Monitoring Minimal Residual Disease in AML with molecular markers

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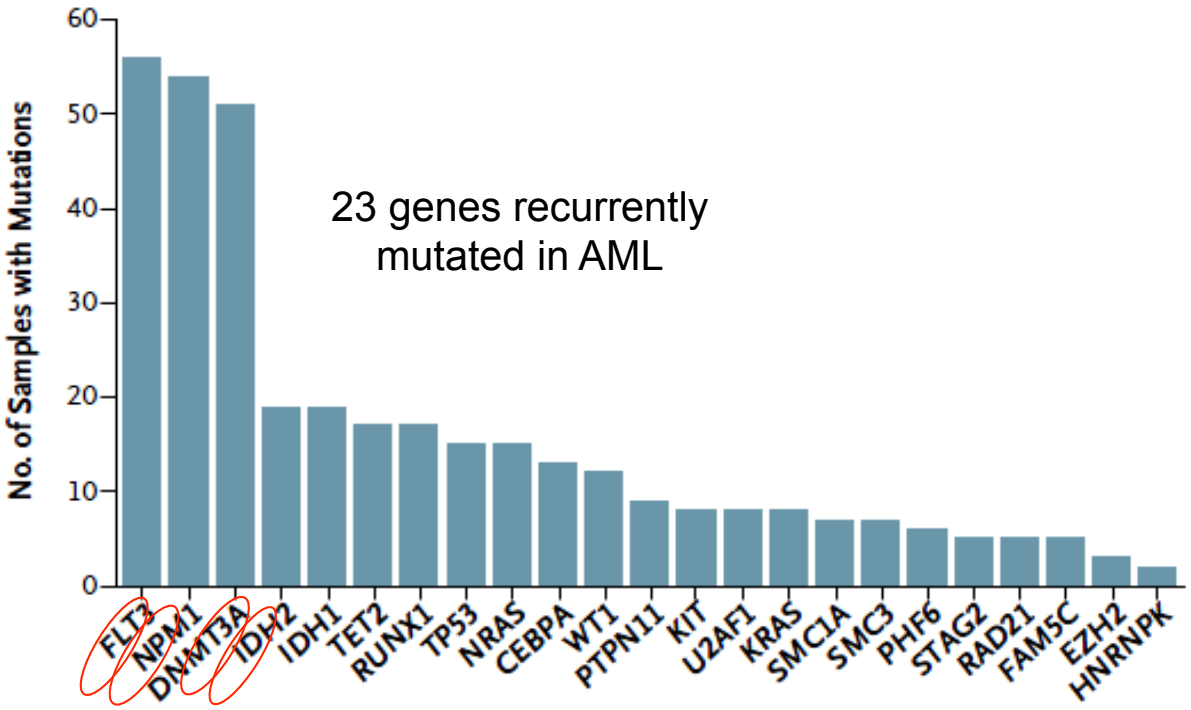
Genomic and Epigenomic Landscapes of Adult De Novo Acute Myeloid Leukemia

The Cancer Genome Atlas Research Network

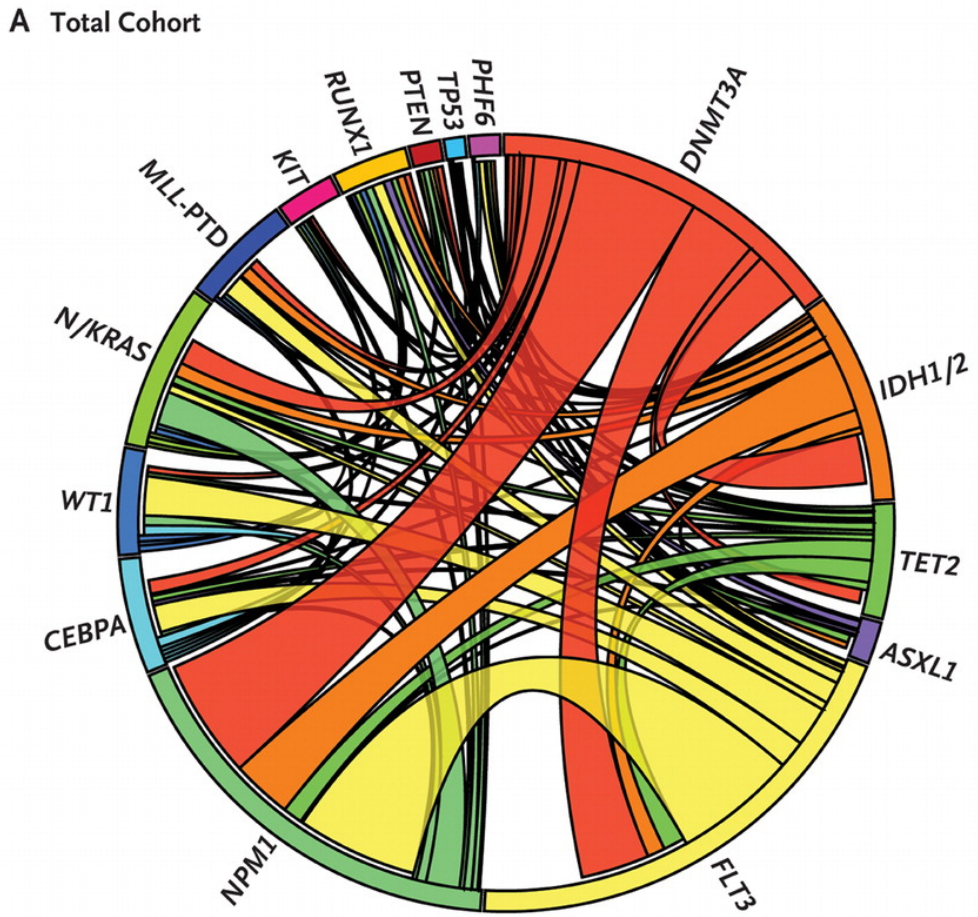


23 genes recurrently mutated in AML

The Cancer Genome Atlas Research Network, *New Engl J Med* 2013;368:2059–2074.

Challenges to molecular targeting

- AML is genetically heterogeneous
- Inhibitors against one target will not suppress all leukemogenic clones
- Clearing all mutations increases overall survival

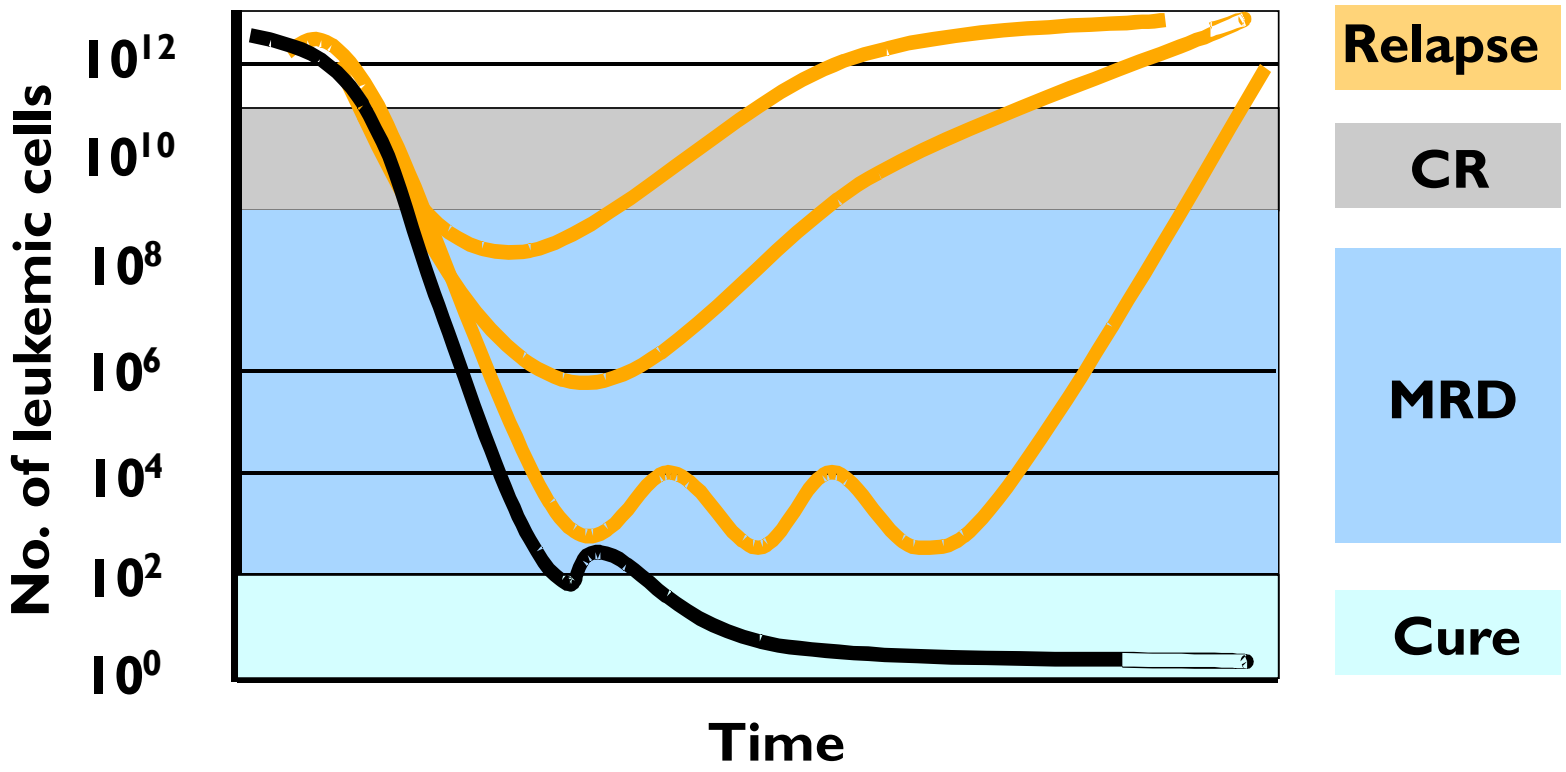


Patel JP, *et al. N Engl J Med* 2012;366:1079–1089.

2017 ELN risk stratification by genetics

Risk category[*]	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}[†]
	Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high}[†]
	Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}[†] (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*[‡]
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214*
	t(v;11q23.3); *KMT2A* rearranged
	t(9;22)(q34.1;q11.2); *BCR-ABL1*
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)*
	−5 or del(5q); −7; −17/abn(17p)
	Complex karyotype,[§] monosomal karyotype[‖]
	Wild-type *NPM1* and *FLT3*-ITD^{high}[†]
	Mutated *RUNX1*[¶]
	Mutated *ASXL1*[¶]
	Mutated *TP53*[#]

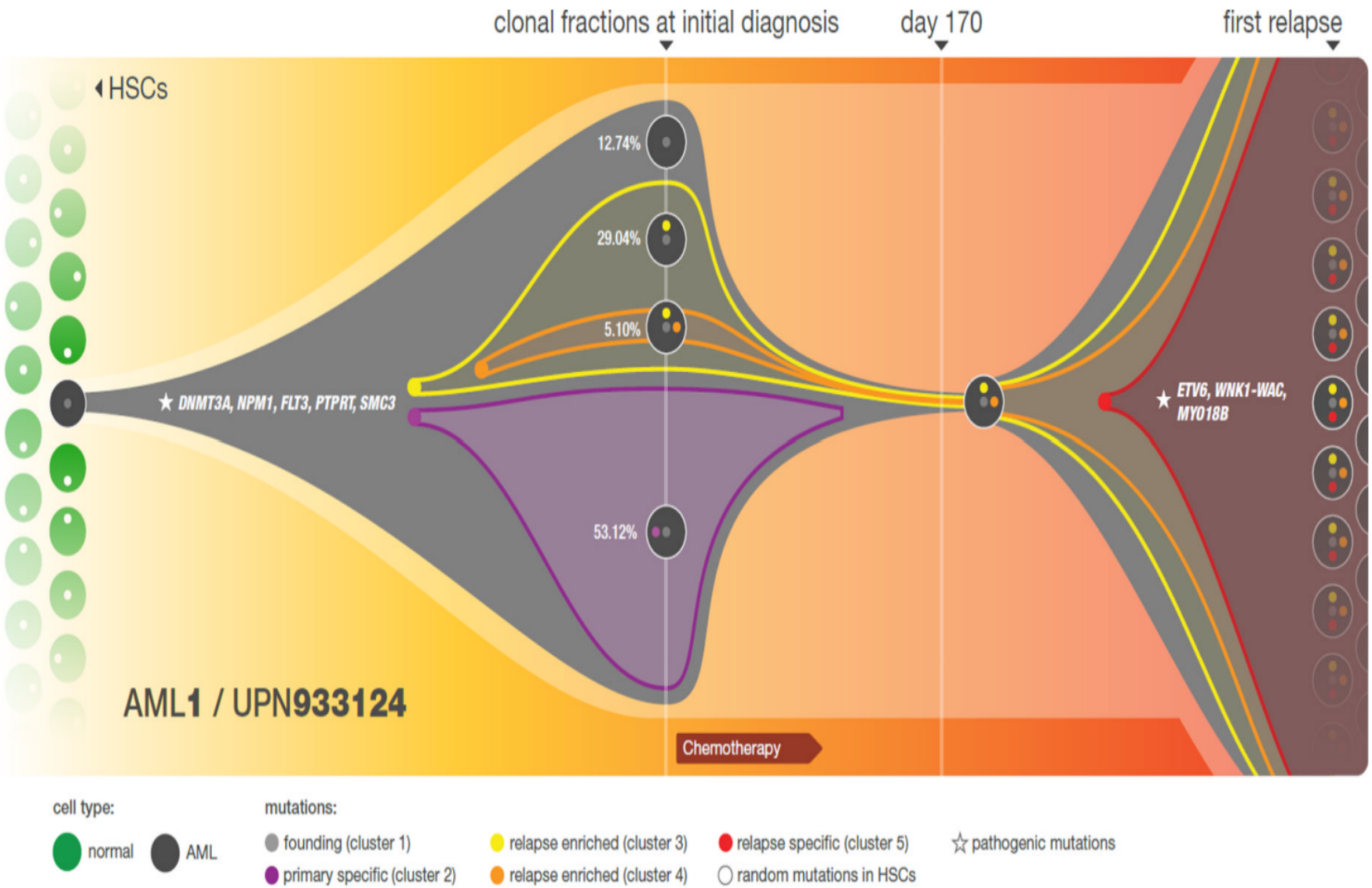
Can MRD improve outcome determination?



a) capture differences in treatment response that reflect an underlying molecular heterogeneity

b) capture inter-patient variability in drug availability and metabolism, which may significantly influence outcome

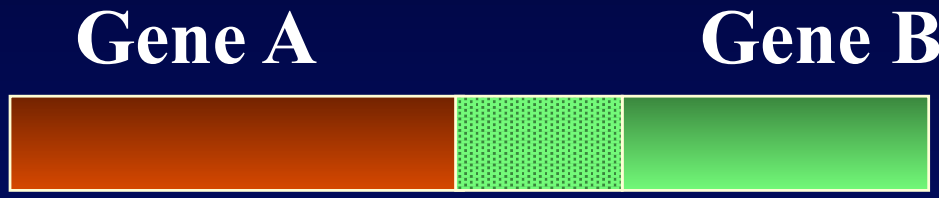
Grimwade, Best Practice 2012



Ding L, Nature 2012

Methods for qualitative RT-PCR in AML

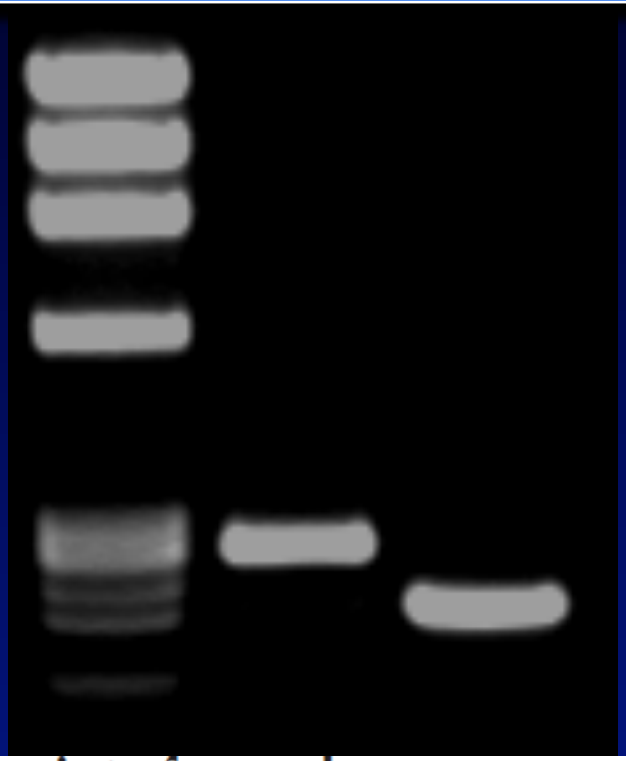
Qualitative PCR analysis



20 cycles →

20 cycles →

Sensitivity potentially achievable: $10^{-4}/10^{-5}$



Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease

Report of the BIOMED-1 Concerted Action: Investigation of minimal residual disease in acute leukemia

JJM van Dongen[1], EA Macintyre[2], JA Gabert[3], E Delabesse[2], V Rossi[4], G Saglio[5], E Gottardi[5], A Rambaldi[6], G Dotti[6], F Griesinger[7], A Parreira[8], P Gameiro[8], M González Diáz[9], M Malec[10], AW Langerak[1], JF San Miguel[9] and A Biondi[4]

Leukemia 1999

APL: golden standard for clinical application of qualitative RT PCR in AML

ELN recommendations, Sanz MA et al., Blood 2009

2.11. Molecular remission in the bone marrow should be assessed at completion of consolidation by RT-PCR assay affording a sensitivity of at least 1 in 10^4.

2.12. Patients with confirmed molecular persistence should be considered for allogeneic HSCT.

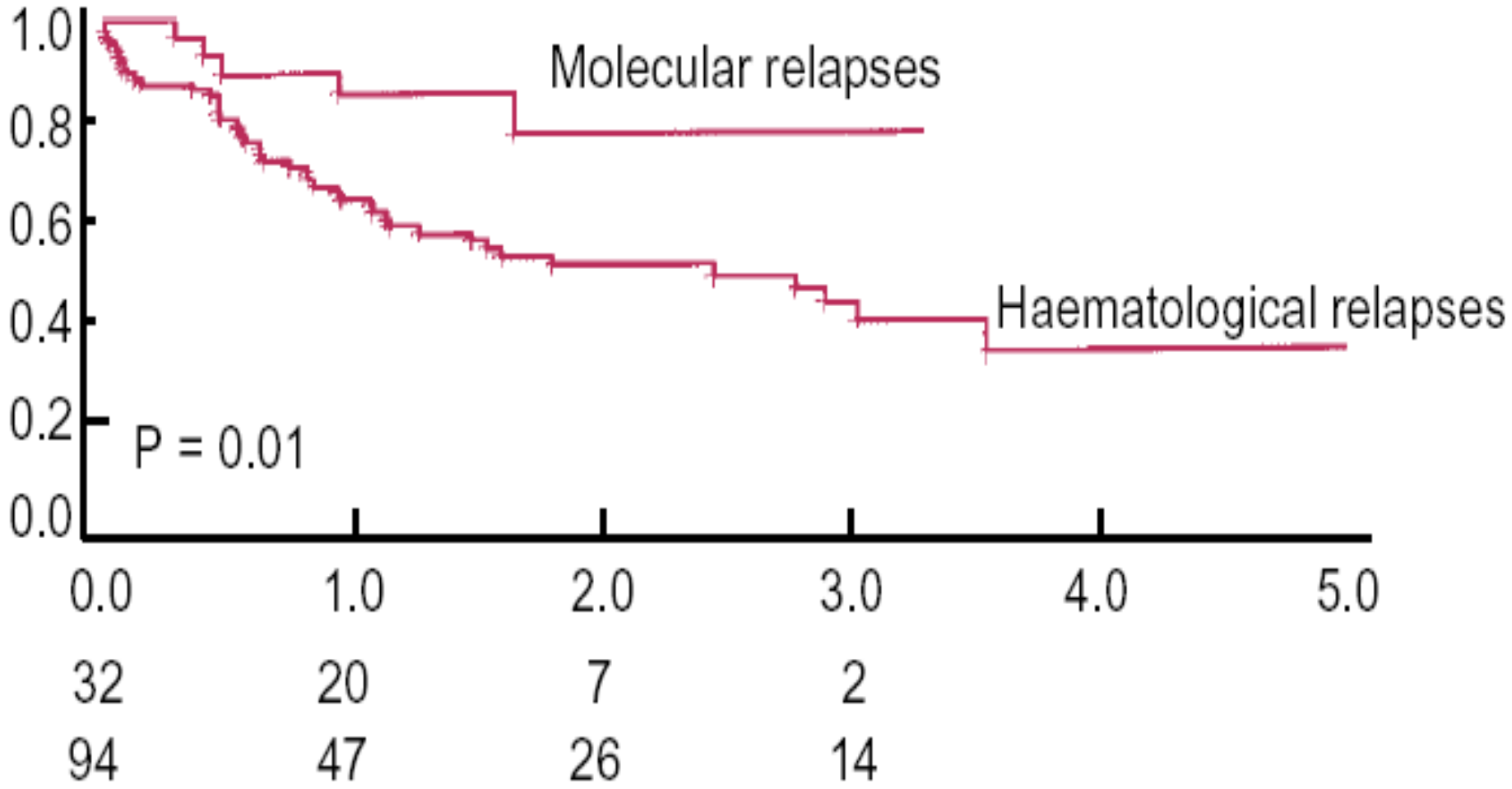
2.13. For patients with molecular persistence who are not candidates for allogeneic HSCT, ATO or gemtuzumab ozogamicin may be considered.

2.15. Because early treatment intervention in patients with evidence of MRD affords a better outcome than treatment in full-blown relapse, every 3 months MRD monitoring of bone marrow should be offered to all patients for up to 3 years after completion of consolidation therapy.

2.16. Bone marrow generally affords greater sensitivity for detection of MRD than blood and therefore is the sample type of choice for MRD monitoring to guide therapy.

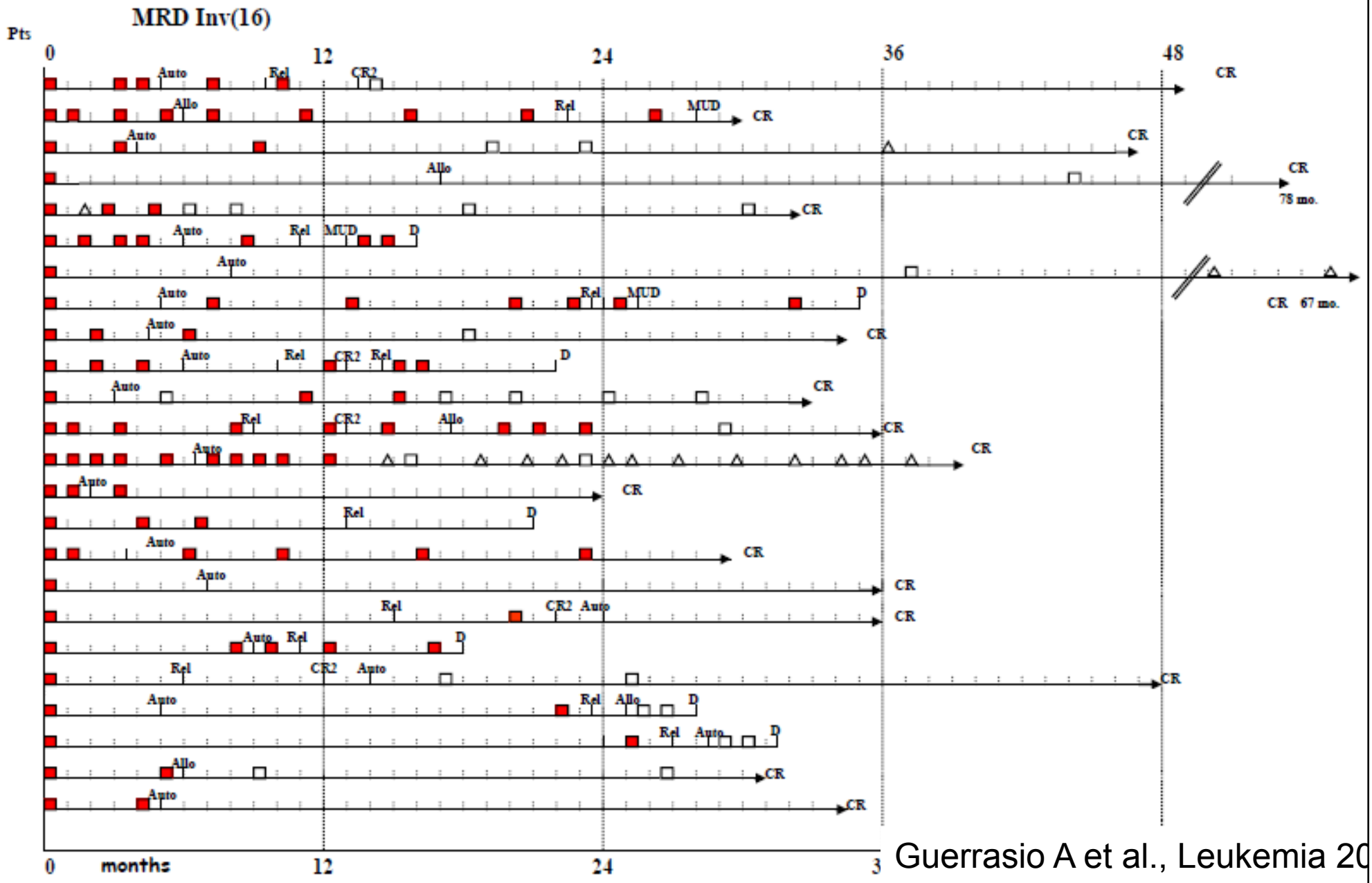
2.17. For patients testing PCR-positive at any stage after completion of consolidation, it is recommended that a bone marrow is repeated for MRD assessment within 2 weeks and that samples are sent to the local laboratory, as well as to a reference laboratory for independent confirmation.

Survival of APL patients treated for haematologic
Vs.molecular relapse (GIMEMA)



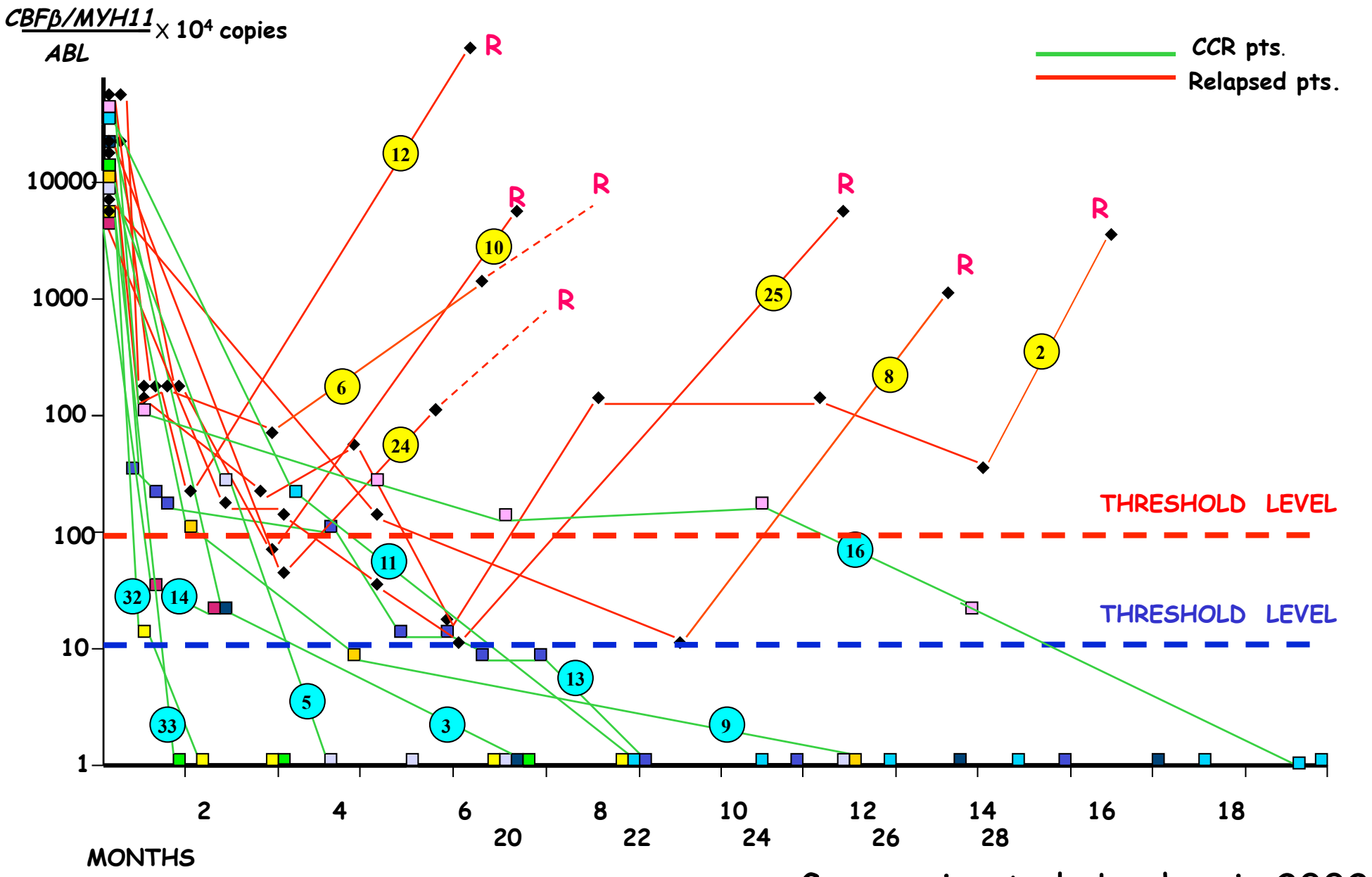
Lo Coco F. et al., Blood 1999

Most patients with CBFs leukemias remain RT PCR positive after completion of therapy, indipendently from the final outcome



Guerrasio A et al., Leukemia 20

Real Time PCR in CBFb-MYH11 positive AML patients



Guerrasio et al., Leukemia 2002

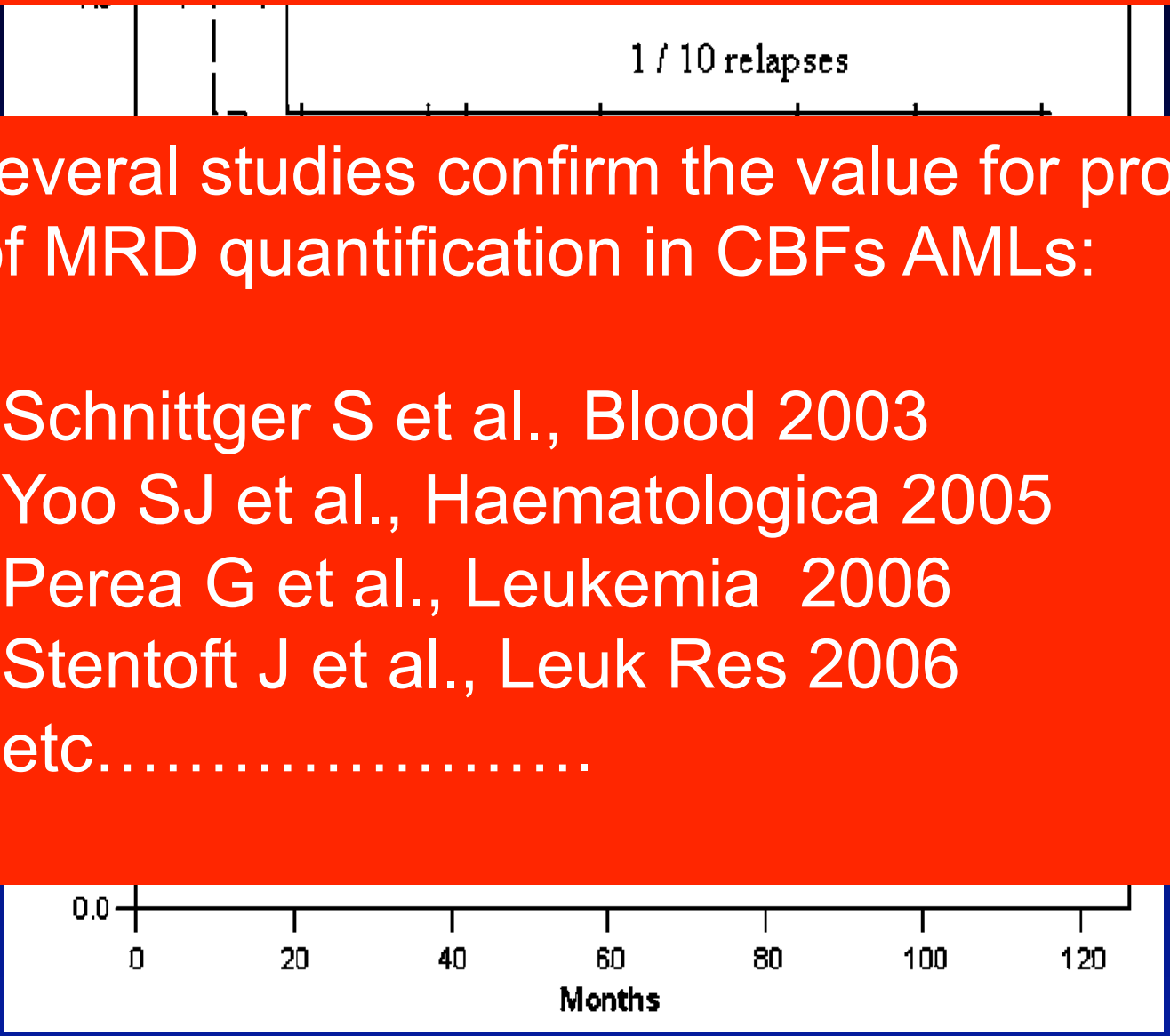
Real Time PCR in CBFb-MYH11 positive AML patients

	Post-induction	Post-consolidation
	<u><100 copies</u>	<u><10 copies</u>
	2/12 relapses	2/12 relapses
	P=0,003	P=0,006
	>100 copies	>10 copies
	6/7 relapses	8/11 relapses

Relapses /total cases

Guerrasio et al., Leukemia 200

Real Time PCR in CBFb-MYH11 positive AML patients



Several studies confirm the value for prognosticati of MRD quantification in CBFs AMLs:

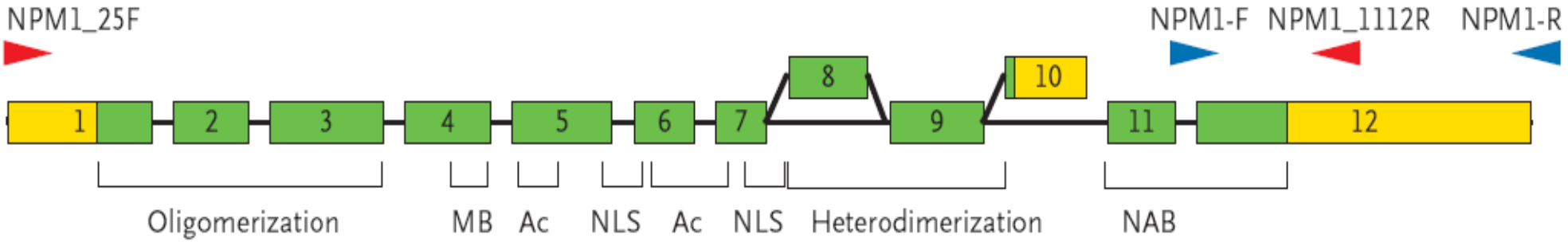
- ✓Schnittger S et al., Blood 2003
- ✓Yoo SJ et al., Haematologica 2005
- ✓Perea G et al., Leukemia 2006
- ✓Stentoft J et al., Leuk Res 2006
- ✓etc……

Leroy H et al., Leukemia 200

FLIT3 ITD and TKD as markers for MRD in AML

- FLIT3 ITD and FLIT-TDK are suitable markers for MRD detection and quantification in AML
  - Stirewalt DL et al., Leuk Res 2001
  - Schnittger S et al., Acta Haematol 2004
  - Scholl S et al., J Lab Clin Med 2005
- Need for a patient-specific probe
- Unstable marker?

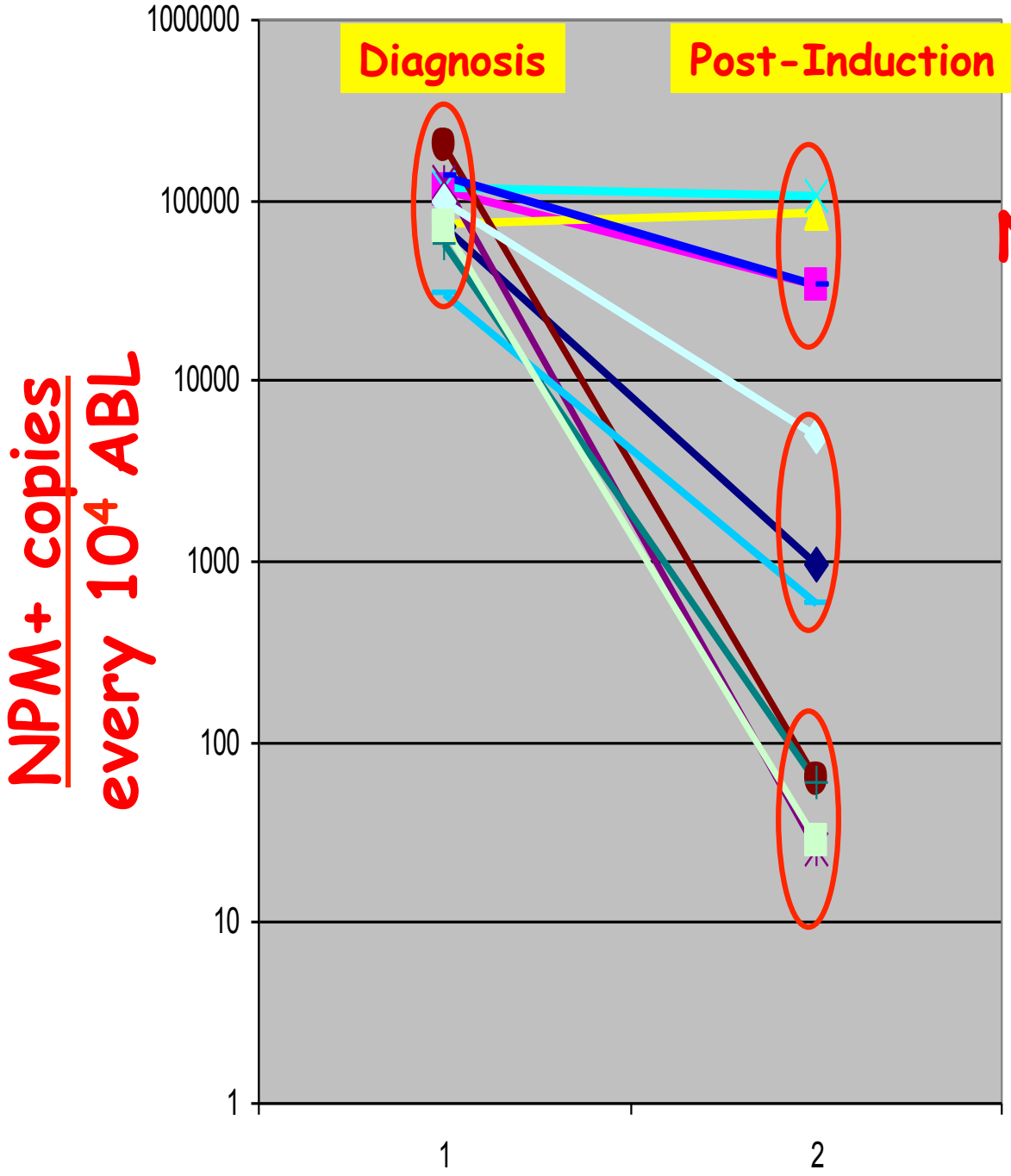
NPM1 as a marker for MRD in AML



Type of Mutation	GenBank Accession No.	Sequence	Predicted Protein
None (wild type)	NM_002520	GATCTCTG....GCAGT....GGAGGAAGTCTCTTTAAGAAAATAG	-DLWQWRKSL
Mutation A	AY740634	GATCTCTGTCTGGCAGT....GGAGGAAGTCTCTTTAAGAAAATAG	-DLCLAVEEVSLRK
Mutation B	AY740635	GATCTCTGCATGGCAGT....GGAGGAAGTCTCTTTAAGAAAATAG	-DLCMAVEEVSLRK
Mutation C	AY740636	GATCTCTGCGTGGCAGT....GGAGGAAGTCTCTTTAAGAAAATAG	-DLCVAVEEVSLRK
Mutation D	AY740637	GATCTCTGCCTGGCAGT....GGAGGAAGTCTCTTTAAGAAAATAG	-DLCLAVEEVSLRK
Mutation E	AY740638	GATCTCTG....GCAGTCTCTTGCCCAAGTCTCTTTAAGAAAATAG	-DLWQSLAQVSLRK
Mutation F	AY740639	GATCTCTG....GCAGTCCCTGGAGAAAGTCTCTTTAAGAAAATAG	-DLWQSLEKVSLRK

	No./No. of Cases (%)	
Mutation A	40/52 (77)	90%
Mutation B	7/52 (13)	

Falini B., NEJM 2005



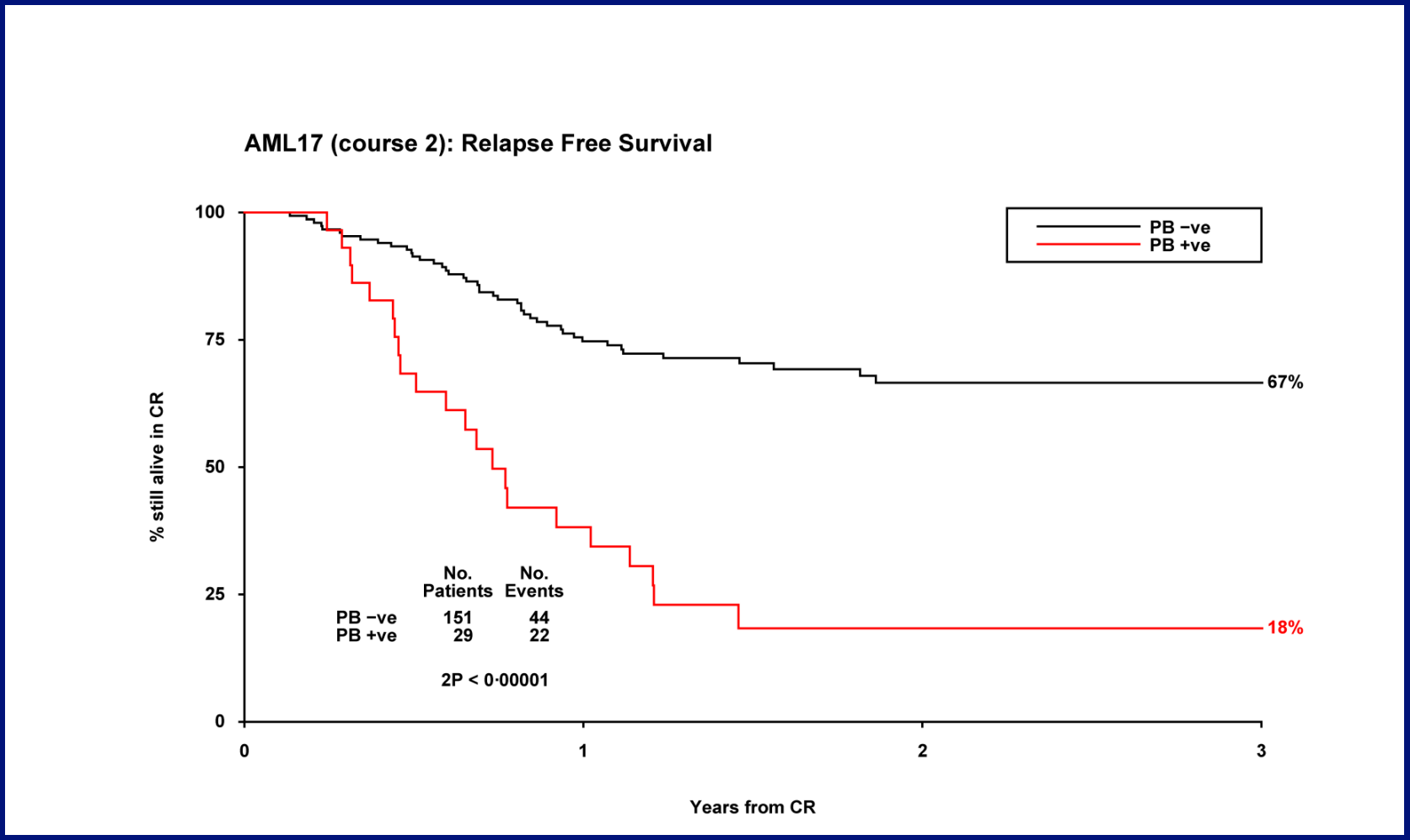
Non responders

Relapses 3/3

Relapses 1/4

Paolo Gorello et al., Leukeima 2006

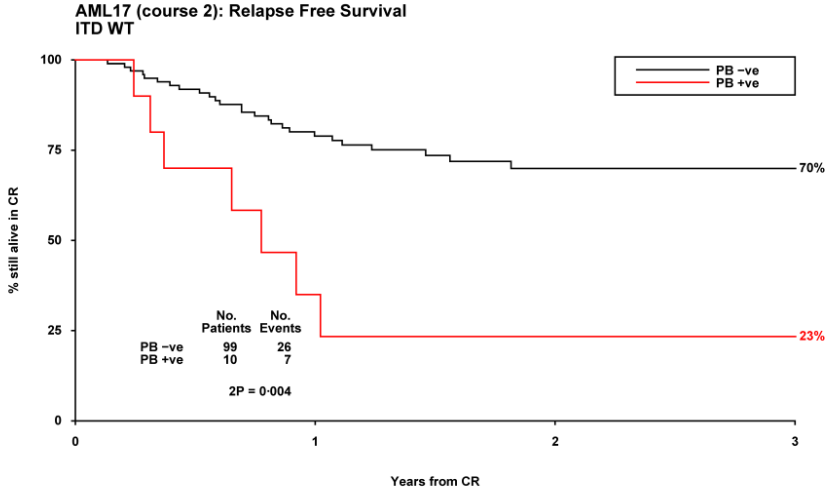
Early assessment of MRD status in *NPM1* mutant AML provides independent prognostic information



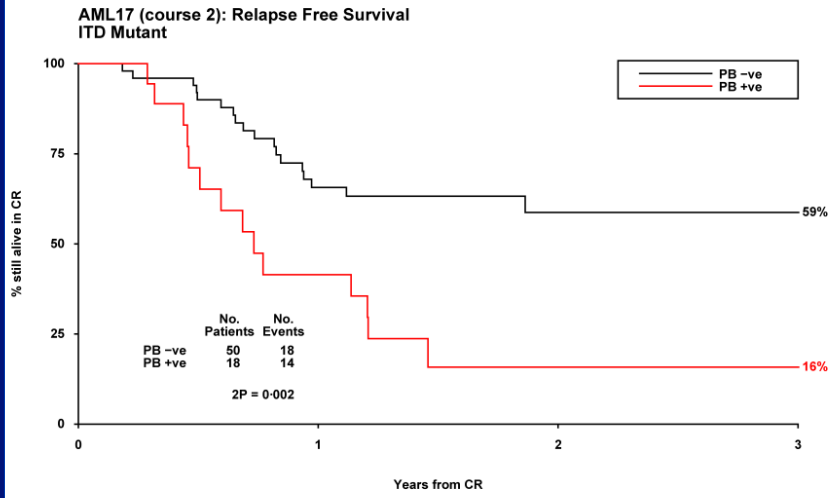
Adam Ivey, Neesa Bhudia, Mandy Gilkes, Rosemary Gale & Robert Hills

Prognostic value of MRD assessment is independent of FLT3-ITD status in NPM1 mutant AML

NPM1 mut/ FLT3-ITD neg

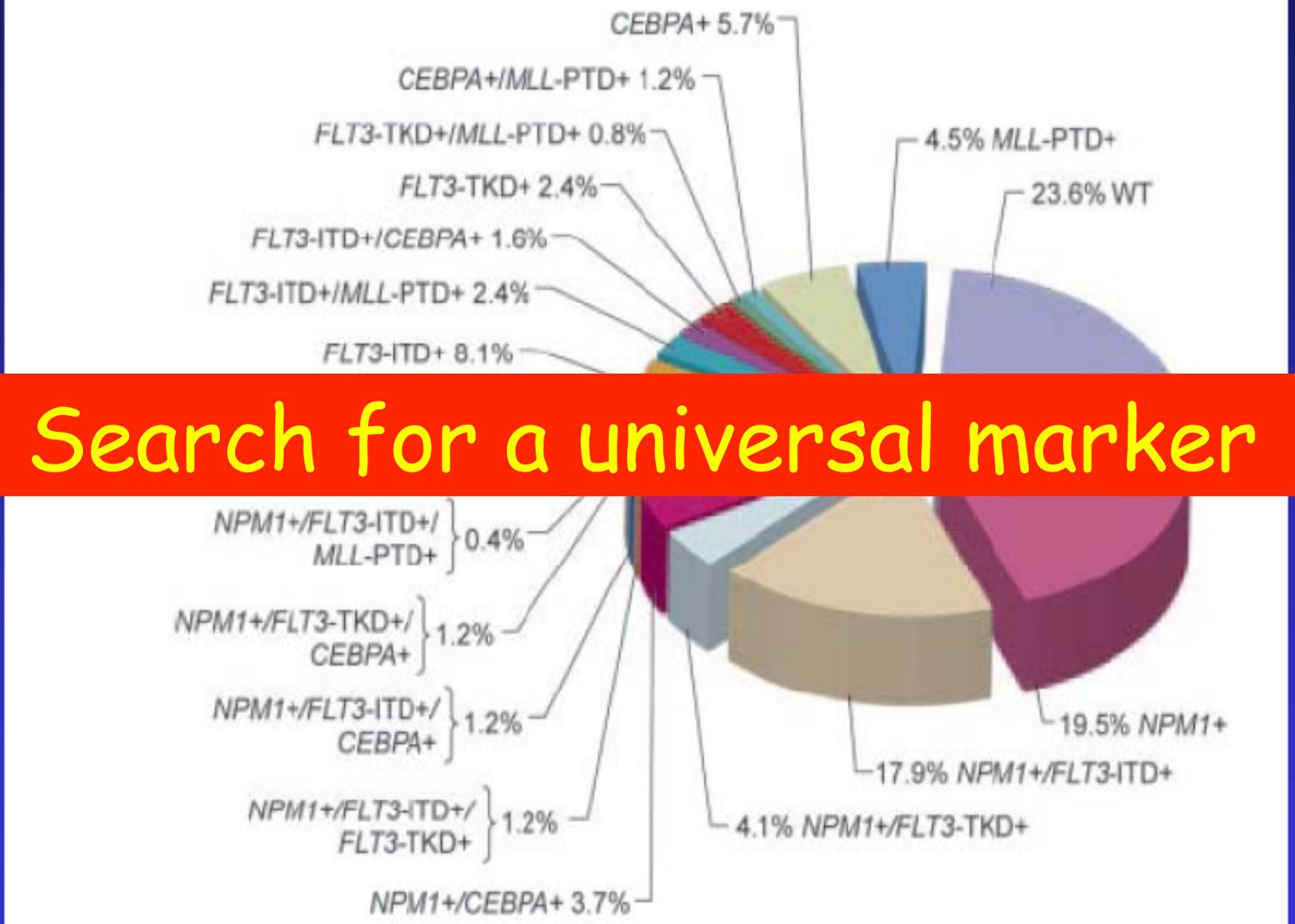


NPM1 mut/ FLT3-ITD +ve



Adam Ivey, Neesa Bhudia, Mandy Gilkes, Rosemary Gale & Robert Hills

Genetic and molecular markers in AML



Search for a universal marker

Mrozek et al., Blood 2008

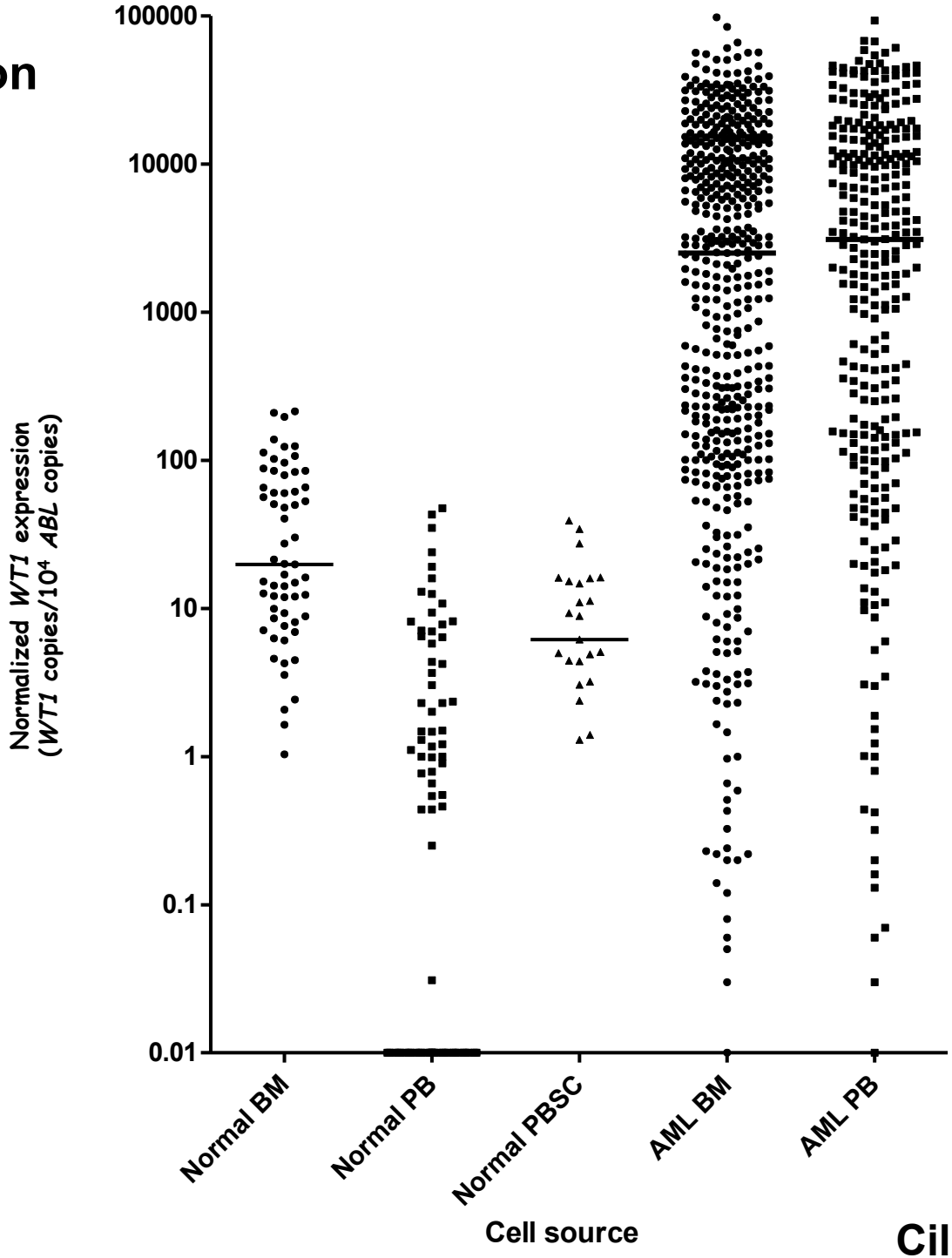
WT1 expression

	mean value (WT1 copies/10000 ABL copies)	range	
Normal BM	35	0-90	
Normal PB	5	0-20	
Conditions associated with WT1 overexpression			**percentage of cases with WT1 overexpression**
Acute myeloid leukemia (AML)	27669	1081-121806	100%
Acute lymphoblastic leukemia (ALL)	13807	318-94682	100%
CML chronic phase and blastic phase	3262	191-54171	100%
Chronic Myelomonocytic leukemia (CMML	4667	1070-23674	100%
Ph negative CML like diseases	9731	890-70980	100%
Primitive Hypereosinophilic Syndromes	280	102-7800	95%
Refractory anaemias	366	100-1289	65%
RAEB	2262	227-11006	100%
RAEB-T	14033	3757-51700	100%

Conditions associated with normal WT1 expression

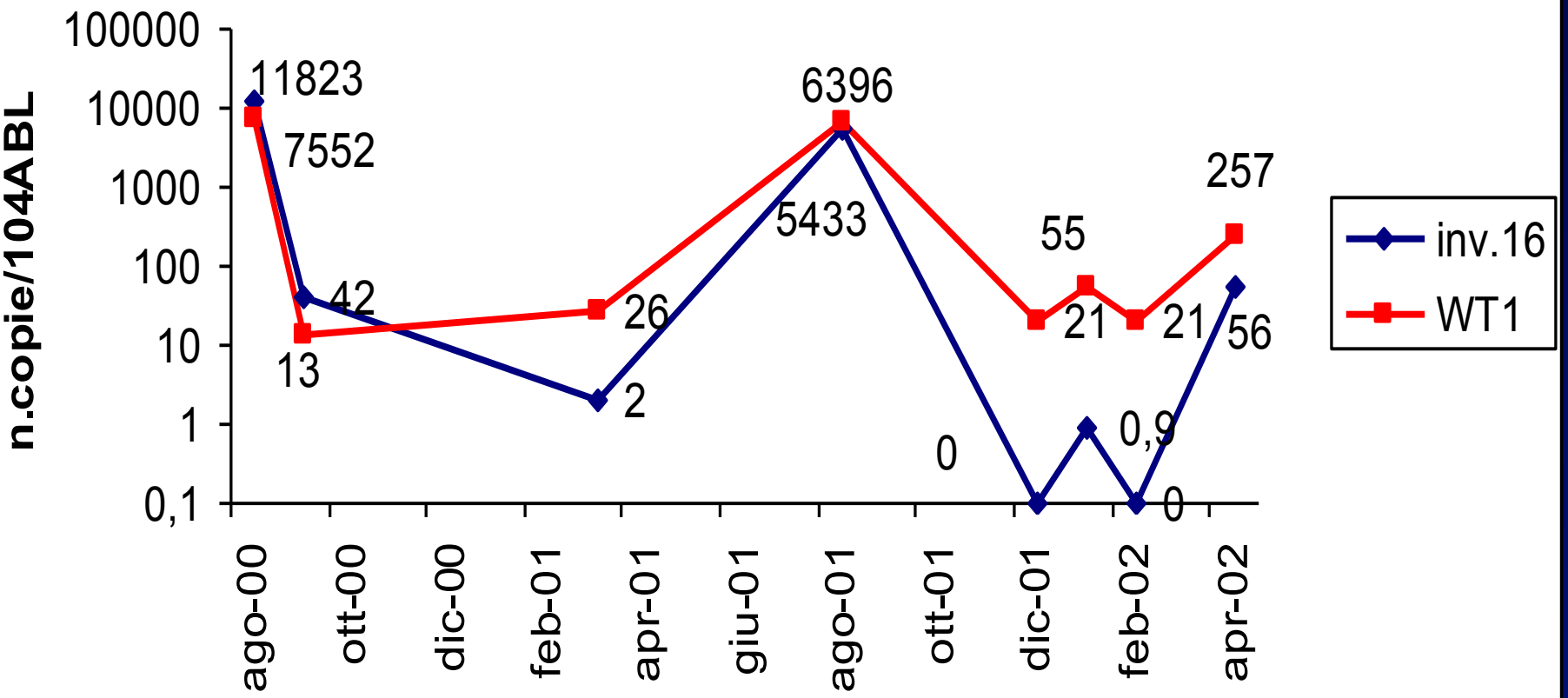
regenerating BM (immature but normal cells)
G-CSF stimulated cells
policlonal anaemias
inflammatory diseases
reactive thrombocytosis

WT1 Standardisation ELN WP12

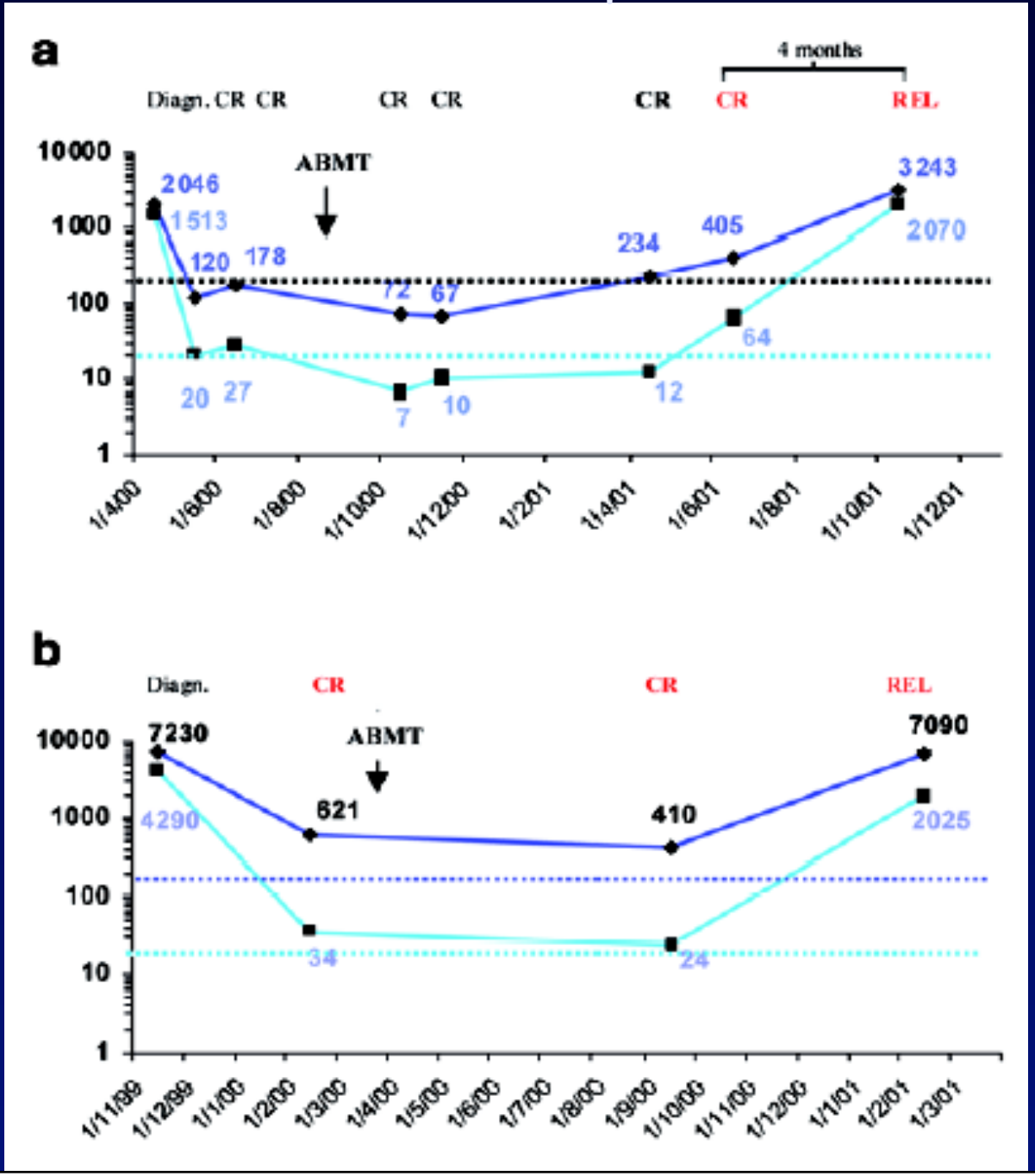


Cilloni et al., JCO 2009

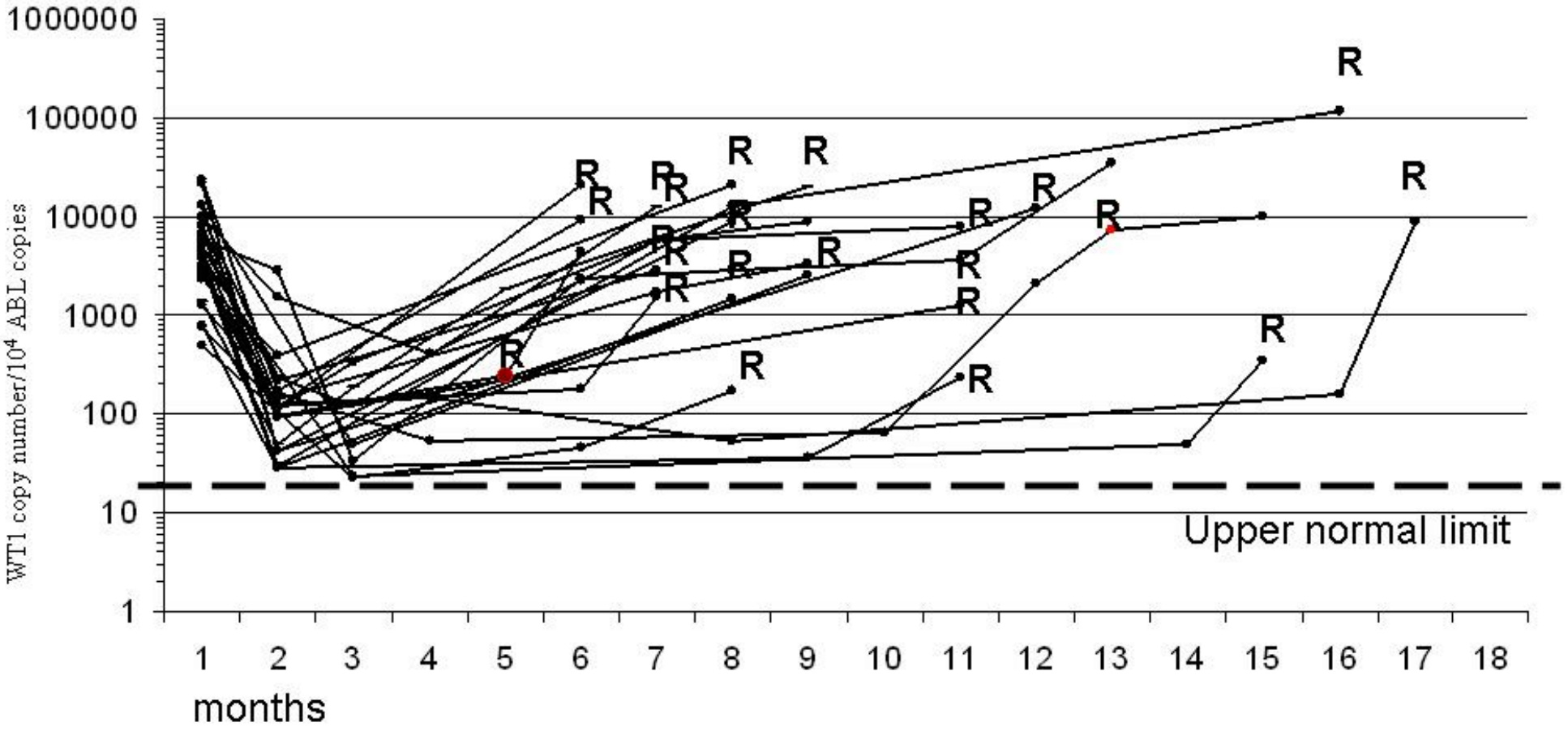
Follow-up of a patient with inv(16) AML



CN AML patients

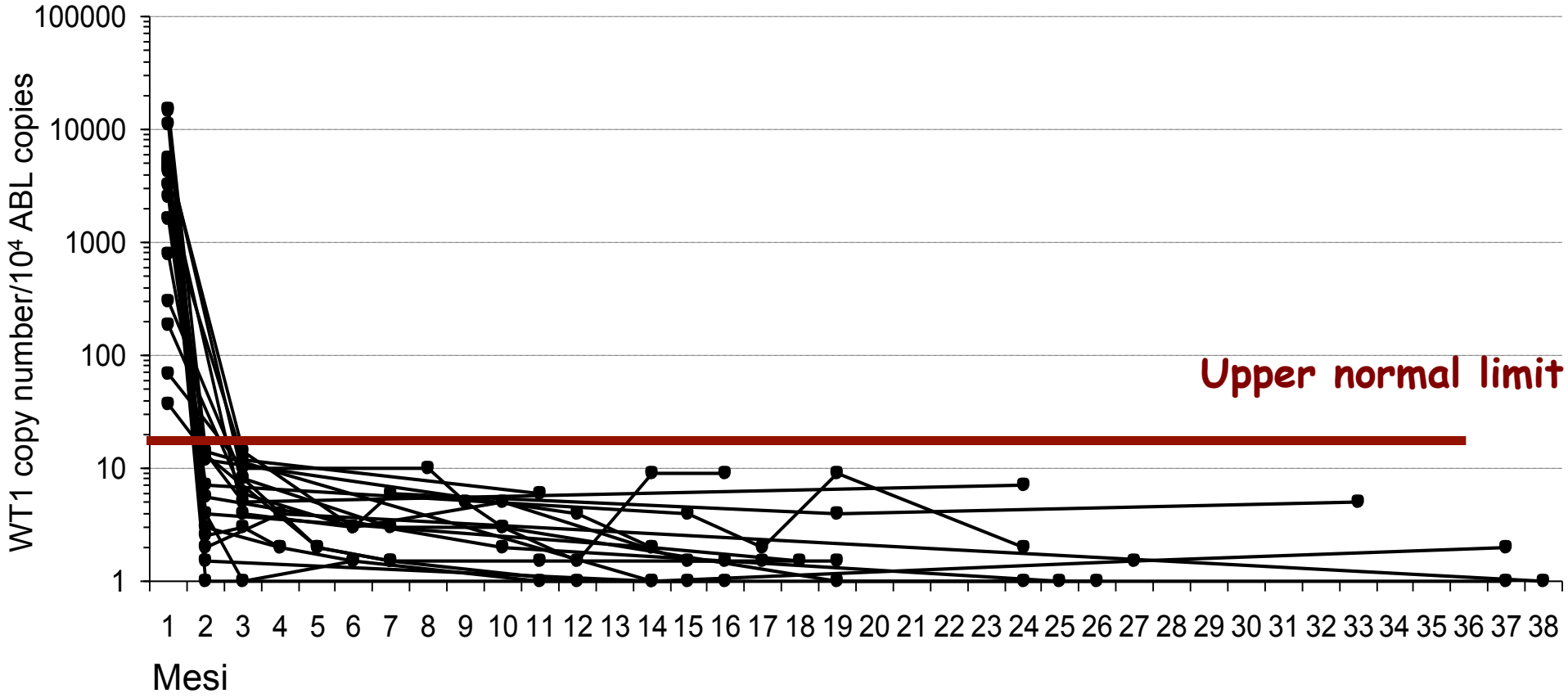


23 CR patients with WT1 above the normal upper limit relapsed after a median of 7 months from diagnosis (range 6-44)



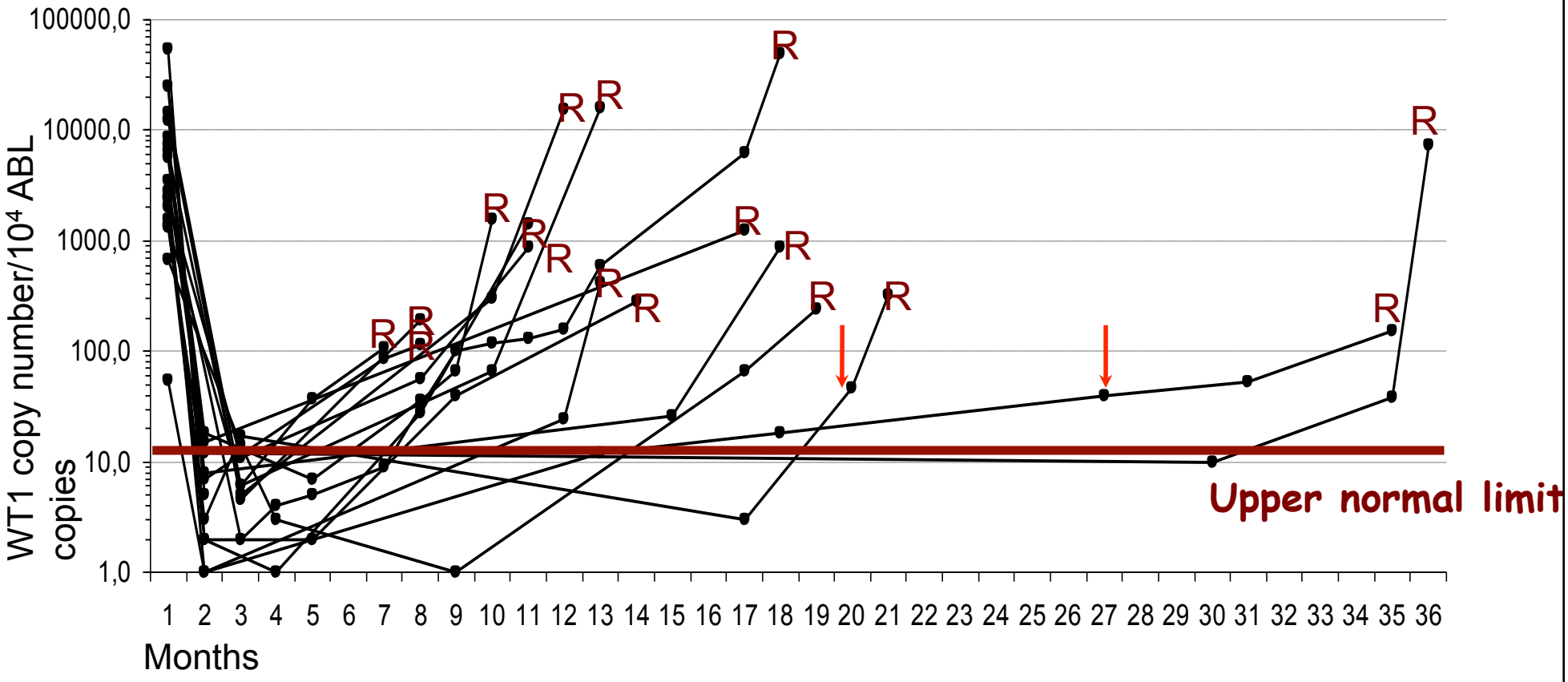
Cilloni et al. Haematologica 2008 ; 93:921

Cilloni et al. Haematologica 2008 ; 93:921



27 pts with WT1 within the normal range after inducion chemotherapy persisted in CR

Cilloni et al. Haematologica 2008 ; 93:921



21 patients with WT1 within the normal range after inducion chemotherapy relapsed after a median of 15 months

1. Menssen HD, Renkl HJ, Rodeck U, Maurer J, Notter M, Schwartz S, Reinhardt R,Thiel E. Presence of Wilms' tumor gene (wt1) transcripts and the WT1 nuclear protein in the majority of human acute leukemias. **Leukemia. 1995** Jun;9(6):1060-7.
2. 2: King-Underwood L, Renshaw J, Pritchard-Jones K. Mutations in the Wilms' tumor gene WT1 in leukemias. **Blood. 1996** Mar 15;87(6): 2171-9.
3. 3: Schmid D, Heinze G, Linnerth B, Tisljar K, Kusec R, Geissler K, Sillaber C,Laczika K, Mitterbauer M, Zöchbauer S, Mannhalter C, Haas OA, Lechner K, Jäger U, Gaiger A. Prognostic significance of WT1 gene expression at diagnosis in adult de novo acute myeloid leukemia. **Leukemia. 1997** May;11(5):639-43.
4. 4: Bergmann L, Maurer U, Weidmann E. Wilms tumor gene expression in acute myeloid leukemias. **Leuk Lymphoma. 1997** May;25(5-6): 435-43. Review.

Until few years ago there were contrasting data in literature

relevance in de novo acute myeloid leukemia. **Leukemia. 1998** Dec;12(12):1886-94.
9. 9: Kreuzer KA, Saborowski A, Lupberger J, Appelt C, Na IK, le Coutre P, Schmidt CA. Fluorescent 5'-exonuclease assay for the absolute quantification of Wilms' tumour gene (WT1) mRNA: implications for monitoring human leukaemias .**Br J Haematol. 2001** Aug;114(2):313-8.
10. 10 Siehl JM, Thiel E, Leben R, Reinwald M, Knauf W, Menssen HD. Quantitative real-time RT-PCR detects elevated Wilms tumor gene (WT1) expression in autologous blood stem cell preparations (PBSCs) from acute myeloid leukaemia (AML) patients indicating contamination with leukemic blasts.**Bone Marrow Transplant. 2002** Mar;29(5):379-81.
11. 11: Trka J, Kalinová M, Hrusák O, Zuna J, Krejcí O, Madzo J, Sedlácek P, Vávra V, Michalová K, Jarosová M, Starý J; For Czech Paediatric Haematology Working Group. Real-time quantitative PCR detection of WT1 gene expression in children with AML:prognostic significance, correlation with disease status and residual diseasedetection by flow cytometry. **Leukemia. 2002** Jul;16(7):1381-9.
12. 12: Menssen HD, Siehl JM, Thiel E. Wilms tumor gene (WT1) expression as a panleukemic marker. **Int J Hematol. 2002** Aug;76(2):103-9. Review.1
13. 13: Cilloni D, Gottardi E, De Micheli D, Serra A, Volpe G, Messa F, Rege-Cambrin G, Guerrasio A, Divona M, Lo Coco F, Saglio G. Quantitative assessment of WT1 expression by real time quantitative PCR may be a useful tool for monitoring minimal residual disease in acute leukemia patients. **Leukemia. 2002** Oct;16(10):2115-21.

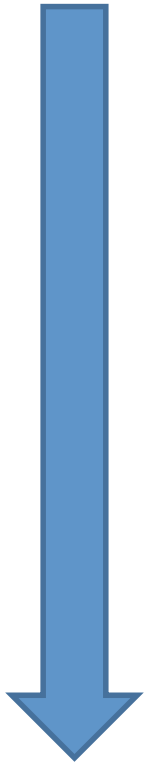
Reasons for discrepancies

The vast majority of the published studies are retrospective

Different populations of patients

Different methods and procedures

Important steps in WT1 monitoring



- RT-PCR
- Quantitative RT-PCR
- Standardization of the methods

2009. Cilloni et al. JCO

Standardization of Real Time procedure for WT1 detection

Turin
London
Manchester
Naples
Prague
Olomouc
Rotterdam
Nijmegen
Aarhus
Munich
Barcelona
Lille

✓9 published and in house *WT1* sets of primers and probe were tested

✓Plasmid containing the full length WT1 sequence was provided by Ipsogen (Marseille, France)

✓Standard curves: plasmid dilutions
✓Normal and diagnostic BM and PB samples

✓The influence of different instruments and reagents was established

✓**ABL** used as housekeeping gene

Assays were excluded basing on:

✓ demonstrated lack of RNA-specificity
✓ location within the 3' region of the gene which has been shown to be subject to deletion or mutations in AML

Selected assay: located within the 5' region

*(J.P. Van Dijk, Br J Haematol 118:1027-1033, **2002**)*

Forward **ccgctattcgcaatcagggtta**

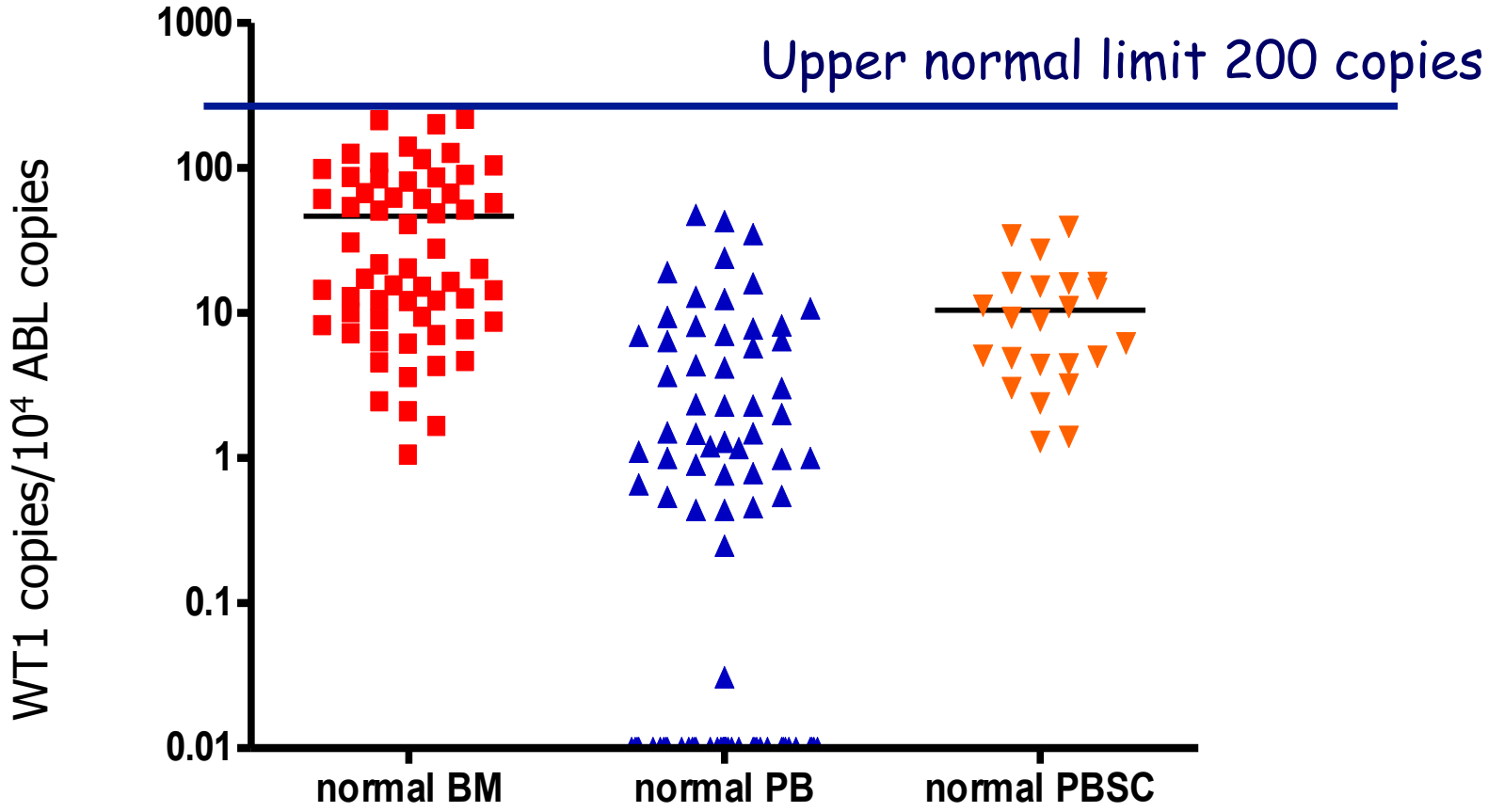
Reverse **gggcgtgtgaccgtagct**

Probe: **cagcacggtcaccttcgacgg**

High level of RNA specificity

High sensitivity

WT1 expression in 204 normal samples (61 BM, 118 PB, 25 PBSC)



	normal BM	normal PB	normal PBSC
Median	19,8	0,01	6,1
Range	0-200	0,01-47	0-39

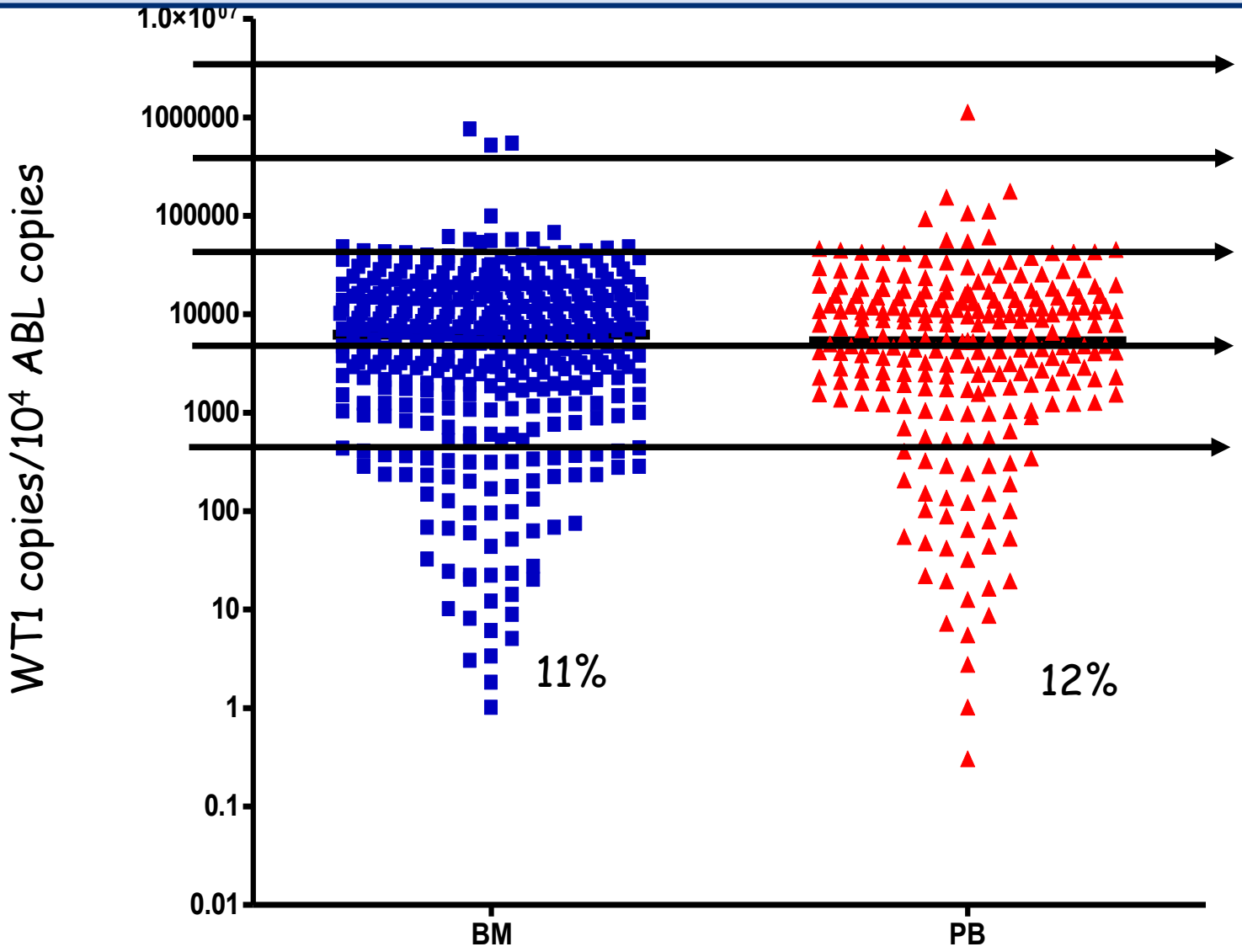
Open issues

- Can WT1 levels after chemotherapy predict the outcome of AML patients?
- If yes at which time point during/after chemotherapy?
- Does WT1 value at diagnosis impact on prognosis?
- Is WT1 useful to predict relapse after allogeneic stem cell transplant?
- BM or PB?

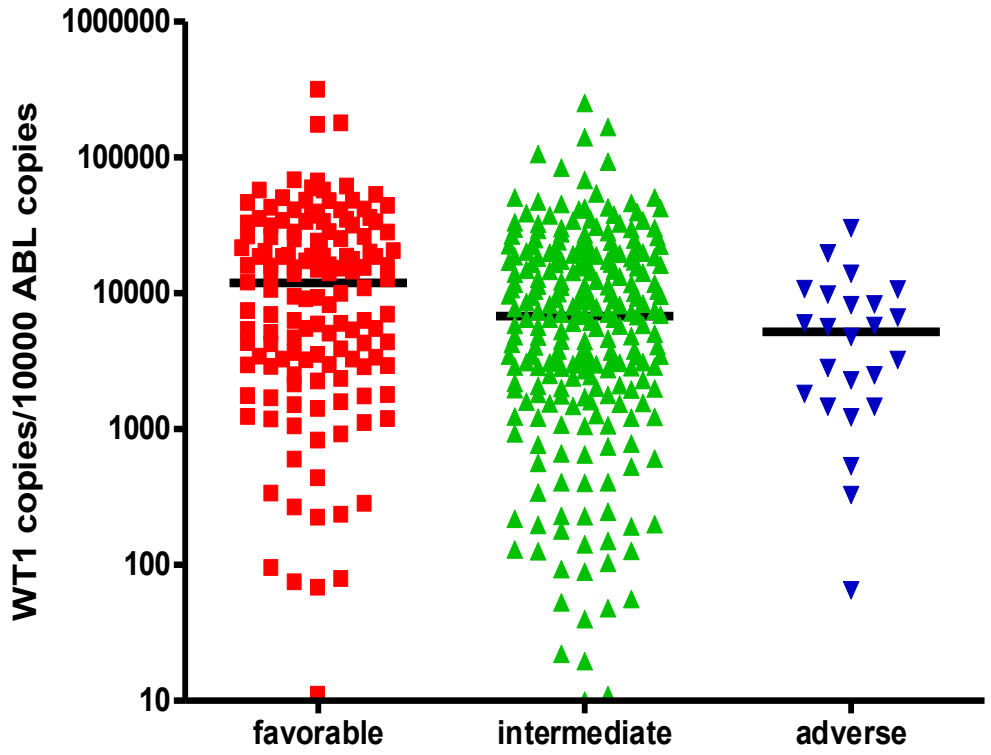
WT1 expression in **729** samples from AML at diagnosis

(collected by the European Leukemia Net)

(588 BM, 141 PB)



- No significant difference in WT1 expression at diagnosis by stratifying the patients according to:
  - cytogenetic risk groups (except for APL patients who show significantly higher WT1 values)
  - mutations of NPM1 or FLT3



Cilloni et al. JCO 2009

AML patients during follow-up (ELN study)

- 114 patients evaluated at diagnosis and during follow-up

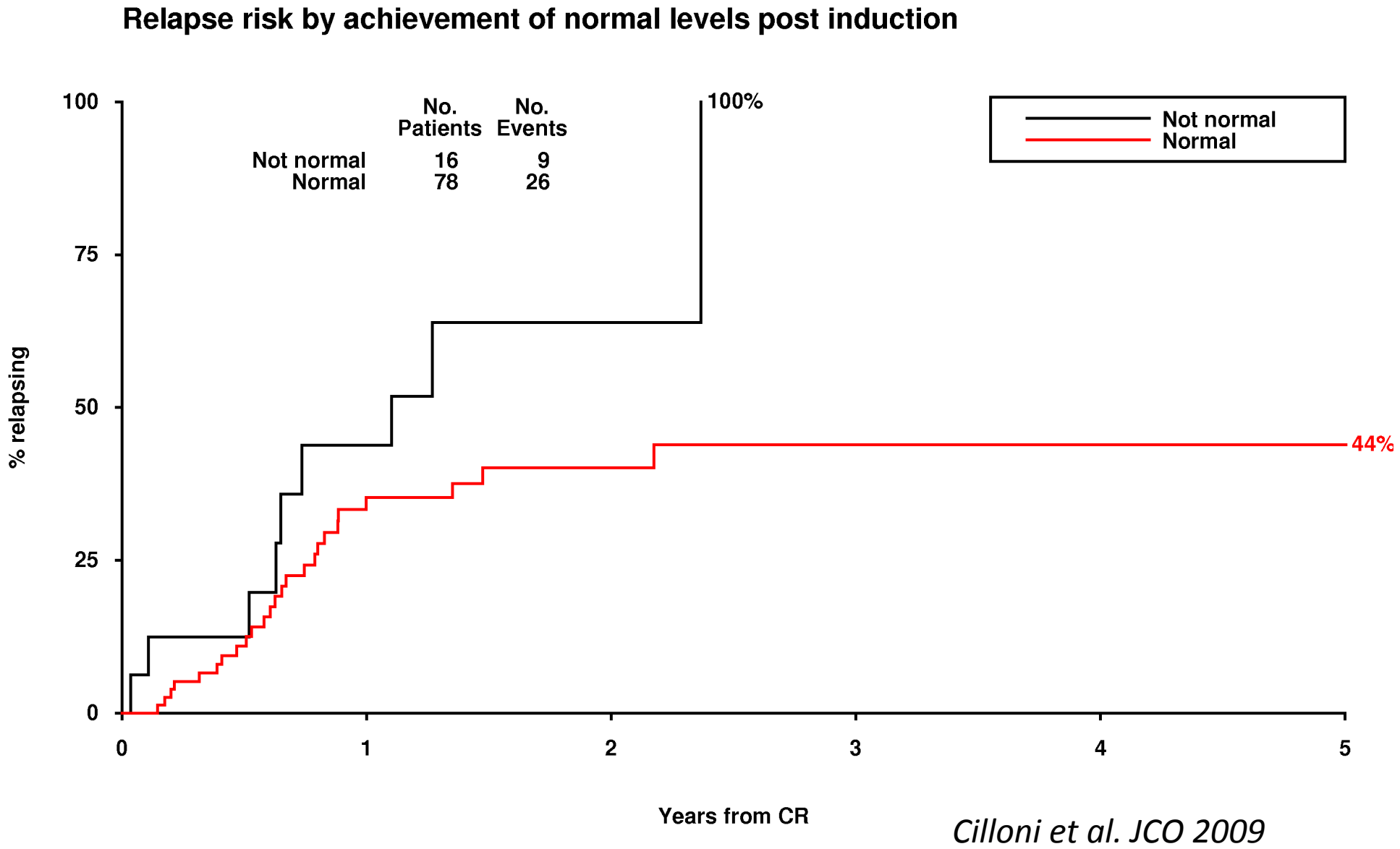
- All the patients included have been previously characterized by cytogenetic and molecular analysis

- Clinical data available

- All the patients were treated with intensive anthracycline and ARA-C

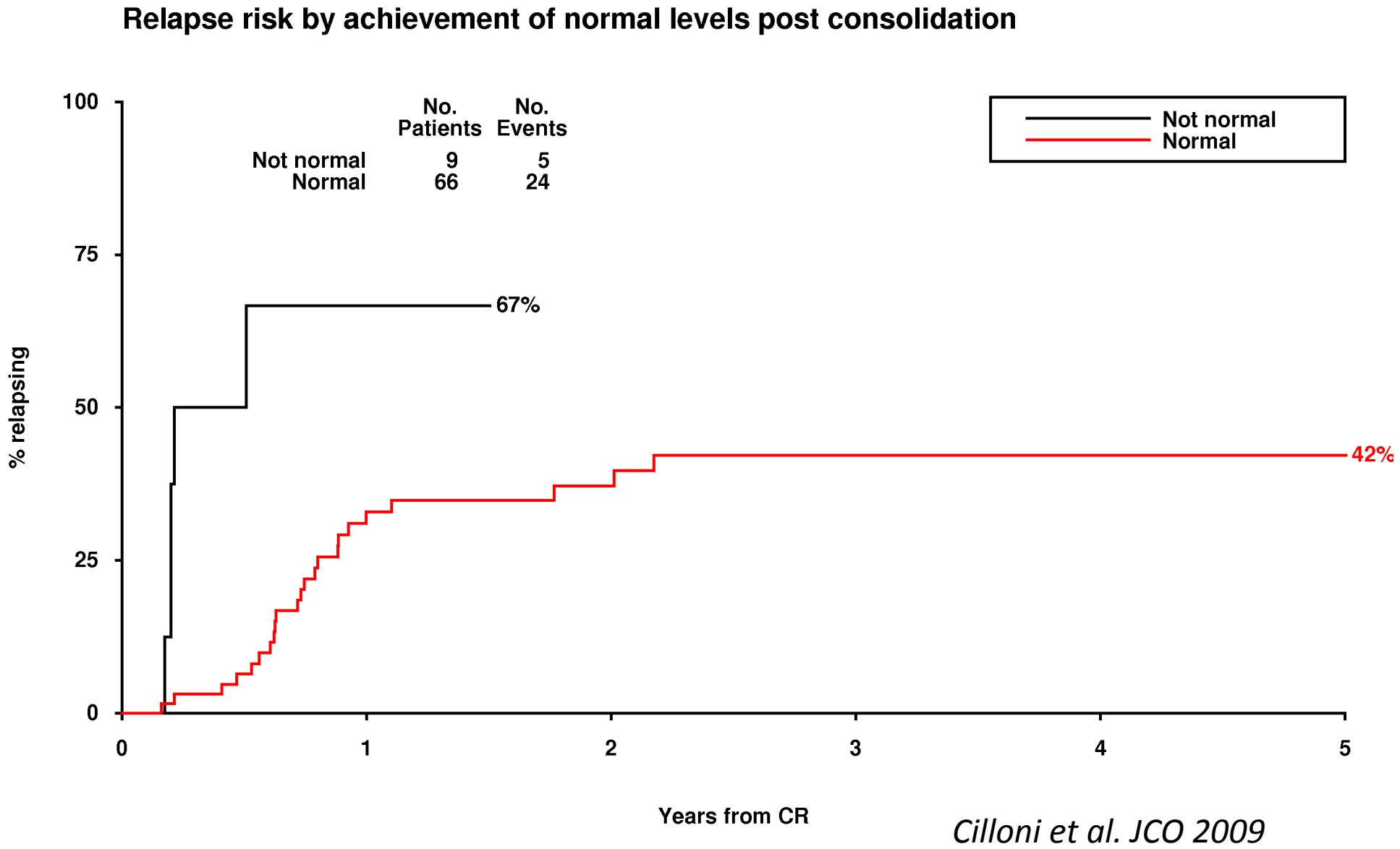
- 91/114 (80%) showed WT1 copies > $20.000/10^4$ ABL at diagnosis (2 logs higher than normal controls)

The achievement of normal WT1 values after induction chemotherapy is predictive of relapse



Cilloni et al. JCO 2009

The achievement of normal WT1 values after consolidation chemotherapy is predictive of relapse



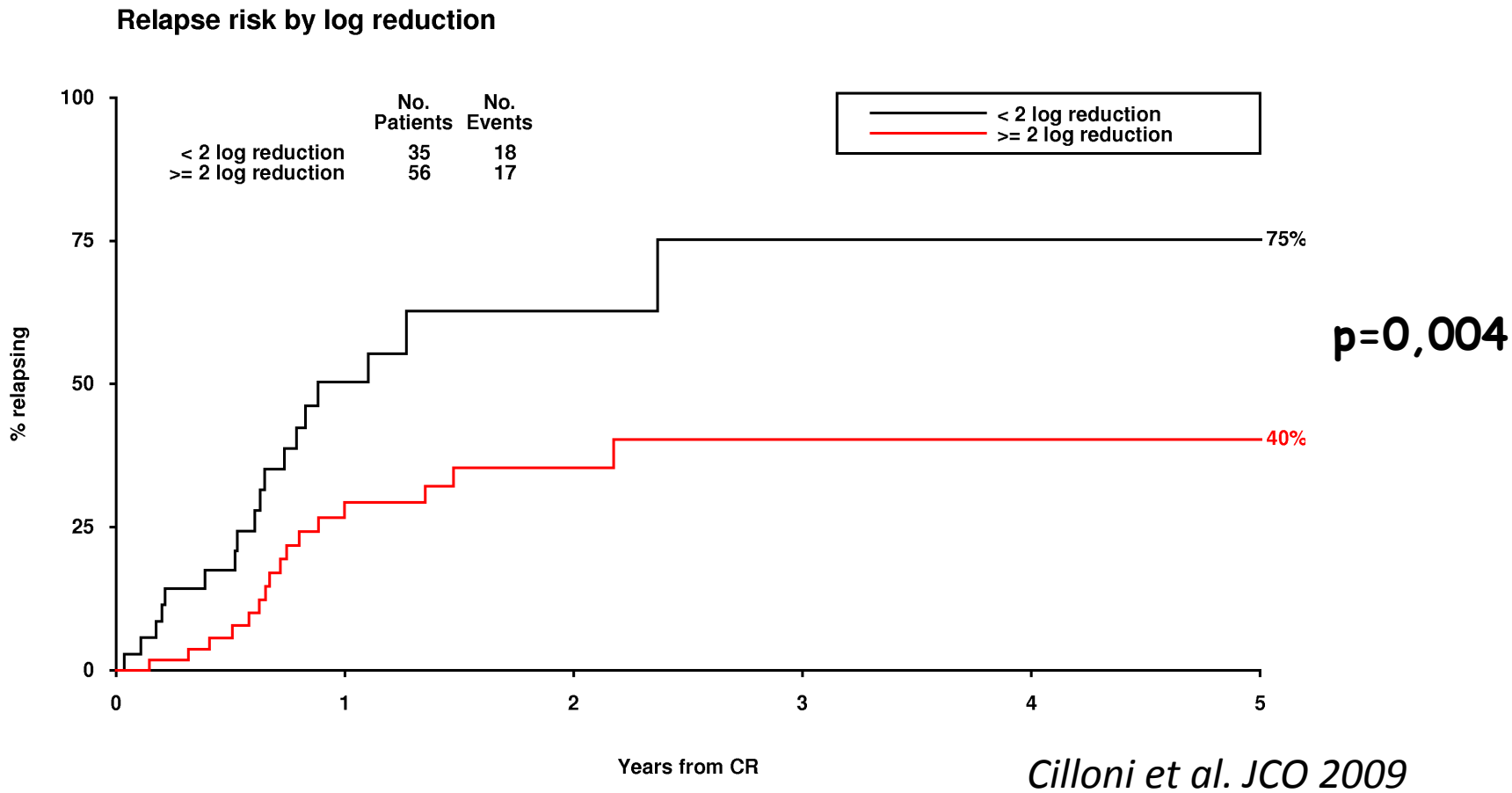
Cilloni et al. JCO 2009

Kinetics of *WT1* response following induction therapy predicts risk of subsequent relapse

Analysis in 91/114 cases with baseline *WT1* > 2 x 10^4 copies/ 10^4 *ABL* copies

Greater reduction in *WT1* decreases risk of relapse

HR 0.54 (0.36-0.83) p=0.004



p=0,004

Cilloni et al. JCO 2009

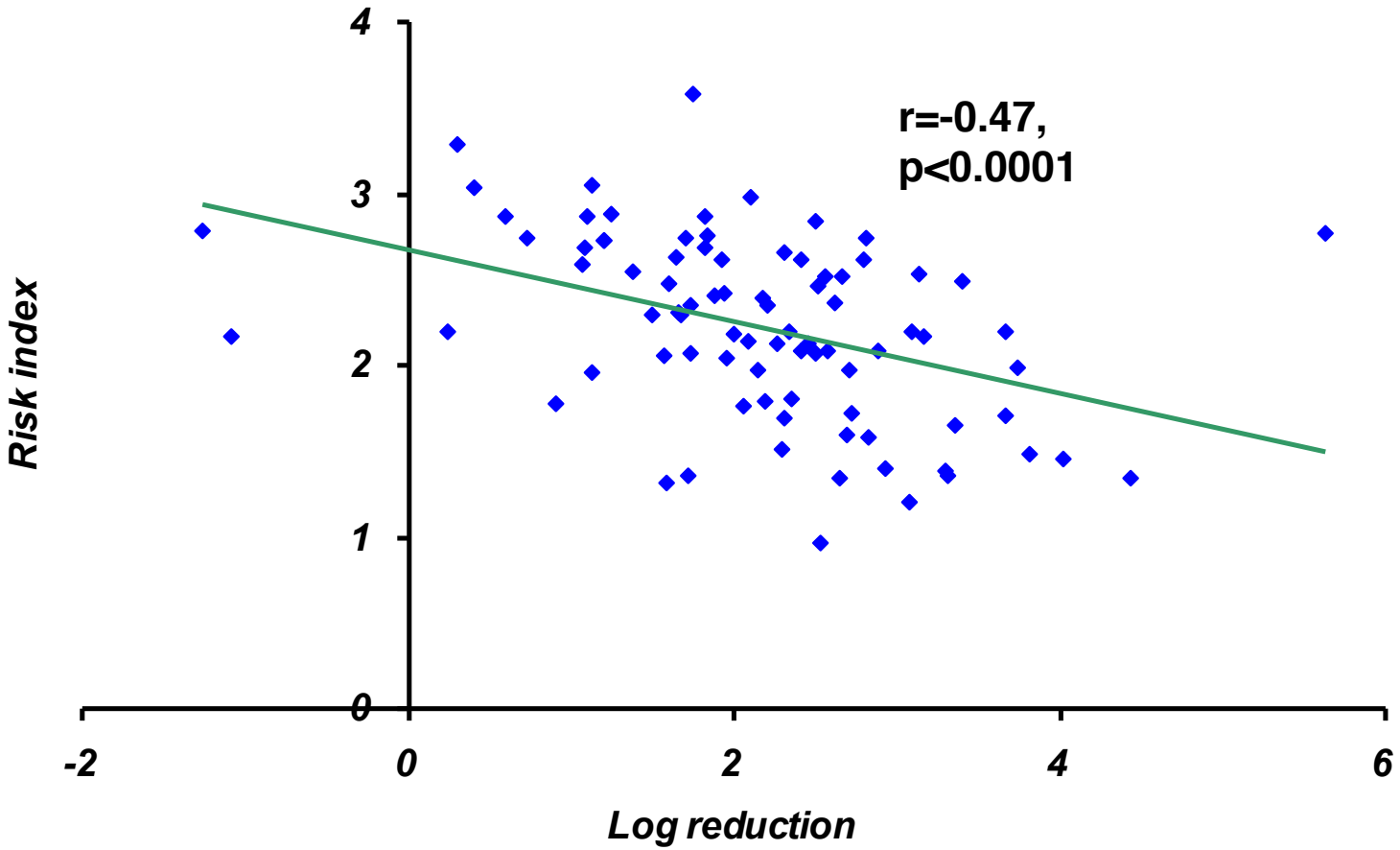
Does log reduction add to the risk score?

- Regression analysis showed that "log reduction" is an independent predictor of relapse

- adjusted for age: HR 0.54 (0.35-0.83) p=0.05
- adjusted for WBC: HR 0.54 (0.35-0.81) p=0.003
- adjusted for cytogenetics: HR 0.63 (0.41-0.98) p=0.04

- **Log reduction remains prognostic even when adjusted for age, WBC, cytogenetics individually**

Kinetics of *WT1* transcript reduction post-induction correlate with MRC risk index



Cilloni et al. JCO 2009

Prospectic study

WT1 at diagnosis and during follow up in AML patients (18-60 years) enrolled in the GIMEMA study treated with the same chemotherapeutical scheme

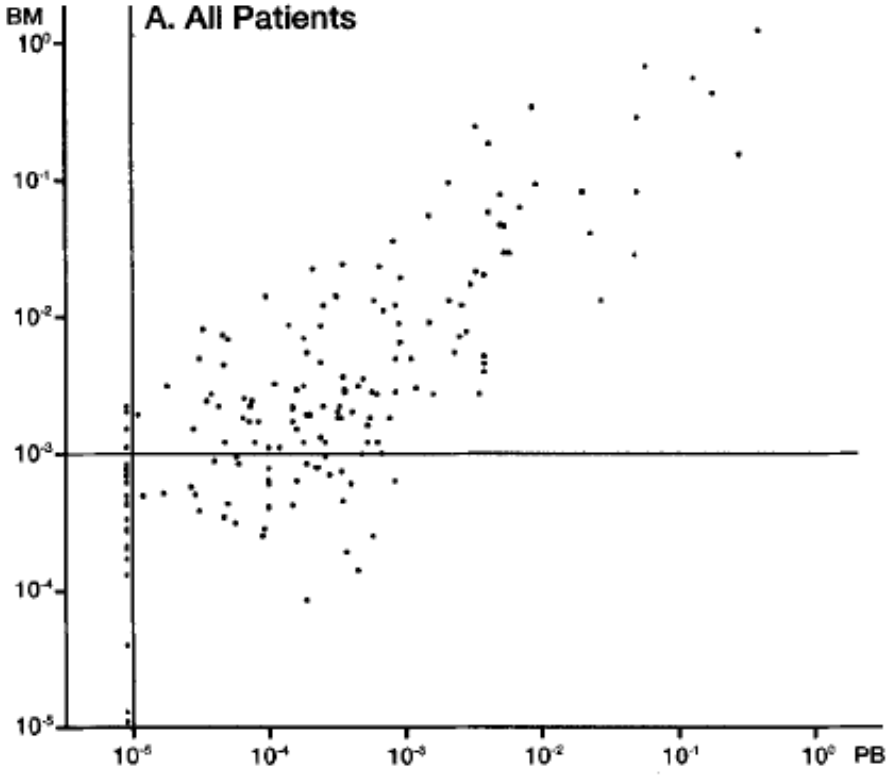
PB or BM for MRD detection?

The vast majority of published papers showed that PB is as sensitive as BM

PB even better?

Long-Term Follow-Up of Minimal Residual Disease in Leukemia Patients by Monitoring WT1 (Wilms Tumor Gene) Expression Levels

By Kazushi Inoue, Hiroyasu Ogawa, Tamotsu Yamagami, Toshihiro Soma, Yoshihiko Tani, Toyoshi Tatekawa, Yusuke Oji, Hiroya Tamaki, Taiichi Kyo, Hiroo Dohy, Akira Hiraoka, Tohru Masaoka, Tadamitsu Kishimoto, and Haruo Sugiyama



Strong correlation between the MRD detected in BM and PB.

Blood. 1996; 88:2267-78.

Conclusions

Detection of WT1 in PB may be even more sensitive than in BM because of the lower level of expression in normal PB samples

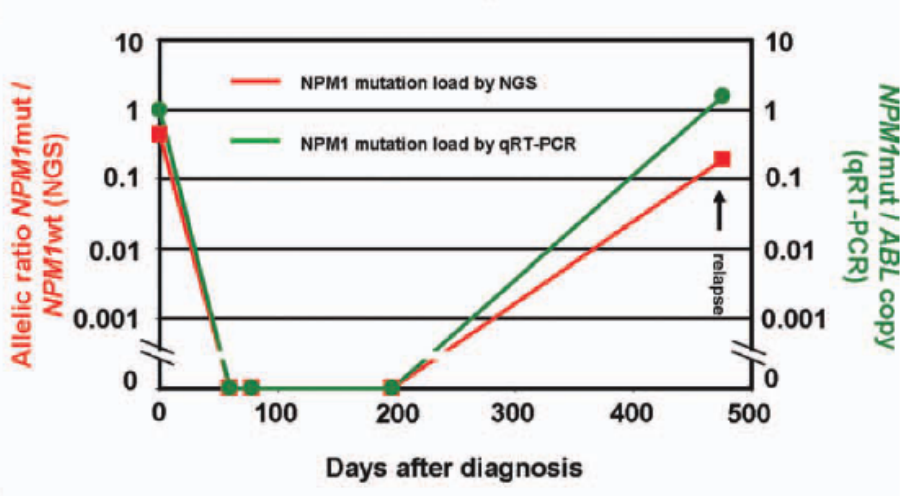
Need for a higher level of standardization

Two different WT1 qRT-PCR assay kits are mainly used in published papers

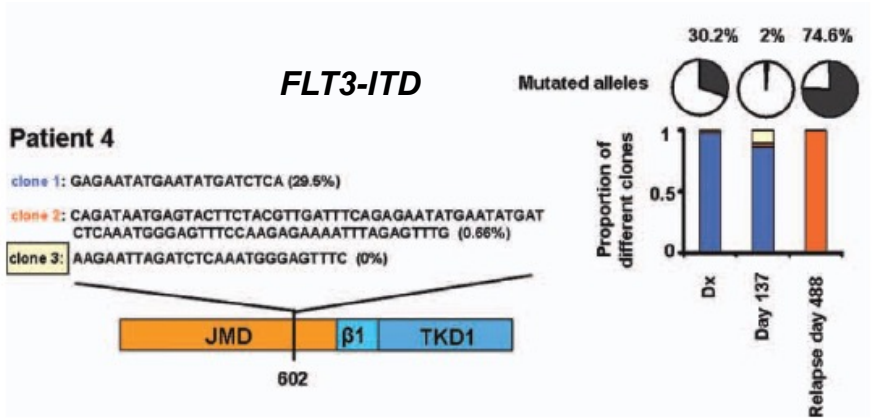
- Otsuka Pharmatheutical Co., Ltd. (Approved in Japan)
- Ipsogen (France) (Qiagen) (Cilloni et al. JCO 2009)
- Additional standardized assays: Willasch et al. Leukemia 2009. (pediatric AML)

Potential of next generation sequencing approaches to track treatment response

***NPM1* mut**

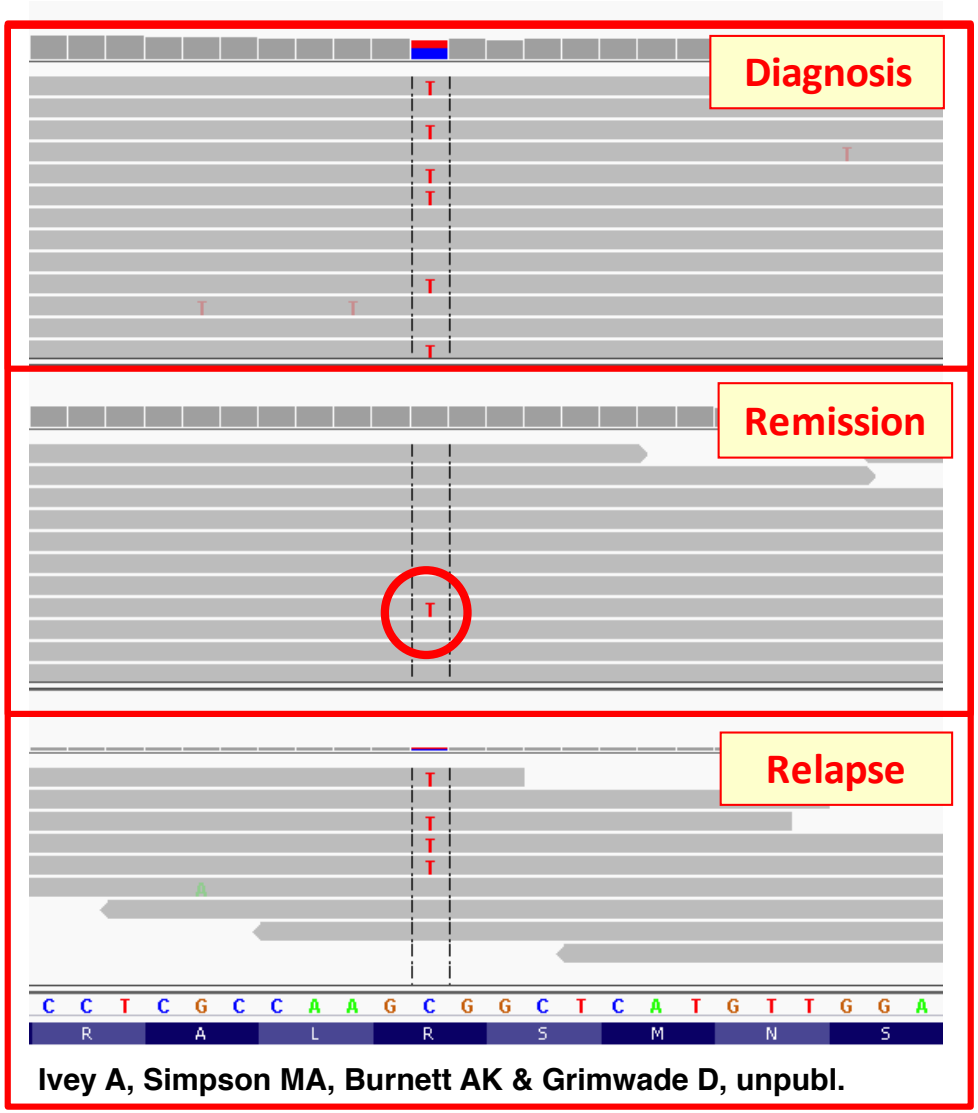


FLT3-ITD

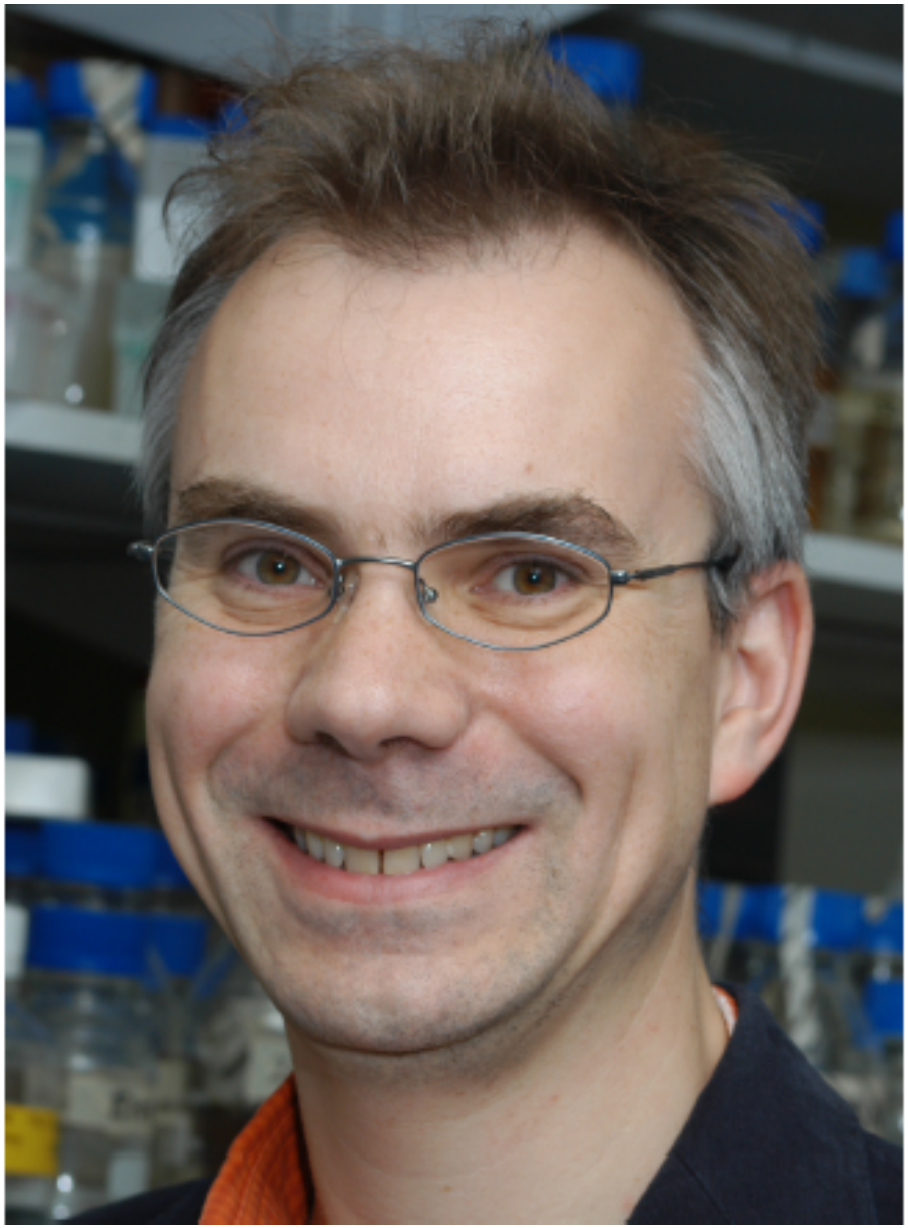


Thol *et al. Genes Chromosomes Cancer* 2012.

***DNMT3A* mut**



Ivey A, Simpson MA, Burnett AK & Grimwade D, unpubl.



Thank you David!