

XXXIV Diada Internacional  
Societat Catalana d'Hematologia i Hemoteràpia.  
Barcelona, 18 de juny de 2010

# **Diagnòstic Integrat de la Leucèmia Limfoblàstica aguda**

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ICO-Hospital Universitari  
Germans Trias i Pujol. Badalona

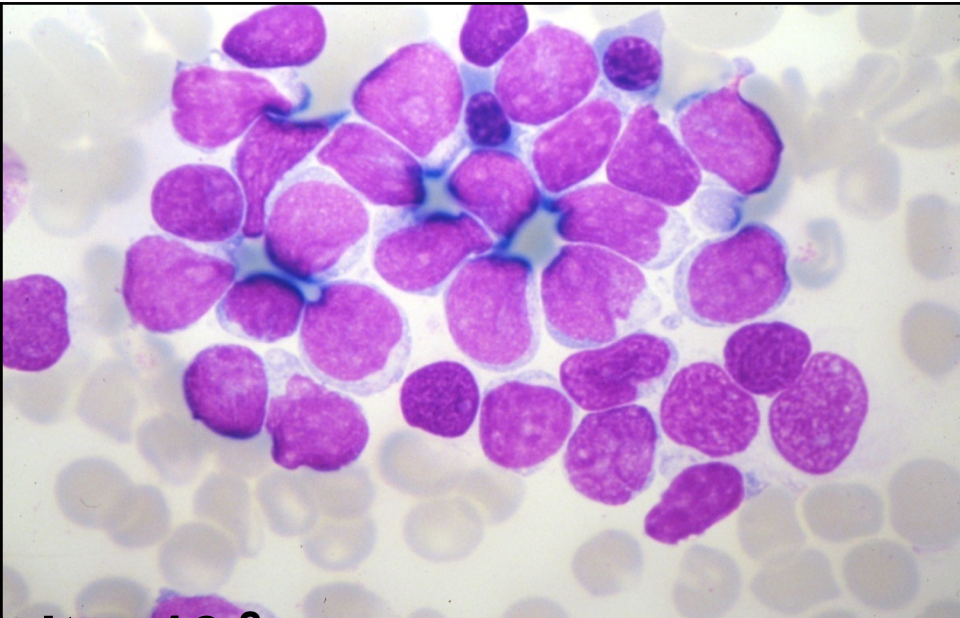
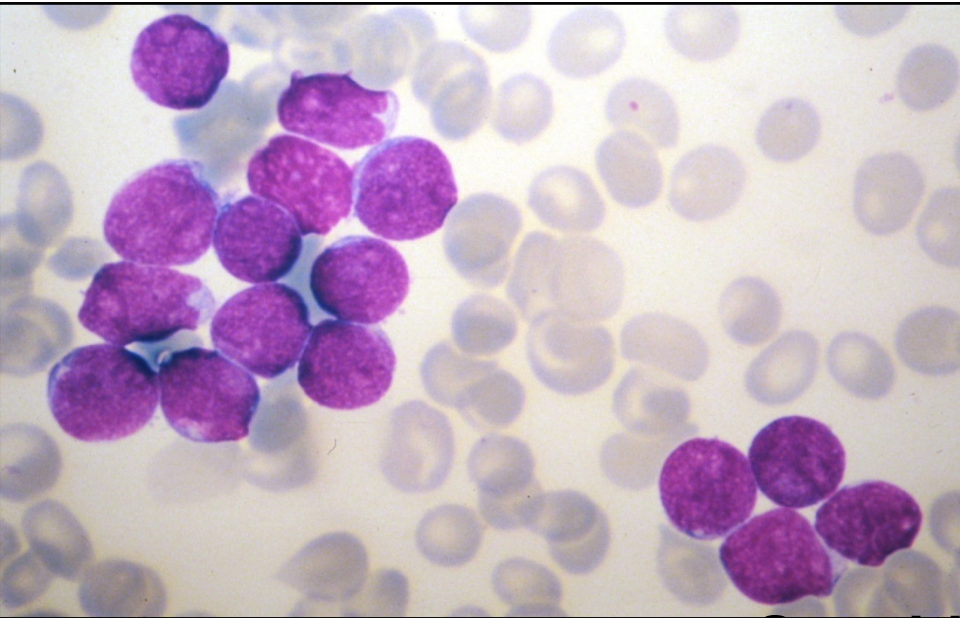
# Diagnostic work-up in ALL

- Anamnesis, physical examination
- Complete blood count , coagulation status, serum biochemical study
- EKG, LVEF (advanced age or history of cardiac disease)
- Chest X-ray film
- Bone marrow smear (morphology, cytochemistry)
- Bone marrow biopsy (only if dry tap)
- Immunophenotypic study (BM, PB)
- Cytogenetics
- FISH
- Study of molecular rearrangements (PCR)
- CSF study
- Storage: cells, DNA, RNA.

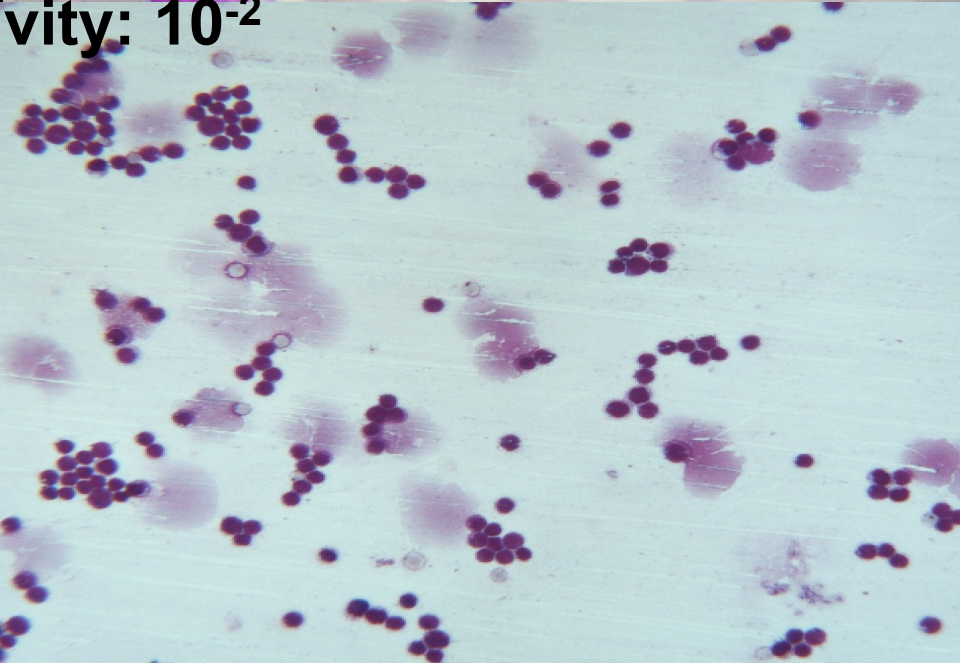
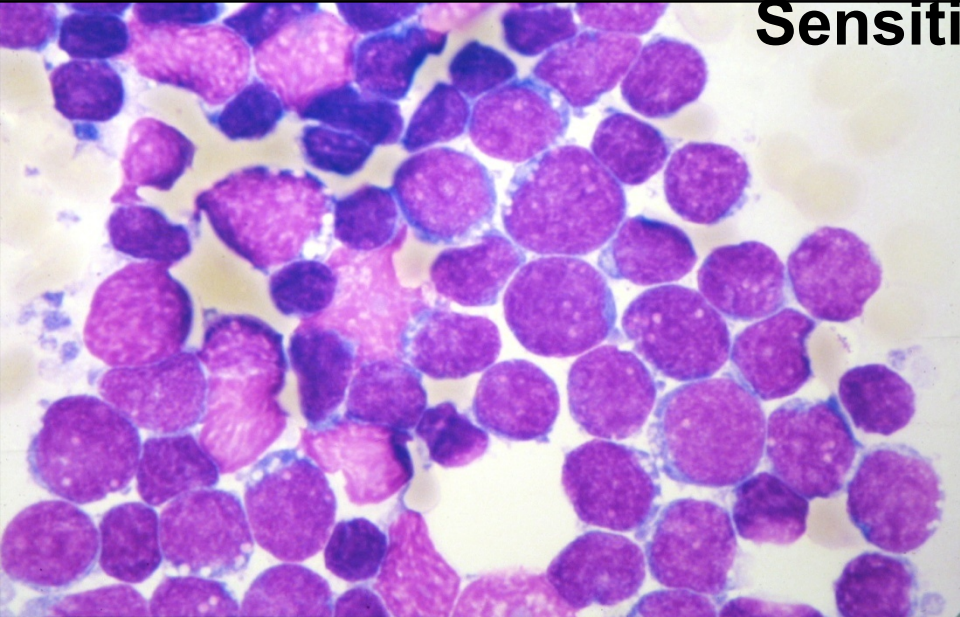
# ALL. WHO Classification

- **B precursor ALL**
  - t(9;22); *BCR/ABL*
  - 11q23; *MLL*
  - t(1;19); *E2A/PBX1*
  - t(12;21); *ETV/CBF alpha*
- **T-ALL**
- **Burkitt-like ALL (mature B-ALL)**
  - t(8;14), t(2;8), t(8;22); *C-MYC*

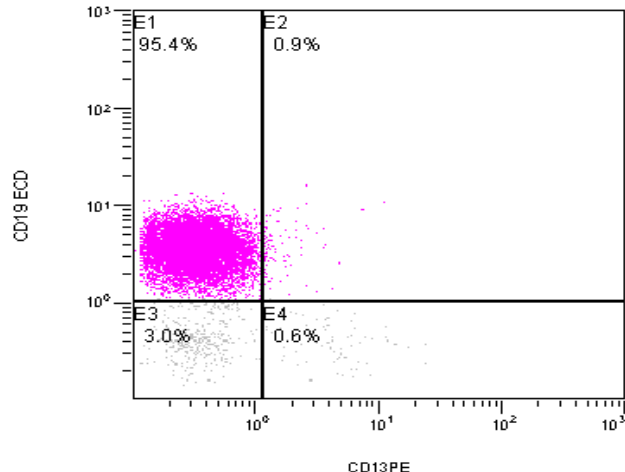
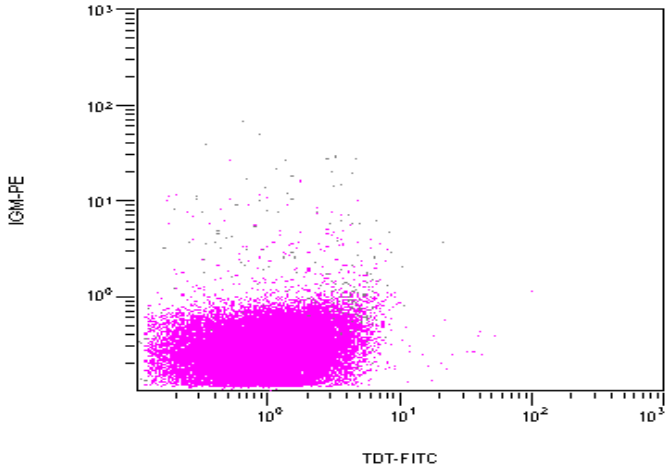
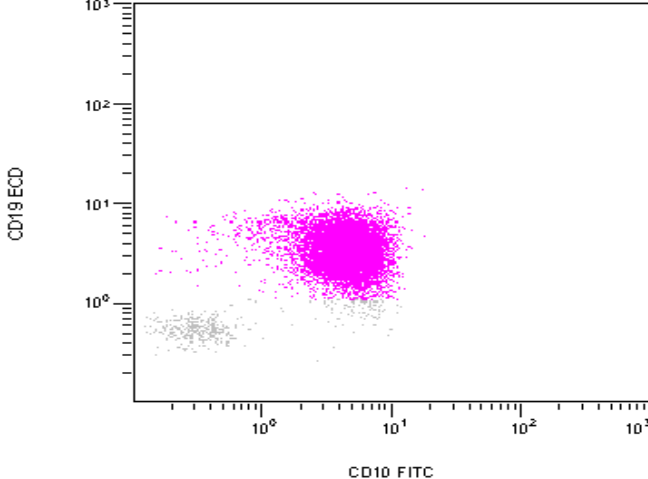
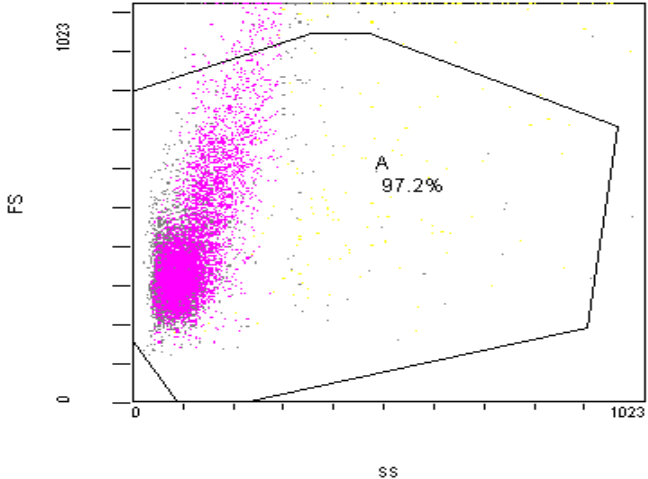
# LAL. Morphology



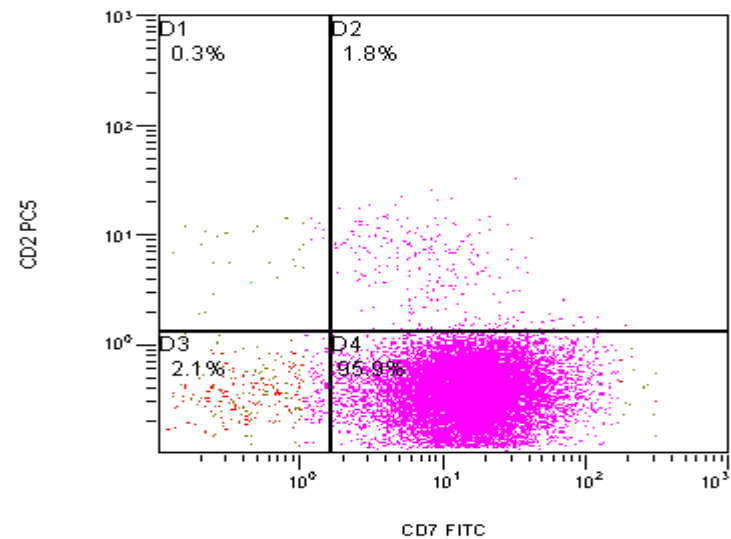
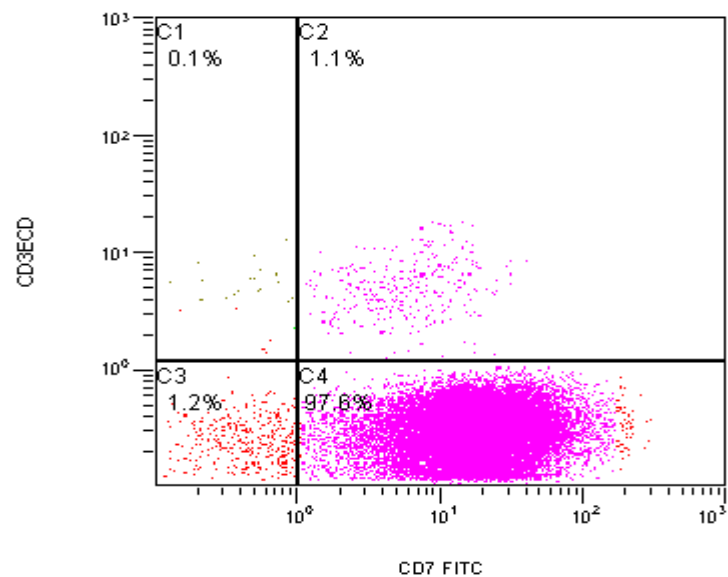
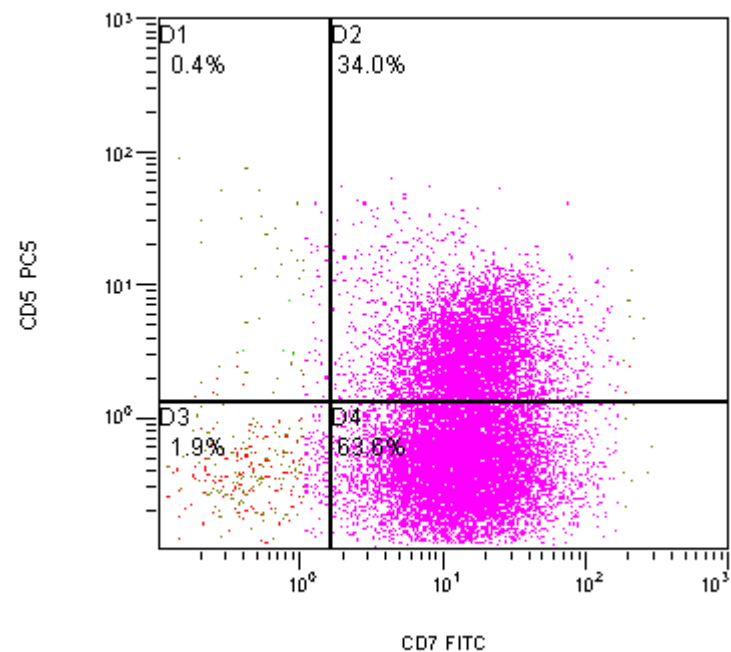
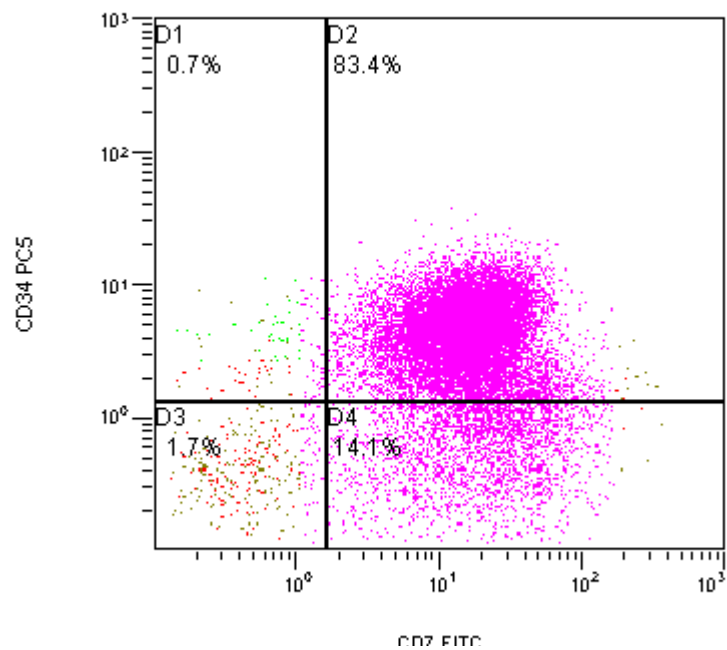
Sensitivity:  $10^{-2}$



# Phenotypic study



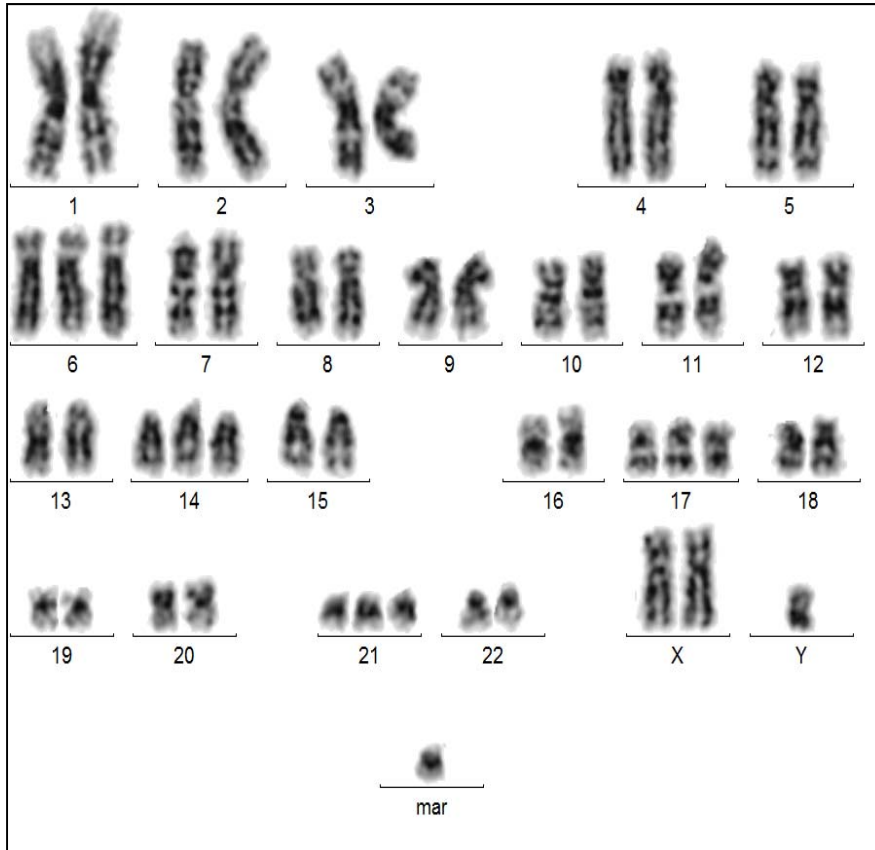
**Sensitivity:**  $10^{-4}$  (4 colors),  $\geq 10^{-5}$  (>4 colors)



# Cytogenetics

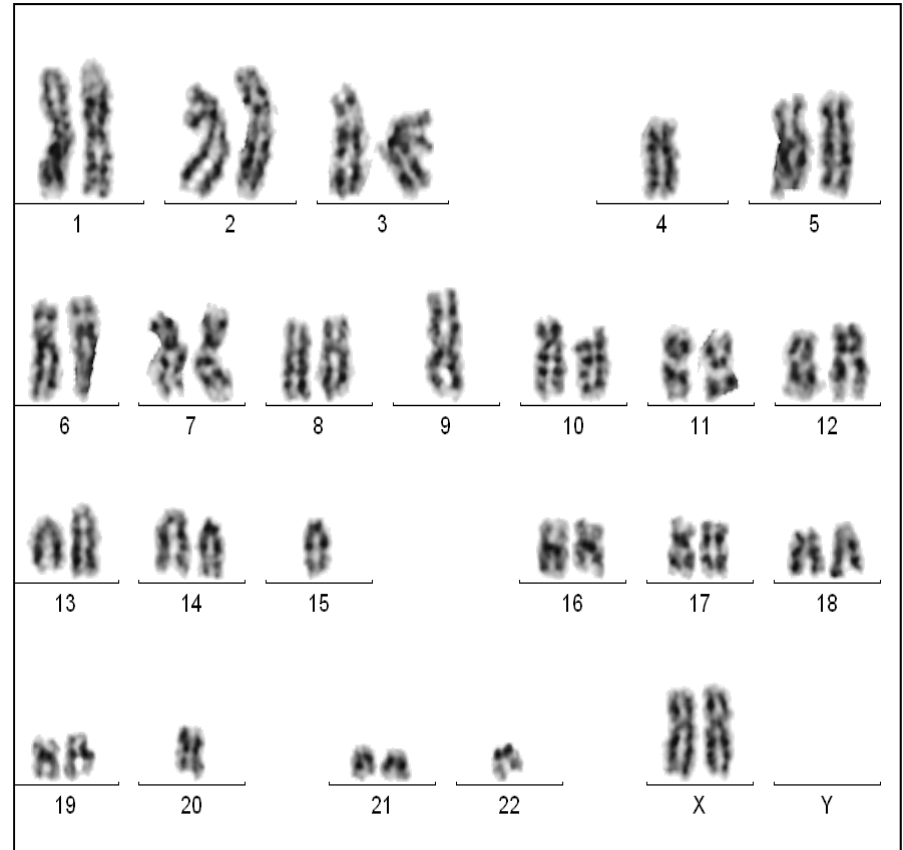
## Hiperdiploidy

52,XY,+X,+6,+14,+17,+21,+mar



## Hipodiploidy

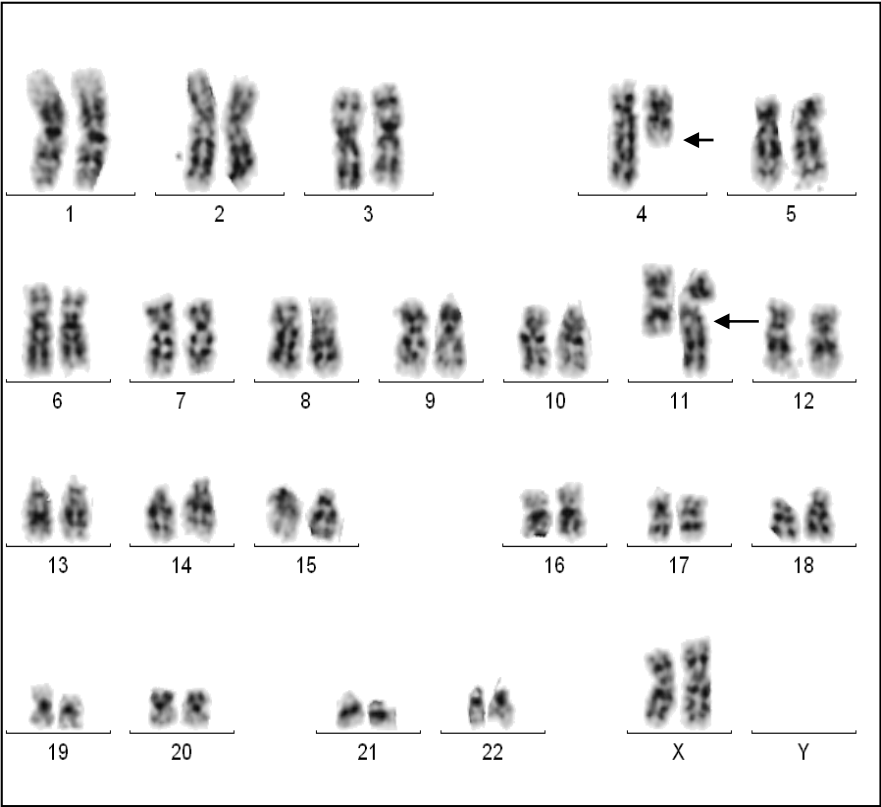
41,XX,-4,-9,add(9)(p21),-15,-20,-22



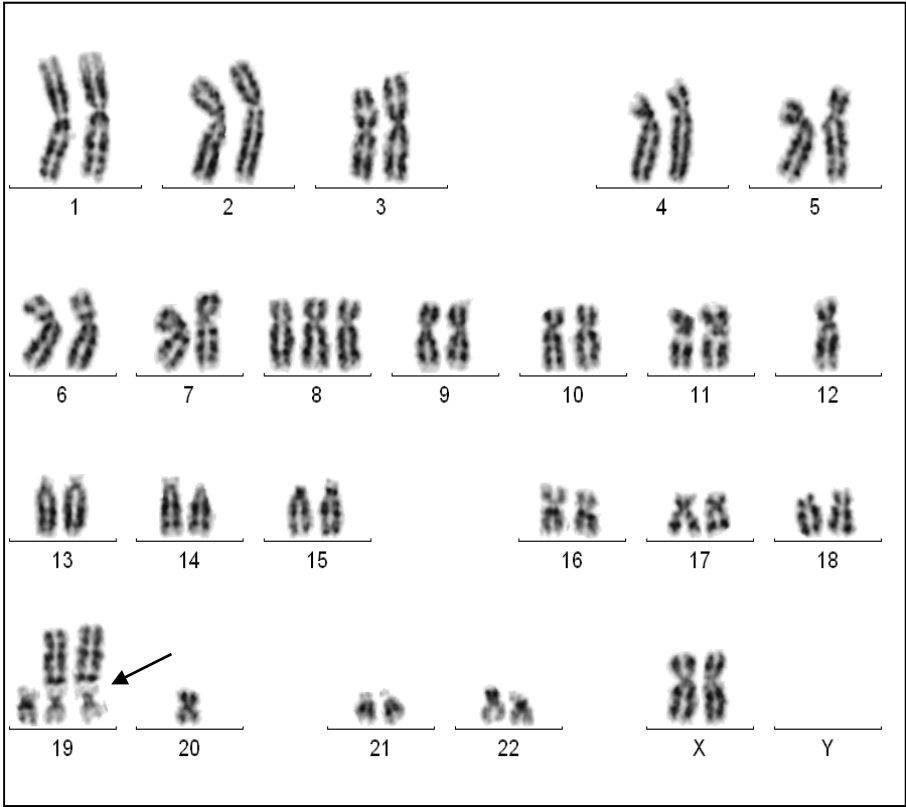
Sensitivity:  $10^{-2}$

# Pseudodiploidy

46,XX,t(4;11)(q21;q23)



46,XX,+8,-12,der(19)t(1;19)(q23;p13.3),  
+der(19)t(1;19)(q23;p13.3),-20

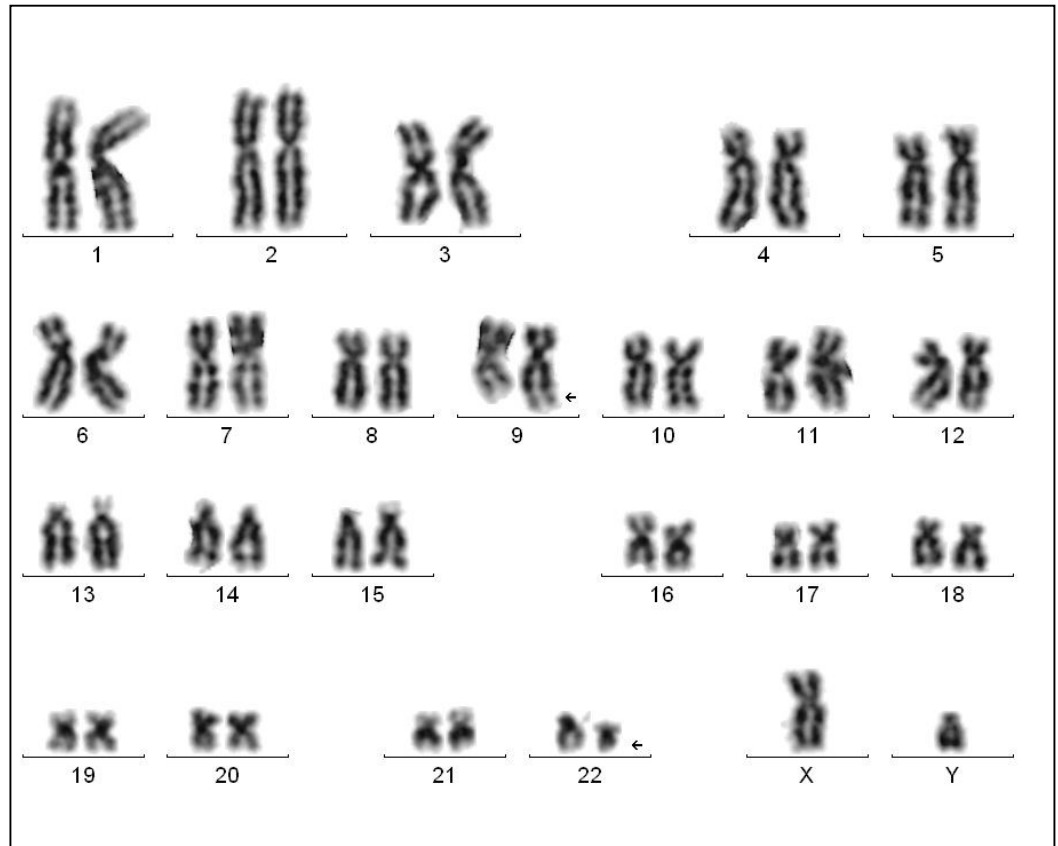
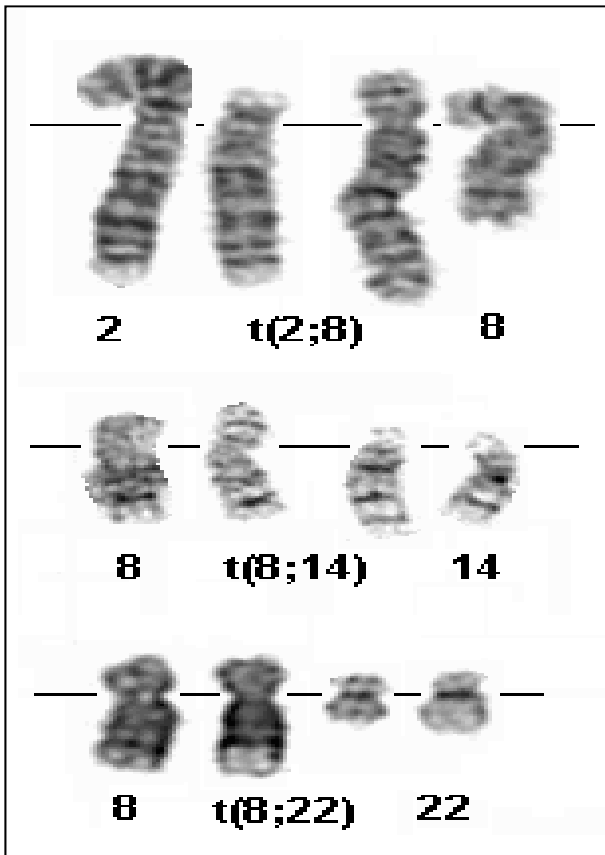


Sensitivity:  $10^{-2}$

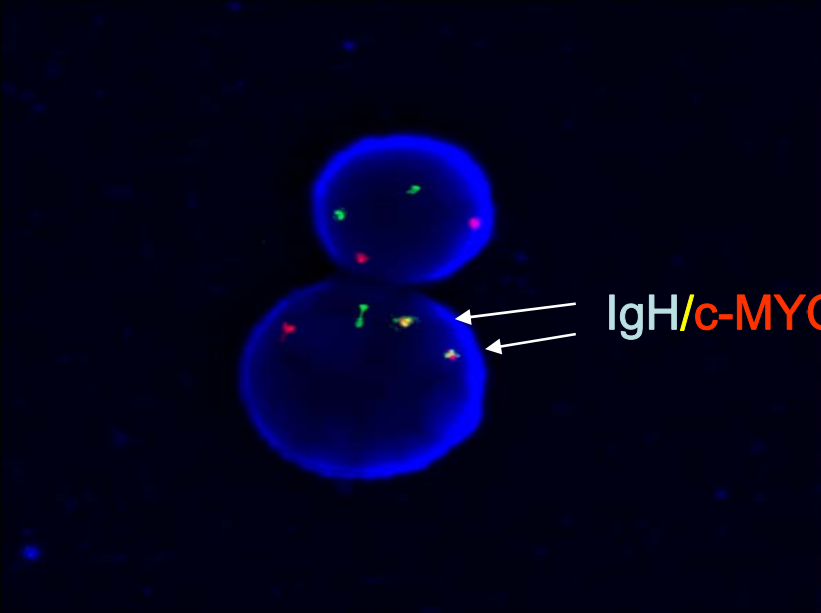
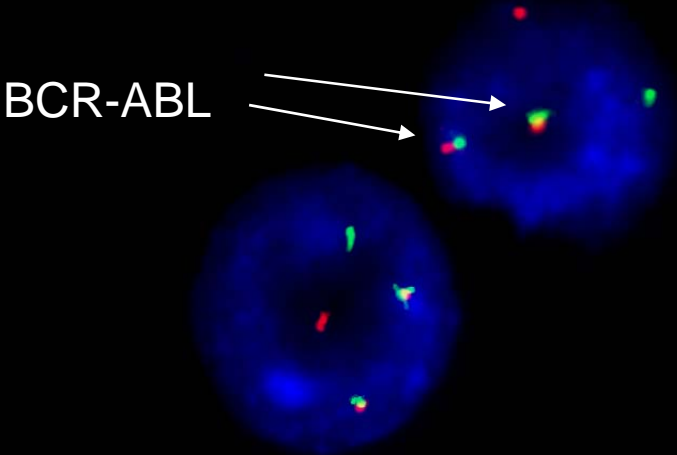


# Pseudodiploidy

46, XY, t(9;22)(q34.1;q11.2)



# ALL. FISH

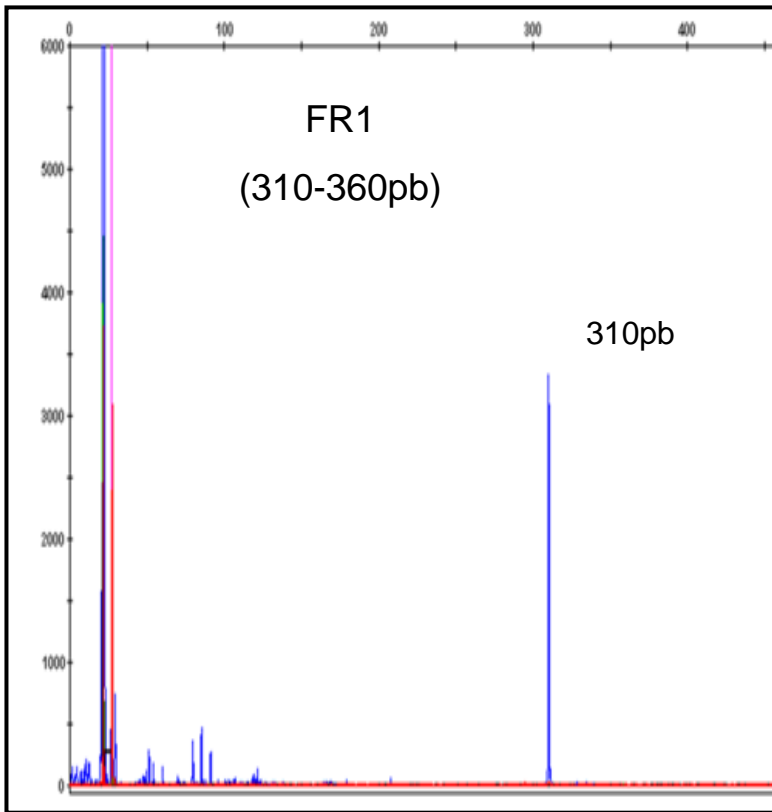


nuc ish(ABL1x3),(BCRx3),(ABL1con BCRx2)[90/100]

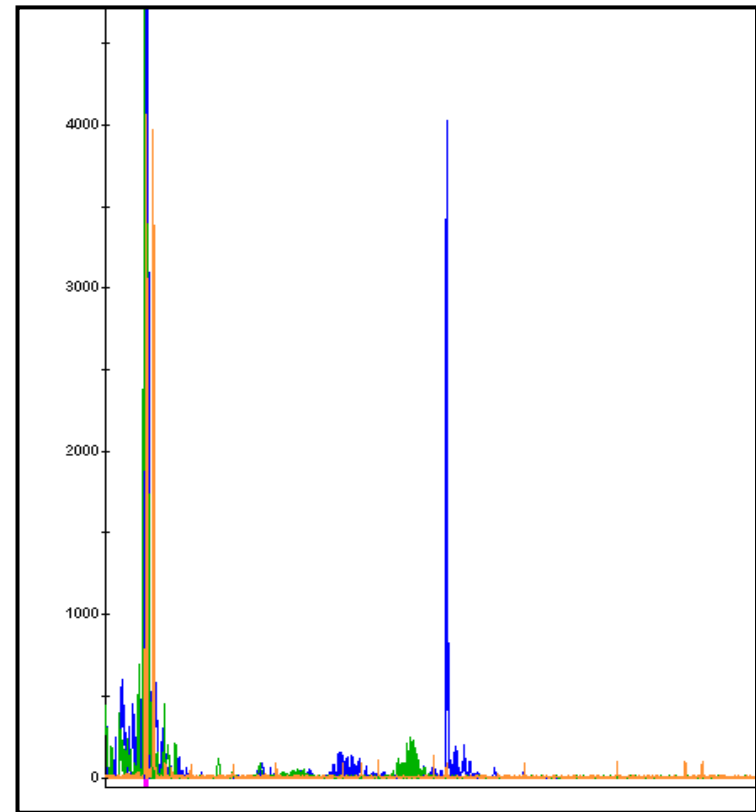
Sensitivity:  $5 \times 10^{-2}$

# Ig & TCR rearrangements

IgH clonal



TCR clonal

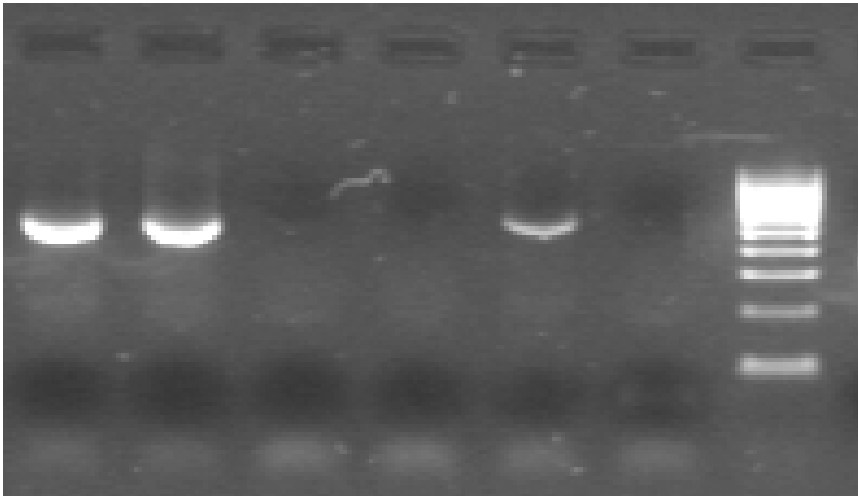


**Sensitivity:**  $10^{-4}$  –  $10^{-5}$  (RQ-PCR)

# Quantification of the amount of mRNA transcripts

## BCR/ABL - t(9;22)(q34.1;q11.2)

1 2 3 4 5 6 7



**1 & 2: Patient 1 (positive p190)**

**3 & 4: Patient 2 (negative p190)**

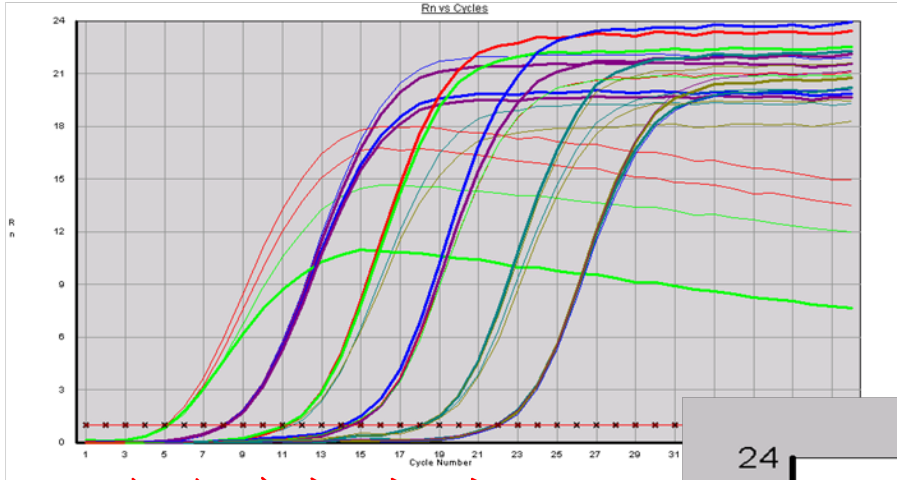
**5: Positive control p190**

**6: Negative control**

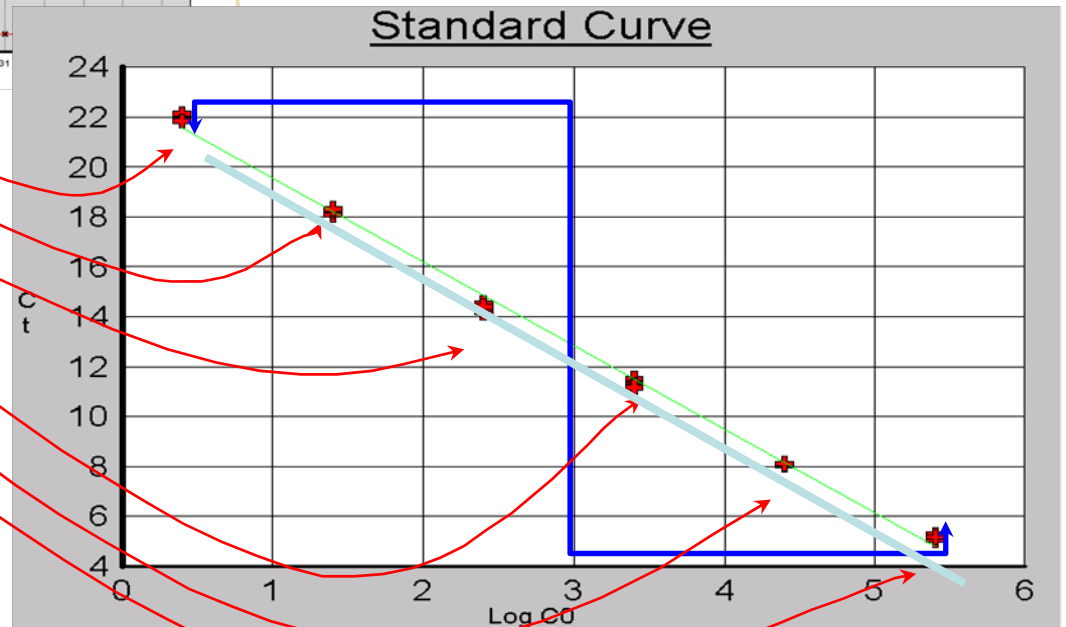
**7: Marker of molecular weight**

# RQ-PCR

Standard curve



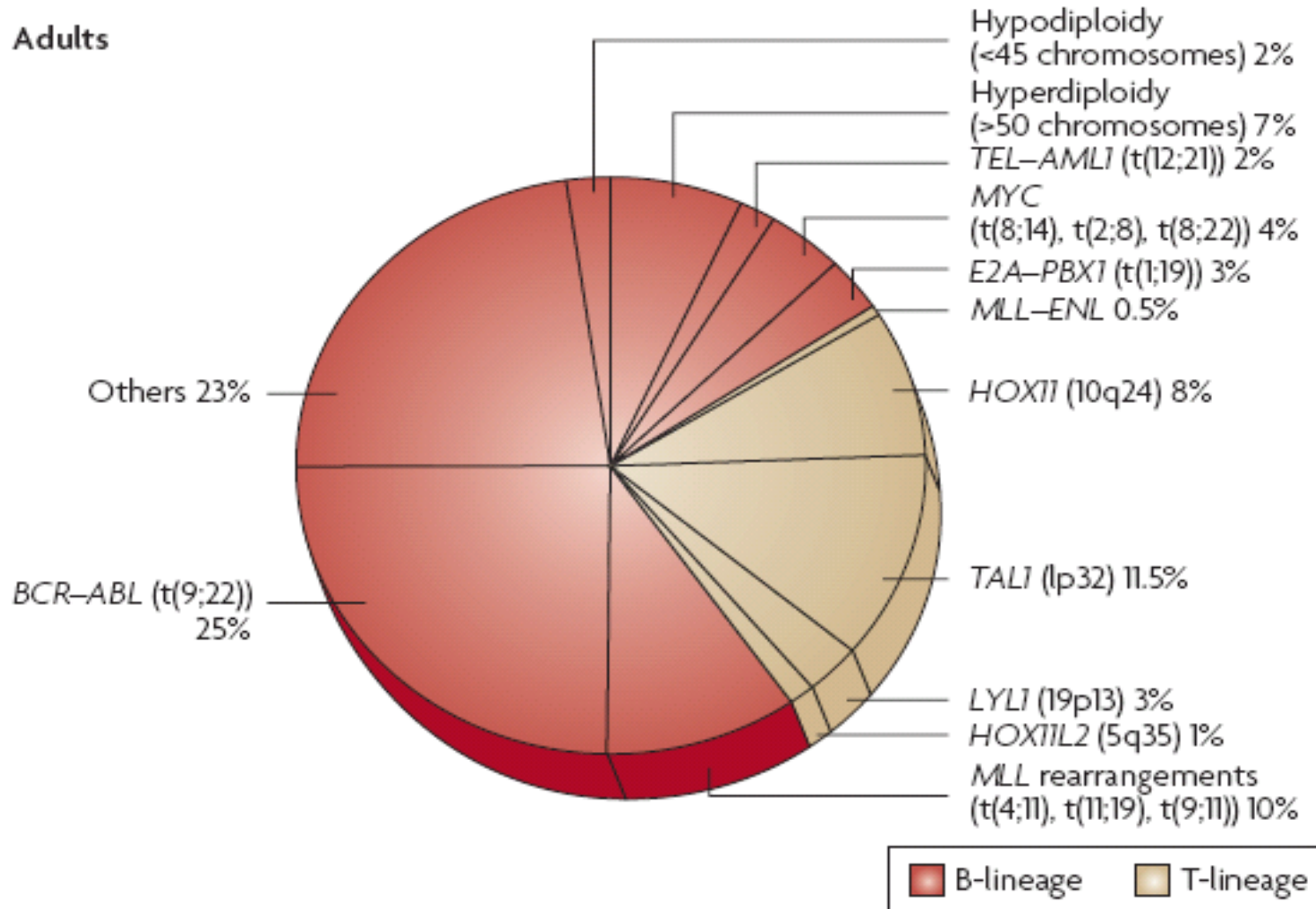
Linear dynamic range (5 Logs)



**Sensitivity:  $10^{-5}$ -  $10^{-6}$**

# Genetic Heterogeneity in Adult ALL

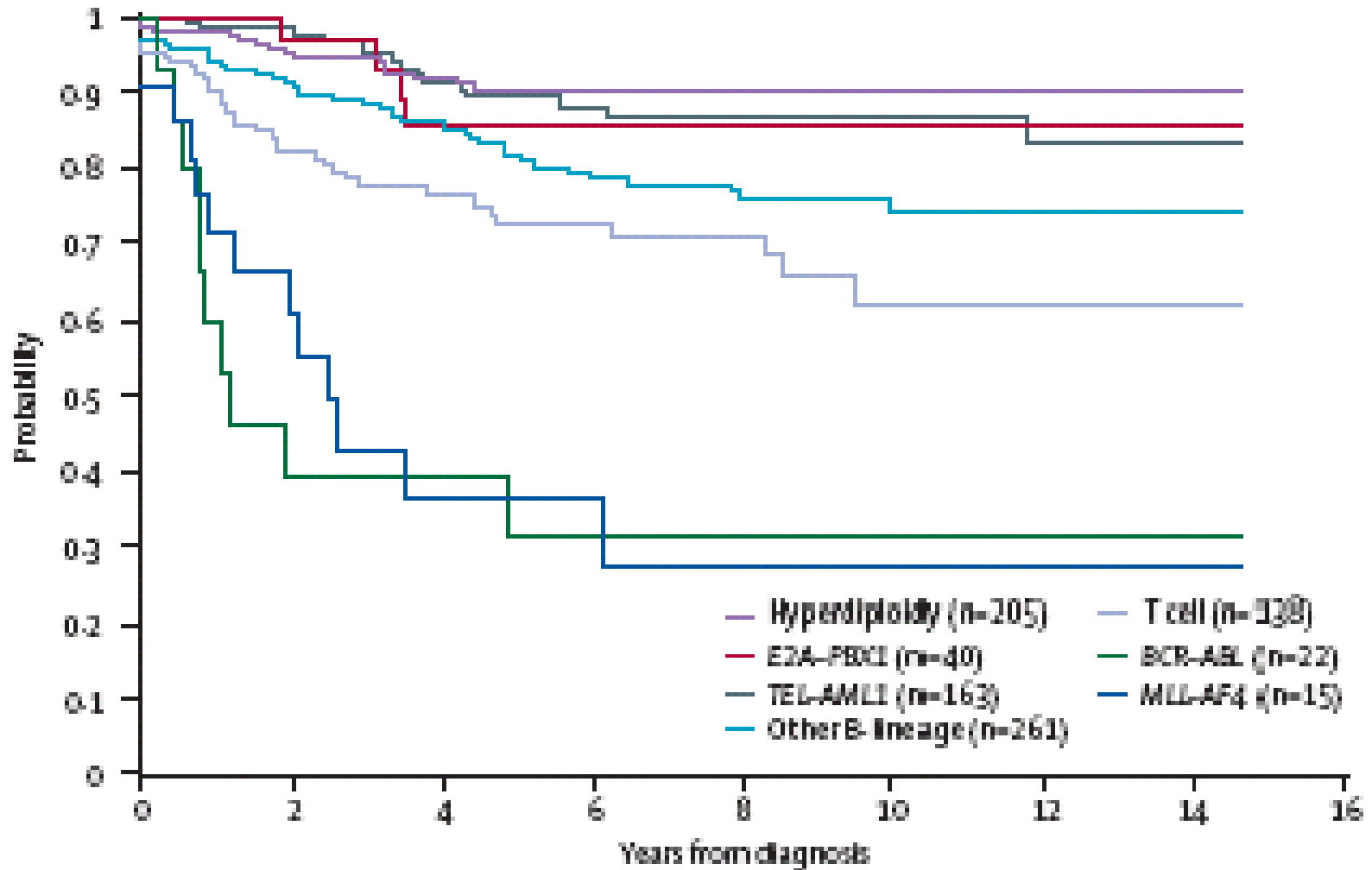
**b Adults**



# Usefulness of diagnostic work-up

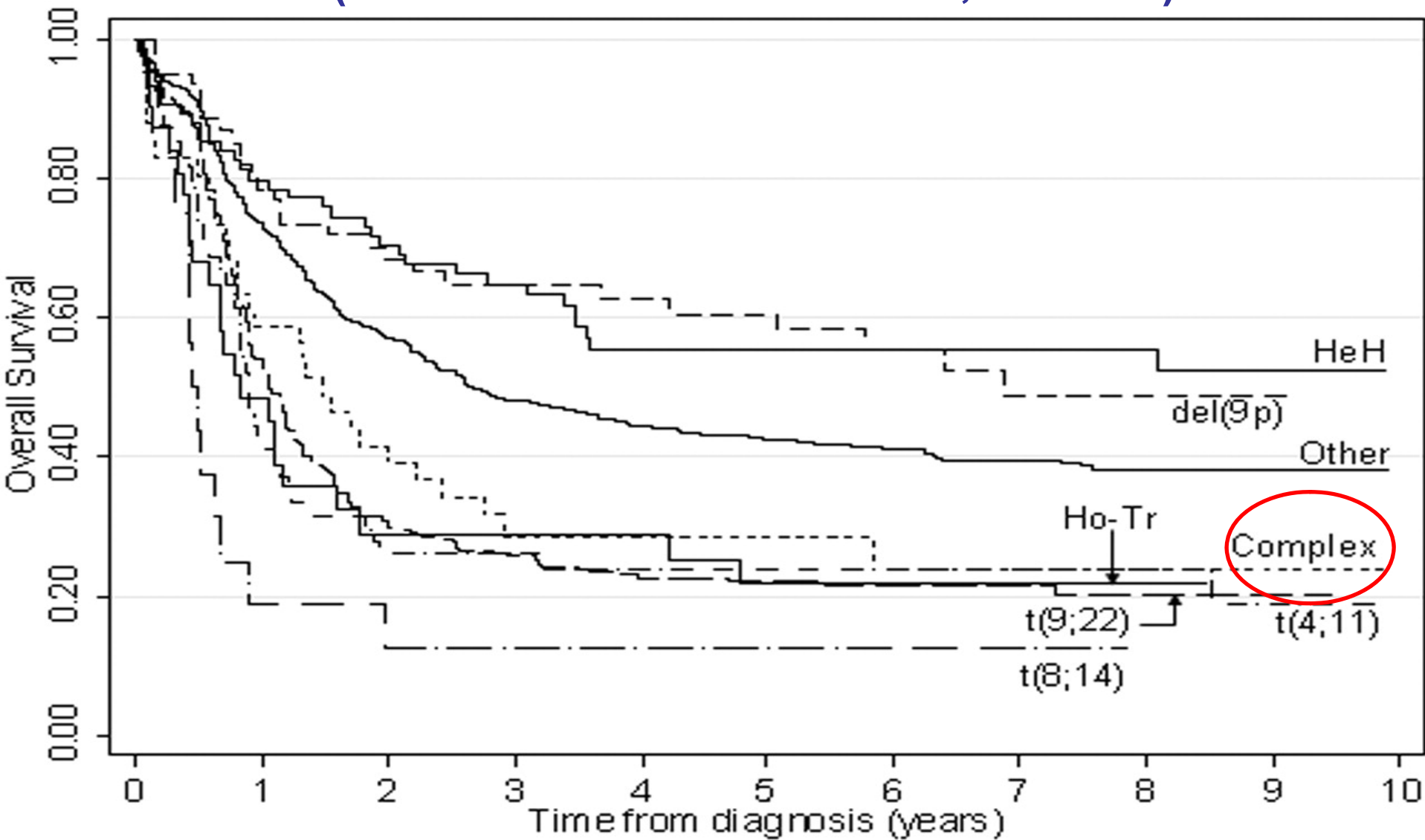
- Diagnosis
- **Prognosis**
- MRD evaluation and follow-up
- Early detection of relapses

# Prognostic impact of genetic and molecular classification of childhood ALL

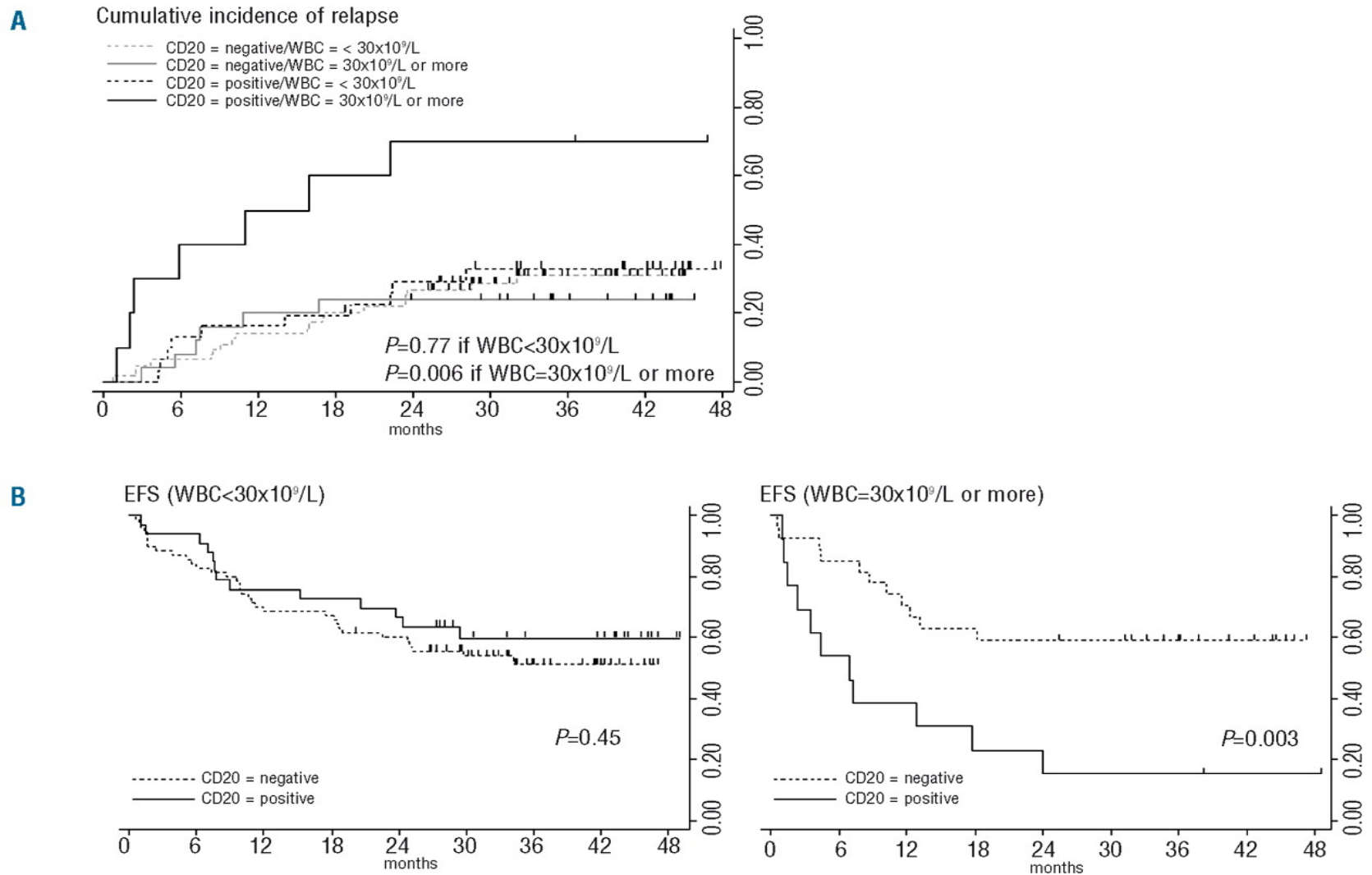




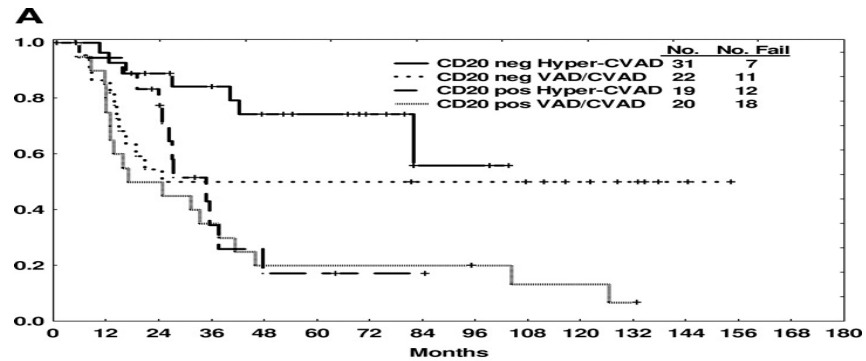
# Genetics and prognosis in adult ALL. (MRC UKALLXII/ECOG 2993, n= 1522)



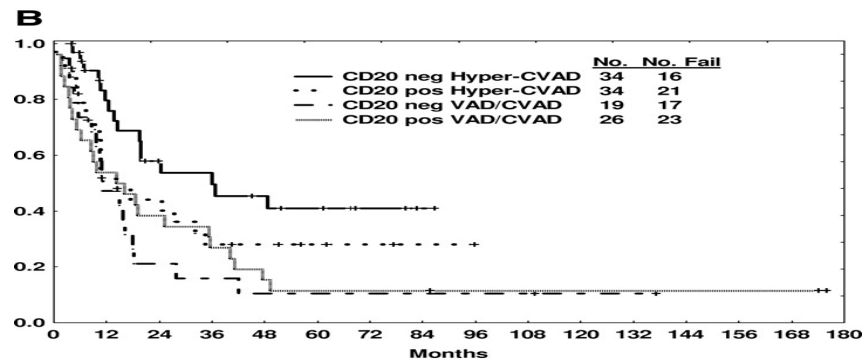
# CIR and EFS according to CD20 expression and WBC in adult ALL



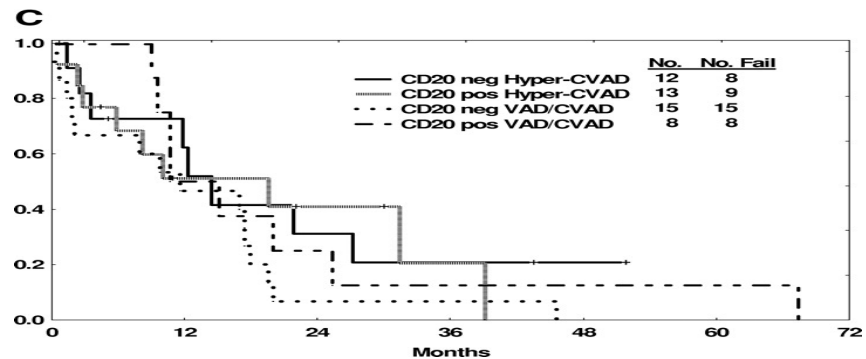
# Outcome by CD20 expression and therapy according to age subgroups



Protocol

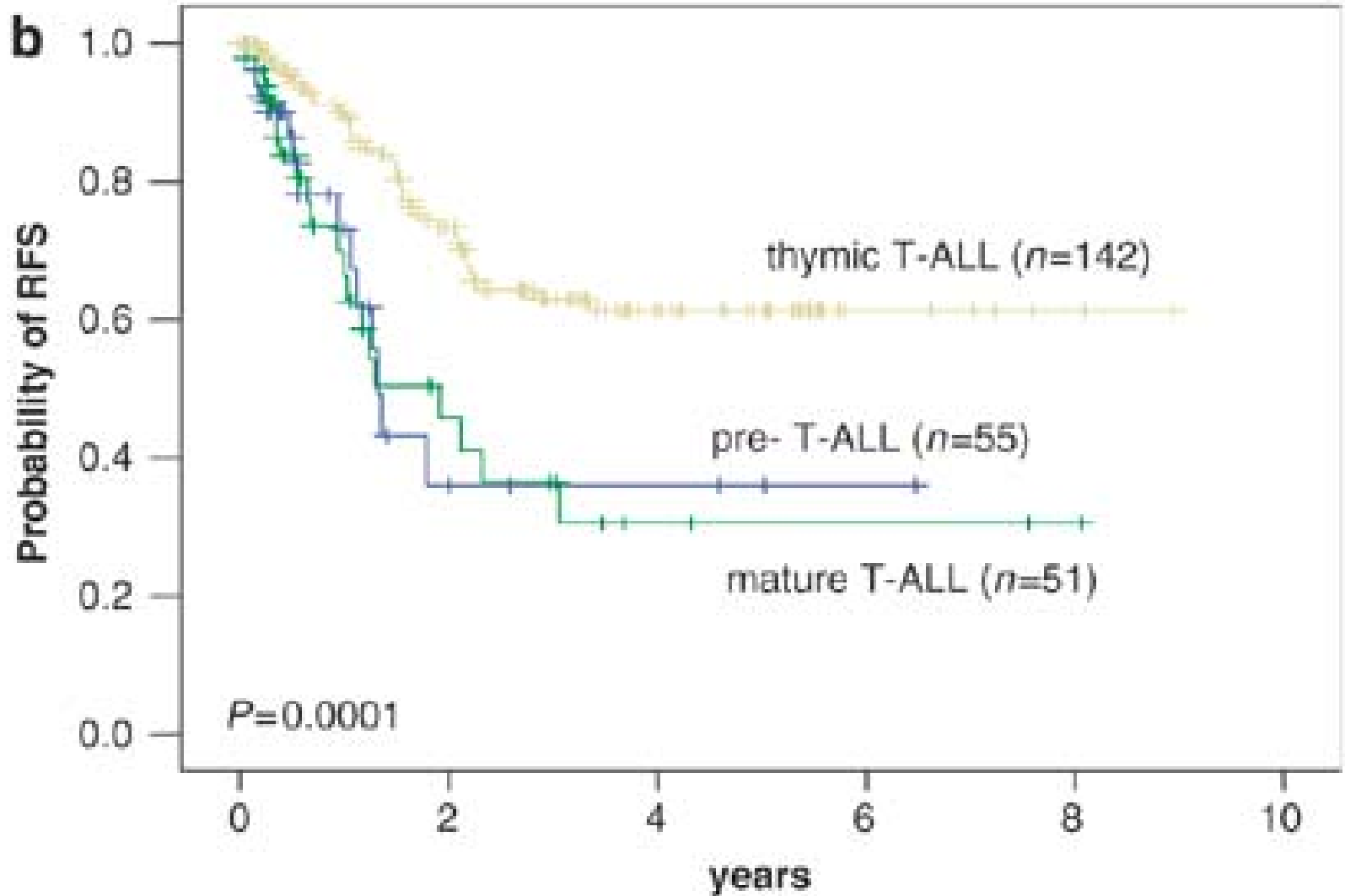


Young

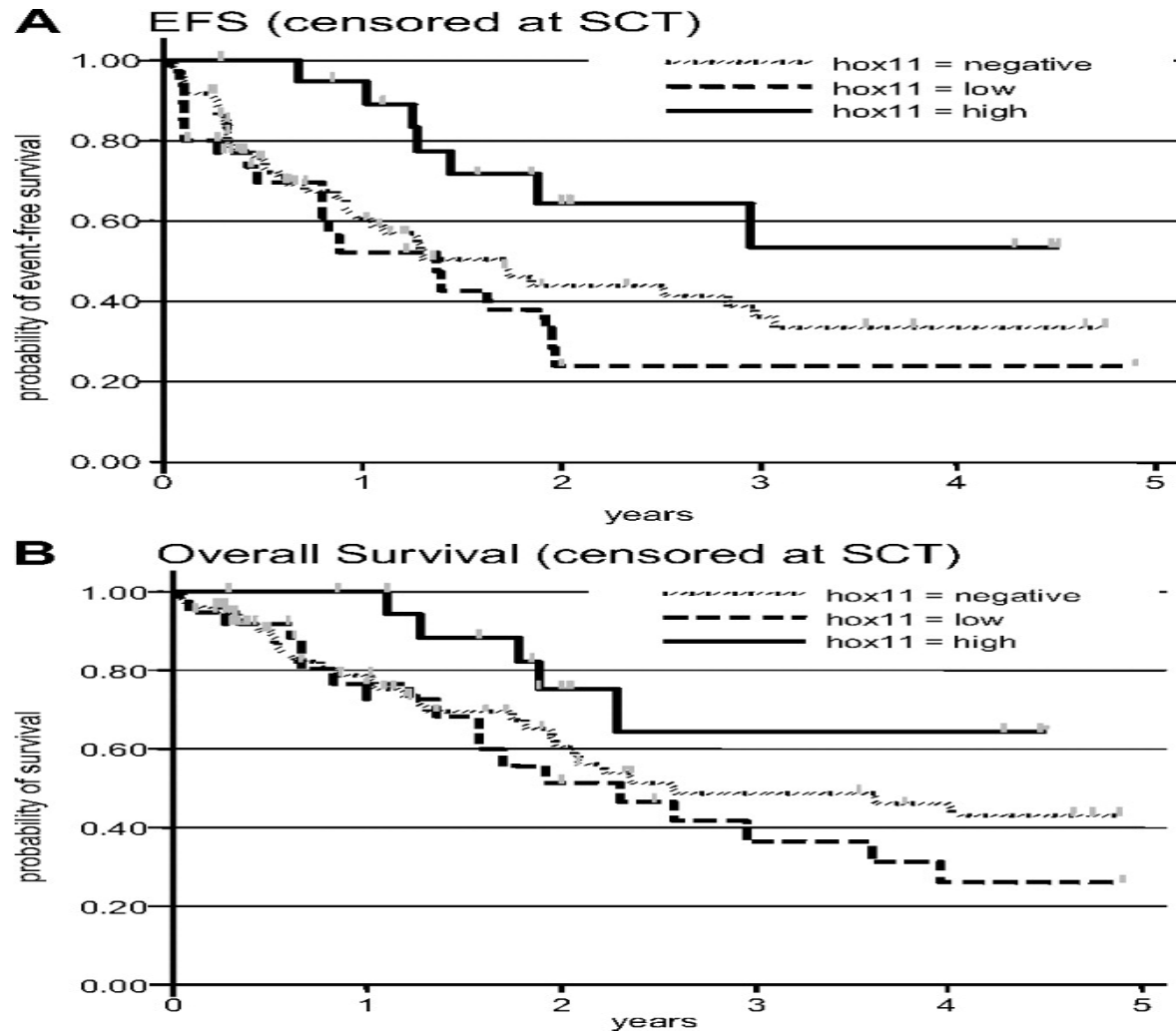


Elderly

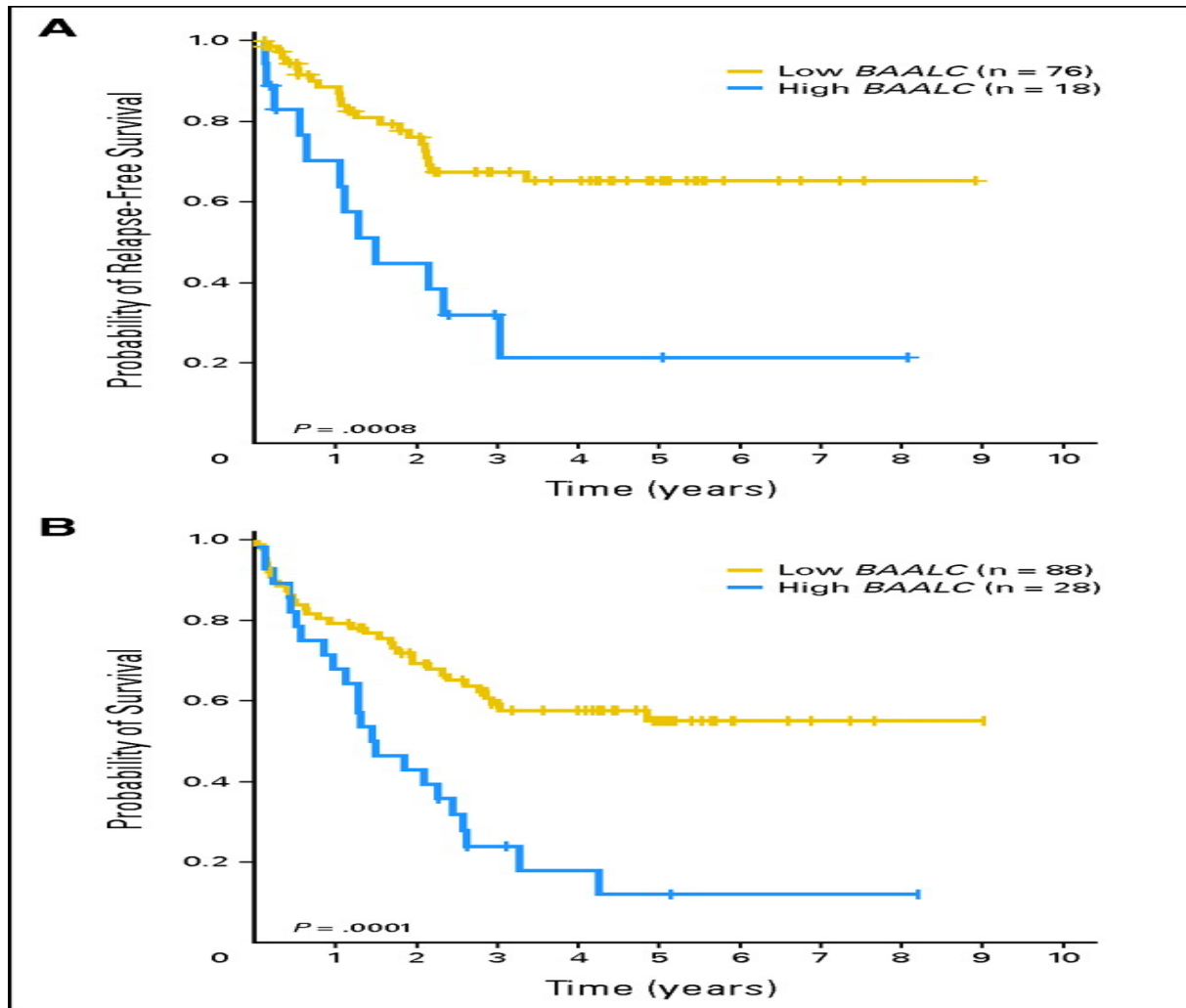
# T-ALL: prognostic value of differentiation stage/phenotype



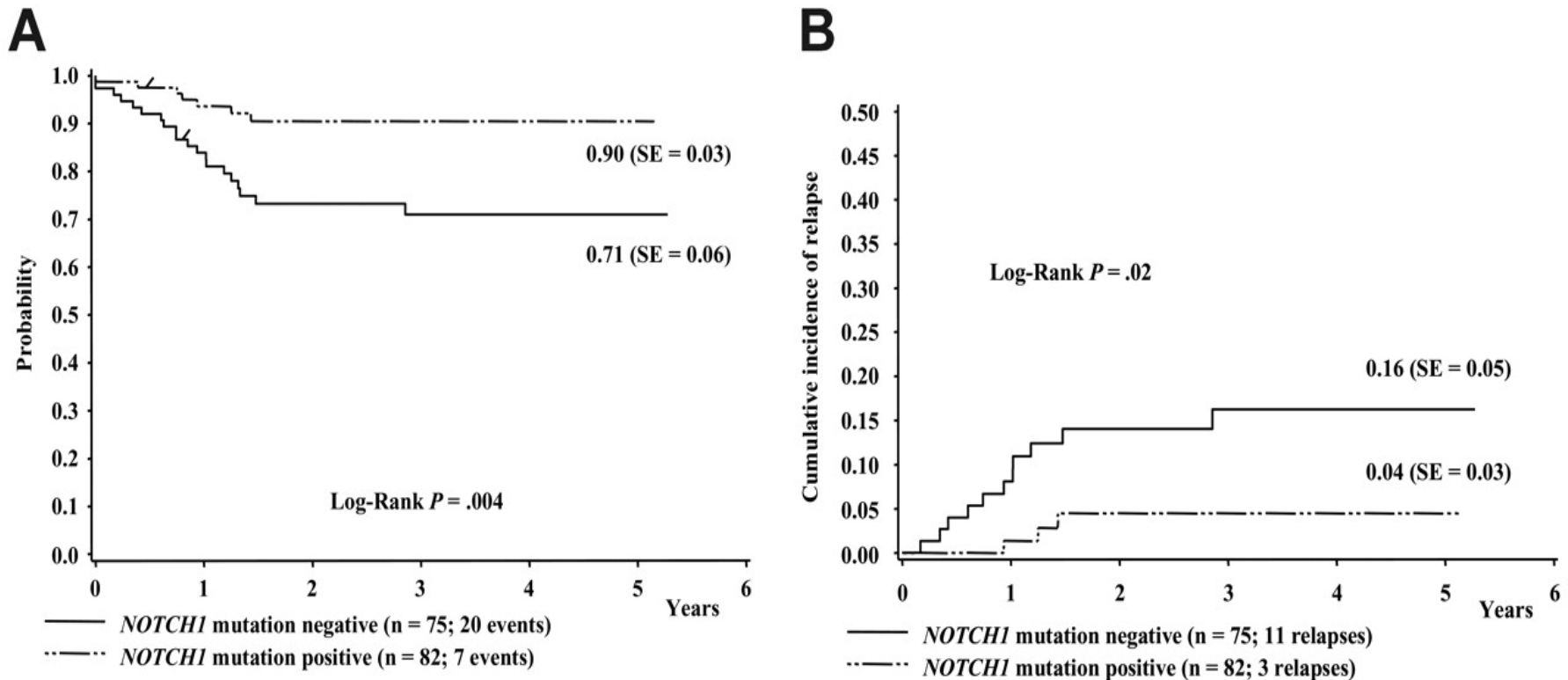
# Prognostic impact of HOX1/TLX1 in adult T-ALL



# Impact of BAALC expression on survival in adult T-ALL

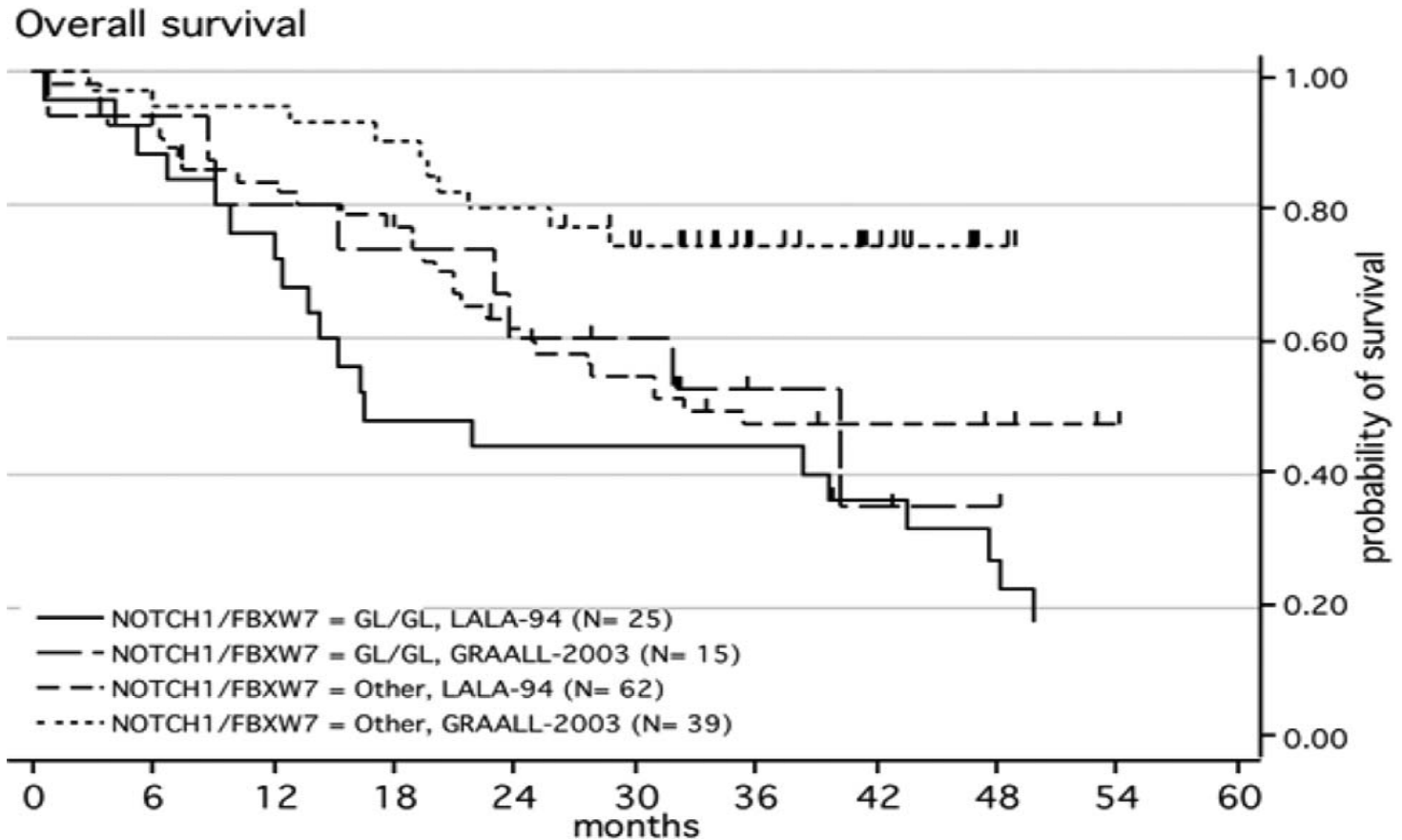


# Effect of NOTCH1 mutation status on long-term prognosis in childhood T-ALL



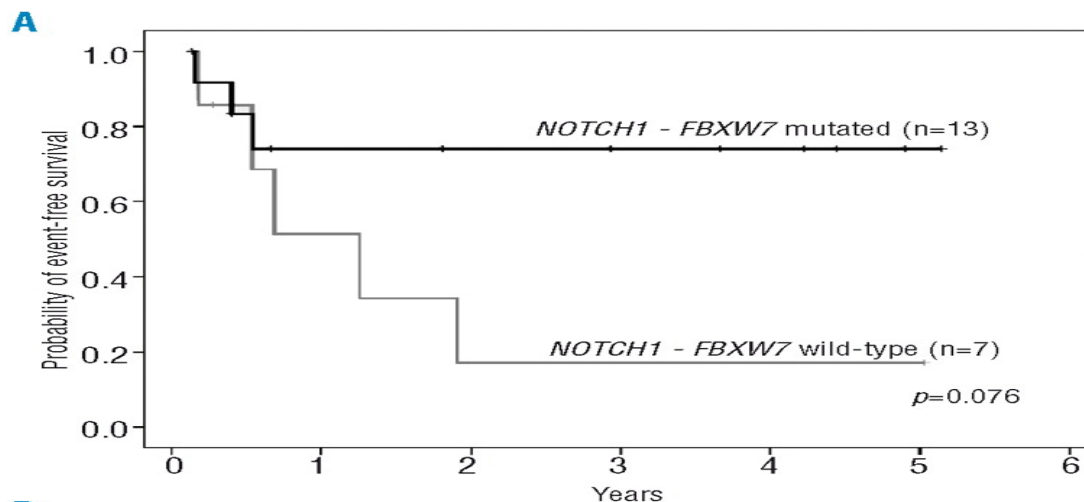
# OS in adult T-ALLL

## according to NOTCH1 and/or FBXW7 mutations and chemotherapeutic protocol

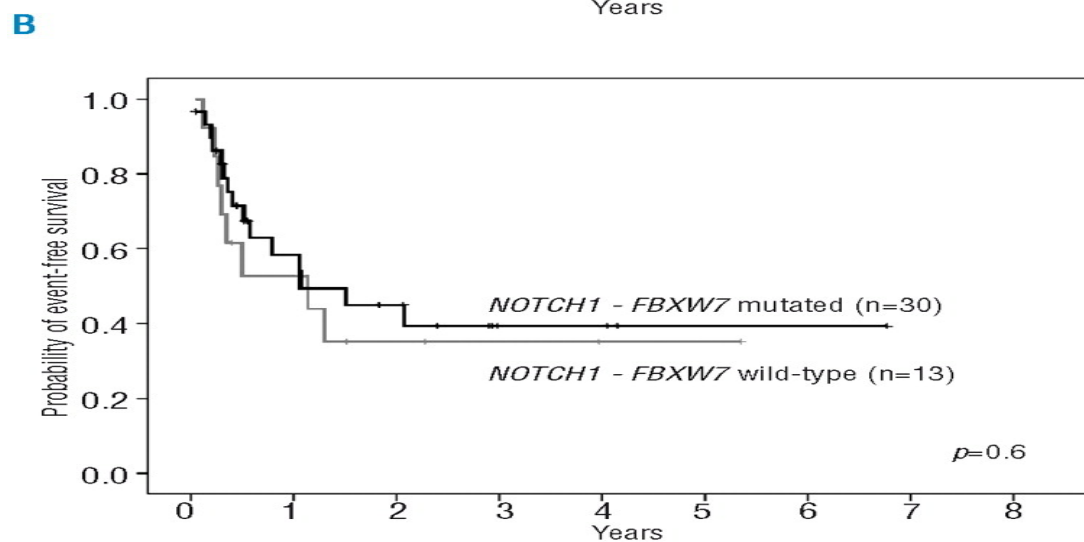




# EFS impact of *NOTCH1-FBXW7* mutations within *ERG/BAALC* expression groups

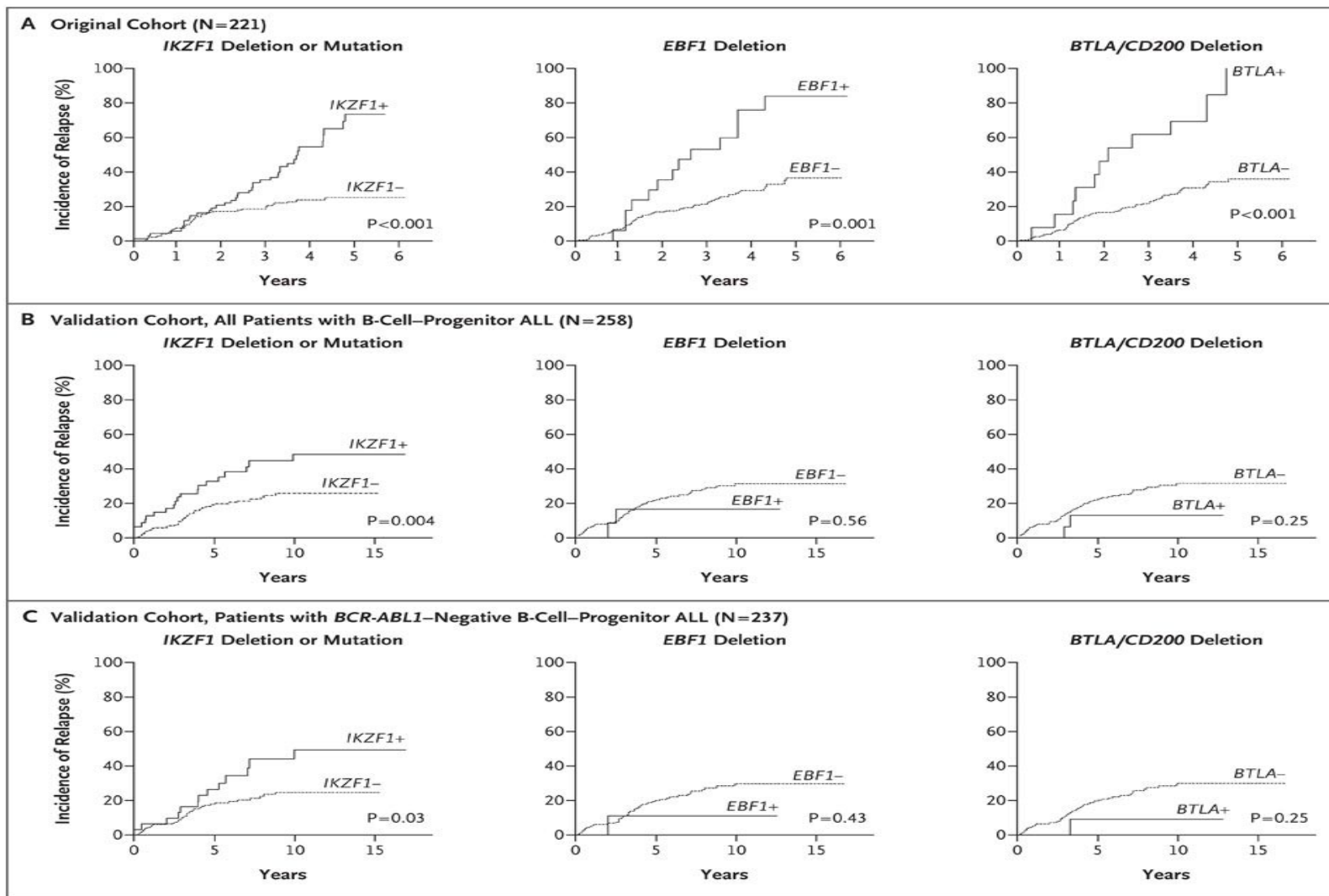


Low *ERG/BAALC*

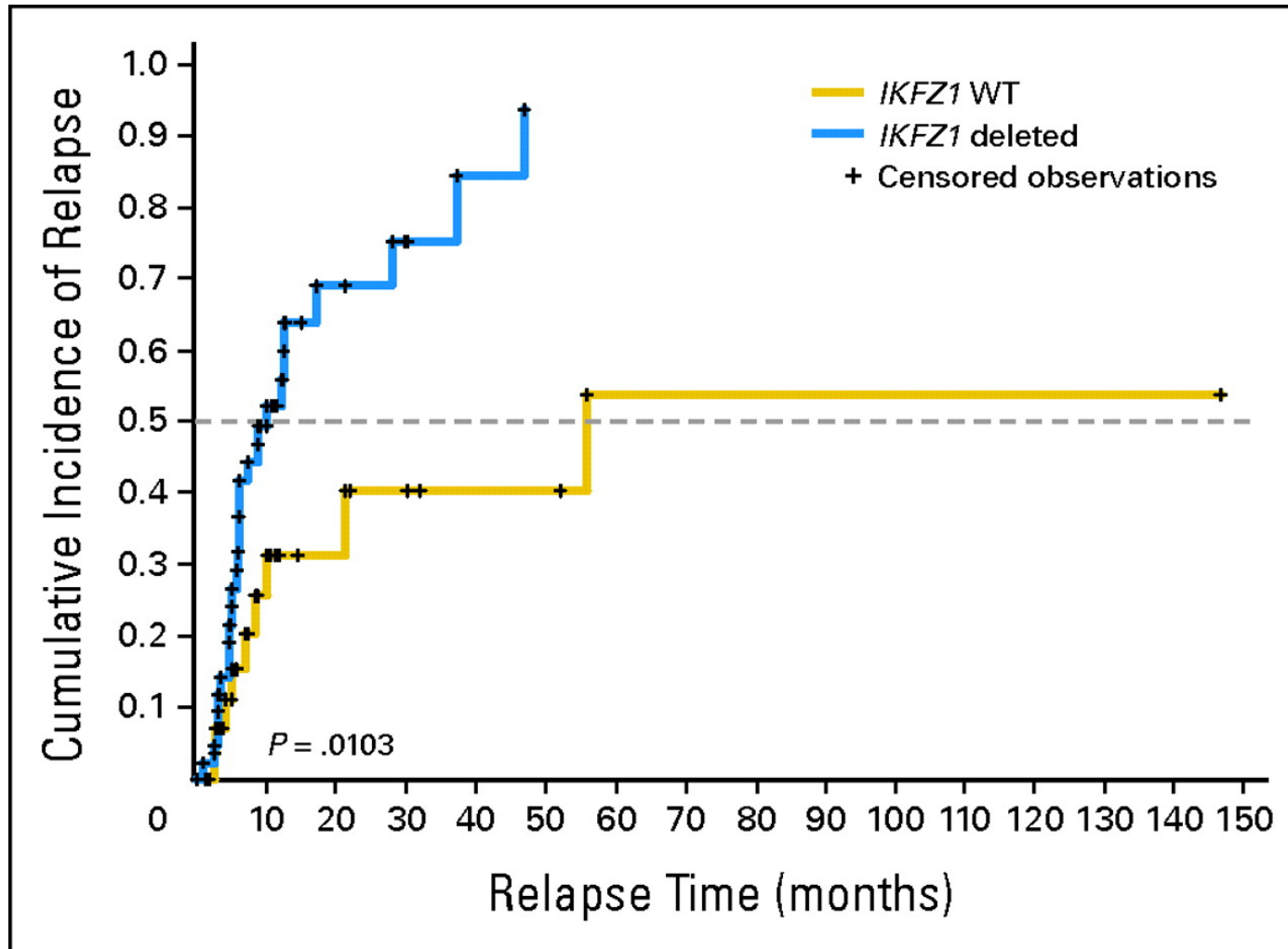


High *ERG/BAALC*

# Genetic Alterations of IKZF1, EBF1, and BTLA/CD200 and the Cumulative Incidence of Relapse in the Original Cohort



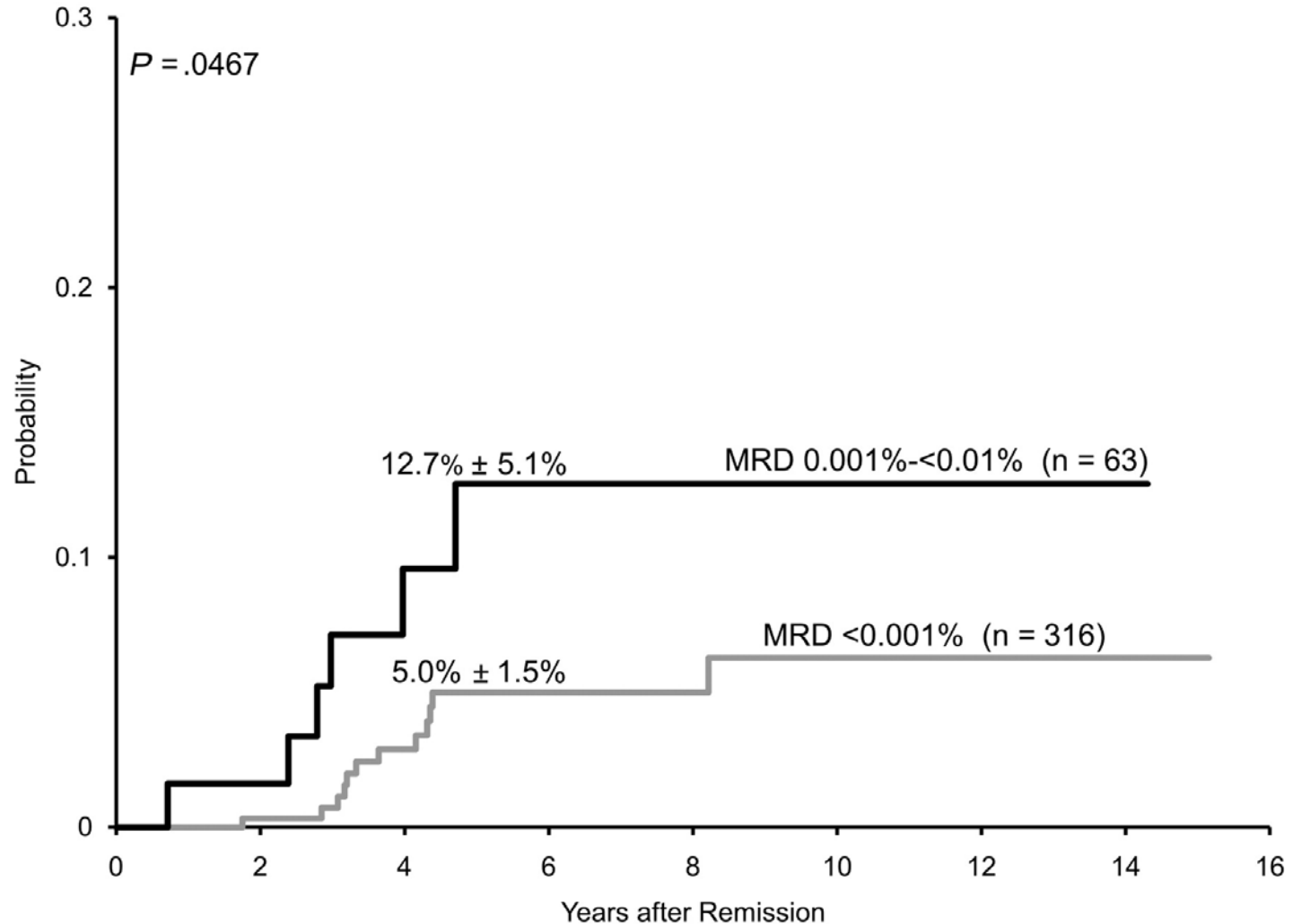
# CIR according to *IKZF1* deletion in *BCR-ABL*+ ALL



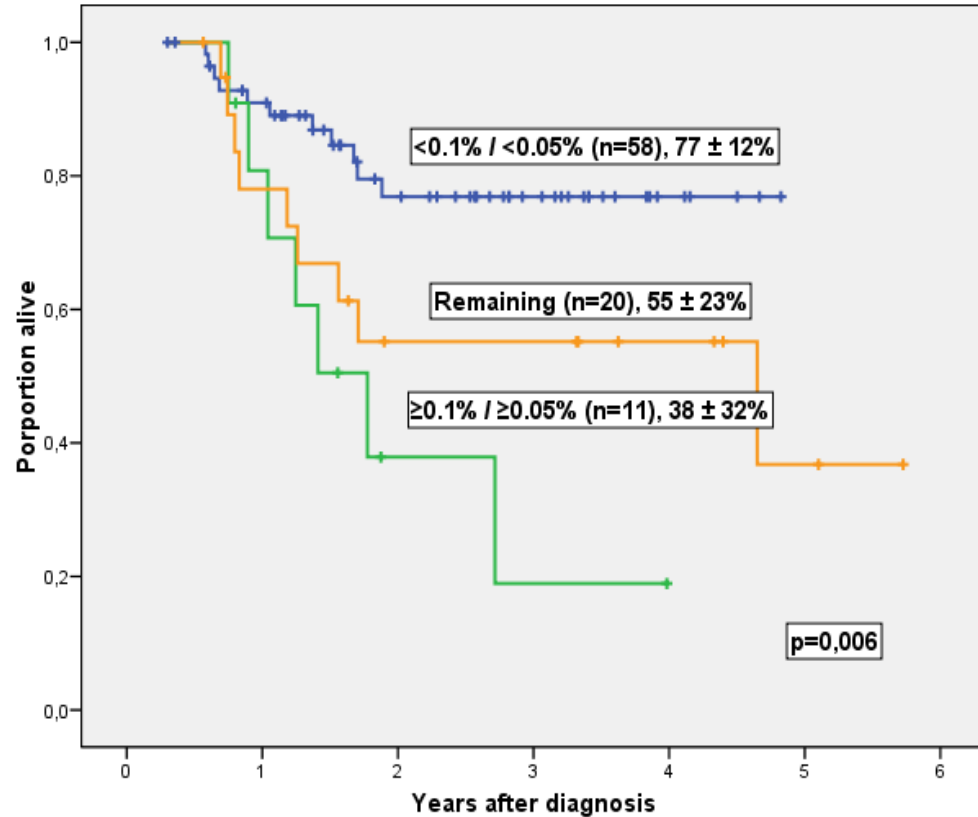
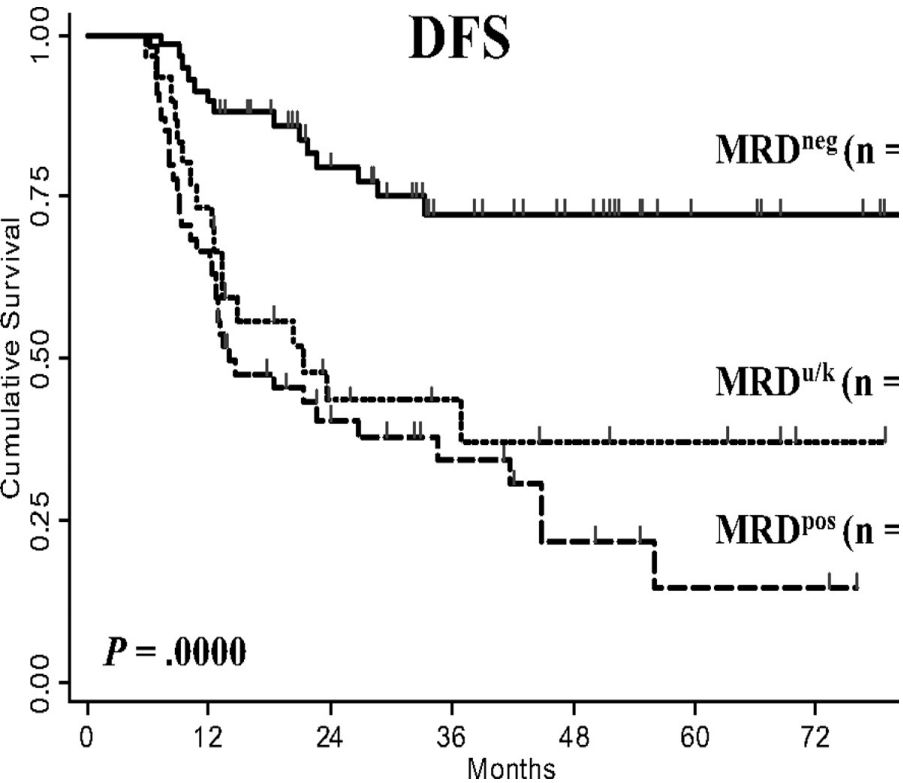
# Usefulness of diagnostic work-up

- Diagnosis
- Prognosis
- **MRD evaluation and follow-up**
- Early detection of relapses

# CIR among 379 children with B-lineage ALL whose MRD levels were less than 0.01% on day 46



# Prognostic significance of MRD in adult ALL



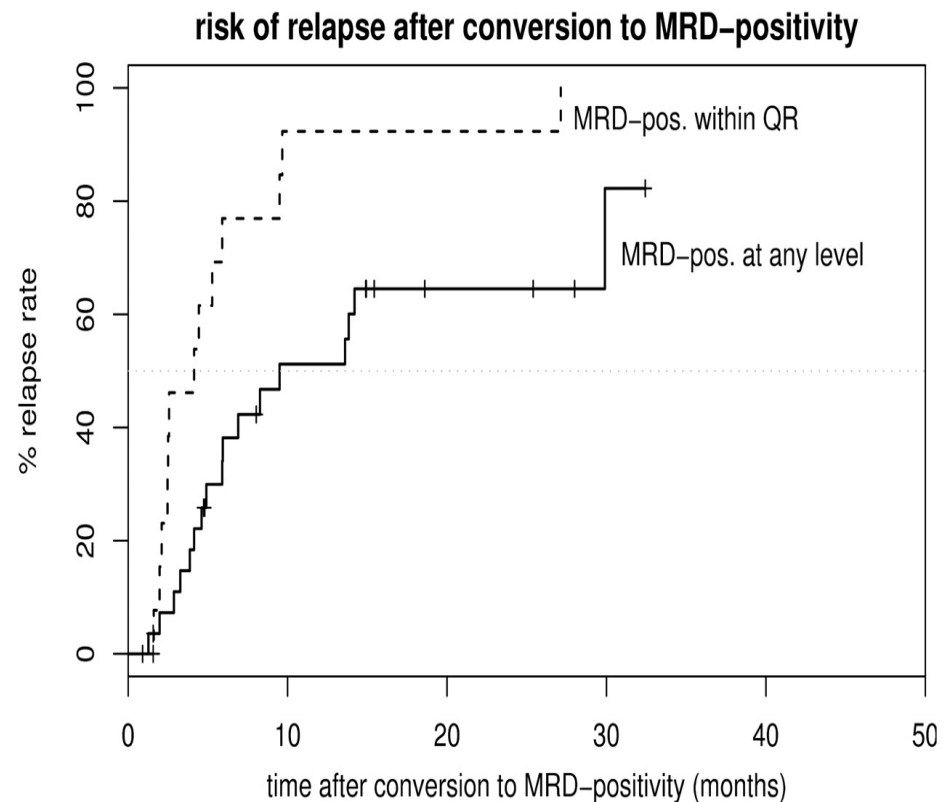
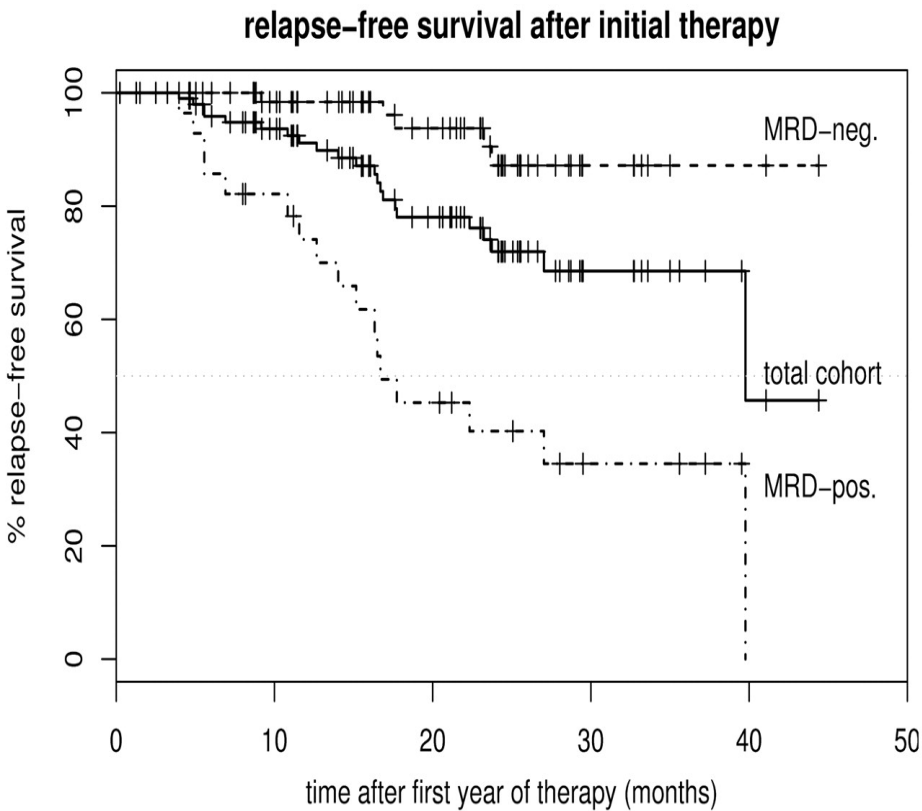
Bassan R, et al. Blood 2009; 113: 4153-4162

JM Ribera et al, ASH 2009

# Usefulness of diagnostic work-up

- Diagnosis
- Prognosis
- MRD evaluation and follow-up
- **Early detection of relapses**

# MRD as a Predictor of Relapse in Adults with Standard-Risk, Ph-negative ALL





# Clinical case

Female, 35 years-old

**Clinical picture:** weakness, gum bleeding and fever in the last 15 days

**Physical exam:** pale, petechiae and ecchymoses in arms and legs, gum bleeding, liver enlargement (3 cm below right costal margin)

### **Complete blood count**

Hb 88 g/L, hematocrit 0,24 L/L, MCV 90fL, WBC count  $48 \times 10^9/L$  (20% N, 30% L, 50% blasts), platelet count  $15 \times 10^9/L$ , coagulation status normal

### **Serum biochemical parameters**

Uric acid 8.8 mg/dL, LDH 2230 U/L.

**Chest X-ray film:** normal

**EKG:** normal

**BM aspirate:** 98% blasts, lymphoid appearance

**Cytochemistry:** peroxidase negative

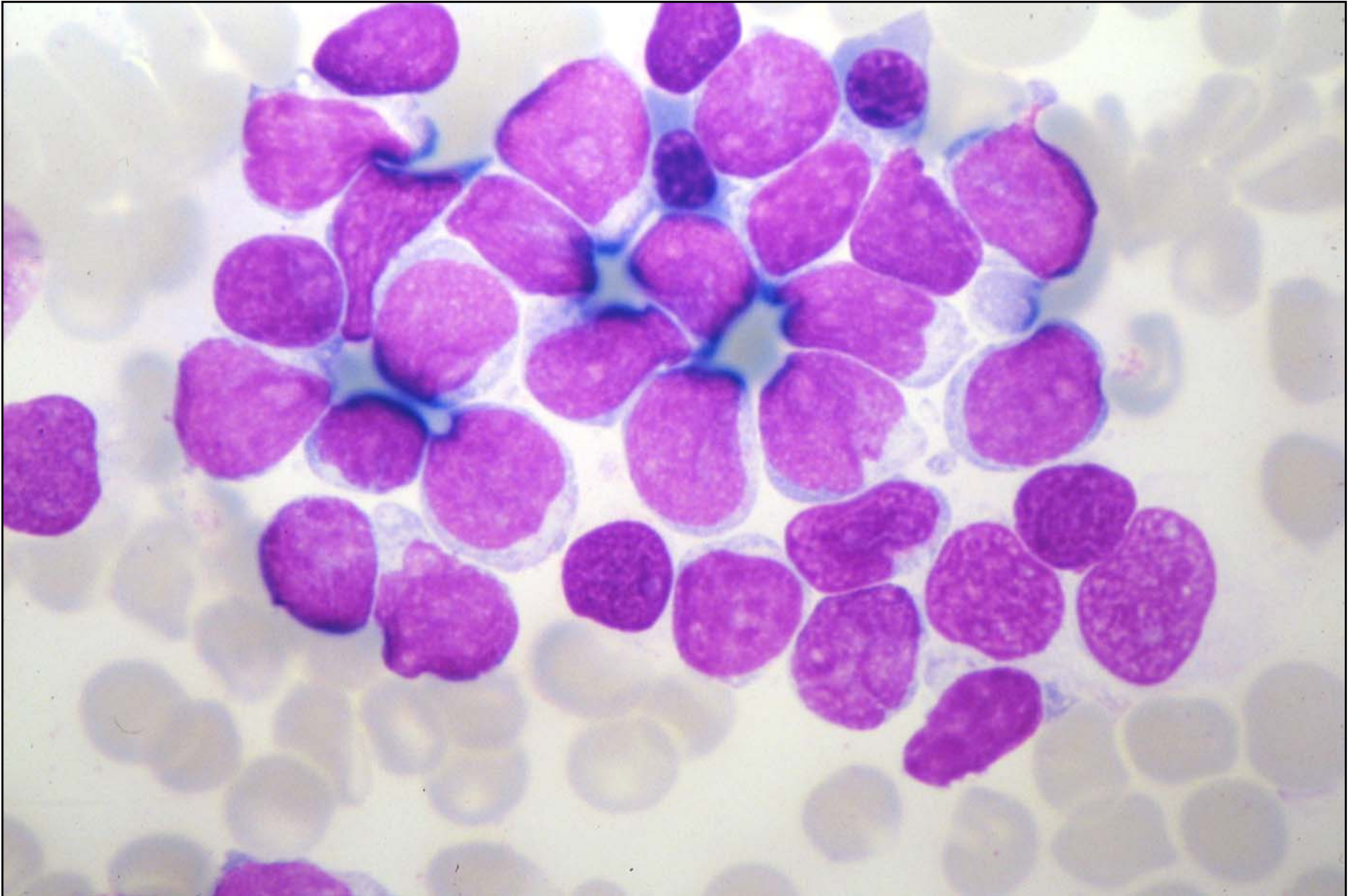
**Citogenetics:** 46, XX, t(9;22)(q34.1;q11.2)[22]

**Immunophenotypic study:** Precursor B-ALL CD20+, with myeloid markers

**Molecular biology:** *BCR-ABL*, p190

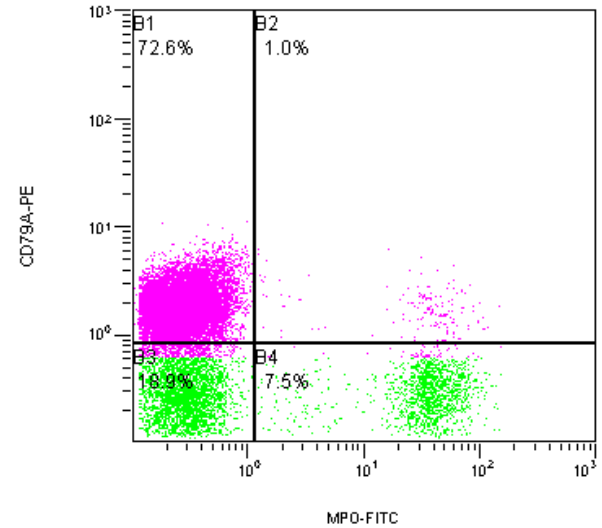
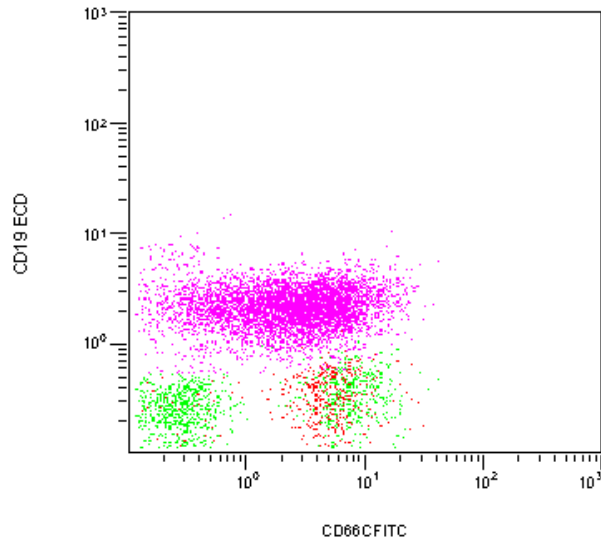
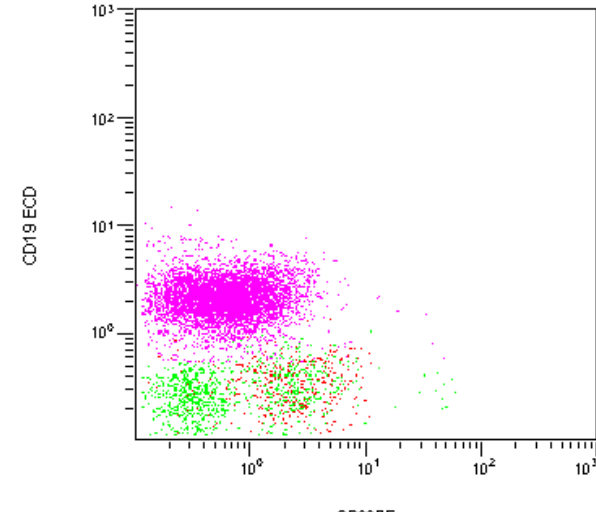
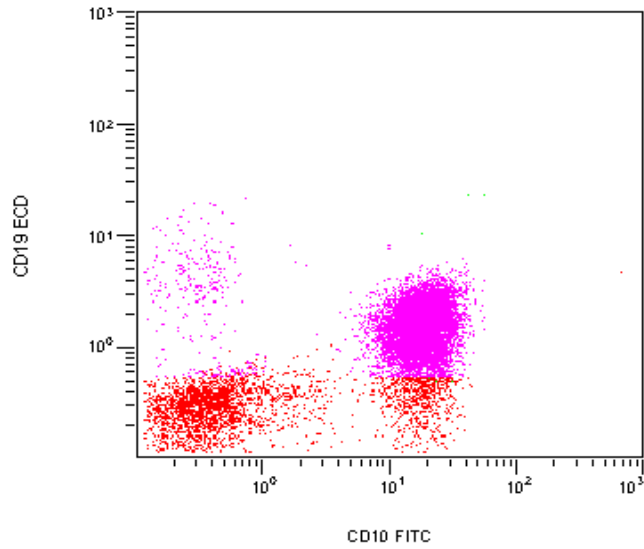
**CSF study:** normal

# May-Grünwald-Giemsa

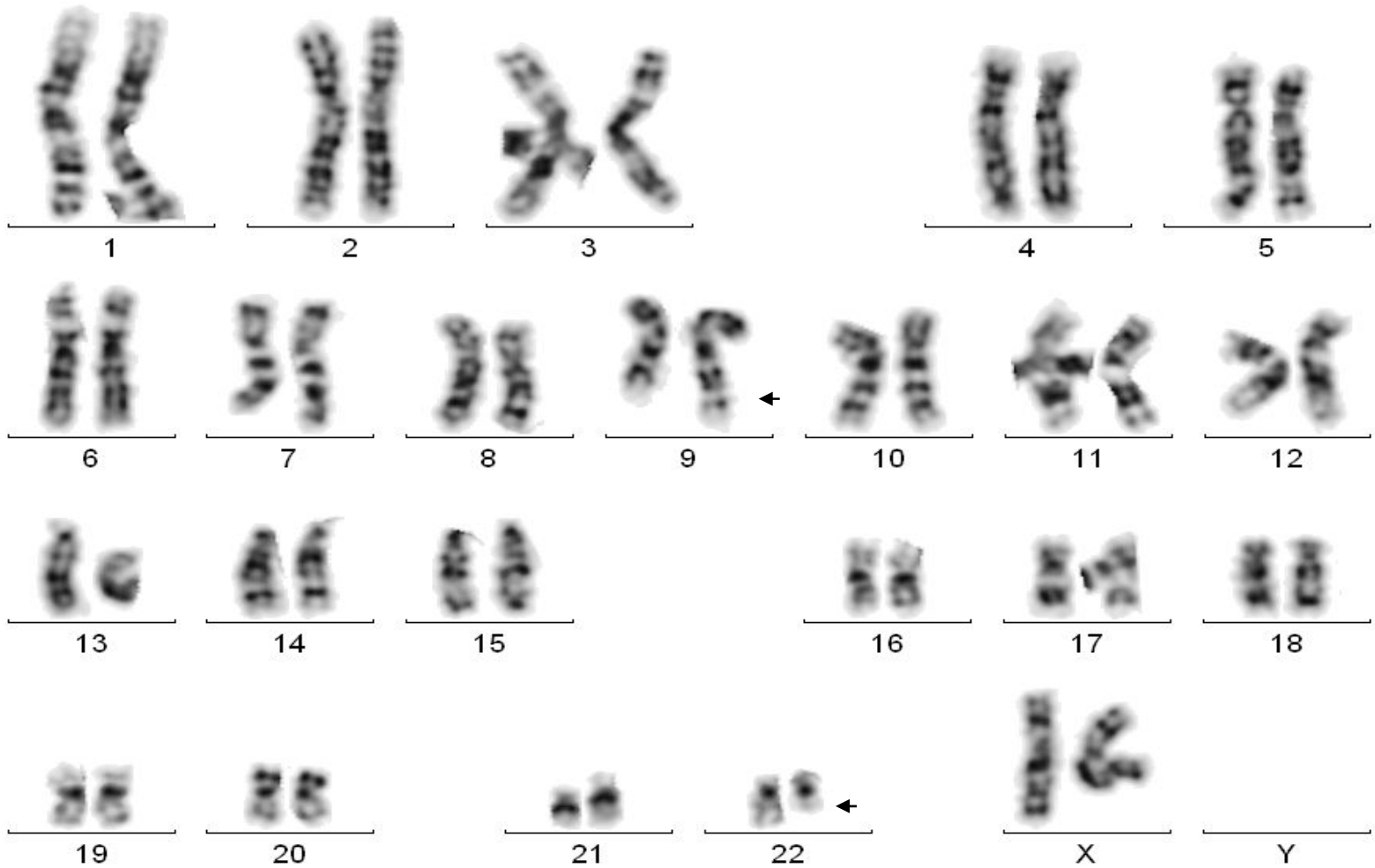


# Flow Cytometry

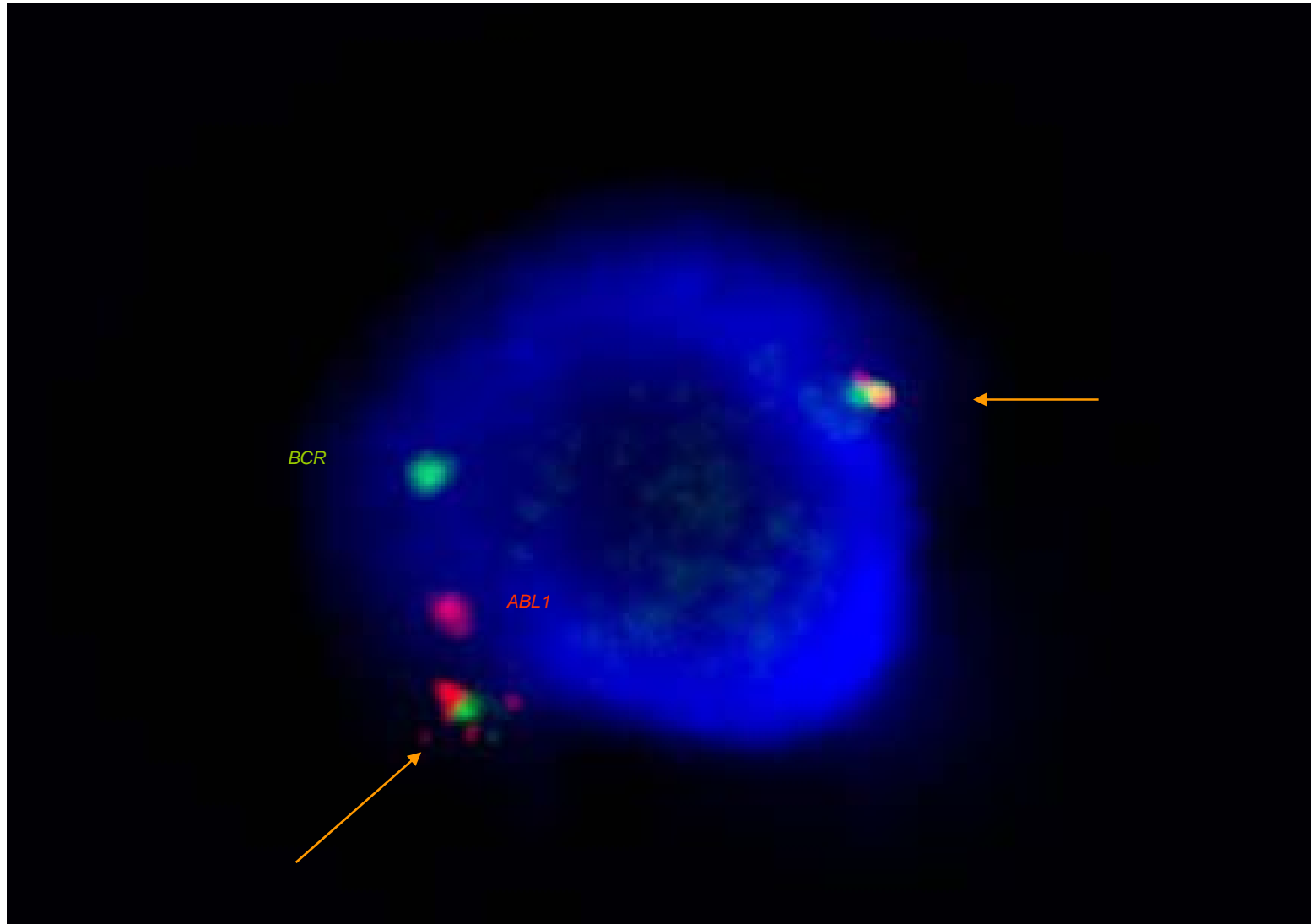
CD20+ ALL with My: CD33+; CD66C++



# 46, XX, t(9;22) (q34.1;q11.2) [22]

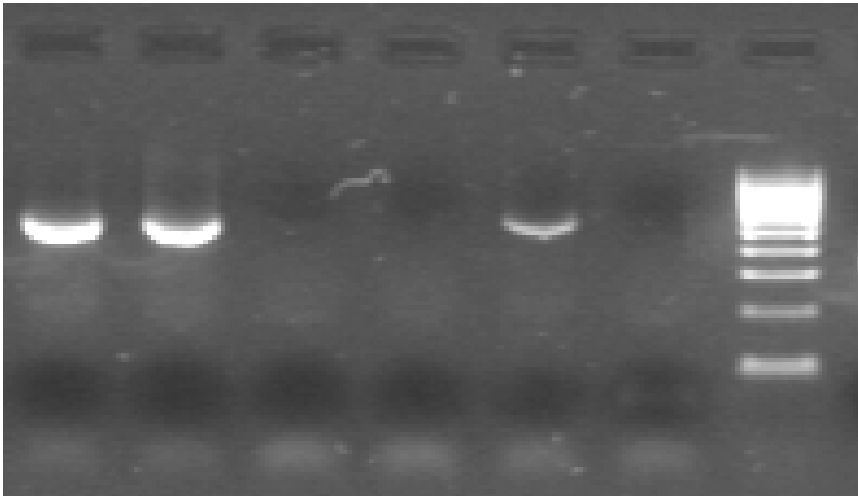


# *FISH. BCR-ABL*



# p190 BCR-ABL

1 2 3 4 5 6 7



- 1 & 2: Patient 1 (positive p190)
- 3 & 4: Patient 2 (negative p190)
- 5: Positive control p190
- 6: Negative control
- 7: Marker of molecular weight

**[(BCR-ABL)/ABL]x100: 130.12**

# Treatment

## Induction:

- Imatinib, VCR, DNR, PDN (clinical trial CSTIBES02)
- **Result: Complete remission**
- **[(BCR-ABL)/ABL]x100: 0.032**

## Consolidation-1

- Imatinib, HD-MTX, HD-ARA-C
- **[(BCR-ABL)/ABL]x100: 0.0079**

## Allogeneic SCT from a HLA-identical sibling

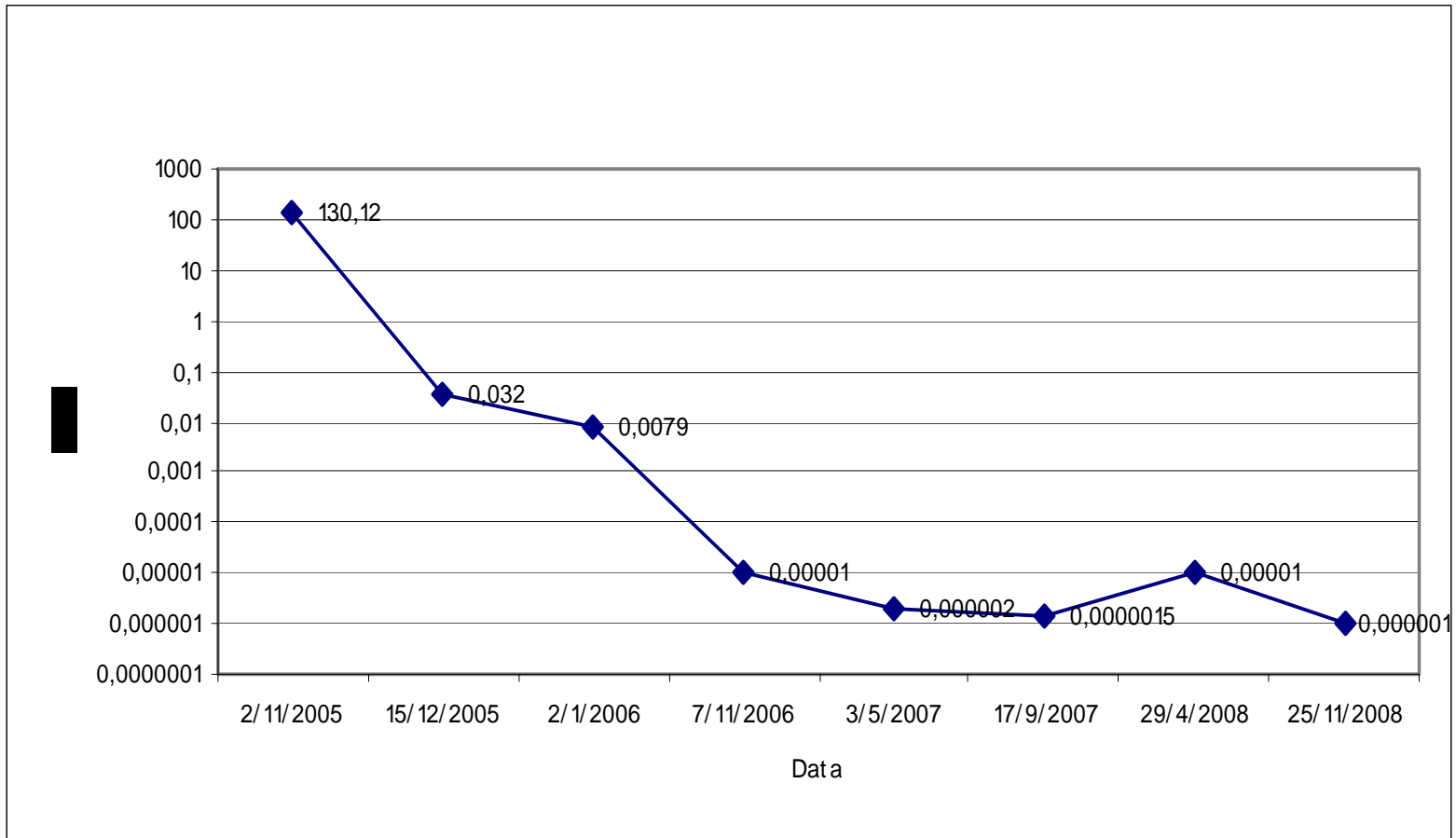
- Conditioning regimen: cyclophosphamide + ICT
- Grade 2 cutaneous acute GVHD
- Chronic GVHD with limited skin involvement
- **[(BCR-ABL)/ABL]x100: 0.00001**

## Imatinib post TPH

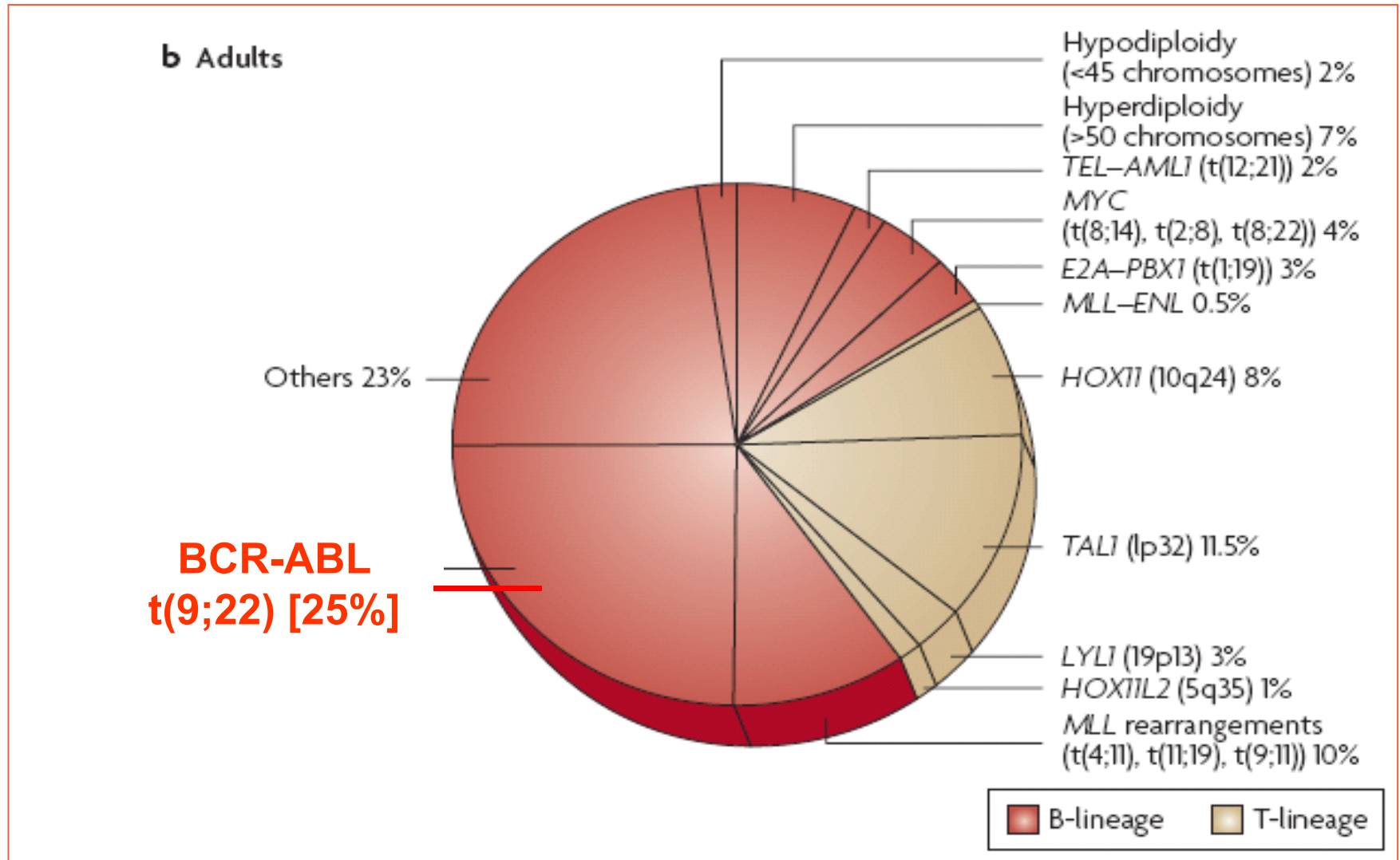
- Well tolerated
- **[(BCR-ABL)/ABL]x100: 0.000003**
- Sustained complete molecular remission

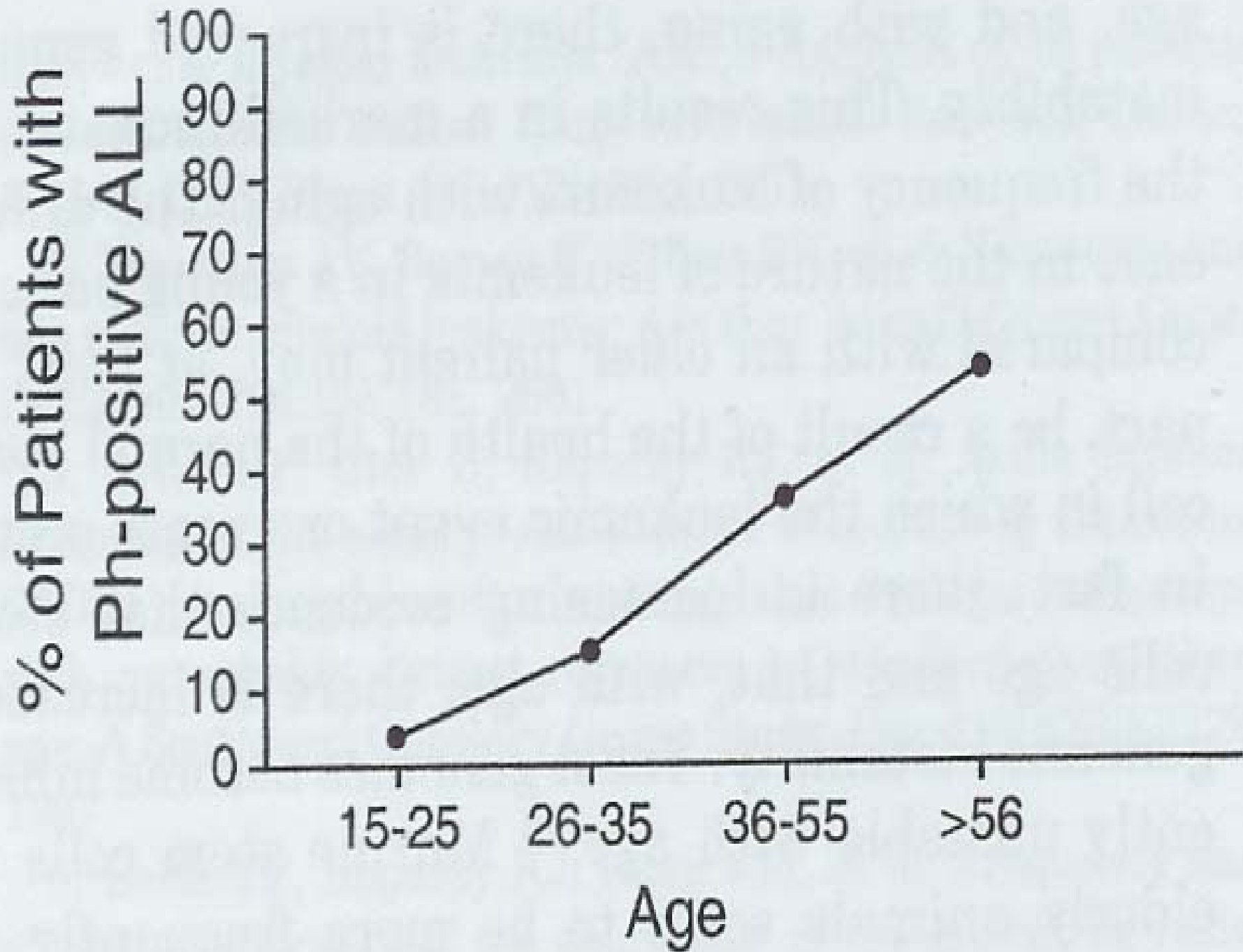


# Molecular follow-up (RQ-PCR)



# Genetic Heterogeneity in Adult ALL



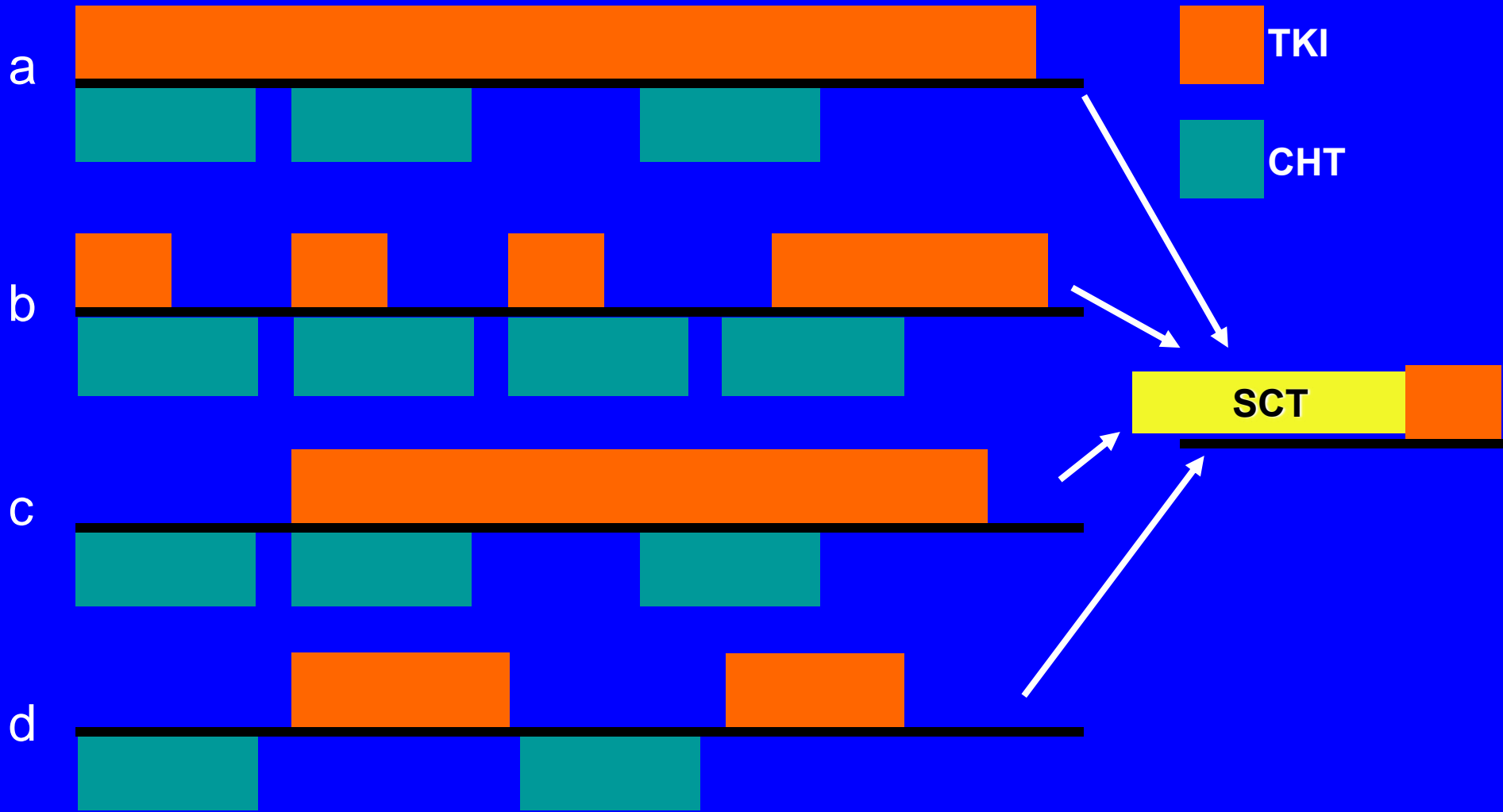


# Ph+ ALL

TKI era

**Imatinib+CHT → Allo-SCT → Tx post TPH**

# TKI+CHT. Combination modalities



# Efficacy of imatinib in combination with chemotherapy in newly diagnosed Ph(+) ALL

	Thomas et al. (n=20)	Towatari et al. (n=24)	Lee et al. (n=20)	Ribera et al. (n=30)
Induction regimen	Hyper-CVAD	CYP, DNR, VCR, PDN	DNR, VCR, PDN, ASP	VCR, DNR, PDN
CR	93%	96%	95%	90%
Induction mortality	NR	4%	5%	7%
Death CR	25%	NR	35%	10%
SCT	50%	63%	85%	70%

Thomas D, et al. *Blood* 2004;103:4396–4407; Towatari et al. *Blood*. 2004;104:3507–12; Lee K-H, et al. *Leukemia* 2005; 19, 1509–16. Ribera JM, *Haematologica* 2010

# PH+ ALL in the TKI era

## Unsolved questions

- **Induction**

- Intensity of CHT, number of cycles?
- Type of TKI. Combination of TKI?

- **SCT**

- Always?
- Modality?
- MRD status at SCT

- **Maintenance after SCT**

- Always or in MRD+ status?
- Type of TKI
- TKI + other cytotoxic/immunomodulatory drugs?
- Duration?



**Thank you!**

White blood cells from a patient with acute lymphoblastic  
leukaemia

Lancet Oncology 2009