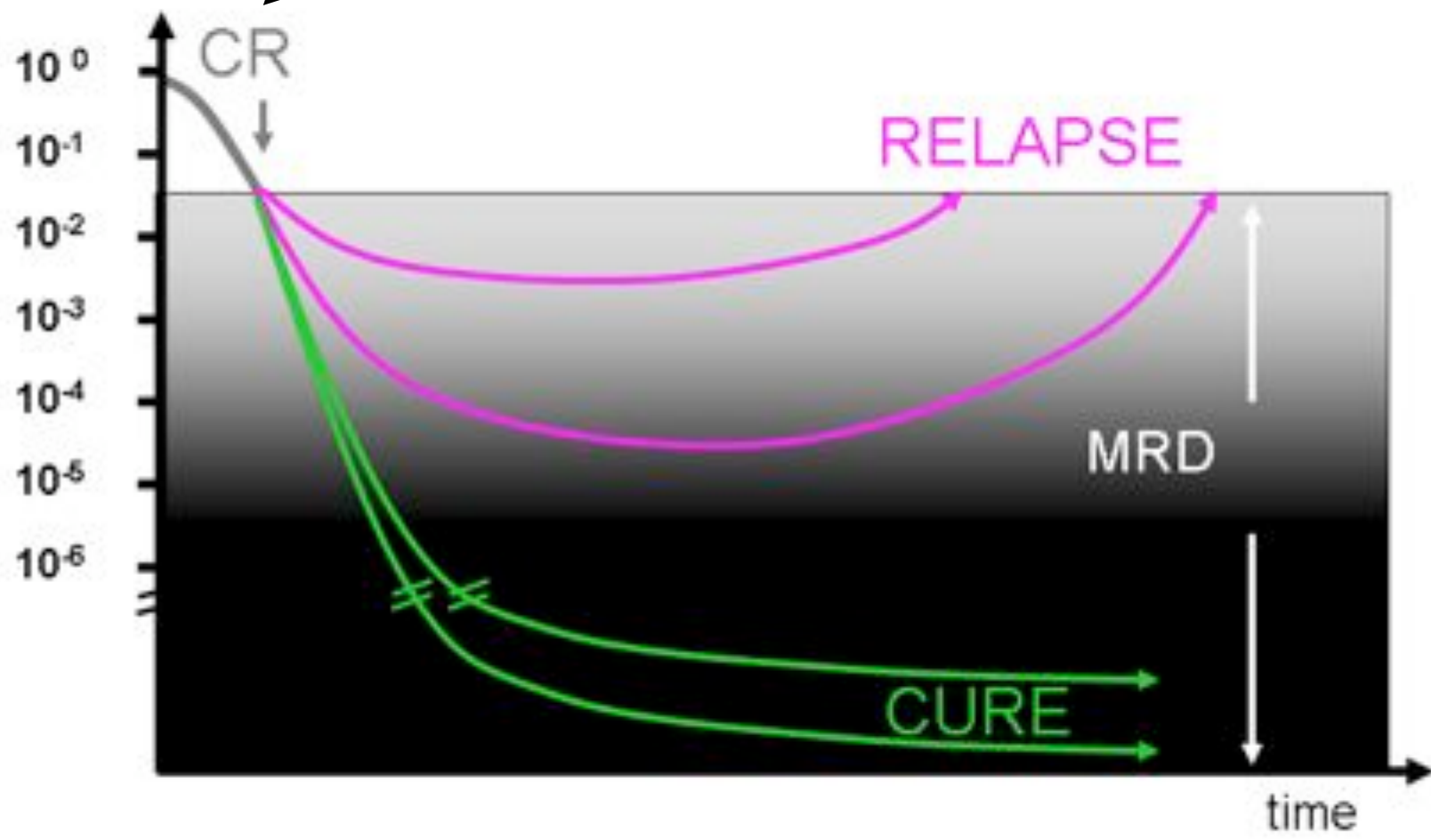

Clinical relevance of MRD studies in acute leukemia

Nicole Straetmans
Hôpital de Jolimont

MRD analysis in acute leukemia

- Why should we assess MRD?
 - Which technique should be used?
 - Is it a prognostic factor of outcome?
 - Is it an independent prognostic factor?
 - Is it predictive of an impending relapse?
 - Does treatment adaptation to MRD results improve the outcome?
-

Restoration of normal blood counts
< 5% blast cells by morphology



⇒ Information on the treatment effectiveness

Factors involved in treatment effectiveness

Characteristics of tumor cells, e.g. :

- Gene expression profile
- Drug sensitivity

Host factors, e.g. :

- GI absorption
- Drug metabolism (polymorphism of enzymes)
- Liver excretion
- Kidney excretion

Treatment protocol/ Compliance, e.g. :

- Drugs
- Drug interaction
- Compliance
- Kind of maintenance
- Dose reduction
- Transplant

OVERALL EVALUATION BY MRD ANALYSIS

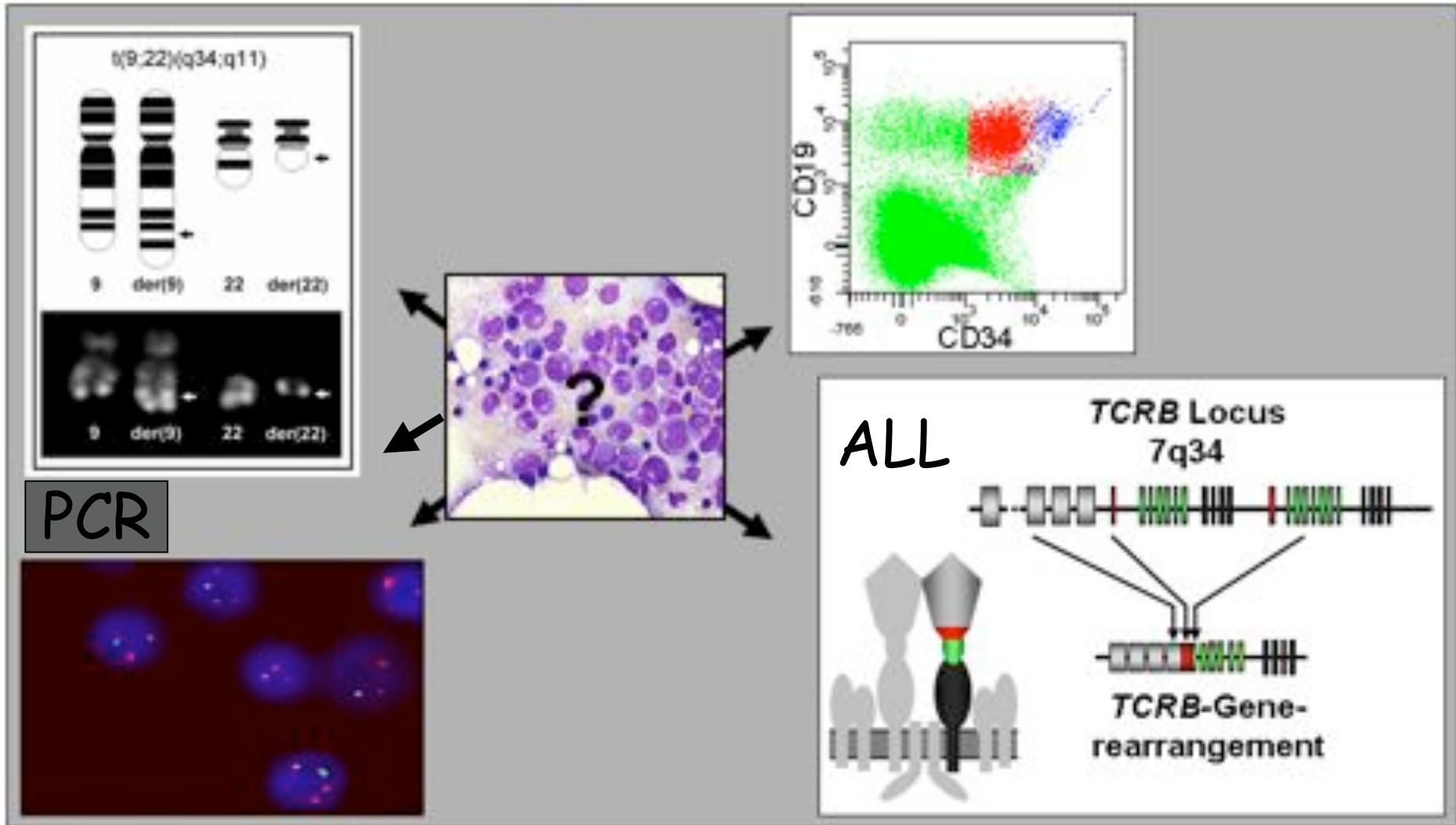
Treatment adaptation

Which technique should
be used?

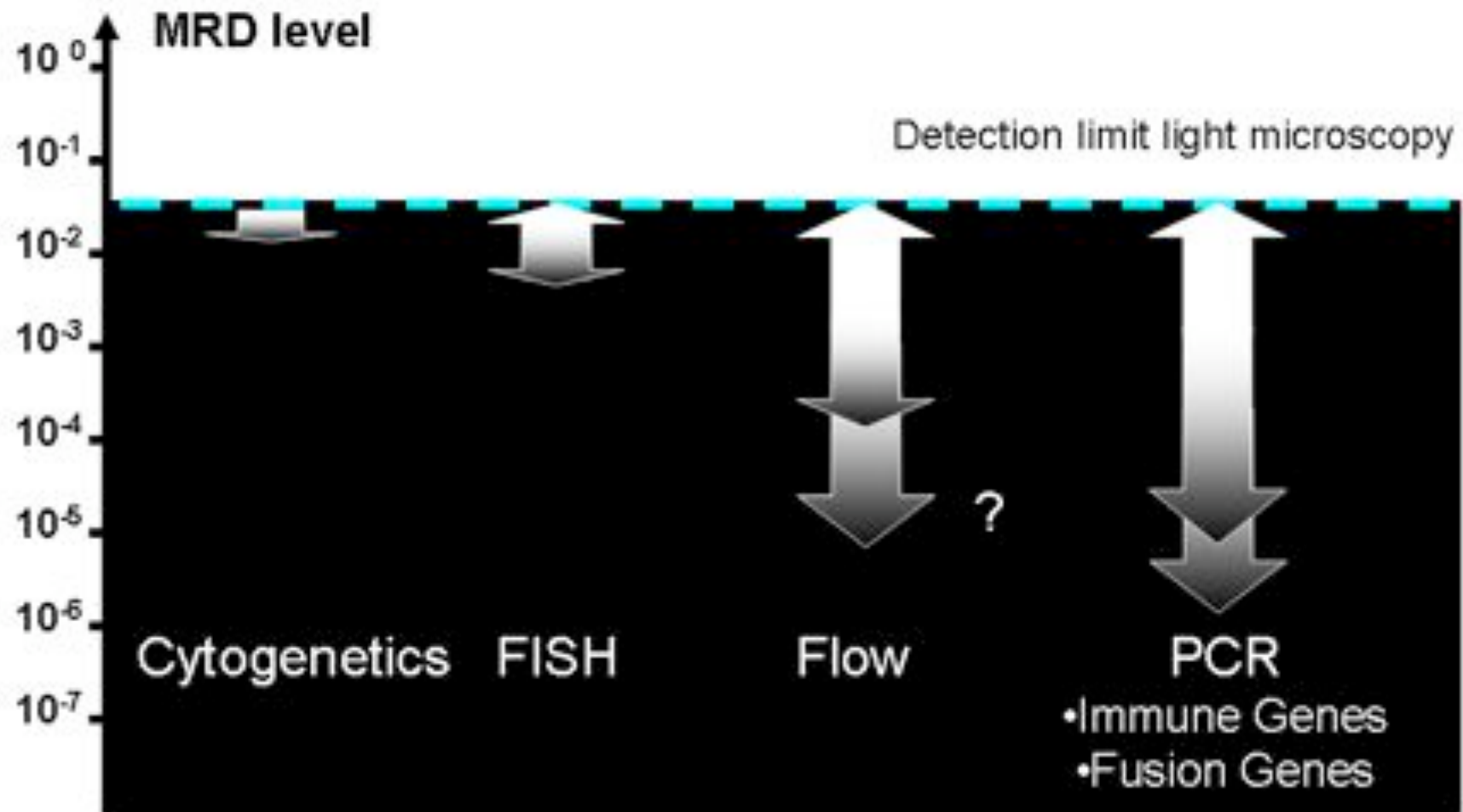
An optimal technique should be :

- Leukemia specific
 - With a sensitivity adapted to clinical endpoint:
 - Low sensitivity (no false positive) : identify patients at high risk of relapse \longrightarrow **treatment escalation**
 - High sensitivity (no false negative) : identify patients at low risk of relapse \longrightarrow **treatment de-escalation**
 - Applicable to the vast majority of patients
 - Intra- and inter-laboratory reproducible results expressed in the same way
 - Quantitative
 - Stable during the course of the disease
-

Leukemic specific



Sensitivity



PCR analysis of chromosome aberration (mainly fusion gene)

- Identification of specific translocation at diagnosis
- PCR analysis of follow-up samples using standardized primer/probe sets

■ ADVANTAGES

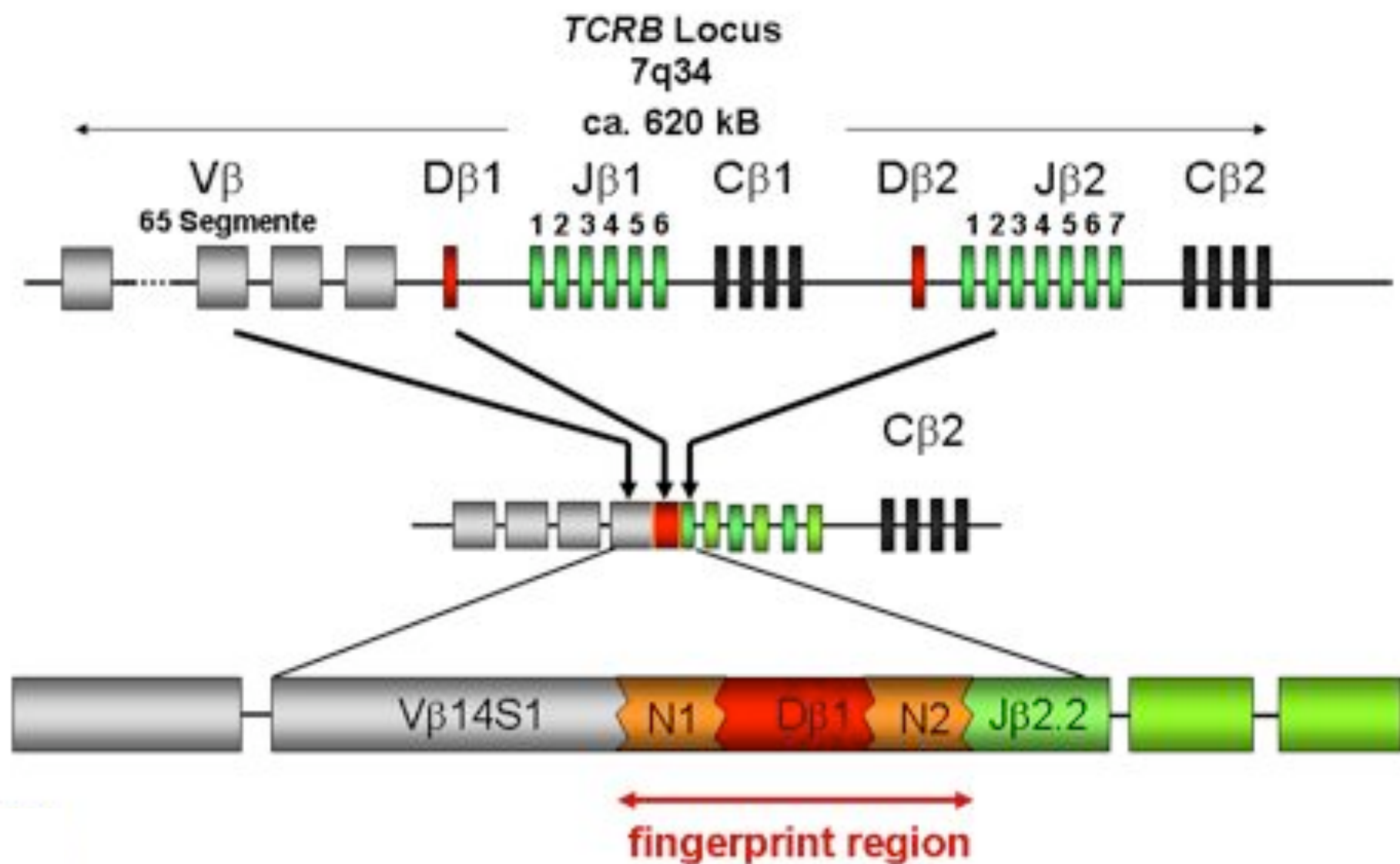
- High sensitivity
- Stable
- Disease-specific
- Fast
- Relatively easy and cheap

■ DISADVANTAGES

- Applicable to only a minority of patients
 - B-ALL : 40-45%
 - T-ALL : 15-35%
 - AML : 20%
- Instability of RNA
- Different expression levels



Ig-/TCR-Gene-Rearrangements for MRD-Analysis



Ig-TCR rearrangement for MRD analysis

■ ADVANTAGES

- High degree of standardization (ESG-MRD-ALL)
 - Design
 - Interpretation
- High sensitivity
- Applicable for most ALL patients (90-95%)
- Stability of DNA (multicenter setting)

■ DISADVANTAGES

- Time consuming
 - Expensive
 - Need for preferably 2 PCR targets because of chance of clonal evolution (2 sensitive targets available in ~80% of patients)
 - Extensive expertise is needed
-

Flow cytometry immunophenotyping

- Identification of leukemia-associated aberrant immunophenotype (LAIP)
 - Based on
 - Cross-lineage expression
 - Lack of expression of an antigen
 - Overexpression
 - Asynchronous expression of antigens
 - Present at low frequency or absent in normal marrow (! regenerating marrow)
-

Flow cytometry immunophenotyping

■ ADVANTAGES

- Rapid
- Applicable to almost all patients
- Quantitative
- Discriminates apoptotic cells

■ DISADVANTAGES

- Immunophenotypic shifts
 - Regenerative marrow
 - Low cellularity after induction
 - Lower sensitivity (10^{-3} - 10^{-4}) than PCR analysis using 3-4 color flow cytometers
 - Interlaboratory standardization
 - Expertise
-

Comparison

- Results are largely concordant in single-center studies (ALL)
- Differences in sensitivity
 - Discrepancies occur at very low level of MRD
 - Hampers the detection of very-low risk patients
 - Usage of different techniques within the same protocol should be avoided
- Costs

	Immunophenotype		Immunogenotype	
	B ALL	T ALL	B ALL	T ALL
Diagnosis	190€ 3h	70€ 3h	250€ 7h	250€ 7h
Follow up	55€ 2h	55€ 2h	55€ 2h	55€ 2h

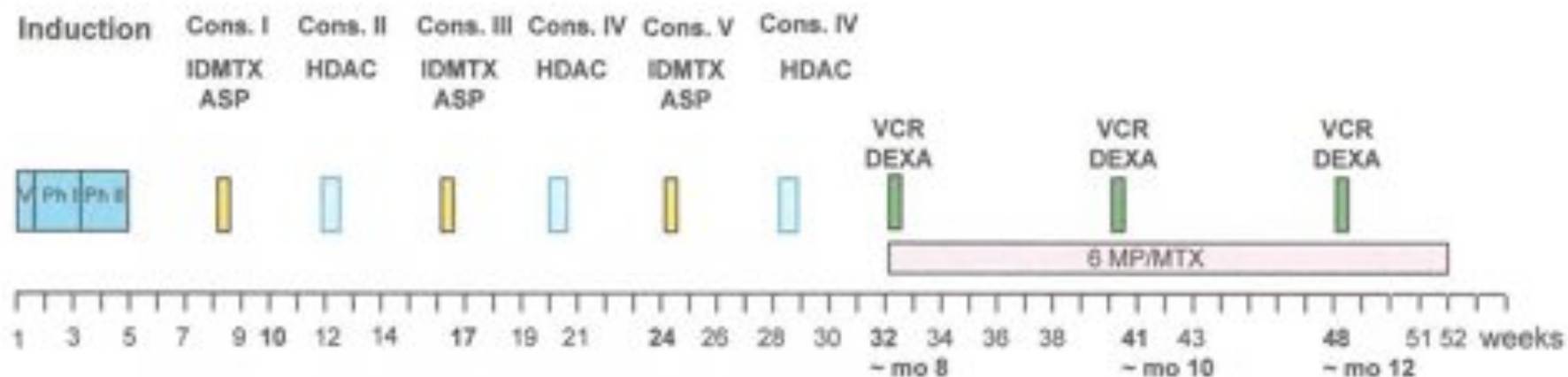
Kerst et al Br J Haematol 2005

Regarding the analysis of clinical relevance...

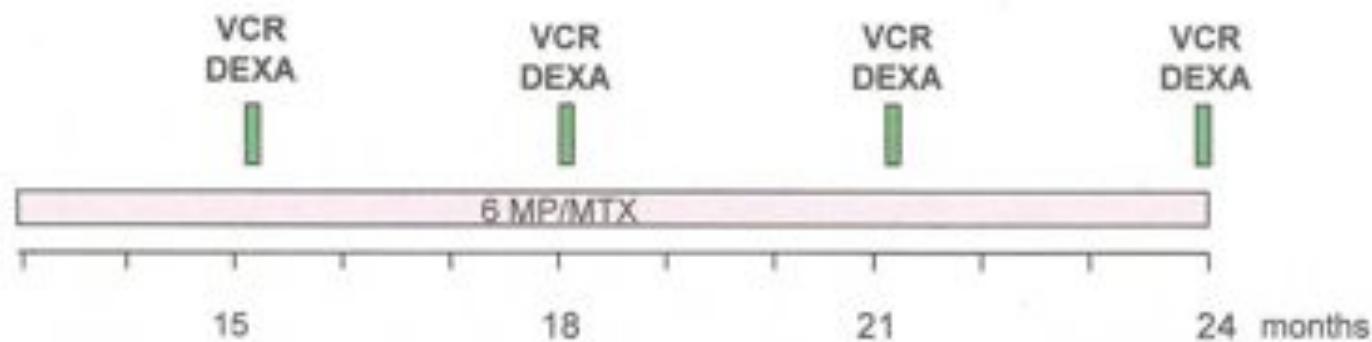
- Difficulties in interpreting the published data
 - Different regimens
 - Different groups of patients
 - Different time points of analysis during therapy
 - Different positivity thresholds used for outcome prediction
-

ALL

Induction and Consolidation Therapy (1st year)

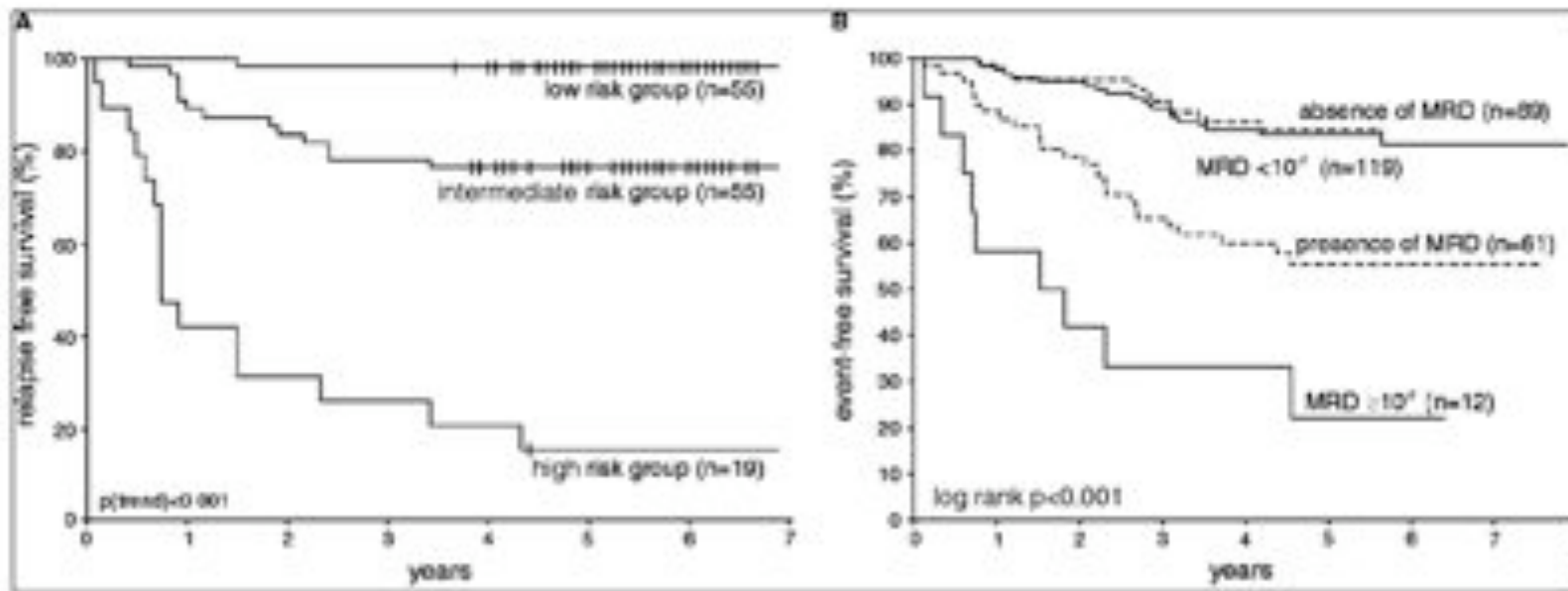


Maintenance Therapy (2nd year)



Childhood ALL

„MRD after induction is most important independent prognostic factor“

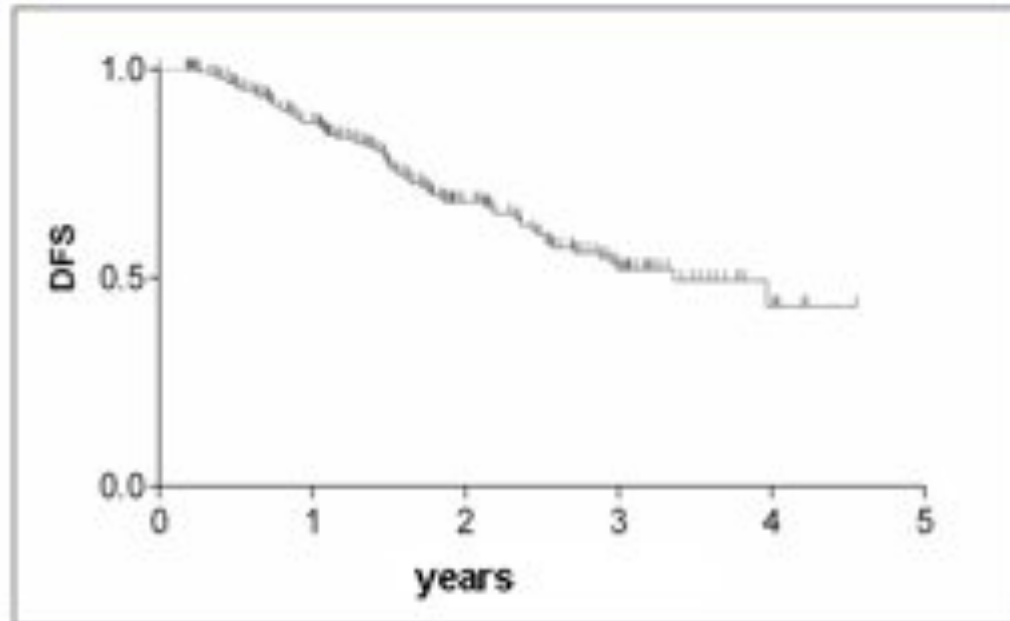


I-BFM-SG

EORTC trial 5881

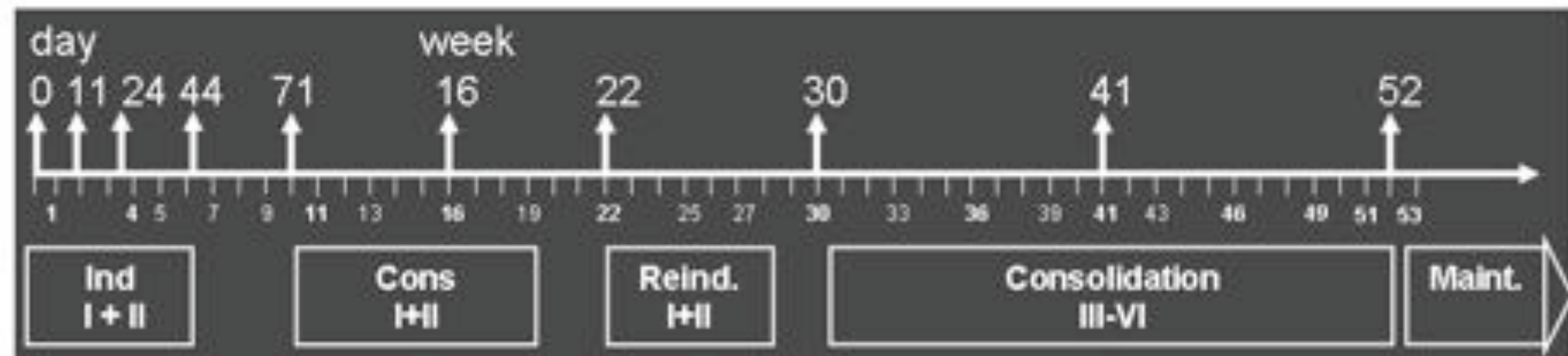
ASH 2002

GMALL : standard risk adult ALL

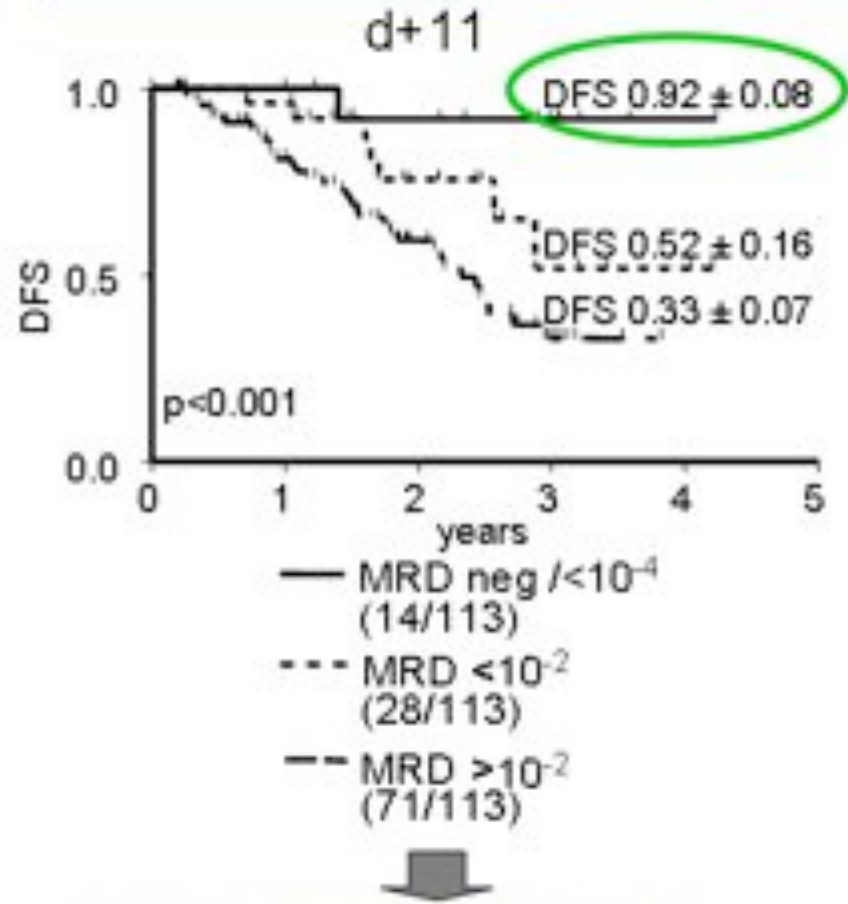


Standard risk group:

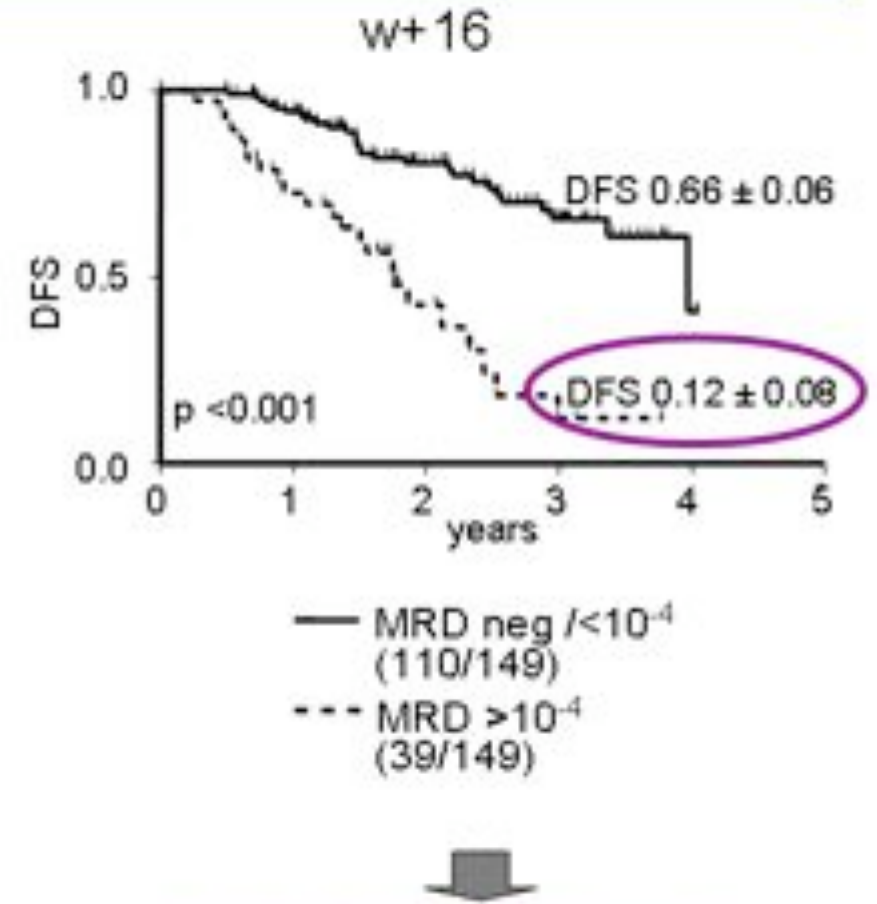
- age 15-55 (65) years
- absence of conventional risk factors
- Standard risk criteria:
 - pre-B-/c-ALL, cortical T-ALL
 - absence of t(4;11)/MLL-AF4
 - absence of t(9;22)/BCR-ABL
 - BCPALL: WBC < 30,000/ μ l
 - CR after Induction I
- account for about 50% of all GMALL patients



GMALL : standard risk adult ALL



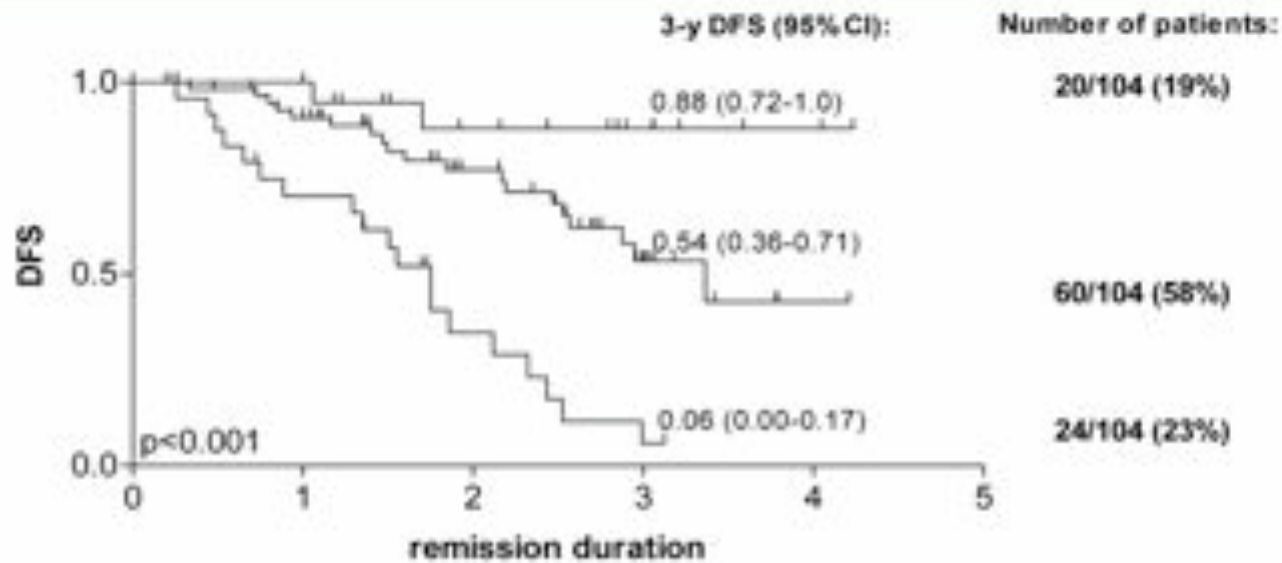
Identification of patients with rapid tumour clearance and good prognosis



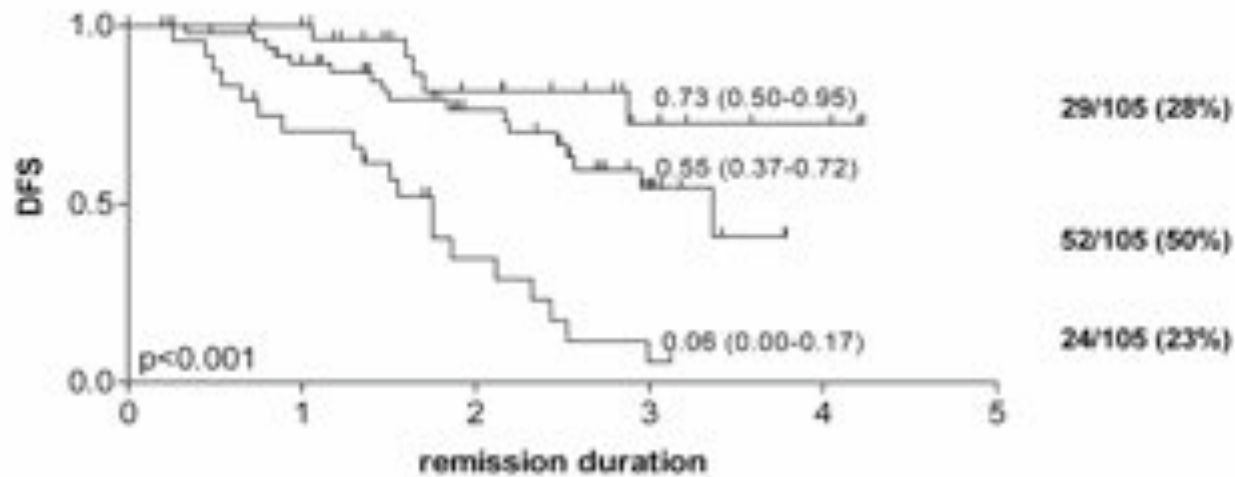
Identification of patients with persistent high level MRD and poor outcome

Standard risk ALL

MRD based risk stratification



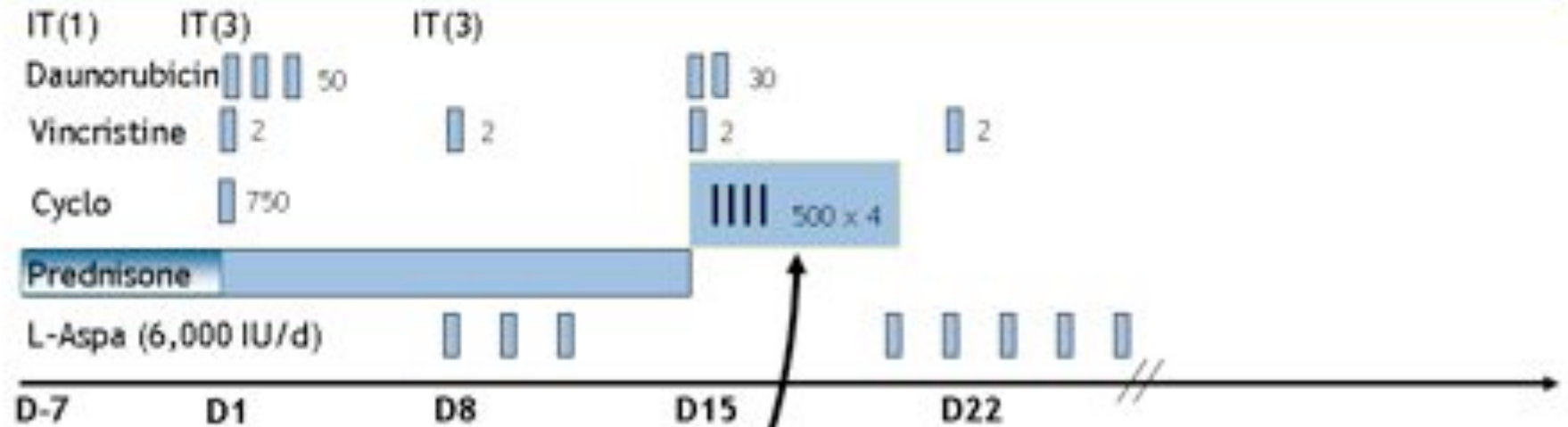
d+11:
 10^{-3}



d+11:
 10^{-2}

Is MRD redundant with
other risk factors?

GRAALL O3



High risk markers

Early Response (ER)
Corticoresistance (CsR)

Chemoresistance (ChR)

Baseline (BL)

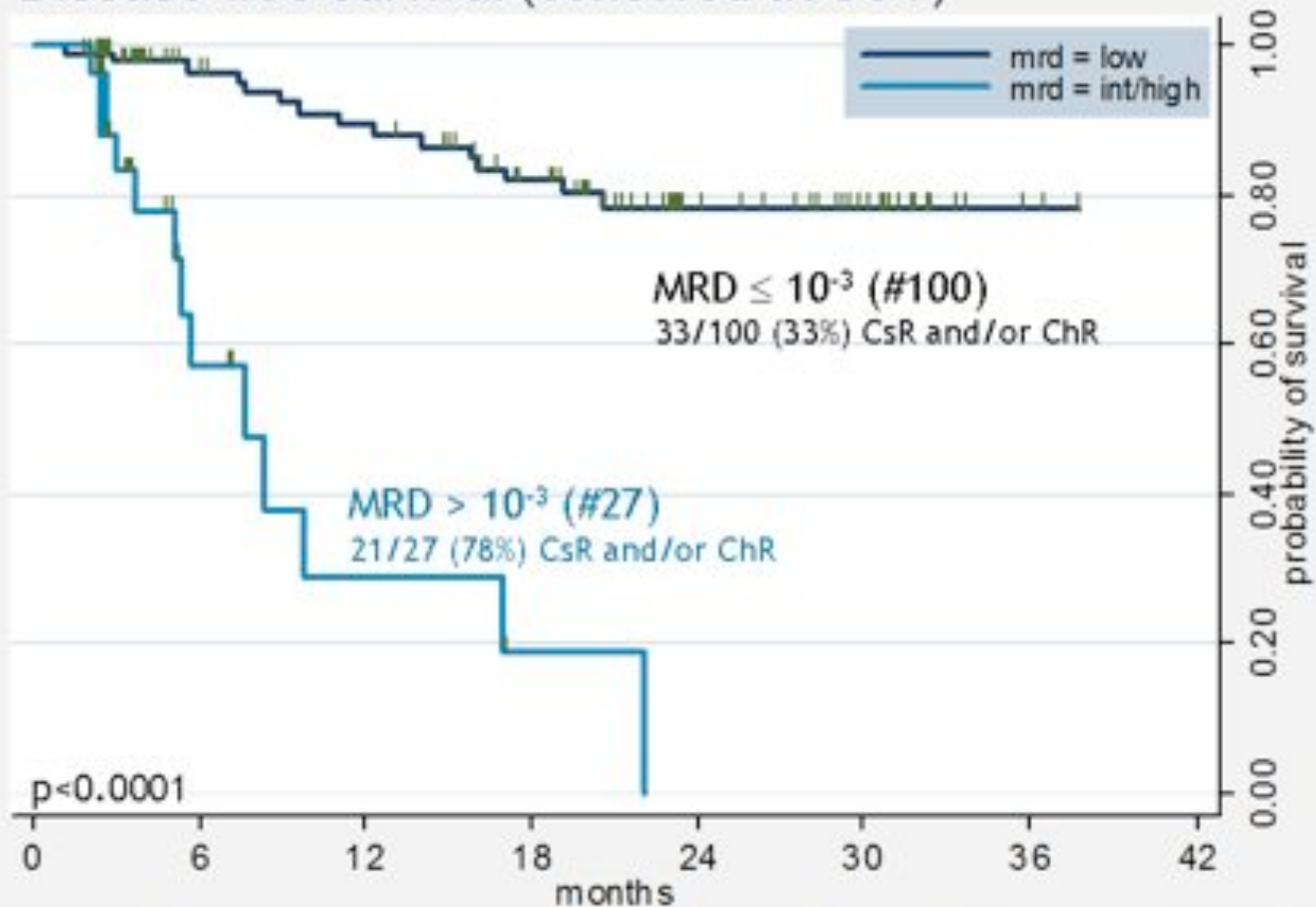
- WBC >30 G/L if BCP-ALL
- CNS involvement
- haplo or near-triploidy
- t(4;11) and/or *MLL-AF4*
- t(1;19) and/or *E2A-PBX1*

Post-induction

- Need of salvage to reach CR
- MRD $\geq 10^{-2}$

SCT in first CR

Disease-free survival (censored at SCT)



F Huguet et al. Blood 2006; 108:147a
K Beldjord et al. Blood 2006; 108:642a

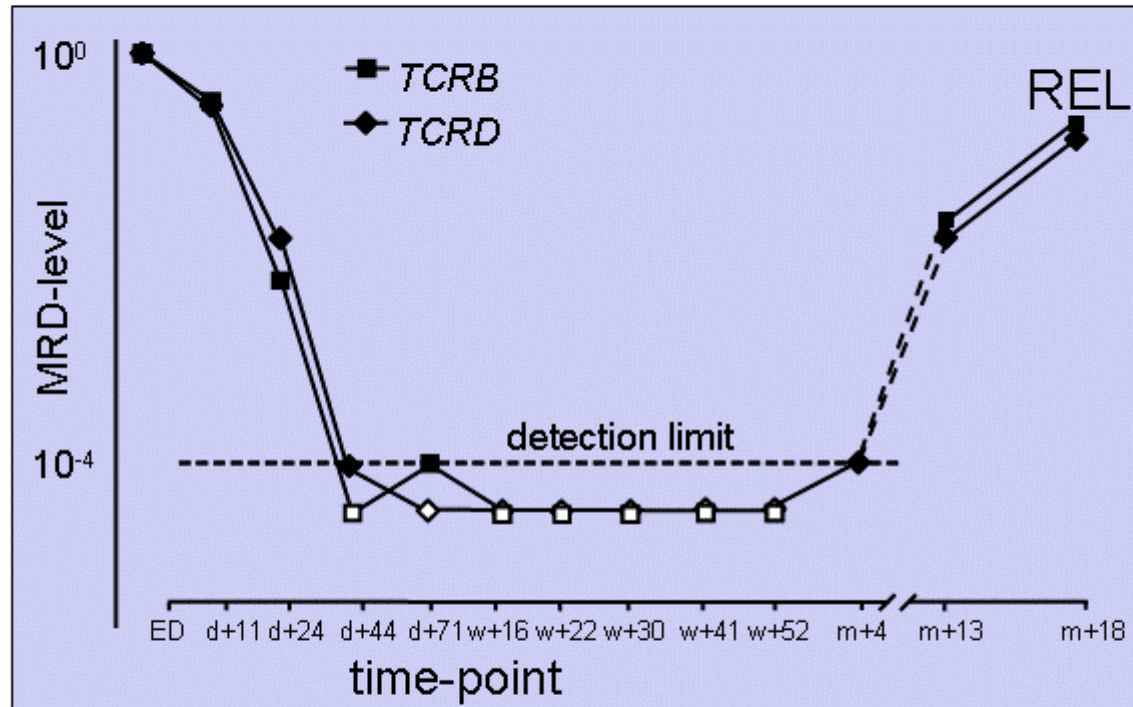
Multivariate analysis

P values	BL markers	Early response	MRD level
Relapse incidence	0.006	0.58	<0.001
DFS	0.001	0.95	<0.001

- BL markers and post-induction MRD level were independently predictive of a worse outcome.
- Post-induction MRD useful to detect patients with low MRD level despite a poor early response (60% of poor early responders belonged to low level MRD group in the context of HyperC reinforcement)

Is it predictive of an
impending relapse?

Diagnosis of a molecular relapse?



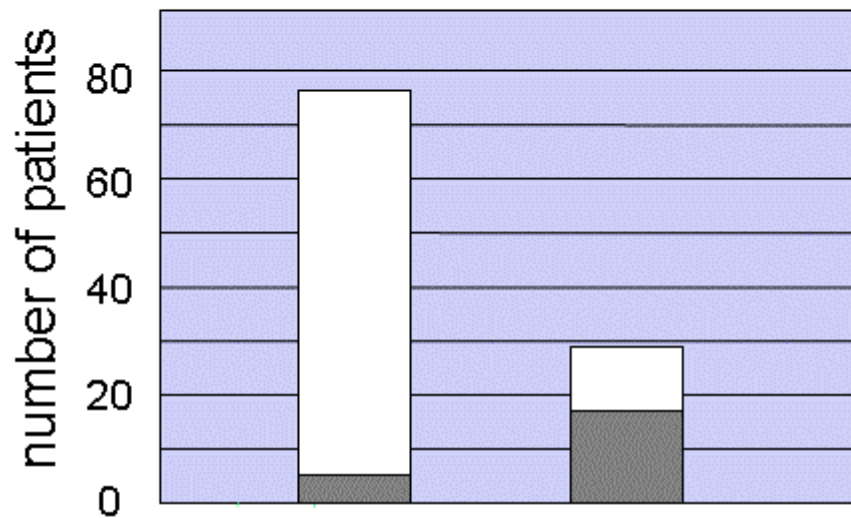
- Prediction of relapse possible?
- time from MRD conversion to relapse?

➔ Prospective MRD analysis in SR-ALL patients after end of consolidation treatment

Inclusion criteria:

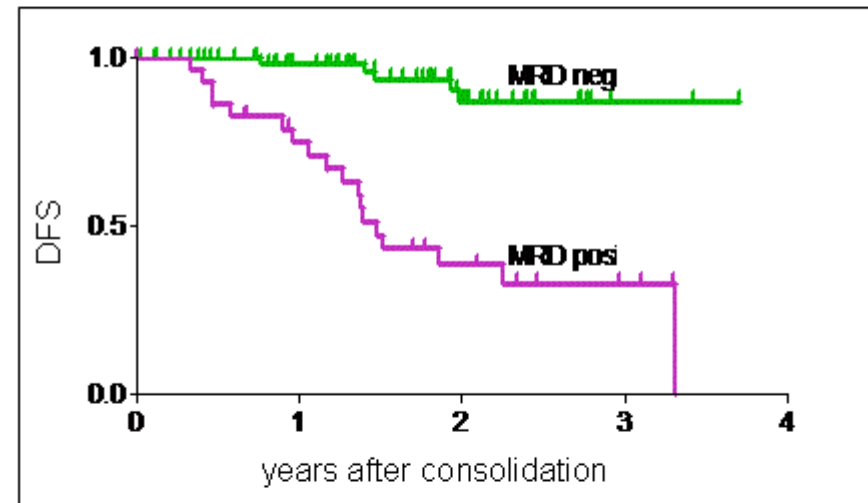
- sensitive molecular marker
- MRD negativity during consolidation

Diagnosis of a molecular relapse?



n=105

	MRD negative	MRD positive	
□ CCR	72	11	83
■ REL	5	17	22
	77	28	



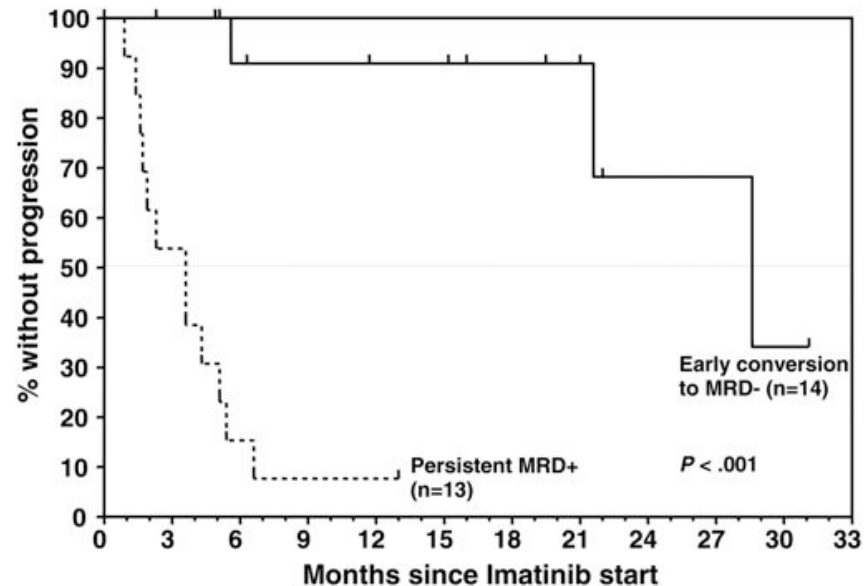
Median time from molecular to clinical relapse : 9.5 months

5/22 relapses not detected by MRD analysis

Clinical intervention
guided by MRD results?

Treatment of MRD post transplant

- 27 Ph+ ALL
- Detection of MRD post transplant (probability of relapse : 90%)
- Start Imatinib in the setting of MRD
- +/- 50 % of these patients experienced prolonged DFS which can be anticipated by the rapid achievement of a molecular remission



Implementation of MRD results into treatment stratification

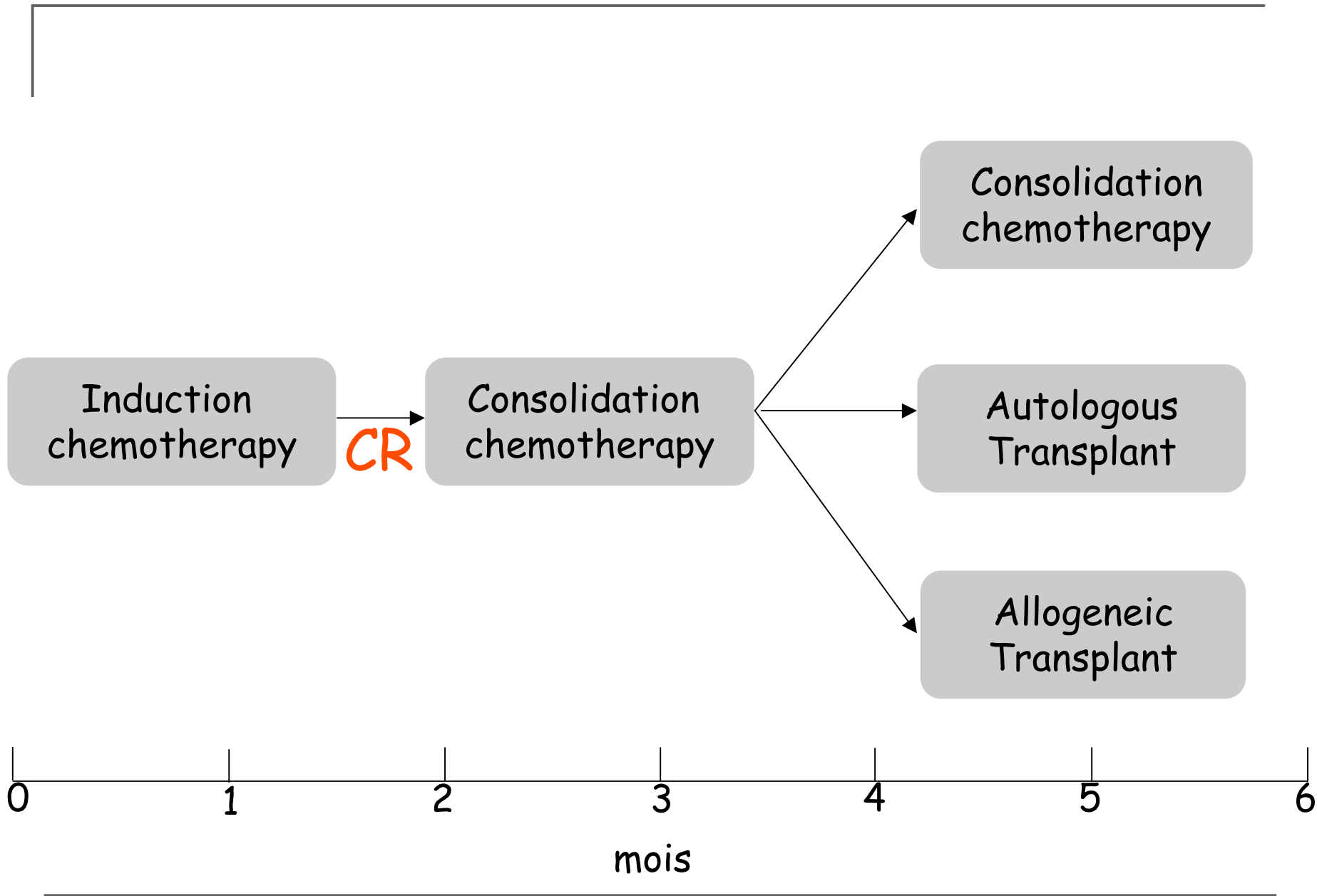
Study	patients	MRD based treatment stratification
GMALL 07/03	SR	<ul style="list-style-type: none"> • MRD LR: Treatment reduction • MRD HR: allocation to SCT • Molecular relapse: salvage treatment
NILG 9-00	t(4;11) ^{neg} t(9;22) ^{neg}	<ul style="list-style-type: none"> • MRD pos: allocation to autologous/allogeneic SCT • MRD neg: standard maintenance regardless of classical risk factors
PETHEMA ALL-AR-03	high risk t(9;22) ^{neg}	<ul style="list-style-type: none"> • No SCT in patients with good d+14 cytologic response and MRD <5x10⁻⁴ after consolidation
GRAALL03	t(9;22) ^{neg}	<ul style="list-style-type: none"> • Implementation of SCT in patients with MRD >1x10⁻² after induction

Goekbuget et al Ann Hematol 2004;83: S129; Bassan et al Blood 2005;106:1836a; Ribera et al Blood 2006;108:1872; Huguet et al Blood 2006:147a; Beldjord et al Blood 2006;108:642a

AML

AML

- No immunogenotype
 - Flow cytometry
 - RQ-PCR
 - Fusion genes : 20%
 - CBFB-MYH11
 - AML1-ETO
 - PML-RAR α
 - Mutated genes
 - Nucleophosmin : 50-60% of AML with a normal karyotype
 - Flt3 : 20-25% AML
 - MLL
 - Overexpression
 - WT1
-

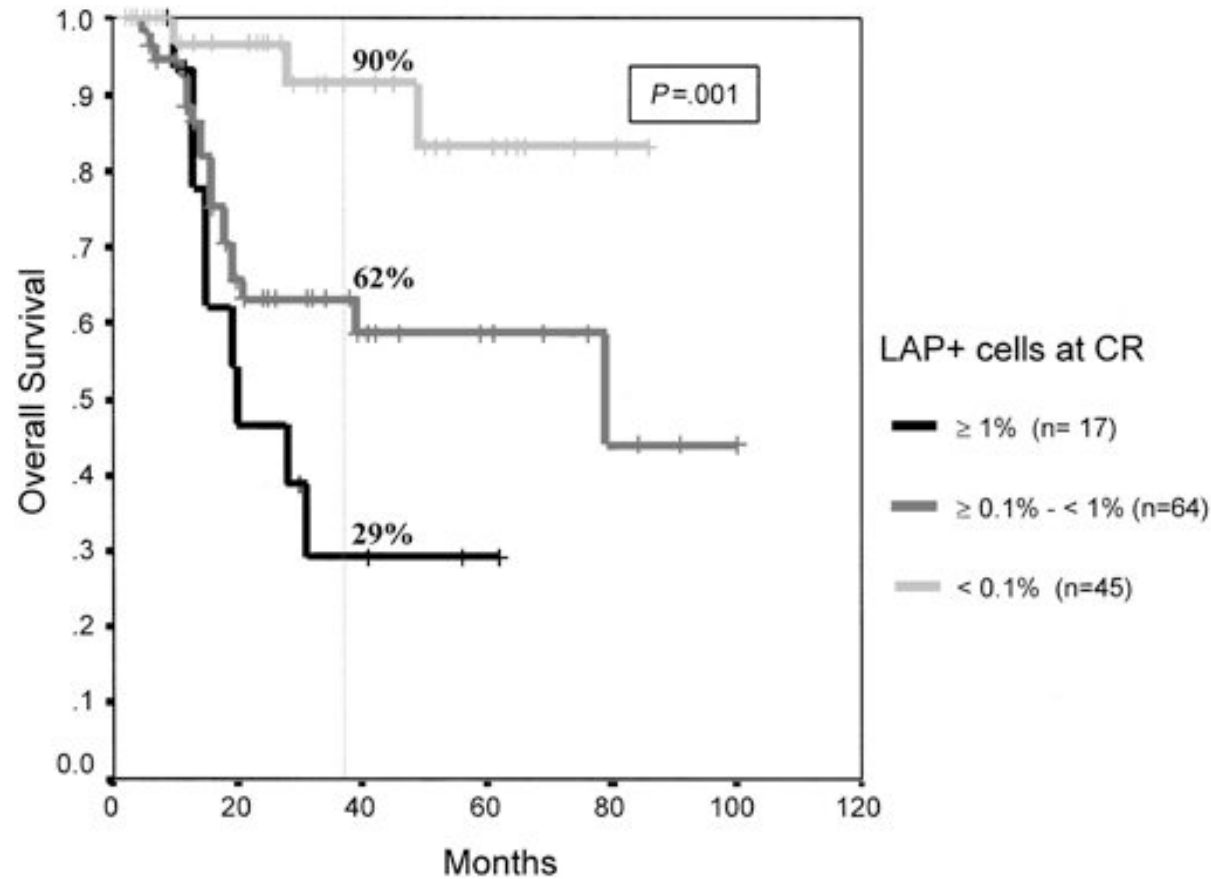


Has MRD a prognostic
impact on outcome?

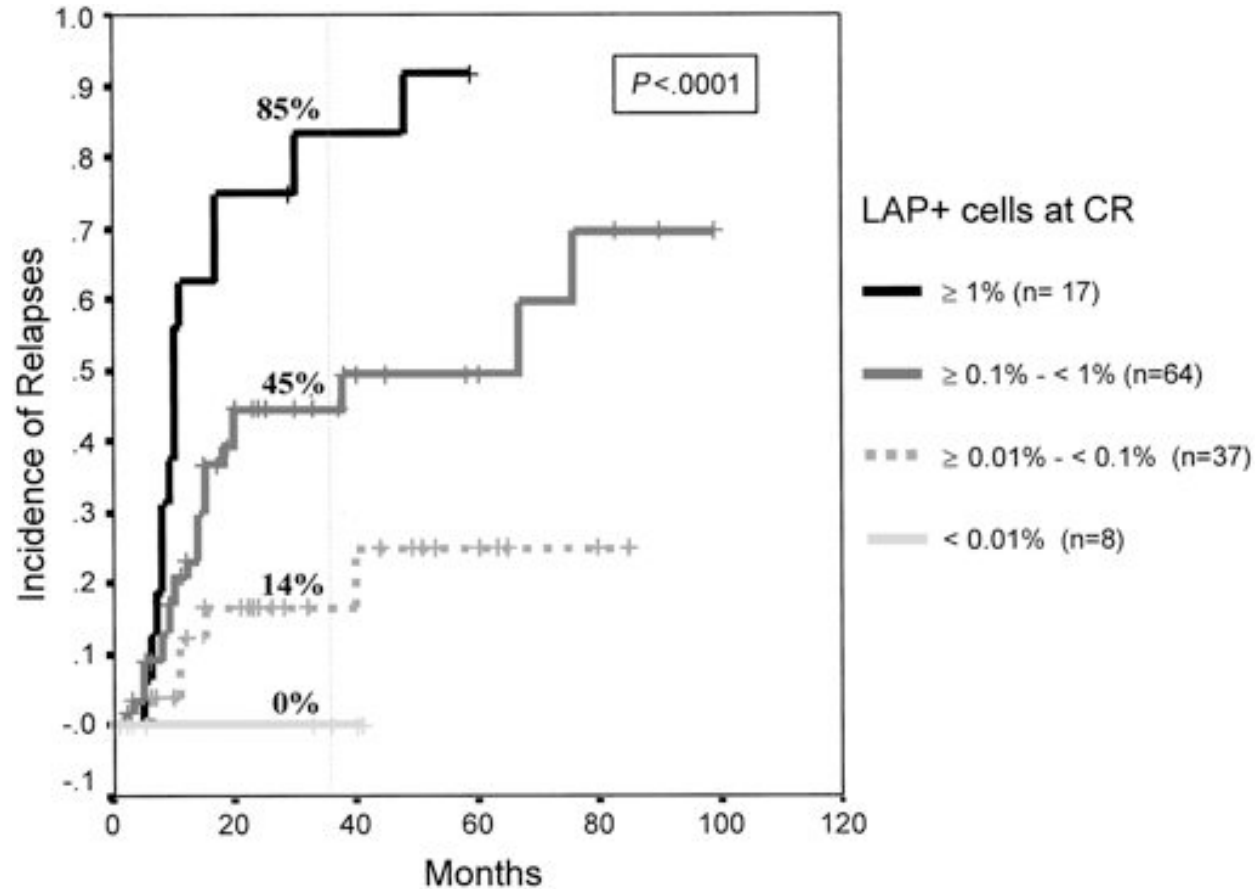
Flow cytometry

- Inclusion :
- LAIP identified
- achievement of a complete remission

53/89



Flow cytometry



Flow cytometry

- Better predictor after consolidation
 - Independent factor in multivariate analysis in some but not all studies
 - Predictive of outcome also in the context of transplantation
-

PCR of fusion genes

- Transcript ratios **at diagnosis**
 - Transcript number reduction **during therapy**
 - Rather slow decline of the leukemic cell mass
 - Evaluation after consolidation
 - Predictive of outcome
 - Independent of pretreatment risk factors
 - **Long term remission is associated with PCR negativity**
 - Increasing transcript ratios indicates **molecular relapse** 1-5 months before hematological and cytogenetic relapse
 - 8/15 relapses detected BUT follow-up intervals < 3 months
 - 7/15 not detected BUT follow-up intervals > 6 months
-

Nucleophosmin gene mutation

- Mutant copy number at CR higher in patients with subsequent relapse
- Relapse is accompanied by significant increase in copy number
- Any rise of copy number during follow-up increase the relapse risk by ~3 fold compared to persistently low or undetectable signals

Chou et al, Leukemia 2007, 21 : 998-1004

MRD guided clinical
intervention?

Impact of transplantation

- Autologous transplantation has no impact on the unfavorable value of high pre-ASCT MRD level

Venditti et al Leukemia 2003, 17 : 2178-2182

- MRD-positive patients who underwent allogeneic SCT had similar RFS to patients who had no detectable MRD

Laane et al 2006 Haematologica 91 : 833-836

AML M3 (PML-RAR α)

- Evaluation at the end of consolidation (misleading at earlier time points)
 - Sensitivity 10^{-3} - 10^{-4} (more sensitive methods are less informative and show positivity in long term remission)
-

AML M3 (PML-RAR α) : persistent MRD after consolidation

- 3.4%
- Have a very bad prognosis unless they are given early salvage therapy including aggressive approaches :

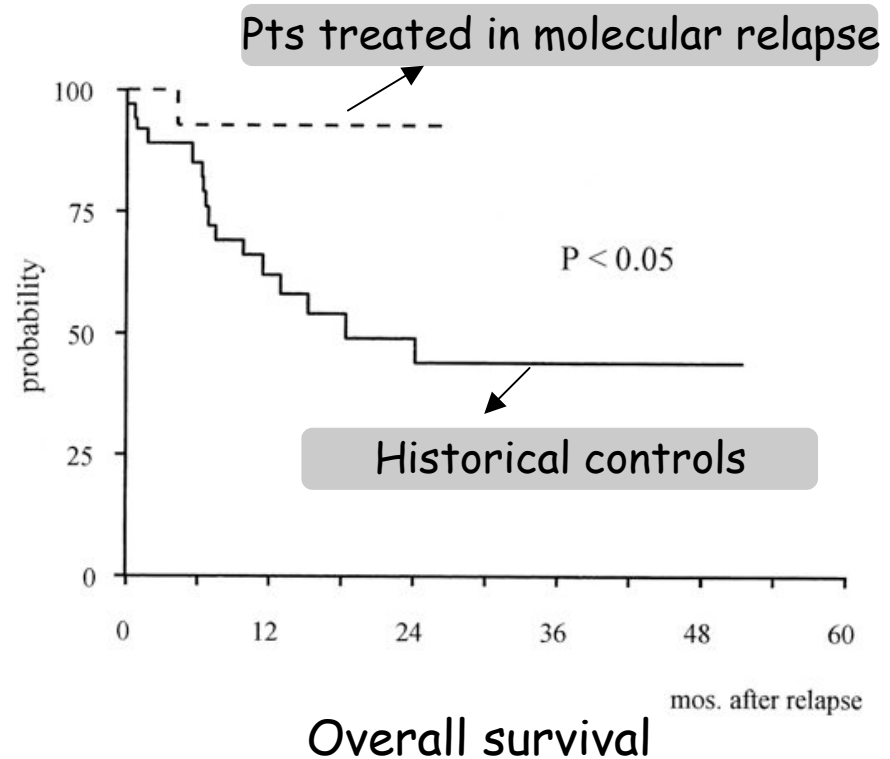
7 patients treated in molecular refractoriness : 7/7 alive
16 patients treated in hematological relapse : 2/16 alive

Breccia et al, haematologica 2004, 89 : 29-33

- Salvage therapy :
 - ATO +/- anti CD33
 - Allogeneic transplant
 - Autologous transplant if PCR negativity is reached before transplant
-

AML M3 (PML-RAR α) : follow-up with RQ-PCR

- Increase in MRD identifies patients at high risk of relapse
- Median time to hematological relapse : 3m (1-14m)
- Administration of pre-emptive treatment at the time of molecular relapse provides survival advantage over treating in overt hematological relapse
- PCR analysis every
 - 3 months during 1st year
 - 4-6 months 2 and 3 years
 - More frequently in high risk



Coco, F. L. et al. Blood 1999;94:2225-2229

Conclusions

- MRD is predictive of outcome
 - in first line
 - in relapsed patients
 - in the context of transplantation
 - MRD can detect relapses earlier
-

Implementation of MRD evaluation in risk stratification of patients in a clinical protocol

- Choice of the MRD technique
 - High sensitivity for detection of low risk
 - Low sensitivity for detection of high risk
 - Same technique throughout the protocol
 - Clearly defined time-points, cut-off levels and technical steps for each treatment protocol
 - Probably at least 2 time-points to improve accuracy
 - Treatment blocks before the MRD sampling cannot be changed because this would directly change the prognostic value of the MRD result
 - It is impossible to extrapolate data from one clinical protocol to another
-

Risk stratification on MRD results

- Added to the other well-known risk factors
 - Numerous subgroups of patients
 - Large cooperative studies
 - Interlaboratory standardization of MRD analysis
 - Need for better active treatment
-

Perspectives

- Gene expression profiling
 - Better markers for follow up
 - Better knowledge of mechanisms of resistance or response
 - Better targeted therapies
-