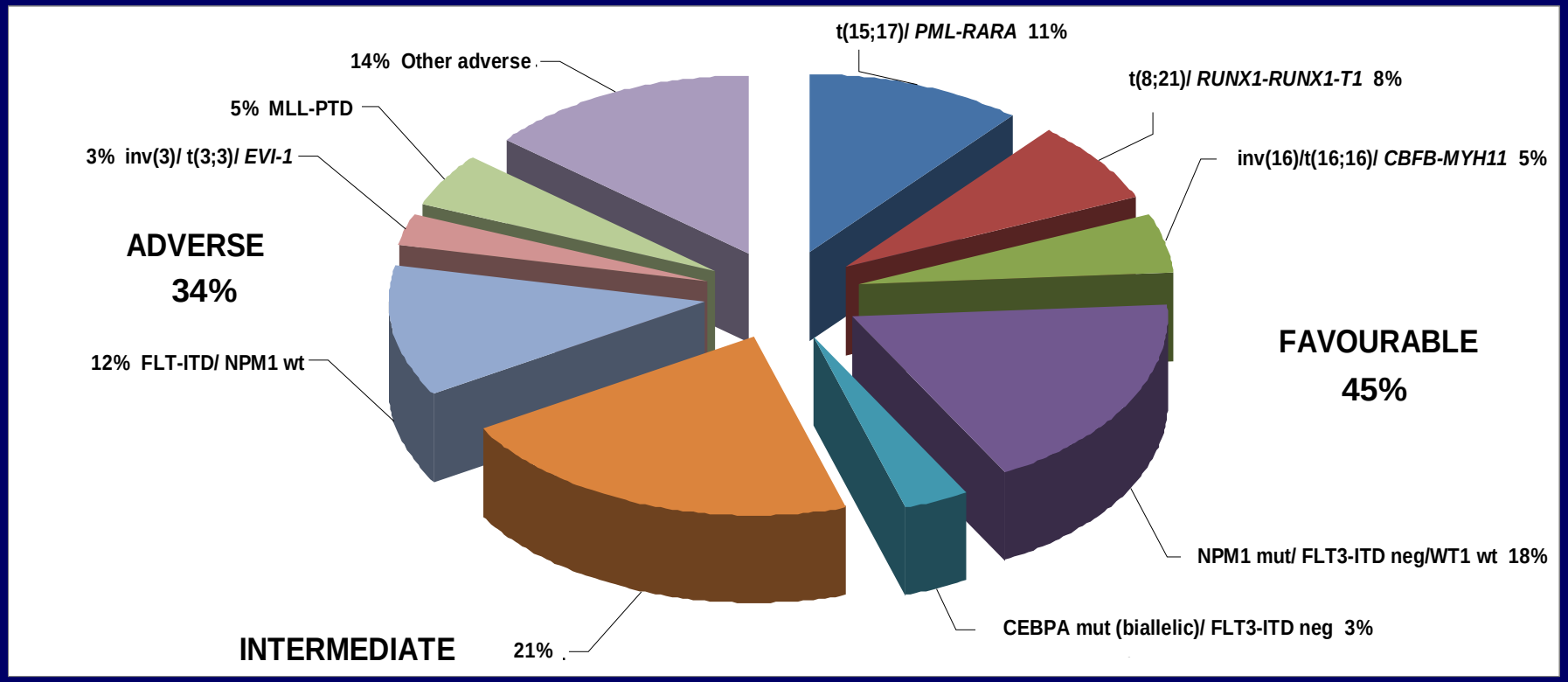
Wilms' Tumour gene (*WT1*) as a potential MRD marker in AML

David Grimwade

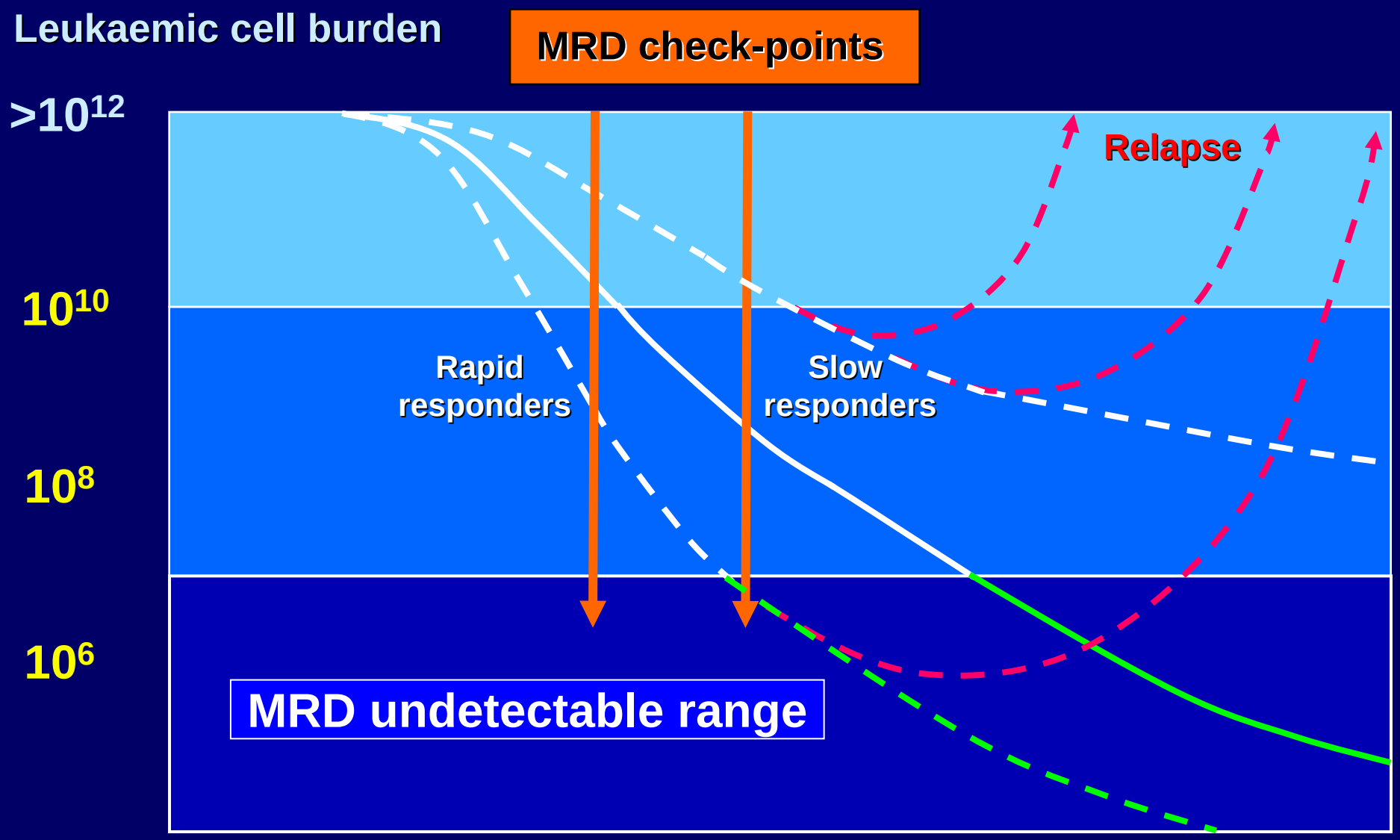
ELN European LeukemiaNet

Standardised leukaemia-specific RT-qPCR assays for MRD detection are currently available in ~50% of AML cases

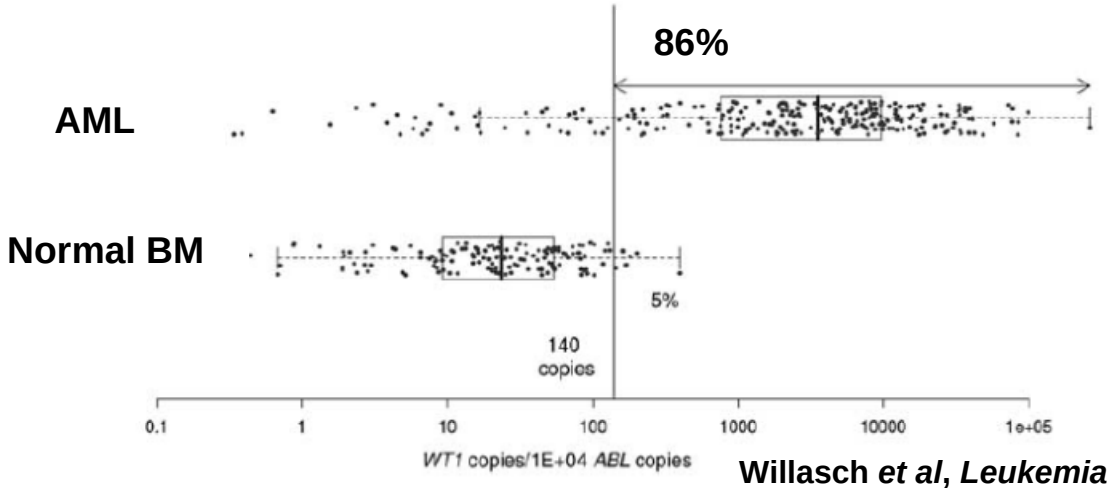


Grimwade & Hills, *Hematology Am Soc Hematol Educ Program* 2009; 385-95.

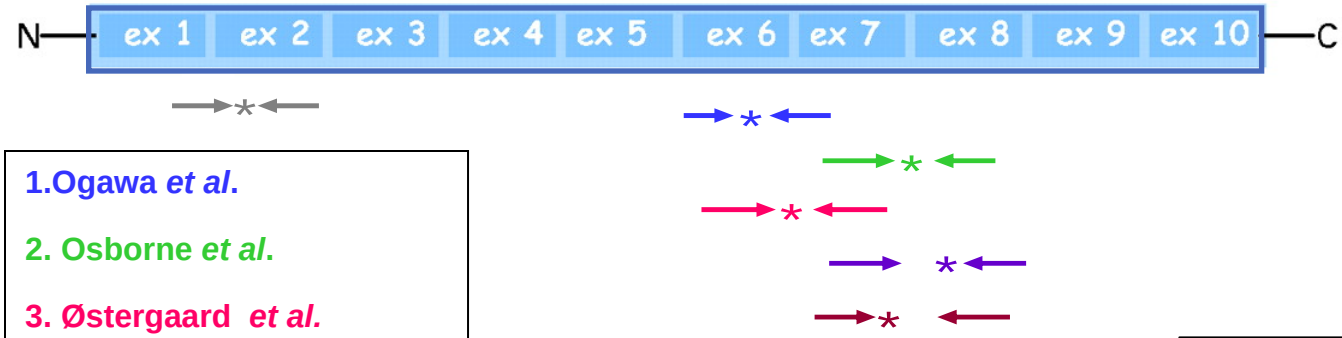
Assessment of kinetics of disease response to inform risk-stratification



Investigation of *WT1* as a potential MRD target in AML



Willasch *et al*, *Leukemia* 2009; 23: 1472-9



1.Ogawa *et al*.

2. Osborne *et al*.

3. Østergaard *et al.*

4-7. Unpublished.

8. Cilloni *et al*.

9. Van Dijk *et al*.

European LeukemiaNet

Cilloni *et al*, *J Clin Oncol* 2009, 27: 5195-201

Development of standardised RT-qPCR assays for detection of *WT1* transcripts

3 assays eliminated:

- Cross-reactivity with genomic DNA (n=1)
- Low sensitivity (n=2)

3 assays eliminated:

- Reduced efficiency & sensitivity (n=3)

Best-performing *WT1* assay selected:

- Designated ELN WT1 assay

Phase I

9 *WT1* assays evaluated:

- Cell line dilutions
- Genomic DNA

→

Phase 2

6 *WT1* assays evaluated:

- Plasmid standards

→

Phase 3

3 *WT1* assays evaluated:

- Cell line dilutions
- Plasmid standards

→

Phase 4

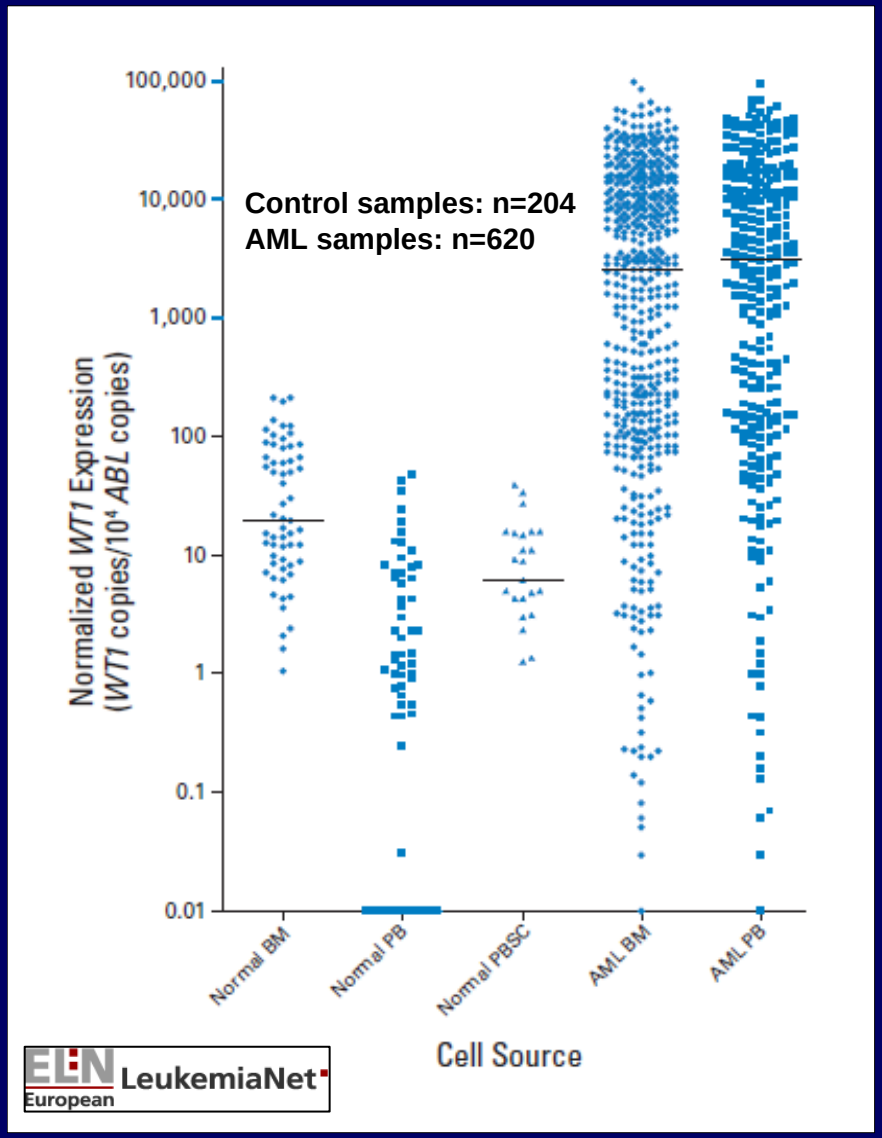
Evaluation of ELN assay

- Normal PB/BM/PBSCs (n=204)
- Diagnostic AML samples (n=620)
- Follow-up samples from 145 pts

ELN European LeukemiaNet

Cilloni *et al*, *J Clin Oncol* 2009, 27: 5195-201

Exploring the use of over-expressed genes to track treatment response in AML



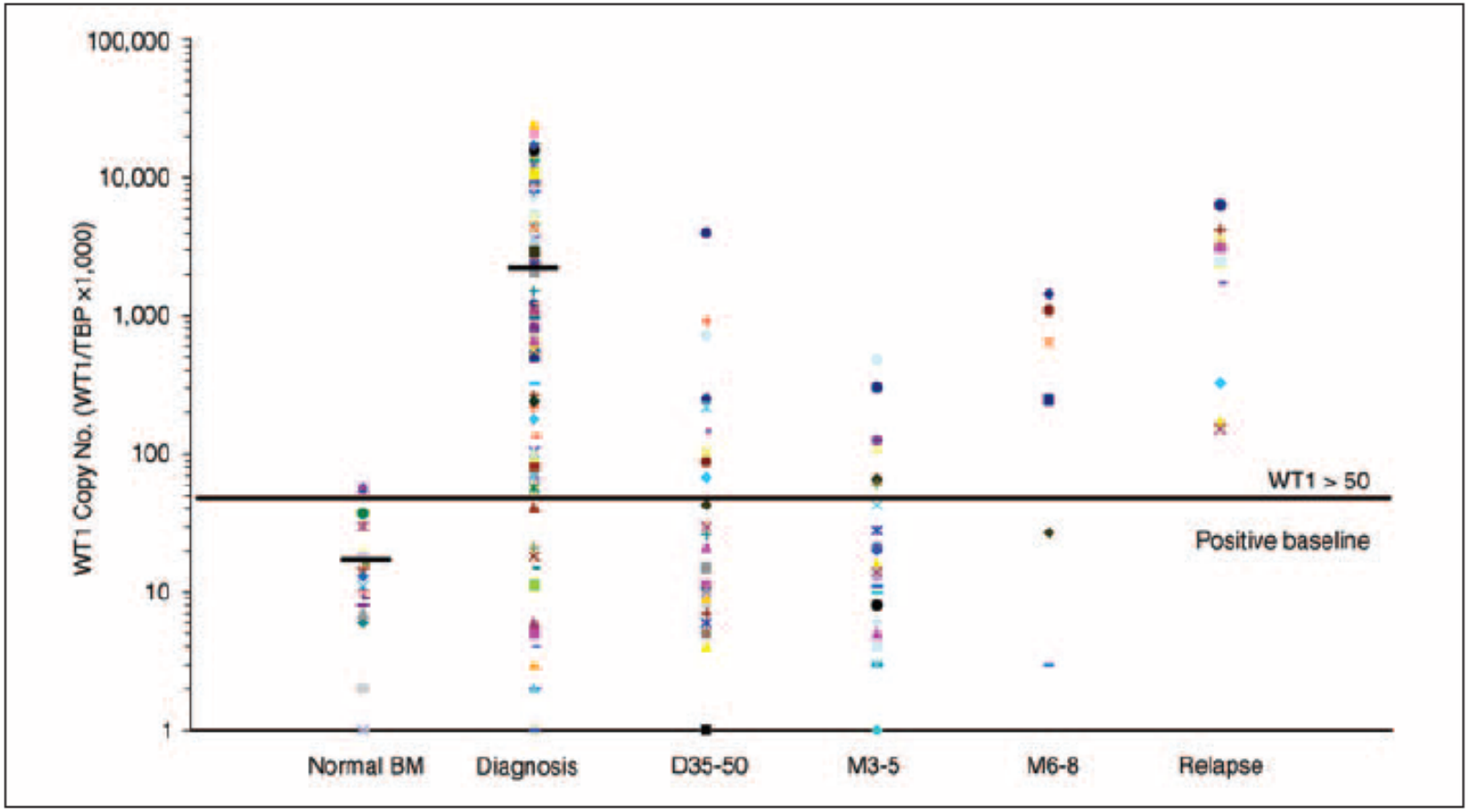
Wilms Tumour gene (*WT1*)

Compared to level of expression in normal PB & BM:

***WT1 is* sufficiently over-expressed to discriminate 2-log reduction in transcripts in 46% and 13% of AML, according to follow-up sample source**

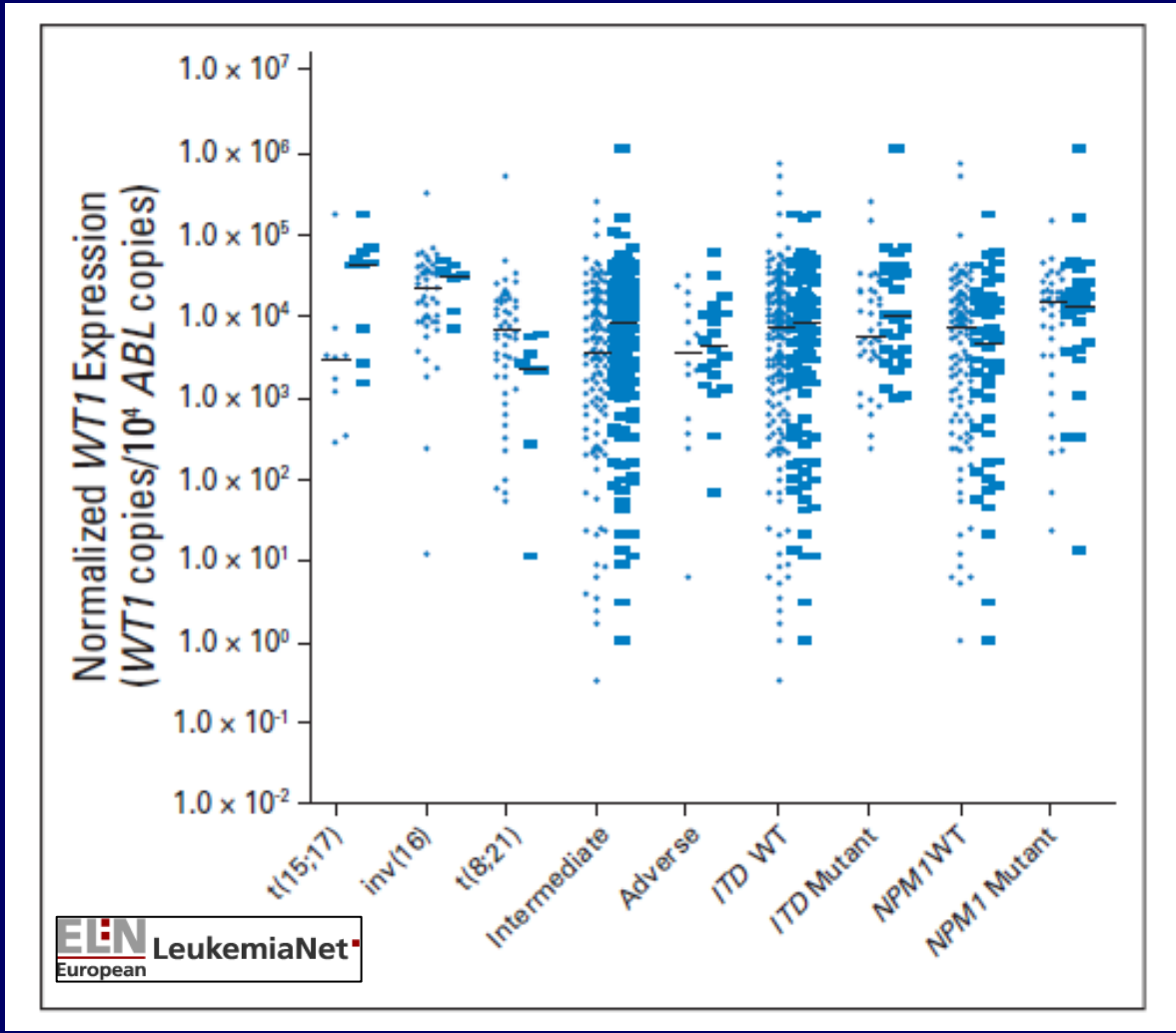
Cilloni *et al*, *J Clin Oncol* 2009, 27: 5195-201

WT1 is over-expressed in majority of cases of paediatric AML



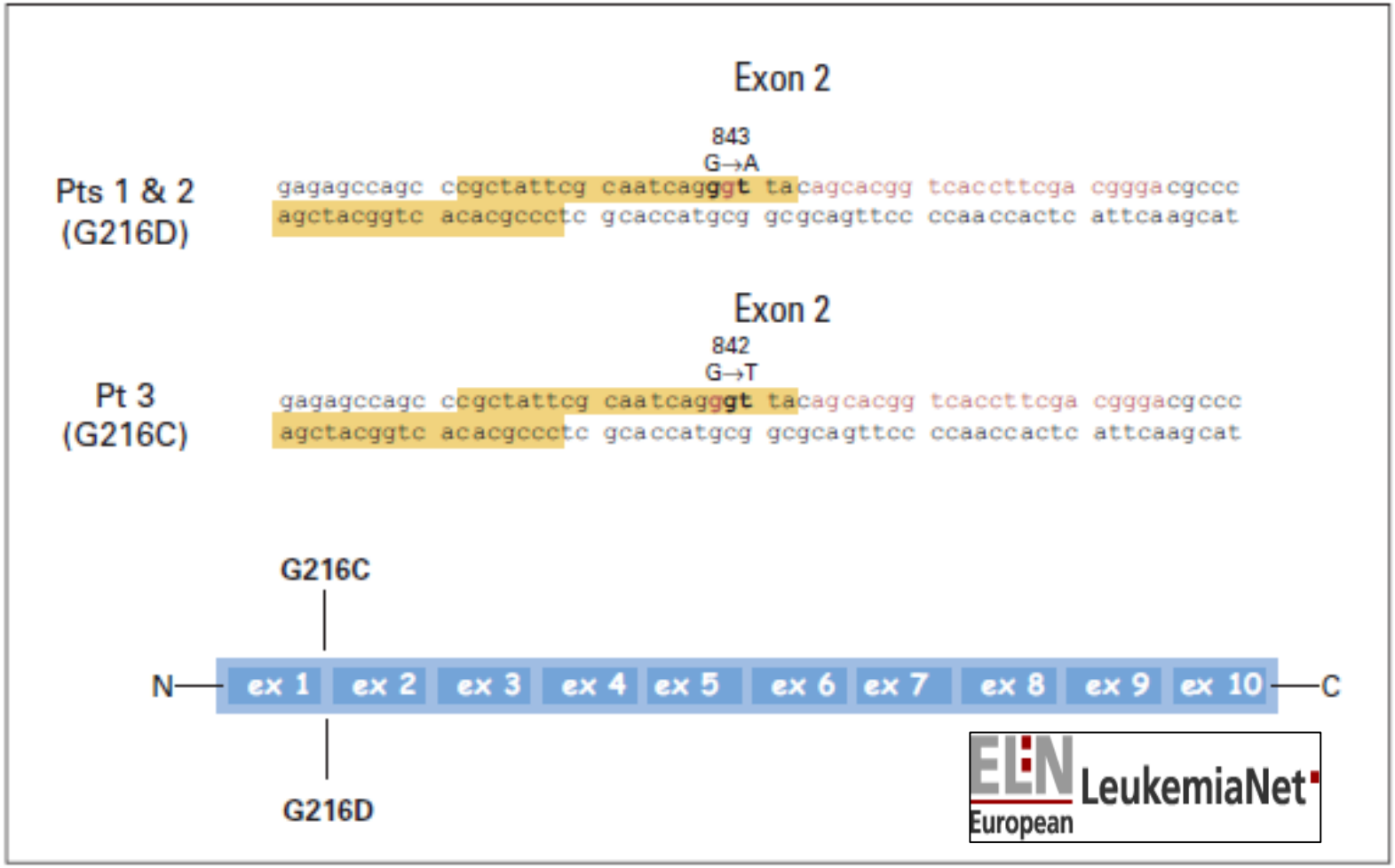
Lapillonne *et al*, *J Clin Oncol* 2006, 24: 1507-15

WT1 expression in cytogenetic and molecular subtypes of adult AML



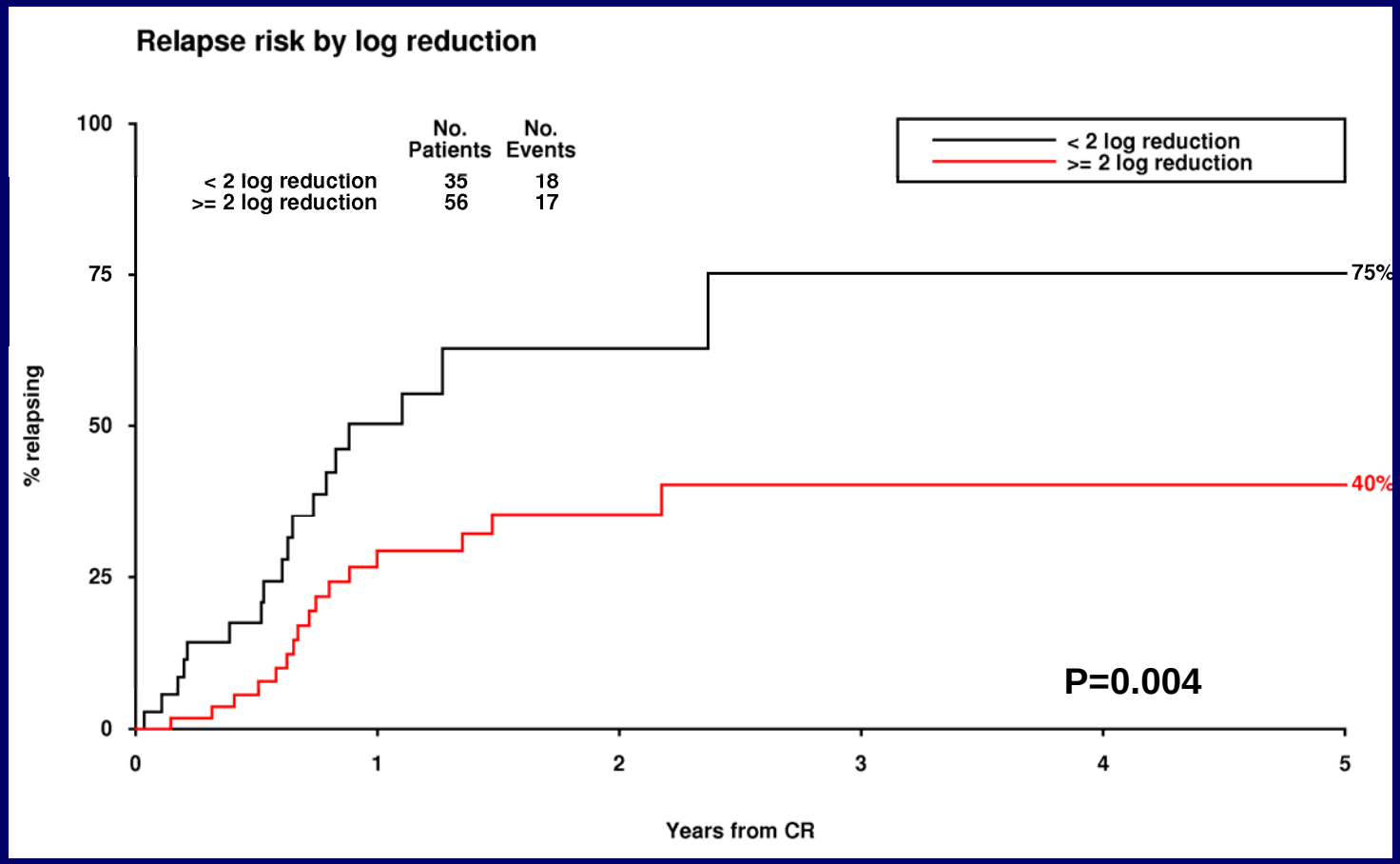
Cilloni *et al*, *J Clin Oncol* 2009, 27: 5195-201

Detection of mutations in the *WT1* gene in cases of AML with "low" *WT1* gene expression by RT-qPCR



Cilloni *et al*, *J Clin Oncol* 2009, 27: 5195-201

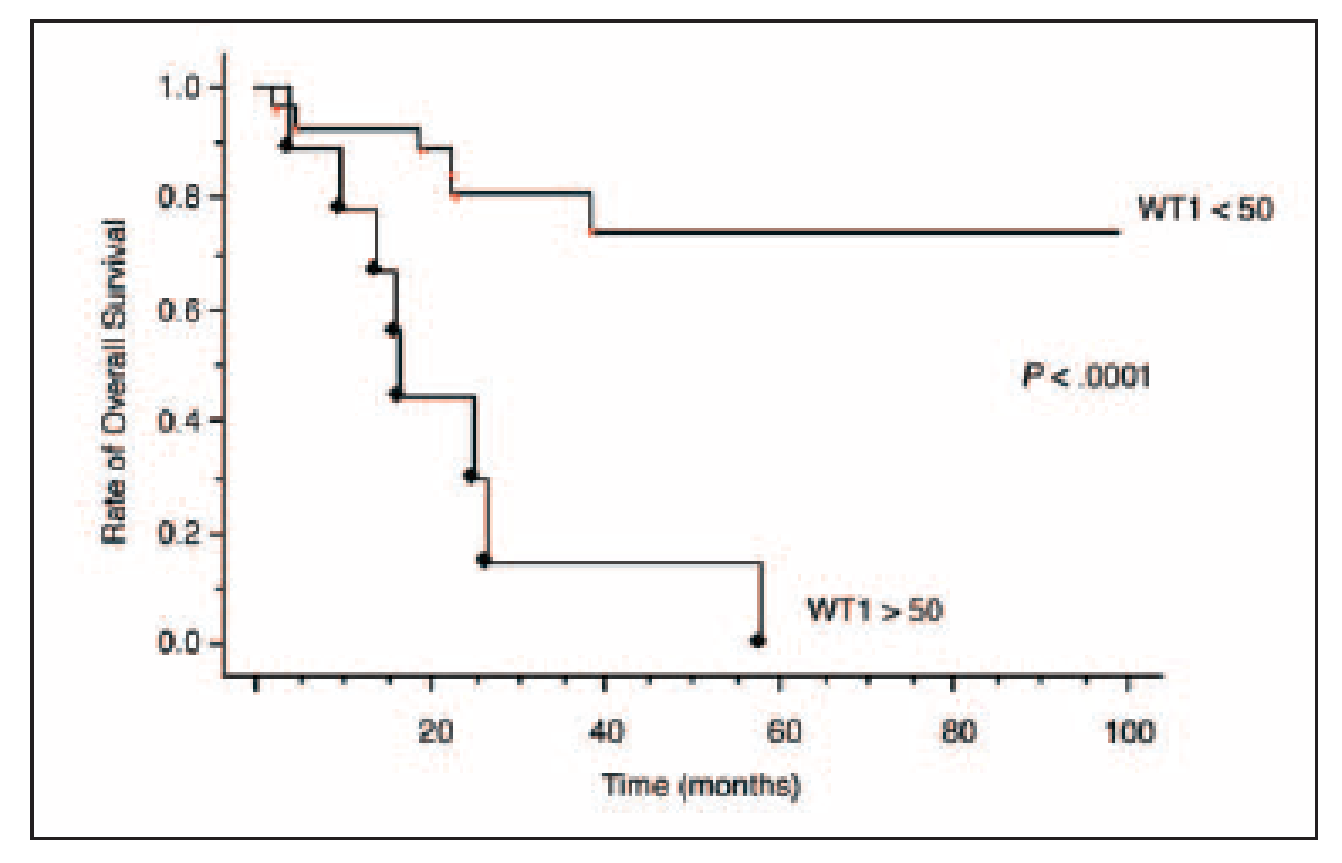
Evaluation of selected ELN *WT1* assay: kinetics of *WT1* transcript reduction predict risk of relapse



HR 0.54 (0.36-0.83) per log-reduction, *p=0.004*

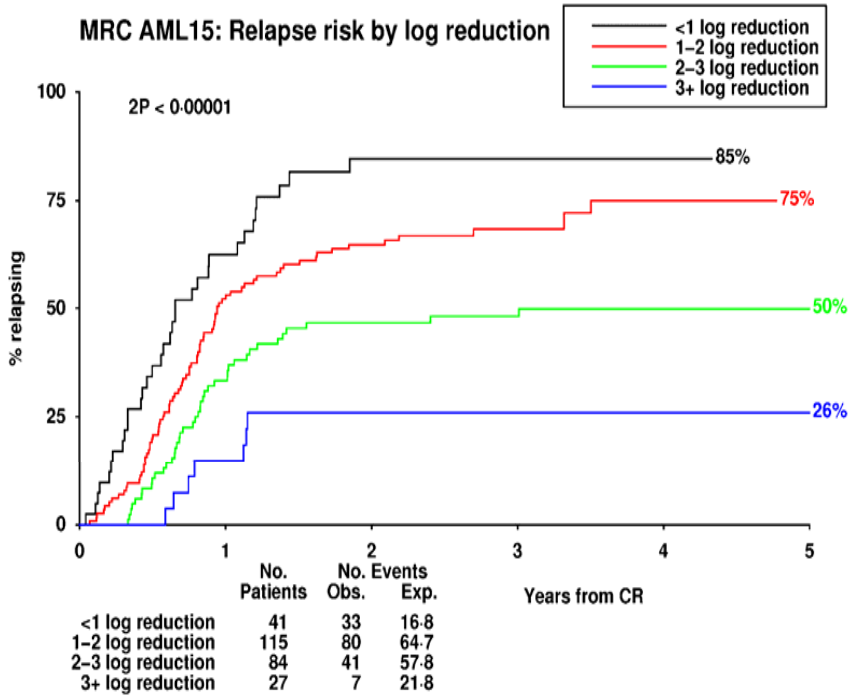
Cilloni *et al*, *J Clin Oncol.* 2009; 27:5195-201.

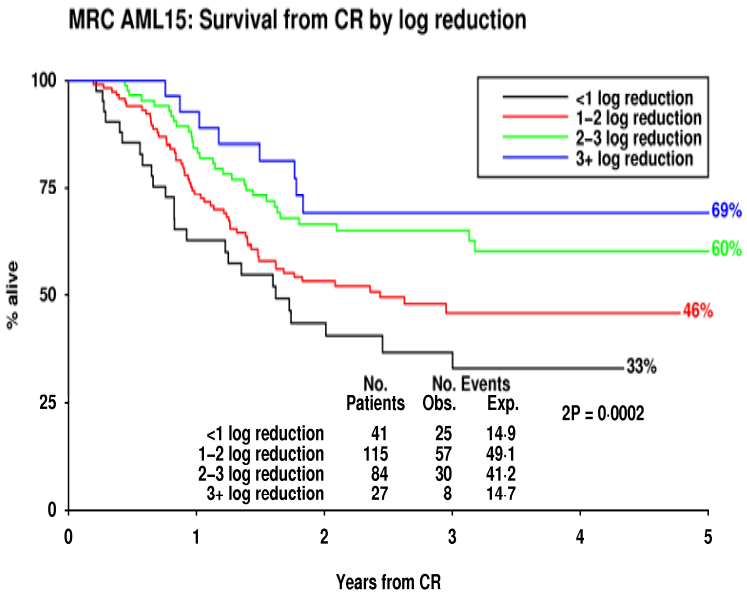
Post-induction assessment of *WT1* status predicts relapse risk in paediatric AML



Lapillonne *et al*, *J Clin Oncol* 2006, 24: 1507-15

Log-reduction in *WT1* transcripts following induction is an independent prognostic factor in AML

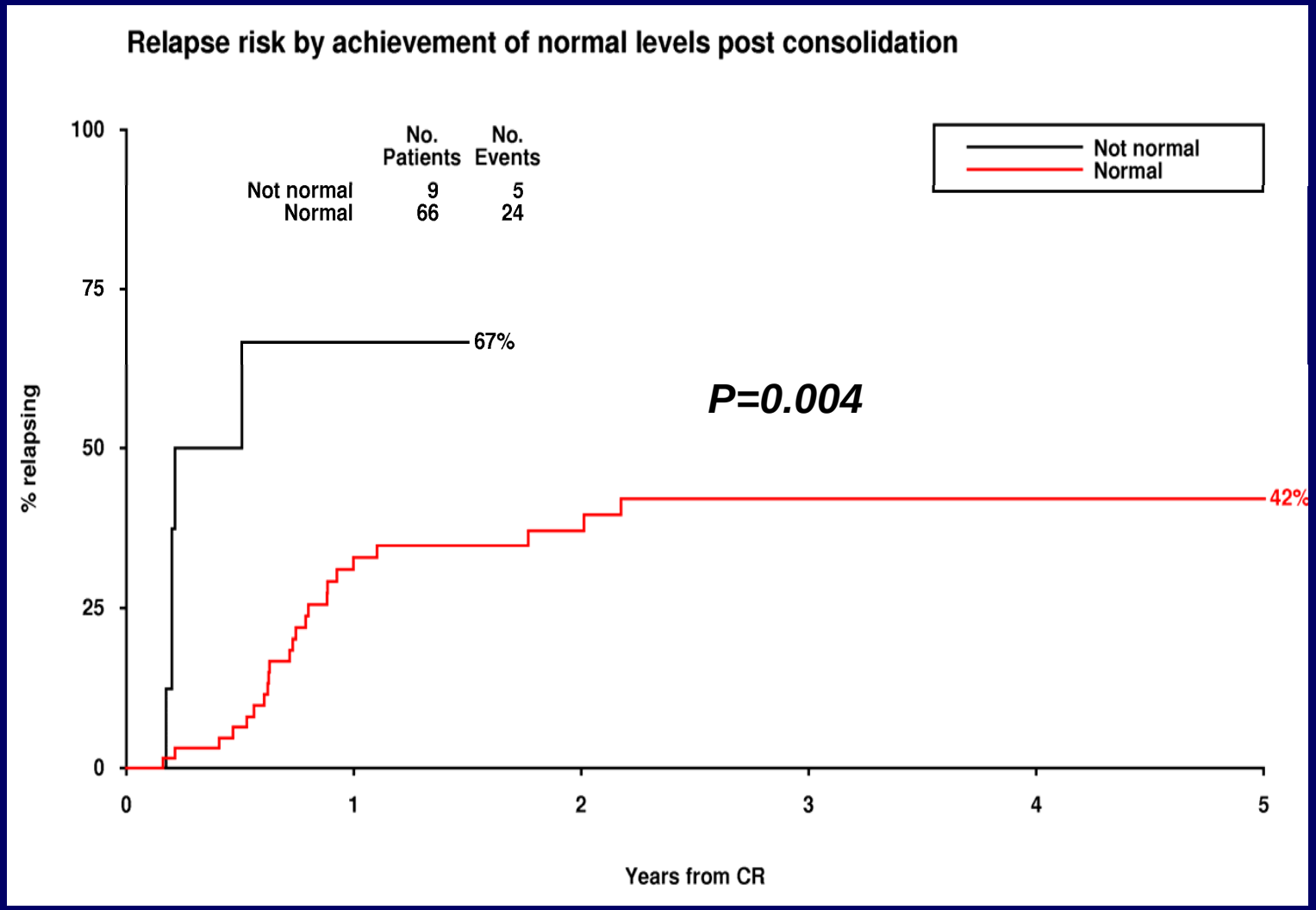




Leukaemia & Lymphoma Research
BEATING BLOOD CANCERS

John Liu-Yin *et al*, ASH 2008 #697

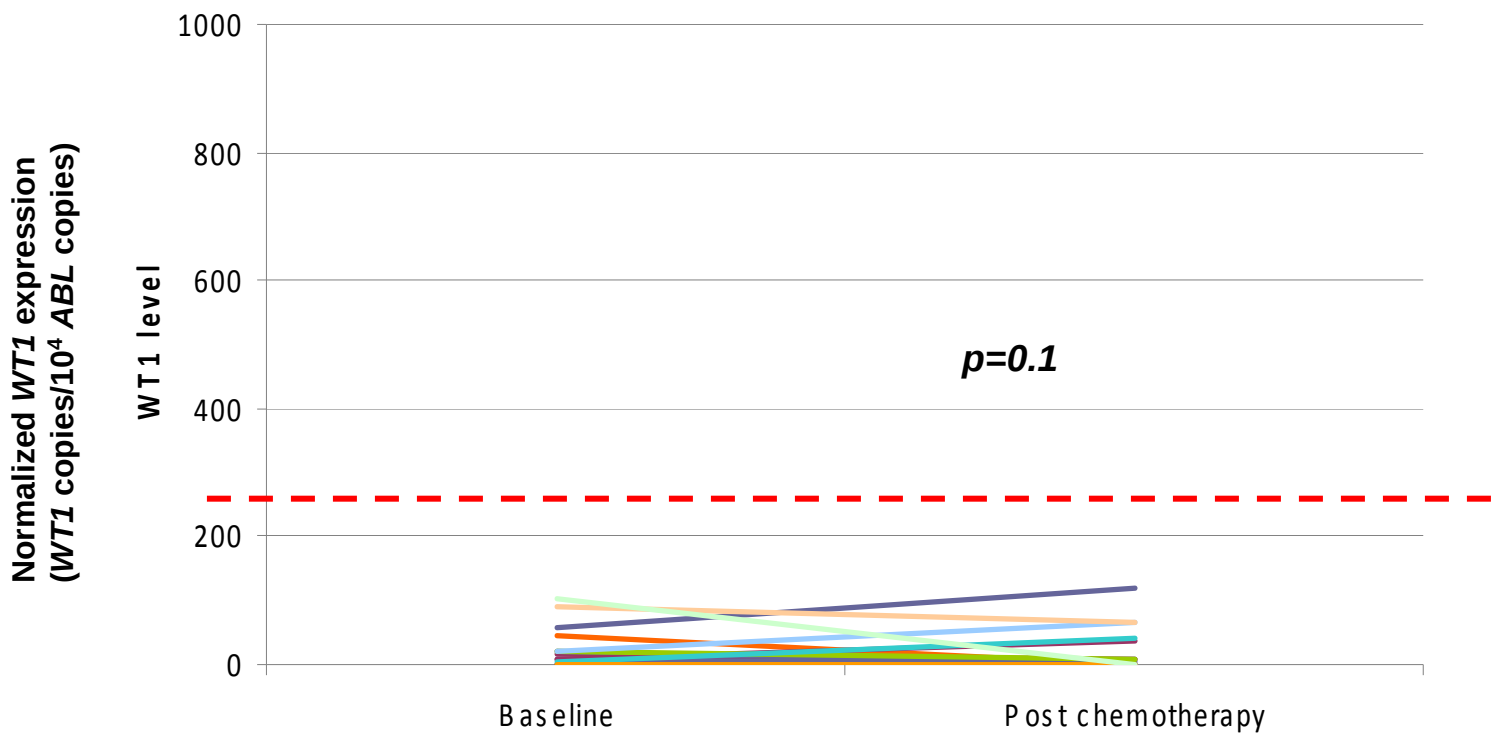
Failure to normalise *WT1* transcripts by the end of consolidation predicts increased relapse risk



Cilloni *et al*, *J Clin Oncol* 2009, 27: 5195-201

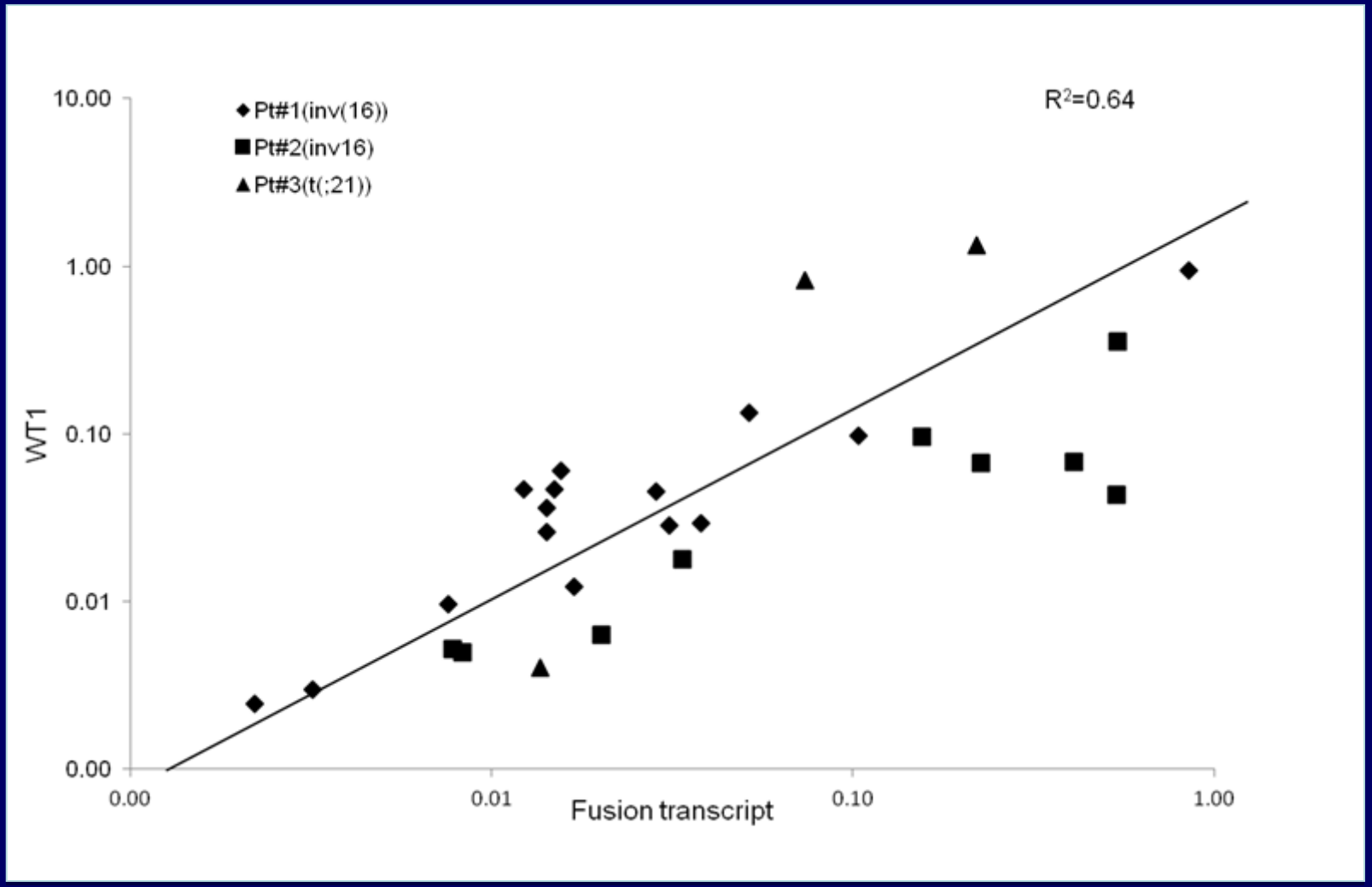
WT1 gene expression is not significantly modulated on regeneration following conventional chemotherapy

Analysis of follow-up samples from 16 cases of AML with *WT1* <250 copies/10^4 *ABL* at diagnosis



Cilloni *et al*, *J Clin Oncol* 2009, 27: 5195-201

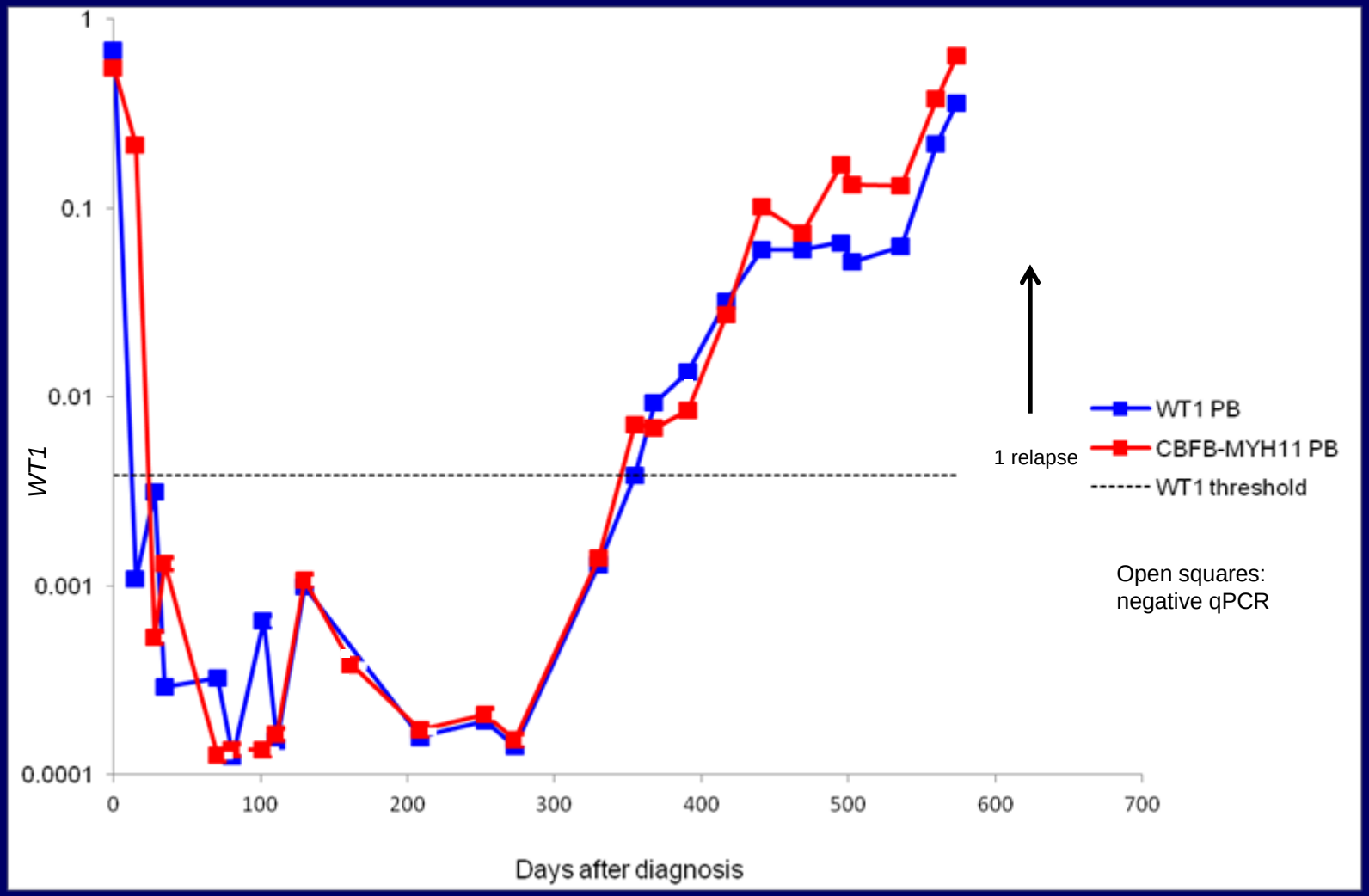
Correlation between W*T1* expression and fusion transcript expression



x=y

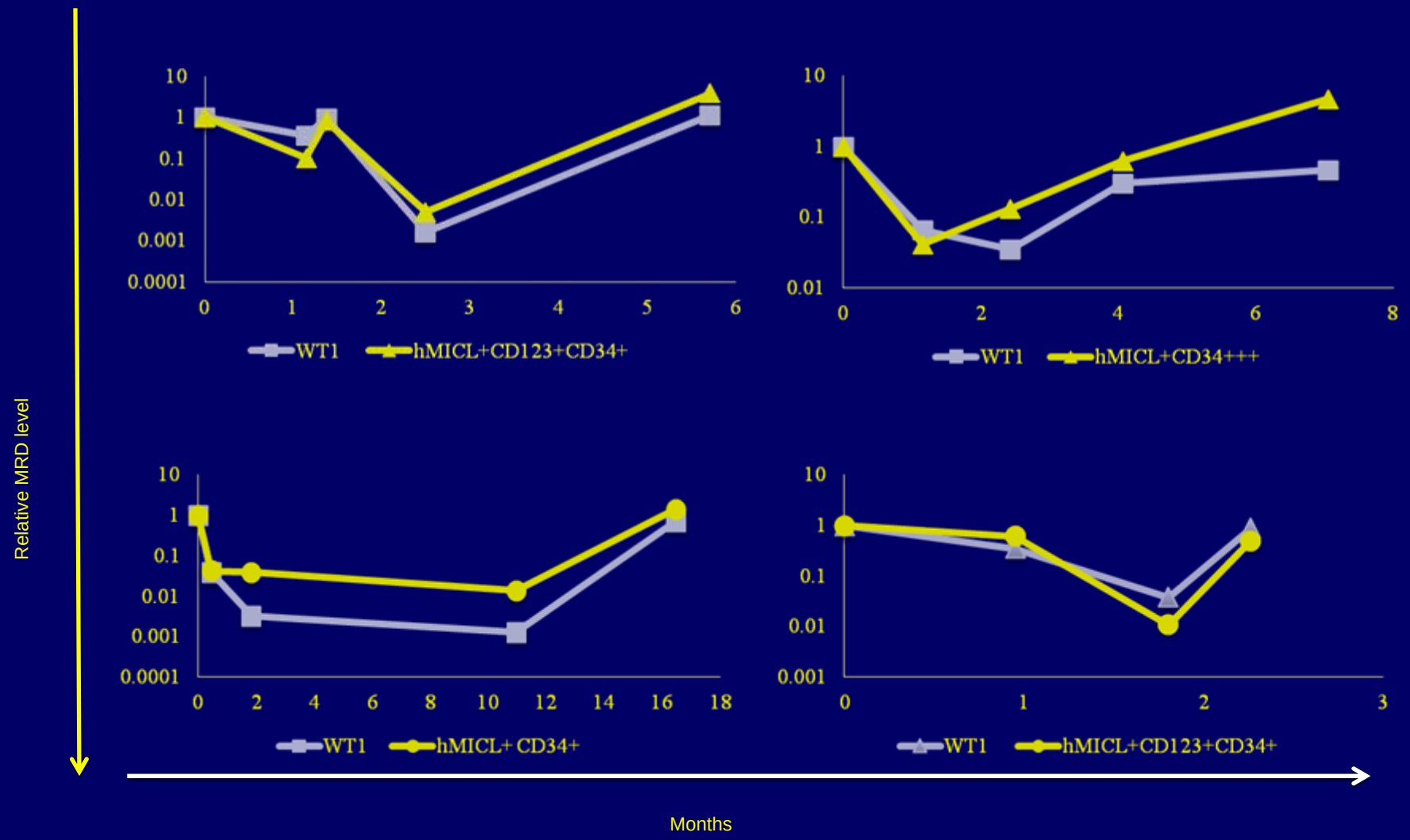
Ommen & Hokland

Correlation between W*T1* expression and fusion transcript expression



Ommen & Hokland

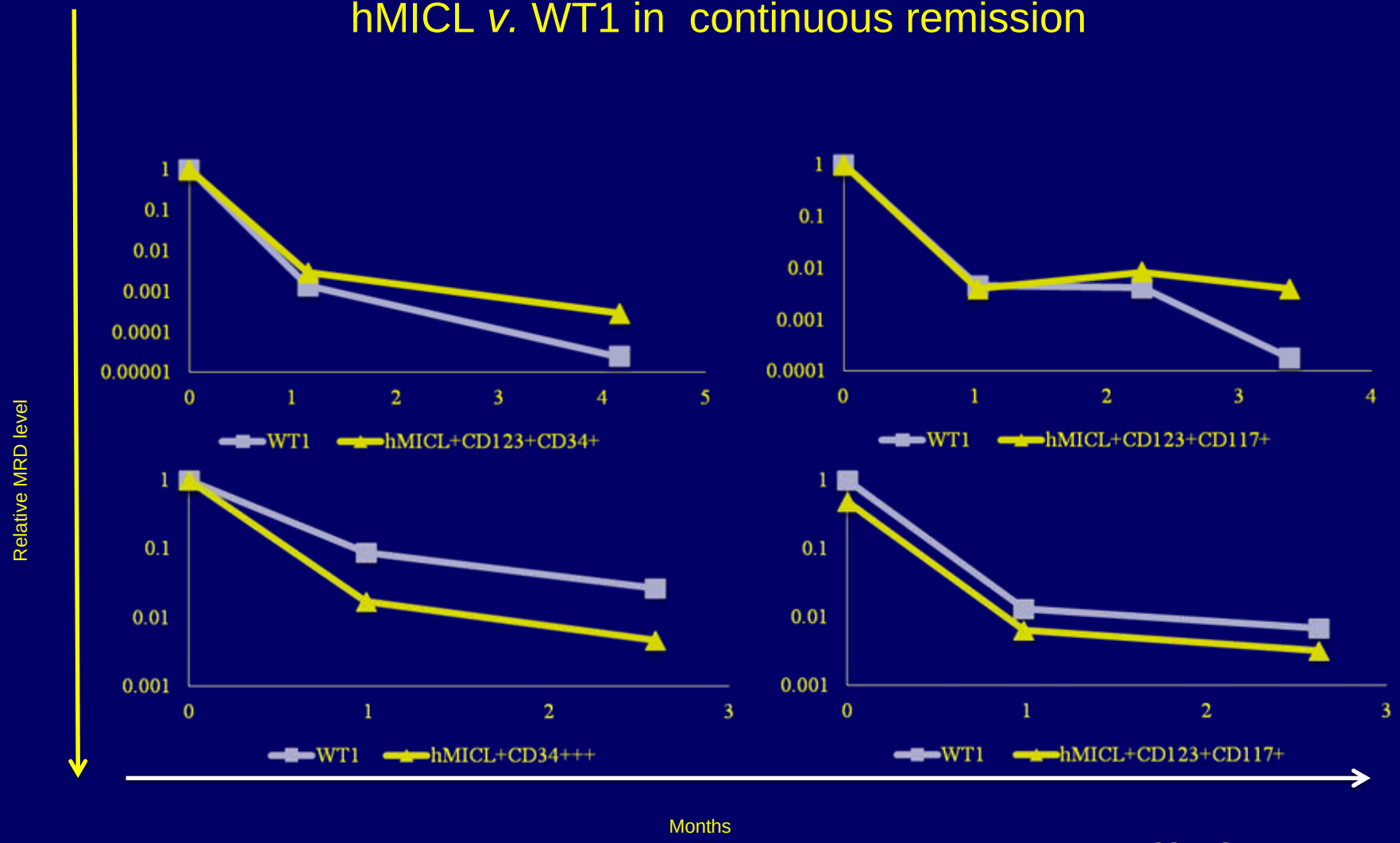
Comparison of relative MRD as measured by qPCR and flow cytometry. hMICL *v.* WT1 in relapse



Roug & Hokland

Aarhus University Hospital

Comparison of relative MRD as measured by qPCR and flow cytometry. hMICL *v.* WT1 in continuous remission

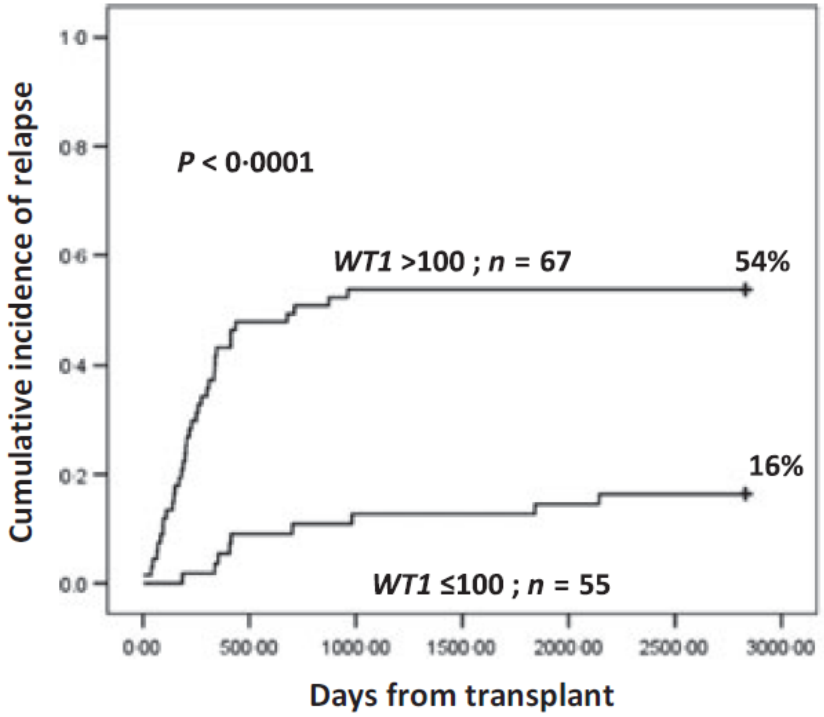


Roug & Hokland

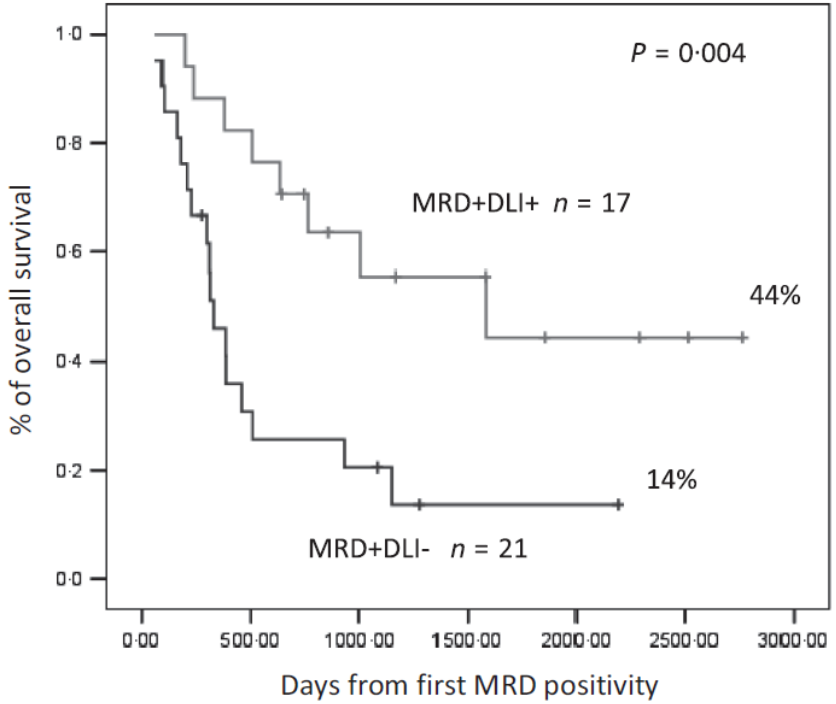
Aarhus University Hospital

Investigation of optimised ELN *WT1* assay to predict outcome & guide therapy post-allogeneic transplant in AML

Association between *WT1* level post-transplant & relapse



Survival following DLI in patients with higher *WT1* levels



BM samples analysed with ELN *WT1* assay

>180 copies *WT1* / 10^4 *ABL* used as definition of MRD positivity

Pozzi *et al*, *Br J Haematol* 2013: 160: 503-9

Conclusions

- ***WT1*** **is over-expressed in a significant proportion of AML**
- ***WT1*** **is expressed in normal marrow and peripheral blood**
  - ***i.e. WT1*** **is not a leukaemia-specific MRD marker**
- **Background *WT1* expression in normal blood and marrow limit assay sensitivity, with PB affording greater sensitivity**
- **Standardised RT-qPCR assays for *WT1* have been established**
- ***WT1*** **detection at early timepoints (applicable in ~50%) of AML patients can provide independent prognostic information**
- ***WT1*** **monitoring can predict relapse risk following allogeneic transplantation**

Acknowledgments

Dept. of Medical & Molecular Genetics, GKT School of Medicine

Daniela Diverio
Rajinder Flora
Elizabeth Nugent
Jelena Jovanovic
Ellen Solomon

MRC Trials Tissue Bank/ Dept. of Haematology, UCL Hospitals

Stephen Langabeer
Sivatharsini Srirangan
Yashma Patel
Helen Walker
Rosemary Gale
David Linch

MRC/NCRI Trials

Alan Burnett
Robert Hills
Tony Goldstone
Liz Brettell
Anthony Moorman
Christine Harrison
Brenda Gibson
Keith Wheatley

Non-APL MRD studies

Sarah Daly
Anna Barfield
Stuart Green
Abida Awan
Khalid Tobal
John Yin

Regional Banks/labs

Steve Austin
Mike Griffiths/Susanna Akiki/ Joanne Mason
Paul Evans/Mike Short
Anthony Bench
Ian Carter
Gill Wilson
Anne Sproul
Vincent van der Velden

European LeukemiaNet

Andreas Hochhaus
Giuseppe Saglio
Nick Cross
Peter Hokland
Torsten Haferlach/ Susanne Schnittger
Francesco Lo Coco
Hartmut Döhner
Rüdiger Hehlmann

NHS National Institute for Health Research

Leukaemia & Lymphoma Research BEATING BLOOD CANCERS

ELN European LeukemiaNet