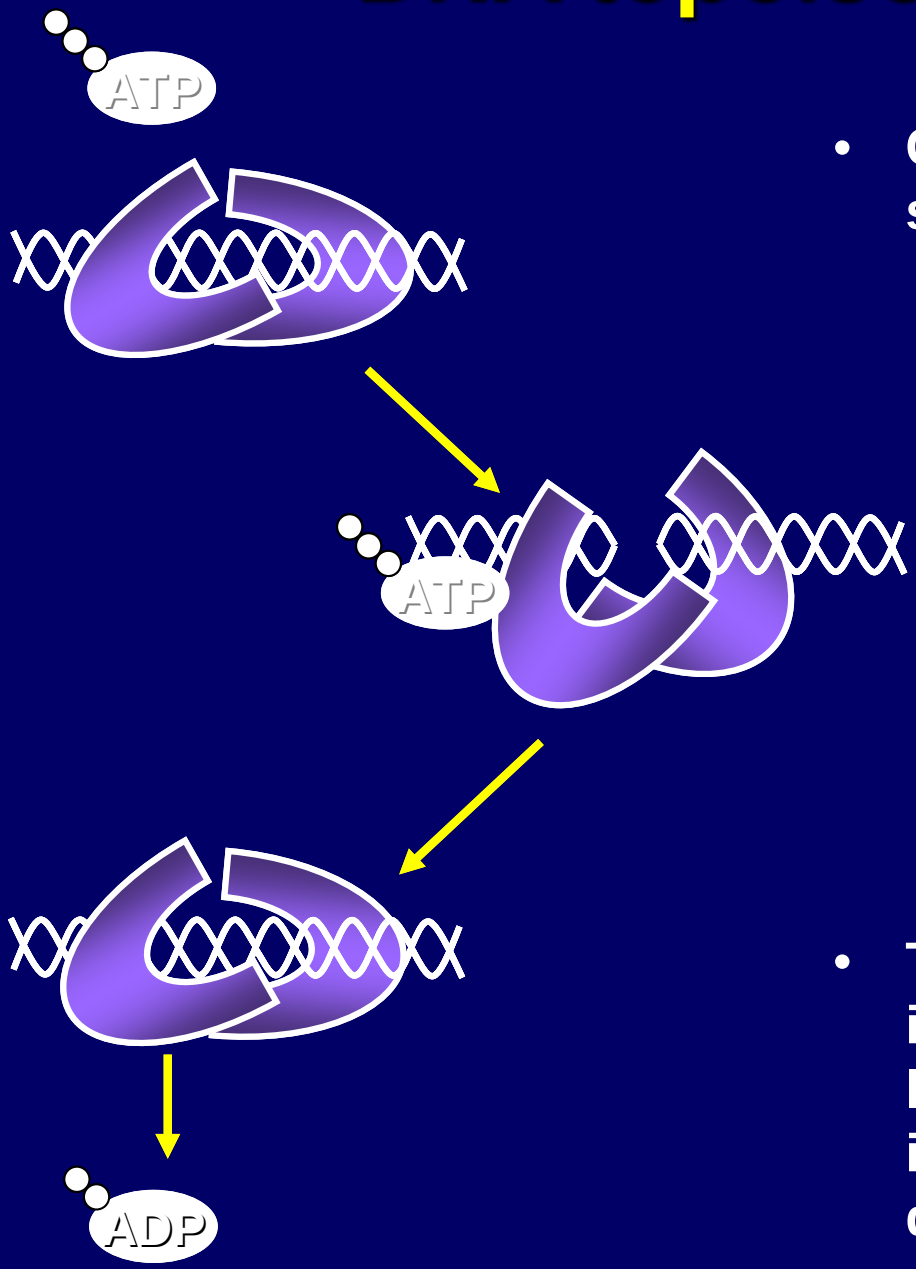


- Transcriptional repression
- Altered retinoid signalling
- Disruption of PML NBs
- Loss of PML growth suppressor & pro-apoptotic effects

Investigating mechanisms underlying formation of leukaemia-associated translocations

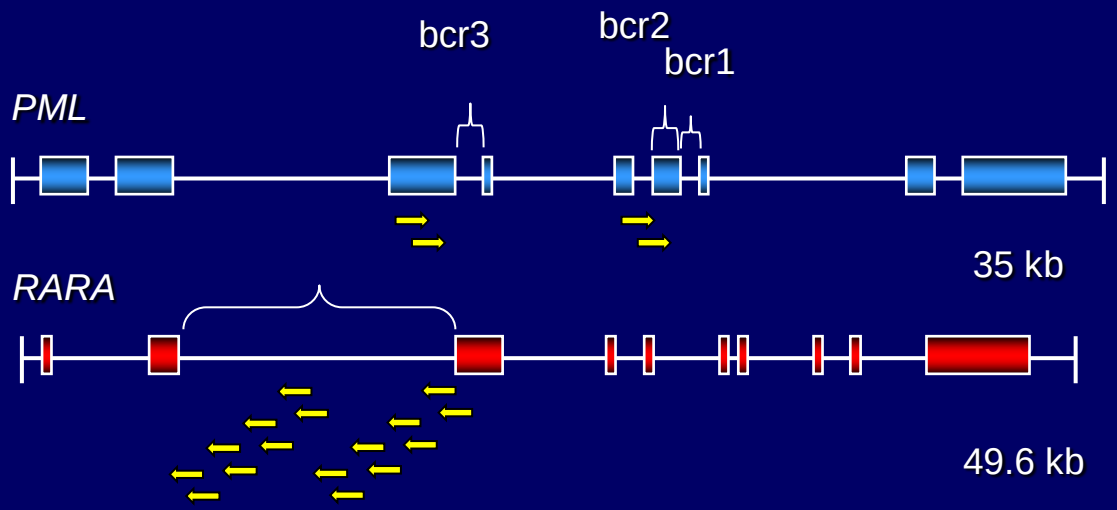
- Insights can potentially be gained from therapy-related leukaemias which have *de novo* counterparts
- Therapy-related leukaemias are becoming an increasing healthcare problem as more patients survive their primary cancers
- Incidence of therapy-related acute promyelocytic leukaemia (t-APL) with t(15;17) rising
- Balanced translocations arise following exposure to chemotherapeutic agents targeting DNA topoisomerase II
- Suggests a role for DNA topoisomerase II in forming translocations, but mechanisms controversial

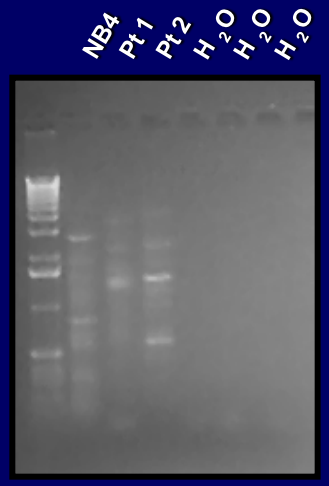
DNA topoisomerase II



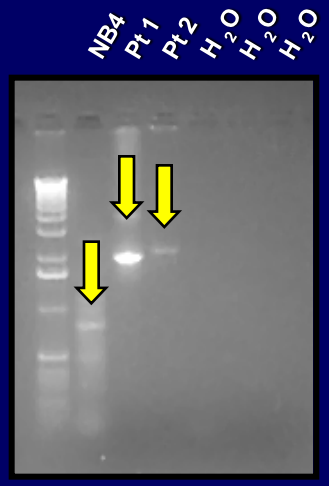
- Catalyses relaxation of supercoiled DNA
  - DNA topoisomerase II homodimer introduces 4-base staggered nicks in DNA as each subunit covalently binds and cleaves one strand
  - A second DNA double helix passes through the cleaved strands
  - Cleaved strands are then re-ligated
- Topoisomerase II-targeted drugs interfere with cleavage-re-ligation equilibrium → net increased cleavage → DNA damage → apoptosis

Characterisation of t(15;17) genomic breakpoint junctions in t-APL





1st Round of Nested PCR



2nd Round of Nested PCR

Patient Breakpoint Sequence

PML 1886

GGATTCCCATAGGTGCACACCCACACCCCTCCCAGCATGCATCCTAGGCAGTTCATAATG

Breakpoint Sequence

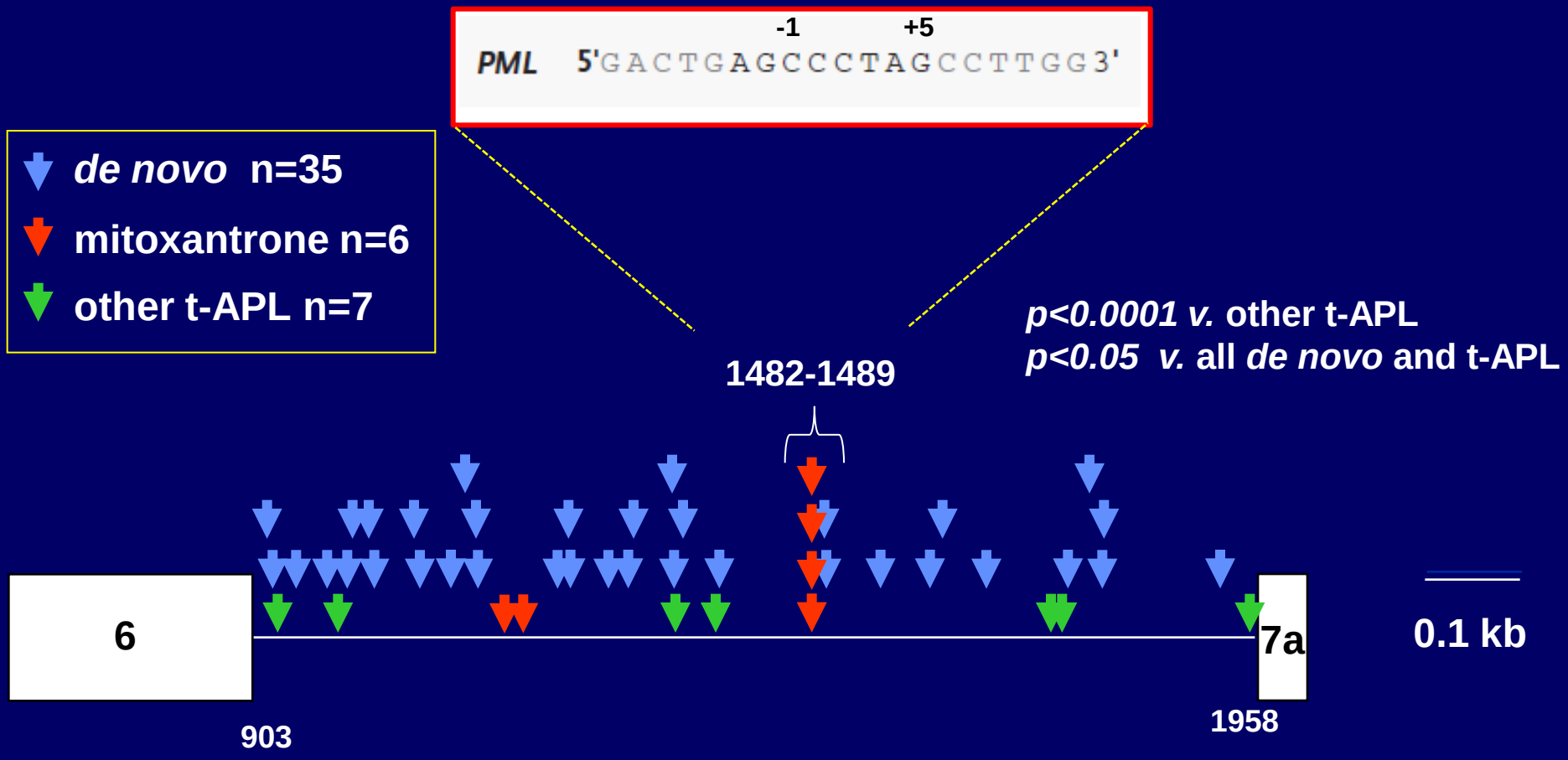
GGATTCCCATAGGTGCACACCCACACCCCTGATGGGGGGTACCCAGAATAATGGGCTTTT

RARA

AGACCCCCTTTGTCATGCCATCTCTCCCCAGATGGGGGGTACCCAGAATAATGGGCTTTT

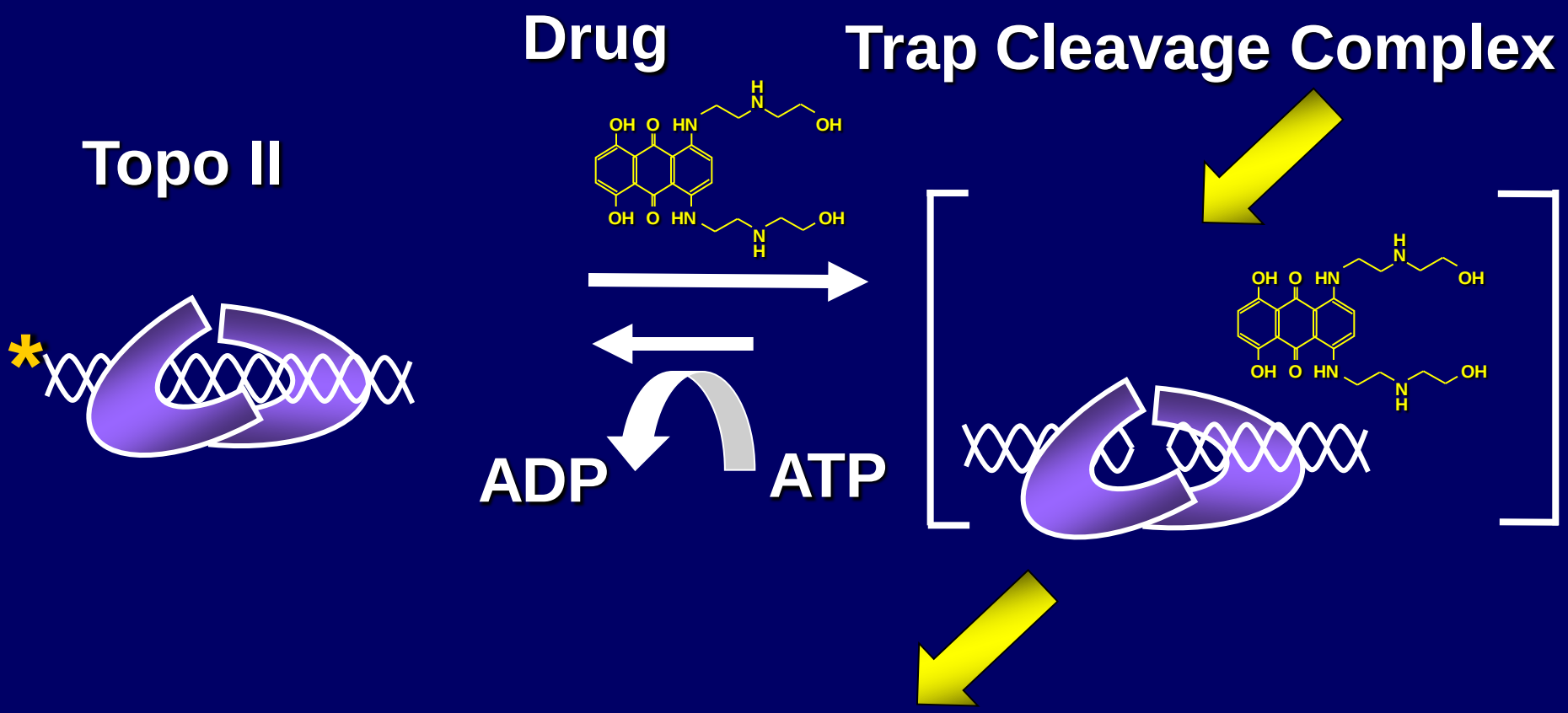
Ashley Mays

Defining mechanisms underlying AML as a complication of cancer therapy: An increasing healthcare problem

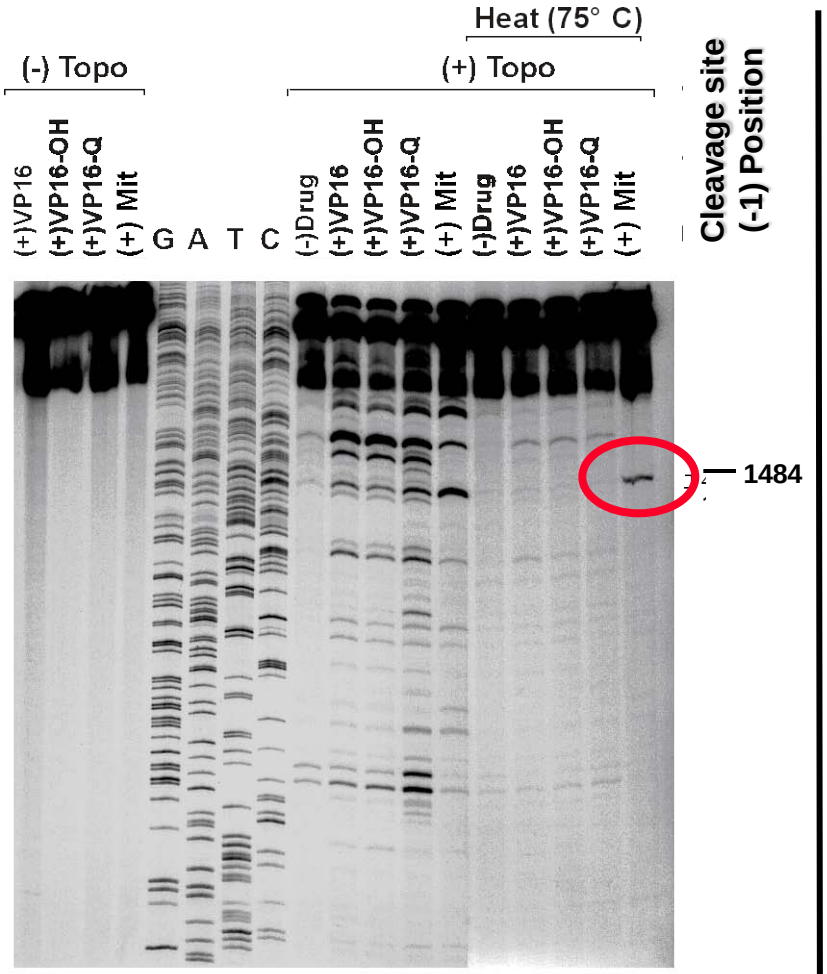


Mistry *et al*, *N Engl J Med* 2005; 352: 1529-1538

DNA topoisomerase II *in vitro* cleavage assay

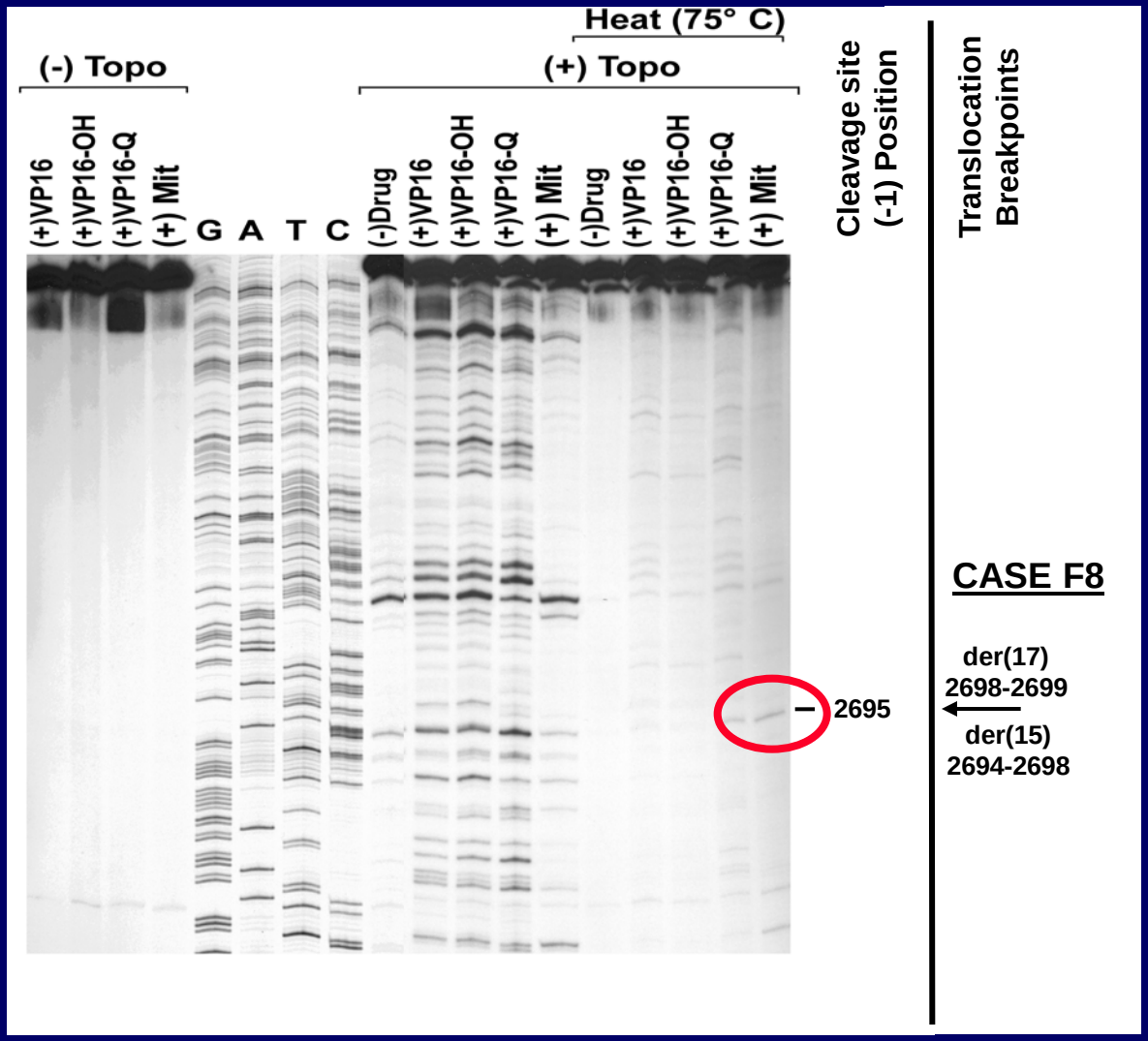


Mitoxantrone induces strong, heat-stable topoisomerase II cleavage at *PML* translocation breakpoint hotspot



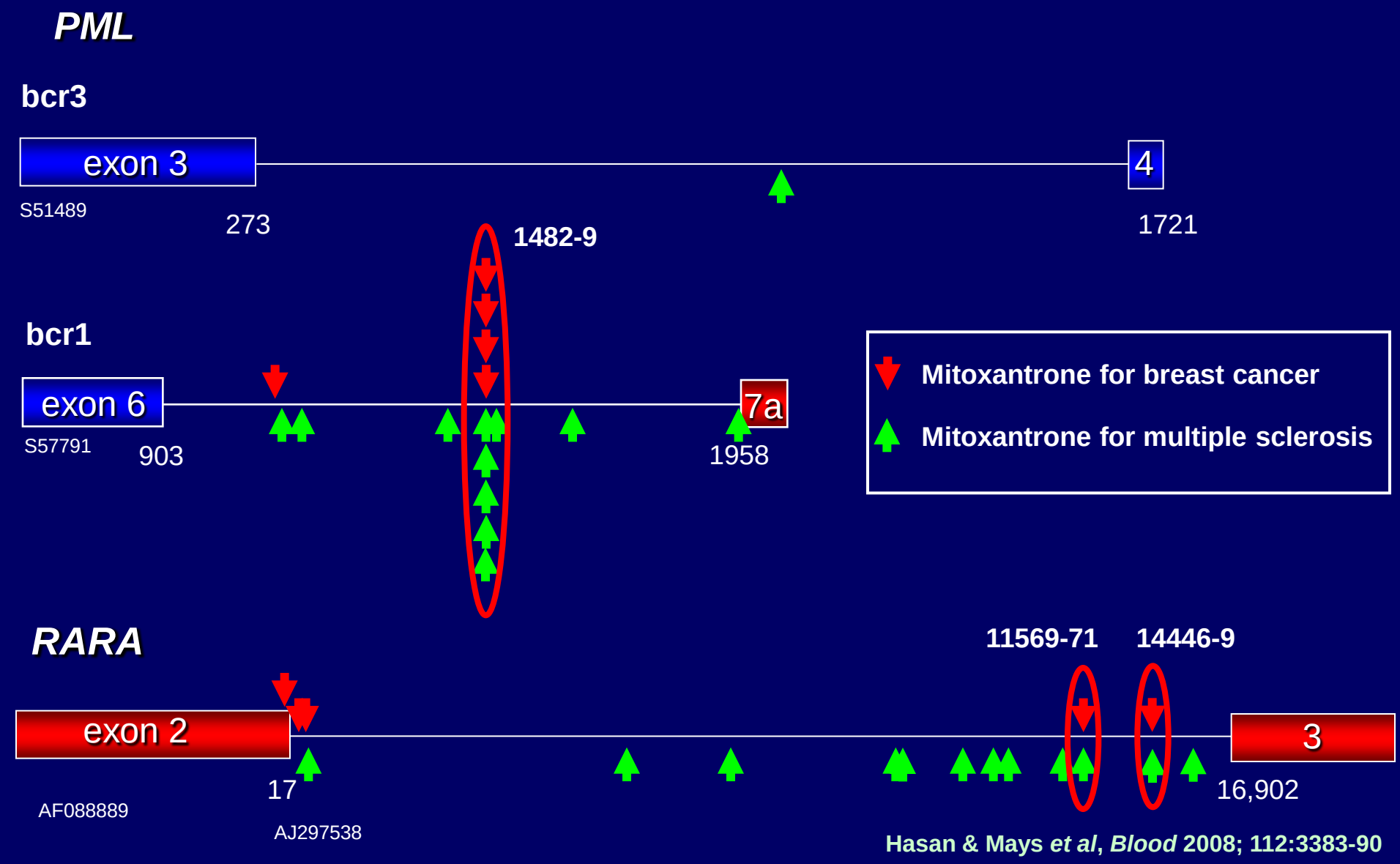
Mistry *et al*, *N Engl J Med* 2005; 352: 1529-1538

Mitoxantrone induces DNA topoisomerase II cleavage at *RARA* translocation breakpoints in t-APL



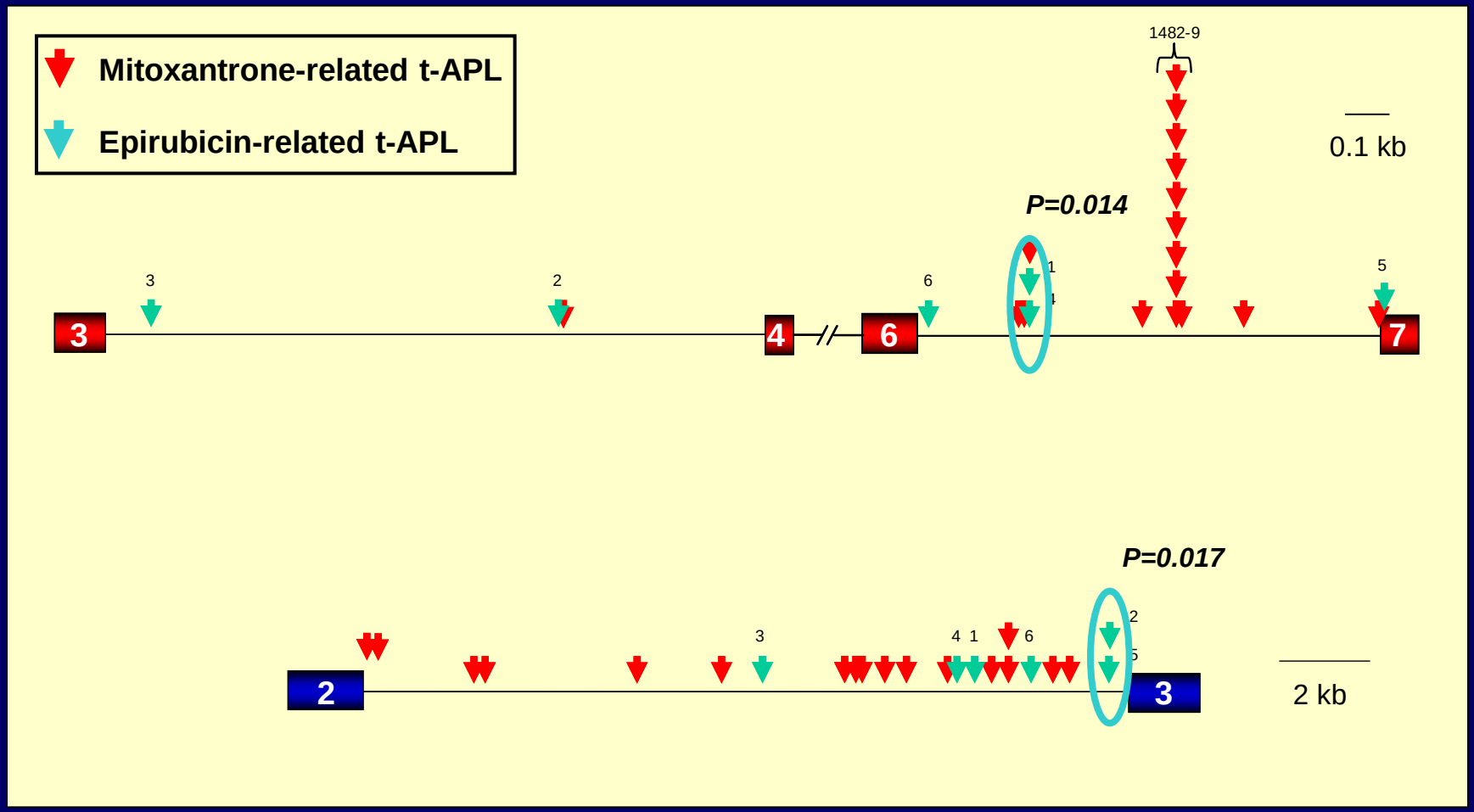
Mistry *et al*, *N Engl J Med* 2005; 352: 1529-1538

PML breakpoint clustering in t-APL arising after mitoxantrone treatment of malignant and benign conditions



Hasan & Mays *et al*, *Blood* 2008; 112:3383-90

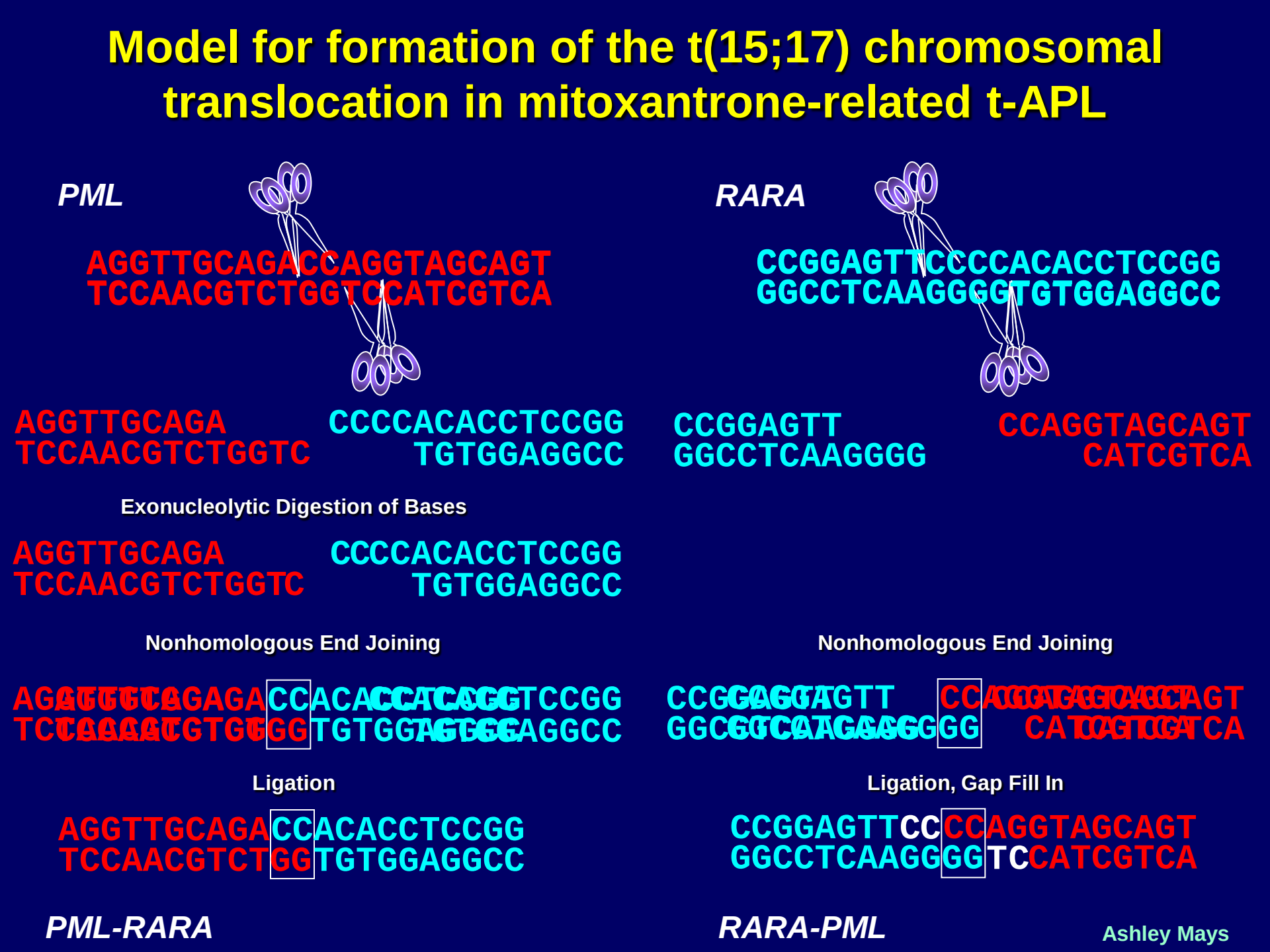
Genomic breakpoints in epirubicin-related t-APL cluster outside mitoxantrone hotspot



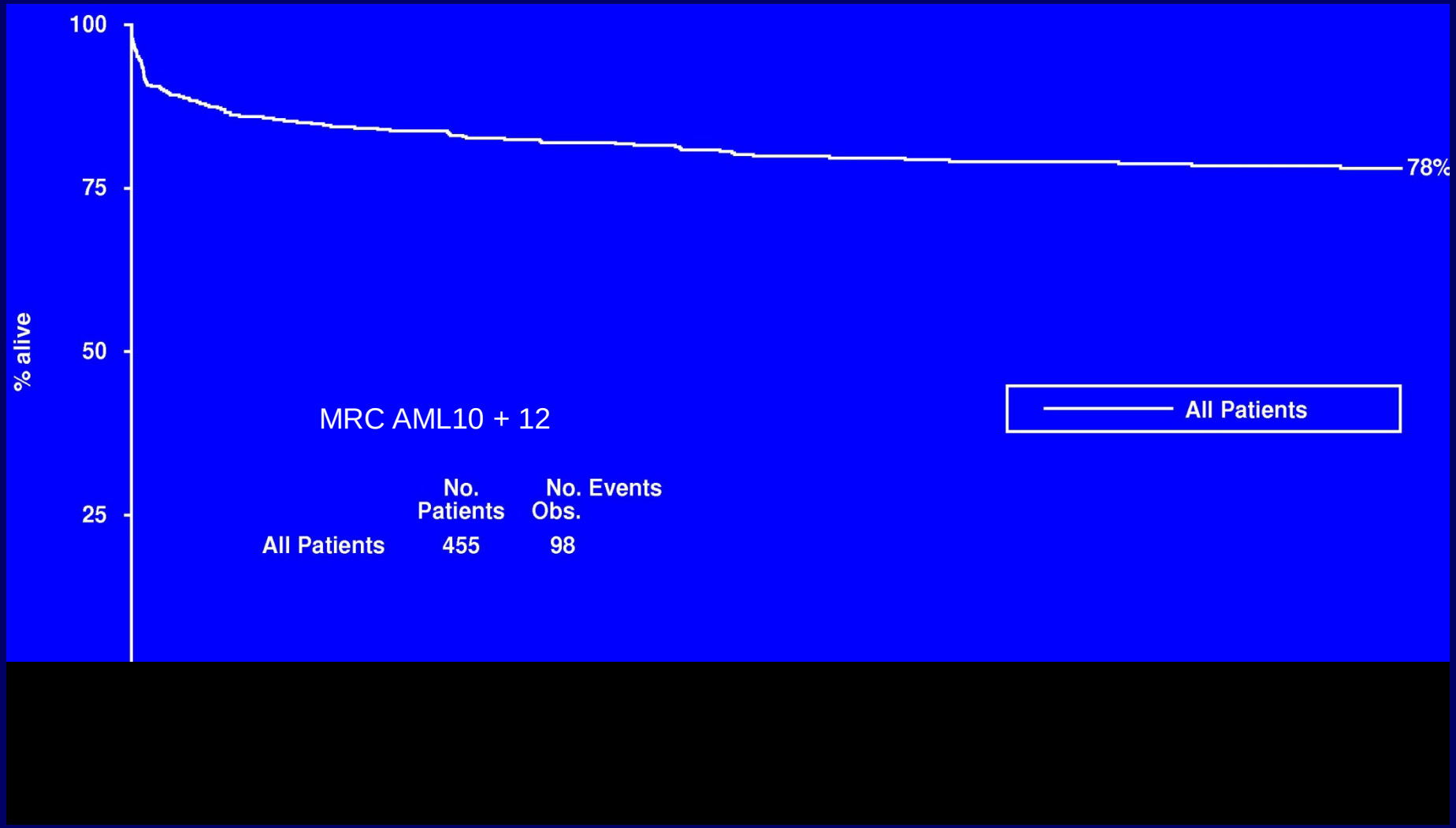
Leukaemia & Lymphoma Research
BEATING BLOOD CANCERS

Mays *et al*, *Blood* 2010; 115:326-30.

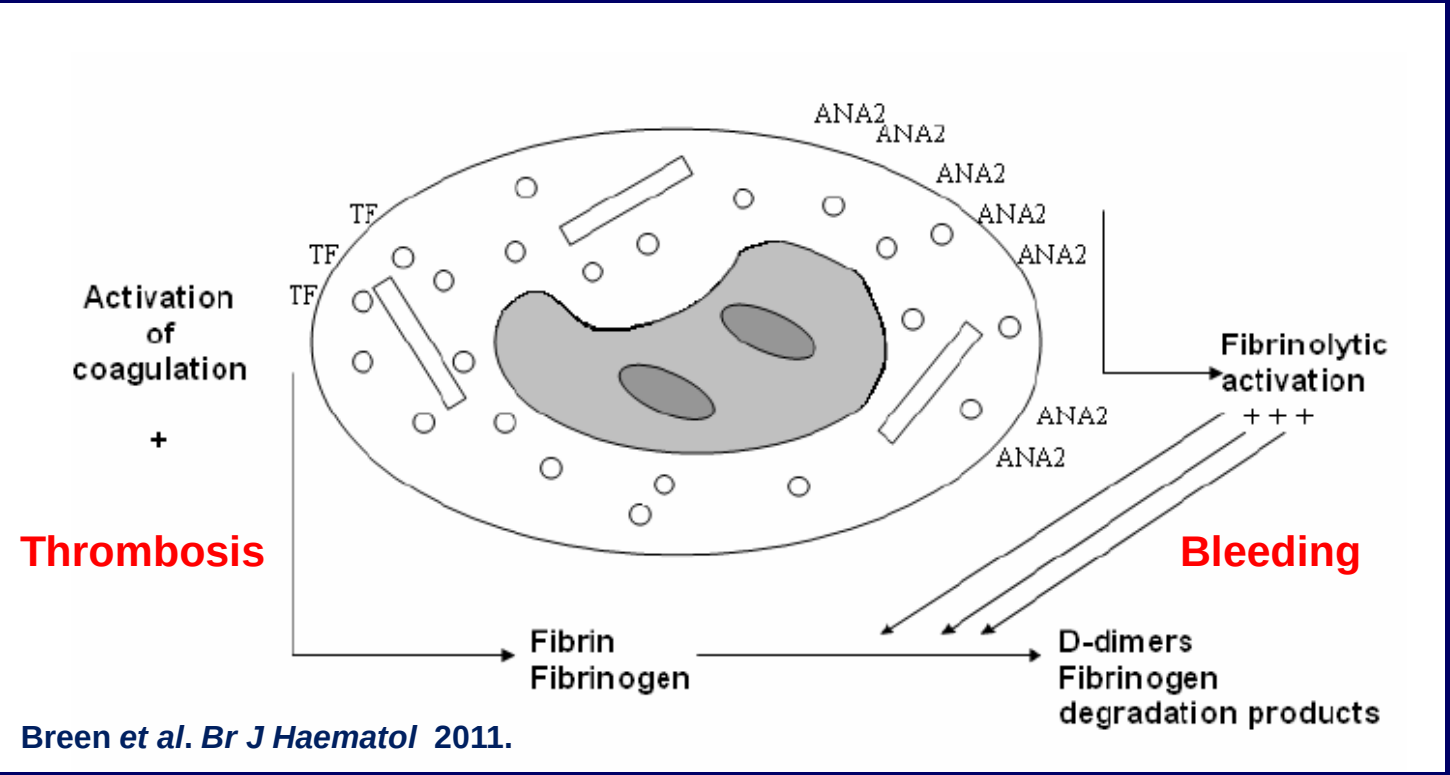
Model for formation of the t(15;17) chromosomal translocation in mitoxantrone-related t-APL



The challenge:
How to improve on outcome already achieved with ATRA & anthracycline-based chemotherapy?



APL coagulopathy



Breen *et al*. *Br J Haematol* 2011.

- **Early death remains a significant problem:**
  - **17% in SEER registry data (Park *et al*, *Blood* 2011; 118: 1248-54)**
  - **30 of 105 APL cases arising between 1997-2006 identified in Swedish population died within 30d (29%) (Lehmann *et al*, *Leukemia* 2011; 25: 1128-34)**

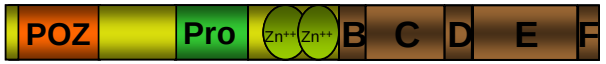
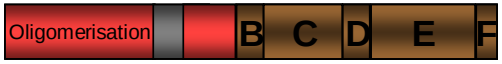
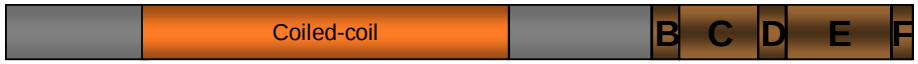
Initial management of APL

- Commence ATRA as soon as diagnosis suspected
- Implementation of supportive care for coagulopathy
- Important to establish presence of PML-RARα fusion, which predicts sensitivity to ATRA & arsenic:
  - PML immunofluorescence
  - Cytogenetics
  - PCR

Milligan *et al*, *Br J. Haematol* 2006
Sanz *et al*, *Blood* 2009

Molecular basis of APL: Implications for targeted therapy

Sensivity to targeted therapy

ATRA	ATO		
			**RARα**
✓	✓		**PML-RARα** ~98% of APL
✘	✘		**PLZF-RARα** ~0.5% of APL
✓	?		**NPM1-RARα**
✓	?		**NuMA-RARα**
✘	?		**STAT5b-RARα**
?	?		**PRKAR1A-RARα**
✓	?		**FIP1L1-RARα**

Cancer Treat Res. 2009;145:219-35.

Is it APL?

- **Case History**
  - 25yr old female
  - Presented – PE (23.12.09)
  - FBC: Hb 10g/dl, WBC 0.5, Plt 86
  - Marrow – APL?
  - Cytogenetics: Normal
  - FISH: No *PML-RARA* fusion signal

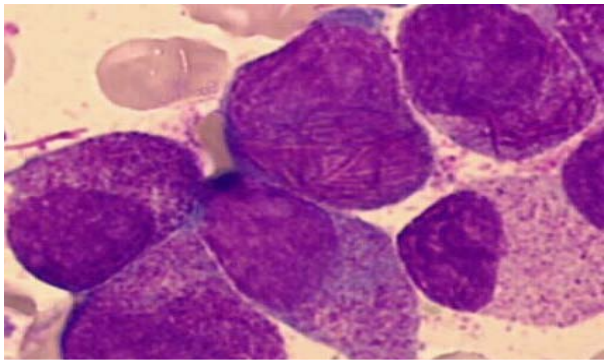
    PML antibody test +ve

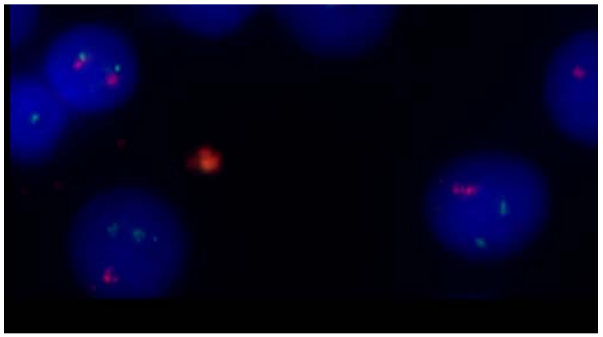
    RT-PCR: *PML-RARA* +ve

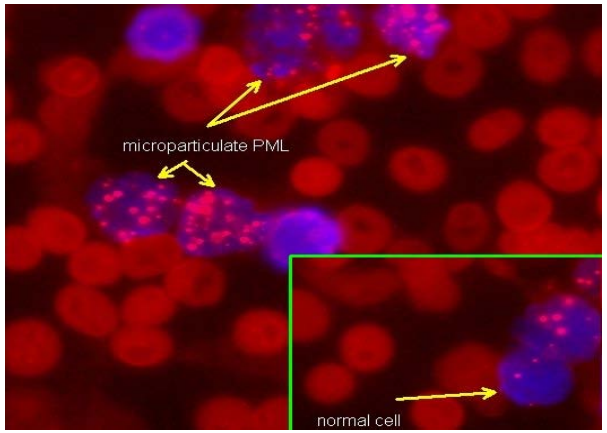
    (reciprocal *RARA-PML* neg)

    Diagnosis: APL secondary to *PML-RARA* insertion

    ➔ ATRA + Idarubicin





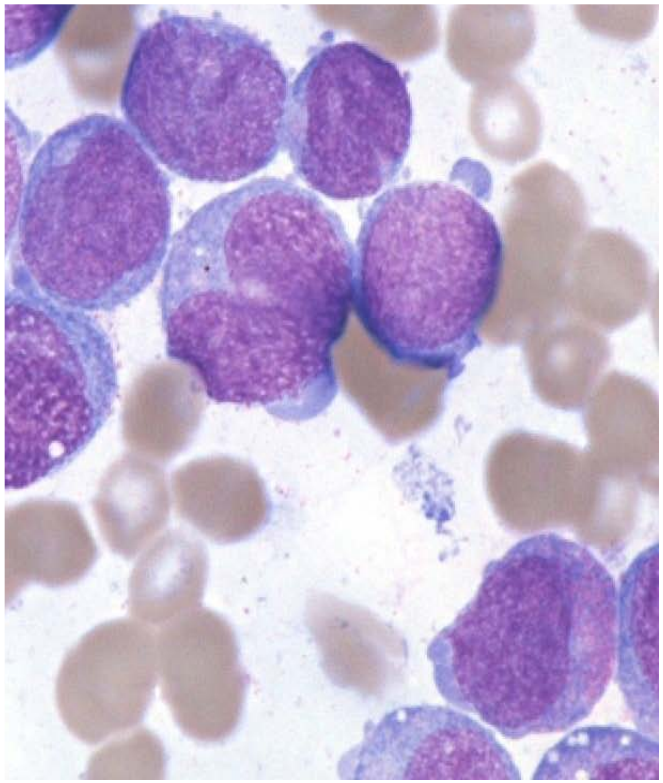


Importance of molecular diagnostics to guide appropriate therapy

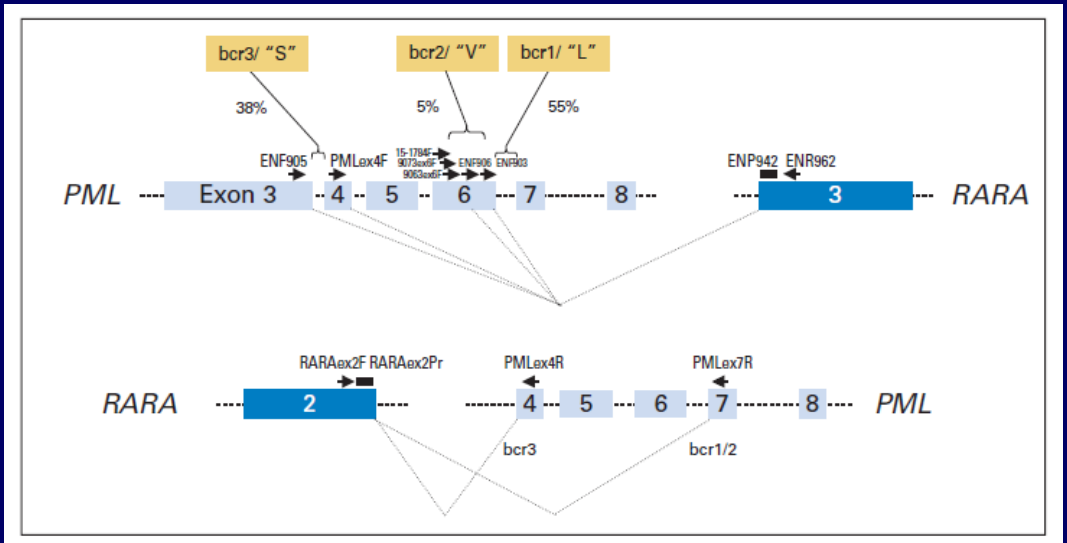
- **Case History**
  - 75yr old female
  - WBC 15 x 10^9/l
  - Suspected M3v
  - Randomised to AIDA in AML17 trial
  - Cytogenetics: Normal
  - FISH: No *PML-RARA* fusion signal

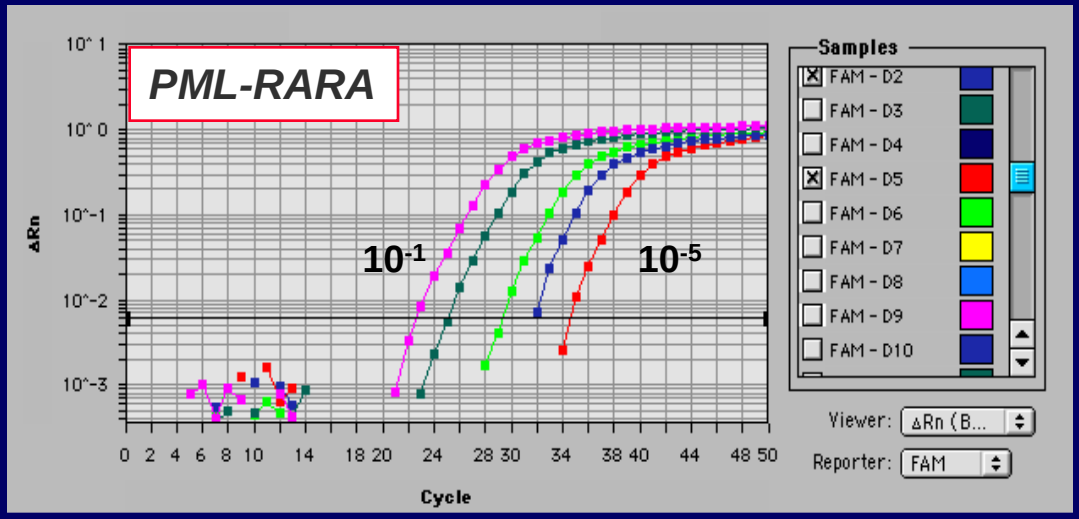
    RT-PCR: *PML-RARA* neg

    Diagnosis: NPM1 mutant AML



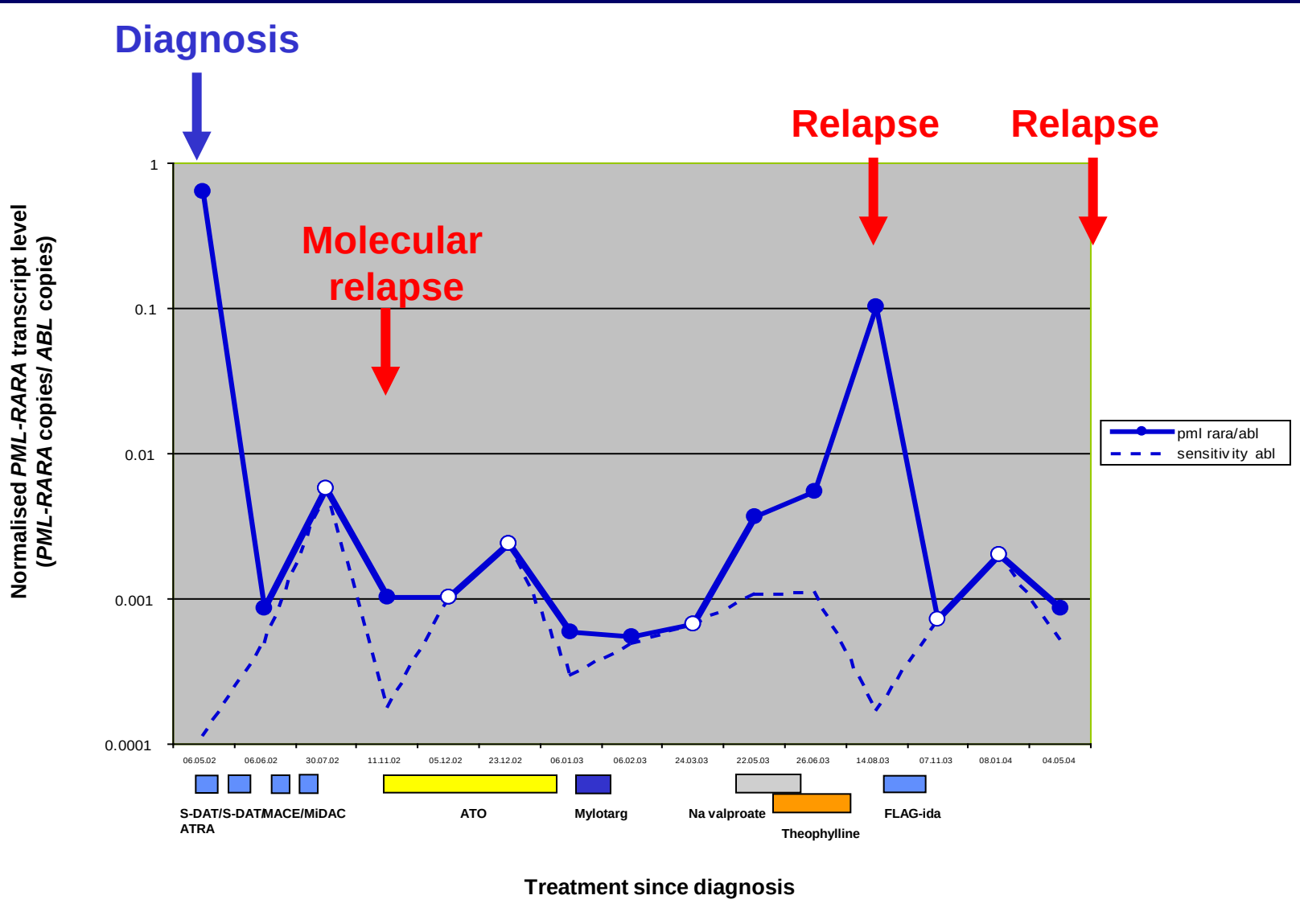
Real-time quantitative PCR (RQ-PCR) amplification of *PML-RARA* transcripts can detect submicroscopic levels of leukaemia



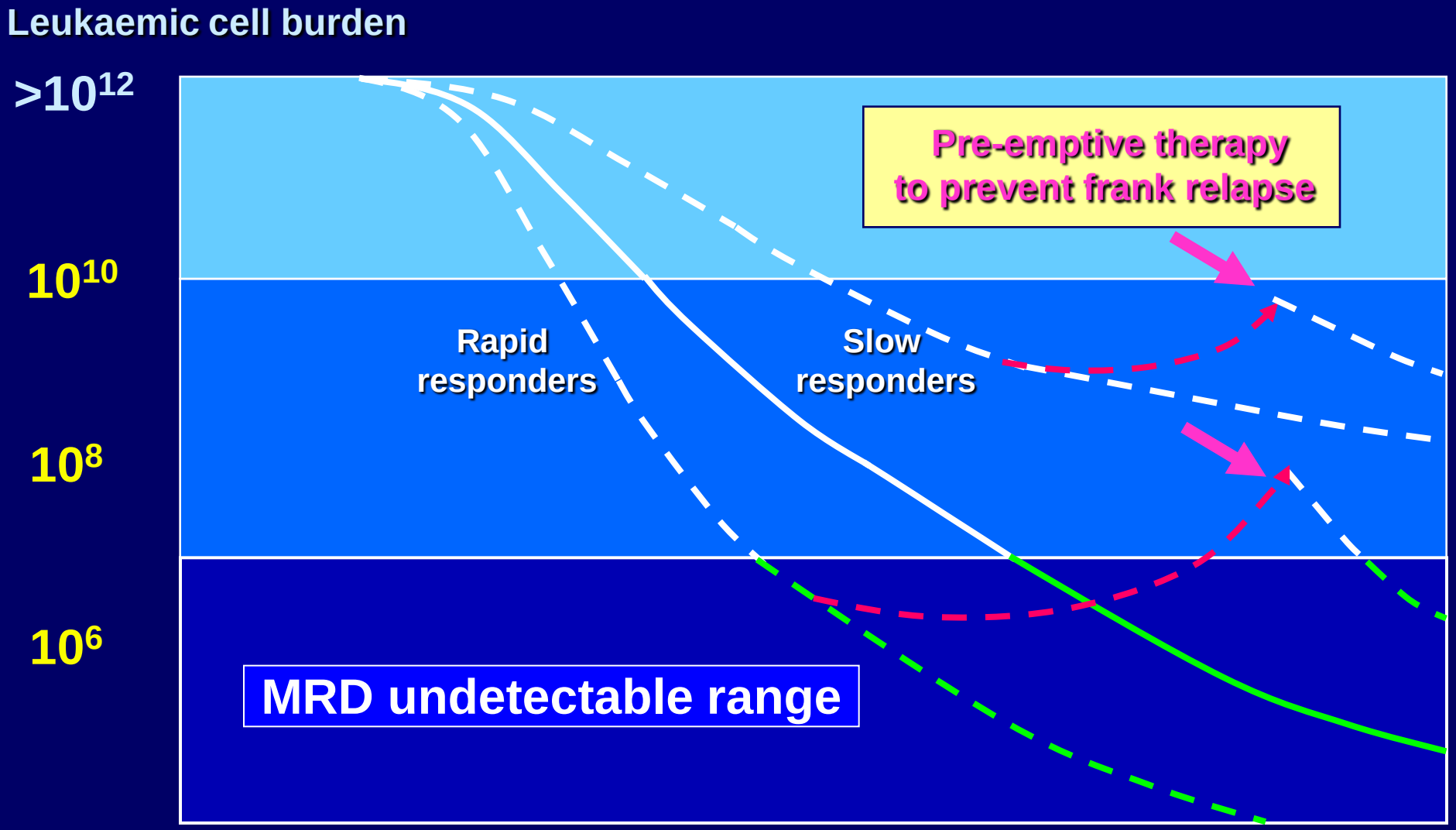


Grimwade *et al, J Clin Oncol* 2009; 27: 3650-8.

MRD monitoring using RQ-PCR to predict relapse in APL

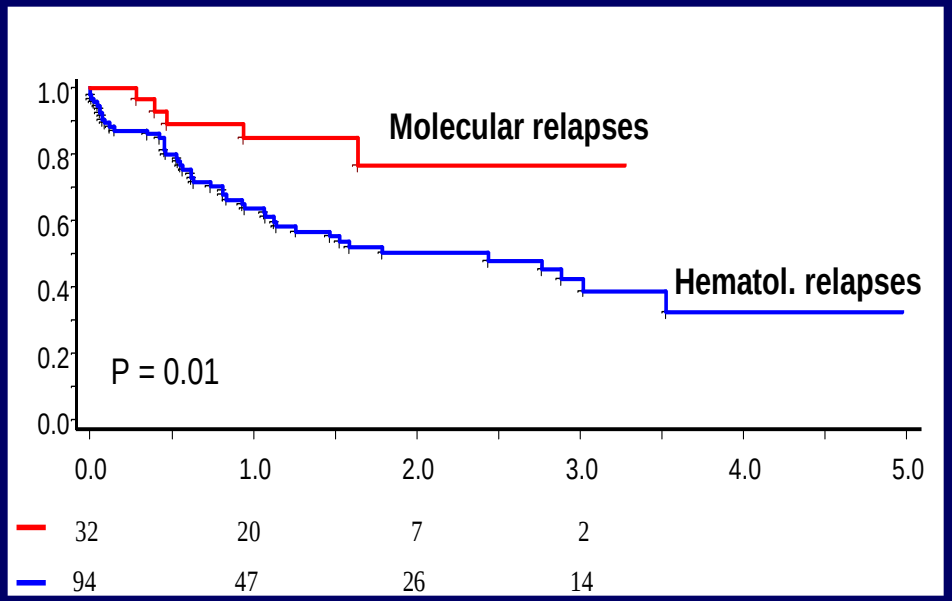


Use of sequential MRD monitoring to direct pre-emptive therapy to prevent impending relapse



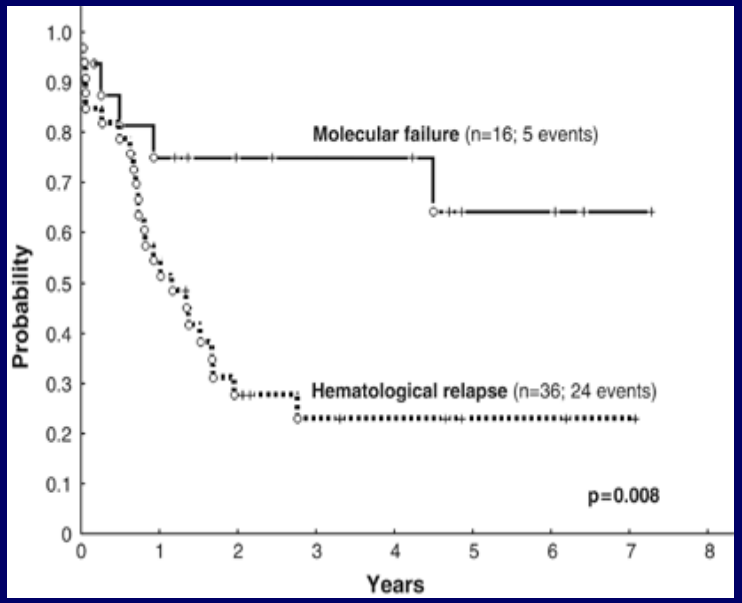
Defining role of MRD monitoring in APL: Outcome according to disease burden at time of relapse

GIMEMA



Lo Coco *et al*, Semin Hematol 2002; 39 (2 Suppl 1): 14-17.

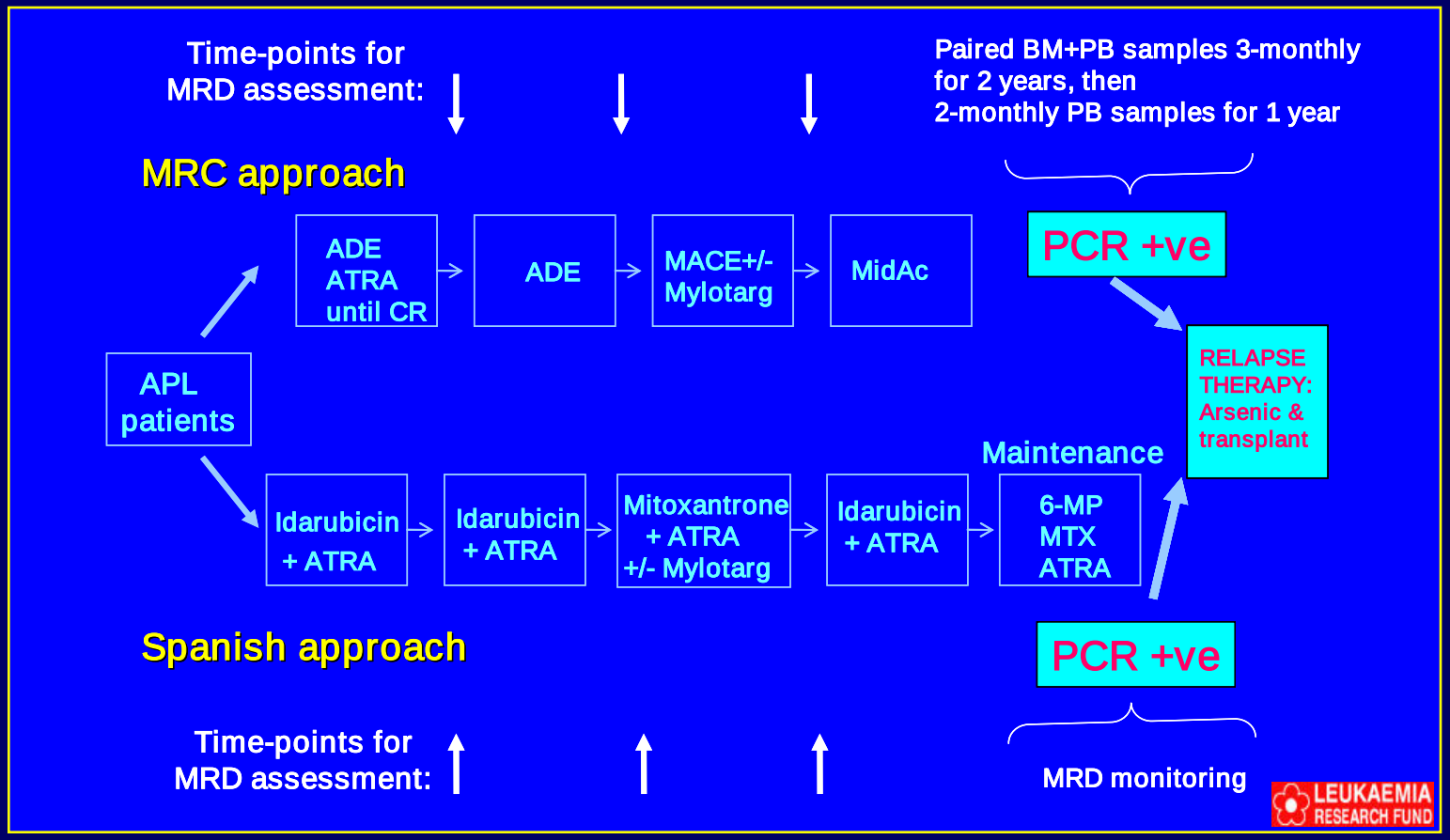
PETHEMA



Esteve *et al*, Leukemia 2007; 21: 446-452

Evaluation of MRD monitoring by RQ-PCR to determine treatment approach in patients with APL: MRC AML15 trial

- 281 patients, median age 42 yrs (16-69), median follow-up 26 months (0-56mo)
- 5,207 samples analysed
  - Including 1,832 paired BM+PB samples, median of 18 samples analysed/pt (range, 1-50)



Serial MRD monitoring by RQ-PCR to detect persistent disease/ molecular relapse is strongest independent predictor for clinical relapse

Parameter	Hazard ratio (95% CI)	P-value
Persistent PCR positivity or molecular relapse	33.56 (4.59-245.27)	<0.0001
PCR positivity at post-course 2 timepoint	8.88 (1.25-63.11)	0.0009
Presenting WBC	1.03 (1.01-1.05)	0.004

Multivariable analysis considering also:

- Pre-treatment *PML-RARA* expression level
- *PML* breakpoint
- Kinetics of *PML-RARA* transcript reduction after induction
- PCR status after any given course of chemotherapy (post #1, #2, #3)

Grimwade *et al, J Clin Oncol* 2009; 27: 3650-8.

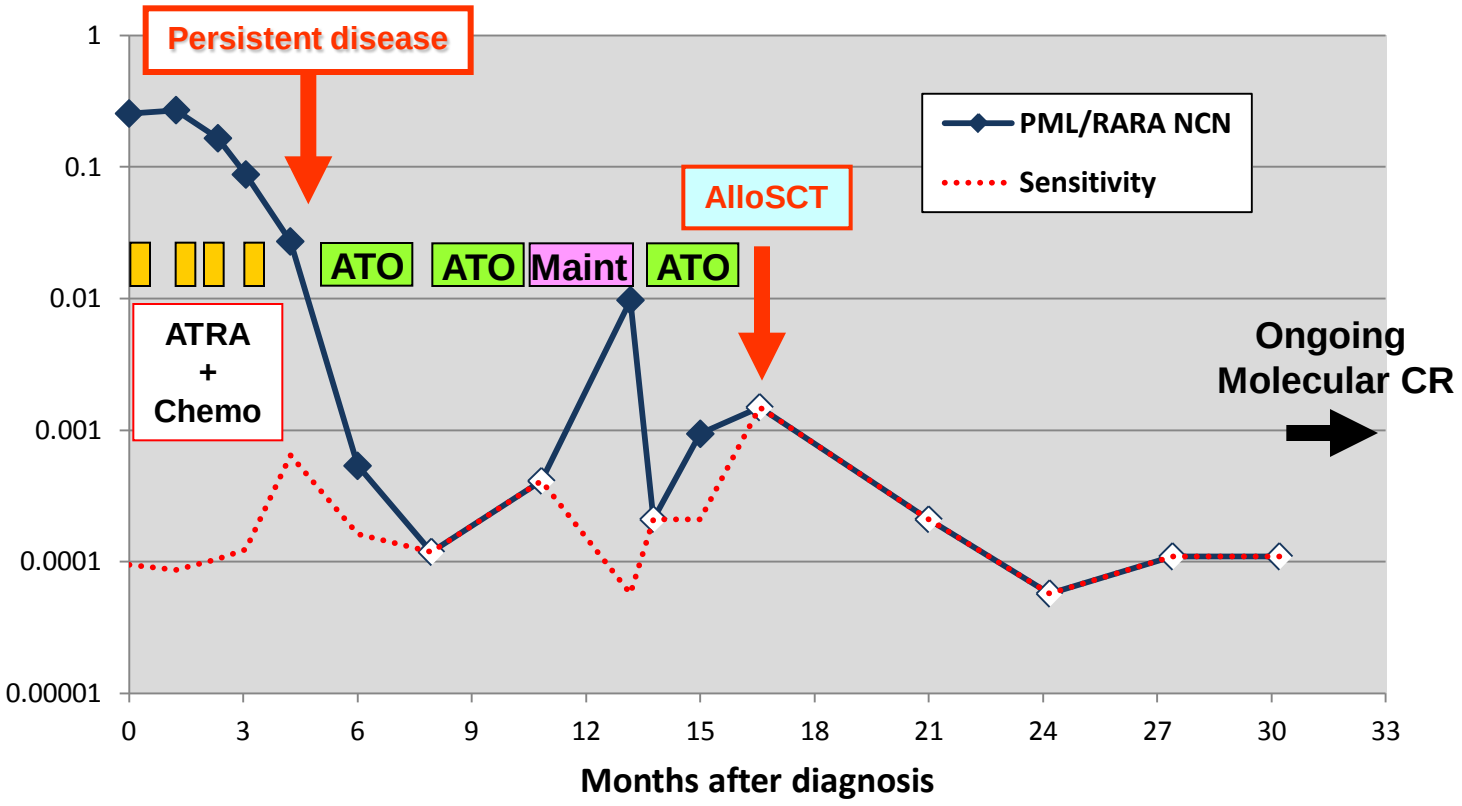
Serial MRD monitoring by standardised RQ-PCR assay to guide patient management in *PML-RARA*+ APL

Case History:

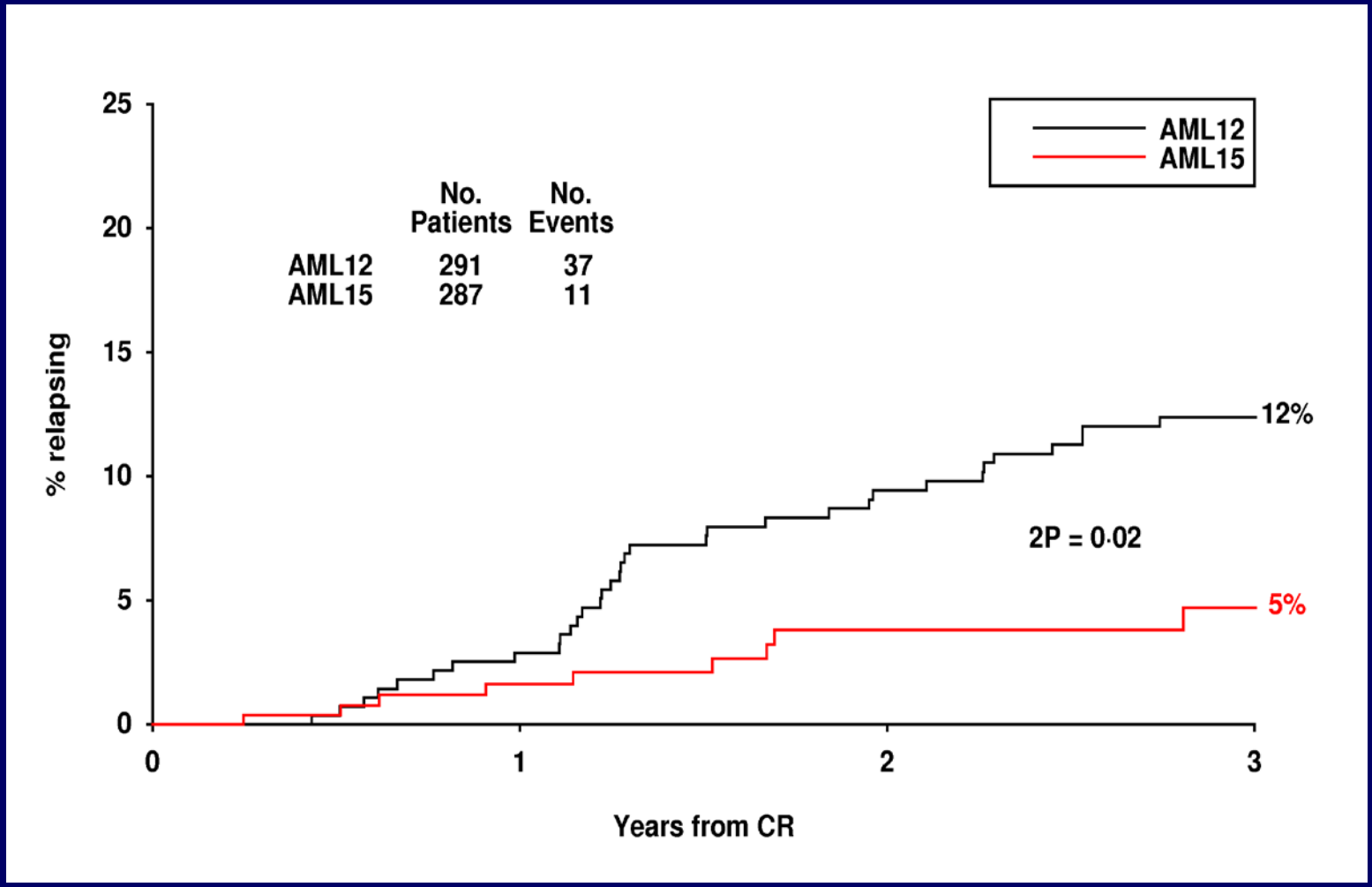
9 month male infant

FBC: Hb 8g/dl, WBC 28.6 x 10^9/l, Plt 12 x 10^9/l

PML-RARA+ APL

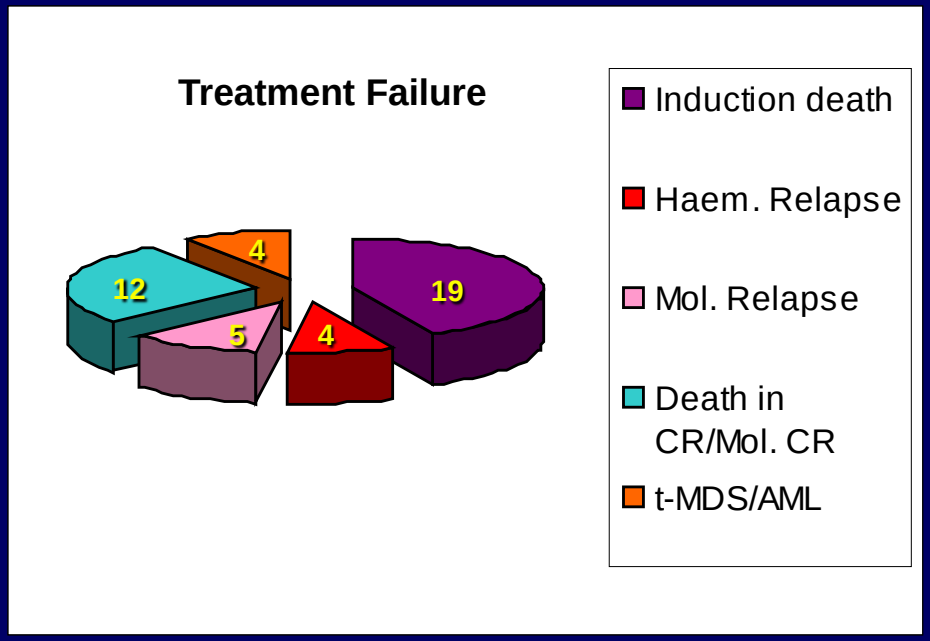


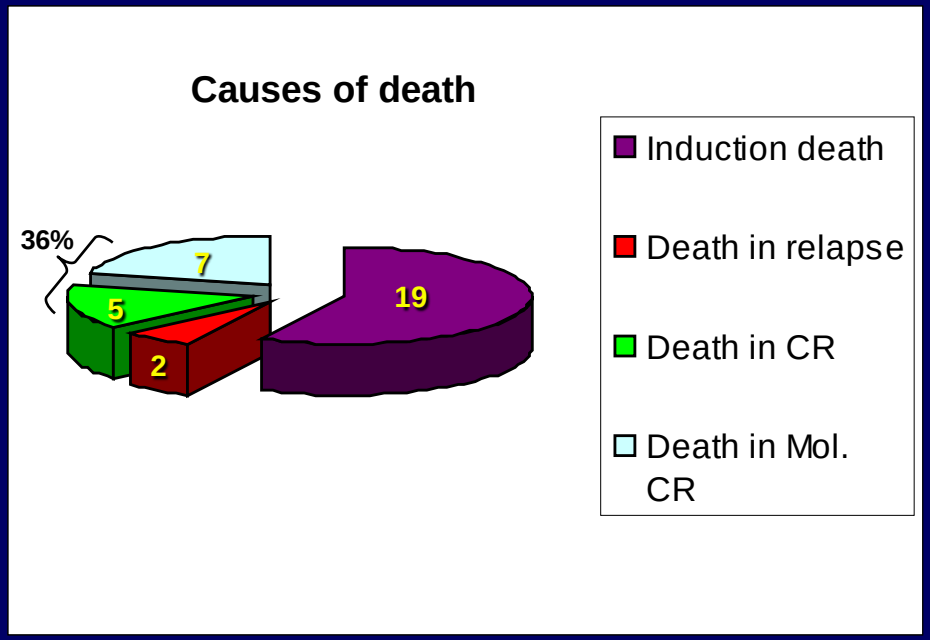
Evaluation of MRD monitoring & pre-emptive therapy to reduce rates of frank relapse in *PML-RARA*+ APL in MRC AML15 trial



Grimwade *et al, J Clin Oncol* 2009; 27: 3650-8.

Causes of treatment failure & death in *PML-RARA*+ APL in MRC AML15





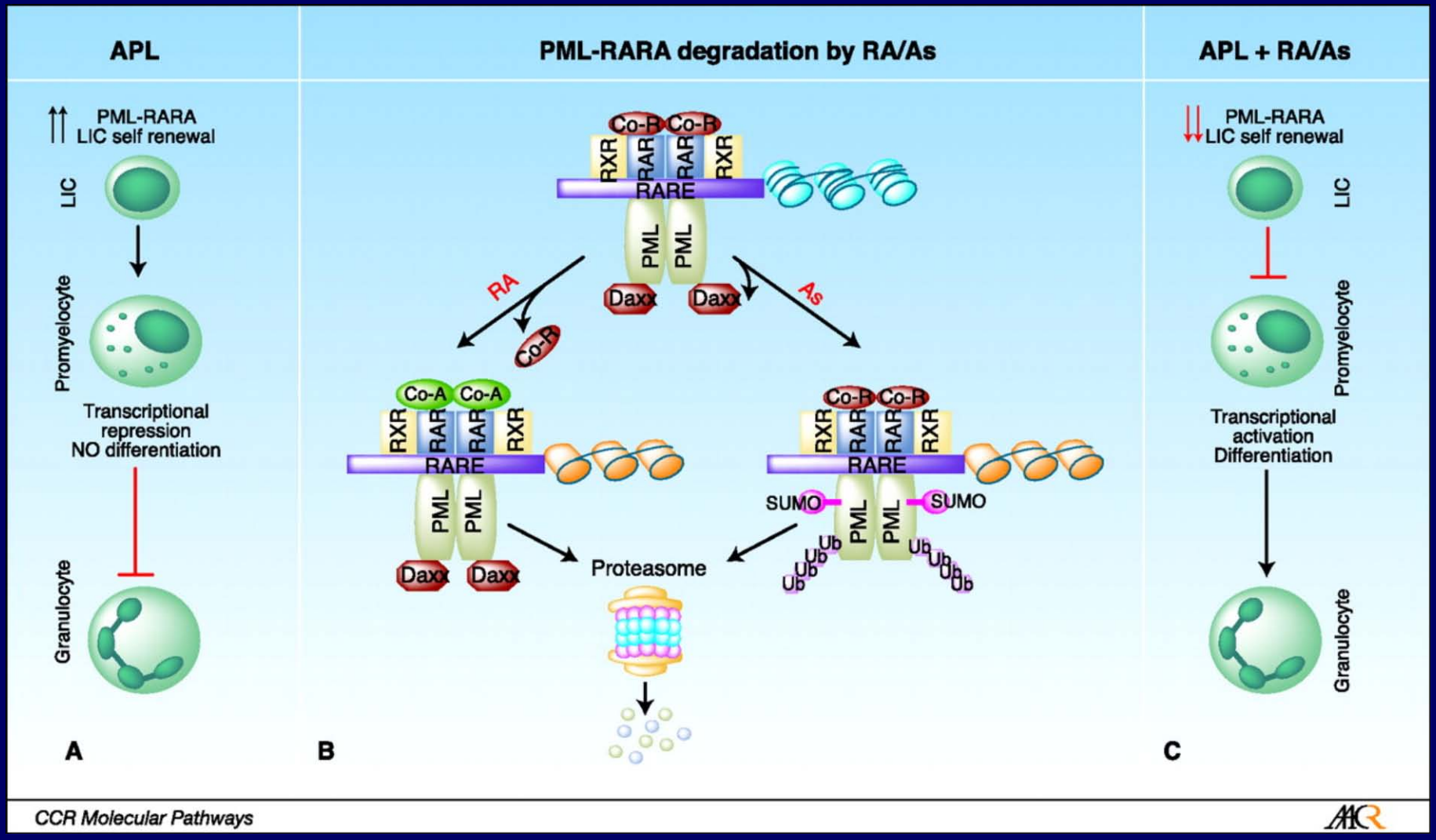
Treatment-related toxicity

~5% Death in CR/Molecular CR

~5% Treatment curtailment/amendment (~3% with significant cardiac toxicity)

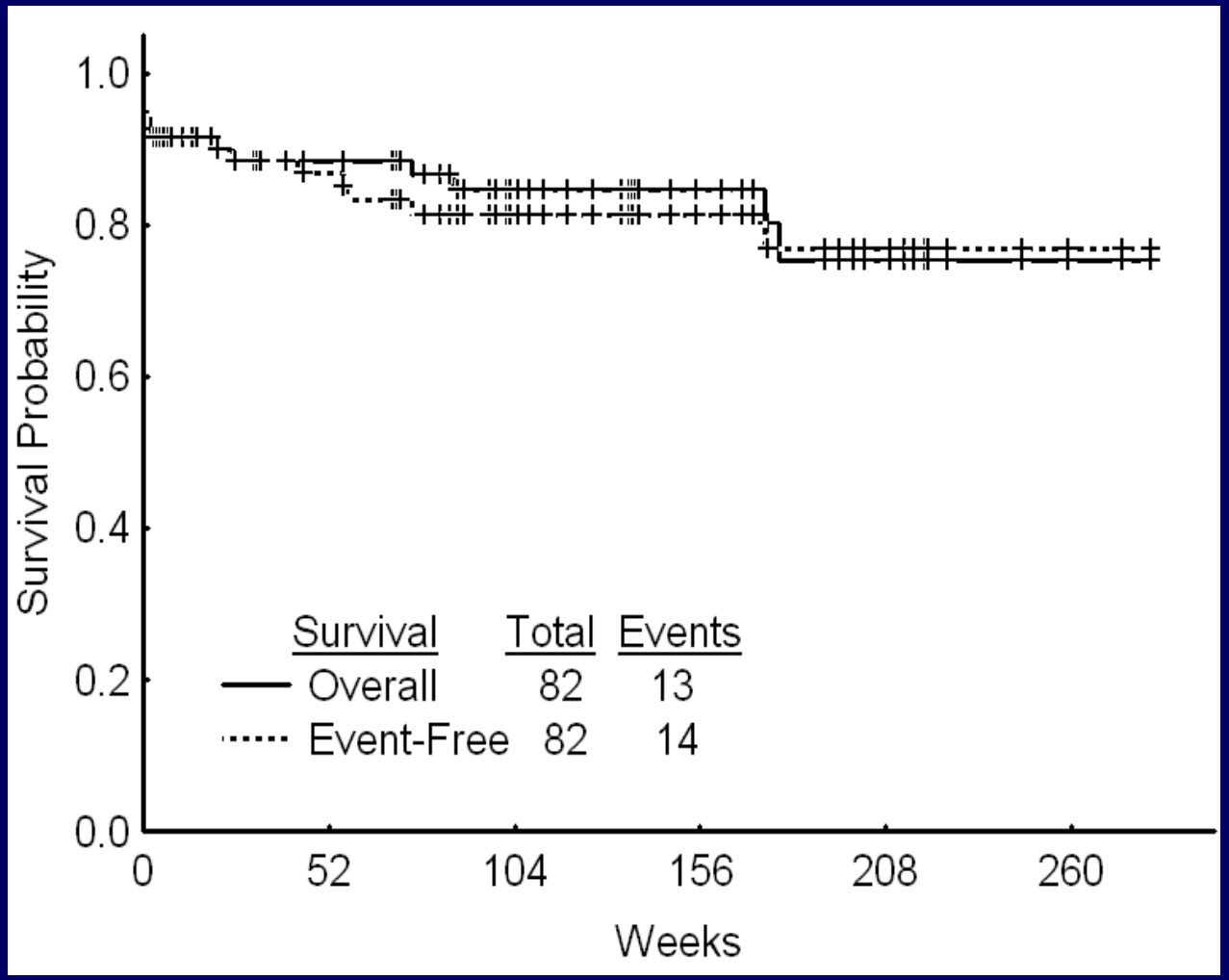
~2% t-MDS/ t-AML

ATO and ATRA exert synergistic activity *in vivo*



Nasr R *et al*. *Clin Cancer Res* 2009;15:6321-6326

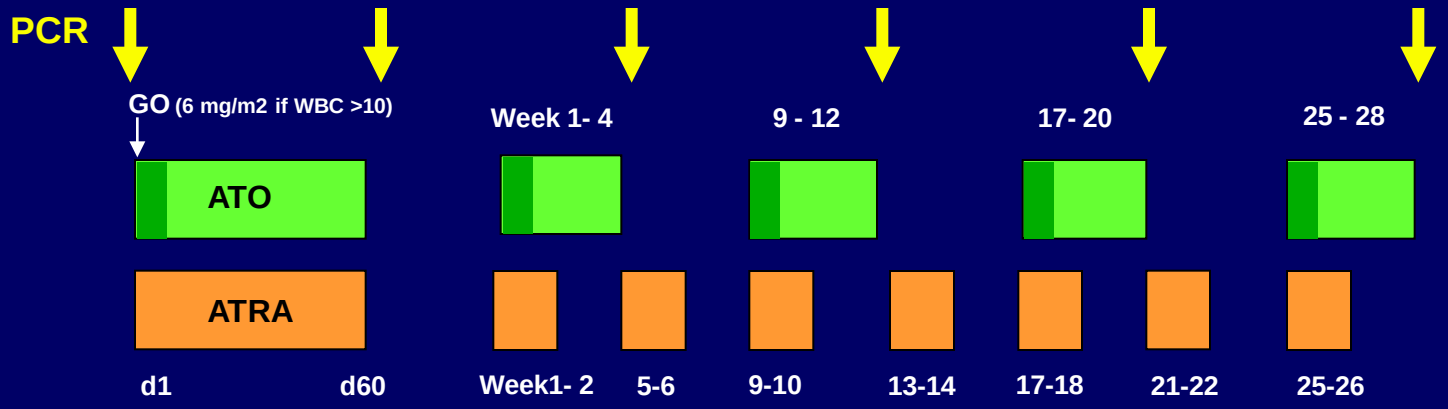
Outcome of *PML-RARA*+ APL following ATRA+ATO based therapy: Updated MD Anderson experience



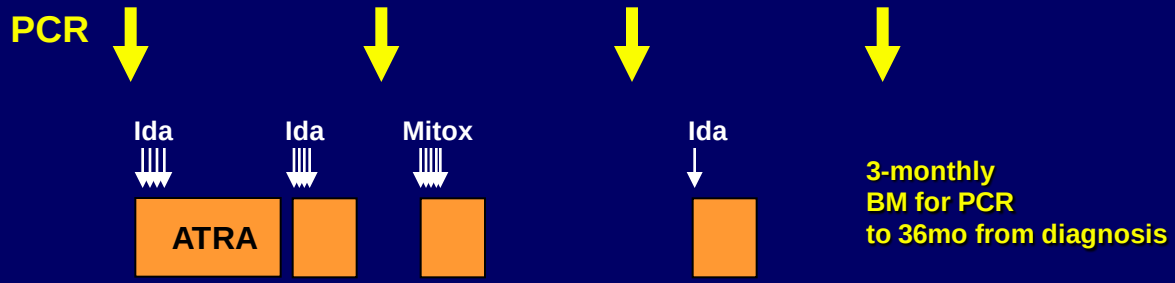
Ravandi, F. *et al*. *J Clin Oncol*; 27:504-510 2009

MRD monitoring to guide de-intensified treatment for *PML-RARA*+ APL in AML17 trial

CHEMO-FREE



AIDA



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